GENETIC IMPROVEMENT OF ADAPTED ETHIOPIAN CHICKPEA (*Cicer arietinum* L.) CULTIVAR FOR DROUGHT TOLERANCE THROUGH CONVENTIONAL AND MARKER-ASSISTED BACKCROSS BREEDING METHODS

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DEDICATION

This thesis is dedicated to my beloved mother, Rufie Genemo Wako, who is naturally wise, humble and kind to all; undefeated by the vagaries of poverty and oppressive cultural marital life, she has raised us with determination, affection and great discipline. She is my icon of high integrity, ethics and humanity. I also dedicate this thesis work to my late brother, Mohammed Jarso, who passed away in his early twenties, at the time he would have ripped the fruit of his educational endeavors. I always miss him!

DECLARATION

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

Signed on 30th of May 2017, The School of Graduate Studies, Faculty of Life Sciences, Addis Ababa University, Arat Kilo.

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ACRONYMS AND ABBRIVIATIONS

AAU = Addis Ababa University

AFLP = amplified fragment length polymorphism

ANCOVA = analysis of covariance

ANOVA = analysis of variance

BC_1 = first backcross

BC_1F_1 = first backcross, first filial generation

BC_2F_1 = second backcross, first filial generation

BC_3F_1 = third backcross, first filial generation

BC_3F_2 = third backcross, second filial generation

BC_3F_3 = third backcross, third filial generation

BLAST = basic local alignment search tool

BMY = biomass yield

BNF = biological nitrogen fixation

Bp = base pair

cDNA = complementary DNA

CEG = Center of Excellence in Genomics

CGIAR = Consultative Group on International Agricultural Research

CIAT = International Center for TropicalAgriculture

CID = carbon isotope discrimination

CIMMYT = International Maize and Wheat Improvement Center

CLSs = clusters

CSA = Central Statistics Authority (of Ethiopia)

CTAB = cetyltriethylammonium bromide

CV = coefficient of variation

D^2 = Mahalanobis D square

DArT = diversity array technology

DEGs = differentially expressed genes

DNA = deoxyribonucleic acid

DNase = dioxyribonuclease: enzyme used in the elimination of DNA from RNA

DSI = Drought susceptibility index

DTF = number of days to flowering

DTGF = number of days to grain filling

DTM = number of days to maturity

DWt = Dry weight

DZARC = Debre Zeit Agricultural Research Center

EIAR = Ethiopian Institute of Agricultural Research

EMA = Ethiopian Mapping Authority

EST = expressed sequence tag

ET = evapotranspiration

F_1 = first filial generation

FAO = Food and Agriculture Organization (of the United Nations)

FAOSTAT = FAO statistics

FPKM = fragments per kilobase of exon per million mapped reads

FW = full water

FWt = fresh weight

GA = genetic advance from selection

GAM = genetic advance as a percentage of the mean

GCV = genetic coefficient of variation

GEBV = genomic estimated breeding value

GG = genetic gain

GM = geometric mean productivity

GO = gene ontology

GS = genomic selection

GYFW = grain yield under full-water condition

GYLD = grain yield

GYWS = grain yield under water-stress condition

H^2_b = broad sense heritaility

HI = harvest index

HM = harmonic mean

HSW = hundred seeds weight

HT1 = hydration buffer

IBM Corp. = International Business Machines Corporation

ICARDA = International Center for Agricultural Research in the dry Areas

ICRISAT = International Crop Research Institute for the Semi-Arid Tropics

InDels = insertions/deletions

IRRI = International Rice Research Institute

ISSR = inter-simple sequence repeat

LAI = leaf area index

LAPP = leaf area per plant

LDWt = leaf dry weight

LSU = learning system unity (of ICRISAT)

μ = population mean

μL = microliter

MABC = marker-assisted backcrossing

MARS = marker-assisted recurrent selection

MAS = marker-assisted selection

MB = molecular breeding

MDB = membrane desalting buffer

MP = mean productivity

mRNA = messenger RNA

N = nitrogen

NaOH = sodium hydroxid

NH^+_4 = ammonium

nM = nanometer

NO_3 = nitrate

OC = organic carbon

PC = principal component

PCR = polymerase chain reaction

PCV = phenotypic coefficient of variation

PE = paired-end read

PHT = plant height

PPP = number of pods per plant

QC = quality control algorithm for filtering single nucleotide polymorphisms (SNPs)

QTL = quantitative trait loci

RA3 = wash buffer RA3

RAPD = random amplified polymorphic DNA

RAW2 = wash buffer RAW2

rDNase = recombinant DNase that is used to remove genomic and template DNA from RNa

RDWt = root dry weight

RFLP = restricted fragment length polymorphism

r_G = genetic correlation

RLD = root-length density

RNA = ribonucleic acid

RNA-seq = ribonucleic acid sequencing (RNA sequencing)

RP = recurrent parent

r_P = phenotypic correlation

RR = relative performance reduction

RWC = relative water content of leaf

σ = standard deviation of the population

SAS = statistical analysis system

SCAR = sequence characterized amplified regions

ShDWt = shoot dry weight

SNP = single nucleotide polymorphism

SPP = number of seeds per pod

SRR = shoot to root ration

SSI = stress susceptibility index

SSR = simple sequence repeat

StDWt = stem dry weight

STI = stress tolerance index

TILLING = targeting induced local lesions in genomes

TL-1 = Tropical Legume Phase 1 project

TOL = tolerance index

Tw = turgid weight

VARCOMPs = variance components

WS = water-stress

WUE = water-use efficiency

WUEBMY = water-use efficiency for biomass yield

WUEGY = water-use efficiency for grain yield

YI = yield index

YSI = yield stability index

ABSTRACT

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume both in area and production next to common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.), grown mainly by small-scale farmers in the semi-arid tropics, West Asia and North Africa, and sub-Saharan regions, including Ethiopia. It an affordable cheap source of protein, one of the export commodities for cash and plays important role in soil amelioration. However, currently its average national yield is 1.91 t/ha when its potential could reach 5.5 t/ha. The low productivity is attributed to biotic and abiotic stresses, of which drought is accounting for 40-50% globally. Genetic manipulation of the crops through breeding for drought tolerance is among the ways to combat this problem. In recent years, tremendous progresses have been made in the development of novel genetic tools such as DNA molecular markers, dense genetic maps, and whole-genome transcription profiling techniques to identify genomic regions and genes underlying plant stress responses. The root traits such as root length-density have been also proposed as the main drought avoidance traits that contribute to yield under terminal drought environments in chickpea. Major genomic region containing QTL for drought related traits contributing up to 36% phenotypic variation have been identified by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). However, these markers have not yet been validated and been deployed into the Ethiopian chickpea breeding program. Even the already released Ethiopian chickpea cultivars for optimum environment have never been systematically evaluated for their response to drought tolerance. Ethiopia has vast area coverge under drought-prone zones and smallholder farmers are growing chichpea under this sub-optimal environment, but not specific breeding program in place for drougt tolerance. In this study, therefore, I have conducted two set of experiments: (1) introgression of drought tolerance genes from a drought tolerant donor parent (ICC 4958 obtained from ICRISAT, India) to the adapted high yielder Ethiopian chickpea cultivar, Ejere and (2) screening the improved cultivars for drought tolrance.

A totao of 208 BC_3F_4 isogenic lines (ILs) were developed through MABC from by crossing ICC 4958 and Ejere, these ILs were tested in multi-location field trial under full-water (FW) and under water-stress (WS) conditions to select ILs tolerant to moisture stress with good agronomic traits. Data were collected on number of days to flowering (DTF), maturity (DTM)

and grain filling (DTGF), number of pods per plant (PPP) and seeds per pod (SPP), plant height (PHT), hundred seed weight (HSW), biomass (BMY) and grain yields (GYLD) and harvest index (HI), and subjected to statistical analyses. The result of the ANOVA indicated that there were significant differences among the ILs for the traits at each location and combined over locations under both FW and WS conditions. Significant difference was also observed beteen the locations and genotype by location interaction for almost all the traits.

There relative reduction (RR) in traits' means due to WS for almost all the traits except DTF and SPP ranged from 0.2% for DTF to 41% for GYLD. The RR of GYLD for different genotypes ranged from 19.0 to 41% with average of 37.5%. The highest RR in GYLD was recorded for the recurrent parent (41%) followed by the ILs without markers (26.1%), while the least (19%) was for best ILs with markers for drought tolerance. Of the yield components, PPP had the highest RR ranging from 23%- 41%. Moderate to high RR were recorded for the rest of the traits too. Under FW condition, ILs 5-9, 7-19, 37-3, and 37-4 with markers and ILs lines 5-10, 18-3, 18-5, and 19-4 without markers were among top 10% selected for high GYLD. Under WS condition, the ILs 9-7, 10-10, 29-6, and 38-2 with markers and ILs 7-14, 27-2, 30-5, and 34-2 without markers were among selected top 10%. The analysis of drought tolerance index (DSI) revealed that HI, GYLD, DTF and PPP had high (above unity) DSI while SPP had the least (below unity) under FW; but under WS, DTF, HI and HSW had high DSI while GYLD, SPP, BMY, and PPP had low DSI. The selected ILs under WS tolerated the stress by low susceptibility of GYLD, BMY, SPP and DTGF.

The cluster analysis has grouped the 208 ILs and the parents into 12 distinct clusters under FW and 10 clusters under WS. There was high genetic divergence for the genotypes under FW than under WS as revealed by the distances among the clusters. The principal component analysis has also revealed that PPP, BMY, HI and GYLDs accounted for larger proportion of the variations in the genotypes under both FW and WS while DTGF under WS only. The variance component analysis has showed the existence of high genetic variations among the tested ILs for studied traits. High genetic variations were recorded for GYLD, BMY and PPP along with high genetic coefficient of variation (GCV) under both FW and WS. Broad sense heritability (H^2_b) of the traits ranged from 69% for DTF to 96% for PHT under FW and 55% for SPP to 85% for PPP under WS. The GCV and H^2_b were higher under FW than under WS condition. The expected genetic gain (GA) from selection and the GA as a

percentage of the mean (GAM) under FW condition were high for PPP (10 pods and 36%), GYLD (14.3g and 29.6%), PHT (7.7cm and 24.2%), HI (6.6% and 21.4%) and HSW (5.3g and 18.4%), respectively. Under WS condition, GA and GAM were high for GYLD (10.7g and 29.9%), PPP (7.2 pods and 25.7%), BMY (27.9g and 24.2%), respectively. Traits with high GCV and H^2_b values have resulted in high GA and GAM as compared to traits with low GCV and / or H^2_b. The genetic correlations (r_G) among traits ranged from -0.41 to 0.98 under FW and -0.67 to 0.96 under WS conditions. Grain yield had strong positive correlations with PPP, BMY, and HI (r_G=0.67 to 0.99) under both FW and WS; it had strong positive correlation with DTM (r_G =0.40) and DTGF (r_G=0.53) whereas strong negative correlation with DTF (r_G=-0.44) only under WS. GYLD had medium positive or negative genetic correlation with SPP, PHT and HSW under FW and WS conditions.

In the sequence analysis of the selected two ILs (5-12 and 38-2) and the two parents, 293.3 million paired end (PE) raw reads were generated from control and stress root tissue samples. A total of 272 (92.8%) million PE reads were aligned of which 88.8% were concordantly aligned and 11.2 % were non-concordantly aligned. A total of 48,698 transcripts were assembled using Cufflinks and subjected to differential gene expression analysis. Gene expression was identified up- and down- regulated and differentially expressed genes. A total of 1,088 differentially expressed unique transcripts were functionally categorized based on gene ontology (GO) into three principal categories: molecular function (401), biological process (436) and cellular component (251). In total, 9,393,545 SNPs identified among different combinations across the eight chickpea chromosomes that could be further exploited for molecular breeding of chickpea.

The ANOVA for the released cultivars tested for drought tolerance showed significant differences between the cultivars for most of the agronomic traits under both FW and WS conditions. There was significant difference between the soils for GYLD, RWC, LAPP, LAI and WUEGY under both FW and WS while for EV and HI under WS condition only. Soil by cultivar interaction was significant for EV, DTF, HI, GYLD and WUEGY under both FW and WS, while for DSI under WS condition only. There was also significant differences between the soils, the crop stages and the cultivars for BMY, leaf, stem, root and shoot dry weights (LDWt, StDWt, RDWt, and ShDWt, respectively), shoot-root ratio (SRR), transpiration (TP) and WUEBM. Almost all the interactions were significant for all the traits. Under WS condition, there was significant difference between the soils, destruction levels and cultivars for all the traits and all the interaction, except for

WUEBM. Mean GYLD of the cultivars under FW ranged from 6.65 to 28.45g per five plants with mean of 15.93g and 3.54 to 27.42g under WS condition. Cultivars Shasho, Marye, Natoli, Ejere, and ICC 4958 had high GYLD while DZ-10-4 and Dz-10-11 had low GYLD under FW; cultivars Arerti, Natoli, ICC 4958 and Minjar had high GYLD while Acos Dubie, Akaki, Akuri and Dz-10-11 had low GYLD under WS. The high GYLD of the cultivars was due to high BMY, PPP and HI under both FW and WS. In addition, these cultivars had high TP, WUEBM and WUEGY under both FW and WS, and high ShDWt, SRR and LAI under FW while high RDWt, LAPP, RWC under WS. For agronomic traits, the highest overall mean reduction (MR) due to WS was scored for GYLD (28%) followed by BMY (24%). The overall MR in DTM, DTGF, SPP, and HSW was negligible. There was clear difference between the cultivars for MR; Mean GYLD reduction for the cultivars ranged from 12 to 70%. For physiological traits, the MR for LDWt (28%), ShDWt (23%), LAPP (23%), LAI (21%), StDWt (20%), WUEGY (16%), RDWt (12%), and SRR (11%). The WUE of the cultivars for GYLD ranged from 2.7 to 9.4% with overall mean of 5.4% under FW and 5.7 to 19.4% with overall mean of 12.2% under WS condition. Cultivar Shasho was the most efficient (9.4%) while Dz-10-4 and Dz-10-11 were the least (2.7% each) for GYLD under FW while Arerti was the most efficient (9.4%) followed by ICC 4958 (8.3%) and the least for Dz-10-11 (1.9%) under WS condition. Water-use efficiency of the cultivars for BMY had similar trend with that of GYLD and it ranged from 5.7 to 19.4% with overall mean of 12.2% under FW and from 4.5 to 19.6% with the overall mean of 10.1% under WS. This result clearly revealed that a cultivar that is water use efficient under FW condition may not necessary be efficient under WS and *vice-versa*. Cluster analysis grouped the 20 cultivars into five distinct clusters under both FW and WS conditions and are recommended based on their efficiency to the two environments. The pair wise generalized square distances among the clusters were significant for most of the cluster pairs indicating genetic divergence between cultivars in the different clusters. Principal component analysis confirmed that trait like GYLD, StDW, ShDWt, WUEBM, WUEGY have accounted for the highest proportion of the genetic variability in the tested cultivars under both stress levels.

In conclusion, the introgression of drought tolerance QTLs into the adapted high yielding Ethiopian cultivar has resulted in ILs with high yield and water-stress tolerance; screening of the already released cultivars for optimum environment has also enabled identification of some cultivars that are relatively better tolerant to water-stress condition with reasonable grain yields.

1. INTRODUCTION

1.1. Background and Justification

Chickpea (*Cicer arietinum* L., 2x = 2n = 16) is the world's third most important food legume both in area and volume of production next to common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (FAOSTAT, 2014). The crop is grown mainly by small-scale farmers in the semi-arid tropics and West Asia and North Africa, and sub-Saharan regions (Gaur et al., 2010). It is cultivated in about 50 countries worldwide on approximately 13.98 million ha of land and total production of 13.73 million tonns, the average productivity being 1.0 t/ha glogally (FAOSTAT, 2014) and 1.91 t/ha in Ethiopa (CSA, 2014/15). According to the same source, Asia alone contributes 85.2% of the total production in the world. The major producing countries include India (9,880,00 t from 9,927,000 ha), Pakistan (399,030 t from 949,513 ha), Turkey (450,000 t from 338,169 ha), Australia (629,400 t from 507,800 ha), Myanmar (562,163 t from 384,217 ha), Ethiopia (458,682 t from 239,755 ha), Malawi (65,451 t from 115,126 ha), and Sudan (14,500 t from 8,000 ha). India alone contributes about 72.0% of the produce worldwide, followed by Turkey (3.3%), Ethiopia (3.3%), Pakistan (2.9%) (FAOSTAT, 2014). Africa annually produces about 757,332 t (about 5.5% of the world total), Ethiopia with around 60.0% of the total production in Africa being the leading producer. The other producers in Africa include Tanzania, Sudan, and Malawi.

In Ethiopia, chickpea grows in wider ranges of agro-ecologies with residual moisture on black *Vertisol* on altitude ranging from as low as 1400 m a.s.l. in the Rift Valley to as high as 2600 m a.s.l. in the Central Highlands (Geletu and Ketema, 2006).

Chickpea is an inexpensive source of protein especially for the majority of the poor people who cannot afford animal products. It is one of the export commodities and cash crops in Ethiopia (Geletu and Ketema, 2006) and many other countries (Gaur *et al*., 2010). Like many other legume crops, it also fixes significant amount of atmospheric nitrogen for itself and other crops grown in rotation or association (Rupela and Saxena, 1987). Chickpea also benefit the subsequent crop by solublizing fixed forms of soil phosphorus with root exudates like citrate and malate (Ali *et al*., 2002). Chickpea is also a good "break" crop to different diseases and pest when grown in rotation with cereals. It is a suitable crop for yield maximization, resource-use optimization and risk aversion as it suits double cropping and can be grown with residual moisture even after the failure of the main crop (Rupela and Saxena, 1987; Gaur *et al*., 2010).

The productivity of chickpea has been very low due partly to the biophysical challenges constraining productivity under the smallholder farmers' conditions on the one hand and inadequate technological interventions made to reverse the situation on the other. In order to improve production and productivity in Ethiopia through developing and transferring of improved cultivars along with improved agronomic and crop protection practices, the commencement of research on chickpea dates back to the 1970's. So far, 24 chickpea varieties have been developed and released for different agro-ecologies and recommendation domains in Ethiopia (Geletu and Ketema, 2006). While chickpea as a crop is assumed to have genetic yield potential of up to 5.5 t (Bejiga and van der Maesen, 2006), the improved varieties released in Ethiopia may on average yield 2.8 t/ha on research stations and 1.8 t/ha on farmers' fields (Geletu et al., 2006). Despite the potential and possibilities, however, the average national yield has not significantly been improved in the past two decades in Ethiopia largely due to abiotic

(drought, poor soil fertility, and poor cultural practices) and biotic (diseases, insect pests and weeds) stresses constraining production.

Among the abiotic stresses, drought is the most important constraint to yield in chickpea, accounting for 40-50% of the yield reduction globally (Ahmad et al., 2005). Among the important ways of confronting this challenge is the genetic manipulation of the crops to be grown and the growing environment itself. Genetic modification of the crop to improve yield is often preferred over manipulation of the growing environments due to concerns for agricultural sustainability and also cost factors (Srivastava et al., 1987; Singh, 2002; Keneni, 2007).

Drought may be managed by the use of moisture conserving structures like tie ridging (Nyakatawa, 1996; Omer and Elgalani, 1997; McHugh, et al., 2007) but use of such structures alone may not lead to better yields from inferior genotypes (Singh, 2005). The breeding of drought tolerant genotypes could be one of the stable alternatives to address problems of drought. A number of authors reported that effective drought management could be achieved from genetic improvement through conventional breeding for drought tolerance in crop cultivars. For instance, in chickpea, selections based on large root systems have resulted in efficient genotypes for drought tolerance (Saxena and Johansen, 1990; Saxena et al., 1993). While conventional breeding continues to have a vital role to play in producing drought tolerant cultivars, it appears that in some systems there will continue to be limitations by large number of genes that control and trait conditioning drought tolerance resulting in large genotype x environment interaction.

Recent years have seen tremendous progresse in the development of novel genetic tools such as DNA molecular markers, dense genetic maps, and whole-genome transcription profiling techniques to identify genomic regions and genes underlying plant stress responses. These new tools have improved the efficiency and effectiveness of crop breeding schemes like marker-assisted backcrossing (MABC) and introgression of desirable traits into a single genotype.

The root traits such as root length and root lenth-density have been proposed as the main drought avoidance traits that contribute to seed yield under terminal drought environments in chickpea (Saxena and Johansen, 1990; Saxena et al., 1993; Kashiwagi et al., 2008; Vadez et al., 2008) thus showing the opportunity for enhancing drought tolerance of chickpea by improving root characteristics. Currently, genetic maps and molecular markers associated with genes conferring tolerance to drought have become available through efforts made by the Tropical Legumes-I Project. Major genomic region containing QTLs for root traits, 13-C-isotope discrimination and yield contributing up to 36% phenotypic variation have been identified from ICC 4958 × ICC 1882 population by the International Crops Research Institute for the Semi-Arid Tropics. However, these markers have not yet been validated and been deployed in the Ethiopian chickpea breeding program and, hence, the need for this study. This has been recommended for use in the improvement of the adapted high yielding cultivars of Asia and Sub-Saharan countries including Ethiopia (Saxena, 2003). A previous investigation about gene action of drought tolerance gene indicated that additive, additive by additive interaction and dominance gene effects play important role in governing the root length and density and root dry weight in the population having this QTL (Singh, 2005; Kashiwagi *et al* 2008).

Even though Ethiopia has vast area coverage under drought-prone zones (EMA, 1988) and small holder farmers are growing chickpea in this sub-optimal environment, there was no strong specific breeding program in place for drought tolerance. Where there is shortage of specifically targeted varieties suitable under such circumstances, starting breeding work just from the scratch may take a long time. Rather, the conversion of the otherwise well adapted varieties into their tolerant versions through incorporation of genes conferring drought tolerance seems to be an immediate strategy (Singh, 2003; Gemechu, 2012).

1.2. Objectives

1.2.1. General Objective

- To improve adapted and farmers preferred Ethiopian chickpea cultivars through conventional and marker-assisted backcrossing methods.

1.2.2. Specific Objectives

This experiment was designed with the following specific objectives:

- To identify suitable parental genotypes from among the adapted Ethiopian chickpea cultivars for marker-assisted backcross breeding;
- To convert adapted Ethiopian chickpea cultivars into their drought tolerant versions through introgression and incorporation of drought tolerance genes/QTLs using marker-assisted backcross technique;
- To evaluate and identify drought tolerant chickpea backcross progenies for potential release for drought-prone environments of Ethiopia;

- To conduct gene expression (transcriptome) analyses of drought challenged roots of marker-assisted backcross lines and thereby, elucidate the magnitude and pattern of gene expression for drought tolerance.
- To assess and evaluate the Ethiopian chickpea cultivars released for optimum environments for stress tolerance and determine their water-use efficiency.

2. LITERATURE REVIEW

2.1. The Role of Legumes in the Farming System

It is a well documented fact that legumes play important role in the farming system (Mannetje et al., 1980; Norman, 1982; Haque and Jutzi, 1984). Their most important role is the fixation of atmospheric nitrogen through their symbiotic relationship with *Rhizobium* spp., that are usually associated with the host's root system, leading to two consequential role, i.e., their capacity to increase soil fertility and high levels of protein in the plant tissue (Mannetje et al, 1980). Mannetje *et al* (1980) reported that crude protein levels in temperate legumes, tropical legumes and tropical grass grown under identical environmental condition was, 23.5, 21.2 and 12.2%, respectively, which is 88 and 74%, respectively, higher over the grass.

The ability of legumes in soil fertility improvement is very well recognized and used by farmers since antiquity (Hirsch, 2009), even before the discovery of symbiotic association between bacteria and the host plants (Lim and Burton, 1982; Hirsch, 2009). Two types of biological nitrogen fixation are recognized based on the level of association between the host plant and the bacteria.

The first is symbiotic nitrogen fixation, resulting from symbiotic association of certain nodule bacteria with the roots of legume plants. Symbiotic host-bacteria association is a relationship between legume host plants and the bacteria whereby both associates mutually benefit from the relationship with each other. The bacteria gets energy from the host plant and reduces atmospheric nitrogen to ammonium, which eventually is exported to plant tissues for use by the plant for protein synthesis (Keyser and Li, 1992). The second type is non-symbiotic association of micro-organisms like *Azotobacter* and *cyanobacteria* (blue-green algae) with non-nodulating host plants like rice. Non-symbiotic nitrogen fixers have been categorized as rhizosphere organisms, facultative endophytes and obligate endophytes (Baldani *et al*., 1997). It is a type of biological nitrogen fixation where the microorganisms freely and independently live in the soil.

Biological nitrogen fixation makes an integral part of sustainable agroecosystems by contributing well over hundred million metric tons of renewable nitrogen per year to the nitrogen economy of the globe (Hardarson and Atkins, 2003; Hubbell and Kidder, 2003; Jensen and Hauggaard-Nielsen, 2003). Symbiotic nitrogen fixation is considered as the most important type of fixation for contribution to agricultural sustainability (Hubbell and Kidder, 2003). The amount of nitrogen annually fixed with symbiotic association is estimated to be far more than the amount industrially produced each year (Ishizuka, 1992; Bockman, 1997; Rengel, 2002; Jensen and Hauggaard-Nielsen, 2003).

A number of studies showed that some legumes may also enhance nutrient availability for associated or subsequently grown cereals (Li *et al*., 1997; Nuruzzaman *et al*., 2005; Jemo *et al*., 2006; Vesterager *et al*., 2007; Kirkegaard *et al*., 2008) by producing exudates like citrate and

malate that solublize soil minerals including fixed forms of soil phosphorus (Ascher *et al*., 2001; Ali *et al*., 2002; Ojo *et al*., 2006; Vesterager *et al*., 2006; Fageria *et al*., 2008). For instance, white lupine (*Lupinus albus*) improved phosphorus uptake for wheat (*Triticum aestivum*) (Gardner and Boundy, 1983); pigeon pea (*Cajanus cajan*) for sorghum (*Sorghum bicolor*) (Ae *et al*., 1990); faba bean, cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) for maize (*Zea mays*) (Li *et al*., 2003b; Vesterager *et al*., 2007; Waddington *et al*., 2007); and soybean, chickpea and faba bean for wheat (Herridge *et al*., 2000; Li *et al*., 2003a; Rao *et al*., 2004; Fan *et al*., 2006).

Nitrogen fixation by faba bean was found to have significant spillover effect to subsequently grown wheat in Ethiopia (Gorfu, 1998). On average the estimated amounts of N fixed by chickpeas under regular precipitation and drought stress conditions are 60 kg/ha (Unkovich and Pate, 2000; Abi-Ghanem, et al., 2012) and 19–24 kg ha^{-1} (Carranca et al., 1999; Abi-Ghanem, et al., 2012), respectively. Abi-Ghanem, et al., (2012) also reported that variety of chickpeas had a greater effect on variation in proportion of nitrogen fixed than did the symbionts strain. Other research has demonstrated a strong influence of plant variety on N fixation. Bello et al. (2001) found 70% and 25% variability in kg N fixed ha^{-1} among three soybean varieties at two sites. Some reports also indicated that growing legumes after cereals with only residual nutrients resulted in harvesting full yields of both crops (Bahl and Pasricha, 1998; Ahlawat *et al*., 2007). Beyond replenishing soil fertility through symbiotic nitrogen fixation and mineral solublization, legumes also play important roles as "break" crops in rotation with cereals as they can break the life cycles of crop-specific pathogens of cereals as non-host crops (Malhotra *et al*., 2004; Kirkegaard *et al*., 2008).

In order to keep the farming system healthy and sustainable, therefore, legumes should occupy a significant portion of area currently under cereals both as components of multiple cropping and in crop rotation (Parr *et al*., 1983; Pearson *et al*., 1995). Despite the importance being already realized, legumes are grown so far as sources of food and feed but not to the level required for soil fertility amendment as their main purpose (Bockman, 1997). Production statistics show that only a small portion of land is allotted to legumes particularly in Sub-Saharan Africa (Serraj *et al*., 2004; Twomlow, 2004). In Ethiopia, for instance, of the total 11,822,786 ha of land currently cultivated to grains, pulses occupy only 1,357,523 (11.48%) while cereals occupy 81.97% (CSA, 2011). Even though Ethiopia has a huge potential for growing and exporting various pulses, the achievements to date are very low. This is due to least share of land and input allotment to these crops, resulted in low production (Adenew, 2009). Besides, the consumption is only 12 kg of pulses per capita per year (FAO, 1996). The trend is more or less the same in many other developing countries (e.g., India) (Commodityonline, 2009).

At farm level, nitrogen fixation from annual legumes may contribute 20 to 120 kg of N ha^{-1} in a growing season in the temperate region (Pearson *et al*., 1995) or 15–120 kg of N ha^{-1} in Africa (Dakora and Keya, 2000) even though higher values were reported for soybean (Keyser and Li, 1992) even in Africa (Shoko *et al*., 2007). The contribution from symbiotic nitrogen fixation may rise by 3-4 folds for perennial forage and tree legumes where leaves can annually be pruned and incorporated into the soil (Pearson *et al*., 1995; Carlsson and Huss-Danel1, 2003; Sanginga, 2003). Non-symbiotic bacteria may also contribute 1-3 kg of nitrogen ha^{-1} in a year (Crouch *et al*., 2004). There is increasing evidence that suggests that more N can be fixed by existing

legume grain crops if they are inoculated more often or with more effective strains of rhizobia (Brockwell, 1989). Significant increases in BNF can result from the use of different commercial or wild strains (Brockwell and Bottomley, 1995).

A number of reports prove that much of the nitrogen may be removed when legumes are harvested with still significant amount remaining in the soil for future crops (Lo´pez-Bellido *et al*., 2006; Liu *et al*., 2010). Studies from different countries in different parts of the world like Syria (Keatinge *et al*., 1998), Australia (Armstrong *et al*., 1997; Holford and Crocker, 1997; Herridge *et al*., 2000; Evans *et al*., 2001; Smith *et al*., 2009), Ghana (Adjei-Nsiah *et al*., 2008), Guinea (Sanginga *et al*., 1997), India (Ghosh *et al*., 2007; Sharma and Behera, 2009), Nigeria (Adeboye *et al*., 2007; Yusuf *et al*., 2007; 2009), Ethiopia (Gorfu, 1998), Tunisia (Romdhane *et al*., 2008) and Denmark (Jorgensen *et al*., 1999) have clearly revealed benefits of different cereals grown after different legumes. Even in dry areas, identification of legume cultivars tolerant to drought for attributes of symbiotic nitrogen fixation was proven to be a possibility of practical significance (Purcell *et al*., 1997; Serraj and Sinclair, 1998; Serraj *et al*., 2004; Devi *et al*., 2010).

Haque and Jutzi (1984) reviewed the factors that may limit biological N fixation and indicated that N_2 fixation is the product of the host legume plant and the bacterium and this may be affected by the reaction of one or the other or both. They generalized that fixation is proportional to the vigor of the host plant and, therefore, is affected by the factors that affect plant growth (water, temperature, nutrients and light), and indicated that this generalization may be changed by factors that specifically affect the activity of the *rhizobium* rather than the host (by factors such as temperature, soil pH, nutritional status and genetic specificity). Therefore, that

measurements of the amounts of N fixed vary quite widely (Haque and Jutsi, 1984) and also the fixed N may accumulate in the soil in organic, biological or inorganic (NO_3, NH^+_4) forms. As a limitation in some situations (e.g. heavy clay soils), total N fertility may increase steadily whilst available N fertility declines because of the buildup of micro-organisms that compete successfully for available N and as a result, a high-fertility soil becomes apparently as infertile as an inherently low-fertility soil.

2.2. The Chickpea Crop

The Genus *Cicer* has eight annual and 34 perennial species (Van der Maesen, 1987) of which *Cicer arietinum* is the only cultivated species (Millan *et al*., 2006). An earlier study on the cytogenetic relationships of different species indicated that the wild *C. reticulatum* was the progenitor of the cultivated chickpea (Ladizinsky and Adler, 1976). Nevertheless, the ancestry of C. *reticulatum* could not be reconfirmed with another study (Ohri and Pal, 1991). The cultivated chickpea belongs to family Fabaceae (formerly Leguminosae) and subfamily Faboideae. Different chromosome numbers were reported from early cytogenetic studies (van der Maesen, 1987) but it was later confirmed that chickpea is a diploid species having chromosome number of 2n = 2x = 16 (van der Maesen, 1987; Upadhyaya *et al*., 2008) with comparatively a small genome size of 740 Mbp (Arumuganathan and Earle, 1991).

Based on the presence of wild relatives, namely *C. reticulatum* and *C.echinospermum*, South-Eastern Turkey and the adjoining areas of Syria was considered as the most probable center of origin for chickpea (van der Maesen, 1987). Archeological evidences show that chickpea was first domesticated in the Middle East before the late Neolithic period (as early as 3500 BC) in

Turkey (Tanno and Willcox, 2006). The crop was probably diffused from where it was originated and first domesticated, i.e. the Middle East, to different continents of the world by the Phoenicians (The Worldwide Gourmet, 2010). As early as 1520 BC, chickpea was known to be grown in Ethiopia (Joshi *et al*., 2001), which is now considered as the secondary center of diversity for the crop (van der Maesen, 1987). Chickpea is a strictly self-pollinated crop (Muehlbauer and Tullu, 1997; Romeis *et al*., 2004; Toker *et al*., 2006) with two types of cultivars, *Desi* and *Kabuli*. The *Desi* type has small darker seeds with a rough seed coat while the *Kabuli* has larger seeds with lighter colour and a smoother seed coat.

Existence of a pea-shaped third type characterized by medium to small seed size and creamy colour was also recognized (Upadhyaya *et al*., 2008), which may be a result of crossing of *Desi* with *Kabuli* types resulting in a sort of intermediate types (Muehlbauer and Tullu, 1997). Currently, about 75% of the area all over the world is covered by the *Desi* and the remaining 25% by the *Kabuli* types (Kassie *et al*., 2009). The *Desi* type is grown mostly in Indian subcontinents, Ethiopia, Mexico and Iran while the *Kabuli* type is grown in Southern Europe, Northern Africa, Afghanistan, Pakistan and Chile (Upadhyaya *et al*., 2008; Kassie *et al*., 2009).

The crop may grow to a height of 30-70 cm depending on the suitability of the growing environment. It has small feathery leaves on both sides of the stem and sometimes with 2-3 bisexual flowers in a node. The flowers may be white, pink, purple or blue in color. The crop may set 1-3 seeds in a pod and the seed is a good source of protein (23%), carbohydrates (64%), fat (5%) and crude fiber (6%). It also contains 340 mg of phosphorus, 190 mg of calcium, 140 mg of magnesium, 7 mg of iron and 3 mg of zinc in 100 g of seed (Bejiga and van der Maesen,

2006). Chickpea needs a subtropical or tropical climate with well drained fertile soils having pH of 5.5-8.6 (Muehlbauer and Tullu, 1997; Kassie *et al*., 2009).

2.3. Chickpea Production Constraints

Chickpea (*Cicer arietinum* L.) is cultivated mostly in the arid and semi-arid regions of the world. Conventional breeding has produced several high-yielding chickpea genotypes without exploiting its potential yield owing to a number of biotic and abiotic constraints. Among the biotic constraints, disease and insect pests and among the abiotic stresses drought, salinity, water logging, high temperature and chilling frequently limit growth and productivity of chickpea. Its production in marginal areas is considered to be constrained by low rainfall. Whish et al., (2007) reported that the supply of water between flowering and maturity was significantly related to yield. They quantified that plant stresses experienced during flowering caused a 50 % loss of potential pods. This entails genes expressed during flowering have important bearing. The genetic complexity of these abiotic stresses and lack of proper screening techniques and phenotyping techniques and genotype-by-environment interaction have further jeopardized the breeding program of chickpea. Therefore, considering all the challenging abiotic stresses, the scientists have to understand the knowledge gap involving the physiological, biochemical and molecular complex network of abiotic stresses mechanism.

Above all emerging ‘omics’ approaches will lead the breeders to mine the ‘wealth of genes’ from wild donors and tailor a genotype harbouring ‘climate resilient’ genes to mitigate the challenges in chickpea production.

2.3.1 Diseases

Although chickpea is susceptible to a large number of diseases (more than 50 have been reported worldwide), few have a significant impact on production globally. The two most important diseases include *Ascochyta* blight, caused by *Ascochyta rabiei*, and Fusarium wilt, caused by *F.oxysporum f. sp.ciceris*. *Ascochyta* blight is an important foliar disease of chickpea worldwide that causes grain yield losses up to 100% (Pande et al., 2005). The spread of the disease is more with cool (15-25^{o}C) and humid weather (>150 mm rainfall) that prevails during the crop season (Pande et al., 2005). The pathogen causing Fusarium wilt is soil borne and can affect all stages of plant growth and development but higher incidence at flowering and podding stage (Maitlo et al., 2014). The severity of the disease is maximum under high temperature and drought conditions. Annually 10–90% losses occur due to chickpea wilt disease (Sharma & Muehlbauer, 2007).

Tolerant cultivars are available for both diseases, but generally these will not be sufficient if an epidemic of Ascochyta blight occurs. Several fungicides are labeled for this disease and should be used in conjunction with genetic resistance, timely scouting, and fungicidal seed treatments. A number of root rot and collar rots are caused by *Fusarium solani*, *Rhizoctonia*, and *Phytophthora* spp. The *Fusarium solani* and *Rhizoctonia* have been observed more commonly in dryland production. Under sprinkler irrigation, Fusarium wilt has been observed as well as a foot rot caused by *Phytophthora*.

The CGIAR Research Program on Grain Legumes where ICRISAT is a leading center together with the National Agricultural Research System (NARS) partners developed advance research

program on high-priority challenges such as development of heat-tolerant chickpea, high nitrogen-fixing chickpea, insect-smart chickpea and extra-early chickpea to maintain chick pea production in view of anticipated climate change in chickpea growing regions.

2.3.2. Drought as a Production Constraint

Drought is a rather difficult meteorological term to define and more difficult to quantify (Sinha, 1986; Singh, 2005). There are several definitions of drought based on the effects of precipitation, distribution, evaporation, temperature, soil water storage, and other factors, but a working definition is the one by Sinha (1986): "the inadequacy of water availability interms of precipitation and soil moisture in quantity and distribution during the crop life cycle restricting crop's expression of its genetic yield potential" is of practical importance, i.e., meets the requirements of agriculture (Khanna-Chopra, (2003).

Chickpea is highly susceptible to climate change, both drought and heat can severely limit its productivity. Drought stress commonly affects chickpea because it is largely grown under rainfed conditions during the post-rainy season on residual soil moisture (Gaur et al. 2008). Terminal drought and heat stress, among other abiotic and biotic stresses, are the major constraints to chickpea production in the warmer short-season semi-arid tropical environments. Inclusion of chickpea in the sequential cropping system resulted in late sowing resulting in prolonged exposure to heat stress during the reproductive phase of chickpea leading to reduction in seed yield. Predicted climate change scenarios for Asia and Africa include an increase in seasonal temperatures and extreme temperature events. Flowering and podding in chickpea found to be very sensitive to temperature changes and exposure to heat stress (Summerfield et

al., 1984). Therefore, breeding for increased heat and drought tolerance are the important priority areas for chickpea research to maintain chickpea productivity sustainable in vulnerable areas.

Drought is the main abiotic stresses which is a primary cause for yield loss in agricultural crops and, as a result, negatively affect crop production. It affects 26% of the total arable land of the world (Singh, 2005). In Ethiopia, for instance, the area coverage under drought-prone zones accounts for 55%, and nearly 40% of the population lives in these zones (EMA, 1988). The wider scope and the tremendous negative impacts of drought in developing countries show that food security is totally unsustainable without increasing yield in marginal environments, especially in drought-prone areas, as 80 % of agriculture worldwide is rain fed (Lorieux, 2005). Therefore, drought will continue to be a problem in crop production, since water limitation causes stress in plants and limits the production of important cultivations worldwide (Boyer, 1982). The need for new alternatives for a sustainable agriculture such as drought-tolerant varieties will provide a practical solution to alleviate the problem of drought.

2.4. Drought Tolerance

2.4.1. Mechanisms of drought Tolerance

Drought tolerance of plants may be defined as heritable modifications in structures or functions that increase the probability of a given plant surviving and reproducing under drought (Kramer, 1980). Drought tolerance is also defined in different ways by different disciplines: it may be defined as survival of individual cells or unicellular organisms through osmo-regulation of water stress by a molecular biologist; as tolerance of biochemical reactions such as protein synthesis to water deficit to a biochemist; as maintenance of growth during water stress and its accelerated

resumption on termination of water stress to a plant physiologist; and as stability in yield performance of a crop or a variety in a water-deficient environment by an agronomist (Sinha, 1987).

A number of workers also defined drought tolerance in terms of the difference in yield performance between stress and non-stress environments (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez, 1992). Nevertheless, definitions by molecular biologists, biochemists and physiologists are based on plant survival and have little economic implication as compared to the definitions by agronomists which is based on productivity or economic yield. Thus rainfall, soil water storage capacity, potential evapo-transpiration and crop phenology and development stage must all be considered when assessing the impact of drought on crop production (Kramer, 1980; Njoroge *et al*., 1997).

The various mechanisms by which a crop can minimize the loss in yield due to drought are grouped into three categories (Singh, 2005):

(1) Dr**ought escape** – the situation where an otherwise drought susceptible cultivar perform well in a drought environment simply by avoiding the period of drought as in the case of early maturing cultivar in terminal-drought prone environments;

(2) **Dehydration avoidance** – is the ability of the plant to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress as a result of physiological, biochemical and metabolic processes. This include reduced transpiration, osmotic adjustment, abscisic acid concentration, cuticular wax, leaf characteristics, and increased water uptake; and

(3) **Dehydration tolerance** - a characteristics of a genotype by which it significantly lower the level of changes induced as compared to other genotype exposed to the same level of dehydration through various measurements of dehydration tolerance such as maintenance of membrane integrity which is usually determined by the leakage of solutes from a cell.

2.4.2. Genetics of Drought Tolerance

Plants have different adaptation strategies to tolerate different biotic and abiotic stresses which are genetically encoded. The biological basis for drought tolerance as summarized by Xoconostle-Cazares et al., (2011) include physiological and anatomical adaptation to water deficit, biochemical and molecular level responses, drought sensing and signal transduction, induced genes at transcriptional level and drought-induced proteins.

The genetic control of traits associated with drought tolerance ranges from oligogenic to polygenic with additive, additive by additive interaction and dominance gene effects (Singh, 2005; Kashiwagi *et al*, 2008). The same source states that available heritability estimate of these traits ranges from low to high. However, many of the traits are reported to be associated with yield under stress, but the level of association is usually low making the selection efficiency for yield low.

2.4.3. Breeding of Chickpea for Drought Tolerance

Activities of chickpea breeding started at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in 1974, focusing on crossing germplasm obtained from diverse regions and distributing the segregating populations and advanced breeding lines to National Agricultural Research Systems for trial. Studies have been done to determine the morphological traits that contribute to drought tolerance or its avoidance in chickpea (Turner et al., 2001). Large root system and smaller leaf area have been suggested to determine drought tolerance, the former is being important for absorption of available soil moisture while the later helps to reduce the amount of water loss through transpiration (Saxena, 2003).

2.4.4. Sources of Drought Tolerance

Sources of drought tolerance in crop may include cultivated varieties, elite breeding lines, improved cultivars and landraces. When the variability present in the elite breeding lines and landraces is exhausted, wild relatives, especially those adapted to arid environments are shown to be rich in drought tolerance characters. But utilization of the wild relatives may be problematic due to undesirable linkage drags among others.

As far as efforts made to develop chickpea varieties for drought tolerance are concerned, over 1500 chickpea accessions and released varieties have been screened for tolerance to drought at ICRISAT, as a result of which the variety ICC 4958 was found to be drought tolerant showing 30% more root volume than a previous popular variety Annigeri (Saxena et al., 1993). This variety has been used to develop promising drought tolerant lines. For instance, a three-way cross involving ICC 4958, Annigeri and ICC 12237 has resulted in varieties with high yield and

better drought tolerance (Saxena, 2003). Efforts were also made to combine the large root traits of ICC 4958 and small leaf area traits of ICC 5680 and several progeny combining these traits with high yield were recovered through subsequent selection (Saxena, 2003).

2.4.5. Successes and Challenges of Breeding for Drought Tolerance

Some successes in developing drought tolerant cultivars using both conventional and molecular breeding and recently, genetic engineering include: Drought tolerant maize developed by CIMMYT thought conventional breeding (Banziger, et al., 2006) that is being used in 13 African countries including Ethiopia; transgenic bread wheat lines containing the *Arabidobsis thaliana* stress inducible promoter developed by CIMMYT; identification of drought-associated QTL in wild barley that allowed better productivity under drought stress (Baum et al., 2003); rice hybrids with high yield under drought stress developed through conventional crosses and the identification of QTLs (Bernier et al., 2008) by the International Rice Research Institute (IRRI); There are many other attempts by CGIAR centers like CIMMYT, ICARDA, ICRISAT, and CIAT on different crops including sorghum, common beans and chickpea.

Despite the efforts made to develop variety for drought tolerance (Richards, 1987; Kashiwagi et al., 2006), the cultivars released in different countries for drought-tolerance are mostly of the escaping types (Kashiwagi et al., 2008) and it is hardly possible to say that these efforts have resulted in varietal breakthrough as such and boosted productivity under drought-prone environments as desired (Kashiwagi et al., 2008) because of technical difficulties encountered in making major advances. There are multitudes of challenges against breeding crop cultivars for drought tolerance as compared to breeding for optimal conditions. The major ones include:

complexity of the problem due to multitude of interacting factors (amount and the distribution of rainfall, soil water storage capacity, potential evapo-transpiration and crop phenological and development stages); inconsistency in pattern of drought scenarios (early, intermittent, terminal and a sort of continuous drought occurrence): high genotype by environment interaction which is greater under stressed environments relative to the non-stressed ones and makes varietal selection under drought conditions difficult; low genetic gain from selection under drought condition due to low genetic variability and low heritability; high risk of genetic vulnerability due to use of narrow genetic base of improved cultivars in drought area; and limited Knowledge of genetic and physiological bases of drought tolerance.

2.4.6. The Role of Molecular Techniques in Breeding for Drought Tolerance

The process of developing new crop varieties through conventional plant breeding methods believed to take long time especially for improving complex traits like yield and abiotic stress tolerance. To date, however, molecular breeding has considerably shortened the time for development of new crop varieties to a few years. It also increases precission and reduces labor. To help identify specific genes, scientists use what are called molecular or genetic markers (Dita et al., 2006), string or sequences of nucleic acid which make up a segment of DNA located near the DNA sequence of the desired gene and are transmitted by the standard laws of inheritance from one generation to the next. These molecular markers include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter-simple sequence repeats (ISSR), and sequence characterized amplified regions (SCAR) for amplification of specific band. Many others such as single nucleotide polymorphism

(SNPs) (Varshney, et al., 2006) and diversity array technology (DArT) (Jacoud, et al., 2001) have also recently been developed. Since the markers and the genes are close together on the same chromosome, they tend to stay together as each generation of plants is produced. This is called genetic linkage. This linkage helps scientists to predict whether a plant will have a desired gene. If researchers can find the markers tightly linked with a gene controlling a trait, it means that the desired gene is present and selection will be done accordingly.

These days plant breeders use molecular breeding methods which includes: marker-assisted selection – the selection of specific alleles for traits controlled by few loci; marker-assisted backcrossing (MABC) – the transfer of limited number of loci from one genetic background to another; marker-assisted recurrent selection (MARS) – the identification and selection of several genomic regions involved in the expression of complex traits within a single population; and genomic selection (GS) - in which genetic markers covering the whole genome are used. Methods for marker-assisted backcrossing were developed rapidly for the introgression of desired genes or segment of a genome and reduction of linkage drag, where molecular markers are used to select those individuals that contained the greatest proportion of favorable alleles from the recurrent genome (Johnson and Mumm, 1996). The main process of GS is the calculation of genomic estimated breeding values (GEBVs) for large breeding population having only genotypic data, resulting in accelerated genetic gains in improving the difficult quantitative traits like drought tolerance. Hence, molecular breeding is currently standard practice in many crops.

2.4.6.1. Chickpea genome sequencing

Sequencing of the chickpea genome has been completed for 90 chickpea genotypes, including several wild species. A collaboration of 20 research organizations, led by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) identified more than 28,000 genes and several million genetic markers (Varshney et al., 2014). Scientists expect this work will lead to the development of superior varieties. The new research will benefit the millions of developing country farmers who grow chickpea as a source of much needed income, as well as for its ability to add nitrogen to the soil in which it grows. Production is growing rapidly across the developing world, especially in West Asia where it has increased four-fold over the past 30 years. India is by far the world largest producer but is also the largest importer (Varshney et al., 2014).

2.4.6.2 Gene expression

The efficiency of MAS approaches is improved by using the information from gene expression studies. Understanding the mechanisms employed by plants to tolerate stresses and a more complete knowledge about the genes involved, will allow a more precise use of MAS. Sequence information is insufficient to answer questions concerning gene function, regulatory networks and the biochemical pathways activated in response to stresses. To address these questions, more comprehensive approaches, including quantitative and qualitative analyses of gene expression products, are necessary at the transcriptomic, proteomic, and metabolomic levels (Dita et al., 2006).

Although the use of MAS may be helpful for crop improvement, its practical application in legumes for the genetic improvement of resistance or tolerance to stress has been limited, being mainly hampered by lack of investment and the genetic complexity of most stress-related traits. There are some exceptions where MAS has already facilitated breeding efforts for several legume crops against important biotic stress. For instance, MAS was successfully used for the breeding of resistant soybean to cyst nematode (Diers, 2004), of resistant into bean to common bacterial blight (Mutlu et al., 2005) and of resistant narrow-leafed lupin (*Lupinus angustifolius* L.) to phomopsis stem blight (Yang et al., 2002) and anthracnose (Yang et al., 2004).

2.4.6.3. Transcriptomics

An important step in the control of stress responses in plants is the transcriptional activation or repression of genes (Chen et al., 2002). Thus, identification of differentially expressed genes is particularly important to understand stress response in plants. To achieve this objective, tools such as microarrays (Schena et al., 1995), suppression subtractive hybridization library (Diatchenko et al., 1996), serial analyses of gene expression (Velculescu et al., 1995) and quantitative measurement of transcription factor (TF) expression patterns have been developed in addition to older techniques such as Northern blotting. In legumes, gene expression patterns following biotic stresses have been more extensively studied than those following abiotic stresses. Large-scale analyses of gene expression patterns in response to pathogens have revealed the differential expression of large numbers of genes. The coupling of these powerful large-scale gene expression profiling methods with recombinant inbred lines or near isogenic lines in legumes that differ in susceptibility/resistance to key stresses will greatly facilitate a more comprehensive understanding of genes involved in the tolerance response of legumes to specific

biotic and abiotic stresses (Dita et al., 2006). With respect to abiotic stress, gene expression analyses have been mainly based on studies with cloned genes (Singh et al., 2004).

2.4.6.4. Proteomics

In parallel to the accumulation of a wealth of genomic and transcriptomic data, recent technological developments have allowed the establishment of valuable methods for quantitative and qualitative protein profiling (C´anovas et al., 2004). This approach is very important in evaluating stress-responses because mRNA levels do not always correlate with protein accumulation (Gygi et al., 1999). Indeed, large differences in protein turnover and post-translational modifications may lead to large variations between transcriptomic and proteomic data. Thus, protein studies are needed to provide information on their levels and activities (Zivy & de Vienne, 2000). To this purpose, proteomic based techniques that allow large-scale protein profiling are powerful tools for the identification of proteins involved in stress-responses in plants (Gygi & Aebersold, 2000).

2.4.6.5. Metabolomics

Transcriptomic and proteomic data are important steps in deciphering a complex biological process, but they are still insufficient to understand them fully since most biological processes are ultimately mediated by cell metabolites. Alternative mRNA splicing, protein turnover rates and post-translational modifications that modulate protein activity imply that changes in the transcriptome or proteome do not always correspond to alterations in the cell metabolome (Sumner et al., 2003). Therefore, the only way to the complete understanding of both gene function and molecular events controlling complex plant processes is to analyze in parallel the

transcriptome, the proteome and the metabolome in an integrative manner (Dixon, 2001). In legumes, this kind of approach has been taken in *M. truncatula* suspension cells to various stimuli (Bell et al., 2001), and with the characterization of metabolic changes during the nitrogen-fixing symbiotic interaction in *L. japonicus* (Colebatch et al., 2004; Desbrosses et al., 2005; Rispail, 2005).

2.4.6.6. Currently available genomic resources to combat Drought in chickpea

To combat negative effects of drought on chickpea through biotechnological tools, Varshney et al., (2009) have assembled drought tolerance ESTs for the entire set of chickpea ESTs including those from the public domain. They provided an overview of expression patterns of 105 genes that were up or down-regulated in response to imposed abiotic stresses, validated either by over expression or TILLING (Targeting Induced Local Lesions IN Genomes). These genes together with 177 SSR markers and 742 genes with SNPs provide a comprehensive resource for integration and development of the transcript map of chickpea. The EST resource generated in their study would significantly impact chickpea genetics, and breeding in general and for improving the crop for drought and salinity tolerance in particular (Varshney et al., 2009).

Grage et al., (2011) have reported for the first time the complete transcriptome of chickpea, generated millions of non-redundant set of transcripts and various analyses, including GC content analysis, sequence similarity/conservation with other plant species, functional categorization and identification of simple sequence repeats (SSRs) have been done. Such data will provide a very useful genomic resource for future studies in chickpea (Grage et al., 2011).

2.5. Rationale and Relevance of the Present Study

Drought is among the major abiotic factor affecting 26% of the arable land worldwide and about 55% of the total area in Ethiopia, where nearly 40% of the population lives. This large proportion of the country is categorized as arid or semi-arid, with low rainfall and recurrent crop failure. Growing of chickpea as an alternative drought tolerant crop is ideal since there is no other additional cost to the farmer once drought tolerant variety is obtained by the farmers.

Conventional breeding methods have been used in similar cases but with a major disadvantage of taking too much time. Recent molecular breeding techniques like marker assisted backcross breeding (MABC) has been known to take a shorter time and enable to undertake a more precise selection. In addition, drought tolerance attributes (such as root length, root length density, high harvest index, and water use-efficiency, yield and drought tolerance indeces) could exist in the elite breeding matrials and in some of the already released cultivars. Identifying and utilizing such sources of drought tolerance is the first priority as affordable and accessible alternative for smallholder farmers.

3. MATERIALS AND METHODS

This study comprises two broad sets of experiments:

I. Marker-assisted introgression of drought tolerance gene/QTLs into adapted Ethiopian chickpea cultivars. This experiment was conducted in a series of four sub experiments. These are: (1) identification of polymorphic recurrent and donor parents for marker-assisted backcrossing; (2) introgression and incorporation of drought related genes/QTLs into adapted Ethiopian chickpea cultivars through marker-assisted backcrossing to develop large isogenic lines; (3) multi-location evaluation and identification of drought tolerant lines from the generated MABC lines; and (4) transcriptome analysis of drought challenged roots of selected marker-assisted backcross lines along with the parents. For this experiment, the main cropping seasons and off-seasons were used to grow more generations per year in order to shorten the time required;

II. Determination of stress susceptibility or tolerance indices and moisture use efficiency of released Ethiopian chickpea cultivars for optimum environments.

3.1. Experiment I: Marker-Assisted Introgression of Drought Tolerance Gene or QTLs into Adapted Ethiopian Chickpea Cultivars

3.1.1. Identification of polymorphic recurrent and donor parents

This study was built on an earlier attempt by the Tropical Legume 1 Project. Based on extensive phenotyping and SSR genotypeing of a recombinant inbred lines (RIL) population, a "QTL-hotspot" genomic region spanned 29 cM) (Varshney et al., 2014) harboring various QTLs for drought related traits (root length, root length density, HI, ^{13}C-isotope discrimination and yield) has bee identifiec

on linkage group four(CaLG04) on the mapping population of ICC 4958 × ICC 1882. A set of six markers (TAA170, ICCM0249, GA24, STMS11, NCPGR21 and NCPGR127) have been identified as associated with drought tolerance related QTLs. The distance between the markers and the QTLs ranged form 5 to 10cM. Genotype ICC 4958 has high root length density and root dry weight under moisture stress conditions and it is found to be the most tolerant to drought as compared to other genotypes and could be used as a parental material for developing drought tolerant genotypes (Saxena, 1984; Ali et al., 2005). The superiority of ICC 4958 over other genotypes was for root proliferation expressed through root-length density (Saxena, 2003). Thus, the variation in root-lenght-density (RLD) can be the most relevant root trait that reflects chickpea's potential for soil moisture or P acquisition (Saxena, 2003).

The application of molecular markers in breeding programs requires preliminary studies to identify and validate potential markers and also considering factors like level of polymorphism existing between parental lines, among others (Yu et al., 2004; Dita et al., 2006). Therefore, marker polymorphism was tested between ICC 4958 and ten elite Ethiopian chickpea cultivars (Akaki, Arerti, Dube, Ejere, Habru, Marye, Natoli, Shasho, Teji, and Worku) for the four markers (ICCM0249, TAA170, GA24 and STMS11). The parental combination with higher marker polymorphism was selected for the MABC. The standard CTAB protocols for DNA isolation, standard PCR and marker analyses were used.

3.1.2. Development of backcross lines

The donor genotype, ICC 4958, was crossed with selected Ethiopian chickpea cultivar, Ejere, in green house at Debre Zeit Research Center to generate at least 100 F_1 seeds. After sowing these

F_1 seeds, DNA was isolated and tested for heterozyosity with the molecular markers mentioned above. Selected heterozygous F_1 plants were backcrossed with the recurrent parent to generate at least 100 BC_1F_1 seeds. After raising the BC_1F_1 plants, DNA was isolated and foreground selection was made based on the above mentioned four SSR markers. Depending on the number of true BC_1F_1 plants, the background selection with the molecular markers was undertaken. After analyzing the data based on foreground and background selection, at least 5 true BC_1F_1 plants (with higher genome recovery) were selected and then used for second cycle of backcrossing. Subsequently, about 100 BC_2F_1 seeds were harvested. As mentioned above, foreground and background selection was performed on BC_2F_1 plants and then the selected BC_2F_1 plants were used for the 3rd cycle of backcrossing. After harvesting BC_3F_1 seeds, at least two selfing cycles was performed to generate large quantity of seeds from the selected BC_3F_3 isogenic lines. A general schematic presentation of the main steps followed starting from the initial crossing to multi-location field testing for yield and other agronomic traits is shown in Figure 1.

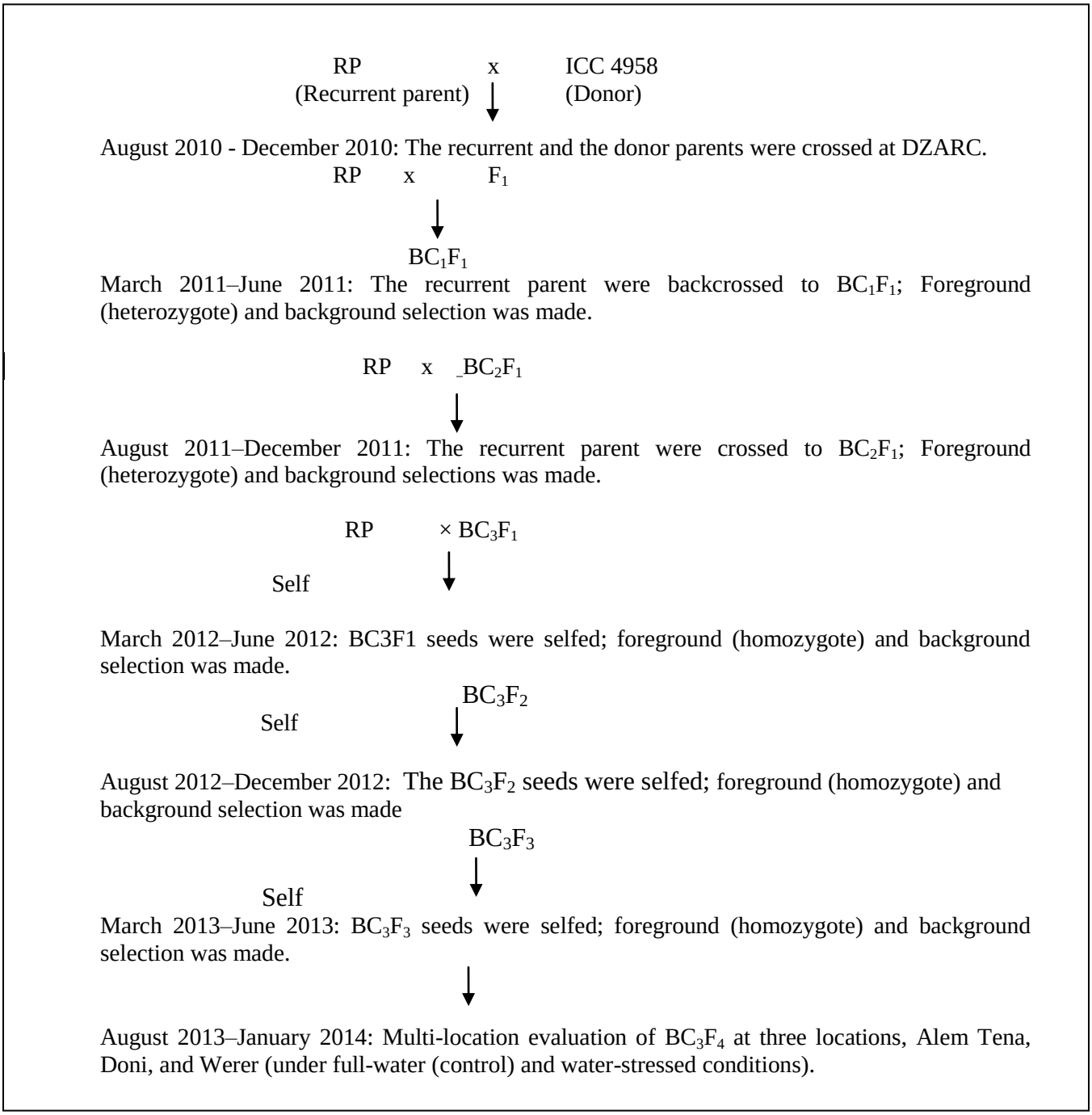


Figure 1. Schematic presentation of drought tolerance gene introgression through marker-assisted backcrossing of adapted Ethiopian cultivar, Ejere, with drought tolerant cultivar, ICC 4958.

3.1.3. Field evaluation of backcross isogenic lines

3.1.3.1. Plant Material and Experimental Design

The recurrent and the non-recurrent parents together with generated BC_3F_4 isogenic lines (ILs) were grown on-station simultaneously under full-water (control) and under 50% water-stress (using irrigation) conditions adjacent to each other at three moisture deficit locations (Alem Tena, Doni and Werer) for one season to phenotypically evaluate and quantify the level of drought tolerance recovered in the progenies. The trial was laid out in incomplete block design with two replications and four blocks within replication with a total of 210 genotypes (208 ILs and the two parents). Recommended crop management practices for chickpea were followed at each location for the stressed and non-stressed sets. The plot size was 2m x 0.6m = $1.2m^2$ (2m length, 2 rows in 30 cm between rows, and 10 cm between plants in a row). Twenty seeds were planted per row.

3.1.3.2. Description of the test locations

The experiment was conducted under field conditions at three locations (Alem Tena, Doni and Werer) in the Rift Valley of Ethiopia for one season at each location (January to May 2014 at Doni and Werer and from August to November 2014 at Alem Tena). Werer lies in the lower altitude in the Rift Valley, having high temperature while Alem Tena is in mid-altitude and Doni is in between. These locations are assumed to represent the moisture-stressed (low rainfall) chickpea production areas of Ethiopia (Figure 2). Climatic data of the test locations during the growing period were taken from each location's meteorology stations (Figure 3). Soil samples from each site were collected from the top 30 cm for physico-chemical characterization (Table

1). The soil of Alem Tena was slightly acidic (pH= 6.3) while that of Doni and Werer were silightly alkaline (pH=7.8 and 7.4, respectively) according to stanadard classification of soil's pH ranges. As chickpea crop is best suited to well drained loam and clay loam soils that are neutral to alkaline (pH 5.5 to 8.6) (Muehlbauer and Tullu, 1997; Kassie *et al*., 2009) and have good water holding capacity, the soils at the test locations are assumed to be appropriate for the normal growth of the crop. In terms of soil percent total N and OC, Doni seems better than the two locations.The average daily temperature of Alem Tena was the lowest (15.8 to 23.8 °C), Doni was intermediate (21.6 to 27.0 °C) and Werer was the highest (22.4 to 34.3 °C). The average temperatures and RH of the locations were almost similar with their respective long-term trends at all the locations (data not shown), indicating the possible reproducibility over seasons of the results in this study.

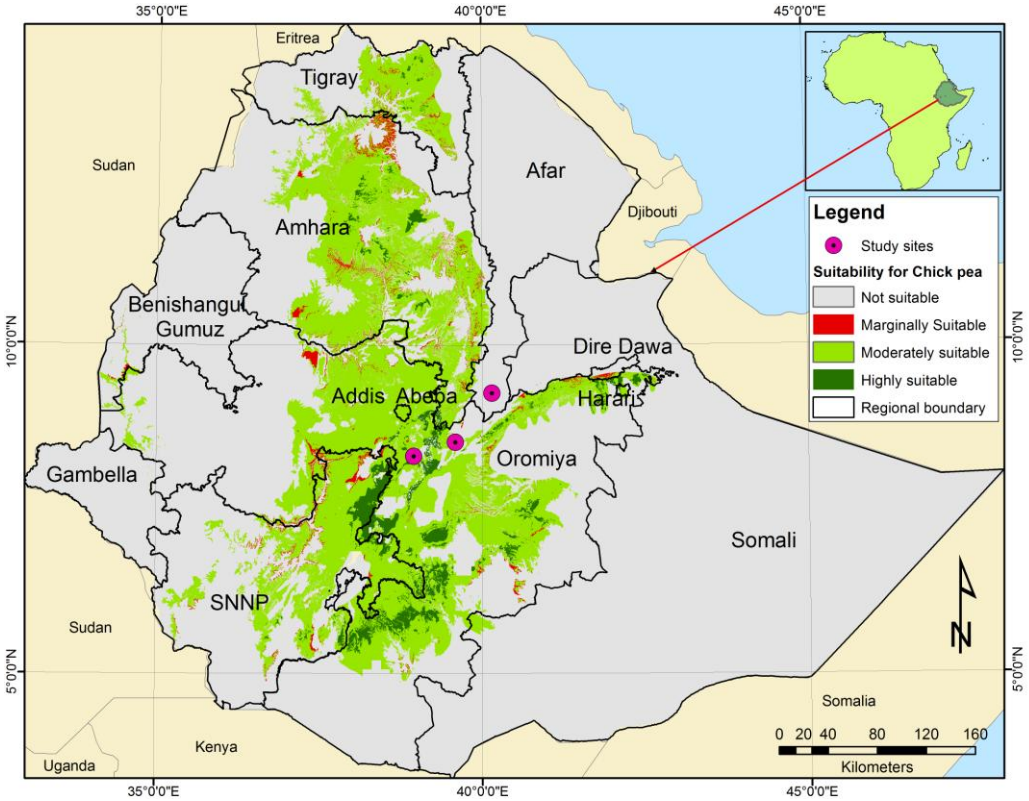


Figure 2. Suitability map of chickpea in Ethiopia and the distribution of test locations (Alme Tena, Doni and Werer) (Source: EIAR, 2016).

Table 1. Characteristics of the test locations

Parameter	Locations		
	Alem Tena	Doni	Werer
Grid	39°57'E: 8°61'N	39°35'E: 8°31'N	40°9'E: 9°16'N
Altitude (m.a.s.l.)	1646	1240	740
Rainfall (mm) during crop season	698.8 (early planting)		
	403.4 (late planting)	306.4	68.2
Mean daily temperature (°C) range	15.8 - 23.8	21.6 - 27.0	22.4 - 34.3
Mean daily relative humidity (%) range	41.9 - 80.2	NI	33.1 - 84.8
Soil pH	6.3	7.8	7.4
Soil phosphorus (ppm)	14.08	5.1	NI
% Total nitrogen	0.07	0.12	NI
%Organic carbon	0.60	1.68	NI

NI = no information

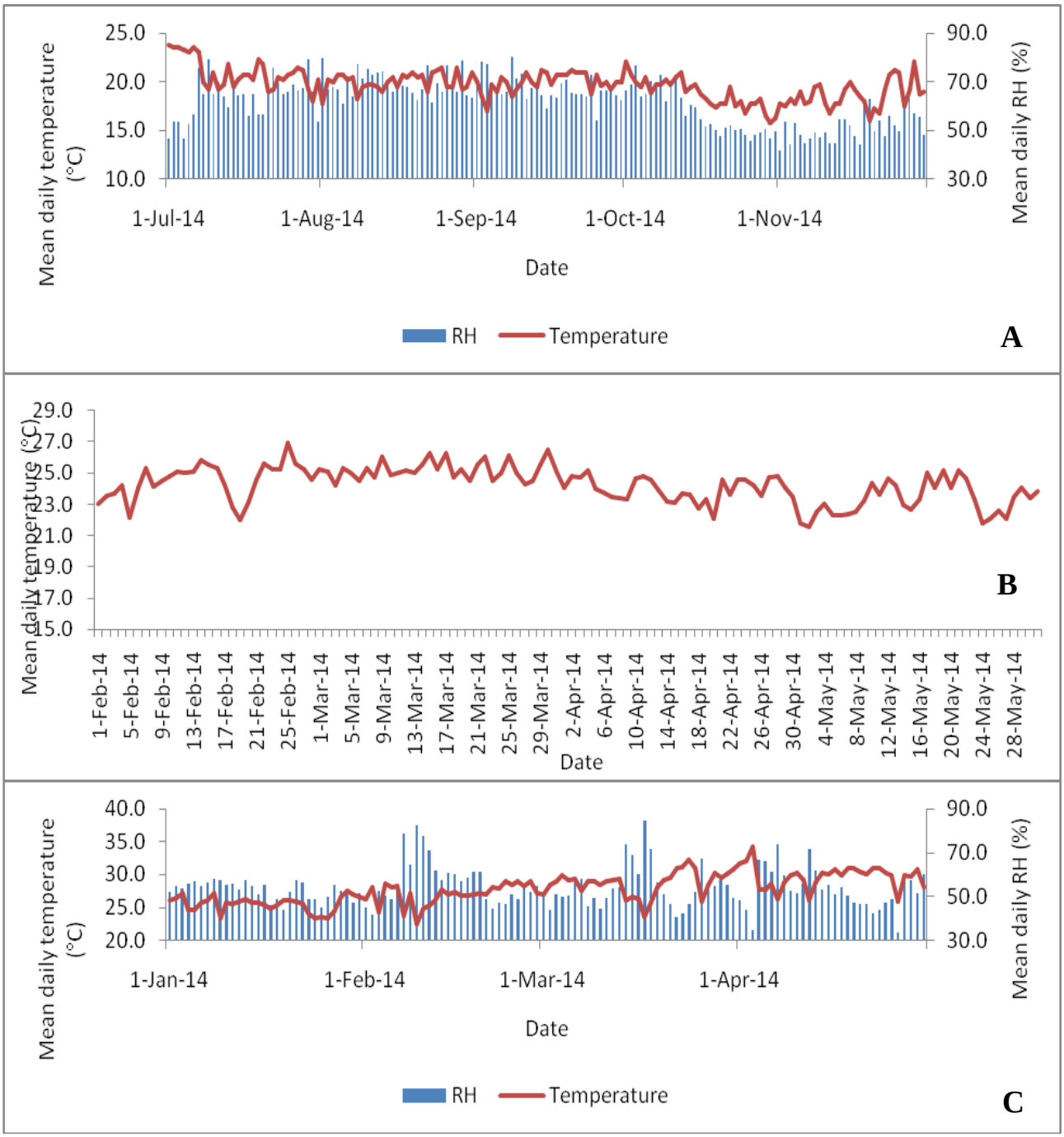


Figure 3. Mean daily temperature (°C) and relative humidity (RH %) at the test locations during the crop season: (A) Adadi, (B) Doni and (C) Werer.

3.1.3.3. Data Collection and Analysis

Data was collected either on plot basis or from randomly selected plants on number of days to flowering (DTF), maturity (DTM) and grain filling (DTGF), number of pods per plant (PPP) and seeds per pod (SPP), ascochyta blight disease (in 1-5 scale), plant height (PHT in cm), biomass yield (BMY g five plants^{-1}), grain yield (GYLD g five plants^{-1}), 100 seed weight (TSW g), harvest index HI %), drought susceptibility index (DSI). As a result of detailed analysis on above mentioned traits, the most desirable BC_3F_4 lines that outperform the recurrent and donor parents in terms of the yield and drought tolerance were selected.

All the data were subjected to statistical analysis like testing for outliers, and normality of residuals, descriptive statistic (mean), univairate analysis (analysis of variance, ANOVA, and variance component analysis) according to Gomez and Gomezusing (1984) and bivariate (associations or correlation) and multivariate (cluster and principal component) analyses according to Hardle and Hlavka (2007) using SAS (SAS Institute, 2002) and SPSS (IBM Corp., 2011, Version 20) computer softwares.

The total variability for each trait was quantified using analysis of variance according to the following model for single location and combined analyses over locations:

For single location analysis:

The linear model of observations in incomplete block design is of the form:

$Y_{ijk} = m + G_i + R_j + B_k\,(R_j) + e_{ijk}$

where m=grand mean; Y_{ijk} denotes the value of the observed trait for *i*-th genotype in the *k*-th block within j-th replicate (superblock), G_i is the fixed effect of the *i*-th genotype ($i = 1,2,\ldots,g$);

R_j is the effect of the *j*-th replicate (superblock) ($j = 1,2,...,r$); B_{jk} is the effect of the *k*-th incomplete block within the *j*-th replicate ($k = 1,2,...s$) and e_{ijk} is an experimental error associated with the observation of the *i*-th treatment in the *k*-th incomplete block within the *j*-th complete replicate;

For combined over locations analysis:

$Y_{ijkr} = m + G_i + R_j + B_k(R_j) + L_r + (GxL)_{in} + e_{ijk}$

where m=grand mean; Y_{ijkr} denotes the phenotypic observation of a trait for *i*-th genotype in the *k*-th block within j-th replicate (superblock) in n-th location; G_i is the effect of the *i*-th genotype ($i = 1,2,...,g$); R_j is the effect of the *j*-th replicate (superblock) ($j = 1,2,...,r$); B_{jk} is the effect of the *k*-th incomplete block within the *j*-th replicate ($k = 1,2,...s$); L_n is the effect of the n-th location (n=1,2,...,l); $(GxL)_{in}$ is the interaction effect between genotype and location; and e_{ijkn} is an experimental error associated with the observation of the *i*-th genotype in the *k*-th incomplete block within the *j*-th complete replicate and n-th location.

Partitioning of the total variation into components due to genotype (σ^2_g), environment (σ^2_e) and genotype by environment interaction (σ^2_{ge}) was performed using the VARCOMP procedure of SAS which is based on expected mean squares. Broad sense heritability (H^2_b) was estimated based on mean basis for each location and combined locations as suggested by Eckebil et al. (1977) as follows:

Single Location:

$$H^2_b\% = \left[\frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 e}{r}} \right] \times 100$$

Combined over location:

$$H^2_b\% = \left[\frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 g \times \ell}{\ell} + \frac{\sigma^2 e}{r\ell}} \right] \text{ x } 1000$$

where σ_g^2 is genetic variance; σ_e^2 is error variance; l is location; rl is replication within location; $\sigma^2_{g\times l}$ is variance due to genotype by location and r is replication.

Genetic advance (GA) was calculated with the method suggested (Allard, 1960; Singh and Chaudhury, 1985)

$$GA = K \times \sigma ph \times H_b^2$$

where GA= genetic advance; K= constant, which is equal to 2.06 at 5% selection intensity; σph = square root of phenotypic variance; and H_b^2 = broad-sense heritability.

GA as % of mean (GAM) = (GA/mean value)*100

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were computed according to Singh and Chaudhury (1985) using the following equations:

$$GCV\ (\%) = \sqrt{\frac{s2g}{x}} \times 100$$

$$PCV\ (\%) = \sqrt{\frac{s2ph}{x}} \times 100$$ where s^2g = genotypic variance; s^2ph = phenotypic variance; and X = sample mean.

Phenotypic and genotypic correlations were estimated using the standard procedure suggested by Miller et al. (1958) from the corresponding variance and covariance components using the following equations:

Phenotypic correlation coefficient:

$$rpxy = \frac{\sigma pxy}{\sqrt{\sigma 2px * \sigma 2py}}$$

Genotypic correlation coefficient:

$$rgxy = \frac{\sigma gxy}{\sqrt{\sigma 2gx * \sigma 2gy}}$$

where, r_{pxy} = phenotypic correlation coefficient between characters X and Y and r_{gxy}= genotypic correlation coefficients between characters X and Y.

Stress susceptibility index of traits: Fischer and Manuer (1978) method, one of the most used methods for evaluation and selection under drought was employed. In this method, average yield of tested genotype in each of the moisture treatment was used to calculate drought susceptibility index of seed yield and yield components grown under water stress and optimum water regime treatments and calculated using the following equations:

Stress susceptibility index (SSI) = 1-(GYWS/GYFW)/SI while SI=1-($\overline{X}$ GYWS/ $\overline{X}$ GYFW), where SI is stress intensity, $\overline{X}$ GYFW and $\overline{X}$ GYWS are means of all genotypes under full-water and water-stress conditions, respectively.

To compare selected subsets of the 10% best genotypes within the whole population, they were sorted and means were independently computed for each character. The absolute value of Student's Z test was calculated to compare genotypic values of the 10% best selected genotypes with the base population as: $Z= \frac{\overline{X} - \mu}{\sigma / \sqrt{n}}$

Where $\overline{X}$ is mean of selected genotypes, μ is mean of the base populations, σ is the standard deviation calculated for the base populations and n is the number of genotypes selected from the

base population for better performance. The significance of the difference between the population parameter (μ) and sample mean ($\overline{X}$) was tested using Z table, i.e. when the calculated value of Z-test was more than the tabulated Z value, the difference was considered significant (Singh, 2001).

3.1.4. Transcriptome Analysis of Selected Backcross lines

3.1.4.1. Experimental Design

Two superior BC_3F_4 isogenic lines (5-12 and 38-2), that were selected for high yield performance and drought tolerance under moisture stress condition, along with two contrasting parental cultivars (Ejere and ICC 4958) were grown in glasshouse, laid down in two set (full-water and water-stress sets) in four replications for each set (4 genotypes x 4 replications x 2 stress levels = 32 experimental units). In the water-stress set, the lines were subjected to controlled stress in the greenhouse at ICRISAT following Varshney et al. (2009). Plastic pots with perforated bottom, 25 cm in radius and 21 cm in height, were filled with black *Vertisol* collected from ICRISAT research field and autoclaved before use. No fertilizer was applied to the pots. The pots were filled with about 10 kg of soil. Two seeds were directly planted in each pot and randomly thinned down to one plant per pot after emergence.

Sowing was done on 22 June 2015 and the plants were grown without stress for 25 days by watering every other day and water-stress was imposed just at the flower initiation for water-stress set. One day before imposing the stress, all pots were saturated with water and allowed to drain excess water in 24 hours to maintain to field capacity so that the soil moisture amount at

each pot is uniform. Then each pot was weighed to know the amount of water at filled capacity. All pots were weighted every other day on a sensitive balance with a resolution of plus or minus 0.1g. The full-water pots (control) were then watered to restore the soil to about 80% of filled capacity by adding a pre-calculated amount of water while the stress pots were remained stressed until the stress level reached 20% of their filled capacity.

3.1.4.2. Root Tissue Sample Collection

As the stress level for each pot of stress treatment reached 20%, root tissues from the non-stressed as well as stressed plants were collected for RNA extraction. For non-stress set, the soil in each pots was washed away by running water, roots were cleaned and tissue was collected. For stress set, the soil in each pot was carefully removed from the pots, broken by hand and the roots were carefully removed and cleaned from soil using only alcohol since it does not stay on the root and affect the weight. To avoid desiccation of the root samples, liquid nitrogen was used for preservation. During RNA extraction, the samples from the four replications were pooled into single sample of each genotype.

3.1.4.3. RNA extraction

RNA was extracted following a standard procedure, NucleoSpin® RNA Plant kit (MACHEREY-NAGELGmbH& Co.KG, 2013), used at Center of Excellence in Genomics (CEG) lab at ICRISAT. Root tissue sample (100 mg) from both the stressed and non-stressed sets of the selected genotypes were crushed using mortar and pestle in the presence of liquid N_2 to disrupt the cells. The cells were lysed by addition of 350 µL of RA1 and 3.5 µL of β-Mercaptoethanol and vortexed vigorously. The lysate was filtered through NucleoSpin® Filter

(violet ring). The NucleoSpin® Filter was placed in a collection tube (2 mL) and centrifuge for 1 min at 11,000 x g. Then the filtrate was transferred to a new 1.5 mL microcentrifuge tube. The NucleoSpin® Filter was discarded and 350 µL ethanol (70%) was added to the homogenized lysate, mixing by pipeting up and down (5 times). DNA was bound using NucloSpin®RNA Plant Column (light blue ring) placed in Collection Tube to load the lysate. Then the silica membrane was desalted by adding 350 µL MDB (Membrane Desalting Buffer) and centrifuge at 11,000 x g for 1 minutes to dry the membrane. A DNA reaction mixture was prepared in 1.5µL microcentrifuge tube and 10 µL reconstituted rDNase added to 95 µL reaction buffer for rDNase and mixed. At last, the dry silica membrane was washed by RAW2 and RA3 buffers to dry the membrane and finally eltuted the RNA in 60 µL RNase-free water and centrifuged. The quantity and quality of the RNA was triple checked using Gel Electrophoresis, NanoDrop and Bioanalyzer.

3.1.4.4. cDNA Library construction and sequencing

The concentration of RNA was checked using Qubit Flouorometer and quality using Agilent Bioanalyzer. Then, the following protocol was used to convert the total RNA into a library of template molecules suitable for subsequent cluster generation and sequencing using the reagents provided in the Illumina®TruSeq®RNA Sample Preparation Kit v2.

First, the poly-A containing mRNA molecule was purified using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This was followed by

second strand cDNA synthesis using DNA polymerase I and RNase H; these cDNA fragments then went through an end repair process, the addition of a single 'A' base, and then followed by ligation of the adapters. The products were then purified and enriched with PCR to create the final cDNA library.

The concentration of the cDNA library was checked using Qubit Fluorometer and the cDNA was diluted using the following formula: (1) Concentration was calculated as ng/µL x 10^6 and this was denoted as A; (2) average base pair size (that was read from the quality test graph) and this was multiplied by a constant (660) and this was denoted as B; (3) from this, concentration in nM was calculated as A/B; (4) this concentration was diluted to 2 nM; and (5) 10µL of this concentration was added to 10µL 0.1N NaOH to get denaturation. After keeping for 5 minutes at room temperature, hybridization buffer (HT1) was added. From this, 20 picomol was taken for cluster generation on c-Bot machine and finally proceeded to sequencing on HiSeq-2500.

3.1.3.5. RNA sequence data analysis

The sequencing machine generated 294 million paired end raw reads. The raw sequence reads were trimmed for low quality bases (PHRED score <20) and adapter contamination. The final processed reads were mapped against the chickpea genome sequence (Varshney et al. 2013, Nature Biotechnology).The reads were mapped using TopHat splice mapper (Trapnell et al. 2009). The data was analyzed for differentially expressed genes (DEGs) for identification of candidate genes involved in drought tolerance between the stressed and non-stressed as well

as the isogenic lines and the parental cultivars were compared; variant analysis for identification of SNPs and insertion/deletion (InDels) was performed.

3.2. Experiment II. Stress Tolerance or Susceptibility Indices and Water Use-Efficiency of Released Chickpea Cultivars

Drought indices which provide a measure of drought tolerance based on the loss of yield under drought conditions in comparison to normal conditions have been used for screening drought tolerant genotypes (Mirta, 2001). These indices are either based on drought resistance or susceptibility of genotypes (Fernandez, 1992)

Water use efficiency (WUE) is defined by different authors in slightly different ways but with similar general implication. It is defined as a measure of a crop's capacity to convert water into plant biomass or grain. It is also defined as the ratio of the seed yield to the amount of measured crop evapo-transpiration, Kg ha^{-1} mm^{-1} (Shouse et al 1981; Boyer, 1996). Under field condition, WUE includes the use of water stored in the soil and rainfall during the growing season. It relies on the soil's ability to capture and store water, the crop's ability to access water stored in the soil and rainfall during the season, the crop's ability to convert water into biomass, and the crop's ability to convert biomass into grain (harvest index).

3.2.1. Plant materials

A pot experiment was carried out at Holetta Agricultural Research Center in greenhouse during the main season (August 2013 to January 2014). Twenty improved chickpea cultivars

of diverse genetic background that were released for optimum environments and under production were tested for their stress susceptibility or tolerance indices and water-use efficiency (Table 2).

Table 2. Year of release, adaptation zone and yield potential of released chickpea cultivars used in screening for stress tolerance indices and moisture use efficiency trial under greenhouse condition at Holetta in 2014.

			Adaptation Zone					Grain yield (t/ha)	
No.	Variety	YR	Altitude (m.a.s.l.)	RF (mm)	DTM	HSW (g)	Seed Color	Research Station	On-farm
1	Acos Dubie	2001	1600-2400	700-1200	136	640		1.1-2.4	1.0-1.3
2	Akaki	1995	1800-2300	700-1200	97-149	210	Golden	1.8-4.2	1.9-2.6
3	Akuri	2011	1800-2300	660-1025	98			2.1-2.6	1.7-2.0
4	Arerti	1999	1800-2300	700-1200	105-155	257	White	2.6-4.6	1.8-4.7
5	Cheffe	1996	1800-2600	700-1200	93-150	310		1.2-4.8	1.8-3.6
6	Dubie	1978	1800-2300	700-1100	110-115	220	Gray	1.7-2.8	1.6-1.7
7	DZ-10-4	1974	1800-2300	700-1100	110-135	102	White	1.6-2.2	1.1-1.4
8	DZ-10-11	1974	1600-2000	700-1100	106-123	130	Brown	1.5-2.8	1.1-1.9
9	Ejere	2005	1800-2600	700-1200	118-129	320	White	2.0-3.5	1.2-1.5
10	Fetenech	2005		660-1025	80-120			1.6-2.5	1.4-1.8
11	Habru	1996	1800-2600	700-1200	91-150	310		1.4-5.0	2.0-4.0
12	ICC 4958*	-	-	-	-	-	-	-	-
13	Kasech	2011		660-1025	100	-	-	2.0-2.5	1.6-2.0
14	Kobo	-		660-1025	-	-	-	-	-
15	Maryye	1985	1800-2300	700-1300	100-120	255	Brown	1.8-3.0	1.4-2.3
16	Minjar	2010	1800-2600	600-1200	86-143	280		2.2-5.0	2.0-4.0
17	Natoli	-	-	700-1200	-	-	-	-	-
18	Shasho	1999	1800-2300	700-1200	97-149	299	White	2.6-5.2	2.0-4.2
19	Worku	1994	1800-2300	700-1200	100-149	330	Brown	1.9-4.0	1.9-2.9
20	Yelibe	2006	-	660-1025	77-106	-	-	1.8-2.5	1.4-2.0

*ICC 4958 is a drought tolerant line introduced from ICRISAT, India and not yet released in Ethiopia; YR=year of release; DTM=number of days to mature; HSW=hundred seeds weight; RF=rainfall

3.2.2. Experimental design

Randomized complete block design in a factorial arrangement in three replications was used. The factorial combination of 20 genotypes, two soil types and two water treatment (full-water and water-stress) resulted in 80 treatment combinations. The two soil types were heavy black *Vertisol* from Ginchi area and light black *Vertisol* from Adadi area. These two soil types represent the two most important chickpea growing soils of the country.

3.2.3. Water treatment

Moisture-stress treatment was applied just at the start of flowering (to simulate terminal drought) and continued throughout the entire period of the crop to maturity. Two water regime (full-water, FW, i.e., the control and water stressed, WS) were applied as follows:

For full-water (FW) or control, the water was maintained during the stress period around 80% field capacity of the available soil water while for water-stress (WS), after imposition of the stress, the plants were not watered until the stress level reached 20% of field capacity of the available water and re-watered to 80% field capacity.

3.2.4. Planting and watering procedure

Plastic pots, with perforated bottom, 25 cm in radius and 21 cm in height were filled with soil collected from Ginchi and Adadi research stations (Table 1). No fertilizer was applied to the soils. Each pots were filled with 7 kg of soil. Fifteen seeds were directly planted in each pot and randomly thinned down to five plants after emergence. The number of plants per pot was

determined on the basis of the area of the pot and the highest alternative chickpea seed rate recommendation (50 plants per meter square) (Geletu and Maesen, 2006).

One day before starting the treatment, pots were saturated with water and allowed to drain excess water in 24 hours to maintain to field capacity so that the soil moisture amount at each pot is uniform. Then each pot was weighed to know the amount of water at filled capacity since the weight of pots and the soil was already known. All pots were weighted every three days on a sensitive balance with a resolution of plus or minus 5 g. The pots were then be watered to restore the soil to the appropriate moisture regime by adding a calculated amount of water. The general principle stated by Boutraa and Sanders (2001) was used for the water treatment application. The amount of water depleted (evapo-transpiration, ET) by the crop was calculated from applied irrigation water and the change in soil moisture at harvest. Irrigation was the only source of water and there was no drainage or rainfall water.

3.2.5. Data collection

Agro-morphological and physiological data collected were number of days to flowering (DTF), maturity (DTM) and grain filling (DTGF), plant height in centimeter (PHT) (for growth stage two and three), number of pods per plant (PPP) and seed per pod (SPP), hundred seeds weight (HSW) in gram, grain yield (GYLD) in gram per five plants, biomass yield (BMY) in gram per five plants, harvest index (HI) in percent, and water-use efficiency (WUE), depending on the respective growth stage (late flowering, late podding and maturity). These data were collected on five plants in the plot. In addition, the physiological data - leaf dry weight (LDWt), stem dry weight (StDWt), shoot dry weight (ShDWt), and root dry weight (RDWt), shoot-root ratio

(SRR), leaf area per plat (LAPP), leaf area index (LAI), and relative water content (RWC) were collected on five plants at all the three plant growth stages (late flowering, late pod setting and maturity):

Shoot dry weight (ShDWt g five plants^{-1}**)**: Above ground shoot of five plants was harvested at late flowering time from the early destruction pots and then oven dry at 70 degree c for 48 hrs and then weighted;

Root dry weight (RDWt g five plants^{-1}): Root from five plants were carefully separated from the soil oven dried at 70 degree centigrade for 48 hrs and then weighted;

Shoot-root ratio (SRR): Ratio of the shoot dry weight to the root dry weight;

Leaf area per plant (LAPP): All leaves from five plants were taken and their leaf area was measured by leaf-area meter.

Leaf area index (LAI) = leaf area divided by soil area ($cm^2_{leaf}cm^{-2}_{soil}$). The measurement was done using LAI meter.

Relative water content (RWC) = [(Fw-Dw)/(Tw-Dw)]*100, where Fw is the fresh weight of the leaflets, Tw is the turgid weight of the leaflets floated on distilled water in petridish for 24 hourss, and Dw is the dry weight of the leaflets oven dried at 80 degree centigrade to constant weight. Leaflets from five randomly selected plants per treatment were excised and used for the

estimation of RWC. Different drought tolerance indices, as suggested by different scientists, were calculated as follows:

1. Tolerance index (TOL) and mean productivity (MP) as done by Rosielle and Hamblin (1981):

   TOL= (GYFW-GYWS); and

   MP = (GYFW+GYWS)/2

   Where GYFW and GYWS are grain yields of a cultivar under full-water and water-stressed conditions, respectively;

2. Harmonic mean (HM) (Kristin et al., 1997):

   HM = 2(GYFW*GYWS)/ (GYFW+GYWS);

3. Stress susceptibility index (SSI) (Fisher and Maurer, 1978):

   SSI = 1-(GYWS/GYFW)/SI while SI=1-($\overline{X}$ GYWS/ $\overline{X}$ GYFW),

   Where SI is stress intensity, $\overline{X}$ GYFW and $\overline{X}$ GYWS are means of all genotypes under full-water and water-stress conditions, respectively.

4. Geometric mean productivity (GM) and stress tolerance index (STI) (Fernandez, 1992; Kristin et al., 1997):

   GMP= $(GYFW*GYWS)^{½}$

   STI= (GYFW*GYWS)/ $(\overline{X}\ GYFW)^2$

5. Yield index (YI) (Gavuzzi et al., 1997; Lin et al., 1986):

   YI = GYWS/ $\overline{X}$ GYWS

6. Yield stability index (YSI) (Bouslama and Schapaugh, 1984):

   YSI = GYWS/GYFW

Determination of water use efficiency - Water use efficiency for grain yield (WUEGY) and biomass (WUEBM) were calculated as the ratio of the grain yield or above-ground biomass to the water transpired. The evapo-transpiration was measured gravimetrically from series of trials in pots with or without plants. Adjacent plant-free pots were used to estimate evaporation. Transpiration was then calculated as the difference between evaporation from the planted pots and plant-free pots.

3.2.6. Statistical data analysis

All the data were subjected to statistical analysis like descriptive statistic (mean), univairate analysis (analysis of variance, ANOVA and variance component analysis) according to Gomez and Gomez using (1984), bivariate (associations or correlation), and multivariate (cluster and principal component) analyses according to Hardle and Hlavka (2007) using SAS (SAS Institute, 2002) and SPSS (IBM Corp., 2011, Version 20) computer softwares.

4. Results and Discussions

4.1. Experiment 1. Marker-Assisted Backcrossing

4.1.1. Identification of polymorphic donor and recurrent parents

Marker polymorphism was tested between chickpea cultivar, ICC 4958 (donor parent) and 10 elite Ethiopian chickpea cultivars (Akaki, Arerti, Dube, Ejere, Habru, Marye, Natoli, Shasho, Teji, and Worku) for four primers of SSR markers mentioned above (Table 3). The parental combination with higher marker polymorphism was selected for undertaking marker-assisted backcrossing (MABC). Cultivar, Ejere, was selected as the best recurrent parent because of its maximum polymorphism with the donor and high yield potential.

Table 3. Screening of the released Ethiopian chickpea cultivars and ICC 4958 (as parent genotypes) for drought tolerance related SSR marker's polymorphism.

Cross	Polymorphism with markers			
	TAA170	ICCM0249	GA24	STMS11
Ejere x ICC 4958	✓	✓	✓	✓
Arerti x ICC 4958	✓	✓	✓	✓
Dubie x ICC 4958	×	✓	×	×
Habru x ICC 4958	✓	×	×	✓
Marye x ICC 4958	×	✓	×	×
Natoli x ICC 4958	✓	✓	×	×
Shasho x ICC 4958	✓	×	×	×
Teji x ICC 4958	✓	✓	✓	✓
Worku x ICC 4958	✓	✓	×	×
Akaki x ICC 4958	×	×	×	×

✓ Mark indicates the presence of polymorphism with the markers

4.1.2. Development of marker-assisted backcross progenies

The backcross breeding method is a standard method that can be employed to introduce a specific trait, such as disease resistance, from a donor parent (usually none improved) to an elite (agronomic) breeding line. It is an effective method to transfer one or a few genes controlling a specific trait. In this regard a drought tolerant line developed by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) was used as a donor parent and crossed with a farmers' preferred improved and released Ethiopian chickpea cultivar, as a recurrent parent. The backcrossing involved making an initial cross between the donor and recurrent parents to obtain the F_1 progeny having 50% of their genetic material from each parent. Then the F1 individuals were crossed to Ejere to develop a backcross one (BC_1) population. Individuals from the BC_1 population were once again crossed to Ejere. Each generation of backcrossing is expected to reduce the proportion of the genome of donor parent present in the population by half. This cycle of crossing backcross progeny to the recurrent parent continues until BC3F1 were obtained (Table 4). These BC3F1 lines are expected to contain about 93.75% genome of the recurrent parent and 6.25% that of the donor parent. The backcrossing process was accelerated using marker-assisted backcrossing. The developed BC_3F_1 lines were selfed two seasons to obtain BC_3F_3 isogenic lines. In this study, 208 isogenic lines were developed for multi-location evaluation. The average crossing success of chickpea in this study was found to be very low (only 26%) in Ethiopia. Previous reports from Ethiopia also indicate that crossing success of chickpea is low, about 10% (Bejiga and Tessema, 1981), as it is influenced by the environment in which a plant grows and by parental identity (e.g., seed size) (Pittman and Levin 1989). Anbesse and Warkentin (2005) reported that the percent crossing success ranged from 8.2 to 42.7 in Canada, depending upon the seed size of the female parent used and the specific leaf area of leaves subtending the flowers.

Table 4. Crossing and backcrossing success of Ejere × ICC 4958 for MABC isogenic lines development.

Season	Number of				Crossing Success* (%)	Total seeds harvested
	Seeds Planted	Seed Germinated	Crossed Flowers	Effective Pods		
Aug2010-Dec 2010	240	218	850	204	24	248 F_1
Mar 2011-June 2011	248	132	347	52	15	98 BC_1F_1
Aug 2011-Dec 2011	98	88	700	210	30	384 BC_2F_1
Mar 2012-Jun 2012	384	169	614	215	35	388 BC_3F_1
Aug 2012-Dec 20112	Selfing of selected BC_3F_1 progenies to obtain BC_3F_2					
Mar 2013-Jun 2013	Selfing of selected BC_3F_2 progenies to obtain BC_3F_3					
Aug 2013-Dec 2013	Selfing of selected BC_3F_3 progenies to obtain BC_3F_4					
Jan 2014 - April 2014	Field evaluation of 208 isogenic lines along with Ejere and ICC 4958 at Doni and Werer under full-watered and stressed environments.					
July 2014 - Oct 2014	Field evaluation of the same lines at Alem Tena under rainfed condition (early planting as full-watered and late planting as water-stressed set)					

*Crossing success=(effective pod)/total crossed flowers) ×100

4.1.2.1. Genotyping of the backcross progenies

Development and use of polymerase chain reaction (PCR)-based molecular markers and genetic maps in chickpea started as early as 1990s (Guar and Slinkard, 1990). Subsequently, several hundred simple sequence repeats (SSR) markers have been developed in chickpea (Varshney, et al., 2007). Genotyping of 256 BC_3F_3 progenies along with the two parents was conducted using six primers of SSR marker (GA24, ICCM0249, NCPGR21, NCPGR127, STMS11 and TAA170) that are related to drought tolerance genome region. Of these progenies, 48 were found to have at least one marker and above (Table 5).

Table 5. List of 48 lines selected for having the markers and included in the multi-location trials

S. No.	Line	GA24	ICCM0249	NCPGR21	NCPGR127	STMS11	TAA170	S. No.	Line	GA24	ICCM0249	NCPGR21	NCPGR127	STMS11	TAA170
1	4-9 (4)	✓		✓	✓		✓	25	29-2 (3)	✓		✓		✓	
2	4-10 (1)		✓					26	29-6 (3)	✓		✓		✓	
3	4-14 (2)			✓		✓		27	30-1 (1)			✓			
4	5-1 (3)			✓		✓	✓	28	30-2 (1)			✓			
5	5-11 (3)		✓		✓		✓	29	33-2 (2)	✓				✓	
6	5-1 (4)		✓	✓		✓	✓	30	33-3 (3)	✓	✓	✓			
7	5-19 (5)		✓	✓	✓	✓	✓	31	34-3 (1)			✓			
8	6-8 (2)		✓				✓	32	34-4 (1)			✓			
9	6-11 (3)		✓	✓		✓		33	35-3 (1)			✓			
10	6-13 (3)			✓		✓	✓	34	35-4 (1)	✓					
11	6-15 (1)						✓	35	36-2 (1)	✓					
12	7-8 (3)	✓		✓		✓		36	36-4 (3)	✓	✓	✓			
13	7-9 (2)		✓		✓			37	37-1 (1)			✓			
14	7-11 (2)					✓	✓	38	37-3 (1)			✓			
15	7-9 (1)				✓			39	37-4 (1)			✓			
16	8-10 (1)				✓			40	37-5 (1)			✓			
17	9-7 (1)			✓				41	38-1 (1)					✓	
18	10-10(3)		✓	✓			✓	42	38-2 (3)	✓		✓		✓	
19	14-2 (1)		✓					43	38-4 (1)			✓			
20	15-5 (1)			✓				44	38-5 (1)			✓			
21	16-7 (1)			✓				45	39-7 (1)			✓			
22	21-1 (2)	✓			✓			46	39-15(1)			✓			
23	25-5 (1)			✓				47	40-4 (2)	✓		✓			
24	28-1 (1)		✓					48	40-8 (2)	✓		✓			

Figures in parenthesis indicate number of markers identified in the line

4.1.3. Field evaluation of backcross progenies in multi-locations

4.1.3.1. Analysis of variance

The independent ANOVA for the three locations indicate that there was significant difference ($P<0.01$) between the tested lines for all the traits under study for both full-water and water-stress conditions (Table 6a). The coefficient of variation for each traits at each location was not high. Under full-water condition, high coefficients of variation were recorded for Ascochyta blight (18.1%) at Alemtena; similarly, under water-stressed condition, high coefficients of variations were recorded for number of pods per plant (19.7%) at Doni; In general, there seems a tendency of high coefficient of variation under water-stressed condition than under full-water condition. The effect of the genotypes on the response variables was high for all the locations under both water conditions, ranging from 0.74 to 0.95 under full-water and 0.80 to 0.93 under water-stressed condition as expressed by R^2 (also called coefficient of determination). This means that 74 to 95% of the variability in the traits studied under full-water conditions and 80 to 93% under water-stressed conditions were accounted for by the variability in the test lines/genotypes.

The combined ANOVA also clearly showed that there was highly significant difference among the location and the genotypes ($P<0.01$) under both full-water and water-stressed conditions (Table 6b). The same holds true for genotype by locations interaction except there was no location by genotype interaction effect ($P>0.05$) for plant height under full-water, and number of seeds per pod under water-stressed conditions. The effect of location, genotype and genotype by location interaction, combined together, was large as explained by R^2, and ranged from 0.90 to 0.94 and 0.83 to 0.94, under full-water and water-stressed conditions, respectively.

Table 6a. Mean squares (MS) , R^2, and CV for separate ANOVA of 11 agro-morphological traits of 210 chickpea genotypes tested at three locations (Alem Tena, Doni and Werer), in 2014/15 under full-water and water-stressed condition

Full-water(control)									
	Alem Tena			Doni			Werer		
Trait	MS	R^2	CV	MS	R^2	CV	MS	R^2	CV
Full-water									
DTF	2.384**	0.87	3.3	2.413**	0.74	3.2	2.987**	0.88	3.4
DTM	5.807**	0.89	3.6	11.604**	0.94	2.7	6.121**	0.91	3.0
DTGF	6.577**	0.88	7.8	10.371**	0.86	7.5	7.098**	0.90	2.5
AB	0.580**	0.80	18.1	-	-	-	-	-	-
PPP	64.639**	0.95	5.0	49.577**	0.92	8.6	96.928**	0.93	8.1
SPP	0.047**	0.83	8.1	0.096**	0.93	6.6	0.055**	0.84	8.2
PHT	36.274**	0.90	6.5	31.380**	0.90	4.8	28.492**	0.94	8.6
HSW	44.453**	0.89	3.3	17.694**	0.91	6.6	9.959**	0.87	5.7
BMYLD	140.662**	0.90	8.9	127.208**	0.82	12.2	142.907**	0.91	5.6
HI	29.544**	0.91	6.0	27.677**	0.88	6.5	29.756**	0.90	5.9
GYLD	133.930**	0.90	8.6	122.209**	0.89	9.2	144.723**	0.94	8.3
Water-stressed									
DTF	6.707**	0.86	2.7	7.438**	0.89	2.7	5.855**	0.90	2.7
DTM	7.595**	0.87	3.3	5.837**	0.86	3.2	3.875**	0.86	2.8
DTGF	9.107**	0.85	4.3	8.148**	0.85	8.6	5.812**	0.85	6.6
AB	0.979**	0.91	13.5	-	-	-	-	-	-
PPP	56.042**	0.89	11.1	39.860**	0.87	19.7	36.550**	0.83	8.8
SPP	0.033**	0.80	7.9	0.054**	0.88	6.8	0.033	0.77	8.3
PHT	25.00**	0.86	7.3	13.939**	0.80	5.8	13.871**	0.79	6.5
HSW	20.123**	0.86	6.0	16.455**	0.79	7.3	5.330**	0.72	5.6
BMYLD	432.719**	0.87	7.6	620.263**	0.90	7.5	915.674**	0.93	7.6
HI	26.769**	0.85	8.2	26.869**	0.86	8.9	25.459**	0.83	7.4
GYLD	85.161**	0.88	10.9	120.991**	0.92	9.4	189.079**	0.92	10.5

DTF, DTM & DTG = number of days to flowering, maturity & grain filling; AB=Ascochyta blight disease; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); BMYLD & GYLD = biomass & grain yield (g); HI=harvest index; R^2 =measure of effect; CV=coefficient of variation (%)

Table 6b. Mean squares (MS), R^2, and CV for combined ANOVA of 11 agro-morphological traits of 210 genotypes tested at locations (Alem Tena, Doni and Werer) in 2014 under full-watered and water-stressed conditions

	Full-watered (Control)					Water-stressed				
	MS					MS				
Trait	Loc	Geno	G x L	R^2	CV	Loc	Geno	G x L	R^2	CV
DTF	299.14**	6.53**	0.63**	0.91	3.2	1924.92**	11.41**	4.30**	0.93	2.5
DTM	784.40**	16.92**	3.31**	0.93	3.1	187.01**	10.08**	3.61**	0.87	3.2
DTGF	906.83**	18.36**	2.84**	0.93	7.7	3297.48**	15.49**	3.79**	0.92	8.2
PPP	3179.19**	168.44**	21.35**	0.94	9.1	9406.11**	101.82**	15.31**	0.91	17.9
SPP	0.34**	0.12**	0.04**	0.92	7.7	0.66**	0.06**	0.03ns	0.83	15.7
PHT	3115.75**	88.22**	4.11ns	0.93	9.5	5014.69**	38.28**	7.44**	0.89	15.4
HSW	592.83**	40.11**	2.90**	0.91	6.5	3242.43**	26.09**	7.91**	0.88	2.9
BMYLD	2611.77**	324.60**	42.09**	0.90	8.0	28978.26**	1443.21**	262.72**	0.92	10.2
HI	440.00**	70.47**	8.25**	0.91	6.2	663.33**	56.52**	11.47**	0.85	7.8
GYLD	2271.60**	324.54**	38.16**	0.92	8.8	6974.97**	270.31**	62.46**	0.92	10.3

DTF, DTM & DTG = number of days to flowering, maturity & grain filling; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); GYLD = grain yield (g); Geno=genotype; G x L = genotype by location interaction; R^2 =measure of effect; CV=coefficient of variation (%)

4.1.3.2. Phenotypic trait performances

There seems some genetic variability in the tested isogenic lines for different traits studied both under full-water and water stressed conditions (Table 7). The number of days to flowering (DTF) for the two parents, Ejere and ICC 4958 was almost the same (52) but that of the isogenic lines has ranged from 49 to 67 days with mean of 53 days that was comparable with the whole population's mean under full-water condition; similarly, for water-stressed condition, the mean DTF was 53 days for Ejere and 51 days for ICC 4958 with average of 52 days whereas it ranged from 49 to 56 days for isogenic lines with mean of 52 days which was similar with mean of the whole population mean. The mean DTF under full-water and water-stress conditions were not significantly different as there was no moistre constrait until the flowering period. The number of days to maturity (DTM) for Ejere and ICC 4958 was 91 and 90 days with mean of 90 days while for the isogenic lines the mean

DTM ranged from 87 to 99 days with mean of 93 days which was similar with the mean of the whole population under full-water condition; similarly, the mean DTM for Ejere and ICC 4958 was 85 and 79 days, respectively, with mean DTM of the two parents being 82 days while the mean DTM of the isogenic lines ranged from 76 to 91 days with mean DTM of 84 days which was close to the mean DTM of the whole population. There was significant difference between the mean DTM under full-water and water-stressed condition in which it was low for the parents, the lines and the whole population under moisture-stress condition. This indicated that water-stress has significantly affected the mean number of DTM. The number of days to grain filling (DTGF) for the two parents, Ejere and ICC 4958 was 35 and 34 days, respectively, with their mean being 34 days while that of the isogenic lines ranged from 30 to 44 days with mean DTGF of 37 days which was similar with mean DTGF of the whole population under full-water condition; similarly, mean DTGF for Ejere and ICC 4958 was 33 and 28 days, respectively, with their mean bing 30 days while for the isogenic lines it ranged from 25 to 40 days with mean of 32 days which was similar with mean DTGF of the whole population under water-stressed condition. Under moisture-stress condition, the drought tolerant parent, ICC 4958, had significantly early grain filling period as compared to Ejere. As of number of DTM, moisture stress has significantly reduced number of DTGF of the tested genotypes in general.

The number of pods per plant (PPP) for Ejere and ICC 4958 was 36 and pods 46 , with their mean being 41 pods while for isogenic lines it ranged from and 20 to 58 pods with mean of 37 pods which was similar with the mean PPP for the whole population under full-water condition; similarly, PPP for Ejere and ICC 4958 was 20 and 35, respectively, with their mean being 28 pods while that of the isogenic lines ranged from 13 to 37 pods with mean of 25 pods that was similar with the mean PPP

of the whole population under water-stressed condition. From the result, it was clear that water-stress has significantly reduced number of PPP of the tested genotypes. The mean number of seeds per pod (SPP) for the two parents was similar, 1.4, while it ranged from 0.9 to 1.7 with mean of 1.3 for the isogenic lines that was similar with the mean SPP for the whole population under full-water condition; similarly, mean SPP for Ejere was 1.6 while that of ICC 4958 was 1.1 with mean of the two parents being 1.3 seeds, whereas the mean SPP of the whole population was 1.2 under water-stressed condition. Here also it seems that the mean number of SPP was reduced under water-stress condition in general.

The mean plant height (PHT) for Ejere was 46cm while that of ICC 4958 was 48cm with the mean of the two parents being 47cm where as the mean PHT of the isogenic lines ranged from 28 to 48cm with mean of 38 that was similar with the mean PHT of the whole population full-water condition; similarly, mean PHT for Ejere was 38 cm and that of ICC 4958 was 34 cm with the mean of the two parents being 36 while that of the isogenic lines ranged from 25 to 40 cm with mean of 32 that was similar with mean PHT of the whole population under water-stressed condition. This result indicated that water-stress significantly reduced the mean PHT of the parents, the isogenic lines and the whole population indiscriminately. The mean hundred seeds weights (HSW) for Ejere and ICC 4958 were very close, 30 and 29g, respectively, with mean of 29.5g while that of the isogenic lines ranged from 24 to 39g with mean of 31g as it was for the whole population under full-water condition; similarly, mean HSW for Ejere was 28g while that of ICC 4958 was 29g with mean HSW of the two parents being 28.7g whereas the mean HSW of the isogenic lines has ranged from 21 to 38g as with the mean of 28.5g that was comparable with that of the whole population under

water-stress condition. For HSW also, there was a tendency of reduction in mean performance under water-stress condition as compared to under full-water condition.

The mean biomass yield (BMY) of the two parents, Ejere and ICC 4958 was 135g five plants^{-1} each while that of the isogenic lines range from and 72 to 198 g five plants^{-1} with mean of 139 g five plants^{-1} that was similar with the mean BMYLD of the whole population under full-water condition; similarly, the mean BMY of Ejere was 93 g five plants^{-1} that of ICC 4958 was 121 g five plants^{-1} whereas the mean BMY of the isogenic lines ranged from 54 to 164 g five plants^{-1} with mean of 106.1 g five plants^{-1} that was similar with mean BMY of the whole population under water-stressed condition. Mean BMYs of the two perents were similar under full-water condition but they significantly varied under water-stress condition in which ICC 4958 had the highest mean BMY. In addition, the water-stress had significantly reduced the mean BMY performance as compared to that under full-water water conditition. The mean grain yield (GYLD) of Ejere was 44.4 g five plants^{-1} and that of ICC 4958 was 42.5 g five plants^{-1} with mean of 43.4 g five plants^{-1} whereas the mean GYLD of the isogenic lines ranged from 23 to 71g five plants^{-1} with mean of 44.5 g five plants^{-1} that was similar with the mean GYLD performance of the whole population under full-water condition; similarly, the mean GYLD performance of Ejere was 26.2 g five plants^{-1} while that of ICC 4958 was 32.9 g five plants^{-1} while that of the isogenic lines ranged from 17 to 56 g five plants^{-1} with mean of 33.3 which was similar with the mean GYLD of the whole population under water-stressed condition. Under the full-water condition, the mean GYLD performance of the two parents were comparable whereas under water-stress condition, mean GYLD of the drought tolerant parent, ICC 4958, was higher as expected. The mean harvest index (HI) of Ejere was 34.3% while that of ICC 4958 was 31.3% with mean HI of the parents being 32.8 whereas that of the isogenic lines

ranged from 22 to 41% with mean of 31% that was comparable with the mean HI of the whole population under full-water condition; similarly, the mean HI of Ejere was 33.9% and that of ICC 4958 was 33.1 while the mean HI of the isogenic lines ranged from 24 to 44% with mean of 35% which was comparable with mean HI of the whole population under water-stressed condition. The mean HI of Ejere was higher under full-water condition as compared to that under water-stress. On the contrary, the mean HI of the drought tolerant parent, ICC 4958, was highest under water-stress condition. The range, and the mean HI of the isogenic lines and the whole population was also higher under water-stress condition. This result was in contrast with other traits that had reduced performances under water-stress condition as compared to the full-water condition indicating that the water-stress positively affected the harvest index.

Under both full-water and water-stressed conditions, the mean performances of the lines and mean of the two parents significantly varied for most of the traits. The mean performances of the isogenic lines for some traits like number of DTF, DTM, DTGF, HSW, BMY and GYLD were higher over the means of the checks while for number of PPP, SPP, PHT and HI, mean of the parents were higher than the means of the isogenic lines under full-water condition. Similarly, under water-stressed conditions, mean of the isogenic lines were higher for some traits like number of DTF, DTM, DTGF, HI and GYLD while for PPP, SPP, PHT and BMY means of the parents were higher than the means of the isogenic lines; Mean HSW of the two parents and that of the isogenic lines were not significantly different. When looking at the range of the performances of the traits for the isogenic lines, there were large number of lines that had significantly higher or significantly lower performances as compared to the mean of the two parents, indicating the existence of transgressive segregation in the lines (Table 7). Therefore, this wide range of variability in mean performances

of the isogenic lines for the observed characters could be further exploited through improvement and selection programs.

Under the stressed condition, ten isogenic lines (7-19, 9-7, 10-10, 12-5, 21-3, 29-6, 36-4, 37-4, 38-2, and 39-7) were found to be better in grain yield than the best check, ICC 4958 (data not shown). Out of these, five (10-10, 21-3, 29-6, 36-4, and 38-2) had three markers for drought tolrerance each while the rest five were found to have only one marker each.

In general, lines with marker had higher mean grain yields under both full-water and water-stress conditions followed by selected lines without markers (Figure 4). Under full-water condition, recurrent parental, Ejere, had higher mean grain yield than the donor parent, ICC 4958, while it was the reverse under water-stress condition. This could be so because, ICC 4958 is a known drought tolerant cultivar better than Ejere under water-stress condition.

There was a clear and significant trait performance reduction under water-stressed condition as compared to under full-water condition for almost all the traits except for number of days to flowering and number of seeds per pod. The average relative grain yield reduction due to water-stress was 37.5%. The relative grain yield reduction of lines with markers for drought tolerance due to water-stress was the lowest (19%) as compared to the best lines without markers (21%), ICC 4958 (25%) and Ejere (40%) indicating that lines with markers have better tolerance to water-stress as they possess the genes for drought tolerance. Out of the 48 isogenic lines having marker for drought tolerance, 19 were better than or at least equal with the best parent in yield and other agronomic trait, including drought tolerance, and hence, could be selected for further

advancement in breeding program; similarly, out of 160 lines without markers, about 70 were better than the two parents in yield and other agronomic traits, especially under full-water conditions; on average, selected lines with markers had higher performance in grain yield than those which were better performing without markers under moisture stressed conditions. Therefore, the introgression of drought tolerance gene from the donor, ICC 4958, to the good agronomic background, Ejere, has resulted in the development of lines (whether they have the markers for drought tolerance or not) with better yield and drought tolerance as compared to the two parental genotypes.

Mean trait performances of the top 10% selected isogenic lines are presented in Tables 8a and 8b for full-water and water-stress conditions, respectively. Twenty one lines for each stress sets were selected. In each selection groups, there are lines that have markers for drought tolerance and also lines that do not have the markers but were found to be best performerers for grain yields under full-water and water-stress conditions.

Under full water condition, two isogenic lines, 5-9, having marker for drought tolerance, and line 4-5, with no marker, were early flowering than the two parents (ICC 4958=52 and Ejere=53 days) and the population mean (53 days) with mean DTF being 51 and 52 days, respectively. Two other lines, 37-4 that has the marker and 24-2 that has no marker were late flowering as compared with the means of the parents and the whole population with mean DTF of 55 days. In terms of number of days to maturity (DTM) almost all the selected lines having the markers and those without markers had higher number of DTM with mean ranging from 93 to 96 as compared to the two parents (ICC 4958=90 and Ejere=91 days) that might have contributed to

better yield due to longer time of translocation of assimilates to the sink. Among lines that had late maturity were 7-19 that has marker and 4-13, 5-10, 6-6, 27-1, 27-5, and 31-1 that have no marker for drought tolerance. Regarding number of days to grain filling (DTGF), two lines with markers (5-9 and 7-19) had high mean DTGF (40 days each) than the two parents (ICC 4958=34 and Ejere=35) and the population mean (37 days) while two other lines with markers (37-3 and 37-4) had mean DTGF of 33 and 35 days that was comparable to the mean DTGF of the parents but less than the population mean. Among lines without markers, 5-9 and 5-10 had higher mean DTGF of 40 and 41 days, respectively, as compared to the mean DTGF of the parents and the whole population mean while 18-4, 18-5, and 33-1 had lower mean DTGF of 33, 30, and 33 days respectively, as compared to the means of the parents and the whole population.

For number of pods per plant (PPP), two lines with markers, 37-3 and 37-4, had mean PPP of 42 and 46 pods, respectively, that was comparable with mean PPP of the best parent (ICC 4958=46 pods) while other two lines with markers, 5-9 and 7-19, had low mean PPP of 39 that was comparable with the mean of the other parent (Ejere) and the mean of the whole population (36.0 days). Among the lines without markers, 18-4, 19-4 and 27-5 had high mean PPP of 52.7, 51.6, and 49.9, respectively, while lines 27-2, 27-1, and 33-1 had low mean PPP of 37.7, 36.1, and 36.5, respectively. Number of seeds per plant (SPP) had no much variability and it ranged from 1 to 2 seeds per pod with population mean of 1.3 seeds and mean of the two parents being 1.4 seeds. The mean SPP of the lines ranged from 0.9 to 1.5 seeds.

Regarding the mean plant height (PHT), all the selected lines had lower mean PHT as compared to the two parents (ICC 4958=48 and Ejere=46cm) but line 5-9, having the marker, and lines 4-7

and 4-13 without markers had mean PHT ranging fromm 39 to 46cm that was significantly higher than the whole population mean of 38cm while line 8-4 that has no marker had comparable mean PHT (46cm) with the two parents (intermediate in between them). There was no much variation for hundred seeds weight (HSW) for the selected lines. One line having the markers (7-19) and two lines without markers (24-2 and 33-1) had mean HSW of 34g each that was higher that the two parents (ICC 4958=29 and Ejere=30g) and the whole population mean (31g) whereas lines 18-4 and 18-5 had smaller mean HSW (26g) which was lower than the mean of the two parents and that of the whole population.

With regard to the biomass yield (BMY), all the selected lines hand higher mean ranging from 140 to 188g as compared to the two parents (135g each) and the population mean (139g) except line 4-5 having no markers and had mean BMY of 119g which was significantly lower than the two parents and the whole population mean. Most of the selected lines had significantly higher mean HI than the two parents. These include two lones with markers (7-19 and 37-3) having mean HI of 36.3 and 37.4%, respectively. Eight lines without markers (18-3, 18-4, 19-4, 4-5, 6-6, 18-5, 27-1 and 27-5) having mean HI ranging from 36 to 41% that was higher than the population mean, implying their overall yield potential. On the other hand, line 5-9 that has marker and lines 24-2, 31-1 and 33-1 without markers had lower mean HI ranging from 27 to 30% than the two parents (ICC 4958=31 and Ejere=34%) and the population mean of 31%. All the selections had significantly higher grain yield (GYLD) as compared to the two parents (ICC 4958=45.4 and Ejere=49.9g) and the mean of the whole population mean (47.5g). The lines with markers had mean GYLD ranging from 62 to 64g while lines without markers had mean GYLD ranging from 57 to 71g. Interestingly, the whole population mean was intermediate between the

means of the two parents. Line 18-5 that has no marker for drought tolerance was the top yielder with mean of 71g followed by line 37-4 having marker for drought tolerance indicating that under full-water condition, a lines with marker for drought tolerance may not necessarily be the best yielder.

Under water-stress condition, most of the selected lines had intermediate number of days to flowering (DTF) between the two parents (ICC 4958= 51 days and Ejere= 53 days). But line 29-6 having mearker for drought tolerance and line 29-3 without markers had comparable mean DTF of 51 and 50 with ICC 4958 (51 days) while lines 7-4, 17-5 and 34-2 having mean DTF of 53 to 54 were late in DTF than Ejere (53 days), the late parent. Out of lines with markers, lines 29-6 and 38-2 were as early in DTF as the early parent, ICC 4958 with mean DTF while line 9-7 had comparable mean DTF with Ejere; line 10-10 had intermediate mean DTF (51 days) between the two parents. In number of days to maturity (DTM), almost all the selections were late maturing as compared to the drought tolerant early maturing parent, ICC 4958 that had mean DTM of 79 days except line 29-3 that has no marker for drought tolerance and had earliest mean DTM of 78 days. All the selected lines having markers (9-7, 10-10, 29-6, and 38-2) had intermediate mean DTM between ICC 4958 (79 days) and Ejere (85 days) with mean DTM ranging from 81 to 84 days. Most of the lines that do not have the markers had late DTM than Ejere with mean ranging from 86 to 88 days while lines 5-8 and 30-5 had earlier DTM with mean of 84 and 82 days, respectively. Like for DTM, almost all the selections had longer number of days to grain filling (DTGF) than the earliest parent ICC 4958 and some of them were even later than Ejere, the late parent. All the lines having markers had intermediate DTGF between the two parents with mean ranging from 31 to 32 days. But most of the lines without markers had

longer DTGF ranging from 33 to 36 days except lines 29-3 and 30-5 that had shorter DTGF comparable wo that of ICC 4958.

With regard to number of pods per plant (PPP), all the selections had better mean PPP than Ejere (20 pods) under water-stress condition. All the lines having markers had intermediate number of PPP between Ejere (20 pods) and ICC 4958 (35 pods) with mean ranging from 27 to 32 pods. Out of lines that do not have markers, lines 9-8, 9-10 and 27-2 had higher mean PPP than the best paren ICC 4958, with mean ranging from 36 to 37 pods. There wan not mauch variation in number of seeds per pod (SPP), ranging from 1 to 2 pods, in general. The best agronomic parent, Ejere, had mean SPP of 1.6 seeds while the drought tolerant parent, ICC 4958 had mean SPP of 1.1. All the selections were intermediate in SPP between Ejere and ICC 4958 ranging from 1.0 to 1.5 seeds per pod with the whole population mean of 1.2. Some lines like 7-12, 10-9, 10-1, 10-10, 29-3 and 34-2 had higher mean SPP of 1.4 to 1.5. In plant height (PHT), all the lines having markers were shorter than the two parents (ICC 4958=34 and Ejere=38cm) and the whole population mena PHT (32cm) with mean PHT ranging from 30 to 31cm. Out of lines without markers, lines 7-12, 7-14, 8-6, 9-8, 10-4 and 27-2 had intermediate mean PHT betweeb the two parents with mena ranging from 35 to 37cm; but lines 8-7 was the tallest of all with mean PHT of 39cm above the tallest parent, Ejere, while lines 5-8, 9-2, 10-1, 10-5, and 10-9 had shorter mean PHT than the two parents.

The variability in hundred seeds weight (HSW) was not much, in general. However, line 29-6 having marker had larger seed weight (32g) than the two parents (ICC 4958=29g and Ejere=28g) and the whole population mean (28.5g). Similarly, line 27-2 that has no marker had significantly

larger mean HSW (38g) as compared to all the selections, the two parents and the population mean. The other lines without markers (5-8, 7-12, 8-7, 9-8, and 30-5) had smaller mean HSW than the two parents and the population mean. The rest of the lines without markers had comparable mean HSWs with the two parents. With regard to biomass yield (BMY), most of the selected lines hand higher mean BMY than the two parents (ICC 4958=121 and Ejere=93g) and the whole population mean (106g). Out of the lines having the markers, line 10-10 and 38-2 had higher mean BMY of 146 and 128g, respectively, while lines 9-7 and 29-6 had intermediate mean BMY between the two parents. Out of the lines without the markers, lines 5-8, 7-12, 7-14, 8-7, 9-8, 9-10, 10-1, 10-5, 10-9, 30-5 and 34-2 had higher BMY ranging from 127 to 164g with line 34-2 being the highest in mean BMY (164g) followed by line 9-10 (155g). On the contrary, lines 9-2, 10-4, 17-5, 27-2, and 29-3 had low BMY ranging from 106 to 123g as comared to ICC 4958 and the population mean but not lower than Ejere, the parent with the lowest BMY of 93g. In the case of HI, all the selections having the markers and without markers had high mean HI than the two parents (ICC4858=33% and Ejere=34%) and the population mean (35%). Among the lines having markers, line 29-6 had the highest mean HI (42%) followed by line 10-10 (42%) whereas among lines without markers, line 29-3 had the highest HI of 44% followed by line 30-5 that had mean HI of 42%. In case of grain yield, all the selected lines having the markers or without markers had significantly high GYLD than the two parents Ejere and ICC 4958. Under the water-stress condition ICC 4958 had highest yield (33.9 g) than Ejere (29.5) as expected. The highest mean GYLD was recorded by line 10-10 that has markers for drought tolerance followed by lines 7-17 (51.8g) and 34-2 (50.2g) that do not have markers. Even under moisture stress conditions, some lines that do not have marker for drought tolerance had comparable grain yield with lines having the markers for drought tolerance.

Table 7. Mean traits performance (over three locations) of 210 chickpea genotypes tested under full-water and water-stress conditions at three locations in 2014.

	Parental genotypes			208 isogenic lines		Pop.
Trait	Ejere	ICC 4958	Mean	Range	Mean	Mean
Under full-water condition						
DTF	52.5	52.0	52.0	49-67	53.0	53.0
DTM	90.5	89.5	90.0	87-99	93.4	93.3
DTGF	35.0	33.5	34.3	30-44	36.6	36.6
PPP	36.0	46.0	41.0	20-58	36.7	36.7
SPP	1.4	1.4	1.4	0.9-1.7	1.3	1.3
PHT	45.7	47.5	47.0	28-48	38.2	38.3
HSW	30.0	29.0	29.5	24-39	31.4	31.3
BMY	135.0	135.0	135.0	72-198	139	138.9
HI	34.3	31.3	32.8	22-41	30.9	31.0
GY	44.4	42.5	43.4	23-71	44.5	44.5
Under water-stress condition						
DTF	52.5	50.8	51.7	49-56	52.4	52.4
DTM	85.0	78.8	81.9	76-91	84.3	84.2
DTGF	32.5	28.0	30.3	25-40	31.9	31.9
PPP	20.2	35.2	27.7	13-37	25.1	25.1
SPP	1.6	1.1	1.3	1.0-1.6	1.2	1.2
PHT	38.1	34.1	36.1	25-40	31.5	31.6
HSW	28.2	29.2	28.7	21-38	28.5	28.5
BMY	93.0	121.1	107.0	54-164	106.1	106.1
HI	33.9	33.1	33.5	24-44	34.8	33.3
GY	26.2	32.9	29.5	17-56	33.3	34.8

DTF, DTM & DTG = number of days to flowering, maturity & grain filling; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); GYLD = grain yield (g); Pop.=population

Table 8a. Mean trrait performances (over three locations) of top 10% selected isogenic lines tested under full-water condition

	Trait									
Lines	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
4-2 (NM)	53.5	94.5	34.5	45.9	1.3	36.7	32.0	140.0	34.5	57.6
4-5 (NM)	51.5	94.0	38.3	42.1	1.2	36.7	30.7	119.0	38.0	58.6
4-7 (NM)	52.0	92.3	35.3	42.7	0.9	39.3	29.0	139.8	28.8	57.4
4-13 (NM)	52.5	96.2	38.0	39.2	1.3	40.0	31.9	145.5	33.7	57.6
5-9 (M)	50.8	93.8	40.0	39.1	1.4	40.0	29.5	181.8	28.9	63.4
5-10 (NM)	52.8	95.8	41.0	45.8	1.4	38.5	30.7	174.3	31.0	63.9
6-6 (NM)	54.5	95.8	36.0	40.8	1.4	41.3	30.4	170.8	35.5	62.1
7-19 (M)	51.8	95.0	39.3	39.4	1.3	38.7	34.4	152.5	36.3	61.5
8-4 (NM)	53.8	92.8	35.8	32.1	1.4	45.8	29.0	188.0	30.0	60.4
18-3 (NM)	53.5	91.5	36.0	57.5	1.2	33.3	29.6	176.9	40.8	65.2
18-4 (NM)	53.0	89.0	32.5	53.6	1.5	38.0	25.7	166.3	41.3	63.1
18-5 (NM)	53.8	87.8	29.8	52.7	1.3	38.0	25.6	174.8	36.7	70.7
19-4 (NM)	53.3	93.2	34.5	51.6	1.3	37.1	32.4	171.3	40.8	63.8
24-2 (NM)	54.8	93.3	33.8	37.7	1.1	33.9	34.2	159.3	29.8	57.8
27-1 (NM)	53.3	95.2	36.8	36.1	1.4	43.8	30.4	143.5	39.2	58.2
27-5 (NM)	53.5	95.2	38.0	49.9	1.0	35.5	31.8	146.3	35.5	58.2
31-1 (NM)	53.0	94.7	37.8	44.4	1.1	39.8	30.9	163.8	26.8	59.3
33-1 (NM)	54.5	90.2	32.5	36.5	1.1	38.3	34.8	171.5	28.8	57.8
37-3 (M)	54.5	92.3	32.8	45.6	1.2	42.2	31.1	172.5	37.4	62.4
37-4 (M)	53.0	92.5	34.5	42.4	1.1	41.0	31.5	160.8	33.5	64.0
39-1 (NM)	53.8	91.0	32.3	46.0	1.4	36.5	33.0	171.0	38.0	58.0
ICC 4958	52.0	89.5	33.5	46.0	1.4	47.5	29.0	135.0	31.3	45.4
Ejere	52.5	90.5	35.0	36.0	1.4	45.7	30.0	135.0	34.3	49.9
Pop. Mean	53.0	93.3	36.6	36.7	1.3	38.3	31.3	138.9	31.0	47.5

DTF, DTM & DTG = number of days to flowering, maturity & grain filling; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); GYLD = grain yield (g); M and NM in the parenthesis indicates lines with and without markers for drought tolerance, respectively.

Table 8b. Mean trait performances (over thre locations) of top 10% selected isogenic lines tested under water-stress condition

	Traits									
Lines	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
5-8 (NM)	51.2	83.8	32.7	24.1	1.2	30.8	25.6	135.9	41.8	48.0
7-12 (NM)	51.5	84.5	33.0	34.3	1.5	35.9	23.9	137.1	37.5	46.6
7-14 (NM)	54.3	86.5	32.2	33.0	1.2	34.8	29.4	126.6	40.4	51.8
8-6 (NM)	52.2	85.8	33.7	31.6	1.2	36.6	26.1	153.1	40.5	45.5
8-7 (NM)	51.7	87.0	35.3	28.3	1.2	39.0	26.8	138.3	40.9	47.9
9-2 (NM)	52.0	88.0	36.0	31.0	1.3	33.2	30.0	122.9	41.2	47.2
9-7 (M)	52.5	84.3	31.8	27.3	1.1	29.9	30.0	119.3	35.7	47.9
9-8 (NM)	52.0	87.5	35.5	36.0	1.0	35.0	25.6	133.5	41.5	46.8
9-10 (NM)	52.5	84.5	32.0	36.4	1.1	32.7	29.1	154.5	38.3	47.7
10-1 (NM)	52.7	85.2	32.5	26.8	1.4	31.5	26.1	129.1	37.2	45.8
10-4 (NM)	52.2	86.7	34.5	31.8	1.1	34.7	28.3	119.7	39.7	46.7
10-5 (NM)	52.2	85.7	33.5	27.8	1.1	32.9	29.4	131.6	36.8	46.0
10-9 (NM)	52.5	86.0	33.5	33.1	1.5	27.9	29.7	133.0	35.8	46.1
10-10 (M)	51.3	82.0	30.7	31.7	1.4	29.6	28.6	145.6	41.5	57.3
17-5 (NM)	53.2	85.2	32.0	29.5	1.1	33.4	30.3	115.3	38.4	45.5
27-2 (NM)	51.8	86.5	34.7	36.5	1.3	36.4	37.5	105.6	40.2	50.2
29-3 (NM)	50.2	78.2	28.0	24.1	1.4	32.8	28.4	119.7	43.7	46.4
29-6 (M)	50.7	81.5	30.8	31.3	1.3	29.8	34.2	108.8	42.0	49.9
30-5 (NM)	52.5	81.8	29.3	22.2	1.3	34.0	27.3	149.3	42.0	48.2
34-2 (NM)	53.7	86.5	32.8	23.9	1.5	33.2	26.0	164.0	32.3	50.0
38-2 (M)	49.8	80.8	31.0	30.3	1.3	31.3	30.7	128.3	37.6	48.1
ICC 4958	50.8	78.8	28.0	35.2	1.1	34.1	29.2	121.1	33.1	33.9
Ejere	52.5	85.0	32.5	20.2	1.6	38.1	28.2	93.0	33.9	29.5
Mean	52.4	84.2	31.9	25.1	1.2	31.6	28.5	106.1	34.8	35.9

DTF, DTM & DTG = number of days to flowering, maturity & grain filling; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); GYLD = grain yield (g); M and NM in the parenthesis indicates lines with and without markers for drought tolerance, respectively.

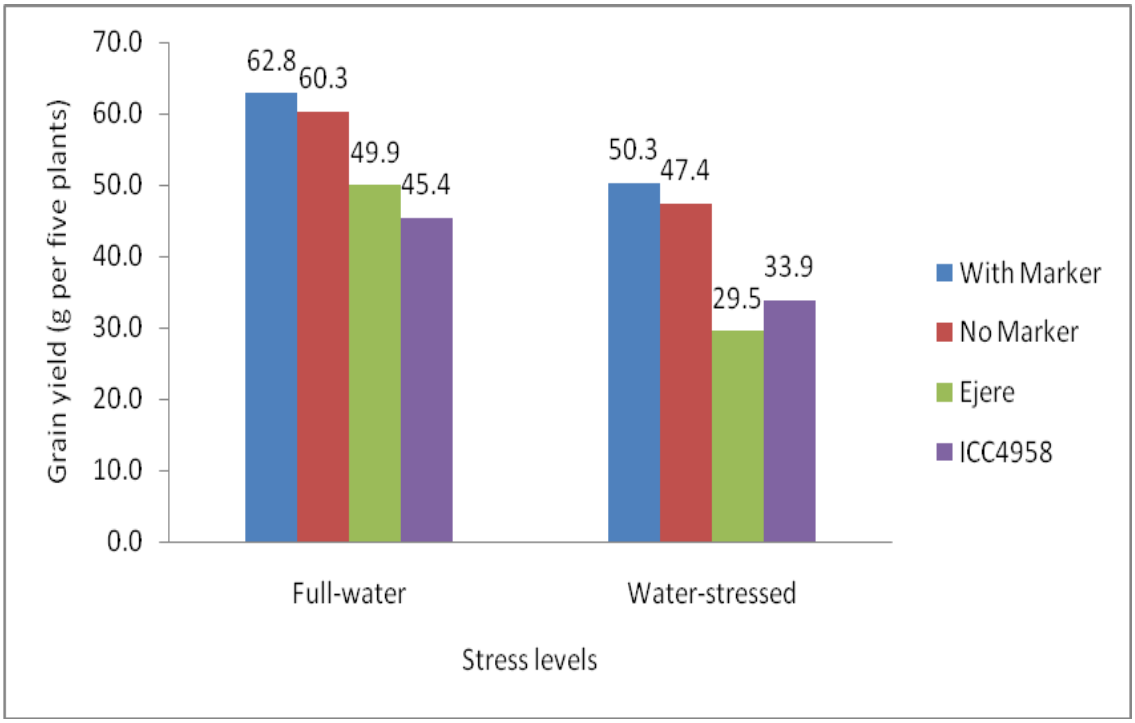


Figure 4. Relative grain yield performances of lines with and without markers as compared to the two parents under full-watr and water-stressed conditons.

4.1.3.3. Effects of locations on traits performances

The three locations have shown significant difference for all the test traits under both water-stress conditions. The mean traits performances at each locations for the traits studied is indicated in Table 9. Under full-water (FW) conditions, mean number of days to flowering (DTF) were 54, 53, and 52 days for Alem Tena, Doni, and Werer, respectively. Similarly, the mean number of days to maturity (DTM) were 98, 96, and 98 days for Alem Tena, Doni and Werer. However, number of days to grain filling (DTGF) were 44, 43, and 46 days, respectively. Because the plants flowered earlier and matured late in Werer, there was longer number of DTGF which could contributes significantly for increased grain production. On the contrary, plants flowered late and matured late at Alem Tena with intermediate number of DTGF while they flowered intermediate at Doni and matured early with lower number of DTGF. Similar trend held true for the phenology traits under water-stressed

(WS) condition. The plants have flowered earlier (50 days) and matured late (87 days) at Werer with longer DTGF (37 days) while they flowered late (55 days) and matured earlier (85 days) at Alem Tena. Doni was intermediate in number of DTF (52 days), DTM (86 days) and DTGF (33 days).

For the yield and yield component traits, Werer had the highest mean performances. Under FW condition, it had the highest mean for number of PPP (42 pods), BMY (150 g five plants^{-1},) and GYLD (50 g five plants^{-1}), whereas it was intermediate in SPP (1.28). Similarly, under WS condition, Werer had the highest mean performances for PPP (33 pods) and GYLD (40 g); whereas it had intermediate SPP (1.23) and BMY (116 g). At Alem Tena, the performances of the traits were poor to intermediate under both stress levels. Number of SPP was the least (1.25) while BMY and GYLD were intermediate, 147 and 47 g, respectively, under full water-condition; whereas number of PPP (24 pods), SPP (1.19), BMY (114 g) and GYLD (32 g) were the least under water-stressed condition. At Doni, the mean PPP (37), BMY and GYLD (145 and 45 g, respectively) were the least while SPP (1.3) was the highest under FW condition; whereas under WS condition, SPP (1.27) and BMY (124g five plants^{-1}) were the highest while PPP (28 pods) and GYLD (35 g) were intermediate.

Mean hundred seeds weight (HSW) was highest at Alem Tena (39 g), intermediate at Doni (38 g) and the least at Werer (37 g) under FW condition; the same trend held true under water-stressed condition (32, 29 and 27 g, respectively). The mean harvest index (HI) was the highest at Werer (33%), intermediate at Alem Tena (32%) and the least (31%) at Donin under FW condition but it was the highest at Werer (32%), intermediate at Doni (31%) and the least at Alem Tena (29%). The

mean plant height (PHT) was the highest at Werer (46 cm), intermediate at Doni (43 cm) and the least at Alem Tena under FW condition; whereas under water-stress condition, the mean PHT was the highest at Doni (35 cm), intermediate at Werer (32 cm) and the least at Alem Tena (28 cm).

Consistently highest mean yield was obtained at Werer both under FW and WS conditions. This might be attributed to high number of days to grain filling as a result of early flowering and late maturity, high number of pod per plant, high biomass yield as a result of increased plant height and also improved harvest index due to increased translocation of assimilate to grain production and also intermediate number of seed per pod and decreased hundred seeds weight under full-water condition. The same trend held true under water-stressed condition with the exception of intermediate plant height under this condition.

At Alem Tena, mean grain yield performance was intermediate under full-water condition but it was the lowest under water-stressed condition. The intermediate performance under the former could be due to intermediate mean performances of number of DTGF as a result of late flowering and intermediate maturity period, intermediate number of PPP, BMY and HI and high HSW under full-water conditions; whereas, low mean GYLD was attributed to low mean number of DTGF, PPP and SPP, significantly reduced PHT, BMY and HI and also increased HSW and high Ascochyta disease pressure that was aggravated by moisture stress.

At Doni, the mean GYLD performances under the two water stress conditions were contrasting with that of the Alem Tena. There was lowest mean GYLD performance under full-water conditions as compared to that of Werer and Alem Tena but it was intermediate under WS condition. The low

yield under FW conditions could be attributed to low mean DTGf as a result of intermediate DTF and early DTM, low PPP, BMY and HI while the intermediate mean GYLD performance under WS condition could be due to intermediate DTGF as result of intermediate DTF and DTM, intermediate number of PPP, HSW, BMY and HI and relatively increased PHT and SPP.

In general, these data in this study suggested that the growth conditions at Werer were more favorable for chickpea than at Alem Tena and Doni and that differences between locations reflects differences in soil type and other environmental conditions prevalent during the study period.

Table 9. Mean of the locations (Alem Tena, Doni and Werer) of 10 traits of 210 chickpea genotypes tested under full-water and water-stressed conditions, in 2014.

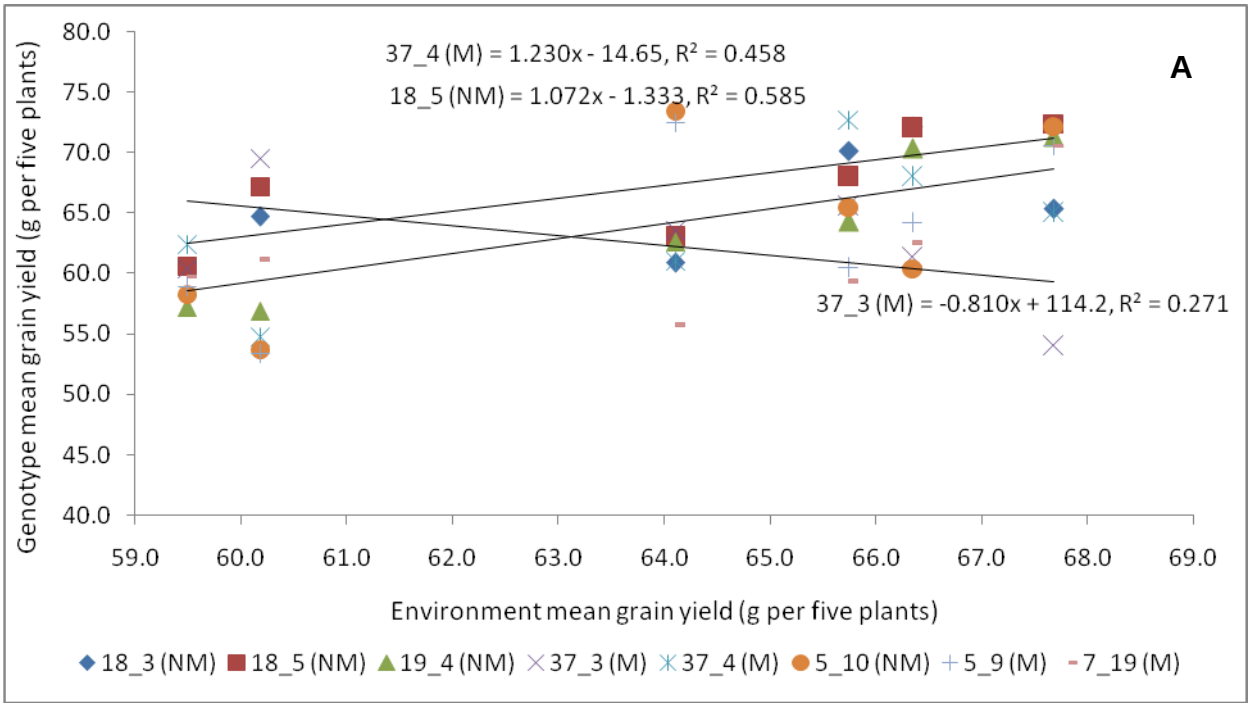
	Full water (control)				Water-stressed			
Trait	Alem Tena	Doni	Werer	Mean	Alem Tena	Doni	Werer	Mean
DTF	53.9a	52.5b	52.3c	52.9	54.6a	52.4b	50.3c	52.4
DTM	97.6b	95.6c	98.3a	97.2	85.1c	85.6b	86.5a	85.7
DTGF	43.8b	43.1c	45.9a	44.3	30.6c	33.2b	36.2a	33.3
AB	2.25	-	-	2.25	2.50	-	-	2.50
PPP	39.6b	36.9c	42.3a	39.6	23.6c	28.0b	33.0a	28.2
SPP	1.25c	1.30a	1.28b	1.28	1.19c	1.27a	1.23b	1.23
PHT	40.5c	43.0b	45.9a	43.1	28.1c	35.0a	31.7b	31.6
HSW	38.9a	37.7b	36.5c	37.7	32.1a	29.2b	26.5c	29.3
BMYLD	146.9b	144.9c	149.8a	147.2	108.2c	114.0b	124.6a	115.6
HI	32.0b	30.9c	33.0a	32.0	29.4c	31.0b	31.9a	30.7
GYLD	47.4b	45.2c	49.8a	47.5	32.0c	35.4b	40.1a	35.8

Figures with similar alphabates within a row (independently for FW and WS conditions) are not significatly (P>0.05) different; DTF, DTM & DTG = number of days to flowering, maturity & grain filling; PPP & SPP = number of pods per plant & seeds per pod; PHT = plant height (cm); HSW= hundred seeds weight (g); GYLD = grain yield (g);

4.1.3.4. Genotype by location interaction effects

Genotype by environment interaction is a well established fact that the phenotype is a joint contribution of both genes and the environments. The genes and the environments are not entirely independent in their action. Any change in environment of a particular genotype does not produce an equivalent change in phenotype and the effect of a particular environment is not exactly the same on the mean performances of two or more genotypes with the result that mean performance and even ranking of the genotypes change from environment to environment. This type of variation arising from the differential performance of genotypes in different environments leading to change of rank or even relative performances is known as genotype by environment interaction (G×E), a concept very well dealt with in depth by several authors (Finlay and Wilkinson, 1963; Alard and Bradshaw, 1964; Eberhart and Russell, 1966; Bowman, 1972; Zobel, 1990; Ceccarelli, 1996; Fox et al., 1997; Annicchiarico, 2002; Chahal and Gosal, 2002)..

As indicated in the combined analysis of variance (section 4.1.3.2) above, there was significant G×E for almost all the traits indicating that genotype that is best at one location might not be the best at the other location for the trait under consideration. For example, the stability analysis using joint regression with some top 10% selected lines for grain yield performance clearly revealed that IL 37-3 performed best at poor environment and poor at potential environment under full-water condition while IL 27-2 was best at potential environment and poor at poor yielding environment under water-stressed condition, and IL 18-5 was stable within each water-stress environment (Figures 5). In fact, all the top 10% selected lines under full-water condition were no more in the top 10% selected under water-stressed condition because the two environments were found to be distinct and different set of genotypes were selected as best lines for each.



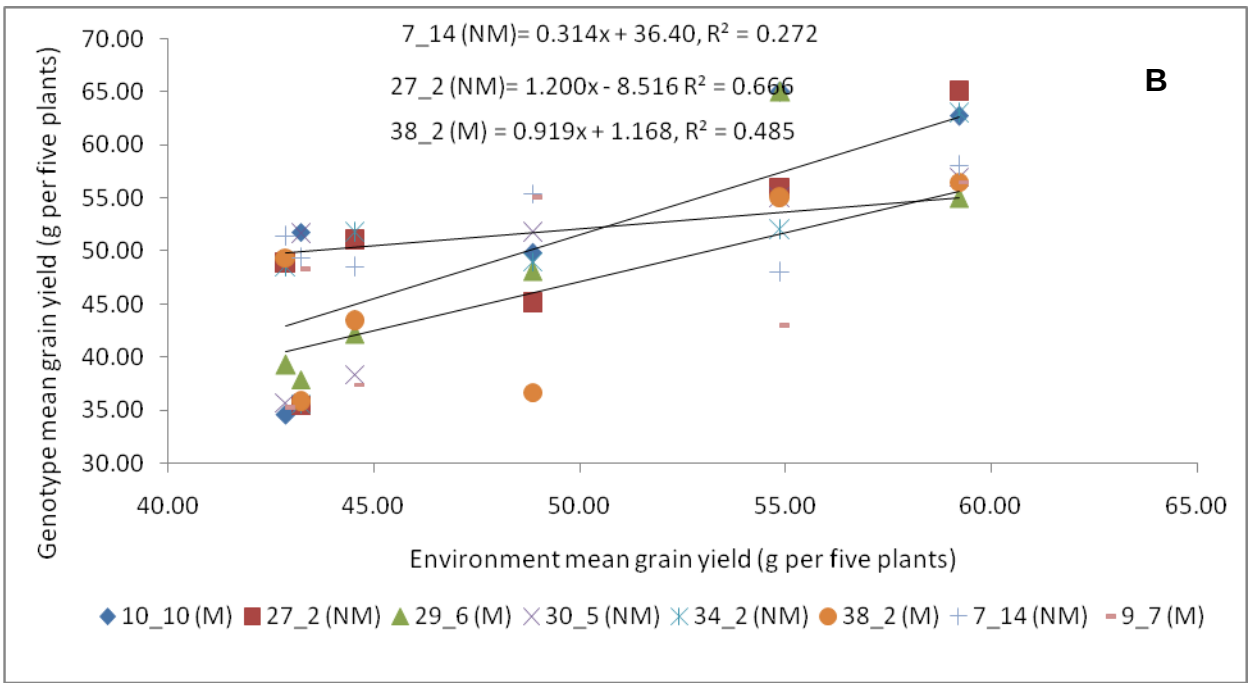


Figure 5. Yield stability (joint regression) of some of the top 10% selected chickpea lines across the test environments: (A) under full-water and (B) under water-stress conditions.

4.1.3.5. Cluster and distance analyses of the isogenic lines

The cluster analysis has grouped the 210 chickpea genotypes into 12 distinct clusters (CLS) under full-water condition and 10 under water-stressed condition (Table 11 and Figures 6a and 6b). It is generally assumed that members within a cluster are more closely related based on the traits under consideration than genotypes within significantly distant clusters. Each cluster comprises different number of genotypes (3 in C_{12} to 33 in C_1 under full-water and 9 in C_9 to 10 in C_{10} under water-stressed conditions). Each cluster has a peculiar characteristics. For example, C_6 was characterized by high mean for number of pods per plant, biomass yield, grain yield and harvest index and intermediate mean for number of days to flowering, grain filling, plant height and hundred seeds weight under full-water condition. Similarly, C_{12} was characterized by high mean for number of pods per plant, biomass yield and grain yield and harvest index but early flowering and maturity and low number of days to grain filling and hundred seeds weight. In the contrary, C_7 was characterized by low mean for number of pods per plant, biomass and grain yield and harvest index.

Under water-stressed condition, C_6 was characterized by high mean for number of pods per plant, biomass and grain yield and harvest index and intermediate mean for number of days to maturity and grain filling, and plant height. In the contrary, C_3 was characterized by low mean for number of pods per plant, biomass and grain yield and harvest index. In general, for further improvement through breeding, selection should be focused on the clusters and individuals within clusters with high mean for desirable traits like number of days to grain filling, number of pods per plant, biomass and grain yield and harvest index.

Table 10. Number of elements in each cluster their mean trait performances of 210 chickpea genotypes grouped into different clusters under the full-water and water-stressed conditions

Cluster	No. of Elements	Mean									
		DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMYLD	GYLD	HI
Under full-water condition											
CLS1	33	53	95	39	39	1.2	38	33	147	45	31
CLS2	15	52	91	35	36	1.4	41	33	139	43	33
CLS3	19	54	95	38	40	1.3	42	29	146	49	34
CLS4	14	52	97	41	35	1.3	40	30	125	37	28
CLS5	20	52	91	36	29	1.3	34	33	112	36	30
CLS6	20	53	92	35	44	1.2	37	32	153	54	37
CLS7	8	53	93	37	29	1.4	43	28	110	34	27
CLS8	21	54	94	36	40	1.2	41	30	168	51	27
CLS9	19	55	95	36	29	1.3	38	31	114	36	29
CLS10	24	52	91	35	37	1.4	34	30	136	46	32
CLS11	14	54	91	34	36	1.2	37	34	149	45	29
CLS12	3	52	88	32	52	1.4	37	25	167	64	38
Under water-stressed condition											
CLS1	21	51	82	31	25	1.2	31	30	119	35	33
CLS2	13	52	86	34	24	1.2	31	26	91	32	40
CLS3	38	52	84	32	21	1.2	30	30	83	25	30
CLS4	37	53	87	35	26	1.2	33	27	108	31	32
CLS5	29	54	83	29	25	1.3	30	28	101	32	35
CLS6	27	53	86	33	30	1.2	33	28	129	44	40
CLS7	14	52	80	28	22	1.2	30	28	96	31	37
CLS8	12	53	84	31	28	1.4	35	27	132	40	34
CLS9	9	52	87	35	27	1.4	35	31	101	35	37
CLS10	10	51	79	28	25	1.3	31	31	121	41	39

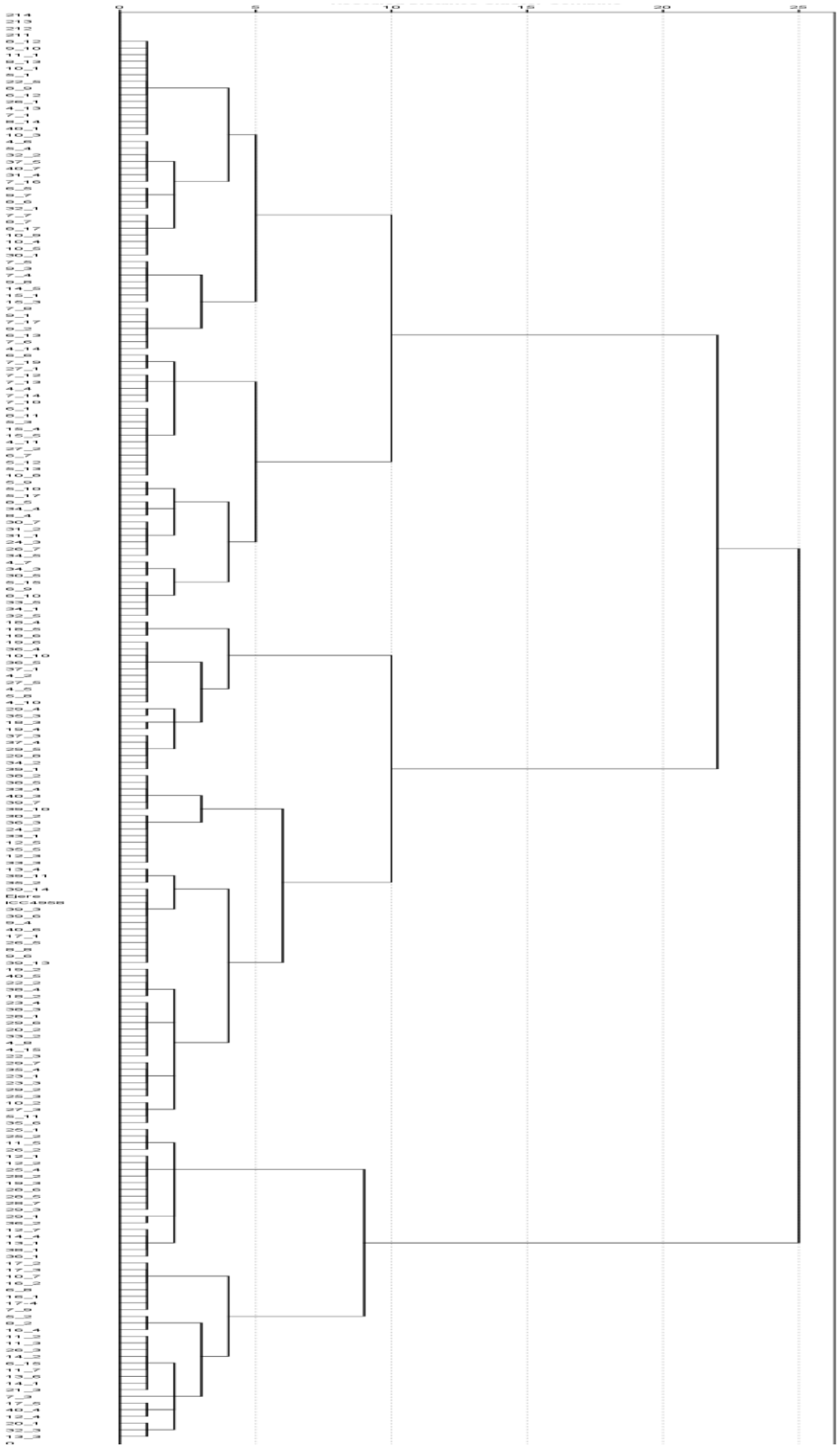


Figure 6a. Dendrogram of 210 chickpea genotypes tested under full-water conditions in multi-locations in 2014 cropping season

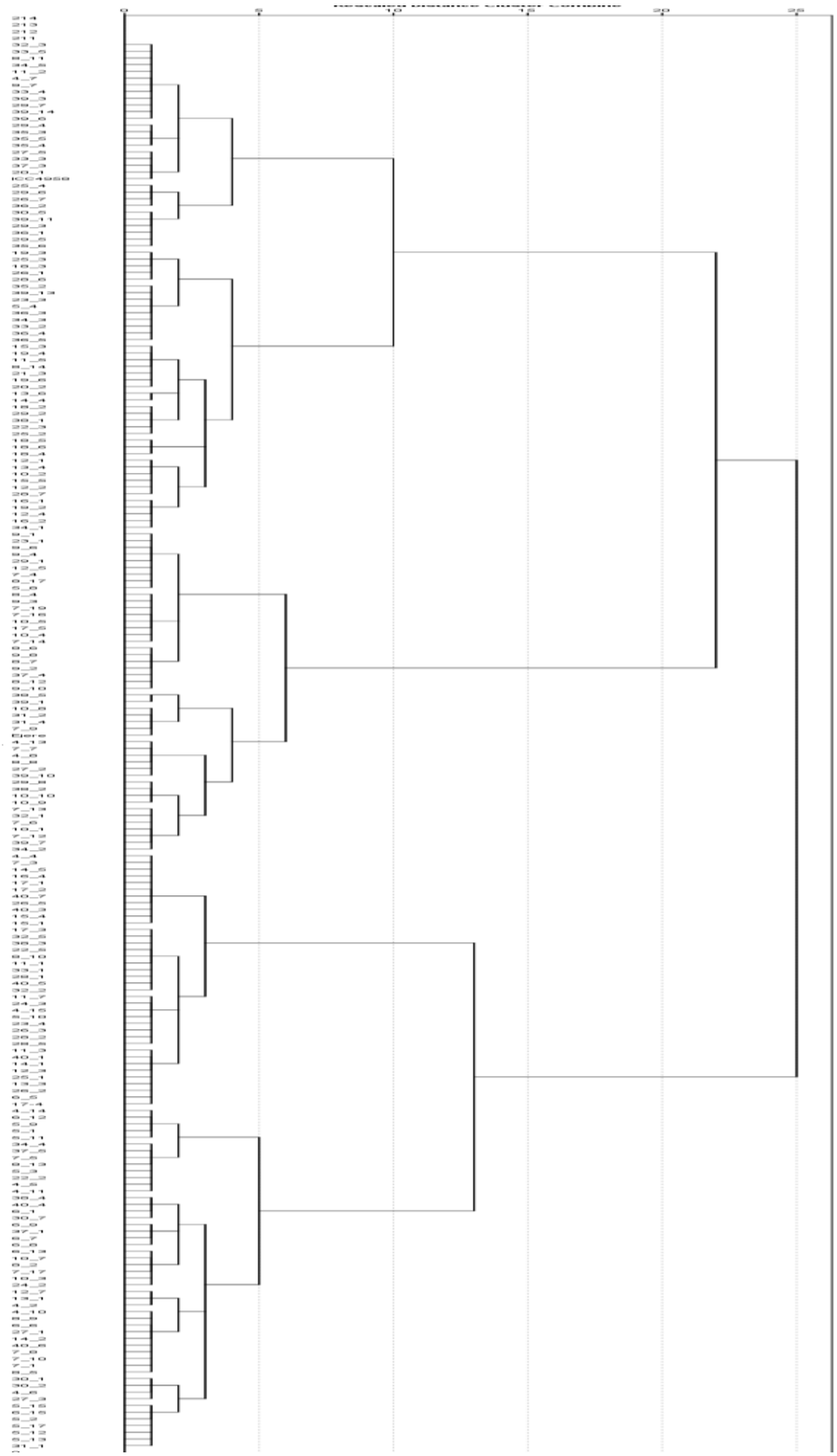


Figure 6b. Dendrogram of 210 chickpea genotypes tested under water-stressed conditions in multi-locations in 2014 cropping season

The pair wise generalized squared distance (D^2) among the 12 clusters under full-water and 10 clusters under water-stressed conditions are presented in Tables 11a and 11b. The maximum distance under full-water condition was between C_9 and C_{12} ($D^2 = 89^{**}$) followed by C_4 and C_{12} ($D^2 = 84^{**}$), C_5 and C_{12} ($D^2 = 78^{**}$) and the least significant distance was between C_3 and C_9 ($D^2 = 19^{*}$) and between C_5 and C_7 ($D^2 = 19^{*}$). Under water-stressed condition, the maximum distance was between C_4 and C_{10} ($D^2 = 37^{**}$) followed by C_3 and C_6 ($D^2 = 35^{**}$), C_3 and C_{10} ($D^2 = 32^{**}$) and the least significant distance was between C_1 and C_8, C_3 and C_9, C_5 and C_9 ($D^2 = 20^{*}$, each). The maximum genetic divergence between the clusters is believed to yield maximum genetic recombination and variation in crossing of parents selected from these clusters. However, the parents from these divergent clusters should be based on a specific merit of both the clusters and the genotypes considered depending on the objectives of the crossing (Singh, 1990; Chahal and Gosal, 2002). There was no significant ($P>0.05$) genetic divergence among some of the clusters under both water level conditions indicating the close relation of the members in these clusters based on the traits considered. Though the 208 chickpea genotypes were isogenic lines developed from the cross of two contrasting genotype (Ejere, Ethiopian improved cultivar with good agromomic traits including grain yield and ICC 4958, ICRISAT identified drought tolerant), large genetic diversity between the lines is expected because the two parents were found to be polymorphic for the markers for drought tolerance and contrasting for agronomic traits (seed size, seed color) including drought tolerance.

Table 11a. Pair wise squared distances (D^2) among 12 clusters of 210 chickpea genotypes tested at under full-water condition

Cluster	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
C_1	0											
C_2	13	0										
C_3	10	12	0									
C_4	8	18	12	0								
C_5	23*	15	35**	21*	0							
C_6	12	12	12	30**	32**	0						
C_7	31**	17	22*	14	19*	43**	0					
C_8	10	16	11	20*	35**	15	31**	0				
C_9	16	15	19*	12	13	33**	13	25**	0			
C_{10}	16	8	18	22**	9	13	21*	17	18	0		
C_{11}	13	10	23*	26**	18	15	34**	11	17	12	0	
C_{12}	66**	47**	45**	84**	78**	28**	76**	46**	89**	36**	59**	0

* and ** indicate significance at P<0.05 and P<0.01, respectively

Table 11b. Pair wise squared distances (D^2) among 10 clusters of 210 chickpea genotypes tested at under water-stressed condition

Cluster	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
C_1	0									
C_2	16	0								
C_3	12	15	0							
C_4	13	9	11	0						
C_5	10	16	11	16	0					
C_6	17	15	35**	15	20*	0				
C_7	7	15	12	21*	6	26**	0			
C_8	12	20*	28**	16	12	9	22*	0		
C_9	18	13	20*	13	20*	15	27**	12	0	
C_{10}	10	28**	32**	37**	15	22*	12	15	25**	0

* and ** indicate significance at P<0.05 and P<0.01, respectively

4.1.3.6. Principal component analysis

Principal component (PC) analysis showed the first four PCs accounted for about 79% of the total variation under full-water condition with PC_1, PC_2, PC_3 and PC_4 contributing 35.5, 20.1, 12.2, and 11.0%, respectively (Table 12 and Appendices 1a-1c). The first PC that accounted for the highest total variation in the tested genotype had high and positive load for number of pods per plant (0.402), biomass yield (0.513), and harvest index (0.514) and grain yield (0.515). The second PC had high and positive load for number of days to maturity and grain filling period (0.665 and 0.619, respectively) while third PC had high and positive load for plant height (0.393) and high and negative load for hundred seeds weigh (-0.485). Similarly, under water-stressed condition, first four PCs accounted for about 74% of the total variation with PC_1, PC_2, PC_3 and PC_4 contributing 37.4, 16.5, 10.3, and 9.6%, respectively. The first PC that accounted for the highest total variation in the tested genotype had high positive load for number of days to grain filling (0.411), number of pods per plant (0.450), biomass yield (0.377), harvest index (0.342) and grain yield (0.481). The second PC had high positive load for number of days to flowering (0.617), days to maturity (0.496) while third PC had high and positive load for plant height (0.458).

Using principal components, it is possible to find out causes of the variation associated with a particular principal component by examining the coefficients of linear combination of original variables (Chahal and Gosal, 2002). The variables with eigenvector of large absolute magnitude (close to unity) reflects a strong influence while those of small magnitude (near zero) reflect little influence for a particular variable provided that the first few principal components accounts for a substantial portion of the variation (Chahal and Gosal, 2002). In

this regard, the traits like PPP, BMY, GYLD and HI with high absolute value of the load (eigenvector) are assumed to have high contribution for the phenotypic variation in the tested genotypes under both stress conditions while DTGF under water-stress condition as they have large load for PC_1. Traits like DTM and PHT have moderate contribution for the variation in the genotypes as the have large load for PC_2.

Table 12. Principal component analysis of ten traits of 210 genotypes, combined over three locations (Alem Tena, Doni, Werer) in 2014 under stressed and unstressed conditions.

	Under water-stressed condition				Under full-watered condition			
PC	1	2	3	4	1	2	3	4
Eigen Value	3.738	1.645	1.03	0.958	3.547	2.007	1.223	1.104
Difference	0.094	0.615	0.071	0.026	1.54	0.784	0.119	0.263
Proportion	0.374	0.165	0.103	0.096	0.355	0.201	0.122	0.110
Cumulative	0.374	0.538	0.641	0.737	0.355	0.555	0.678	0.788
Eigen vectors								
Trait	Prin1	Prin2	Prin3	Prin4	Prin1	Prin2	Prin3	Prin4
DTF	-0.186	0.617	0.159	0.029	0.103	0.093	0.514	-0.691
DTM	0.289	0.496	-0.145	0.093	-0.047	0.665	-0.097	-0.185
DTGF	0.411	-0.007	-0.256	0.067	-0.098	0.619	-0.361	0.167
PPP	0.450	-0.000	0.038	0.002	0.402	0.076	0.018	0.060
SPP	0.070	-0.191	0.806	-0.197	-0.076	-0.015	0.427	0.632
PHT	0.124	0.331	0.458	0.091	0.015	0.290	0.393	0.139
HSW	-0.021	-0.355	0.126	0.821	-0.133	-0.273	-0.485	-0.176
BMYLD	0.377	-0.244	-0.014	-0.357	0.513	0.001	-0.097	-0.048
HI	0.342	0.190	0.098	0.368	0.514	0.002	-0.093	0.045
GYLD	0.481	-0.073	0.042	-0.045	0.515	0.016	-0.078	0.057

4.1.3.7. Genetic variation and heritability of the isogenic lines

There was a tremendous genetic variations among the tested chickpea lines tested in multi-locations for morpho-agronomic traits. The frequency distribution clearly showed the existence of high level of variation in terms of range among the tested genotypes for all the traits (Appendix 2a & b). The estimate of combined over location phenotypic (PCV) and genotypic (GCV) variances and broad-sense heritability (H^2_b) and expected genetic gains (GA) are shown in Tables 13.

Under FW condition, the magnitude of genotypic variance ranged from 0.01 to 53.6 while the phenotypic variances ranged from 0.02 to 61.9. Relatively high genetic and phenotypic variances were recorded for traits like GYLD (53.6 and 61.9), BMY (53.0 and 60.0), PPP (27.3 and 30.8), PHT (14.5 and 15.2) and HI (11.5 and 12.8), respectively. Accordingly, the GCV and PCV for these traits were, 15.4 and 16.6% for GYLD, 6.3 and 6.7% for BMY, 18.6 and 19.8 for PPP, 12.0 and 12.3% for PHT, and 11.0 and 11.6% for HI, respectively. The high GCVs and PCVs indicate the presence of high genetic variations among the tested lines for the traits studied.

Broad sense heritability (H^2_b) for the traits studied ranged from 69.3% to 95.5%. Relatively highest broad sense heritability was recorded for PHT (95.5) followed by HSW (93.7%), number of DTF (90.4%), HI (89.4%), number of PPP (88.4%) while low heritability scores were recorded for number of DTF (69.3%). This could be due to high environmental effect and low phenotypic and genotypic coefficients of variations of these traits under full-water condtion.

Under water-stressed condition, the magnitude of genotypic variance ranged from 0.01 to 201.6 while the phenotypic variances ranged from 0.01 to 245.7. Relatively high genetic and phenotypic variances were recorded for traits like BMY (201.6 and 245.7), GYLD (34.5 and 45.0), number of PPP (14.4 and 17.0) and HI (7.1 and 9.6), respectively. Accordingly, the GCVs and PCVs for these traits were, 16.4 and 18.7% for GYLD, 12.3 and 13.6% for BMY, 13.5 and 14.7 for number of PPP and 9.0 and 10.0% for HI, respectively. The high GCVs and PCVs indicate the existence of high genetic variations among the tested lines for the traits studied. Broad sense heritability (H^2_b) for these traits ranged from 54.8% to 85.0%. The highest H^2_b was recorded for number of PPP (85.0%) followed by BMY (82.1%), PHT (80.7%), HI (80.0%). Low H^2_b scores were recorded for number of SPP (54.8%), number of DTF (63.1%), number of DTM (64.5%) and number of DTGF (75.8). This could be due to high environmental effect and low PCV and GCV for these traits.

The magnitude of H^2_b was generally high for most of the characters at each location except for number of seeds per pod (Appendices 3a, b and c) and for combined across location (Table 13) under full-water conditions; whereas under water-stress condition, most of the characters had high heritability for individual locations but across location it was low for DTF, DTM, and Spp. The GCV and PCV and H^2_b were higher under full-water condition than under water-stress indicating that the water-stress has negatively affected these parameters.

4.1.3.8. Genetic advance and gain from selection

Under full-water condition, expected genetic gain from selection (GA) and the gains as a percentage of the mean (GAM) were high for number of PPP (10.2 and 36.0%), GYLD (14.3g

five plants^{-1} and 29.6%), PHT (7.67cm and 24.2%), HI (6.60% and 21.4%) and HSW (5.28g and 18.4%), respectively. Under water-stressed condition, GA and GAM were high for GYLD (10.7g five plants^{-1} and 29.9%), number of PPP (7.2 and 25.7%), BMY (27.9g five plants^{-1} and 24.2%), respectively; the least was for number of DTM (2.4 and 2.8%), respectively.

As observed from the data, traits with high genetic coefficient of variation (GCV) and high heritability (H^2_b) resulted in high expected GA as compared to traits with low GCV and / or H^2_b. Therefore, it is important to jointly consider both high GCV and H^2_b than depending solely on any one of the two for achieving high expected genetic gain from selection of individuals. Yield could be indirectly improved throught selection for PPP, BMY, and HI as these traits have high (H^2_b) and GCV in addition to selecting for grain yield *per se*.

High H^2_b reflects the large heritable variance which may offer the possibility of improvement through selection. However, Johnson et al. (1955) reported that H^2_b estimates together with GA are more important than H^2_b alone to predict the resulting effect of selecting the best individuals. The estimates of H^2_b and GAM considered together will better help in drawing conclusion about the nature of gene action governing a particular character. This is due to the fact that combined study of H^2_b and GA is more reliable in forecasting the effect of selection (Johnson et al., 1955). Based on the findings, it could be concluded that selection will be effective in the test lines used in this study as revealed by the significant substantial variations among the lines for all the characters observed in each (Appences 3a to c) and combined locations (Table 13). Therefore,

the significant genetic variability for these characters in the lines over locations could be further exploited through improvement and selection programs.

Table 13. Combined over location genetic (V_G), environmental (V_E), and phenotypic (V_P) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability (H^2_b), genetic gain from selection (GA) and genetic gain as a percentage of mean (GAM) of 10 agro-morphological traits of 210 chickpea genotypes tested under full-water and water-stress conditions

Trait	Mean	V_G	V_{GxL}	V_E	V_P	PCV	GCV	H^2_b	GA	GAM
Under full-water condition										
DTF	52.6	0.983	0.124	0.381	1.088	2.0	1.9	90.4	1.94	3.7
DTM	85.3	2.335	1.184	0.937	2.886	2.0	1.8	80.9	2.83	3.3
DTGF	32.8	2.683	0.906	1.028	3.156	5.4	5.0	85.0	3.11	9.5
PPP	28.1	27.264	7.914	5.559	30.829	19.8	18.6	88.4	10.12	36.0
SPP	1.233	0.014	0.016	0.006	0.021	11.6	9.7	69.3	0.20	16.6
PHT	31.7	14.512	0.155	3.803	15.198	12.3	12.0	95.5	7.67	24.2
HSW	28.7	6.864	0.461	1.864	7.328	9.4	9.1	93.7	5.28	18.4
BMY	115.5	53.001	12.363	16.985	59.953	6.7	6.3	88.4	14.10	12.2
HI	30.9	11.482	2.197	3.789	12.846	11.6	11.0	89.4	6.60	21.4
GYLD	47.4	53.593	15.249	19.479	61.923	16.6	15.4	86.5	14.03	29.6
Under water-stress condition										
DTF	52.5	1.226	1.603	1.088	1.942	2.7	2.1	63.1	2.0	3.7
DTM	85.5	1.096	1.148	1.316	1.698	1.5	1.2	64.5	2.4	2.8
DTGF	32.9	1.985	1.130	1.535	2.618	4.9	4.3	75.8	3.4	10.3
PPP	28.1	14.439	4.076	7.182	16.995	14.7	13.5	85.0	7.2	25.7
SPP	1.23	0.006	0.010	0.009	0.011	8.4	6.2	54.8	0.1	9.9
PHT	31.6	5.182	1.605	4.210	6.419	8.0	7.2	80.7	4.2	13.4
HSW	28.9	3.187	2.230	3.469	4.509	7.3	6.2	70.7	4.5	15.7
BMY	115.5	201.618	96.153	72.004	245.670	13.6	12.3	82.1	27.9	24.2
HI	30.9	7.656	2.826	5.823	9.569	10.0	9.0	80.0	5.7	18.5
GYLD	35.9	34.518	24.665	13.577	45.003	18.7	16.4	76.7	10.7	29.9

4.1.3.9. Comparison of selected genotypes with population mean and the parents

Under full-water conditions, the mean traits performances of some of the selected top 10% lines with markers is presented in Table 14a. Out of these lines with markers (5-9, 7-19, 37-3, and 37-4), lines 7-9, 37-3, and 37-4 were late flowering as compared to the two parents and the population mean while line 5-9 was early flowering; lines 7-19 and 5-9 were late maturing where the two parents were early maturing; lines 5-9, 7-9, and 37-4 had higher number of DTGF as compared to the two parents by 2.4 to 15.4%; all the selected lines had higher number of pod per plant than one of the parents, Ejere, by 4.5 to 17.2% while they had less number of pods per plant than ICC 4958, the second parent, by 0.9 to 11.7%; lines 5-9 and 7-19 had higher number of SPP while lines 37-3 and 37-4 had low number of PPP; All the selected lines had 9.4 to 12.5% shorter plant stature as compared to the parents; they were smaller in HSW than the large seeded Ejere and larger that the small seeded parent, ICC 4958, except line 7-19 which was smaller in HSW than both the parents; all the selected lines were 15.1 to 25.7% better in HI, 7.1 to 11.9% in BMY, and 23.2 to 40.9% in GYLD than the two parents. The high yield performance of the selected lines stem from high HI, intermediate HSW (smaller than Ejere and larger than ICC 4958), shorter PHT, intermediate number of PPP (higher than that of Ejere and lower than that of ICC 4958), higher DTGF (as a result of early flowering and late maturity) as compared to the two parents.

Similarly, the selected lines without markers (5-10, 18-3, 18-5, and 19-4) under full-water condition had high yield as compared to the two parents (Table 14b); this was due to high HI (18.1 to 30.0%), BMY (7.6 to 13.7%), shorter plant stature (16.6 to 26.5%), higher number of PPP (0.8 to 33.1%), longer number of DTGF (4.5 to 15.0% for three lines out of four), and number of DTM (1.8 to 6.4%) over the two parents of the lines.

Under water-stressed conditions, the mean traits performances of some selected top 10% lines with markers is presented in Table 15a. Out of the lines (9-7, 10-10, 29-6, and 38-2), lines 29-6, and 38-2 were early flowering by 1.7 to 6.9% as compared to the two parents while line 9-7 and 10-10 were earlier in number of DTF than Ejere, the earliest parent, by 1.3 to 4.7%; lines 10-10 and 29-6 were intermediate between Ejere, the latest parent, and ICC 4958, the early parent; whereas, 9-7 and 38-2 were late in maturity than the two parents; but all the selected lines had higher number of DTGF as compared to the two parents by 5.1 to 14.9%; all the selected lines had higher number of PPP than the two parents by 19.6 to 44.2% with lower number of SPP than Ejere by 9.8 to 26.8% and higher SPP by 14.3 to 20.9% than ICC 4958 except line 9-7; again, all the selections had shorter PHT by 8.6 to 21.4% as compared to the two parents; lines 10-10, 29-6 and 38-2 had high BMY by 5.7 to 30.7% while 9-7 had lower BMY by 9.1% as compared to the parents; lines 9-7, 29-6, and 38-2 had highest HSW by 5.5 to 16.0% than the two parents while 10-10 had lower HSW by 2.9% than ICC 4958 which had the highest HSW of the two parents; all the selected lines had higher HI by 19.4 to 44.9% and GYLD by 35.4 to 94.0% as compared to the parents. The high yield in this group was due to high number of DTGF, number of PPP, HI and short plant height.

Similarly, the selected lines without markers (7-14, 27-2, 30-5, and 34-2) under water-stressed condition had high GYLD as compared to the two parents (Table 15b) as a results of high HI (23.1 to 48.9%), number of PPP (21.4 to 51.7%), number ofDTGF (9.1 to 20.4%), and shorter PHT by 0.7 to 17.4% over the two parents. In general, high HI, number of PPP and shorter PHT were consistently related to high grain yields under all conditions for all lines with and without marker. Number of DTGF was related to higher grain yield also most often.

Table 14a. Mean trait performance and relative advantages of frour top selected lines out of the top 10% chickpea lines with markers, tested under full-water conditions

Selected		Relative advantage (%) over				Selected		Relative advantage (%) over			
Lines	Mean	MM	MP1	MP2	MPop	Lines	Mean	MM	MP1	MP2	MPop
	DTF						DTM				
37_4	53.2cd	0.3	0.3	0.6	0.5	37_4	95.8hij	-1.1	1.1	2.1	-1.4
5_9	50.8h	-4.1	-4.1	-3.8	-3.9	5_9	98.2cde	1.3	3.5	4.6	1.0
37_3	54.7a	3.1	3.1	3.5	3.3	37_3	96.3ghi	-0.6	1.6	2.7	-0.8
7_19	54.7a	3.1	3.1	3.5	3.3	7_19	98.7bcd	1.8	4.0	5.2	1.6
MM	53.0cde	0.0	0.0	0.3	0.2	MM	96.9fgh	0.0	2.2	3.2	-0.3
MP1	53.0cde	0.0	0.0	0.3	0.2	P1	94.8kl	-2.1	0.0	1.1	-2.4
MP2	52.8cde	-0.3	-0.3	0.0	-0.2	P2	93.8kl	-3.1	-1.1	0.0	-3.4
MPop	52.9cde	-0.2	-0.2	0.2	0.0	MPop	97.2efg	0.3	2.5	3.5	0.0
	DTGF						PPP				
37_4	42.8ghi	-2.4	2.4	4.5	-3.3	37_4	44.4fg	9.9	10.6	-6.6	11.9
5_9	47.3a	7.8	13.1	15.4	6.9	5_9	43.4g	7.4	8.1	-8.7	9.4
37_3	41.8ij	-4.7	0.0	2.0	-5.5	37_3	47.0def	16.5	17.2	-0.9	18.7
7_19	44.0def	0.2	5.2	7.3	-0.6	7_19	41.9gh	3.8	4.5	-11.7	5.7
MM	43.9def	0.0	4.9	7.1	-0.8	MM	40.4hi	0.0	0.6	-15.0	1.8
MP1	41.8ij	-4.7	0.0	2.0	-5.5	MP1	40.1hi	-0.6	0.0	-15.5	1.2
MP2	41.0k	-6.6	-2.0	0.0	-7.4	MP2	47.5cde	17.6	18.4	0.0	19.8
MPop	44.3de	0.9	5.8	8.0	0.0	MPop	39.6i	-1.8	-1.2	-16.5	0.0
	SPP						PHT				
37_4	1.1fg	-9.1	-15.2	-17.3	-10.4	37_4	46.5bc	10.6	-9.4	-10.9	7.8
5_9	1.4ab	11.3	3.9	1.32	9.73	5_9	45.7cde	8.6	-11.0	-12.46	5.8
37_3	1.2def	-5.6	-11.8	-14.1	-6.9	37_3	46.2bcd	9.8	-10.1	-11.5	7.0
7_19	1.4ab	11.9	4.4	1.8	10.3	7_19	46.5bc	10.6	-9.4	-10.9	7.8
MM	1.3cd	0.0	-6.6	-9.0	-1.4	MM	42.1def	0.0	-18.1	-19.4	-2.5
MP1	1.4ab	7.1	0.0	-2.5	5.6	MP1	51.3a	22.1	0.0	-1.6	19.0
MP2	1.4ab	9.9	2.6	0.0	8.3	MP2	52.2a	24.1	1.6	0.0	20.9
MPop	1.3cd	1.5	-5.3	-7.7	0.0	MPop	43.1c-f	2.6	-15.9	-17.3	0.0

Table 14a. Continued.

Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop	Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop
	HSW						BMYLD				
37_4	36.7efg	-2.0	0.7	5.7	-2.7	37_4	163a-d	10.5	8.2	11.9	10.8
5_9	35.3ghi	-5.8	-3.2	1.5	-6.5	5_9	163a-d	10.4	8.1	11.8	10.7
37_3	36.4e-h	-2.8	-0.1	4.8	-3.5	37_3	161a-g	8.9	6.6	10.3	9.3
7_19	34.2i	-8.6	-6.0	-1.4	-9.3	7_19	162a-g	9.4	7.1	10.7	9.7
MM	37.4def	0.0	2.8	7.8	-0.8	MM	148i	0.0	-2.1	1.3	0.3
MP1	36.4e-h	-2.7	0.0	4.9	-3.4	MP1	151h	2.1	0.0	3.4	2.5
MP2	34.7hi	-7.2	-4.7	0.0	-7.9	MP2	146jk	-1.2	-3.3	0.0	-0.9
MPop	37.7de	0.8	3.6	8.6	0.0	MPop	147ij	-0.3	-2.4	0.9	0.0
	HI						GYLD				
37_4	39.2a-d	21.5	18.5	25.7	22.4	37_4	64.0a-c	33.5	28.2	40.9	34.8
5_9	38.8a-f	20.4	17.3	24.5	21.2	5_9	63.4a-e	32.1	26.9	39.4	33.4
37_3	38.8a-f	20.3	17.2	24.4	21.1	37_3	62.4a-e	30.1	25.0	37.3	31.4
7_19	38.0a-g	18.1	15.1	22.1	18.9	7_19	61.5a-e	28.2	23.2	35.4	29.5
MM	32.2i	0.0	-2.5	3.4	0.7	MM	48.0fg	0.0	-4.0	5.6	1.0
MP1	33.1h	2.6	0.0	6.1	3.3	MP1	49.9f	4.1	0.0	9.9	5.2
MP2	31.2j	-3.3	-5.7	0.0	-2.6	MP2	45.4g	-5.3	-9.0	0.0	-4.3
MPop	32.0i	-0.7	-3.2	2.7	0.0	MPop	47.5fg	-1.0	-4.9	4.5	0.0

MM, MP1, MP2, MPop=mean of genotypes with marker(s), the recurrent parent (Ejere), the donor parent (ICC 4958), and the whole population, (genotypes with and without markers together and the two parents), respectively.

Table 14b. Mean trait performance and relative advantages of frour top selected lines out of the top 10% chickpea lines without markers tested under full-water conditions

Selected		Relative advantage (%) over				Selected		Relative advantage (%) over			
Lines	Mean	MM	P1	P2	MPop	Lines	Mean	MM	MP1	MP2	MPop
	DTF						DTM				
18_5	54.7a	3.4	3.1	3.5	3.3	18_5	93.8l	-3.5	-1.1	0.0	-3.4
18_3	52.7def	-0.4	-0.6	-0.3	-0.5	18_3	96.5gh	-0.8	1.8	2.8	-0.7
5_10	53.0cde	0.2	0.0	0.3	0.2	5_10	99.8a	2.6	5.3	6.4	2.8
19_4	54.0ab	2.1	1.9	2.2	2.1	19_4	97.7def	0.4	3.0	4.1	0.5
MNM	52.9cde	0.0	-0.2	0.1	-0.1	MNM	97.3efg	0.0	2.6	3.7	0.1
MP1	53.0cde	0.2	0.0	0.3	0.2	MP1	94.8kl	-2.5	0.0	1.1	-2.4
MP2	52.8cde	-0.1	-0.3	0.0	-0.2	MP2	93.8kl	-3.5	-1.1	0.0	-3.4
MPop	52.9cde	0.1	-0.2	0.2	0.0	MPop	97.2efg	-0.1	2.5	3.5	0.0
	DTGF						PPP				
18_5	39.2l	-11.8	-6.4	-4.5	-11.5	18_5	50.0bc	27.0	24.7	5.4	26.2
18_3	43.8d-g	-1.3	4.8	6.9	-1.0	18_3	53.7b	36.4	33.9	13.2	35.6
5_10	47.2ab	6.2	12.7	15.0	6.5	5_10	47.9cde	21.5	19.3	0.8	20.8
19_4	43.7e-h	-1.7	4.4	6.5	-1.4	19_4	49.4bcd	25.5	23.2	4.1	24.7
MNM	44.4de	0.0	6.2	8.3	0.3	MNM	39.4i	0.0	-1.8	-17.1	-0.6
MP1	41.8ij	-5.8	0.0	2.0	-5.5	MP1	40.1hi	1.9	0.0	-15.5	1.2
MP2	41.0k	-7.7	-2.0	0.0	-7.4	MP2	47.5cde	20.6	18.4	0.0	19.8
MPop	44.3de	-0.3	5.8	8.0	0.0	MPop	39.6i	0.7	-1.2	-16.5	0.0
	SPP						PHT				
18_5	1.4bc	5.5	0.1	-2.4	5.7	18_5	43.5c-f	0.4	-15.3	-16.6	0.8
18_3	1.2def	-7.1	-11.8	-14.1	-6.9	18_3	38.3g	-11.5	-25.3	-26.5	-11.2
5_10	1.4ab	10.2	4.6	1.9	10.4	5_10	42.7ef	-1.5	-16.9	-18.2	-1.1
19_4	1.3cd	-0.6	-5.7	-8.1	-0.4	19_4	41.2fg	-5.0	-19.8	-21.1	-4.6
MNM	1.3cd	0.0	-5.1	-7.5	0.2	MNM	43.3	0.0	-15.6	-17.0	0.4
MP1	1.4ab	5.4	0.0	-2.5	5.6	MP1	51.3a	18.5	0.0	-1.6	19.0
MP2	1.4ab	8.1	2.6	0.0	8.3	MP2	52.2a	20.4	1.6	0.0	20.9
MPop	1.3cd	-0.2	-5.3	-7.7	0.0	MPop	43.1c-f	-0.4	-15.9	-17.3	0.0

Table 14b. Continued.

Selected		Relative advantage (%) over				Selected		Relative advantage (%) over			
Lines	Mean	MM	P1	P2	MPop	Lines	Mean	MM	MP1	MP2	MPop
	HSW						BMYLD				
18_5	31.4j	-17.0	-13.8	-9.6	-16.8	18_5	166a	12.8	9.9	13.7	12.7
18_3	32.2j	-14.8	-11.5	-7.2	-14.6	18_3	164ab	11.2	8.4	12.1	11.1
5_10	36.3e-h	-4.0	-0.3	4.6	-3.7	5_10	163abc	11.1	8.3	12.0	11.0
19_4	38.8bcd	2.6	6.6	11.8	2.9	19_4	162a-f	10.4	7.6	11.3	10.3
MNM	37.8cd	0.0	3.8	8.9	0.3	MNM	147ij	0.0	-2.5	0.9	-0.1
MP1	36.4e-h	-3.7	0.0	4.9	-3.4	MP1	151hi	2.6	0.0	3.4	2.5
MP2	34.7hi	-8.2	-4.7	0.0	-7.9	MP2	146jk	-0.8	-3.3	0.0	-0.9
MPop	37.7de	-0.3	3.6	8.6	0.0	MPop	147ij	0.1	-2.4	0.9	0.0
	HI						GYLD				
18_5	40.5a	26.8	22.5	30.0	26.5	18_5	67.2a	41.9	34.6	47.9	41.5
18_3	39.8ab	24.7	20.5	27.9	24.5	18_3	65.2ab	37.7	30.6	43.6	37.4
5_10	39.0a-e	22.2	18.1	25.3	22.0	5_10	63.9abc	35.0	28.0	40.7	34.6
19_4	39.2abc	22.8	18.7	25.9	22.6	19_4	63.8a-d	34.6	27.7	40.4	34.3
MNM	31.9i	0.0	-3.4	2.5	-0.2	MNM	47.4fg	0.0	-5.2	4.2	-0.2
MP1	33.1h	3.5	0.0	6.1	3.3	MP1	49.9f	5.4	0.0	9.9	5.2
MP2	31.2j	-2.5	-5.7	0.0	-2.6	MP2	45.4g	-4.1	-9.0	0.0	-4.3
MPop	32.0i	0.2	-3.2	2.7	0.0	MPop	47.5fg	0.3	-4.9	4.5	0.0

MNM, MP1, MP2, MPop=mean of genotypes with no markers, the recurrent parent (Ejere), the donor parent (ICC 4958), and the whole population, (genotypes with and without marker and the two parents together), respectively

Table 15a. Mean trait performance and relative advantages of frour top selected lines out of the top 10% chickpea lines with markers tested under water-stressed conditions

Selected		Relative advantage (%) over				Selected		Relative advantage (%) over			
Lines	Mean	MM	MP1	MP2	MPop	Lines	Mean	MM	MP1	MP2	MPop
DTF						DTM					
10_10	50.3c-g	-2.2	-4.7	0.0	-4.15	10_10	83.8ef	-2.2	-2.3	4.1	-1.8
29_6	49.2g	-4.4	-6.9	-2.3	-6.4	29_6	83.3ef	-2.8	-2.9	3.5	-2.4
38_2	49.5efg	-3.8	-6.3	-1.7	-5.7	38_2	86.8bcd	1.3	1.2	7.9	1.7
9_7	52.2bc	1.4	-1.3	3.6	-0.7	9_7	86.8bcd	1.3	1.2	7.9	1.7
MM	51.4bcd	0.0	-2.6	2.2	-2.0	MM	85.8de	0.0	-0.1	6.5	0.5
MP1	52.8b	2.7	0.0	5.0	0.6	MP1	85.8de	0.1	0.0	6.6	0.6
MP2	50.8c-g	-2.2	-4.7	0.0	-3.2	MP2	82.5g	-6.1	-6.2	0.0	-3.3
MPop	52.5bc	2.1	-0.6	3.3	0.0	MPop	85.3de	-0.5	-0.6	3.5	0.0
DTGF						PPP					
10_10	33.5def	-2.4	1.5	11.0	2.0	10_10	34.1a-f	17.7	27.4	39.3	21.4
29_6	34.2b-e	-0.4	3.5	13.3	4.1	29_6	34.0a-f	17.4	27.0	38.9	21.1
38_2	37.3a	8.8	13.1	23.8	13.7	38_2	35.3a-d	21.9	31.8	44.2	25.7
9_7	34.7b-e	1.0	5.1	14.9	5.6	9_7	32.1b-f	10.5	19.6	30.8	14.0
MM	34.3b-e	0.0	4.0	13.7	4.5	MM	29.0g	0.0	8.2	18.3	3.1
MP1	33.0ef	-3.8	0.0	9.4	0.5	MP1	26.8i	-7.6	0.0	9.4	-4.7
MP2	32.2fg	-12.1	-8.6	0.0	-2.6	MP2	29.5g	-15.5	-8.6	0.0	4.9
MPop	32.8ef	-4.3	-0.5	2.7	0.0	MPop	28.1h	-3.0	4.9	-4.7	0.0
SPP						PHT					
10_10	1.3abc	6.0	-14.0	15.4	6.6	10_10	31.6e-h	-1.4	-16.4	-10.4	-0.2
29_6	1.3a-d	5.0	-14.7	14.3	5.7	29_6	29.7hi	-7.3	-21.4	-15.8	-6.2
38_2	1.4abc	11.1	-9.8	20.9	11.8	38_2	31.6e-h	-1.3	-16.3	-10.4	-0.1
9_7	1.1fgh	-9.9	-26.8	-1.9	-9.3	9_7	32.2d-g	0.7	-14.6	-8.6	1.9
MM	1.2cf	0.0	-18.8	8.8	0.6	MM	32.0	0.0	-15.2	-9.2	1.2
MP1	1.5a	23.2	0.0	34.1	23.9	MP1	37.8	18.0	0.0	7.1	19.4
MP2	1.1fgh	-8.1	-25.4	0.0	-7.5	MP2	35.3	10.2	-6.6	0.0	11.4
MPop	1.2cf	-0.6	-19.3	8.2	0.0	MPop	31.6	-1.2	-16.2	-10.3	0.0

Table 15a. Continued.

Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop	Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop
HSW						BMYLD					
10_10	28.3d-g	-0.4	0.6	-2.9	-1.9	10_10	145ab	20.7	37.1	12.5	25.8
29_6	32.7b	14.8	16.0	11.9	13.1	29_6	137a-e	13.3	28.8	5.7	18.2
38_2	30.8bcd	8.3	9.3	5.5	6.6	38_2	139a-d	15.0	30.7	7.2	19.9
9_7	31.7bc	11.5	12.6	8.7	9.8	9_7	117f	-2.6	10.7	-9.1	1.6
MM	28.4d-g	0.0	1.0	-2.5	-1.5	MM	120g	0.0	13.6	-6.8	4.3
MP1	28.2d-g	-1.0	0.0	-3.5	-2.5	MP1	106j	-12.0	0.0	-17.9	-8.2
MP2	29.2c-f	2.6	3.6	0.0	1.0	MP2	129c-f	7.2	21.9	0.0	11.8
MPop	28.9d-f	1.6	2.6	-1.0	0.0	MPop	116h	-4.1	9.0	-10.6	0.0
HI						GYLD					
10_10	34.9a-e	10.6	25.6	33.8	13.1	10_10	57.3abc	47.5	94.0	69.0	59.4
29_6	34.9b-e	10.6	25.6	33.8	13.1	29_6	49.9bc	28.6	69.1	47.4	39.0
38_2	33.2de	5.1	19.4	27.2	7.5	38_2	48.1bc	24.0	63.1	42.1	34.1
9_7	37.8ab	19.7	36.0	44.9	22.4	9_7	45.9c	18.2	55.4	35.4	27.7
MM	31.6f	0.0	13.6	21.0	2.3	MM	38.8d	0.0	31.5	14.6	8.1
MP1	27.8h	-12.0	0.0	6.5	-10.0	MP1	29.5f	-24.0	0.0	-12.8	-17.8
MP2	26.1i	-17.4	-6.1	0.0	-15.5	MP2	33.9e	-12.8	14.7	0.0	-5.7
MPop	30.9g	-2.2	11.1	18.3	0.0	MPop	35.9e	-7.5	21.6	6.0	0.0

MM, MP1, MP2, MPop=mean of genotypes with marker(s), the recurrent parent (Ejere), the donor parent (ICC 4958), and the whole population (genotypes with and without marker and the two parents together), respectively

Table 15b. Mean trait performance and their relative advantages of frour top selected lines out of the top 10% chickpea lines without markers, tested under water-stressed conditions

Selected		Relative advantage (%) over				Selected		Relative advantage (%) over			
Lines	Mean	MM	MP1	MP2	MPop	Lines	Mean	MM	MP1	MP2	MPop
DTF						DTM					
7_14	52.0bc	-1.5	-1.6	3.3	-1.5	7_14	88.0abc	3.2	2.5	9.3	3.2
27_2	50.0d-g	-5.3	-5.4	-0.7	-5.3	27_2	86.3cd	1.2	0.6	7.2	1.2
34_2	52.0bc	-1.5	-1.6	3.3	-1.5	34_2	87.5abc	2.6	1.9	8.7	2.6
30_5	51.8bc	-1.8	-1.9	3.0	-1.8	30_5	84.8de	-0.5	-1.2	5.4	-0.5
MNM	52.8b	0.0	-0.1	4.9	0.0	MNM	85.3de	0.0	-0.7	5.9	0.0
MP1	52.8b	0.1	0.0	5.0	0.1	MP1	85.8de	0.7	0.0	6.6	0.7
MP2	50.8c-g	-4.6	-4.7	0.0	-4.6	MP2	82.5g	-5.6	-6.2	0.0	-3.3
MPop	52.5bc	-0.5	-0.6	3.3	0.0	MPop	85.3de	0.1	-0.6	3.5	0.0
DTGF						PPP					
7_14	36.0a-d	10.8	9.1	19.3	10.8	7_14	35.3a-d	26.5	31.8	44.2	26.5
27_2	36.3abc	11.8	10.1	20.4	11.8	27_2	37.2ab	33.1	38.7	51.7	33.1
34_2	35.5a-d	9.3	7.6	17.7	9.3	34_2	32.5a-f	16.5	21.4	32.8	16.5
30_5	33.0ef	1.6	0.0	9.4	1.6	30_5	35.6a-d	27.3	32.6	45.1	27.3
MNM	32.5ef	0.0	-1.6	7.7	0.0	MNM	27.9h	0.0	4.2	14.0	0.0
MP1	33.0ef	1.6	0.0	9.4	1.6	MP1	26.8j	-4.0	0.0	9.4	-4.0
MP2	32.2g	-7.1	-8.6	0.0	-1.6	MP2	29.5g	-12.3	-8.6	0.0	-1.6
MPop	32.8ef	1.1	-0.5	2.7	0.0	MPop	28.1i	0.7	4.9	-4.7	0.0
SPP						PHT					
7_14	1.2d-g	-4.2	-22.9	3.4	-4.2	7_14	34.3bcd	9.0	-9.1	-2.7	9.0
27_2	1.3abc	9.5	-11.9	18.1	9.5	27_2	35.0abc	11.2	-7.3	-0.7	11.2
34_2	1.5a	18.7	-4.5	28.1	18.7	34_2	31.2e-i	-1.0	-17.4	-11.6	-1.0
30_5	1.3abc	7.6	-13.4	16.1	7.6	30_5	33.5c-f	6.4	-11.3	-5.0	6.4
MNM	1.2cf	0.0	-19.5	7.9	0.0	MNM	31.5e-h	0.0	-16.6	-10.7	0.0
MP1	1.5a	24.3	0.0	34.1	24.3	MP1	37.8a	19.9	0.0	7.1	19.9
MP2	1.1efg	-7.3	-25.4	0.0	-7.3	MP2	35.3abc	12.0	-6.6	0.0	12.0
MPop	1.2cf	0.2	-19.3	8.2	0.0	MPop	31.6e-h	0.5	-16.2	-10.3	0.0

Table 15b. Continued.

Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop	Selected Lines	Mean	Relative advantage (%) over MM	MP1	MP2	MPop
HSW						BMYLD					
7_14	29.4c-f	1.3	4.3	0.7	1.3	7_14	126def	10.3	18.9	-2.4	10.3
27_2	36.2a	24.9	28.6	24.1	24.9	27_2	134a-e	17.6	26.7	4.0	17.6
34_2	25.7hi	-11.6	-8.9	-12.1	-11.6	34_2	145ab	26.9	36.8	12.3	26.9
30_5	27.6fgh	-4.8	-2.0	-5.4	-4.8	30_5	139abc	21.8	31.3	7.7	21.8
MNM	29.0d-f	0.0	3.0	-0.6	0.0	MNM	114i	0.0	7.8	-11.6	0.0
MP1	28.2d-g	-2.9	0.0	-3.5	-2.9	MP1	106j	-7.2	0.0	-17.9	-7.2
MP2	29.2c-f	0.6	3.6	0.0	0.6	MP2	129c-f	13.1	21.9	0.0	13.1
MPop	28.9d-f	-0.4	2.6	-1.0	0.0	MPop	116h	1.1	9.0	-10.6	0.0
HI						GYLD					
7_14	38.9a	26.4	39.8	48.9	26.4	7_14	51.8ab	47.9	75.4	52.9	47.9
27_2	37.1abc	20.8	33.7	42.4	20.8	27_2	50.2abc	43.5	70.2	48.3	43.5
34_2	34.2cde	11.4	23.2	31.2	11.4	34_2	50.0abc	42.7	69.3	47.5	42.7
30_5	34.5cde	12.3	24.2	32.3	12.3	30_5	48.2bc	37.8	63.4	42.4	37.8
MNM	30.7g	0.0	10.6	17.8	0.0	MNM	35.0e	0.0	18.6	3.4	0.0
MP1	27.8h	-9.6	0.0	6.5	-9.6	MP1	29.5f	-15.7	0.0	-12.8	-15.7
MP2	26.1i	-15.1	-6.1	0.0	-15.1	MP2	33.9e	-3.2	14.7	0.0	-3.2
MPop	30.9g	0.4	11.1	18.3	0.0	MPop	35.9e	2.6	21.6	6.0	0.0

MM, MP1, MP2, MPop=mean of genotypes with marker(s), the recurrent parent (Ejere), the donor parent (ICC 4958), and the whole population, (genotypes with and without marker and the two parents together), respectively

4.1.3.10. Association of traits with grain yield

The association of nine traits with grain yield under both full-water and water-stress levels is presented in Figure 7. Under full-water conditions, number of PPP, BMY and HI had strong linear association with GYLD. Similarly, under water-stress conditions these traits had linear associations with GYLD. Under stressed condition, in addition to these traits, number of DTM and DTGF seem to have linear associations with GYLD. This indicates the traits that have linear associations with GYLD are similarly affected with water stress like that of the GYLD. The

other traits had no or little linear association with GYLD under both water level conditions indicating that they are differently affected by the stress.

4.1.3.11. Phenotypic and genetic correlations among traits

The phenotypic (r_P) and genotypic (r_G) correlations among traits for each location are shown in Appendices 4a, b and c while for combined over location in Table 16. Under full-water condition, the phenotypic and genotypic correlations coefficients among traits ranged from -0.38 to 0.99 and -0.41 to 0.98, respectively. Grain yield had strong positive genetic and phenotypic correlations ($P<0.01$) with number of PPP ($r_P=0.57$; $r_G=0.67$), BMY ($r_P=0.98$; $r_G=0.99$) and HI ($r_P=0.98$; $r_G=0.99$), respectively. It had also a weak positive genetic correlation ($P<0.05$) with number of DTF ($r_G=0.16$) and weak negative genetic correlation ($P<0.05$) with number of SPP ($r_G=-0.15$) and HSW ($r_G=-0.15$), respectively. With other traits, grain yield had none significant ($P>0.05$) genotypic and phenotypic correlations. Other traits that had strong positive genetic and phenotypic correlation ($P<0.01$) between each other were number of PPP and BMY ($r_P=0.57$; $r_G=0.67$), number of PPP and HI ($r_P=0.56$; $r_G=0.66$), BMY and HI ($r_P=0.97$; $r_G=0.98$) and number of DTM and DTGF ($r_P=0.81$; $r_G=0.80$), respectively. Traits that had medium ($P<0.01$) but negative genetic and phenotypic correlations were number of DTF and DTGF ($r_P=-0.41$; $r_G=-0.38$) and number of PPP and HSW ($r_P=-0.38$; $r_G=-0.26$).

Under water-stressed conditions, the same trend held true with few exceptions. The phenotypic and genotypic correlations coefficients among traits under this water-stress condition ranged from -0.61 to 0.83 and -0.67 to 0.96, respectively (Table 16). Grain yield had strong positive phenotypic and genetic correlations ($P<0.01$) with number of DTM ($r_P=0.30$; $r_G=40$), DTGF ($r_P=0.64$; $r_G=53$), PPP ($r_P=0.96$; $r_G=0.81$), BMY ($r_P=0.85$; $r_G=0.83$) and HI ($r_P=0.67$; $r_G=0.68$),

respectively, but negative correlation with number of DTF (r_P=-0.44; r_G=-0.34), respectively. It had also a weak positive genetic correlation (P<0.05) with number of SPP (r_G=0.16) and PHT (r_G=0.14), respectively. With HSW, GYLD had none significant (P>0.05) genotypic and phenotypic correlations. Other traits that had strong positive genetic and phenotypic correlation (P<0.01) between eachother were number of PPP and BMY (r_P=0.72; r_G=0.60), number of PPP and HI (r_P=0.75; r_G=0.63), number of DTM and DTGF (r_P=0.63; r_G=0.59), whereas, strong negative correlation between number of DTF and DTM (r_P=-0.67 and r_G=-0.61), respectively, whereas, medium positive correlation (P<0.01 between DTM and number of PPP (r_P=0.35 and r_G=0.27), DTM and BMY (r_P=0.36; r_G=0.26), number of DTGF and BMY (r_P=0.56; r_G=0.46), number of DTGF and HI (r_P=0.40; r_G=0.34) and medium negative correlation (P<0.01) between number of DTF and number of PPP (r_P=-0.40; r_G=-0.27) and number of DTF and BMY (r_P=-0.38 and r_G=-0.29), respectively. There was small positive or negative (P<0.05) correlation or none significant correlation (P>0.05) among the rest of the traits other than the grain yield.

In general, it is assumed that traits that have positive correlation move in the same direction during selection, i.e., when one is improved, the other also improves positively. In this regard, traits that have strong positivel correlation with grain yield are useful in indirect selection for grain yield especially when they have also high heritability. For negatively associated traits, there should be a compromise between selections for both traits or the breeder should set a minimum standard for one trait while selecting for the other, or separate breeding for such traits should be an alternative (Gemechu, et. al., 2006). For traits with weak association, there may be an independent genetic control between the two traits and improvement in any one of the two would have little effect on the other.

Under full-water Under water-stress

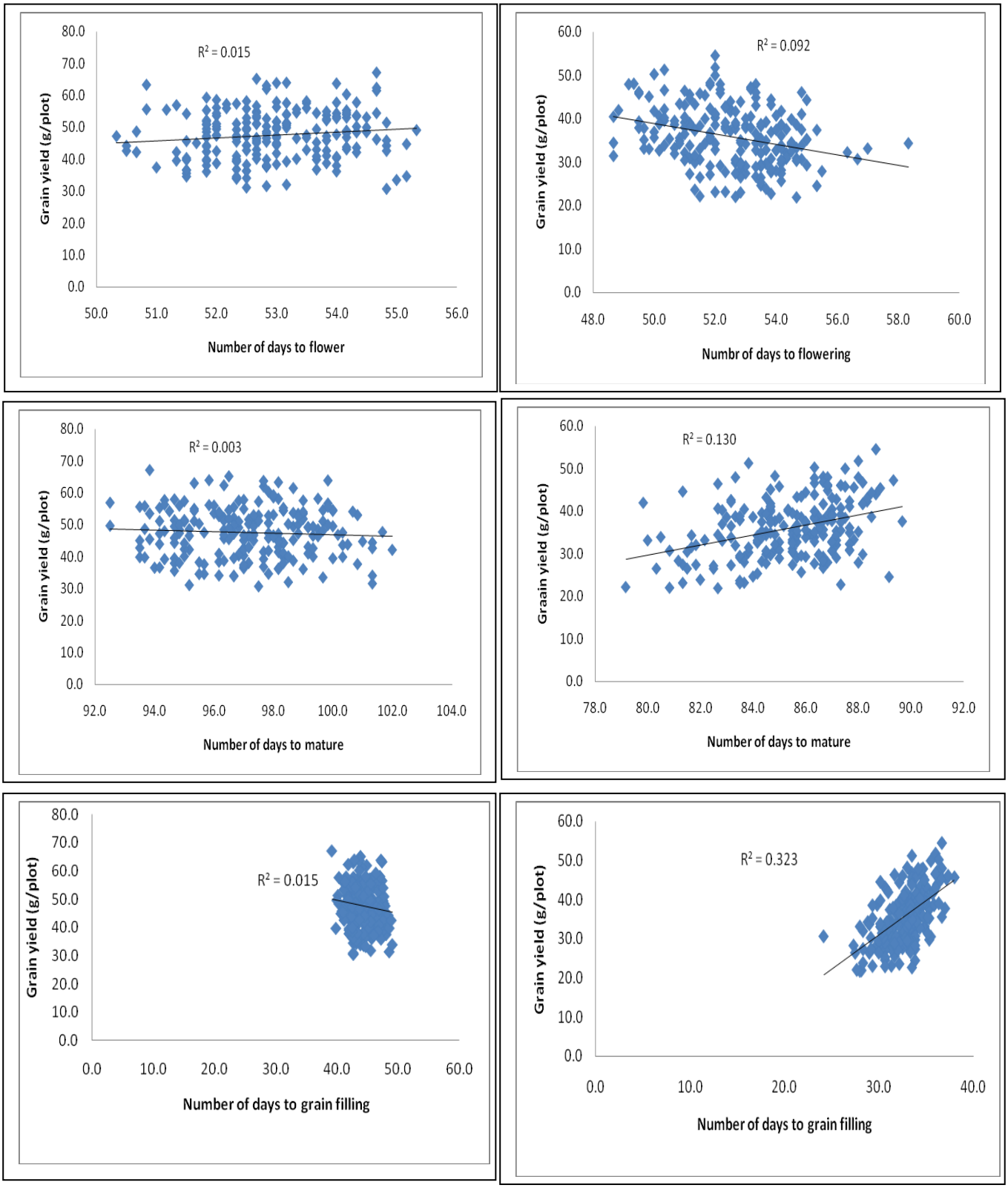


Figure 7. Association of nine chickpea traits with grain yield under full-water and water-stress conditions

Under full water Under water-stressed

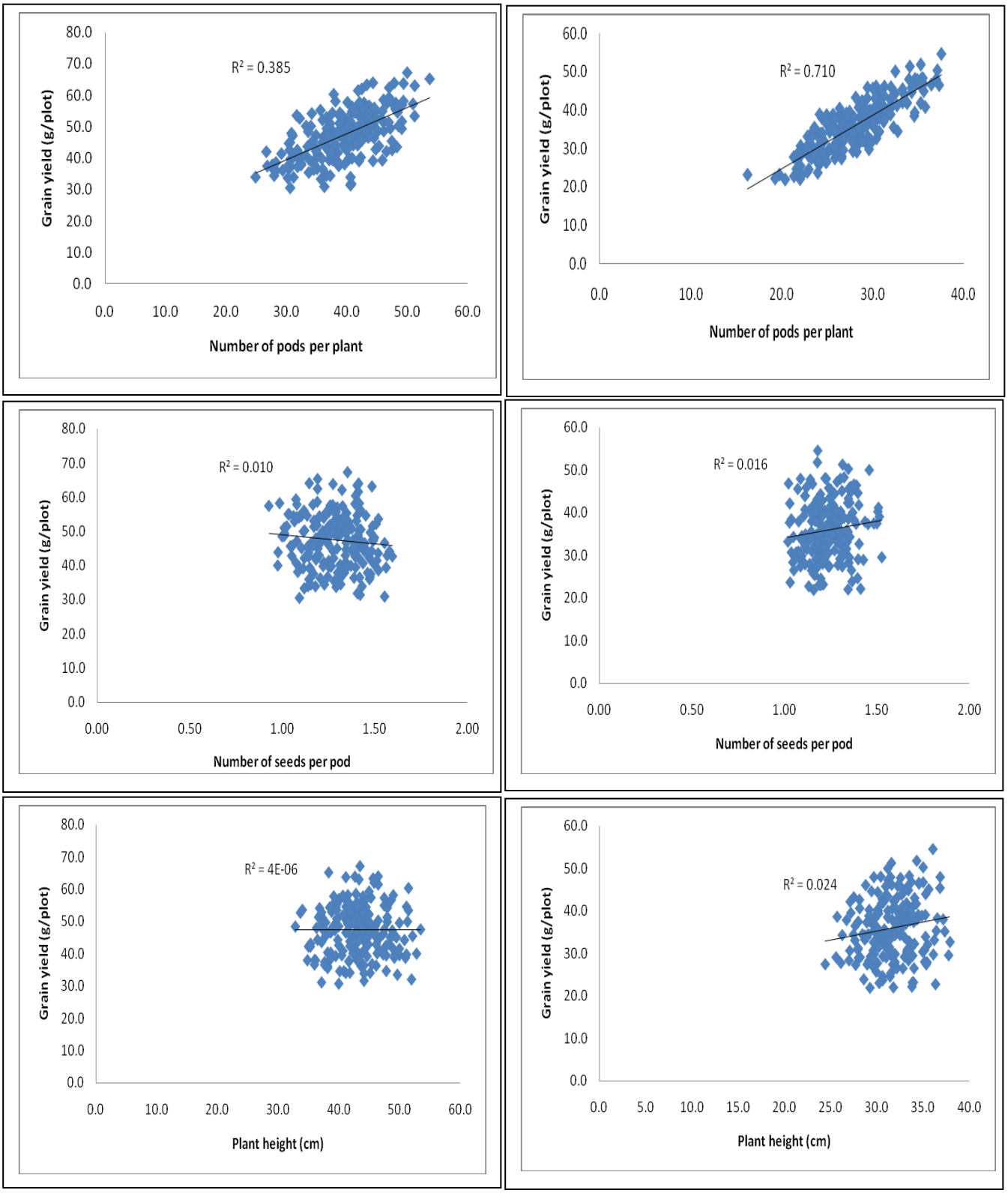


Figure 7. Continued.

Under full water Under water-stressed

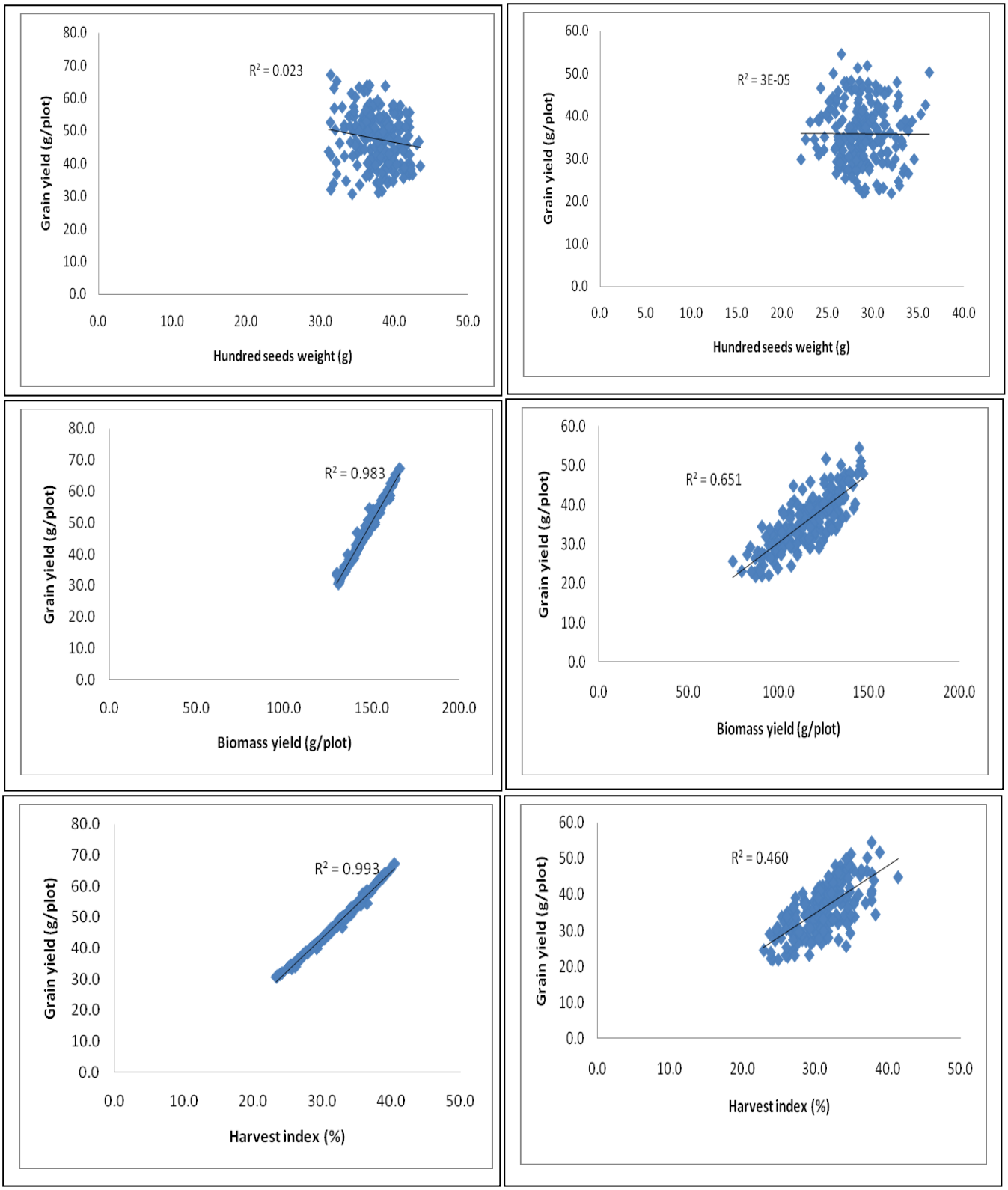


Figure 7. Continued.

Table 16. Combined phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients for ten agro-morphological traits of 210 chickpea genotypes tested at three locations under full-water and water-stressed conditions in 2014.

Trait	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
Under full-water										
DTF	1	*0.23***	*-0.38***	*0.09*^{ns}	*-0.07*^{ns}	*0.03*^{ns}	*-0.11*^{ns}	*0.13*^{ns}	*0.13*^{ns}	*0.13*^{ns}
DTM	0.22**	1	*0.81***	*0.08*^{ns}	*-0.12*^{ns}	*0.07*^{ns}	*-0.04*^{ns}	*0.01*^{ns}	*0.01*^{ns}	*0.01*^{ns}
DTGF	-0.41**	0.80**	1	*0.03*^{ns}	*-0.08*^{ns}	*0.05*^{ns}	*0.03*^{ns}	*-0.07*^{ns}	*-0.07*^{ns}	*-0.07*^{ns}
PPP	0.11^{ns}	0.11^{ns}	0.04^{ns}	1	*-0.13*^{ns}	*0.11*^{ns}	*-0.26***	*0.57***	*0.56***	*0.57***
SPP	-0.09^{ns}	-0.14*	-0.08^{ns}	-0.16*	1	*0.09*^{ns}	*-0.12*^{ns}	*-0.11*^{ns}	*-0.11*^{ns}	*-0.11*^{ns}
PHT	0.03^{ns}	0.08^{ns}	0.06^{ns}	0.12^{ns}	0.12^{ns}	1	*-0.10*^{ns}	*0.08*^{ns}	*0.08*^{ns}	*0.08*^{ns}
HSW	-0.13^{ns}	-0.04^{ns}	0.04^{ns}	-0.38**	-0.15*	-0.12^{ns}	1	*-0.11*^{ns}	*-0.13*^{ns}	*-0.13*^{ns}
BMY	0.15*	0.00^{ns}	-0.09^{ns}	0.67**	-0.15*	0.09^{ns}	-0.13^{ns}	1	*0.97***	*0.99***
HI	0.16*	0.01^{ns}	-0.08^{ns}	0.66**	-0.15*	0.09^{ns}	-0.14*	0.98**	1	*0.99***
GYLD	0.16*	0.00^{ns}	-0.09^{ns}	0.67**	-0.15*	0.09^{ns}	-0.15*	0.98**	0.98**	1
Under water-stress										
DTF	1	*0.28***	*-0.61***	*-0.27***	*-0.03*	*0.06*	*-0.12*	*-0.29***	*-0.21***	*-0.34***
DTM	0.15*	1	*0.59***	*0.27***	*-0.05*	*0.15***	*0.01*	*0.26***	*0.20***	*0.30***
DTGF	-0.67**	0.63**	1	*0.45***	*-0.02*	*0.07*	*0.11*	*0.46***	*0.34***	*0.53***
PPP	-0.40**	0.35**	0.58**	1	*0.07*	*0.15**	*-0.05*	*0.60***	*0.63***	*0.81***
SPP	-0.02^{ns}	-0.06^{ns}	-0.01^{ns}	0.13^{ns}	1	*0.04*	*0.02*	*0.08*	*0.07*	*0.10*
PHT	0.13^{ns}	0.23**	0.08^{ns}	0.20**	0.05^{ns}	1	*-0.04*	*0.06*	*0.13*	*0.12*
HSW	-0.17*	0.03^{ns}	0.16*	-0.09^{ns}	0.01^{ns}	-0.04^{ns}	1	*-0.04*	*-0.01*	*-0.03*
BMY	-0.38**	0.36**	0.56**	0.72**	0.14*	0.06^{ns}	-0.10^{ns}	1	*0.16**	*0.83***
HI	-0.26**	0.26**	0.40**	0.75**	0.11^{ns}	0.16*	-0.05^{ns}	0.17*	1	*0.68***
GYLD	-0.44**	0.40**	0.64**	0.96**	0.16*	0.14*	-0.08^{ns}	0.85**	0.67**	1

* and ** indicate significance (P<0.05 and 0.01, respectively); DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP and SPP= number of pods per plant and seeds per pod, respectively; PHT=plant height (cm); HSW=hundred seeds weight (g); BMY=biomass yield (g/plot); HI= harvest index (%); GYLD=grain yield (g/plot).

4.1.3.12. Relative traits' mean performance reductions due to water-stress

The ability of a genotype to give high and stable yield over a wide range of stress and non-stress environments is very important. However, high yield potential and stability over environments are almost independent characters (Finlay, 1968). Sojka *et al.* (1981) pointed out that a high yield scenario that allows a cultivar to do well over a range of environments may not imply drought resistance. Drought stress affects all phenological growth stages, reduces the normal growth and development periods, dry matter production and final yield. Many authors define drought resistance as the ability to minimize yield loss in the moisture scarcity. Under such conditions an ideal genotype might be the one that is stable with high yield potential (Finlay & Wilkinson, 1963).

Relative reduction (RR) of traits' mean performances due to moisture stress for different population category, i.e., 10% selected lines with and without markers, total population with and without markers, the whole population of 210 genotypes and the two parents tested under full-water and water-stressed conditions is presented in Table 17. All the traits considered in this study were affected by the water-stress, albeit at different magnitude. In general, the mean relative traits performance reduction ranged from 0.2 to 41%. For grain yield, the relative reduction ranged from 19.0 to 40.9% for different population category indicated in Table 17. The highest relative reductions in grain yield were recorded for the agronomic parent, Ejere, (40.9%) followed by the group of lines without molecular markers for drought tolerance (26.1%), and the drought tolerant parent, ICC 4958 (25.5%). The relative reduction for the isogenic lines was intermediate ranging from 19.0 to 21.4%, whereas, for the whole population was 24.4%, indicating the presence of some promising lines that better tolerate the effects of water-stress as compared to the parents. Especially, genotypes with markers for drought tolerance and the top 10% selected from this

group had the least relative reduction in grain yield (19.0 to 19.9%). Even top 10% selected genotypes without markers had better tolerated the stress as compared to the parents with overall mean relative reduction of 21.4%.

Another trait with high relative reduction in mean performance due to the stress was number of pods per plant, ranging from 23.3% for the genotypes with marker for drought tolerance to 40.6% for Ejere with overall mean reduction for the different population being 29%. Similarly, moderate to high relative reduction were recorded for number of DTGF (ranged from 20.5 to 26.8% with the whole population mean of 25.6, plant height (23.7 to 32.4% with mean of 26.7%), biomass yield (11.4 to 29.7% with mean of 21.5%), hundred seeds weight (13.3 to 24.0% with mean of 23.4%). The least relative reduction was recorded for number of days to flowering (0.2 to 5.7%, with mean of 0.8), number of seeds per pod (0.8 to 4.1%, with mean of 3.7%)), and harvest index (2 to 16.3%, with mean of 3.5%). In general, grain yield and yield component traits like number of pods per plant, biomass yield, hundred seeds weight and number of days to grain filling and plant height were found to be very sensitive to water-stress. This may indicate that a significant portion of the yield reduction could be attributed to the sensitivity of these traits to drought. Number of days to flowering, number of seeds per pod and harvest index were less sensitive to changes in environment. It seems from the result that number of days to grain filling, number of pods per plant, plant height, biomass yield and hundred seeds weight are good indicator of tolerance/resistance to drought stress and future selection of genotypes for water-stress tolerance should target an ideotype with consistent performance of these traits. However, this should be confirmed with more diversified genotypes in more multi-environment trials.

Table 17. Mean performances of different population category: 10% selected lines with (MSM) and without (MSNM) markers, population with (MM) and without (MNM) markers, the whole population of 210 genotypes (MPop) and the two parents, MP1 and MP2 for Ejere and ICC 4958, respectively) for 10 trait of chickpea tested under full-water and water-stressed conditions and their relative performance reductions (RR in %) due to the water-stress.

Population	DTF			DTM			DTGF			PPP			SPP		
category	FW	WS	RR	FW	WS	RR	FW	WS	RR	FW	WS	RR	FW	WS	RR
MSM	53.3	50.3	5.7	97.3	85.2	12.4	44.0	34.9	20.6	44.2	33.9	23.3	1.3	1.3	0.8
MSNM	53.2	51.9	2.5	97.0	86.8	10.5	43.9	34.9	20.5	45.9	34.4	25.2	1.3	1.2	0.8
MM	53.0	51.4	3.0	96.9	85.8	11.5	43.9	34.3	21.8	40.4	29.0	28.2	1.3	1.2	1.7
MNM	52.9	52.8	0.2	97.3	85.3	12.3	44.4	32.5	26.8	39.4	27.9	29.1	1.3	1.2	4.1
MP1	53.0	52.8	0.3	94.8	85.8	9.5	41.8	33.0	21.1	45.3	26.8	40.6	1.4	1.4	1.7
MP2	52.8	50.8	3.8	93.8	82.5	12.1	41.0	32.0	22.0	43.5	29.5	32.2	1.4	1.4	1.6
MPop	52.9	52.5	0.8	97.2	85.3	12.2	44.3	32.8	25.8	39.6	28.1	29.1	1.3	1.2	3.7
Population	PHT			HSW			BMY			HI			GYLD		
category	FW	WS	RR	FW	WS	RR	FW	WS	RR	FW	WS	RR	FW	WS	RR
MSM	46.2	31.3	32.3	35.6	30.9	13.3	162	134	17.1	38.7	35.2	9.0	62.8	50.3	19.9
MSNM	43.5	33.2	23.7	36.5	28.4	22.2	160	136	14.9	37.6	35.0	7.0	60.3	47.4	21.4
MM	42.1	32.0	23.9	37.4	28.4	24.0	148	120	18.4	32.2	31.6	2.0	48.0	38.8	19.0
MNM	43.3	31.5	27.3	37.8	29.0	23.3	147	114	22.3	31.9	30.7	3.8	47.4	35.0	26.1
MP1	51.3	37.8	26.5	36.4	28.2	22.7	151	106	29.7	33.1	27.8	15.9	49.9	29.5	40.9
MP2	52.2	35.3	32.4	34.7	29.2	15.9	146	129	11.4	31.2	26.1	16.3	45.4	33.9	25.5
MPop	43.1	31.6	26.7	37.7	28.9	23.4	147	115	21.5	32.0	30.9	3.5	47.5	35.9	24.4

RR=relative reduction (%), calculated as (1-WSt/FW)*100; the rest of the abbreviations are as indicated in the text and other tables above.

4.1.3.13. Drought susceptibility index and relative yield of selected lines

Many authors have reported that drought susceptibility index (DSI) and relative yield (RY) are the most important criterion to select high yielding and stable genotypes under drought conditions (Fisher and Maurer, 1978; Langer *et al.*, 1979; Blum *et al.*, 1989; Pinter *et al.*, 1990; Ahmad *et al.*, 1999). Relative yield (yield of an individual genotype under drought relative to that of the highest yielding genotype in the population) could be used to assess the yield potential

of a genotype under water-stressed conditions. Higher RY shows that the genotype performed relatively well under drought. Pinter *et al.* (1990) and Ahmad *et al.* (1999) found relative grain yield to be a useful criterion for assessing drought response of wheat genotypes. Stability in grain yield for each genotype is estimated by the DSI, derived from the yield difference between stress and non-stress environments (Blum *et al.*, 1989). Fisher and Maurer (1978) and Langer *et al.* (1979) have used DSI to characterize the yield stability between two environments.

The DSI of the traits of top 10% selected ILs and the two parents under full-water and water-stress conditions are shown in Tables 18a and 18b, respectively. Under FW conditions, almost half of the selections had low (below 1) DSI for DTF, DTM, DTGF, SPP, PHT, and HSW while almost all the selections had high (above 1) DSI for HI and GY indicating that genotypes best under FW were highly susceptible to moisture stress (Table 18a). Unde WS condition, most of the selections had low (below 1) DSI for DTM, DTGF, PPP, SPP and all the selections had low (below 1) DSI for grain yield; of the two parents, Ejere had high (abouve 1) DSI indicating it is susceptiblie to moisture scarcity whil ICC 4958 had average DSI under WS (Table 18b). The low susceptibility of grain yield to moisture stress for the selections under WS could be partly due to low susceptibility of the phenological traits (DTM and DTGF) and yield component traits (PPP and SPP) and partly due to low susceptibility of yield it self.

Under FW, lines 5-9, 4-7, 6-6, 8-4, 19-4, and Ejere had low DSI for DTGF while lines 4-5, 37-4, 37-3, and 7-19, had high DSI for DTGF. For DTM, lines 37-4 and 4-2 had low DSI while lines 27-5 and 31-1 had high DSI. Line 7-19 had the least DSI of while 31-1 had highest DSI for DTGF; lines 6-6, 8-4 and 7-19 had low DSI while 18-5 and 18-3 had high DSI; for SPP, lines 18-3, 33-1, and 19-

4 had low DSI while lines 4-7, 31-1, 27-1, and 6-6 had high DSI; for PHT, lines 18-3, 27-5, 7-19 had low DSI while lines 18-5 and 4-7 had high DSI; lines 4-2, 18-3, and 4-7 had low DSI while 33-1 and 39-1 had high DSI for HSW; for BMY, ICC 4958 (the donor parents) had the least DSI followed by lines 4-7 and 4-13, whereas, lines 18-5, 18-3, and 4-2 had hight DSI; lines 6-6 and 7-19 had low DSI for HI but lines 33-1, 5-10, 31-1, 37-3, 18-4 hight DSI; for GYLD, the donor parent, ICC 4958, had the least DSI followed by line 4-13 while lines 18-5, 5-10, 5-9, 18-4, 31-1, and 33-1 had high DSI.

Under WS conditions, line 9-2, 34-2, and Ejere had low DSI, whereas lines 10-10, 5-8, and 29-6 had high DSI for DTF; for DTM, lines 34-2, 38-2, and Ejere had lower DSI, whereas lines 10-10, 10-1, and ICC 4958 had high DSI; lines 38-2 and 34-2 had low DSI while line 10-9 had the highest DSI for DTGF; with regard to PPP, line 10-10, 8-6, and 8-7 had low DSI, while ICC 4958, Ejere and line 5-8 had high DSI; seed per pod had low DSI for lines, 10-4 and 7-12 and high DSI for lines 30-5, 17-5, 10-10, 9-8, ICC 4958, and Ejere. For PHT, line 29-3 had the lowst DSI while lines 34-2 and ICC 4958 had high DSI; hundred seeds weight had low DSI for line 27-2 while lines 34-2, 29-3, 10-1, and 8-7 had high DSI. For BMY, lines 8-7, 8-6, and 38-2 had low DSI while Ejere had the highest DSI. Lines 10-1 and 30-5 had low DSI for HI whereas lines, 29-3, 9-8, 9-7, 38-2, and 27-2 had high DSI. For GYLD, lines 9-7, 10-10, and 10-5 had low DSI while Ejere, ICC 4958, and 23-9 had high DSI. In general, under WS conditions DTF, HI and HSW had high (abouve 1) DSI of 3.46, 2.43, and 1.02 while GYLD, SPP, BMY, and PPP had the low (below 1) DSI of 0.03, 0.23, 0.40, and 0.62, respectively (Table 18b). In general, genotypes with low DSI for the studied traits were stable while lines with high DSI were unstable in their performance under the two stresses. In the case of grain yield, all the selections under WS could be said stable as they have low (below 1)

DSI for grain yield. Their stable mean performance could partly be due to their stability in DTGF, BMY, and SPP under the two stress environments and partyl due to stability in mena performance in grain yield itself under the two stress conditions.

The mean relative yields for genotypes selected for high yield under full-water condition were 0.84 under full-water and 0.64 under water-stress with average of 0.74 under the two conditions. While the mean relative yields of genotypes selected for high yield under moisture-stress were 0.65 under full-water and 0.85 under moisture-stress conditions with average of 0.75 under the two conditions. This indicates that lines that were selected as high yielders under full-water condition had higher mean RY under full water, while lines selected as high yielders under water-stress had higher mean RY under water-stress conditions as expected.

Out of the top 10% selected ILs for high mean grain yield under full-water conditions (Table 18a), nine lines each under full-water and water-stress conditions had high RY above the average (0.84 and 0.64, respectively). Among these, ILs 5-9, 5-10, 18-3, 18-5, 19-4 under full-water and 4-13, 6-6, 7-19, 8-4, 19-4, 27-1, and 27-5 under water-stress were the top in their relative mean RY indicating that these lines were high yielders under moisture scarce environments. Out of the top 10% selected ILs for high mean grain yield under water-stress conditions (Table 18b), 12 lines under full-water and 13 lines under water-stress had high RY above average (0.65 and 0.85, respectively). Among these lines 5-8, 7-12, 10-10, 29-6, 34-2 and Ejere under the full-water and ILs 8-6, 7-14, 10-10, 27-2, and 34-2 under water-stress were the top in RY value indicating that they were high yielding under moisture scarce environments.

Table 18a. Drought susceptibility index (DSI) for grain yield and agro-morphological traits and relative yield (RY) of top 10% selected chickpea genotypes for their high mean yield performances under full-water conditions

	DSI of traits										Relative yield		
Genotype	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GY	FW	WS	Mean
4_2(NM)	1.64	0.74	0.73	1.64	4.40	0.81	0.21	1.91	3.32	1.98	0.80	0.55	0.67
4_5(NM)	-5.07	1.05	1.23	1.46	-2.46	0.82	1.06	1.39	5.67	1.81	0.82	0.60	0.71
4_7(NM)	-0.42	0.78	0.84	1.40	-7.68	1.44	0.51	0.59	7.15	1.43	0.80	0.68	0.74
4_13(NM)	3.30	0.94	0.86	0.97	-1.74	1.10	0.59	0.68	4.15	1.09	0.80	0.78	0.79
5_9(M)	0.43	1.06	1.02	1.30	2.31	1.39	1.09	1.84	3.63	2.01	0.91	0.61	0.76
5_10(NM)	3.73	1.06	0.96	1.85	3.41	1.37	0.93	1.34	9.37	2.17	0.90	0.56	0.73
6_6(NM)	-0.42	0.86	0.89	0.64	5.86	0.96	0.94	1.72	0.39	1.58	0.87	0.70	0.79
7_19(M)	7.23	0.82	0.60	0.70	3.07	0.78	0.71	1.04	1.38	1.10	0.86	0.83	0.85
8_4(NM)	0.00	0.78	0.83	0.64	3.94	1.38	0.72	1.32	3.11	1.44	0.83	0.71	0.77
18_3(NM)	-3.34	1.09	1.25	1.97	-0.34	0.31	0.36	2.14	5.71	2.34	0.91	0.51	0.71
18_4(NM)	-1.66	1.16	1.30	1.50	4.86	1.09	0.97	1.22	8.86	2.02	0.88	0.59	0.73
18_5(NM)	4.02	1.10	1.07	1.84	2.20	1.64	0.56	2.34	5.08	2.54	1.00	0.50	0.75
19_4(NM)	0.00	0.98	1.03	1.64	0.81	1.20	0.95	0.92	7.73	1.74	0.90	0.69	0.79
24_2(NM)	-1.21	0.93	1.04	1.36	-3.01	0.98	1.07	0.77	8.66	1.71	0.81	0.62	0.71
27_1(NM)	2.87	1.12	1.05	0.85	7.45	1.28	0.94	0.80	6.26	1.45	0.81	0.69	0.75
27_5(NM)	3.76	1.32	1.19	1.29	-4.39	0.65	0.78	1.26	3.34	1.43	0.81	0.70	0.75
31_1(NM)	-4.24	1.22	1.36	1.82	-6.96	0.93	1.04	1.22	9.25	2.07	0.83	0.54	0.68
33_1(NM)	1.22	0.76	0.78	1.65	-0.08	1.23	1.29	1.03	10.02	2.05	0.81	0.53	0.67
37_3(M)	9.24	0.88	0.62	1.35	1.06	1.11	0.98	1.12	7.11	1.77	0.87	0.65	0.76
39_1(NM)	-4.97	0.90	1.13	1.37	3.95	0.39	1.18	1.15	5.45	1.61	0.81	0.65	0.73
ICC 4958	6.23	1.17	1.02	1.66	4.81	1.21	0.68	0.53	4.58	1.05	0.63	0.62	0.63
Ejere	0.41	0.78	0.82	1.14	-3.53	0.99	0.97	1.38	4.49	1.68	0.70	0.54	0.62
Pop Mean											0.84	0.64	0.74

DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP, SPP=number of pods per plant and seeds per pod; PHT=plant height (cm); HSW=hundred seeds weight (g); BMYLD=biomass yield (g); HI=harvest index (%); and GYLD=grain yield (g five plants^{-1}); FW and St=full-water and water-stressed conditions, respectively; M and NM in the parenthesis indicates lines with and without markers for drought tolerance, respectively.

Table 18b. Drought susceptibility index (DSI) for grain yield and morpho-agronomic traits and relative yield (RY) of top 10% selected genotypes for their high mean yield performances under water-stressed conditions

	DSI of Traits										Relative yield		
Genotype	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GY	FW	WS	Mean
5_8(NM)	9.12	0.89	0.67	1.01	1.13	1.14	0.60	0.17	0.92	0.32	0.73	0.88	0.80
7_12(NM)	7.41	1.12	0.88	0.85	0.54	1.22	1.38	0.86	-1.33	0.62	0.76	0.85	0.81
7_14(NM)	1.25	0.99	0.94	0.87	0.82	1.18	0.64	0.73	-4.96	-0.29	0.67	0.95	0.81
8_6(NM)	1.25	0.80	0.76	-0.12	-2.50	0.98	1.42	0.04	-4.81	-0.65	0.66	1.00	0.83
8_7(NM)	3.68	0.95	0.86	-0.04	2.32	0.67	1.48	-0.01	-3.31	-0.48	0.60	0.88	0.74
9_2(NM)	-0.41	0.97	0.97	0.48	1.80	1.05	0.75	0.22	-3.41	-0.30	0.61	0.87	0.74
9_7(M)	-1.71	0.95	1.00	0.85	0.63	1.00	1.02	0.91	-5.93	-0.02	0.64	0.84	0.74
9_8(NM)	-0.83	0.99	1.04	0.47	5.20	1.08	1.13	0.36	-7.75	-0.73	0.55	0.86	0.71
9_10(NM)	1.26	1.06	1.02	0.60	3.66	0.84	0.95	0.44	-1.76	0.11	0.68	0.88	0.78
10_1(NM)	5.32	1.19	1.03	0.84	-1.48	0.64	1.42	0.31	0.19	0.30	0.69	0.84	0.76
10_4(NM)	4.08	1.07	0.94	0.38	-0.20	0.67	1.03	0.30	-5.03	-0.43	0.59	0.86	0.72
10_5(NM)	2.03	1.05	1.01	0.86	1.04	1.00	1.29	0.41	-2.28	0.07	0.65	0.84	0.75
10_9(NM)	-7.63	0.92	1.17	0.70	-1.80	1.30	0.77	0.29	-2.86	-0.17	0.62	0.84	0.73
10_10(M)	9.70	1.22	0.93	0.00	-4.66	1.08	1.23	0.16	-1.43	-0.08	0.70	0.94	0.82
17_5(NM)	7.30	0.98	0.75	0.82	7.70	1.01	0.74	0.40	-1.06	0.22	0.67	0.84	0.75
27_2(NM)	6.67	1.09	0.87	0.45	1.15	0.71	0.27	0.41	-5.25	-0.38	0.64	0.92	0.78
29_3(NM)	1.28	1.04	1.03	-0.37	-2.29	0.35	1.37	0.05	-8.61	-1.42	0.48	0.85	0.67
29_6(M)	8.76	0.95	0.70	0.69	-0.86	0.96	0.72	0.44	-0.90	0.12	0.69	0.88	0.78
30_5(NM)	7.21	0.85	0.69	0.84	-7.87	0.88	0.89	0.37	-0.57	0.22	0.71	0.88	0.80
34_2(NM)	-0.42	0.58	0.62	0.90	-3.68	1.32	1.53	0.31	1.03	0.44	0.78	0.92	0.85
38_2(M)	7.53	0.75	0.54	0.38	-7.31	1.01	1.16	0.01	-5.85	-0.85	0.53	0.85	0.69
ICC 4958	6.23	1.17	1.02	1.66	4.81	1.21	0.68	0.53	4.58	1.05	0.63	0.62	0.63
Ejere	0.41	0.78	0.82	1.14	-3.53	0.99	0.97	1.38	4.49	1.68	0.70	0.54	0.62
Mean											0.65	0.85	0.75

DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP, SPP=number of pods per plant and seeds per pod; PHT=plant height (cm); HSW=hundred seeds weight (g); BMYLD=biomass yield (g five plants^{-1}); HI=harvest index (%); and GYLD=grain yield (g five plants^{-1}); FW and St=full-water and water-stressed conditions, respectively

4.1.3.14. Association of means of a trait under full-water and water-stress conditions

The linear association between mean performances of a trait under FW and WS condition are presented in Figure 8. The association between mean performances of DTF under the two conditions was weak (R^2 =0.03); for DTM, there was strong association (R^2 =0.34**) between the performances under the two stress conditions; there was moderate association (R^2 =0.13*) between mean performances of DTGF; performances of PPP under the two stress conditions had weak association (R^2 =0.08); there was no association between mean performances of SPP (R^2 =0.00); there was moderate association between mean performances of PHT (R^2 =0.15*) under the two stress conditions; however, there was no association between the mean performances of BMY, HI and GYLD (R^2 =0.03). In general, there was strong association between mean performances of DTM; moderate association between mean performances of DTGF and HSW; but weak or no association between mean performances of DTF, PPP and SPP, PHT, BMY and GYLD and HI. The strong linear association between means of DTM under full-water and water-stress conditions indicated that genotypes that mature late under full-water condition almost do so under water-stress condition too and *vice versa*. The non existence association or weak association between the mean performances of a trait under the FW and WS conditions could be due to the distinctness of the two stress conditions and the differential responses of the traits for the two stresses.

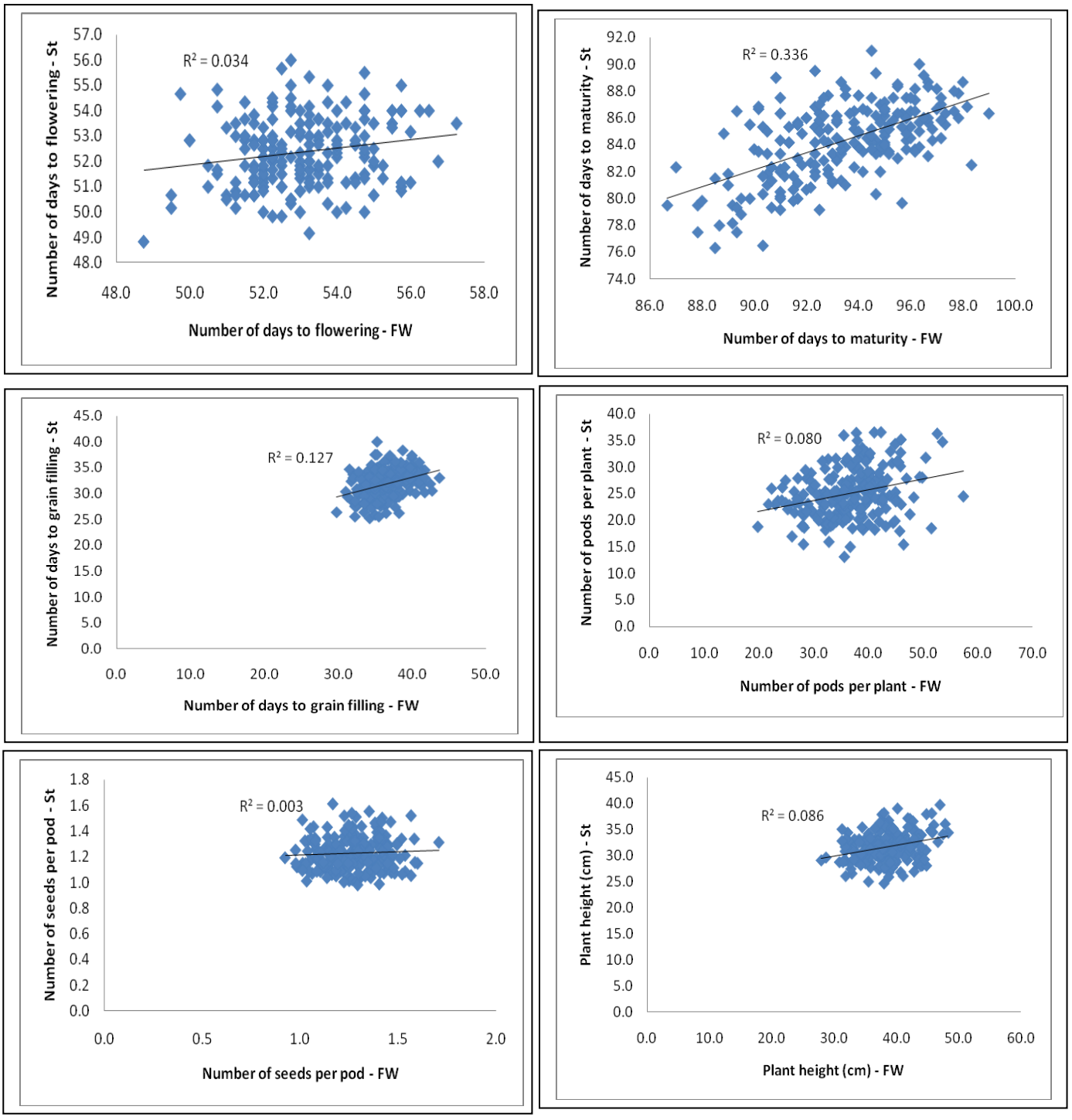


Figure 8. Association of mean performances of a trait under full-water and water-stress conditions

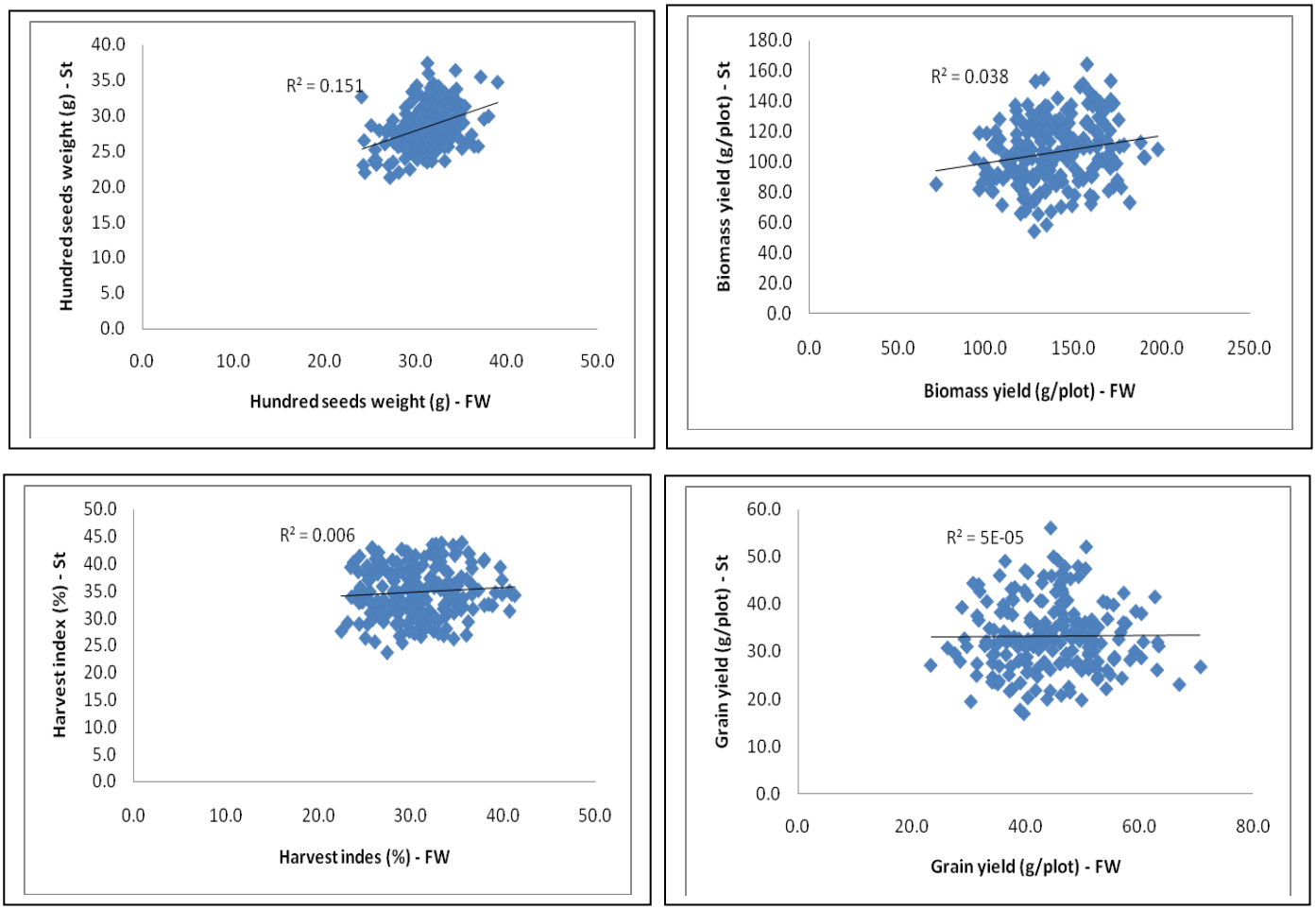


Figure 8. Continued.

4.1.3.15. Transcriptome analysis of selected isogenic lines

(a) Generation of RNA-seq data:

To understand the expression profiles during drought stress in chickpea ILs developed through marker-assisted method, RNA-Seq analysis was performed. A total of 294.8 million paired end (PE) raw reads were generated from control and stress root tissue samples of selected four genotypes: the two parents (Ejere, the recurrent parent and ICC 4958, the donor parent for drought tolerance) and to selected BC_3F_4 ILs (5-12 and 38-2) for their yield

performances and drought tolerance under water-stress condition. After stringent quality control (QC) filtering, a total of 293.3 million PE reads were retained for subsequent analysis. The statistics of the RNA-Seq data generated and QC filtering is given in Table 19.

Table 19. Mapping statistics of RNA-Seq data generated from four drought responsive chickpea genotypes/lines

S. No.	Sample ID	Total no. raw read pairs	Total no of processed read pairs	Mapped read pairs concordantly	% Mapped concordant read pairs	Overall reads mapping rate (%)
1	38-2 control	45613556	45358438	39987482	87.9	92.2
2	38-2 stress	36197211	35991570	32496355	90.0	93.9
3	5-12 control	38372807	38185216	33654235	87.9	92.0
4	5-12 stress	23195589	23069566	20833094	90.0	93.8
5	Ejere control	35536529	35368266	31150464	87.8	92.1
6	Ejere stress	33687958	33521649	29922546	89.0	92.8
7	ICC 4958 control	44301869	44072062	38886561	88.0	92.1
8	ICC 4958 stress	37915725	37739826	34036600	90.0	93.7
	Total	294821244	293306593	260967337	88.8	92.8

Sequence read length ranged from 125 to 20bp. The 293.3 million PE reads were subjected to mapping against the chickpea genome and finally a total of 272 (92.8%) million PE reads were aligned. Of these 272 million PE reads, 88.8% were concordantly aligned (i.e, reads mapped in the expected read orientation and within the insert size) and 11.2 % of the reads were non-concordantly aligned.

(b) Differential gene expression analysis:

Of the aligned 272 PE reads, 48,698 transcripts were assembled using Cufflinks (Trapnell et al. 2012). These 48,698 transcripts were subjected to differential gene expression analysis. Based on FPKM (fragments per kilobase of exon per million mapped reads) gene expression was identified. Up- and down- regulated genes include differentially expressed genes (DEGs) identified from control and stressed samples of the same genotype as well as different combination of genotypes (Table 20).

Table 20. Differential gene expression of two selected isogenic lines and the two parents tested under full-water and water-stress conditions

S. No.	Sample/ genotype combination	DEGs identified	Up-regulated	Down-regulated
1	5-12_C vs 5-12_S	12	5	7
2	38-2_C vs 38-2_S	34	4	30
3	Ejerie_C vs Ejerie_S	5	2	3
4	ICC 4958_C vs ICC 4958_S	10	7	3
5	5-12_C vs 38-2_C	26	3	4
6	5-12_S vs 38-2_S	91	15	24
7	Ejerie_C vs 38-2_C	40	7	3
8	Ejerie_S vs 5-12_S	55	16	1
9	Ejerie_S vs 38-2_S	68	25	29
10	ICC 4958_C vs 5-12_C	91	16	8
11	ICC 4958_C vs 38-2_C	51	10	2
12	ICC 4958_S vs 5-12_S	35	8	2
13	ICC 4958_S vs 38-2_S	49	4	4
14	ICC 4958_S vs Ejerie_S	91	13	18

*****C= control; S-stress; $ The DEGs which are not represented under Up- or down- regulated genes are showing minimal log2 fold change (≥ -2.5 and ≤ 2.5 folds)

(c) Functional annotation, categorization according to Gene Ontology (GO) descriptions:

All differentially expressed genes were annotated using BLASTX against Uniprot database. These were functionally categorized based on GO descriptions. As a result, 1,088 differentially expressed unique transcripts from different genotype combinations were assigned to three principal categories: molecular function (401), biological process (436) and cellular component (251). Details of Gene Ontology descriptions were presented in Table 21.

Table 21: Gene Ontology descriptions of differentially expressed genes (DEGs) with their expressed numbers

S. No.	Sample/ genotype combination	Molecular function	Biological Process	Cellular component
1	5-12_C vs 5-12_S	8	10	7
2	38-2_C vs 38-2_S	24	25	15
3	Ejerie_C vs Ejerie_S	3	4	3
4	ICC 4958_C vs ICC 4958_S	5	8	5
5	5-12_C vs 38-2_C	15	15	10
6	5-12_S vs 38-2_S	55	59	33
7	Ejerie_C vs 38-2_C	26	26	21
8	Ejerie_S vs 5-12_S	35	37	19
9	Ejerie_S vs 38-2_S	41	43	19
10	ICC 4958_C vs 5-12_C	46	56	32
11	ICC 4958_C vs 38-2_C	34	37	20
12	ICC 4958_S vs 5-12_S	28	30	15
13	ICC 4958_S vs 38-2_S	33	34	23
14	ICC 4958_S vs Ejerie_S	48	52	29
	Total	401	436	251

C=control (full-water) treatement; S=stress(water-stress) treatment

(d) Large scale mining of SNPs and InDels:

The RNA-Seq data generated from four genotypes (under full-water and water-stress conditions) was analyzed for identification of SNPs and InDels. The sequence data from control and stress samples of all genotypes was aligned against the reference chickpea genome in order to identify SNPs. Details of SNPs identified among different combinations across the eight chickpea chromosomes (Ca1 to Ca8) as well as scaffolds and contigs (Ca0) were presented in Table 22.

Table 22. Mining of SNPs and InDels of two selected isogenic lines and two parents tested under full-water and water-stress conditions

Chrom.	ICC 4958		Ejerie		IL 5-12		IL 38-2	
	FW	WS	FW	WS	FW	WS	FW	WS
Ca1	150042	134057	135247	129829	142741	117294	147663	138196
Ca2	101240	92128	90521	89659	96186	80048	99746	94293
Ca3	137591	123440	123617	120077	130330	107308	135122	127247
Ca4	194785	176917	176330	172684	186795	155198	192310	181316
Ca5	162730	147812	146076	142843	155297	127814	160000	151797
Ca6	189591	169902	171087	166478	181043	148627	186322	174117
Ca7	160029	142089	144075	138306	152689	124536	157367	147006
Ca8	81553	73597	73917	71966	78043	64471	80533	75291
Ca0	106783	92639	95593	90585	101323	79497	104802	95362
Total SNPs	1284344	1152581	1156463	1122427	1224447	1004793	1263865	1184625

FW=full-water (control); WS=water-stress; IL=isogenic line

4.2. Experiment 2. Stress Susceptibility or Tolerance Indices and Water Use-Efficiency of Released Chickpea Cultivars

4.2.1. Weather data of the test locations

The weather condition (temperature, °C, and relative humidity, RH %) in the greenhouse during the experiment period (July to December 2014) is indicated in Figure 9. The temperature and RH in the greenhouse were not controlled, but daily record of the condition was taken. The average minimum temperature in the greenhouse during the crop growing period was 17.1°C while the average maximum temperature was 20.9°C (mean daily temperature ranging from 14.0 to 22.1°C), while the RH ranged from 45.8 to 64.2%. In general, there was low temperature and high RH in August, whereas in October there was relatively high temperature and low RH.

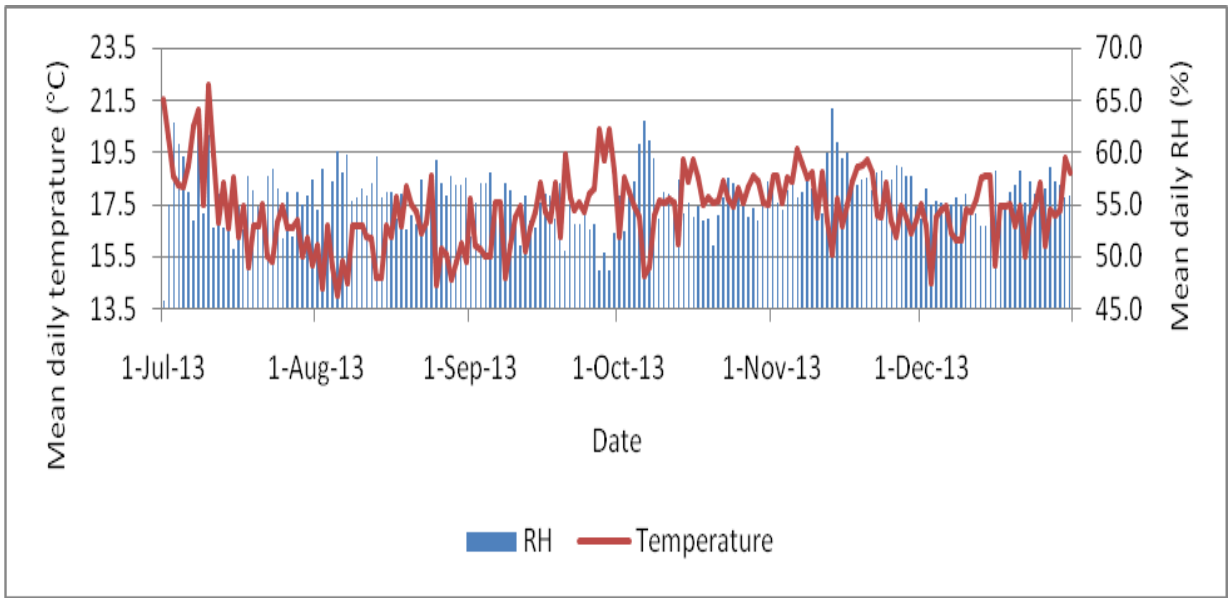


Figure 9. Weather data in the greenhouse at Holetta during the experiment period (July to December 2014: Mean daily temperature (°C) and relative humidity (RH%).

4.2.2. Analysis of variance

The results of ANOVA for 16 agro-morphological traits under full-water and water-stress conditions are showen in Tables 23 while for eight morpho-physiological traits are shown in Table 24. In agro-morphological traits, the data was analized over two soils types while in the physiological raits the data was analized over two soils and three destruction levels. Under full-water condition, the ANOVA showed that there was significant difference between the soils ($P<0.01$) for grain yield, relative water content, leaf area per plant, leaf area index and water use efficiency for grain yield while it was not significant ($P>0.05$) for the rest of agro-morphological traits (Table 23). Similarly, there was significant difference between the genotypes ($P<0.01$) for early vigor number of days to flowering, hundred seeds weight, harvest index, grain yield, water use-efficiency for grain yield and drought susceptibility index; but significant ($P<0.05$) for number of days to grain filling, leaf area per plant, and leaf area index. The soil by genotype interaction effect was also highly significant ($P<0.01$) for early vigor, number of days to flowering, harvest index, grain yield, water use-efficiency for grain yield and drought susceptibility index.

Under water-stress condition, there was highly significant difference between the soils ($p<0.01$) for early vigor, grain yield, harvest index, relative water content, leaf area per plant, leaf area index and water use-efficency for grain yield (Table 23); similarly, there was highly significant difference between the genotypes ($P<0.01$) for early vigor, number of days to flowering, hundred seeds weight, grain yield and water use-fficiency for grain yield; it was significant ($P<0.05$) for plant height and number of seeds per pod. The soil by genotype interaction effect was highly significant ($P<0.01$) for early vigor, number of days to flowering, grain yield, harvest indes and water use-efficieny for grain yield.

In general, traits like grain yield and water use-efficiency for grain yield were highly significantly affected by soil, genotype and their interaction while traits like early vigor, number of days to flowering, hundred seeds weight, and harvest index were highly significantly affected by genotype and soil by genotype interaction effects. The rest of the agro-morphological and physiological traits like DTM, DTGF (except for genotype under full-water), PHT (except for soil under full-water and genotype under water-stress), PPP, SPP, HSW (except for genotype under full-water and genotype under water-stress), and LAI (except for soil and genotype under full-water and soil under water-stress) were not. The RWC was not affected by genotype under both FW and WS and by soil by genotype interaction under WS. The LAI was not affected by genotype effect under WS and the interaction effects of soil by genotype under FW and WS conditions. The DSI was not affected by soil, but it was highly significantly ($p<0.01$) affected by genotype and soil by genotype interactions.

Under FW condition, for physiological traits, there was highly significant difference between the soils, the destruction levels and the genotype ($P<0.01$) for leaf, stem, root and shoot dry weights (LDWt, StDWt, RDWt, ShDWt, respectively), transpiration and WUEBM but significant ($P<0.05$) for BMY and shoot-root ration (SRR). There was also highly significant interaction effect for all the interactions for all the traits (except for S×D interaction for ShDWt and S×G, D×G and S×D×G for Transpiration) (Table 24). There was significant difference ($P<0.05$) for these exceptions. There was non-significant soil by genotype, destruction by genotype, and soil by destruction by genotype interaction for transpiration.

Similarly, under WS condition, there was highly significant difference ($P<0.01$) between the soils, the destruction levels and the genotype for all the traits and all the interaction as well, except there

was no significant difference between the soils (P>0.05) for WUEBM (Table 24). In general, all the traits were highly affected by the main factors (soil, destruction and genotypes) and their interactions under water-stress than under full-water conditions.

Table 23. Mean square of ANOVA of 16 morpho-agronomic and physiological traits of 20 chickpea cultivars tested on two soil types in greenhouse at Holetta in 2014

	Mean Square					
	Full-water			Water-stressed		
Trait	Soil	Geno	Soil*Geno	Soil	Geno	Soil*Geno
EV	0.352 ^{ns}	3.963 **	0.260 **	1.008 **	4.305 **	0.133 **
DTF	21.511 ^{ns}	647.444 **	93.482 **	16.469 ^{ns}	464.487 **	63.662 **
DTM	57.408 ^{ns}	208.419 ^{ns}	77.391 ^{ns}	42.008 ^{ns}	110.629 ^{ns}	121.464 ^{ns}
DTGF	29.008 ^{ns}	580.998 *	122.745 ^{ns}	307.200 ^{ns}	304.870 ^{ns}	153.761 ^{ns}
PHT	1300.208 *	287.401 ^{ns}	121.261 ^{ns}	929.633 ^{ns}	556.318 *	294.633 ^{ns}
PPP	61.347 ^{ns}	89.766 ^{ns}	42.553 ^{ns}	13.333 ^{ns}	96.271 ^{ns}	46.373 ^{ns}
SPP	0.077 ^{ns}	0.049 ^{ns}	0.034 ^{ns}	0.009 ^{ns}	0.080 *	0.054 ^{ns}
HSW	21.505 ^{ns}	347.140 **	9.790 ^{ns}	25.947 ^{ns}	378.705 **	11.055 ^{ns}
HI	44.774 ^{ns}	286.107 **	245.002 **	2185.387 **	391.992 **	151.576 **
GYLD	177.363 **	171.067 **	83.923 **	51.234 **	198.026 **	53.416 **
RWC	158.011 **	22.759 ^{ns}	33.397 *	137.816 *	25.965 ^{ns}	19.111 ^{ns}
LAPP	15207.757 **	776.496 *	488.259 ^{ns}	9133.330 **	337.528 ^{ns}	220.362 ^{ns}
LAI	2.628 **	0.134 *	0.084 ^{ns}	1.578 **	0.058 ^{ns}	0.038 ^{ns}
WUEGY	82.287 **	19.113 **	10.588 **	56.115 **	27.363 **	7.057 **

Table 24. Mean squares from combined ANOVA of eight morpho-physiological traits of 20 chickpea cultivars tested on two soil (S) types and three destruction (D) levels in greenhouse at Holetta in 2014

	Mean Square						
Trait	Soil (S)	Destruction (D)	Genotype (G)	S*D	S*G	D*G	S*D*G
Full-water							
LDWt	70.522**	300.640**	14.617**	6.535**	1.450**	4.635**	3.597**
StDWt	51.105**	3879.489**	35.513**	9.370**	12.364**	27.033**	11.831**
RDWt	5.355**	55.209**	2.700**	0.852**	0.543**	0.880**	0.259*
ShDWt	241.921**	6004.008**	75.556**	7.779*	16.656**	42.936**	21.840**
BMY	17.744*	29179.932**	220.018**	93.256**	65.188**	140.925**	80.472**
SRR	4.912*	363.139**	2.971**	4.432**	2.784**	3.348**	3.333**
Trans	484880.40**	21396493.74**	11348.37*	301655.43**	8861.11^{ns}	3408.36^{ns}	7886.84^{ns}
WUEBM	3.014**	1027.238**	25.130**	83.891**	6.448**	13.992**	8.516**
Water stressed							
LDWt	25.547**	146.127**	8.078**	7.632**	2.735**	4.644**	2.085**
SDWt	100.107**	2675.498**	38.938**	162.046**	20.517**	33.404**	17.496**
RDWt	0.767**	1.920**	2.599**	8.695**	0.329**	0.570**	0.351**
ShDWt	226.797**	4065.157**	75.011**	115.365**	29.551**	56.101**	25.400**
BMY	127.365**	17325.352**	245.964**	47.457**	84.509**	218.245**	73.984**
SRR	38.781**	574.814**	5.716**	7.078**	6.334**	5.175**	6.482**
Trans	467280.28**	15534258.31**	12543.67**	289564.84**	12605.52**	10709.94**	8089.62**
WUEBM	2.248^{ns}	828.463**	26.529**	12.484**	9.652**	20.702**	8.358**

4.2.3. Phenotypic performances of traits

4.2.3.1. Agro-morphological traits

The mean agro-morphological traits performances under FW and WS conditions are shown in Tables 25a and b. Under full-water condition, early vigor (EV) of the cultivars ranged from 1.2 to 4.5. Acos Dube and Yelibe were the most vigorous while DZ-10-4 and DZ-10-11 were the least vigorous during germination. Number of DTF of the genotypes ranged from 57 to 76 days with

mean of 63 days. Cultivar Acos Dube had early DTF while Shaso was late DTF. Number of DTM ranged from 158 to 185 days with mean of 168 days. Cultivar Akaki was early in DTM while Worku was late. The number of DTGF ranged from 77 to 110 days with mean of 97 days. Cultivar Yelibe had shorter DTGF while Ejere took longer DTGF. Plant height ranged from 62 to 90cm with mean of 76cm. Cultivar Yelibe was shorter while Acos Dube was the tallest. Number of PPP ranged from 10 to 22 pods with mean of 15 pods. Cultivar Acos Dube had few number pods while Arerti had highest number of PPP; number of SPP ranged from 1.0 to 1.3. Cultivar Shasho had low number of SPP while Arerti had more number of SPP. Mean HSW of the cultivars ranged from 16.3 to 48.7g with mean of 30.6g. Cultivar DZ-10-4 and DZ-10-11 had the lease seeds weight while Acos Dube had the highest HSW. Biomass yield of the cultivars ranged from 16.8g to 56.2g per five plants with average of 36.9g. DZ-10-4 and DZ-10-11 had low BMY while Shasho had the highest BMY. Harvest index of the cultivars ranged from 30 to 51% with mean of 41%. Cultivar Fetench had the least HI while Marye had the highest HI. The mean GYLD of the cultivars ranged from 6.65 to 28.45 g per five plants with mean of 16.51. Cultivar DZ-10-11, DZ-10-4, and Habru had low GYLD while Shasho, Mariye, Natili, Ejere, and ICC 4958 had high GYLD. Their high GYLD seems due to high BMY, PPP and HI.

Under WS condition, EV of the cultivars ranged from 1.3 to 4.8 with mean of 3.2. Like under FW conditon, Acos Dube and Yelibe were the most vigorous ones while DZ-10-4 and DZ-10-11 were the least. Number of DTF of the genotypes ranged from 57 to 72 days with mean of 65. Cultivar Ejere was early in DTF while Arerti was late. Number of DTM ranged from 159 to 176 days with mean of 168 days. Like under FW condition, cultivar Akaki was early in DTM while Dubie was latest. The number of DTGF ranged from 84 to 109 days with mean of 99 days. Cultivar Akaki had

shorter DTGF while Worku took longer days for grain filling. Plant height ranged from 54 to 90 cm with mean of 67.9 cm. Cultivar Akuri was the shortest while Minjar was the tallest. Number of PPP ranged from 9 to 18 with mean 13.6. Like under FW condition, cultivar Acos Dube had least PPP while Arerti had highest PPP; number of SPP ranged from 1.0 to 1.3. Cultivar Fetenech had the least SPP while Natoli had the highest SPP. Mean HSW of the cultivars ranged from 14.2 to 51.7g with mean of 29.6g. Like under FW condition, cultivar DZ-10-4 and DZ-10-11 had the least HSW while Acos Dube had the highest HSW. Biomass yield of the cultivars ranged from 12.0 g to 51.4 g per five plants with average of 26.5 g. DZ-10-11 and Akuri had low BMY while Arerti had the highest BMY. Harvest index of the cultivars ranged from 22.1 to 48.7% with mean of 37.4%. Cultivar Acos Dubie had the least HI while Minjar had the highest HI. The mean GYLD of the cultivars ranged from 5.0 to 24.9 g per five plants with mean of 11.5. Cultivar Acos Dubie, Akaki, Akuri, Dz-10-11 and Fetenech had low GYLD while Arerti, Natoli, ICC 4958, Minjar and Shasho had high GYLD. Their high GYLD seem to be due to high BMY, PPP and HI.

4.2.3.2. Physiological traits

The mean morpho-physiological traits performances under FW and WS are indicated in Tables 26a and b. Under FW conditions, the mean performance of leaf dry weight (LDWt) ranged from 2.7 to 5.9g with mean of 4.5g. Cultivar Dz-10-4 had the least LDWt while Acos Dubie had the highest mean performance. Mean stem dry weight (StDWt) of the cultivars ranged from 6.0 to 11.1g with overall mean of 8.5g. Cultivar Dz-10-4 had the lowest mean StDWt while cultivar Natoli had the highest. Mean shoot dry weight (ShDWt) of the cultivars ranged from 8.7 to 16.0 g with overall mean of 10.0g. Cultivar Dz-10-4 again had the least mean ShDWt while Shasho and Natoli had the heighest mean ShDWt. Root dry weight (RDWt) ranged from 2.0 to 3.5g with overall mean of 2.7g.

Cultivar Dz-10-4 again had the least RDWt while Natoli had the highest RDWt. Mean shoot-root ratio (SRR) of the cultivars ranged from 4.2 to 5.4g with overall mean of 4.8g. Cultivar Arerti had the least mean SRR while Shasho had the highest. Mean leaf area per plant (LAPP) of the cultivars ranged from 43.4 to 75.0 with overall mean of 57.2. Cultivar Yelibe had the leas LAPP while Minjar had the highest mean LAPP. Mean leaf area index (LAI) of the cultivars ranged from 0.57 to 0.88 with overall mean of 0.72. Cultivar Yelibe and Habru had the least while Shasho had the highest LAI. The relative water content (RWC) of the cultivars ranged from 53.6 to 61.6% with overall mean of 57.6%. Cultivar Yelibe had the least RWC while Acos Dubie the highest. The mean transpiration (Trans) of the cultivars ranged from 753 to 854 with overall mean of 796. Cultivar Dz-10-11 had the least transpiration while Shasho had the highest. The mean water-use efficiency (WUE) of the cultivars ranged from 5.7 to 19.4 with overall mean of 12.2. Cultivar Dz-10-11 had the least mean WUE for BMY while cultivars Shasho, Marye, and ICC 4958 had the highest. Mean WUE for GYLD ranged from 2.7 to 9.4 with overall mean of 5.4. Cultivars Dz-10-4 and Dz-10-11 had the least mean while cultivars Shasho and ICC 4958 had the highest mean WUE for GYLD. Mean drought susceptibility index (DSI) ranged from 0.24 to 2.76 with overall mean of 1.03. Cultivar Akaki had the least DSI while Arerti and Dz-10-4 had the highest mean value for DSI. Under this FW, cultivar Shasho had the highest ShDWt, SRR, LAPP and LAI, transpiration, and WUE for both BMY and GYLD while cultivar Natoli has the highest mean performance in StDW, ShDWT, and RDWt. On the contrary, cultivar Dz-10-4 had the least mean performances in LDWt, StDWt, ShDWt, RDWt, and WUE for GYLD while Dz-10-11 had the least mean performances in transpiration and WUE for both BMY and GYLD.

Under WS conditions, the mean performance of LDWt ranged from 2.0 to 4.2g with mean of 3.2g. Cultivar Dz-10-11 had the least LDW while Arerti, Kobo and ICC 4958 had the highest mean performance. Mean StDWt of the cultivars ranged from 4.9 to 10.1g with overall mean of 6.6g. Cultivar Dz-10-11 had the lowest mean StDWt while cultivar Arerti had the highest mean StDWt. Mean ShDWt of the cultivars ranged from 6.9 to 14.2 g with overall mean of 9.8g. Cultivar Dz-10-11 again had the least mean ShDWt while Arerti had the highest mean ShDWt. Root dry weight ranged from 1.4 to 2.7g with overall mean of 2.3g. Cultivar Dz-10-11 again had the least RDWt while Arerti and Yelibe had the highest RDWt. Mean SRR of the cultivars ranged from 3.4 to 5.2g with overall mean of 4.3g. Cultivar Ejere had the least mean SRR while Worku and Minjar had the highest SRR. Mean LAPP of the cultivars ranged from 29.6 to 54.2 with overall mean of 43.2. Cultivar Dz-10-4 had the leas LAPP while Minjar and Natoli had the highest mean LAPP. Mean LAI of the cultivars ranged from 0.41 to 0.71 with overall mean of 0.57. Cultivar Habru had the least while Natoli and Arerti had the highest LAI. The RWC of the cultivars ranged from 55.4 to 62.9% with overall mean of 59.8%. Cultivar Marye had the least RWC while Yelibe, Natoli, and Dz-10-11 had the highest. The mean transpiration of the cultivars ranged from 649 to 768 with overall mean of 698. Cultivar Kasech had the least transpiration while Arerti had the highest. Under this WS condition, cultivar Arerti had the highest LDWt, StDWt, ShDWt and RDWts, LAI and transpiration. On the contrary, cultivar Dz-10-11 had the least LDWt, StDWt, ShDWt, RDWts and LAPP.

4.2.4. Water-use efficiency of the cultivars

Under FW condition, the WUE of the cultivars tested ranged from 2.7 to 9.4% with overall mean of 5.4% (Table 26a). Cultivar Shasho had utilized the moisture most efficiently (9.4%) followed by

ICC4858 (8.2%) and Maryie (7.2%) for GYLD. In contrast, cultivar Dz-10-4 and Dz-10-11 were the least efficient in utilizing the available moisture (2.7% each) for GYLD. Water-use efficiency of the cultivars for BMY had similar trend with that of GYLD; it ranged from 5.7 to 19.4% with overall mean of 12.2%. Cultivars Shasho, Marye, ICC 4958, Ejere were the most efficient (15.5% to 19.4) while cultivars like Dz-10-11, Dz-10-4, Fetenech and Akuri were low in WUE (5.5 to 9.5%).

Under WS condition, WUE of the cultivars for GYLD ranged from 1.9 to 9.4 with overall mean of 4.4%. Genotype Arerti was the most efficient user of the available moisture (9.4%) followed by ICC 4958 (8.3%) while cultivars Dz-10-11, Akuri, Akaki, and Acos Dubie had low WUE ranging from 1.9 to 3.0%. For BMY, the WUE ranged from 4.5 to 19.6% with the overall mean of 10.1%. The most efficient moisture user was the cultivar Arerti (19.6%), followed by Natoli (16.1%) whereas low water use efficiensts were Akaki (4.5%), Akuri (5.3%), and Dz-10-4 (5.4%).

This result clearly revealed that a cultivar that is water use efficient under FW condition may not necessary efficient under WS and *vice-versa*. For example, cultivar Shasho was the most efficient under FW condition while Arerti was the most use efficient under WS condition; similarly, cultivars least efficient under FW condition may not necessarily be least efficient under WS condition. Cultivar ICC 4958 was constantly moisture use efficient under both stress conditions while cultivar Dz-10-11 was the least moisture use efficient.

Table 25a. Mean performance of agro-morphological traits of 20 chickpea cultivars tested in greenhouse at Holetta under FW condition in 2014

	Trait										
Genotype	EV	DTF	DTM	DGF	PHT	PPP	SPP	HSW	BMYLD	HI	GYLD
AcosDubi	1.2i	57l	167bc	110ab	90abcde	9.9h	1.0de	48.7a	35.35cdef	33.0de	11.02fgh
Akaki	3.6cd	70e	158c	83cde	88a	19.0b	1.2abc	24.5ij	37.06cdef	50.0ab	18.27cd
Akuri	3.4ed	64i	166bc	103abcd	86a	10.9gh	1.1ed	32.1def	29.52fg	45.8abc	13.85efg
Arerti	3.5cd	75abc	163bc	77e	81a	15.8cde	1.3a	30.8efg	31.97defg	48.6ab	15.46de
Chefe	2.8fg	61j	175ab	110a	81ab	17.3bcd	1.2abc	35.7bcd	43.30bcde	31.2e	14.74def
Dubie	4.0b	64i	166bc	92abcde	78ab	15.7cde	1.1de	25.3hij	31.73defg	39.1abcde	13.23efg
Dz-10-4	4.5a	70ef	171abc	93abcde	78ab	13.8efg	1.3a	16.3k	19.82gh	38.5bcde	7.06i
Dz-1-11	4.5a	67g	163bc	81de	75ab	14.8def	1.1bcde	16.9k	16.77h	39.8abcde	6.65i
Ejere	2.6g	58kl	174abc	110a	75ab	15.4cde	1.0de	39.1b	47.66abc	48.3ab	22.08b
Fetench	3.4de	73cd	169abc	86bcde	75ab	15.6cde	1.1bcde	24.0j	29.40fg	29.5e	10.68gh
Habru	2.8fg	65hi	169abc	99abcde	75ab	14.4def	1.1bcde	33.2de	31.24efg	30.2e	9.23hi
ICC 4958	2.7g	59k	173abc	106ab	74ab	12.8efg	1.0de	38.1bc	46.88abc	45.0abcd	20.65bc
Kasech	2.6g	66gh	169abc	94abcde	74ab	12.1fgh	1.0bcd	35.0cd	32.92def	40.2abcde	13.95efg
Kobo	2.6g	63i	166bc	96abcde	74ab	14.4def	1.1bcde	30.0efg	31.70defg	39.7abcde	13.80efg
Marye	3.8bc	73d	167bc	90abcde	74ab	18.2bc	1.1bcde	28.5fg	54.03ab	50.9a	26.62a
Minjar	3.1ef	69f	173abc	93abcde	73ab	13.8efg	1.2bcd	24.1j	35.35cdef	46.6ab	16.39de
Natoli	3.8bcd	75abc	176ab	102abcd	72ab	19.2b	1.1bcde	35.1cd	44.55abcd	44.7abcd	22.425b
Shaso	3.4de	76a	176ab	97abcde	70ab	22.0a	1.0e	33.8ghi	56.19a	47.8ab	28.45a
Worku	3.8bc	74bcd	185a	106abc	65b	12.0fgh	1.0ed	28.0ghi	38.85cdef	34.3cde	14.24efg
Yelibe	2.2h	66gh	171abc	101abcd	62b	15.2de	1.2bcde	32.0def	43.59bcde	45.8abc	19.89bc
Range	1.2-4.5	57-76	158-185	77-110	62-90	9.9-22.0	1.0-1.3	16.3-48.7	16.77-56.19	29.5-50.9	6.65-28.45
G. Mean	3.2	67.3	167.8	96.5	76.0	15.1	1.12	30.6	36.89	41.4	15.93
CV (%)	9.7	3.7	7.1	17.6	19.1	14.9	9.3	10.1	25.7	21.4	18.1
R^2	0.91	0.94	0.33	0.38	0.41	0.80	0.63	0.90	0.74	0.63	0.91
MSE	0.31	2.47	12.05	16.95	14.51	2.247	0.104	3.078	9.493	8.864	2.876

Table 25b. Mean performance of agro-morphological traits of 20 chickpea cultivars tested in greenhouse at Holetta under WScondition

	Traits										
Genotype	EV	DTF	DTM	DGF	PHT	PPP	SPP	HSW	BMYLD	HI	GYLD
AcosDubi	1.3g	57i	165ab	99ab	75abc	8.7f	1.01f	51.7a	15.93hi	22.1h	3.54k
Akaki	3.7bc	69c	159b	84b	59bc	14.4bcd	1.02f	24.3f	11.99i	29.8fgh	3.73k
Akuri	3.4c	62fg	165ab	106a	55c	13.3bcde	1.07ef	26.3ef	13.85hi	31.9defgh	4.03k
Arerti	3.6bc	72a	176a	95ab	80ab	18.0a	1.08def	32.8bcd	51.41a	46.1ab	27.42a
Chefe	2.8de	61gh	167ab	98ab	60bc	13.5bcde	1.10cdef	33.5b	18.25gh	31.1efgh	6.04ijk
Dubie	3.8b	62fgh	176a	107a	70abc	15.7abc	1.00f	24.1f	27.47e	39.6abcdef	11.39efg
Dz-10-4	4.7a	71ab	168ab	92ab	78abc	15.2abc	1.28ab	14.2g	26.84e	48.2ab	13.16de
Dz-1-11	4.8a	67d	162ab	90ab	55c	15.8abc	1.08def	17.3g	14.67hi	37.2bcdefg	5.243jk
Ejere	2.5ef	57i	168ab	108a	59bc	12.7cde	1.02f	34.5b	27.27e	42.4abcd	12.40ef
Fetench	3.4c	67d	169ab	101ab	78abc	12.6cde	1.02f	26.3ef	24.87ef	26.8gh	5.64ijk
Habru	2.8de	70bc	168ab	102ab	60bc	10.2ef	1.20abc	33.5bc	21.59fg	29.8fgh	6.90hijk
ICC 4958	2.6ef	61gh	171ab	106a	74abc	12.8cde	1.06ef	35.3b	37.07c	42.6abcd	16.94c
Kasech	2.6ef	63ef	172ab	108a	68abc	10.8ef	1.18bcde	34.6b	17.83gh	34.3cdefg	6.21ijk
Kobo	2.6ef	60h	173ab	109a	71abc	12.3cde	1.10cdef	33.7bc	33.49cd	27.0gh	9.23fghi
Marye	3.8bc	69c	164ab	93ab	54c	11.7def	1.00f	25.0ef	18.03gh	44.7abc	7.97ghij
Minjar	3.0d	63ef	168ab	98ab	90ab	15.5abc	1.19abcd	23.2f	35.40dc	48.7a	16.22cd
Natoli	3.8b	72a	168ab	94ab	72abc	14.6abcd	1.29a	33.2bc	41.35b	43.9abc	21.47b
Shaso	3.4c	72a	167ab	92ab	69abc	12.8cde	1.07ef	30.9bcd	32.74cd	47.1ab	16.05cd
Worku	3.9b	70bc	171ab	106a	68abc	16.3ab	1.08def	27.2def	32.29d	34.5cdef	10.14efgh
Yelibe	2.3f	65e	169ab	103ab	63bc	14.9abcd	1.06ef	29.4cde	27.70e	41.2abcde	11.994ef
Range	1.3-4.8	57-72	159-176	84-109	54-90	8.7-18.0	1.0-1.3	14.2-51.7	12.0-51.4	22.1-48.7	3.54-27.42
G. Mean	3.23	65.4	168.2	99.5	67.9	13.6	1.09	29.6	26.50	37.4	10.79
CV (%)	8.6	4.0	6.4	14.6	25.0	18.7	7.9	11.9	13.3	22.0	27.5
R^2	0.94	0.90	0.42	0.44	0.52	0.67	0.74	0.88	0.95	0.71	0.90
MSE	0.27	2.61	10.78	14.52	16.97	2.58	0.087	3.524	3.525	8.247	2.963

Table 26a. Mean performance of physiological traits of 20 chickpea cultivarst tested in greenhouse at Holetta under FW condition in 2014

	Traits										
Genotype	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	Trans	WUEBM	WUEGY
AcosDubi	5.93a	8.38efg	14.31b	2.99bcd	4.81abcdefg	55.9abcd	0.72abcd	61.6a	787cd	13.5bcde	4.7fg
Akaki	3.42i	7.59ghi	11.01f	2.18ij	5.26abcd	65.8abcd	0.82abc	59.8ab	798abcd	11.6cde	5.2ef
Akuri	5.19bcd	7.05ij	12.24ed	2.43ghi	5.13abcde	46.5cd	0.61cd	59.8ab	816abc	9.2ef	4.5fg
Arerti	3.68hi	7.35hi	11.03f	2.64efgh	4.19g	67.5abc	0.82abc	59.5abc	767cd	10.7cdef	5.3ef
Chefe	4.25g	9.56cd	13.81bc	2.91bcde	4.56efg	51.5bcd	0.69abcd	59.3abc	785cd	14.3bcde	5.1efg
Dubie	3.88h	7.77ghi	11.65ef	2.38hi	4.84abcdefg	46.3cd	0.61cd	58.8abc	799abcd	10.0def	4.3fg
Dz-10-4	2.65k	6.03k	8.68g	1.99j	4.32fg	47.1cd	0.62cd	58.5abc	774cd	6.3f	2.7h
Dz-1-11	2.99j	6.24jk	9.23g	2.06j	4.61defg	53.0abcd	0.69abcd	58.2abc	753d	5.7f	2.7h
Ejere	5.44bc	10.05bc	15.49a	3.11bc	4.75bcdefg	51.2bcd	0.65bcd	58.0abc	809abcd	15.6abc	7.4bc
Fetench	5.13cd	8.01ghi	13.14cd	2.74def	4.74cdefg	47.6cd	0.61cd	57.9abc	778cd	9.5ef	3.6gh
Habru	4.53fg	9.22cdef	13.76bc	2.69defg	5.27abcd	44.3d	0.58d	57.5abc	848ab	10.0def	4.5fg
ICC 4958	5.34bc	8.52efg	13.87bc	3.17b	4.18g	59.2abcd	0.75abcd	57.1abc	776cd	15.7abc	8.2ab
Kasech	3.89h	7.95ghi	11.83ef	2.49fgh	4.62defg	58.3abcd	0.78abcd	57.1abc	792bcd	10.3def	4.3fg
Kobo	5.46b	7.83ghi	13.29bc	2.85cde	4.56efg	59.5abcd	0.77abcd	56.9abc	801abcd	10.2def	4.6fg
Marye	4.99ed	10.93ab	15.92a	2.82ced	5.43ab	67.3abc	0.80abc	56.8abc	806abcd	17.4ab	7.9b
Minjar	3.66hi	8.31fgh	11.97ef	2.38hi	4.95abcdef	75.0a	0.85ab	56.8abc	773cd	12.0cde	5.6def
Natoli	4.89ed	11.12a	16.01a	3.54a	4.32fg	61.9abcd	0.81abc	56.5abc	813abcd	15.1abcd	6.8bcd
Shaso	5.19bcd	10.15bc	15.34a	2.90bcde	5.44a	71.9ab	0.88a	54.7bc	854a	19.4a	9.4a
Worku	4.71ef	9.01def	13.71bc	2.49fgh	5.39abc	71.6ab	0.80abc	54.7bc	808abcd	12.5bcde	5.2ef
Yelibe	4.46fg	9.33cde	13.79bc	2.68efgh	4.97abcdef	43.4d	0.57d	53.6c	793bcd	14.1bcde	6.4cde
Range	2.7-5.9	6.0-11.1	8.7-16.0	2.0-3.5	4.2-5.4	43.4-75.0	0.57-0.88	53.6-61.6	753-854	5.7-19.4	2.7-9.4
G. Mean	4.5	8.5	13.0	2.7	4.8	57.2	0.72	57.6	796.5	12.2	5.4
CV (%)	9.8	15.6	11.1	15.0	18.0	28.2	21.0	7.5	9.7	31.9	20.9
R^2	0.97	0.96	0.97	0.85	0.86	0.68	0.71	0.74	0.97	0.71	0.87
MSE	0.441	1.333	1.446	0.400	0.869	16.162	0.151	4.350	77.329	3.873	1.132

Table 26b. Mean performance of physiological traits of 20 chickpea cultivars tested in greenhouse at Holetta under WS condition in 2014

	Trait										
Genotype	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	Trans	WUEBM	WUEGY
AcosDubi	4.016a	6.41e	10.47e	2.58abc	4.29def	42.3abc	0.55bcdef	56.9ab	688cde	6.0hij	2.0j
Akaki	2.43gh	5.28ghi	7.71k	1.75f	4.59bcd	41.5abc	0.55bcdef	60.0ab	676cde	4.5j	1.9j
Akuri	3.57b	5.97ef	9.54f	2.34d	4.46cde	53.7a	0.67abcd	60.6ab	690cde	5.3ij	2.2ij
Arerti	4.17a	10.015a	14.22a	2.73a	4.78abc	54.0a	0.71ab	57.7ab	768a	19.6a	9.4a
Chefe	2.86ef	5.69fgh	8.55hi	2.40cd	3.64ghij	38.0abc	0.50cdef	61.1ab	702cd	7.5ghi	2.8hij
Dubie	2.45gh	5.95ef	8.40hij	2.05e	3.87fghi	36.8abc	0.48cdef	60.0ab	713bcd	10.3ef	3.6fgh
Dz-10-4	2.301h	5.62fgh	7.93ijk	1.74f	4.73bcd	29.6c	0.39f	60.8ab	700cd	9.6efg	4.8de
Dz-1-11	1.96i	4.91i	6.87l	1.40g	4.87abc	39.0abc	0.51cdef	62.7a	676cde	5.4ij	2.2ij
Ejere	3.06de	5.64fgh	8.71gh	2.51abcd	3.47ij	45.9abc	0.60abcd	59.0ab	694cde	10.1efg	4.6def
Fetench	3.50b	6.02ef	9.50f	2.70ab	3.56hij	49.5ab	0.65abcd	60.8ab	691cde	9.9efg	3.6fgh
Habru	3.19cd	6.07ef	9.26fg	2.31d	4.03efgh	31.0bc	0.41ef	59.6ab	694cde	8.1fgh	3.5gh
ICC 4958	4.07a	7.86c	11.93cd	2.69ab	4.28def	50.2ab	0.67abcd	59.2ab	695cde	13.9bc	8.3b
Kasech	2.82ef	5.21hi	8.04ijk	2.31d	3.74ghij	41.8abc	0.55bcdef	61.6ab	649e	8.1fgh	3.0ghij
Kobo	4.18a	9.15b	13.32b	2.65ab	4.86abc	45.5abc	0.60abcde	59.5ab	722bc	12.1cde	4.0efg
Marye	2.62fg	5.13hi	7.75jk	2.07e	3.79ghij	40.3abc	0.53bcdef	55.4b	687cde	6.7hij	3.1ghi
Minjar	3.06de	7.22d	10.27e	2.07e	5.02ab	54.2a	0.67abcd	60.1ab	690cde	13.8bc	6.7c
Natoli	3.40bc	8.18c	11.58d	2.72a	4.06efg	53.8a	0.74a	62.8a	706bcd	16.1b	7.5bc
Shaso	3.502b	8.01c	11.53d	2.54abcd	4.51cde	39.6abc	0.52bcdef	55.9b	749ab	11.0de	5.4d
Worku	3.96a	8.28c	12.24c	2.48bcd	5.24a	40.9abc	0.54bcdef	60.3ab	694cde	13.0cd	4.7de
Yelibe	2.94de	5.86efg	8.80gh	2.71ab	3.35j	36.0abc	0.47def	62.9a	667de	10.7def	4.6def
Range	2.0-4.2	4.9-10.1	6.9-14.2	1.4-2.7	3.4-5.2	29.6-54.2	0.41-0.71	55.4-62.9	649-768	4.5-19.6	1.9-9.4
G. Mean	3.2	6.6	9.8	2.3	4.3	43.2	0.57	59.8	697.6	10.1	4.4
CV (%)	11.7	12.0	9.4	13.0	15.4	32.1	24.9	7.6	9.0	20.0	16.6
R^2	0.96	0.98	0.98	0.84	0.95	0.60	0.70	0.72	0.97	0.89	0.92
MSE	0.374	0.798	0.291	0.305	0.656	13.855	0.141	4.568	62.465	2.019	0.862

4.2.5. Relative reduction of traits' mean performance due to water-stress

For agro-morphological traits, the highest overall mean performance reduction (MPR) was recorded for GYLD (28%) followed by BMY (24%), PHT (10%), HI (9%), and PPP (8%). The overall mean reductions in DTM, DTGF, SPP, and HSW were negligible (less than 5%) (Table 27a). In general, there was clear difference between the cultivars for MPRs. In DTM, cultivar Worku and Natoli scored 8 and 5% reduction, respectively, while the rest of the cultivars scored low reduction. For DTGF, cultivars Cheffe and Natoli scored 11 and 8% reduction, respectively, while most of the cultivars had negligible reductions. On the contrary, cultivars Areti, Dube, Fetenech, Kasech and Kobo scored 14 to 23% increase in DTGF. The MPR for PHT ranged from 17 to 36% for cultivars Akuri, Akaki, Dz-10-11, Marye, Habru, Ejere, and Acos Dubie. On the other hand, cultivars Minjar and Worku had 23 and 5% increase in PHT. Cultivars Shasho, Marye, Habru, Akaki, Chefe, Natoli, Fetenech, Ejere and Kobo scored 15 to 42% reduction in PPP due to water-stress while cultivars Worku, Akuri, Arerti, and Minjar increased by 13 to 32% in PPP. The reduction in SPP was 15 and 18% for cultivars Akaki and Arerti, respectively; on the contrary, SPP was increased by 11 and 20% for cultivars Habru and Natoli, respectively. The mean reductions in HSW due to water-stres for cultivars Akuri, Dz-10-4, Ejere, and Marye were ranged from 9 to 18%. On the other hand, HSW of two cultivars, Koba and Fetenech, increased by 12 and 9%, respectively. The mean performance reduction for HI ranged from 12 to 41% for cultivars, Akaki, Acos Dubie, Kobo, Akuri, Ejere, Kasech, Marye, with the highest reduction was for Akuri, followed by Acos Dubie. Of the 20 cultivars, 12 of them had no significant reduction in HI due to WS except one cultivar (Dz-10-4 which had increased HI by 25% under WS condition). The MPR in BMY for cultivars, Marye, Cheffe, Acos Dubie, Akuri, Kasech, Shasho, and Ejere ranged from 42 to 68% while for cultivars, Arerti, Dz-10-4, and Kobo, there

was increase in BMY by 61 and 35, and 6%, respectively. Except for these three cultivars and two other cultivars (Minjar and Natoli which had 0 and 7% reduction, respectively), almost all the cultivars were severely affected by WS condition. Similar trend has taken place with respect to GYLD; of 20 cultivars, Akaki, Marye, Akuri, Acos Dubie, Cheffe, Kasech, Shasho, Ejere, Yelib, Habru, Dubie, Worku, Kobo, Dz-10-11, Fetenech, and ICC 4958 had mean GYLD reduction ranging from 12 to 70% (the highest being for cultivar Akaki and the least for ICC 4958); two cultivars, Minjar and Natoli, had non significant reduction. On the contrary, Dz-10-4 and Arerti had mean GYLD increase by 58 and 57%, respectively.

For morpho-physiological traits, the high overall MPR due to WS was for LDWt (28%), ShDWt (23%), LAPP (23%), LAI (21%), SDWt (20%), WUEGY (16%), RDWt (12%), and SRR (11%) (Table 26b). There was variation among the cultivars for the MPRs of the traits. Highest reduction in LDWt was for the cultivar Marye (47%) and the least for Dz-10-4 (13%). Cultivars that had high reduction in LDWt were Ejer (44%), Dubie (37%), Dz-10-11 (34%) while those with low reduction were Worku and Minjar (16% each), and DZ-10-4 (13%). Cultivar Arerti exceptionally had increased LDWt by 13% under WS condition. The same trend held true for StDWt reduction of the cultivars. The MPR of this trait ranged from 7% for cultivar Dz-10-4 to 53% for Marye. Two cultivars, Arerti and Kobo had increased StDWt under WS condition by 37 and 17%. Reduction in ShDWt ranged from 0 for cultivar Kobo to 51% for Marye. Among cultivars with high MPR were Ejere (44%), Cheffe (38%), Yelibe (36%) and Habru (33%). Among cultivars with low MPR were cultivars Worku, Minjar, IC4958, and Dz-10-4 (9 to 14%). Cultivar Areti, again, had exceptionally increased ShDWt under WS condition by 29%. The high RDWt reductions were recorded for cultivars Dz-10-11 (32%), Marye (27%), Natoli (23%),

Akaki (20%), Ejere (19%) and Cheffe (18%); intermediate reductions were recorded for cultivars ICC 4958 (15%), Acos Dubie, Habru and Dubie (14% each), Dz-10-4 and Minjar (13% each), and Shaso (12%); low reductions were recorded for cultivars Worku (0%), Yelibi and Fetenech (1% each), Arerti (3%), Akuri (4%) and Kobo and Kasech (7% each). The high SRR reductions were recorded for the cultivars Yelibe (33%), Marye (30%), Ejere (27%), Fetenech (25%), Cheffe and Dubie (20% each) and Kasech (19%); intermediate reductionS were scored for cultivars Shaso (17%), and Akaki and Akuri (13% each); low reductions were for cultivars, Worku (3%) and Natoli (6%). Increase in SRR was recorded for cultivars Arerti (14%), Kobo (7%), and Dz-10-11 (6%). High reductions for LAPP were scored for cultivars Shasho (45%) Worku (43%), Marye (40%), Akaki (37%), Dz-10-4 (37%), Habru (30%), Kasech and Minjar (28% each), Cheffe and Dz-10-11 (26% each); intermediate reductions were scored for cultivars Acos Dubie and Kobo (24% each), Arerti and Dubie (20% each); and smaller reduction were recorded for cultivars Yelibei (17%), ICC4950 (15%), Natoli (13%), and Ejere (10%). On the contrary, increased LAPP were recorded for Akuri (15%) and Fetenech (4%). The same trend held true for LAI as for LAPP. The change in RWC of the genotypes due to WS was small in general. The reduction in RWC for cultivars, Akaki, Marye, Arerti, and Acos Dubie were 0, 2, 3 and 8% respecively. For the rest of the cultivars, RWC has increased by 1% for cultivar Akuri to 17% for Yelibe. Under WS, the RWC of the cultivars seems to increas. The overall mean increase in RWC of the cultivars under WS condition was 4%. The reduction in WUE for BMY was highest for cultivar Marye (62%), followed by Akaki (61%), Acos Dubie (56%), Cheffe (47%), Shasho (43%); it was intermediate for cultivars Ejere (35%), Kasech (22%), Yelibe (24%), Habru (19%); low for cultivars ICC 4958 (11%) and Dz-10-11 (6%). On the contrary, there was increased WUE due to WS for cultivars Arerti (83%) and Dz-10-4 (51%); it was

intermediate for Kobo (19%) and Minjar (15%) and low for Natoli (7%) Worku (5%), Dubie (3%). Almost the same trend held true for WUEGY as for WUEBY except for cultivar ICC 4958 which had relative reduction of 0%, Kobo which had 13% reduction, and Worku which had relative WUE reduction of 9% under WS.

Table 27a. Relative performance reduction of nine agro-morphological traits of 20 chickpea cultivars tested under full- and water-stressed conditions in greenhouse at Holetta in 2014

Genotype	DTM	DGF	PHT	PPP	SPP	HSW	BMYLD	HI	GYLD
Acosdubi	0.01	0.10	0.17	0.13	0.02	-0.06	0.55	0.33	0.58
Akaki	-0.01	-0.01	0.33	0.25	0.15	0.01	0.68	0.41	0.70
Akuri	0.01	-0.03	0.36	-0.22	-0.01	0.18	0.53	0.30	0.61
Arerti	-0.08	-0.23	0.01	-0.14	0.18	-0.07	-0.61	0.05	-0.57
Chefe	0.05	0.11	0.26	0.22	0.09	0.00	0.58	0.00	0.58
Dubie	-0.06	-0.16	0.10	0.00	0.06	0.05	0.13	-0.01	0.31
Dz-10-4	0.02	0.01	0.00	-0.10	0.02	0.13	-0.35	-0.25	-0.58
Dz-1-11	0.01	-0.11	0.27	-0.07	0.03	-0.02	0.13	0.06	0.21
Ejere	0.03	0.02	0.21	0.18	0.02	0.12	0.43	0.12	0.45
Fetench	0.00	-0.17	-0.04	0.19	0.06	-0.09	0.15	0.09	0.21
Habru	0.01	-0.03	0.20	0.30	-0.11	-0.01	0.31	0.01	0.35
ICC 4958	0.01	0.00	0.00	0.00	-0.02	0.07	0.21	0.05	0.12
Kasech	-0.02	-0.15	0.08	0.10	-0.02	0.01	0.46	0.15	0.53
Kobo	-0.04	-0.14	0.04	0.15	0.01	-0.12	-0.06	0.32	0.25
Marye	0.02	-0.03	0.27	0.36	0.08	0.12	0.67	0.12	0.66
Minjar	0.03	-0.05	-0.23	-0.13	-0.02	0.04	0.00	-0.04	-0.01
Natoli	0.05	0.08	0.00	0.24	-0.20	0.05	0.07	0.02	0.04
Shaso	0.05	0.05	0.01	0.42	-0.07	0.09	0.42	0.01	0.43
Worku	0.08	0.00	-0.05	-0.36	-0.02	0.03	0.17	0.00	0.27
Yelibe	0.01	-0.02	-0.02	0.02	0.08	0.08	0.36	0.10	0.40
Mean	0.01	-0.04	0.10	0.08	0.02	0.03	0.24	0.09	0.28

Table 27b. Relative performance reduction of 10 physiological traits of 20 chickpea cultivars tested under full- and water-stressed conditions in greenhouse at Holetta in 2014

Genotype	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	WUEBM	WUEGY
Acosdubi	0.32	0.24	0.27	0.14	0.11	0.24	0.24	0.08	0.56	0.58
Akaki	0.29	0.30	0.30	0.20	0.13	0.37	0.33	0.00	0.61	0.64
Akuri	0.31	0.15	0.22	0.04	0.13	-0.15	-0.09	-0.01	0.42	0.51
Arerti	-0.13	-0.37	-0.29	-0.03	-0.14	0.20	0.13	0.03	-0.83	-0.77
Chefe	0.33	0.40	0.38	0.18	0.20	0.26	0.28	-0.03	0.47	0.45
Dubie	0.37	0.23	0.28	0.14	0.20	0.20	0.20	-0.02	-0.03	0.16
Dz-10-4	0.13	0.07	0.09	0.13	-0.09	0.37	0.37	-0.04	-0.51	-0.77
Dz-1-11	0.34	0.21	0.26	0.32	-0.06	0.26	0.26	-0.08	0.06	0.16
Ejere	0.44	0.44	0.44	0.19	0.27	0.10	0.07	-0.02	0.35	0.37
Fetench	0.32	0.25	0.28	0.01	0.25	-0.04	-0.06	-0.05	-0.04	0.01
Habru	0.30	0.34	0.33	0.14	0.24	0.30	0.30	-0.04	0.19	0.23
ICC 4958	0.24	0.08	0.14	0.15	-0.02	0.15	0.11	-0.04	0.11	0.00
Kasech	0.28	0.34	0.32	0.07	0.19	0.28	0.28	-0.08	0.22	0.32
Kobo	0.23	-0.17	0.00	0.07	-0.07	0.24	0.22	-0.05	-0.19	0.13
Marye	0.47	0.53	0.51	0.27	0.30	0.40	0.34	0.02	0.62	0.60
Minjar	0.16	0.13	0.14	0.13	-0.01	0.28	0.21	-0.06	-0.15	-0.18
Natoli	0.30	0.26	0.28	0.23	0.06	0.13	0.08	-0.11	-0.07	-0.09
Shaso	0.32	0.21	0.25	0.12	0.17	0.45	0.41	-0.02	0.43	0.43
Worku	0.16	0.08	0.11	0.00	0.03	0.43	0.33	-0.10	-0.05	0.09
Yelibe	0.34	0.37	0.36	-0.01	0.33	0.17	0.17	-0.17	0.24	0.27
Mean	0.28	0.20	0.23	0.12	0.11	0.23	0.21	-0.04	0.12	0.16

4.2.6. Stress tolerance indices of the cultivars

4.2.6.1. Analysis of variances

The individual soil ANOVA revealed highly significant differences ($P<0.01$) for GYLDs under FW and WS conditions for all stress tolerance indices (Table 28a) indicating that the tested chickpea cultivars were different for genes controlling these traits. In addition, the contribution of the genotypes to the total variation in the experimental design, as explained by R^2, was high ranging from 75 to 96%.

The combined ANOVA over the soils showed highly significant difference ($P<0.01$) between soils for GYLD under FW and WS conditions and the stress susceptibility indices: mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), and stress tolerance index (STI) indicating that the two soils were distinct. There was also significant different between the soils ($P<0.05$) for yield index (YI); but there was no significantly different between the two soils ($P>0.05$) for stress tolerance (TOL), yield stability index (YSI), and stress susceptibility index (SSI). There was highly significant difference ($P<0.01$) among cultivars and soil by cultivars interaction for all the tested traits (Table 27b), indicating that the genotypes were differing for genes controlling GYLD and STIs under the two stress conditions and also there was a differential responses of the genotype for the two soils and *vice-versa*. The effects of the factors included in the experimental design (soil, genotypes, and their interaction) together, as expressed by R^2, was high (0.81 to 0.94) indicating that these factors have explained substantial amount of the variations in the tested cultivars. The mean performances of most of the tested traits (GYLDs, MP, GMP, YI, YSI, and STI) were significantly high on Ginchi soil while TOL, HM, and SSI were similar on both soils (Table 28b).

Table 28a. Mean squares and effect size (R^2) from single location soil ANOVA of 10 traita (grain yield and stress-tolerance indices) of 20 chickpea cultivars tested on two soil types (Adadi and Ginchi) in greenhouse at Holetta in 2014

S. No	Trait[1]	Adadi			Ginchi		
		Rep	Genotype	R^2	Rep	Genotype	R^2
1	GYFW	2.191^{NS}	145.534^{**}	0.91	43.768^{**}	199.562^{**}	0.92
2	GYWS	22.649^{NS}	177.400^{**}	0.91	25.849^{*}	162.895^{**}	0.92
3	TOL	10.752^{NS}	210.953^{**}	0.87	27.805^{NS}	194.598^{**}	0.85
4	MP	9.732^{NS}	108.728^{**}	0.93	27.693^{**}	132.556^{**}	0.96
5	GMP	9.520^{NS}	106.071^{**}	0.93	23.608^{**}	130.498^{**}	0.96
6	HM	10.547^{NS}	106.402^{**}	0.93	20.313^{**}	133.977^{**}	0.96
7	YI	0.195^{NS}	1.525^{**}	0.91	0.219^{*}	1.400^{**}	0.92
8	YSI	0.074^{NS}	0.930^{**}	0.89	0.291^{NS}	0.605^{**}	0.75
9	SSI	0.709^{NS}	8.907^{**}	0.89	2.783^{NS}	5.799^{**}	0.96
10	STI	0.123^{NS}	1.025^{**}	0.91	0.277^{**}	2.064^{**}	0.96

[1]Abbriviations of the names of the traits are indicated in the text.

Table 28b. Mean square and effect size (R^2) from combined ANOVA of 10 traits (grain yields and stress-tolerance indices) of 20 chickpea cultivars tested on two soil types (Adadi and Ginchi) in greenhouse at Holetta in 2014.

S. No	Traits[1]	Mean Square				R^2
		Rep	Soil (S)	Genotype (G)	G*S	
1	GYFW	23.791^{NS}	150.056^{**}	214.875^{**}	130.221^{**}	0.91
2	GYWS	6.314^{NS}	51.627^{**}	243.252^{**}	96.997^{**}	0.90
3	TOL	5.593^{NS}	25.649^{NS}	282.888^{**}	122.663^{**}	0.85
4	MP	13.657^{*}	94.429^{**}	158.341^{**}	82.943^{**}	0.93
5	GMP	15.876^{*}	101.835^{**}	155.117^{**}	81.453^{**}	0.94
6	HM	17.747^{*}	103.786^{**}	156.953^{**}	83.427^{**}	0.94
7	YI	0.054^{NS}	0.444^{*}	2.091^{**}	0.834^{**}	0.91
8	YSI	0.036^{NS}	0.000^{NS}	1.283^{**}	0.251^{**}	0.81
9	SSI	0.0342^{NS}	0.000^{NS}	12.298^{**}	2.409^{**}	0.81
10	STI	0.193^{*}	1.310^{**}	1.953^{**}	1.135^{**}	0.94

[1]Abbriviations of the names of the traits are as indicated in the text.

4.2.6.2. Genetic variation and heritability

Phenotypic and genotypic coefficient of variation (PCV% and GCV %, respectively), broad sense heritability (H^2), genetic advance from selection (GA) and genetic advance as a percentage of the mean (GAM) for individual soils are indicated in Table 29a and for combined over the two soils on Table 29b. On both Adadi and Ginchi soils, the GYLD unde FW was significantly higher than that under WS by 46 and 49%, respectibely. The dada combined over the two soil types indicate that mean GYLD under FW condition was still significantly higher than GYLD under WS condition by 47.8% indicating that the WS has significantly reduced the GYLD of the cultivars on each soil and across the soils.

On individual soils, under FW condition, genetic variance (V(G)), genetic coeeficient of variation (GCV), genetic gain (GA) and genetic gain as a percentageof the mean (GAM) for GYLD were high on Ginchi soil than on Adadi soil while under WS condition, these parameters were high on Adadi soil than on Ginchi soil (Table 29a). Combined over the two soils, the V(G), GCV, and GAM for GYLD were high under WS condition than under FW conditions while V(GxS) was high under FW (Table 29b). The GAM for GYLD on Adadi and Ginchi soils under FW conditions were 17.3 and 20.9%, respectively, while under WS condition it was 89.7 and 62.7%, respectively. The high GAM under WS condition indicated that there is high potential of improvement for stress tolerance under WS conditions as compared to under FW conditions. In addition, this improvement also seems higher on a light *Viertisol* of Adadi soil than on heavy *Verisol* of Ginchi. Broad-sense heritabilities (H_b^2) for GYLD on Adadi and Ginchi soils under both FW and WS conditions were high (ranging from 92 to 94%). The data combined over the two soil types indicated that the mean V(G), GCV, GAM and H_b^2 were all high under WS

condition than under FW condition (Table 29b) indicating that substantial improvement in GYLD could be achieved through selection under WS conditions for this trait both for individual soils and combined over the soil.

For stress tolerance indices (TOL, MP, HM, SSI, GMP, STI, YI and YSI), the GAM ranges from 22 to 433% on Adadi soil; high values for this parameter were recorded for SSI (443%), TOL (334%), STI (120%), YI (90%), and YSI (84%) while the least was for MP (22%). Similarly, on Ginchi soil, the GAM ranged from 21 to 276%, the highest being for STI (276%) followed by TOL (240%), STI (140%) while the least was again for MP (21%). The V(G) was high on Adadi soil for TOL (65), SSI (2.79) and YI (0.48) wihile on Ginchi soil, it was high for MP (43), HM (44), GMP (43) and STI (0.67). The GCV is high on Adadi soil for almost all the traits (ranging from 52 for GMP to 224 for SSI) except it was high for STI on Ginchi soil than on Adadi soil. The GCV for the stress tolerance indices on Ginchi soils range from 50 (for GMP) to 186 (for SSI). The H_b^2 was high for all the traits, ranging from 90% (for TOL) to 94% (for MP, HM and GMP each) on Adadi soil while on Ginchi soil it ranged from 76% (for SSI and TSI each) to 97 (for MP, HM, GMP and STI each). Combined over the two soils, the V(G) ranges from 0.14 (for STI) to 28 (for TOL; the GCV was ranged from 26 (for MP) to 102 (for TOL); and the H_b^2 ranged from 42% (for STI) to 81% (for SSI and YSI each). In general, the V(G), GCV and H_b^2 for combined data were low as compared to the individual soils for majority of the stress tolerance indices except for SSI and YSI. This could be due to high soil by cultivar interaction and low V(G) for these traits (Table 28b). On the contrary, the H_b^2 for SSI and and YSI were high (81% each) as a result of relatively low soil by cultivar interaction and high V(G) for these traits.

Table 29a. Individual soil means, variance component and genetic advances from selection of 10 traits of 20 chickpea cultivars tested on two soil types (Adadi and Ginchi) under FW and WS condition in greenhouse at Holetta in 2014

Trait	Mean	V(G)	V(E)	V(P)	PCV	GCV	H^2_b	GA	GAM
Adadi soil									
GYFW	14.81	46.00	7.53	49.77	47.6	45.8	92.4	17.38	17.3
GYWS	10.13	56.14	8.90	60.59	76.8	74.0	92.7	19.22	89.7
TOL	4.68	65.20	15.35	72.88	182.3	172.4	89.5	20.35	334.5
MP	12.47	34.77	4.42	36.98	48.8	47.3	94.0	15.24	22.2
HM	10.69	34.04	4.30	36.19	56.3	54.6	94.1	15.08	41.0
SSI	0.78	2.79	0.54	3.06	223.7	213.5	91.1	4.25	443.2
GMP	11.48	33.98	4.13	36.05	52.3	50.8	94.3	15.08	31.4
STI	0.66	0.32	0.05	0.35	89.3	85.9	92.4	1.46	120.0
YI	0.94	0.48	0.08	0.52	76.9	74.0	92.6	1.78	89.8
YSI	0.75	0.29	0.06	0.32	75.9	72.4	91.1	1.37	84.2
Ginchi soil									
GYFW	17.05	63.76	8.28	67.90	48.3	46.8	93.9	20.62	20.9
GYWS	11.44	52.01	6.83	55.43	65.1	63.0	93.8	18.62	62.7
TOL	5.61	58.89	17.94	67.86	146.9	136.8	86.8	19.05	239.7
MP	14.25	43.16	3.07	44.70	46.9	46.1	96.6	17.20	20.8
HM	12.55	43.75	2.71	45.11	53.5	52.7	97.0	17.36	38.3
SSI	0.78	1.60	1.02	2.10	185.9	161.9	75.9	2.93	275.8
GMP	13.33	42.58	2.77	43.97	49.8	49.0	96.8	17.11	28.4
STI	0.87	0.67	0.05	0.70	95.7	94.1	96.7	2.15	146.5
YI	1.06	0.45	0.06	0.48	65.0	63.0	93.9	1.73	62.7
YSI	0.75	0.17	0.11	0.22	62.6	54.5	75.8	0.95	26.4

Abbriviations of the traits' names are as indicated in the text; V(G)=genetic variance; V(E)=environmental variance; V(P)=phenotypic variance; PCV and GCF=phenotypic and genotypic coefficient of variation, respectively; H^2_b=broad sense heritability (%); GA=genetic advance from selection; GAM=genetic advance as percent of the mean;

Table 29b. Combined means, variance components and genetic advances from selection of 10 traits of 20 chickpea cultivars tested under FW and WS condition in greenhouse at Holetta in 2014

	Parameters									
Trait	Mean	V(G)	V(GxS)	V(E)	P(V)	PCV	GCV	H^2	GA	GAM
GYFW	15.93	14.11	40.65	8.27	35.812	37.6	23.6	39.4	6.3	39.4
GYWS	10.78	24.75	28.65	8.78	40.542	59.0	46.1	61.1	10.4	96.1
TOL	5.15	27.51	33.58	17.07	47.148	133.4	101.9	58.4	10.7	207.5
MP	13.36	12.57	26.23	4.26	26.390	38.5	26.5	47.6	6.5	48.8
HM	11.62	12.25	26.56	3.75	26.159	44.0	30.1	46.8	6.4	54.9
SSI	0.78	1.67	0.48	0.84	2.050	183.3	165.4	81.4	3.1	397.6
GMP	12.41	12.28	25.88	3.80	25.853	41.0	28.2	47.5	6.4	51.9
STI	0.77	0.14	0.36	0.05	0.325	74.3	48.0	41.8	0.6	82.7
YI	1.00	0.21	0.25	0.08	0.349	59.0	46.2	61.1	1.0	96.2
YSI	0.75	0.17	0.05	0.09	0.214	61.8	55.8	81.4	1.0	134.1

The abbreviations of the parameters are as indicated in Table 29a while that of the traits' names are as indicated in the table; V(GxS)=genotype by soil interaction variance..

4.2.6.3. Mean performances of stress tolerance indices

The mean performances of grain yield (GYLD) and stress tolerance indices (STI) clearly indicated that the genotypes have different response to the two stress environments. On Adadi soil, cultivars Arerti and Shaso were least susceptible or the most tolerant to water-stress according to stress susceptibility index (SSI) and stress tolerance index (STI), i.e., they had low score for SSI and high score for STI (Table 30a); cultivars Dz-10-4, Dz-10-11, ICC 4958, Minjar and Natoli had low SSI but not high in STI while cultivars Kobo, Marye Worku, and Yelibe had high STI but not low in SSI indicating that the two indices (SSI and STI) not necessarily select the same cultivar as tolerant or susceptible. On the contrary, cultivars Acos Dubie, Akaki, Akuri, Chefe, Fetenech, Habru and Kasech, were the most WS susceptible as well as least tolerant on Adadi soil since they had high

(above unity) SSI and low (below unity) STI (Table 30a) whereas cultivars Marye and Ejere had high SSI and STI.

On Ginchi soil, genotypes Arerti, Minjar and Natoli were found to be low in SSI and high in STI whereas cultivars Dz-10-4, Dubie, Habru and Kasech were found to be low in SSI but not high in STI; cultivars ICC 4958, Ejere, and Shasho were high in STI but not low in SSI (Table 30b). On the contrary, cultivars Akaki, Acos Dubie, Akuri, Dz-10-11, Fetenech, Marye, Kobo and Worku were high in SSI but low in STI whereas cultivar Cheffe, Ejere, ICC4958 and Shasho, were low in SSI and STI; cultivars Dz-10-4, Habru, and Kasech were low in SSI and STI.

In combined mean over the two soil types, cultivars Arerti, ICC 4958, Minjar and Natoli were low in SSI as well as high in STI (Table 30c) indicating that these cultivars were the most suitable for WS tolerance out of tested cultivars; cultivars Dubie, Dz-10-4, Dz-10-11, and Worku were only low in both SSI and STI whereas cultivars Ejere and Shasho were high in both SSI and STI. On the contrary, cultivars Akaki, Akuri, Fetenech, and Kasech were high in SSI and low in STI indicating that these cultivars are the most unsuitable for SW condition; cultivars Cheffe, Ejere, Marye and Yelibe were high in SSI and STI while cultivars Acos Dubie, Dz-10-4, Dz-10-11, and Habru, were low in STI and SSI. This study also showed that there was genotype by soil type interaction. Genotypes selected as one of the best on Ginchi soil (for example, Minjar) was no more in the best list on Adad soil. The drought tolerant cultivar, ICC 4958, was also one of the WS tolerant in overall performances. It is generally expected that genotype high in STI would be low in SSI and *vice versa*. However, this is not always so as observed in this study. Therefore, using the two parameters in combination would be appropriate in slectionof individuals.

Table 30a. Mean performances of 20 chickpea cultivars for 10 traits (grain yields and stress tolerance indices) tested under FW and WS conditions on Adadi soil in greenhouse at Holetta in 2014.

	Trait									
Genotype	GYFW	GYWS	TOL	MP	HM	SSI	GMP	STI	YI	YSI
AcosDubi	5.50hi	2.25e	5.50hi	3.88j	3.19h	1.80abcde	3.51i	0.05e	0.21e	0.42efghi
Akaki	23.69ab	5.00de	23.69ab	14.35cde	8.24ef	2.43ab	10.87ef	0.47de	0.46de	0.21hi
Akuri	14.00ef	3.34e	14.00ef	8.67gh	5.18fgh	2.34abc	6.57ghi	0.19e	0.31e	0.24ghi
Arerti	14.25ef	31.68a	14.25ef	22.96a	19.42ab	-3.88i	21.10ab	1.76a	2.94a	2.25a
Chefe	9.98fgh	3.44e	9.98fgh	6.71hij	5.07fgh	2.02abcd	5.82hi	0.14e	0.32e	0.35fghi
Dubie	8.68ghi	7.2de	8.68ghi	7.94ghi	7.40efg	0.48efgh	7.65fgh	0.26e	0.67de	0.84bcde
Dz-10-4	6.85hi	13.86c	6.85hi	10.35fgh	9.16e	-3.22i	9.74efg	0.39de	1.28c	2.04a
Dz-1-11	6.38hi	6.83de	6.38hi	6.60hij	6.56efgh	-0.17gh	6.58ghi	0.18e	0.63de	1.05bc
Ejere	16.10de	6.95de	16.10de	11.52efg	9.69e	1.77abcde	10.57ef	0.45de	0.64de	0.43efghi
Fetench	6.23hi	2.81e	6.23hi	4.52ij	3.65gh	1.62abcde	4.05hi	0.07e	0.26e	0.48efghi
Habru	4.58i	2.11e	4.58i	3.34j	2.82h	1.63abcde	3.06i	0.04e	0.20e	0.47efghi
ICC 4958	13.22efg	13.11c	13.22efg	13.17def	13.15d	0.00fgh	13.16de	0.71cd	1.22c	1.00bcd
Kasech	18.36cde	2.52e	18.36cde	10.44fgh	4.40fgh	2.65a	6.76ghi	0.19e	0.23e	0.14i
Kobo	22.03bc	14.65c	22.03bc	18.34b	17.57bc	1.04bcdefg	17.95bc	1.27b	1.36c	0.67cdefgh
Marye	27.76a	9.42cd	27.76a	18.59b	13.98cd	2.05abcd	16.10cd	1.04bc	0.87cd	0.337fghi
Minjar	17.74cde	13.88c	17.74cde	15.81bcd	15.50cd	0.66efgh	15.65cd	0.98bc	1.29c	0.780bcdef
Natoli	16.86de	13.33c	16.86de	15.09bcde	14.67cd	0.51efgh	14.88cd	0.89bc	1.24c	0.84bcde
Shaso	22.00bc	23.79b	22.00bc	22.89a	22.10a	-0.37h	22.49a	2.01a	2.21b	1.12b
Worku	19.89bcd	13.92c	19.89bcd	16.90bcd	16.31bcd	0.95cdefgh	16.60cd	1.12bc	1.29c	0.69bcdefg
Yelibe	22.21bc	12.51c	22.21bc	17.36bc	15.83bcd	1.33abcdef	16.57cd	1.09bc	1.16c	0.57defghi
Mean	14.81	10.12	4.68	12.47	10.69	0.66	11.48	0.66	0.94	0.75
CV (%)	18.5	29.6	83.7	16.9	19.4	94.4	17.7	34.6	29.6	31.9

GYFW and GYWS= grain yields (g five plants^{-1}) under full-water and water-stressed conditions; TOL=tolerance index; MP=mean productivity; HM=harmonic mean; SSI=stress susceptibility index; GMP= geometric mean productivity; STI=stress tolerance index; YI= yield index; and YSI= yield stability index.

Table 30b. Means of 20 chickpea cultivars for 10 traits (grain yields and stress tolerance indices) tested under FW and WS conditions on Ginchi soil in greenhouse at Holetta in 2014.

	Traits									
Genotype	GYFW	GYWS	TOL	MP	HM	SSI	GMP	STI	YI	YSI
AcosDubi	16.54cd	4.84hi	11.70c	10.69fgh	7.47klm	2.20ab	8.93gh	0.32ghij	0.45hi	0.29fg
Akaki	12.84def	2.46i	10.38cde	7.65hi	4.12o	2.51a	5.60ij	0.13j	0.23i	0.19g
Akuri	13.70def	4.72hi	8.98cdef	9.21gh	7.02lmno	2.02abc	8.04hi	0.26hij	0.44hi	0.35efg
Arerti	16.66cd	23.16b	-6.49k	19.91c	19.18cd	-1.29f	19.54cd	1.52c	2.15b	1.42b
Chefe	19.51c	8.63fgh	10.87cd	14.07de	11.95ghi	1.71abcd	12.96f	0.67efg	0.80fgh	0.45defg
Dubie	17.77cd	15.58de	2.20efghij	16.67d	15.53ef	0.08cdef	16.09e	1.03de	1.44de	0.97bcde
Dz-10-4	7.26g	12.47ef	-5.21jk	9.87fgh	8.73jkl	-3.02g	9.26gh	0.34ghij	1.16ef	1.98a
Dz-1-11	6.93g	3.66hi	3.27defghi	5.30i	4.77mno	1.47abcd	5.03j	0.10j	0.34hi	0.53defg
Ejere	28.05b	17.85cd	10.21cde	22.95bc	21.45bc	1.07abcde	22.18bc	1.94b	1.66cd	0.65cdefg
Fetench	15.12cd	8.48fgh	6.64cdefg	11.80efg	10.86ghij	1.36abcd	11.32fg	0.54fghi	0.79fgh	0.56defg
Habru	13.88de	11.68ef	2.20efghij	12.78ef	12.66fgh	0.47bcdef	12.72f	0.66efgh	1.08ef	0.85bcdef
ICC 4958	28.08b	20.77bc	7.31cdefg	24.42b	23.63b	0.75abcde	24.02b	2.27b	1.93bc	0.76cdefg
Kasech	9.53efg	9.90fg	-0.37ghijk	9.72fgh	9.48ijkl	-0.18def	9.60gh	0.37ghij	0.92fg	1.06bcd
Kobo	5.56g	3.81hi	1.75fghij	4.69i	4.33no	0.99abcde	4.49j	0.08j	0.35hi	0.68cdefg
Marye	25.48b	6.51ghi	18.96b	16.00d	10.35hijk	2.31ab	12.86f	0.66efgh	0.60ghi	0.25fg
Minjar	15.04cd	18.56cd	-3.52ijk	16.80d	16.56de	-0.74ef	16.68de	1.10d	1.72cd	1.24bc
Natoli	28.00b	29.61a	-1.61hijk	28.80a	28.63a	-0.19def	28.72a	3.26a	2.75a	1.06bcd
Shaso	34.89a	8.30fgh	26.59a	21.60bc	13.41fg	2.36ab	17.02de	1.18cd	0.77fgh	0.24fg
Worku	8.60fg	6.36ghi	2.24efghij	7.48hi	7.17lmn	0.70abcde	7.32hij	0.21ij	0.59ghi	0.77cdefg
Yelibe	17.57cd	11.48ef	6.09cdefgh	14.53de	13.79efg	1.02abcde	14.15ef	0.81def	1.06ef	0.67cdefg
Mean	17.05	11.44	5.61	14.25	12.55	0.78	13.33	0.87	1.06	0.75
CV (%)	16.9	22.8	75.5	12.2	13.1	129.2	12.5	24.5	22.8	43.5

GYFW and GYWS= grain yields (g five plants^{-1}) under full-water and water-stressed conditions; TOL=tolerance index; MP=mean productivity; HM=harmonic mean; SSI=stress susceptibility index; GMP= geometric mean productivity; STI=stress tolerance index; YI= yield index; and YSI= yield stability index.

Table 30c. Combined means of 20 chickpea cultivars for 10 traits (grain yields and stress tolerance indices) tested under FW and WS conditions in greenhouse at Holetta in 2014.

	Trait									
Genotype	GYFW	GYWS	TOL	MP	HM	SSI	GMP	STI	YI	YSI
AcosDubi	11.02fgh	3.54k	7.47cdef	7.28gh	5.327i	2.00abc	6.22h	0.186i	0.329k	0.35fgh
Akaki	18.27cd	3.73k	14.53ab	11.00de	6.18hi	2.47a	8.24gh	0.297i	0.346k	0.20h
Akuri	13.85efg	4.03k	9.82bcd	8.94efg	6.099hi	2.18ab	7.30gh	0.222i	0.374k	0.30gh
Arerti	15.46de	27.42a	-11.96i	21.44a	19.296b	-2.59h	20.32ab	1.642b	2.542a	1.84a
Chefe	14.74def	6.04ijk	8.71cde	10.39def	8.507gh	1.86abcd	9.39fg	0.402ghi	0.560ijk	0.40efgh
Dubie	13.23efg	11.39efg	1.84g	12.31d	11.464e	0.28efg	11.87e	0.641fgh	1.056efg	0.91bcd
Dz-10-4	7.06i	13.16de	-6.11h	10.11def	8.945fg	-3.12h7	9.50fg	0.364hi	1.221de	2.01a
Dz-1-11	6.65i	5.243jk	1.41g	5.95h	5.664i	0.65defg	5.80h	0.138i	0.486jk	0.79bcde
Ejere	22.08b	12.40ef	9.68bcd	17.24bc	15.572cd	1.42abcde	16.37cd	1.194c	1.150ef	0.54defgh
Fetench	10.68gh	5.64ijk	5.03defg	8.16fgh	7.259ghi	1.49abcde	7.69gh	0.304i	0.523ijk	0.52defgh
Habru	9.23hi	6.90hijk	2.33fg	8.06fgh	7.739ghi	1.05bcdefg	7.89gh	0.349i	0.639hijk	0.66bcdefg
ICC 4958	20.65bc	16.94c	3.71efg	18.79b	18.388b	0.38efg	18.59bc	1.489b	1.571c	0.88bcd
Kasech	13.95efg	6.21ijk	7.74cdef	10.08def	6.942ghi	1.24bcdef	8.18gh	0.280i	0.576ijk	0.60cdefg
Kobo	13.80efg	9.23fghi	4.56defg	11.51de	10.946ef	1.01bcdefg	11.22ef	0.679efg	0.856fghi	0.67bcdefg
Marye	26.619a	7.97ghij	18.652a	17.29bc	12.162e	2.18ab	14.48d	0.849def	0.739ghij	0.30gh
Minjar	16.39de	16.22cd	0.17g	16.30bc	16.031cd	-0.04g	16.17d	1.041cd	1.504cd	1.01b
Natoli	22.425b	21.47b	0.96g	21.95a	21.654a	0.156g	21.80a	2.077a	1.991b	0.95bc
Shaso	28.45a	16.05cd	12.40bc	22.25a	17.754bc	0.99bcdefg	19.75ab	1.592b	1.488cd	0.68bcdefg
Worku	14.24efg	10.14efgh	4.10efg	12.19d	11.736e	0.82cdefg	11.96e	0.666efg	0.940efgh	0.74bcdef
Yelibe	19.89bc	11.994ef	7.89cde	15.942c	14.811d	1.17bcdefg	15.36d	0.946cde	1.112ef	0.62bcdefg
Mean	15.93	10.79	5.15	13.36	11.62	0.78	12.41	0.77	1.00	0.75
CV (%)	18.1	27.5	80.3	15.4	16.7	117.4	15.7	30.1	27.5	39.6

GYFW and GYWS= grain yields (g five plants^{-1}) under full-water and water-stressed conditions; TOL=tolerance index; MP=mean productivity; HM=harmonic mean; SSI=stress susceptibility index; GMP= geometric mean productivity; STI=stress tolerance index; YI= yield index; and YSI= yield stability index.

4.2.6.4. Correlations among grain yields and stress tolerance indices

The genotypic correlation (r_g) analysis revealed that grain yields under full-water (GYFW) and water-stress (GYWS) conditions have a weak association (r_g=0.32) showing that high yield potential under best possible conditions does not predict superior yield under stress condition and *vice-versa*. Therefore, indirect selection for stress environment based on the performances of non stress conditions would not be effective for grain yield. Grain yield under full-water condition was highly significantly (P<0.01) and positively correlated with MP, HM, GMP, and STI (r_g=0.63 to 0.75); similarly, GYWS was highly significantly (P<0.01) and positively correlated with MP, HM, GMP, STI, YI, YSI (r_g=0.75 to 1.00); it was highly significantly (P<0.01) but negatively correlated with TOL and SSI (r_g=-0.72 and -0.75, respectively). This indicates that as tolerance index (difference between grain yields under full-water and water stress condition) and SSI increase, yield under water-stress condition tend to decrease since the two traits are positively associated with each other, but they both are negatively associated with the grain yield under water-stress condition. Yield index (YI) and YSI were positively significantly correlated (r_g=1.00 and 0.75, respectively) with grain yield only under water-stress condition unlike they were not significant associated under full-water condition. These results clearly revealed that MP, HM, GMP, and STI could be considered as better predictors of the potential yield under both full-water and water-stress conditions than, TOL, SSI, YI, and YSI while YI and YSI could be considered as better predictor of grain yield under water-stressed conditions in addition to MP, HM, GMP, and STI; Tolerance index and SSI could be suitable indices to identify chickpea genotypes with low grain yield and tolerant to water-stress conditions because, under stress, yield decreases with increasing TOL and SSI.

Tolerance index (TOL) had highly significant (P<0.01) and positive genetic correlation with SSI, YI and YSI (r_g=0.95, 0.72 and 0.95, respectively), but it had no significant correlation with MP, HM, GMP, and STI. Mean productivity had highly significant (P<0.01) and positive genetic correlations with HM, GMP, STI and YI (r_g=0.86 t0 1.0), but had no significant correlation with SSI and YSI. Harmonic mean (HM) also had highly significant (P<0.01) and positive correlation with GMP, STI and YI; it had also significant positive (P<0.05) correlation with YSI (r_g=0.45), but it had no significant correlation with SSI. Stress susceptibility index (SSI) had highly significant and negative genetic correlation with YI and YSI (r_g=-0.75 and -1.00) but had no significant correlation with GMP and STI indicating that SSI and STI are genetically independent and controlled by different sets of genes. Similarly, GMP had highly significant positive genetic correlation with STI and YI (r_g=1.00 and 0.90, respectively) but had no significant correlation with YSI. Stress tolerance index had highly significant positive genetic correlation with YI (r_g=0.90) but had no significant correlation with YSI. Yield index (YI) had highly significant positive genetic correlation with YSI (r_g=0.75).

The genotypes' grain yields under both stress conditions (GYFW and GYWS) and STI were used to draw a three-dimensional graph to find drought resistant genotypes using Fernandez (1992) model. Accordingly, the genotypes were categorized into four groups based on their performances under full-water and water-stress conditions (Figure 10): genotypes Natoli, Shasho, ICC 4958, Ejere and Yelibe were categorized in group A, that had high grain yields under both water conditions (full-water and water-stress); genotypes Marye and Akaki placed in group B, having high grain yield only under full-water condition; genotypes Arerti, Minjar and Dz-10-4 put in group C that had high grain yield only under water-stressed conditions; and

genotypes Dz-10-11, Acos Dubie, Fetenech, Akuri, Habru, Chefe, Dubie, Kasech, Kobo and Worku were placed in group D having low grain yields under both full-water and water-stress conditions.

From the genetic variability and correlation studies, it could be concluded that under water-stress condition, SSI, YI and YSI could be used as indirect selection criteria for grain yield as they have high heribability and strong and positive genetic correlation with grain yield. Stress susceptibility indix (SSI) has strong and negative genetic correlation with grain yield and hence, genotype low in SSI is associated with high grain yield.

Table 31. Phenotypic (above diagonal in bold) and genetic (below diagonal) correlation coefficients among grain yields under full-water and water-stress conditions and stress tolerance indices of 20 chickpea cultivars tested in greenhouse at Holetta in 2014

Traits[1]	GYFW	GYWS	TOL	MP	HM	SSI	GMP	STI	YI	YSI
GYFW	1	**0.32****	**0.49****	**0.78****	**0.63****	**0.30****	**0.70****	**0.70****	**0.32****	**-0.30****
GYWS	0.32^{NS}	1	**-0.67****	**0.84****	**0.90****	**-0.73****	**0.88****	**0.89****	**1.00****	**0.73****
TOL	0.42^{NS}	-0.72**	1	**-0.16**^{NS}	**-0.33****	**0.90****	**-0.26****	**-0.26****	**-0.67****	**-0.90****
MP	0.75**	0.86**	-0.28^{NS}	1	**0.95****	**-0.31****	**0.98****	**0.98****	**0.84****	**0.31****
HM	0.63**	0.91**	-0.42^{NS}	0.96**	1	**-0.41****	**0.99****	**1.00****	**0.90****	**0.41****
SSI	0.31^{NS}	-0.75**	0.95**	-0.36^{NS}	-0.40^{NS}	1	**-0.37****	**-0.36****	**-0.73****	**-1.00****
GMP	0.68**	0.90**	-0.36^{NS}	0.99**	0.99**	-0.41^{NS}	1	**1.01****	**0.88****	**0.37****
STI	0.73**	0.91**	-0.34^{NS}	1.02**	1.04**	-0.41^{NS}	1.04**	1	**0.89****	**0.36****
YI	0.32^{NS}	1.00**	-0.72**	0.86**	0.91**	-0.75**	0.90**	0.91**	1	**0.73****
YSI	-0.31^{NS}	0.75**	-0.95**	0.36^{NS}	0.45*	-1.00**	0.41^{NS}	0.41^{NS}	0.75**	1

[1] The abbriviations of the traits are as indicated in the text and in Table 30 above.

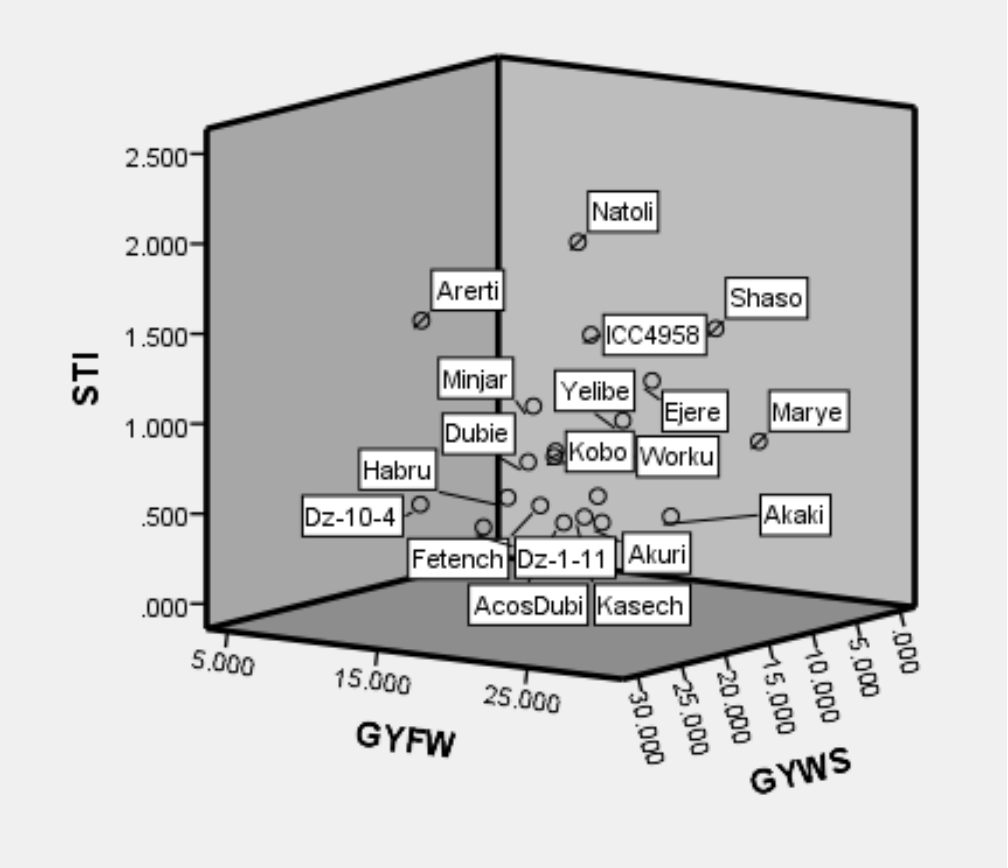


Figure 10. Grouping of the tested 20 chickpea cultivars based on their mean performances of GYLDs under FW (GYFW) and under WS (GYWS) conditions and stress tolerance index (STI) (Fernandez, 1992).

4.2.7. Association of agro-morphological and physiological traits

Associations of mean performances of the same trait under the water stress conditions are presented in Figure 11. There were no associations between mean performances of number of days to maturity and between plant height of the genotypes scored under full- and water-stress conditons. But, there were weak association for mean performances between number of pod per plant (R^2=0.04) and between biomass yield (R^2=0.04) of genotypess scored under full-water and water-stressed conditons. Similarly, there were moderate association between pefromances of seed per pod (R^2=0.12) and between performanes of grain yield (R^2=0.21). There was strong association between mean performances for number of days to grain filling period (R^2=0.26),

between performances of hundered seeds weight (R^2=0.90), and between performances of harvest index (R^2=0.39) under full-water and water-stressed conditions, respectively.

There was no associations between mean performances of shoo-root ratio of the genotypes scored under full- and water-stress conditons. However, there were weak association between mean performances of stem dry weight (R^2=0.02), between relative water content (R^2=0.03) and between water-use efficiency of grain yield (R^2=0.04) scored under full-water and water-stressed conditons. There were moderate association between pefromances of shoot dry weight (R^2=0.21), between performanes of leaf area per plat (R^2=0.16), and between performanes of leaf area index (R^2=0.18) and between performanes of water-use efficiency of grain yield (R^2=0.19). Whereas, there were strong association between mean performances of leaf dry weight (R^2=0.47) and between performances of root dry weeight (R^2=0.60) scored under full-water and water-stressed conditions, respectively.

The strong association between mean performances under two stress conditions indicates stablity of trait performances under the two stress conditions. Selection of genotypes under full-water for improving hundred seeds weight, harvest index, leaf dry weightt and root dry weight will result in the idirect improvement of these traits under water-stress conditions as well. Therefore, selection for these traits in one environment will result in selecting the same genotype in another environment and *vice versa*.

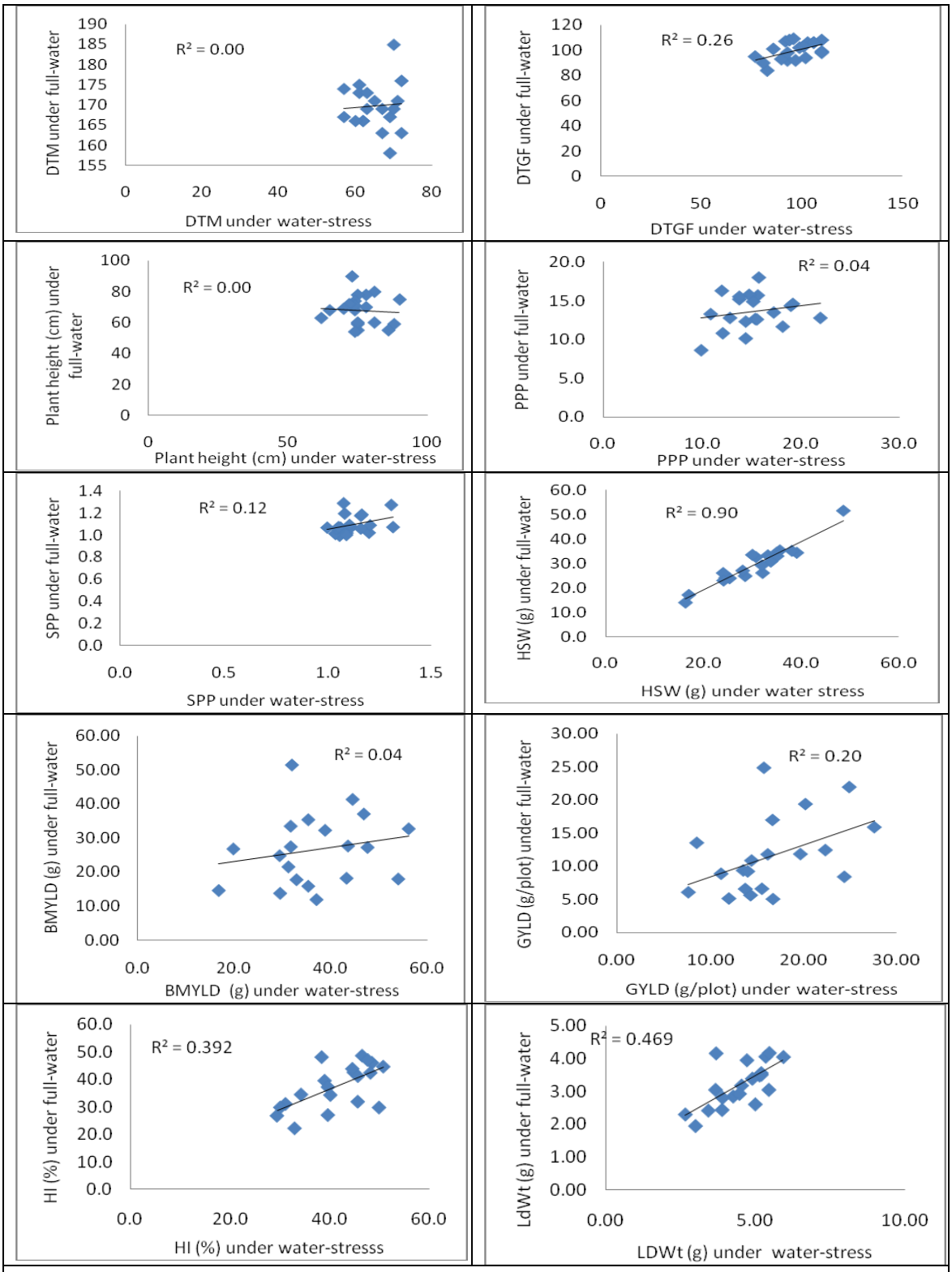


Figure 11. Interrelationship among means of traits taken under full-water and water-stress conditions on 20 released chickpea cultivars tested in greenhouse at Holetta in 2014.

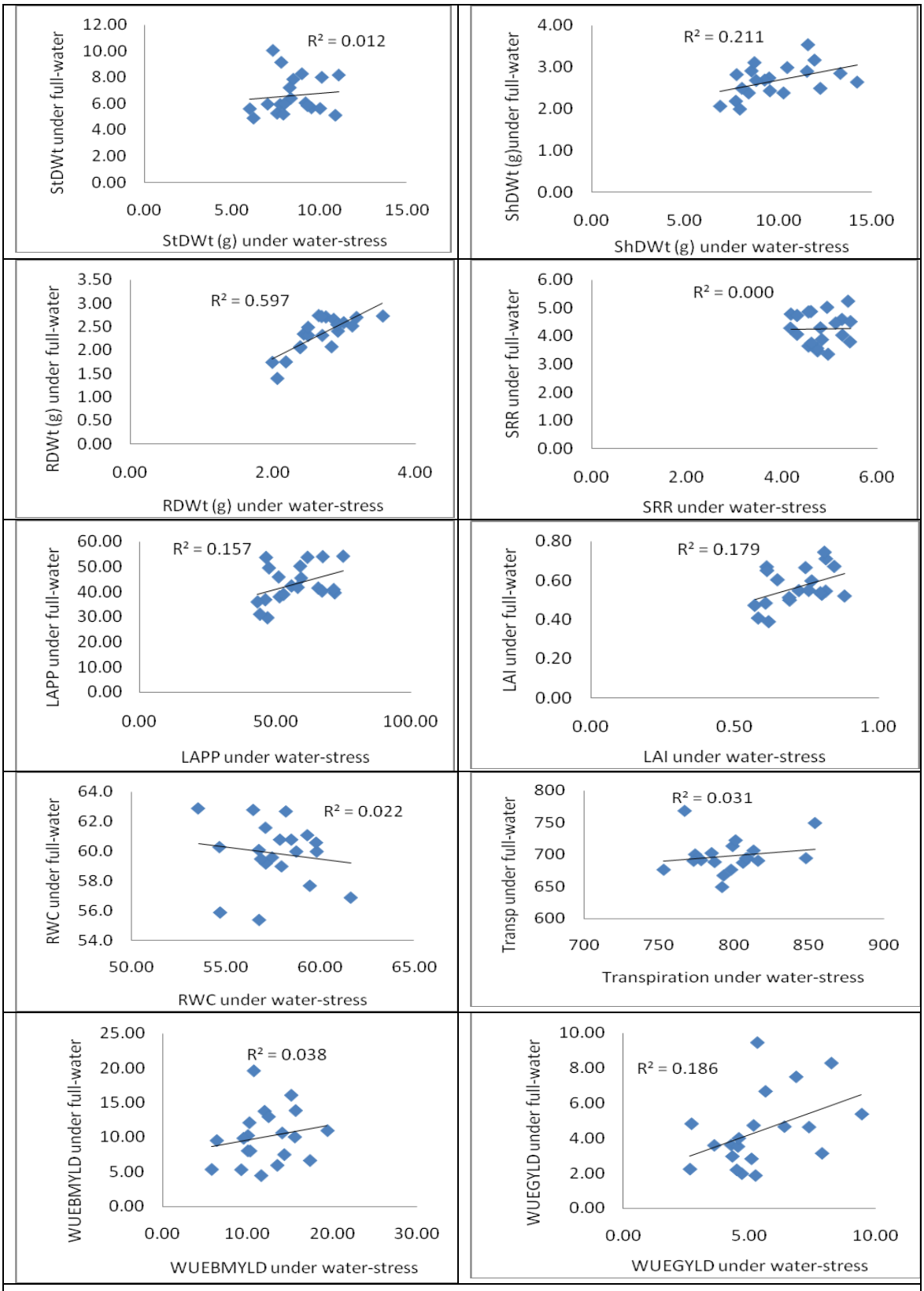


Figure 11. Continued…

4.2.8. Genetic correlations among agro-morphological and physiological traits

The genetic correlation coefficients (r_G) among the traits studied are indicated in Table 32. Grain yield had highly significant and positive genetic correlation (P<0.01) with biomass yield (BMY), stem (StDWt) and shoot (ShDWt) dry weights, WUE for both grain and biomass yields both full-water (FW) and water-stress (WS) conditions (r_G=0.73 to 0.99) and HI (r_G=0.69) under WS condition. It had moderately significan and positive genetic correlation (P<0.05) with PPP and root dry weight (RDWt) under both FW and WS condition (r_G=0.45 to 0.61), with leaf dry weight (r_G=0.48) and HI (r_G=0.61) under FW and with LAI, plant height (PHT) and number of DTM (r_G=0.45, 0.58 and 0.51, respectively under WS condition). It had moderately significant negative genetic correlation (P<0.05) with RWC (r_G=-0.49) under FW condition. It had non-significan correlation (P>0.05) with early vigor, number of DTF and number of SPP.

There were significant genetic correlations among the other traits as well. Early vigor (EV) had significant genetic correlation (P<0.01) with number of DTF (r_G=0.67 and r_G=0.63), number of DTGF (r_G=-0.51 and r_G=-0.56), hundred seeds weight (r_G=-0.85 and r_G=-0.82), leaf dry weight (r_G=-0.50 and r_G=-0.61), and root dry weight (r_G=0.61 and r_G=-0.48) under FW and WS conditions, respectively, and number of PPP (r_G=0.64) under WS. Number of DTF had significant genetic correlations with few traits like number of DTGF (r_G=-0.61 under WS and r_G=-0.56 under FW), number of PPP (r_G=0.53* under FW), hundred seeds weight (r_G=-0.47 under WS and r_G=-0.49* under FW), and leaf area per plant (r_G=0.50* under FW). Number of DTM had significant positive genetic correlation with number of DTGF (r_G=0.63 and r=0.64), plant height (r_G=0.50 and r=-0.61), StDWt (r=0.56 and r=0.52), ShDWt (r=0.55* and r=0.48), under FW and WS conditions respectively. It had also significant genetic correlation with RDWt,

RWC and WUE for grain yield (r_G=0.51, -0.59 and 0.54, respectively) under FW and biomass yield, WUE biomass yield, and DSI (r_G=0.68, 0.7 and 0.54, respectively) under WS. Number of DTGF had significant negative correlation with number of SPP (r_G=-0.50* under FW); it had significantly positive genetic correlation with number of root dry wight (rG=0.50 and 0.55 both under FW and WS) and HSW, leaf dry weight, StDW, ShDWt, and WUE for BMY (r_G=0.69, 0.47, 0.59, and 0.46, respectively) under FW.

Plant height had positive genetic correlation with RWC (r_G=0.96) under FW; it had significant positive genetic correlation with BMY, StDWt, ShDWt, WUE for both biomass and grain yields (rG=0.64, 0.54, 0.52, 0.65, 0.60 and 0.61, respectively, under WS condition). Number of PPP had negative correlation with HSW (r=-0.61* under WS); it had positive correlation with biomass yield, WUE for both biomass and grain yields und both FW and WS conditions (r_G=0.46 to 0.50); it had also positive genetic correlation with StDwt (r_G=0.48 under FW) and DSI and HI (r_G=0.62 and 0.53, respectively) under WS condition. Number of SPP had significan positive genetic correlation with DSI under both FW and WS conditions (r_G=0.53 and 0.52, respectively); it had significant negative genetic correlation with HSW, LDWt, ShDWt, RDWt, WUE for both biomass and grain yields (r_G=-0.46 to -0.77, respectively) under FW condition. Hundred sees weight had significant positive genetic correlations with LDW and RDWt (r_G=0.0.64 to 0.75) buth under FW and WS conditions; it had also strong positive genetic correlation with biomass yield, StDwt, ShDWt, WUE for both biomass and grain yields (r_G=0.55, 0.52, 0.69, 0.60 and 0.49, respectively,) under FW. Biomass yield had positive genetic correlation with WUE both for biomass and grain yields (r_G=0.51 to 0.99) both under FW and WS; it had also positive genetic correlation with LDWt, StDWt, ShDWt and RDWt (r_G=0.56 to 0.87, respectively, under FW)

and HI and DSI (r_G=0.55 and 0.64, respectively, under WS). Harves index had positive genetic correlation with WUE for grain yield under both FW and WS (r_G=0.58 and 0.66, respectively) and postitive genetic correlation with LAI (r_G=0.46) under FW and with WUE for biomass yield and DSI (r_G=0.51 and 0.64, respectively,) under WS condition.

Leaf dry weight had negative genetic correlation with DSI (r_G=-0.50 under WS); it had positivegenetic correlations with StDW, ShDWt, RDWt, and WUE for biomass yield (r_G=0.53 to 0.90 both FW and WS conditions) WUE for grain yield (r_G=0.51 under FW) and LAPP and LAI (r_G=0.53 and 0.55, respectively, under WS). Stem dry weight had negative genetic correlation with RWC (r_G=-0.46) under FW condition; it had positive genetic correlation with ShDWt, RDWt, LDWt, WUE for both biomass and GYLD (r_G=0.77 to 0.98 under both FW and WS conditions), SRR, LAPP, LAI and DSI (r_G=0.49, 0.49, 0.53 and 0.49, respectively, under WS condition). Shoot dry weight had positive genetic correlation with RDWt and WUE for both BMY and GYLD under both FW and WS conditions (r_G=0.60 to 0.87) and SRR, LAPP, LAI and DSI (r_G=0.49, 0.49, 0.53 and 0.49, respectivelt, under WS). Root dry weight had positive genetic correlation with WUE for bot BMY and GYLD (r_G=0.46* to 0.72 under both FW and WS conditions) and LAI (r_G=0.47* under water-stress). Shoot-root ratio had negative genetic correlation with DSI (r=-0.58* under water-stress). Leaf area per plant had positive genetic correlations with LAI and WUE for GYLD (r=0.46 to 0.99** under both FW and WS conditions) and WUE for BMY (r=0.47* under water-stress). Leaf area index had positive genetic correlation with WUE for GYLD under bot FW and WS conditions (r=0.52* and r=0.47, respectively) and WUE for BMY (r=0.52* under WS). Water-use efficiency for BMY had positive genetic correlations with WUE for GYLD (r=0.94** under both stresses) and DSI (r=0.71** under WS).

Table 32. Genetic correlation coefficient of 22 agro-morphological and physiological traits of 20 released chickpea cultivars tested under full-water (below the diagonal) and water-stressed (above the diagonal, in italics) conditions at multi-location field trial in 2014

	Ev	DTF	DTM	DGF	PHT	PPP	SPP	HSW	BMY	HI	GYLD
Ev	1	*0.67***	*-0.14*	*-0.50**	*-0.11*	*0.64***	*0.24*	*-0.85***	*0.05*	*0.41*	*0.08*
DTF	0.63**	1	*-0.08*	*-0.61***	*0.04*	*0.41*	*0.39*	*-0.47**	*0.28*	*0.38*	*0.32*
DTM	-0.05	0.12	1	*0.63***	*0.50**	*0.24*	*0.14*	*0.16*	*0.68***	*0.19*	*0.51**
DGF	-0.56*	-0.56*	0.64**	1	*0.05*	*-0.24*	*-0.09*	*0.35*	*0.12*	*-0.24*	*-0.01*
PHT	-0.11	-0.34	-0.61*	-0.10	1	*0.21*	*0.32*	*0.08*	*0.64***	*0.25*	*0.58**
PPP	0.36	0.53*	-0.01	-0.29	-0.18	1	*0.17*	*-0.61***	*0.49**	*0.53**	*0.45**
SPP	0.31	0.35	-0.25	-0.50*	0.06	0.02	1	*-0.21*	*0.32*	*0.37*	*0.37*
HSW	-0.82**	-0.49*	0.23	0.69**	0.17	-0.18	-0.51*	1	*0.07*	*-0.43*	*0.03*
BMY	-0.30	0.07	0.43	0.43	-0.30	0.50*	-0.43	0.55*	1	*0.55**	*0.94***
HI	0.17	0.23	-0.21	-0.24	-0.05	0.38	0.06	-0.04	0.43	1	*0.69***
GYLD	-0.18	0.12	0.36	0.31	-0.36	0.50*	-0.42	0.43	0.94**	0.61*	1
LDWt	-0.61*	-0.30	0.29	0.61*	-0.04	-0.10	-0.77**	0.75**	0.56*	-0.06	0.48*
StDWt	-0.27	0.12	0.52*	0.47*	-0.40	0.48*	-0.42	0.52*	0.87**	0.17	0.76**
ShDWt	-0.45*	-0.05	0.48*	0.59*	-0.29	0.29	-0.63**	0.69**	0.84**	0.09	0.73**
RDWt	-0.48*	-0.16	0.40	0.55*	-0.16	0.22	-0.54*	0.75**	0.68**	0.04	0.61*
SRR	0.01	0.23	0.09	0.06	-0.13	0.18	-0.27	-0.03	0.32	0.08	0.26
LAPP	0.12	0.50*	0.21	-0.19	-0.12	0.29	-0.01	0.01	0.41	0.44	0.44
LAI	0.09	0.45*	0.13	-0.19	-0.04	0.36	-0.02	0.08	0.41	0.46*	0.45
RWC	-0.09	-0.40	-0.59*	-0.11	0.96**	-0.28	0.08	0.13	-0.42	-0.18	-0.49*
WUEBM	-0.36	0.05	0.44	0.46*	-0.26	0.47*	-0.47*	0.60*	0.99**	0.39	0.92**
WUEGY	-0.23	0.11	0.37	0.33	-0.32	0.49*	-0.46*	0.49*	0.94**	0.58*	0.99**
DSI	0.39	0.34	0.08	-0.34	-0.15	0.01	0.53*	-0.34	-0.29	0.14	-0.14

Table 32. Continued….

	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	WUEBM	WUEGY	DSI
Ev	*-0.50**	*-0.08*	*-0.22*	*-0.61***	*0.39*	*-0.13*	*-0.11*	*0.21*	*0.03*	*0.06*	*0.39*
DTF	*-0.14*	*0.21*	*0.10*	*-0.16*	*0.29*	*-0.15*	*-0.10*	*-0.01*	*0.27*	*0.29*	*0.40*
DTM	*0.43*	*0.56**	*0.55**	*0.51**	*-0.04*	*0.14*	*0.16*	*0.01*	*0.71***	*0.54**	*0.54**
DGF	*0.42*	*0.14*	*0.24*	*0.50**	*-0.26*	*0.13*	*0.11*	*0.12*	*0.17*	*0.03*	*-0.13*
PHT	*0.39*	*0.54**	*0.52**	*0.28*	*0.32*	*0.36*	*0.35*	*-0.05*	*0.65***	*0.60**	*0.61***
PPP	*-0.17*	*0.31*	*0.17*	*-0.21*	*0.41*	*0.17*	*0.17*	*0.33*	*0.50**	*0.46**	*0.62***
SPP	*-0.07*	*0.19*	*0.12*	*-0.06*	*0.25*	*-0.04*	*-0.02*	*0.44*	*0.34*	*0.38*	*0.52**
HSW	*0.64***	*0.28*	*0.41*	*0.68***	*-0.28*	*0.20*	*0.23*	*-0.32*	*0.09*	*0.05*	*-0.29*
BMYLD	*0.53**	*0.86***	*0.79***	*0.53**	*0.25*	*0.44*	*0.48**	*-0.09*	*0.99***	*0.94***	*0.73***
HI	*-0.22*	*0.19*	*0.06*	*-0.12*	*0.11*	*0.09*	*0.09*	*-0.10*	*0.51**	*0.66***	*0.64***
GYLD	*0.44*	*0.75***	*0.68***	*0.48**	*0.23*	*0.44*	*0.48**	*-0.14*	*0.92***	*0.99***	*0.74***
LDWt	1	*0.79***	*0.90***	*0.82***	*0.22*	*0.53**	*0.55**	*-0.34*	*0.53**	*0.45*	*0.07*
StDWt	0.56*	1	*0.98***	*0.60**	*0.49**	*0.49**	*0.53**	*-0.24*	*0.84***	*0.74***	*0.49**
ShDWt	0.82**	0.93**	1	*0.70***	*0.42*	*0.53**	*0.56**	*-0.28*	*0.78***	*0.68***	*0.37*
RDWt	0.77**	0.77**	0.87**	1	*-0.30*	*0.43*	*0.47**	*-0.16*	*0.55**	*0.46**	*-0.01*
SRR	0.19	0.31	0.30	-0.19	1	*0.17*	*0.14*	*-0.10*	*0.22*	*0.20*	*0.37*
LAPP	0.02	0.26	0.19	0.10	0.20	1	*0.99***	*-0.03*	*0.47**	*0.48**	*0.08*
LAI	0.03	0.26	0.19	0.19	0.08	0.97**	1	*-0.01*	*0.52**	*0.52**	*0.09*
RWC	-0.10	-0.46*	-0.35	-0.17	-0.28	-0.23	-0.16	1	*-0.02*	*-0.08*	*0.05*
WUEBM	0.59*	0.86**	0.85**	0.72**	0.28	0.43	0.45	-0.37	1	*0.94***	*0.71***
WUEGY	0.51*	0.77**	0.75**	0.65**	0.24	0.46*	0.47*	-0.45	0.94**	1	*0.73***
DSI	-0.50*	-0.32	-0.44	-0.17	-0.58*	0.13	0.12	-0.05	-0.27	-0.13	1

4.2.9. Cluster Analysis of the cultivars

Cluster analysis grouped the 20 cultivars into five distinct clusters under both water-stress and full-water conditions (Figures 12a and b). Under FW condition, the first cluster consisted of 13 cultivars (Akaki, Akuri, Cheffe, Dubie, Ejere, Fetenech, Habru, ICC 4958, Kasech, Kobo, Minjar, Worku, and Yelibe) that constitute 65% of the tested cultivars (Figure 12a). This cluster

was characterized by intermediate performances of all the agro-morphological and physiological traits including the GYLD (Table 33a); the second cluster consisted of three cultivars (Natoli, Marye, and Shasho) that were characterized by high DTF and DTM, PPP, BMY, HI, GYLD, StDWt, ShDWt, RDWt, SRR, LAPP, transpiration and WUEBM and WUEGY; the third cluster had a single unique cultivar, Acos Dubie, that had high early vigor DTGF, PHT, HSW, LDWt, RWC and susceptibility to drought; but low mean DTF, PPP; the fourth cluster consists of two cultivars (Arerti ad Dz-10-4) which had intermediate trait performances for all the traits except they have low mean in SRR and high performances in drought tolerances; and the fifth cluster had also one unique cultivar, Dz-10-11, which had weakest in early vigor but early flowering and low in HSW, BMY, GYLD, LDWt, StDWt, ShDWT, LAPP, LAI, RWC, transpiration, BMY, WUEBM and WUEGY; but this cultivar had intermediate performances in the rest of the traits tested.

Under WS condition, the first cluster consisted of 11 cultivars (Akaki, Akuri, Cheffe, Dubie, Dz-10-11, Ejere, Fetenech, Habru, Kasech, Marye, and Yelibe)) that make 55% of the tested cultivars (Figure 12b); this cluster was characterized by high mean for PHT and StDWt; low mean transpiration and SRR; and intermediate mean for the rest traits (Table 33b); the second cluster had one unique cultivar, Acos Dubie, characterized by low mean HI, RWC, WUEBM and WUEGY and susceptible to drought; high in mean early vigor, DTF, DTM, PPP, SPP, HSW, BMY and GYLD; and intermediate in the rest of the traits; third cluster also had one cultivar, Dz-10-4, which was the weakest in early vigor and low in mean HSW, LDWt, StDWt, ShDW, RDWt, LAPP, LAI; but high in mean DTF, HI, RWC, DTI; the fourth consists of six cultivars (Shasho, Worku, Natoli, ICC 4958, Minjar and Kobo) which make up 30% of the tested cultivars

and characterized by intermediate mean performances of the traits tested except it was high in DTGF; fifth cluster consists of only one cultivar, Arerti, that had high mean DTF, DTM, PPP, BMY, GYLD, LDWt, StDWt, ShDWt, SRR, LAPP, LAI, transpiration, both WUEBM and WUEGY, and the most drought tolerant.

The pair wise generalized square distance among the clusters are indicatd in Table 34. Under FW condition the distance between clusters ranged from 23,050 to 206,369; the maximum distance between clusters under FW condition was that between C2 and C3 (206,369) followed by C3 and C4 (206,076) and C3 and C5 (205,595) while the least was between C1 and C4 (23,050). Under WS condition, the distance between the clusters ranged from 42,967 to 1,298,359; the maximum distance was between C1 and C5 (1,298,359) followed by C3 and C5 (546,555) and between C4 and C5 (454,455) while the least was between C2 and C5 (42,967). In general, it was clear from the data that there was high divergence between the clusters under WS condition than under FW. The maximum inter-cluster distance suggests the wider genetic divergence among the cultivars belonging to different clusters. This might be useful in selecting parental cultivars for crossing that results in maximum heterosis and wide variability of genetic architecture (Souroush *et al*., 2004) that would be good for further selection and improvement.

Under both FW and WS conditions there was no tendency of clusturing of the Kabuli types together or the Desis types together. Rather, there was a random distribution of the two types in different cluster groups.

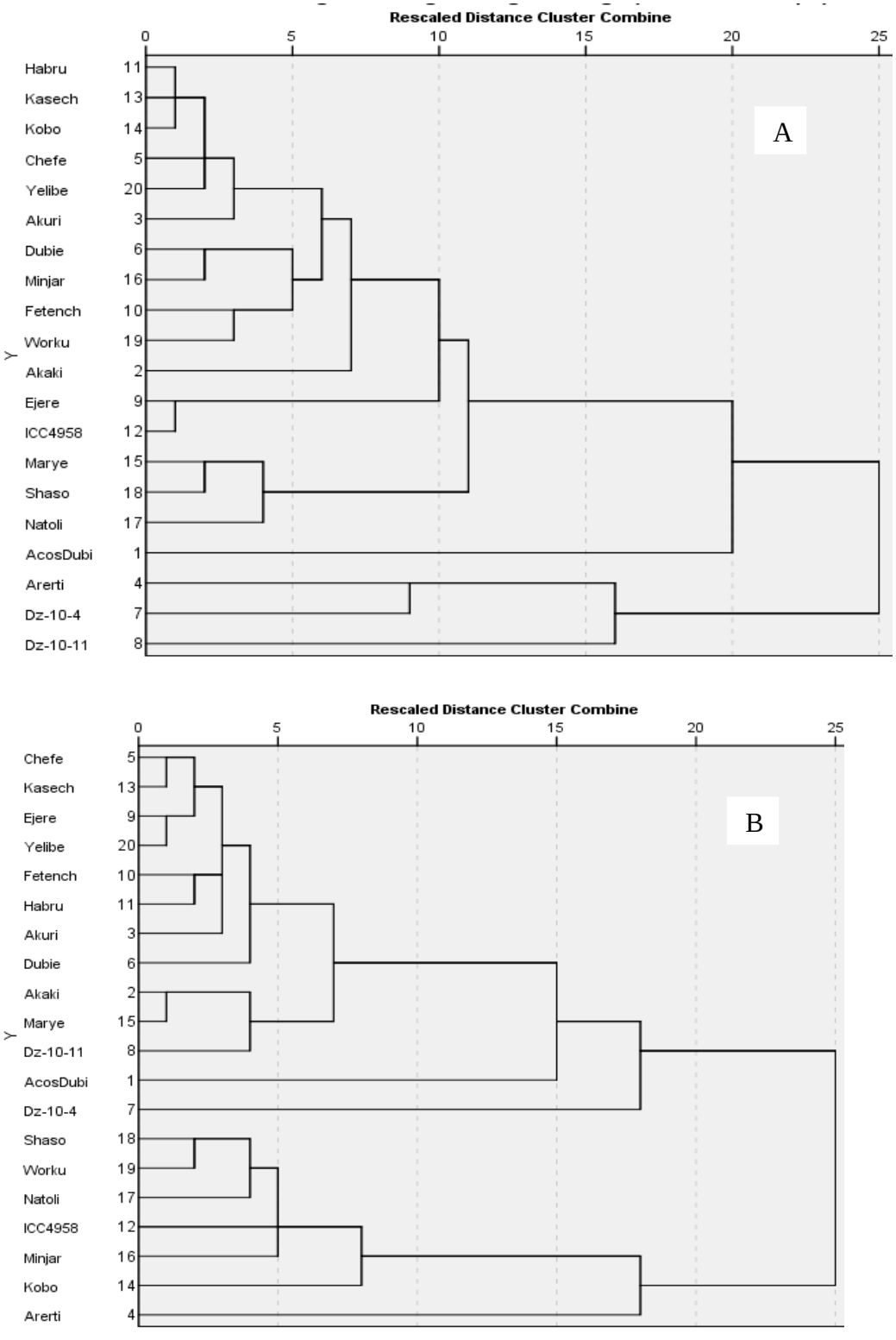


Figure 12. Dendrogram using average linkage for 20 released chickpea genotypes tested under (A) full-water and (B) under water-stressed conditions in greenhouse at Holetta in 2014.

Table 33a. Means of agro-morphological and physiological traits of five cluster of 20 chickpea cultivars tested under FW conditions in greenhouse. 014

	Agro-morphological traits										
Cluster	EV	DTF	DTM	DGF	PHT	PPP	SPP	HSW	BMY	HI	GYLD
C1	3.05	66	170	98	75	14.5	1.10	30.9	36.9	40.4	16.4
C2	3.67	75	173	96	72	19.8	1.07	32.5	51.6	47.8	24.1
C3	1.20	57	167	110	90	9.9	1.00	48.7	35.4	33.0	12.0
C4	4.00	73	167	85	80	14.8	1.30	23.6	25.9	43.6	12.2
C5	4.50	67	163	81	75	14.8	1.10	16.9	16.8	39.8	7.7

	Physiological traits											
Cluster	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	Trans	WUEBM	WUEGY	DSI
C1	4.57	8.48	13.0	2.65	4.86	55.4	0.70	57.5	798	11.92	5.30	0.84
C2	5.02	10.73	15.8	3.09	5.06	67.0	0.83	56.0	824	17.30	8.03	0.94
C3	5.93	8.38	14.3	2.99	4.81	55.9	0.72	61.6	787	13.50	4.70	0.41
C4	3.17	6.69	9.9	2.32	4.26	57.3	0.72	59.0	771	8.50	4.00	2.66
C5	2.99	6.24	9.2	2.06	4.61	53.0	0.69	58.2	753	5.70	2.70	1.00

Table 33b. Mean agro-morphological and physiological traits performances of five cluster of 20 chickpea cultivars tested under WS conditions in greenhouse at Holetta in 2014

	Agro-morphological traits										
Cluster	EV	DTF	DTM	DGF	PHT	PPP	SPP	HSW	BMYLD	HI	GYLD
C1	3.26	65	167	100	62	13.2	1.07	28.1	20.3	35.3	8.2
C2	1.30	57	165	99	75	8.7	1.01	51.7	15.9	22.1	5.1
C3	4.70	71	168	92	78	15.2	1.28	14.2	26.8	48.2	13.5
C4	3.22	66	170	101	74	14.1	1.13	30.6	35.4	40.6	16.1
C5	3.60	72	176	95	80	18.0	1.08	32.8	51.4	46.1	24.9

	Morpho-physiological traits											
Cluster	LDWt	StDWt	ShDWt	RDWt	SRR	LAPP	LAI	RWC	Trans	WUEBM	WUEGY	DSI
C1	2.85	5.61	8.47	2.23	3.94	41.2	0.54	60	685	7.87	3.19	0.70
C2	4.02	6.41	10.47	2.58	4.29	42.3	0.55	57	688	6.00	2.00	0.41
C3	2.30	5.62	7.93	1.74	4.73	29.6	0.39	61	700	9.60	4.80	2.56
C4	3.70	8.12	11.81	2.53	4.66	47.4	0.62	60	709	13.32	6.10	1.18
C5	4.17	10.02	14.22	2.73	4.78	54.0	0.71	58	768	19.60	9.40	2.76

Table 34. Pair wise generalized square distance among five clusters of 20 chickpea cultivars tested under FW (above the diagonal) and water-stressed condition (below the diagonal, in italics)

Cluster	C1	C2	C3	C4	C5
C1	0	40,511	137,597	23,050	79,575
C2	*299,986*	0	206,369	26,443	188,127
C3	*288,346*	*92,597*	0	206,076	205,595
C4	*396,303*	*115,381*	*221,294*	0	170,227
C5	*1,298,359*	*42,967*	*546,555*	*454,455*	0

4.2.10. Principal component analysis of the cultivars

Principal component (PC) analysis showed only the first four PCs out of 22 characters accounted for 78.8% of the total variation with PC_1, PC_2, PC_3 and PC_4 contributing 40.4, 19.6, 10.2, and 8.6%, respectively (Table 35). The first PC that accounted for 40.4% of the total variation in the tested genotype had high and almost similar positive weights (eigenvectors) for six traits: BMY (0.308), GYLD (0.289), StDWt (0.298), ShDWt (0.311), WUEBM (0.310), and WUEGY (0.294); while PC_2 that accounted for 19.6% of the total variation had high and positive weight for early vigor (0.317), DTF (0.395), PPP (0.297), and LAPP (0.294) but high negative weight for DTGF (-0.288); PC_3 that accounted for 10.2% of the total variation had high and positive weight for PHT (0.456), HI (0.305), and RWC (0.411), but hight and negative weight for DTM (-0.361); and PC4 that accounted for 8.6% of the total variation had high and positive weight for DSI (0.468) and negative for SRR (-0.577).

Under WS condition, the first four PCs accounted for about 77.7% of the total variation with PC_1, PC_2, PC_3 and PC_4 contributing 39.9, 22.1, 8.5, and 7.2%, respectively (Table 35). The

first PC had high and almost similar positive weights for five traits: GYLD and StDWt (0.303 each), ShDWt (0.290), WUEBM (0.319), and WUEGY (0.305); PC_2 had high and positive weight for early vigor (0.394), DTF (0.301), PPP (0.302), negative high weight for HSW (-0.382) and RDWt (-0.302); PC_3 had high and positive weight for DTM (0.330), DTGF (0.392) and RWC (0.479), but high negeative weight for SRR (-0.332); and PC4 had high and positive weight for LAPP (0.564) and LAI (0.548).

Though PCs do not have identifiable separate existence and are only mathematical artifact, it is sometimes possible to find out causes of the variation associated with a particular principal component by examining the coefficients of linear combination of original variables (Chahal and Gosal, 2002). The variables with eigenvector of large absolute magnitude (close to unity) reflects a strong influence while those of small magnitude (near zero) reflect little influence for a particular variable provided that the first principal component accounts for a substantial portion of the variation (Chahal and Gosal, 2002). A positive and high eigenvector for a given trait indicate that positive correlation between that trait and the given PC while high and negative eigenvector indicates negative correlation between the trait and a given PC. In this respect, the trait ShDWt had strong positive correlation with PC1 followed by WUEBM in FW condition and these two traits have contributed the most for the variability in the tested cultivars. Under WS condition WUEBM followed by WUEGY and GYLD itself have contributed the most for the variability in the tested cultivars. Under both stress levels, trait like GYLD, StDW, ShDWt, WUEBM, WUEGY have accounted for the highest proportion for the genetic variability in the stested cultivars.

Table 35. Principal component analysis of 22 traits of 20 released chickpea cultivars tested under full-water and water-stress conditions in greenhouse at Holetta in 2014.

Full-water condition					Water-stress condition				
PC	Eigenv	Diff.	Prop.	Cum.	PC	Eigenv	Diff.	Prop.	Cum.
1	9.290	4.778	0.404	0.404	1	9.178	4.097	0.399	0.399
2	4.512	2.166	0.196	0.600	2	5.081	3.117	0.221	0.620
3	2.346	0.370	0.102	0.702	3	1.964	0.315	0.085	0.705
4	1.976	0.765	0.086	0.788	4	1.649	0.376	0.072	0.777
Eigenvector									
Trait	PC1	PC2	PC3	PC4	Trait	PC1	PC2	PC3	PC4
Evigor	-0.135	0.317	-0.150	-0.095	Evigor	-0.005	0.394	-0.106	0.094
DTF	-0.002	0.395	-0.116	-0.064	DTF	0.076	0.301	-0.200	-0.115
DTM	0.174	0.021	-0.361	0.312	DTM	0.218	-0.073	0.330	-0.246
DGF	0.189	-0.288	-0.133	0.161	DGF	0.048	-0.272	0.392	-0.041
PHT	-0.117	-0.178	0.456	-0.235	PHT	0.223	0.015	0.113	0.007
PPP	0.118	0.297	0.062	-0.156	PPP	0.140	0.302	0.056	0.173
SPP	-0.205	0.152	0.161	0.229	SPP	0.079	0.180	0.269	0.022
HSW	0.217	-0.274	0.210	0.110	HSW	0.054	-0.382	-0.054	-0.119
BMY	0.308	0.082	0.089	0.000	BMY	0.321	0.047	0.077	-0.063
HI	0.088	0.258	0.305	-0.044	HI	0.148	0.266	0.102	-0.092
GYLD	0.289	0.143	0.103	0.024	GYLD	0.303	0.085	0.049	-0.038
LDWt	0.243	-0.228	0.029	-0.041	LDWt	0.220	-0.280	-0.167	0.018
StDWt	0.298	0.039	-0.060	0.010	StDWt	0.303	-0.059	-0.162	-0.020
ShDWt	0.311	-0.074	-0.029	-0.012	ShDWt	0.290	-0.135	-0.171	-0.009
RDWt	0.261	-0.115	0.105	0.232	RDWt	0.193	-0.302	0.081	-0.053
SRR	0.113	0.064	-0.196	-0.557	SRR	0.110	0.165	-0.332	0.132
LAPP	0.112	0.294	0.220	0.032	LAPP	0.185	-0.117	-0.090	0.564
LAI	0.118	0.277	0.291	0.043	LAI	0.195	-0.113	-0.080	0.548
RWC	-0.152	-0.214	0.411	-0.132	RWC	-0.053	0.115	0.479	0.354
WUEBM	0.310	0.063	0.120	0.029	WUEBM	0.319	0.036	0.126	-0.014
WUEGY	0.294	0.129	0.129	0.044	WUEGY	0.305	0.074	0.091	-0.002
DSI	-0.123	0.206	0.029	0.468	DSI	0.227	0.242	0.147	-0.168

PC=principal component; Eigenv=eigenvalue; Diff.= difference; Cum=cumulative; Prop=proportion

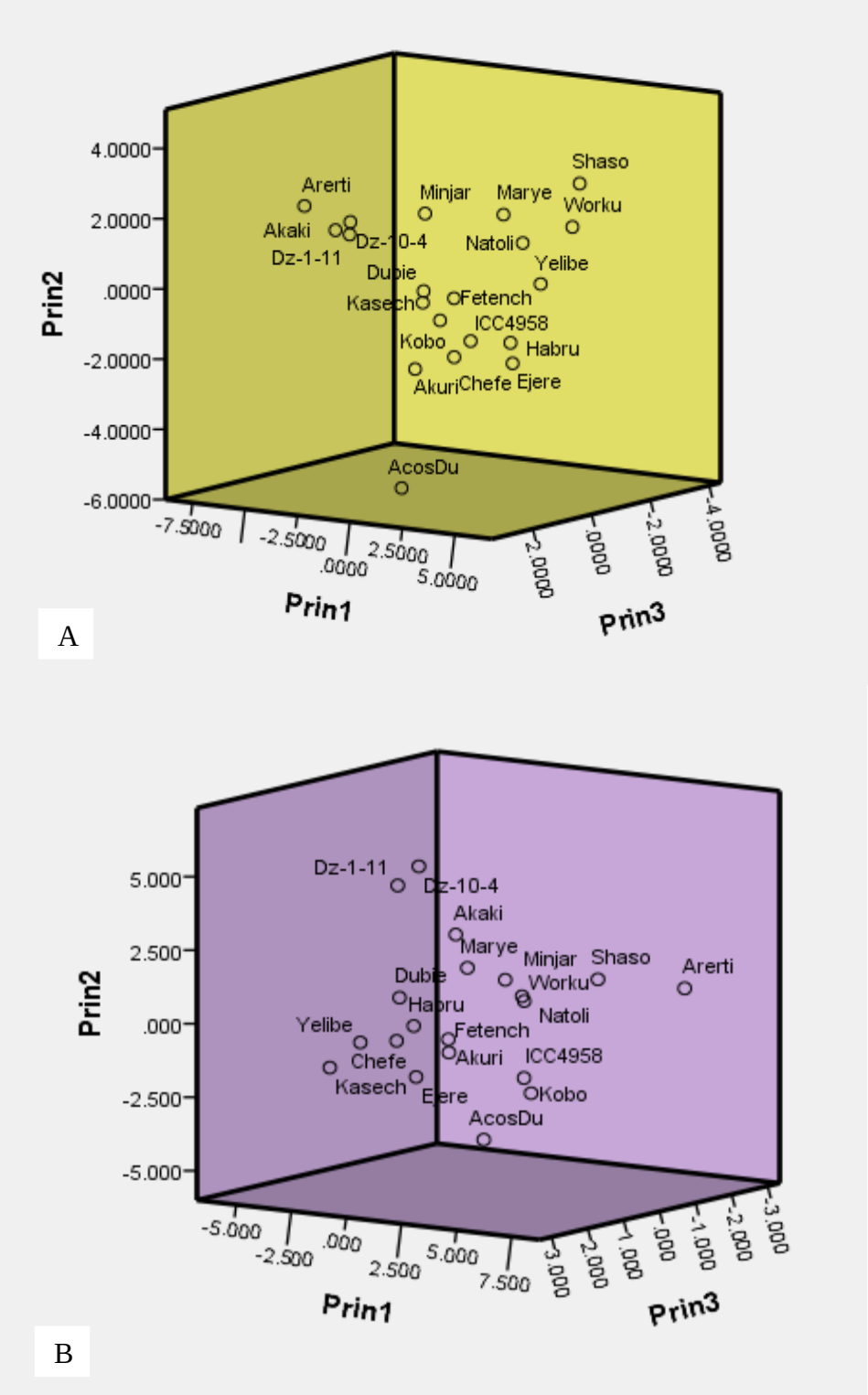


Figure 13. Three dimensional plot of the tested genotypes: (A) under FW and (B) under WS conditions of the first three rincipal components.

5. CONCLUSION AND RECOMMENDATIONS

Chickpea is one of the world's most important food legume, largely grown by small-scale farmers in the developing countries not only for its nutritional value as food and feed but also for its ecological importance in sustaining soil fertility in different farming system. In Ethiopia, it grows in wide agro-ecology with residual moisture on black *Vertisol*, serving as an insurance crop, especially when planting of major crops like *tef* fail due to delayed onset of rain. Despite its economic and ecological importance, the world average seed yield of chickpea is around 1.0 t ha-1 when the potential yield could reach 5.5 t ha-1. In many parts of the world, drought is identified as an important physical limitation to chickpea yield among others. Therefore, enhancing the drought tolerance in chickpea would help to stabilize and increase production.

This study clearly showed that markers developed elsewhere for improving difficult traits like drought tolerance could be used in our breeding program to develop varieties; The introgression of drought tolerance gene from ICC 4958 to Ejere, using MABC has resulted in generation of drought tolerant and high yielding ILs that could be further used for selection and release of variety for drought prone areas of the country;

This study has clearly indicated that WS poses tremendous relative mean performance reduction. Grain yield and PPP were the most affected traits by WS. Among the tested genotypes, Ejere was the most susceptible to WS while the selected ILs with markers for drought tolerance were the most tolerant.

It is believed that a substantial variation in drought tolerance or drought avoidance is available in chickpea gene pool globally. The deployment of such genes into the already released cultivars of Ethiopia is expected to generate drought tolerant breeding materials.

The crossing and series of marker-assisted backcrossing of the recurrent parent (Ejere) and the ICRISAT identified drought tolerant (ICC 4958) has resulted in development of useful ILs of which 48 have expressed the markers for drought tolerance while the rest did not express the markers. These ILs field tested along with the two under full-water (FW) and water-stress (WS) conditions demonstrated the existence of ILs that could be further selected, tested and released for chickpea growing environments with terminal moisture stress areas of Ethiopia. This study revealed that the expected genetic gain (GA) from selection and the gains as a percentage of the mean (GAM) were high for PPP, GYLD, PHT, HI and HSW under FW while under WS condition, they were high for GYLD, PPP, BMY. In general, higher genetic coefficient of variation and genetic gains from selection for the traits were observed under FW than under WS conditions indicating that the WS has negatively affected these parameters.

This study also demonstrated that grain yield had strong positive genetic correlations (r_G) with PPP, BMY, and HI (r_G= 0.67 to 0.99) under both FW and WS; it had strong positive correlation with DTM (r_G=0.40) and DTGF (r_G=0.53), and strong negative correlation with DTF (r_G=-0.44) only under WS. This indicates that under WS high grain yield id associated with early flowering, late maturity with longer DTGF as opposed to under FW where late flowering is positively correlated with high yield while DTM and DTGF had no association with grain yield.

Cluster analysis also showed high genetic divergence for the ILs under FW than under WS as revealed by the distances among the clusters. This high genetic divergence is believed to yield maximum genetic recombination and segregation in crossing of parents selected from these clusters. The PCA has showed that PPP, BMY, HI and GYLDs accounted for larger proportion of the variations in the ILs under both FW and WS while DTGF under WS only.

The analysis also clearly indicated relative reduction (RR) in traits' mean due to WS for almost all the traits. The highest RR was for number of pods per plant (23 to 41%) followed by grain yield (19 to 41 %) for different genotypes in general, indicating high susceptibility of these traits for moisture stress. The highest RR in these traits was for the recurrent parent while the least RR was for the ILs with molecular markers for drought tolerance (19% for GYLD and 23% for PPP). This has clearly indicated that the introgression of drought tolerance gene has resulted in the development of lines with better agronomic traits and moisture scarcity tolerant. Under FW condition, ILs 5-9, 5-10, 7-19, 18-3, 18-5, 19-4, 37-3 and 37-4 under FW and the ILs 7-14, 9-7, 10-10, 27-2, 29-6, 30-5, 34-2 and 38-2 under WS were identified as high yielding. The high mean yield of these ILs stem from increased number of days to grain filling, number of pods per plant, harvest index, and reduced plant height under both FW and WS condition and increased biomass yield under FW condition. The analysis of drought tolerance index (DSI) has revealed that the selected ILs under WS tolerated the stress by relatively low susceptibility of GYLD, BMY, SPP and DTGF.

The sequence analysis of the selected lines to understand the expression profiles during drought stress in chickpea ILs developed through marker-assisted method has generated a total of 293.3 million paired end (PE) raw reads from control and stress root tissue samples of which 272 (92.8%)

million PE reads were aligned 88.8% concordantly and 11.2 % non-concordantly. A total of 48,698 transcripts were also assembled from this study. The gene expression analysis has enabled identification of up- and down- regulated and differentially expressed genes. All the differentially expressed genes were annotated using BLASTX against Uniprot database and functionally categorized based on GO descriptions assigning 1,088 differentially expressed unique transcripts into three principal categories: molecular function (401), biological process (436) and cellular component (251). From this study a total of 9,393,545 SNPs were identified among different combinations of the stress and genotypes across the eight chickpea chromosomes. These large number of SNPs identified in this or other previous studies offer a cost-effective way to further develop functional markers for marker-assisted breeding schemes.

It is generally believed that drought resistance attributes could exist in the elite breeding materials and even in some of the already released cultivars of different crops. The first priority of a breeder should be to identify and utilize such sources of drought resistance since this is the least problematic of other sources. In this regard, 20 released Ethiopian chickpea cultivars along with ICRISAT identified drought tolerant cultivar, ICC 4958, in search of some promising cultivar under moisture stress condition. The analysis of variance clearly showed the existence of genetic difference between the cultivars. Cluster analysis has grouped these cultivars into five distinct clusters under both FW and WS conditions. The pair wise generalized square distances also revealed significant distances among cluster pairs indicating genetic divergence between cultivars in the different clusters. Principal component analysis confirmed that traits like GYLD, StDW, ShDWt, WUEBM, and WUEGY have accounted for the highest proportion for the genetic divergence in the released cultivars for different traits under both stress levels. Cultivars

Shasho, Marye, Natoli, Ejere, and ICC 4958 were identified as high yielding under FW while Arerti, Natoli, ICC 4958 and Minjar under WS. Cultivar ICC 4958 had high and stable yield performances under both FW and WS conditions. The high GYLD of the cultivars was due to high BMY, PPP, HI, TP, WUEBM and WUEGY under both FW and WS and high ShDWt, SRR and LAI under the FW only and high RDWt, LAPP, and RWC under the WS. Cultivar Shasho was the most water-use efficient for GYLD under FW while Arerti was the most water-use efficient followed by ICC 4958 under WS conditions. This result clearly revealed that cultivars that are water use efficient under FW condition may not necessary do so under WS and vice-versa.

In general, the gene introgression using marker-assisted backcrossing to develop ILs and the screening of these ILs using conventional breeding method has enabled the identification of drought tolerant lines that could further be used improving chickpea for drought tolerance. The sequence analysis has also enabled detection of transcripts that provide in-depth view of gene expression under drought condition in chickpea and enabled the identification of SNP marker that could be employed in marker-assisted breeding in developing drought tolerant chickpea varieties. The screening of the already released cultivars for optimum environment has also highlighted the existence of some cultivars that could be further exploited under moisture-stress as well.

The conventional marker-assisted selection (MAS) has been integrated into plant breeding programs with for almost three decade with ambitious expectations, to revolutionize plant breeding. However it is not to the expectation and the genetic gain from MAS was little. MAS

(including marker-assisted backcrossing, MABC) has been found to be effective only for identified genes or QTLs of large effects as it undermines many QTLs with small effect that could contribute significant portion of the phenotypic variation when combined. In this study, a "QTL-hotspot" genomic region that accounts for about 36% of the phenotypic variation was used for the introgression of drought tolerance related QTLs into the recurrent parent disregarding many QTLs with small effect that could contribute significant portion of the phenotypic variation when summed altogether. This has obviously undermined the effectiveness of MABC used in this study in identification superior lines for drought tolerant. On the contrary, genomic selection or genome-wide selection (GS) is a form of MAS that simultaneously estimates all QTL effects across the entire genome to calculate genomic-estimated breeding values (GEBVs) and selects favorable individuals (Meuwissen et al., 2001; Hefner et al., 2009; Jannink et al., 2010; Nakaya and Isobe, 2012). Genomic selection has become possible very recently with the availability of 10s of thousands of markers (e.g., SNP and DArT) and high throughput genotyping technology. Hence, future chickpea breeding effort should gear towards the use of genomic selection for improving chickpea for difficult (polygenic) traits like drought and heat.

References

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

Ahmad, F., Gaur, P.M., and Croser, J.S. (2005). Chickpea (*Cicer arietinum* L.). In: Singh, R.J., and Jauhar, P.P. (Eds.). Genetic resources, chromosome engineering, and crop improvement series, Vol 1, Grain Legumes. CRC Press, Tylor & Francis Group, Boca Raton. Scope for genetic manipulation of mineral acquisition in chickpea, Plant and Soil. 245: 123-134.

Ahmad, R., Qadir, S., Ahmad, N. and Shah, A. H. (2003). Yield potential and stability of nine wheat varieties under water stress conditions. Int. J. Agri. Biol., 5 (1): 6-9.

Ahmad, R., Stark, J.C. Tanveer, A. and Mustafa, T. (1999). Yield potential and stability indices as methods to evaluate spring wheat genotypes under drought. *Agric. Sci.,* 4: 53–9.

Ali, M.Y., Johansen, C., Krishnamurthy, L., and Hamid, A. (2005). Genotypic variation in root systems of chickpea (*Cicer arietinum* L.) across environments. J.Agronomy and Crop Science 191, 464-472.

Allard R.W. (1960). *Principles of plant breeding*. New York: Wiley.

Allard, R.W. and Bradshaw, A.D. (1994). Impication of genotype-environment interactions in applied plant breeding. Crop Sci. 4: 503-508.

Anbessa, Y. and Warkentin, T. (2005). On the improving crossing success in chickpea. Plant Breeding 124, 608—609.

Ansari, BA, Ansari, KA. Khund, A. (2004). Extent of heterosis and heritability in some quantitative characters of bread wheat. Indus. J. Pl. Sci.3:189–192.

Banziger, M., Setimela, P., Hodson, D., and Biniganavil, V. (2006). Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agric. Eater Manage. 80:212-224.

Baum, M., Grandol, S., Baker, G., Jahoor, A., Sabbagh, A., and Ceccarelli, S. (2003). QTLs for agronomic traits in the Medeterranean environment identified in recombinant inbred lines of

the cross Arta H.spontaneum 41-1. Theor. Applied Genet. 107: 1215-1225.

Bejiga, G. and van der Maesen, L.J.G. (2006). *Cicer arietinum* L. *In*: Brink, M. and Belay, G. (Eds.). Plant resources of Tropical Africa 1. Cereals and Pulses. PROTA Foundation, Wageningen, Netherlands. Pp. 42-46.

Bejiga, G., and Tessema, T. (1981). Identification of the optimum time of emasculation and pollination to increase percentage of hybrid seed set in chickpea. Eth. J. Agric. Sci. 3, 129-134.

Bell, C.J., R.A. Dixon, A.D. Farmer, R. Flores, J. Inman, R.A. Gonzales, M.J. Harrison, N.L. Paiva, A.D. Scott, J.W. Weller & G.D. (2001). The Medicago Genome Initiative: a Model legume database. Nucl Acids Res 29: 114–117.

Bello, A. B., Ceron-Dias, W. A., Nickell, C. D., Elsheriff, E. O. and Davis, L. C. (1980). Influence of cultivar, between-row spacing and plant population of fixation of soybeans, *Crop Science*, 20: 751–775.

Bernier, J., Kumar, A., Serraj, R., Spaner, D., and Atlin, G. (2008). Review: breeding upland rice for drought resistance. Sci. Food Agric., 88: 927-939.

Blum, A., Shipler, L. Golan, G. and Mayer, J. (1989). Yield stability and canopy temperature of wheat genotypes under drought stress. *Field Crops Res.*, 22: 289–96 {*Wheat, Barley and Triticale*, 7(4): 3216; 1990).

Bowman, J.C. (1972). Genotype × environment interactions. Ann. Genet. Sel. Anim. 4(1):117-123.

Bouslama, M. and Schapaught, W.T. (1984). Stress tolerance in soybean. Part 1. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci., 24: 933-937.

Boutraa, A., and Stander, F.E. (2001). Influence of water stress on grain yield and vegetative growth of two cultivars of bean (Phaseolus vulgaris L.). Journal of Agronomy and Crop Science, 187(4): page 251.

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

Boyer, J.S. (1996). Advances in drought tolerance in plants. Advances in Agronomy, 56: 187-218.

Brockwell, J., Gault, R. R., Herridge, D. F., Morthorpe, L. J. and Roughly, R. J. 1989. Studies on alternative means of legume inoculation: microbiological and agronomic appraisals of commercial procedures for inoculating soybeans with *Bradyrhizobium japonicum*, Australian

Journal of AgriculturalResearch, 40: 753–762.

Brockwell, J. and Bottomley, P. J. (1995). Recent advances in inoculant technology and prospects for the future, *Soil Biology and Biochemistry*, 27 (4-5): 683–697.

C´anovas, F., Dumas-Gaudot, E. Recorbet, G. Jorrin, J. Mock H.P. and Rossignol, M. (2004). Plant proteome analysis. Proteomics 4: 285–298.

Chahal, G.S. and Gosal, S.S. (2002). Principles and procedures of plant breeding: biometrical and conventional approach. Narosa Publishing House, New Delhi.

Chen,W.Q., Provart, N.J. Glazebrook, J. Katagiri, F. Chang, H.S. Eulgem, T. Mauch, F. Luan, S., Zou, G.Z. Whitham, S.A., Budworth, P.R., Tao, Y., Xie, Z.Y., Chen, X., Lam, S., Kreps, J.A., Harper, J.F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J.L., Wang and Zhu, X. T. (2002). Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14: 559–574.

Colebatch, G., Desbrosses, G., Ott, T., Krusell, L., Montanari, O., Kloska, S., Kopka J. and Udvardi, M.K. (2004). Global changes in transcription orchestrate metabolic differentiation during symbiotic nitrogen fixation in *Lotus japonicus*. Plant J 39: 487–512.

DeLacy, I.H. and Cooper, M. (1990). Pattern analysis for the analysis of regional variety trials. pp. 301-334. In: Kag, M.S. (Ed.) Genotype-by-environment interaction and plant breeding. Lousiana State University Agricultural Center.

Desbrosses, G.G., Kopka J. and Udvardi, M.K. (2005). *Lotus japonicas* metabolic profiling. Development of gas chromatography-mass spectrometry resources for the study of plant-microbe interactions. Plant Physiol 137: 1302–1318.

Diatchenko, L., Lau, Y.F.C., Campbell, A.P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov E.D. andSiebert, P.D. (1996). Suppression subtractive hybridization: A method for generating differentially regulated or tissuespecific cDNA probes and libraries. Proc Natl Acad Sci USA 93: 6025–6030.

Diers, B. (2004). Soybean genetic improvement through conventional and molecular based strategies. In 5th European Conference on Grain Legumes, Djion, France, 7–11 June, 2004; AEP, pp 147–148.

Dita, M.A., Rispail1, N., Prats1, E., Rubiales, D. and. Singh, K.B. (2006). Biotechnology

approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147: 1–24.

Dixon, R.A. and Sumner, L.W. (2003). Legume natural products: understanding and manipulating complex pathways for human and animal health. Plant Physiol 131: 878–885.

Eberhart, S.A. and Russell, W.A. (1966). Stability parameters for estimating comparing varieties. Crop Sci. 6: 36-40.

Eckebil, J.P., Ross, W.M., Gardner, C.O. Maranville, J.W. (1977). Heritability estimates, genetic correlations and predicted gain from S1 progeny tests in three grain sorghum random mating population. Crop Sci. 17:363–377.

EMA. (1988). National Atlas of Ethiopia. Ethiopian Mapping Authority (EMA), Addis Ababa.

FAOSTAT (2011) http://faostat.fao.org/. Accessed 11 July 2011.

Fernandez, G.C.J. (1992). Effective selection criteria for assessing plant stress tolerance. pp. 257-270. *In*: C.G. Kuo (Ed.). *Adaptation of Food Crops to Temperature and Water Stress*. Asian Vegetable Research and Development Center, Shanhua.

Field, A. (2005). *Discovering statistics using SPSS* (2nd Ed.). SAGE Publications Ltd, London.

Finlay, K.W. and Wilkinson, G.N. (1993). The analysis of adaptation in plant breeding program. Aust. J. Agric. Res. 14: 742-754.

Fischer, R.A. and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield response. Australian Journal of Agricultural Research 29: 897-912.

Fox, P.N., Crossa, J. and Romagosa, I. (1997). Multi-environment testing and genotype ×environment interaction. pp. 117-138. In: Kempton, R.A. and Fox, P.N. (Eds) Statistical methods for plant variety evaluation. Chapman and Hall, London.

Garg, R., Patel, R.K., Tyagi, A. K., and Jain, M. (2011). De Novo assembly of chickpea transcriptome using short reads for gene discovery and marker identification. DNA Research pp. 1–11.

Gaur, M.P., Tripathi, S., Gowda, C.L.L., Ranga Rao, G.V., Sharma, H.C., Pande, S., and Sharma, M. (2010). Chickpea Seed Production Manual. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India. 28 pp.

Gavuzzi, P., Rizza, F., Palumbo, M., Campalin, R.G., Ricciardi, G.L. and Borghi, B. (1997). Evaluation of field and laboratory pridictors of drought and heat tolerance in winter

cereals. *Can. J. Plant Sci*., 77 : 523-531.

Geletu Bejiga and Van der Maesen, L.J.G. (2006). Cicer arietinum L:*In*: Brink, M. and Belay, G. (Eds.), Plant Resource of Tropical africa 1. Cereals and pulses. PRPTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands / CTA, Wageningen, Netherlands. pp. 42-46.

Geletu Bejiga and Ketema Daba. (2006). Breeding chickpea for wide adaptation. *In* Ali, Kemal;Keneni, Gemechu; Ahned, Seid; Malhotra, Rajendra; Beniwal, Surendra; Makouk, Khaled;and Halila, M.H. (Eds). Food and Forage Legumes of Ethiopia: Progress and Prospects. Proceedings of the Workshop on Food and Forage Legume, 22-26 September 2003, Addis Ababa, Ethiopia. International Center for Agricultural Research in the dry Areas (ICARD), Aleppo, Syria. pp. 59-66.

Gemechu Keneni. (2012). Genetic potential and limitations in Ethiopian chickpea (*Cicer arietinum* L.) germplasm for improving attributes of symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and Adzui bean beetle (*Callosobruchus chinensis* L.) resistance. A PhD Thesis, Addis Ababa University.

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

Gygi, S.P.and Aebersold, R. (2000). Mass spectrometry and proteomics. Curr Opin Chem Biol 4: 489–494.

Gygi, S.P., Rochon, Y., Franza B.R. and Aebersold, R. (1999). Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19: 1720–1730.

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

Härdle, W. and Hlávka, Z. 2007. *Multivariate Statistics*: Exercise and Solutions. Springer Science + Business Media, LLC, New York. ISBN 978-0-387-70784-6.

Hiremath, P.J., Farmer, A., Cannon, S.B., Woodward J., Kudapa, H., Tuteja, R. (2011). Large-scale transcriptome analysis in chickpea (Cicer arietinum L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. Plant Biotechnology Journal, 9: 922–

931.

IBM Corp. Release. (2011). IBM Statistics for Window, Version 20. Armonk, NY: IBM Corp.

Illumina. (2012). Illumina TruSeq®RNA Sample Preparation v2 Guide, Low sample protocol.

Jacked, D., Peng, K., Feinstein, D., and Kilian, A., 2001. Diversity array: a solid state technology for sequence information independent genotyping. Nucleic Acid Research, Vol. 29, No. 4e25. Oxford University Press.

Johnson, G.R., Mumm, R.H. (1996). Marker assisted maize breeding. Proceedings of the 51st Corn and Sorghum Conference, Chicago, Illinois, USA. 11-12 December 1996. American Seed Trade Association, Washington, D.C.

Johnson, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimation of genetic and environmental variability in soybeans. Agronomy J. 47: 314–318.

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

Kashwagi, J., Krishnamurthy, L., Gaur, P.M., Chandra, S., and Upadhyaya, H.D. (2008). Estimation of gene effects of the drought avoidance root characteristics in chickpea (Cicer arietinum L.). Field Crops Research 105: 64-69.

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

Keneni, G. (2007). Concerns on mismatches between environments of selection and production of crop varieties in Ethiopia. *East AfricanJournal of Sciences* **1**:93-103.

Khanna-Chopra, R. (2003). Physiology of Drought and Low Nitrogen Tolerance in Crop Plants. In: Zaidi, P., Harrington, K., de Meyer, J. and Singh, N.N. (Eds.). Improving maize productivity under abiotic stresses. International Maize and Wheat Improvement Center (CIMMYT)

Kramer, P.J. (1980). Drought, stress and the origin of adaptation. pp. 7-20. In N.C. Turner and P.J. Kramer (eds.). *Adaptation of Plants to Water and High Temperature Stress*. John Wiley & Sons. New York.

Kristin, A.S., Senra, R.R., Perez, F.I., Enriquiz, B.C., Gallegos, J.A.A., Vallego, P.R., Wassimi, N. and Kelly. J.D. (1997). Improving common bean performance under drought stress. *Crop*

Sci., 37: 43-50.

Langer, I., Frey, K.J. and Bailey, T. (1979). Association among productivity, production response and stability indices in oat varieties. *Euphytica*, 28: 17–24.

Lin, C.S., Binns, M.R. and Lefkovich, L.P. (1986). Stability analysis: where do we stand? *Crop Sci*., 26: 894-900.

Lorieux, M. (2005). Comparative QTL mapping for drought tolerance. A Project proposal, IRD/CIAT. Colombia.

Maitlo, S.A., Syed, R.N., Rustamani, M.A., Khuhro R.D. and Lodhi, A.M. (2014). Comparative efficiency of different fungicides against Fusarium wilt of chickpea (Cicer arietinum L.). Pak. J. Bot., 46(6); 2305-2312.

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

McHugh, O.V., Steenhuis, T.S., Berihun, A. and Fernandes, E.C.M. (2007). Performance of in situ rainwater conservation tillage gechniques on dry spell mitigation and erosion control in the drought-prone North Wello zone of the Ethiopian Highlands. Soil and Tillage Research 97: 19-36.

Meuwissen, T.H.E., Hayes, B.J., and Goddard, M.E. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-1829.

Miklas, P.N., Kelly, J.D., Beebe, S.E. and Blair, M.W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* 147:105–131.

Miller, .PA., Williams, J.C., Robinson, H.F., & Comstock, R.E. (1958). Estimates of genotypic and environmental variances and covariance's in Upland cotton and their implications in selection. *Agronomy Journal*, 50, 126-131. http://dx.doi.org/10.2134/agronj1958.00021962005000030004x

Mirta, J. (2000). Genetics and genetic improvement of drought resistance in crop plants. *Curr. Sci*., 80: 758-762.

Muehlbauer, F.J. and Tullu, A. (1997). *Cicer arietinum* L: New crop fact sheet (http://www.hort.purdue.edu/newcrop/cropfactsheets/chickpea. html#Origin).

Mutlu, N., Miklas, P., Reiser, J. and Coyne, D. (2005). Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). Plant Breed 124: 282–287.

Njoroge, K., Wafula, B. and Ransom, J.K. (1997). Characterization of drought stress in the major maize production zones of Kenya. pp. 35-38. In G.O. Edmeades, M. Banzinger, H.R. Mickelson, and C.B. Pena-Valdivia (Eds.). *Developing drought and Low N-tolerant Maize*.

Norman. M.J.T. (1982). A role for legumes in tropical agriculture. In: Grahan P H and Harris (Eds). *Biological nitrogen fixation technology for tropical agriculture*. CIAT, Columbia. pp. 9-26.

Nyakatawa, E.Z. (1996). Rain water and soil fertility management for sustainable cropping on sandy soils in semi-arid low-veld of Zimbabwe. www.atnesa.org/.../Empowering99-CHILIMBA-MW-WWW.pdf

Omer, M.A., and Elgalani. (1997). Effect of tillage and counter diking on sorghum establishment on sandy clay loam in Sudan. Soil & Tillage Research 43:239-240.

Pande, S., Siddique, K.H.M., Kishore, G.K., Baya, B., Gaur, P.M., Gowda, C.L.L., Bretag, T. and Crouch, J.H. (2005). Ascochyta blight of chickpea: Biology, Pathogenicity, and Disease Management. Aust. J. Agric. Res., 56: 317-332.

Pinter, Jr. P.J., Zipoli, G.R. Reginato, J. Jackson, R.D. and Idso, S.B. (1990). Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. *Agric. Water Manag.*, 18: 35–48.

Pittman, K. E., and Levin, D. A. (1989). Effects of parental identities and environment on components of crossing success in phlox Drummondii. Amer. J. Bot. 76, 409—418.

Philologos (October 21, 2005). "Chickpeas — On Language". The Jewish Daily Forward. Retrieved 2009-03-28.

Richards, R.A. (1987). Physiology and the breeding of winter-grown cereals for dry areas. pp. 71-190. *In*: Srivastana, J.P., Porceddu, E., Acevedo E. and arma S. V. (eds.). *Drought*

Tolerance in Winter Cereals. Proceedings of an international workshop 27-31 Oct. 1985, Capri, Italy.

Rispail, N. (2005). Molecular and metabolic characterisation of symbiotic interactions in *Lotus japonicus* PhD Thesis Institute of Grassland and Environmental Research (IGER), University of Wales, Aberystwyth.

Rosielle, A.A. and Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science* 21: 943-946.

Rupela, O.P. and Saxena, M.C. (1987). Nodulation and nitrogen fixation in chickpea. *In*: Saxena and K.B. Singh (eds.), The Chickpea. C.A.B. International Cambrian News Ltd, Aberystwyth, UK

SAS Institute. (2002). SAS/STAT guide for personal computers, version 9.0 edition. SAS Institute Inc., Cary, NC.

Saxena, N. P., (1984). The chickpea. *In*: Goldsworthy, P. R. and Fisher, N. M. (Eds.) Physiology of Tropical Field Crops, pp. 419—452. Wiley, New York.

Saxena,N.P. and Johansen, C., (1990). Chickpea ideotypes for genetic enhancement of yield and yield stability in South Asia. In: van Rheenen, H.A., Saxena, M.C. (Eds.), Chickpea in the ineties: Proceedings of the Second International Workshop on Chickpea Improvement. 4–8 December 1989, ICRISAT Center, Patancheru 502324, A.P., India, pp. 81–85.

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

Saxena, N.P., Krishnamurthy, L. and Johansen, C. (1993). Registration of a drought resistant chickpea germplasm. Crop Sci 33:1424.

Schena, M., Shalon, D., Davis, R.W. and Brown, P.O. (1995). Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. Science 270: 467–470.

Shouse, P.S., Dasberg, J.W.A. and Stolzy, L.H. (1981). Water deficit effects on water

potential, yield and water use of cowpea. Agronomy Journals, 75: 570-572.

Sharma, K.D. and Muehlbauer, F.J. (2007). Fusarium wilt of chickpea: physiological specialization genetics of resistance and resistance gene tagging. Euphytica, 157: 1-14.

Singh, B.D. (2005). Plant breeding: Principles and methods. Kalyni Publishers, New Delhi. 1041 pp.

Singh, B.N., Mishra, R.N., Agarwal, P.K., Goswami, M., Nair, S., Sopory S.K. and Reddy, M.K. (2004). A pea chloroplast translation elongation factor that is regulated by abiotic factors. Biochem Biophys Res Commun 320: 523–530.

Singh, R.K. and Chaudhury, B.D. (1985). Biometrical Methods in Quantitative Genetic Analysis, Kalyani Publishers, New Delhi, ISBN 81-7663-0, pp 318.

Sinha, S. K. (1986). Drought resistance in crop plants: A physiological and biochemical analysis. *In*: Chopra, V.L. and Paroda, R.S. (Eds.) Approaches for Incorporating Drought and Salinity Resistance in Crop Plants. Oxford, IBH, Delhi. pp. 56-86.

Souroush, H.R., Mesbah, M., Hossainzadeh, A. and Bozorgipour, R. (2004). Genetic and phenotypic variability and cluster analysis for quantitative and qualitative traits of rice. Seed and Plant Karaj, Iran 20: 167-182.

Srivastava, J.P., Porceddu, E., Acevedo, E. and Varma, S. (1987). *Drought Tolerance in winter Cereals*. Proceedings of an International Workshop, 27-31 October 1985, Capri, Italy.

Toker, C., Canci, H. and Ceylan, F.O. (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn. *Euphytica* **151**:201–205.

Sumner, L.W., Mendes P. and Dixon, R.A. (2003). Plant metabolomics: Large-scale phytochemistry in the functional genomics era. Phytochemistry 62: 817–836.

Trapnell, C., Pachter, L., Salbzberg, S.L. (2009). TopHat: discovering junctions with RNA-Seq. Bioinformatics doi:10.1093/bioinformatics/btp120.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelly, R.R., Pimentel, H., Salzberg, S., Rinn, J.L. and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature protocols 7 (3): 562-578.

Turner, N.C., Wright, G.C. and Siddique, K.H.M. (2001). Adaptation of grain legumes (pulses) to water-limited environments. Adv Agron 71: 123–231.

Vadez, V. Rao, S. Kholova, J., Krishnamurthy, L., Kashiwagi, J. Ratnakumar, P., Sharma, K.K., Bhatnagar-Mathur, P. and Basu, P.S. (2008). Roots research for legume tolerance to drought: Quo vadis? Journal of Food Legumes 21(2); 77-85

Varshney, R.K., Hiremath, P.J., Lekha, P., Kashiwagi, J., Balaji, J., Deokar, A.A., Vadez, V., Xiao, Y., Srinivasan, R., Gaur, P.M., Siddique, K.H.M., Town, C.D. and Hoisington, D.A. (2009a). A comprehensive resource of drought- and salinity- responsive ESTs for gene discovery and marker development in chickpea (Cicer arietinum L.). BMC Genomics, 10, 523.

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

Varshney et al. (2014). *Chickpea: An ancient crop for the modern world* http://exploreit.icrisat.org/page/chickpea/685/60. ICRISAT. Downloaded 26 January 2014.

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

Velculescu, V.E., Zhang, L., Vogelstein, B. and Kinzler, K.W. (1995). Serial analysis of gene-expression. Science 270: 484–487.

Vijayalakshmi, Y., Rao, M.R. and Reddy, E.N. (1989). Genetic variability in some quantitative characters in chilli. Indian Cocoa Arec. Spices J. 1: 84-86.

Whish, J. P. M., Castor, P., Carberry, P. S. and Peake, A. S. (2007). ON-farm assessment of

constraints to chickpea (Cicer arietinum) productioon in marginal areas of northern Australia. Experimental Agriculture, 43 (04): 505-520.

Xoconostle-Cazares, B., Ramirez-Ortega, F.A., Flores-Elenes L. and Ruiz-Medrano, R. (2010). drought tolerance in crop plants Am. J. Plant Physiol., 5: 241-256.

Yang, H., Boersma, J.G., You, M.P., Buirchell, B.J. and Sweetingham, M.W. (2004). Development and implementation of a sequencespecific PCR marker linked to a gene conferring resistance to anthracnose disease in narrow-leafed lupin (*Lupinus angustifolius* L.). Mol Breed 14: 145–151.

Yang, H., Shankar, M. Buirchell, B.J. Sweetingham, M.W. Caminero C. and Smith, P.M.C. (2002). Development of molecular markers using MFLP linked to a gene conferring resistance to *Diaporthe toxica* in narrow-leafed lupin (*Lupinus angustifolius* L.). Theor Appl Genet 105:265–270.

Yu, K.F., Park, S.J. Zhang, B.L. Haffner M. and Poysa, V. (2004). An SSR marker in the nitrate reductase gene of common bean is tightly linked to a major gene conferring resistance to common bacterial blight. Euphytica 138: 89–95.

Zivy, M. and de Vienne, D. (2000). Proteomics: A link between genomics, genetics and physiology. Plant Mol Biol 44: 575–580.

Zobel, P.W. (1990). A powerful statistical model for understanding genotype-by-environent interactions. pp 126-140. *In*: Kang, M.S. (Ed.) Genotype-by-envorunment interaction and plant breeding. Luisiana State University Agricultural Center.

APPENDICES

Appendix 1a. Principal Component analysis for 11 traits of 210 genotypes tested at Alem Tena under water stress and full-water condition in 2014 cropping season.

	Under water-stressed condition				Under full-watered condition			
PC	1	2	3	4	1	2	3	4
Eigen Value	3.321	1.718	1.244	1.078	3.609	1.935	1.131	1.064
Difference	1.602	0.474	0.166	0.101	1.673	0.804	0.068	0.097
Proportion	0.302	0.156	0.113	0.098	0.328	0.176	0.103	0.097
Cumulative	0.302	0.458	0.571	0.669	0.328	0.504	0.607	0.704
Eigen vectors								
Trait	Prin1	Prin2	Prin2	Prin4	Prin1	Prin2	Prin3	Prin4
DTF	-0.180	0.471	-0.339	0.291	0.147	-0.079	0.567	-0.646
DTM	0.267	0.522	-0.229	-0.365	-0.015	0.670	0.066	-0.247
DGGF	0.396	0.146	0.040	-0.585	-0.084	0.682	-0.226	0.093
AB	0.122	0.385	0.372	0.154	-0.207	-0.170	-0.070	0.101
PPP	0.446	-0.025	-0.168	0.247	0.363	0.093	0.088	0.060
SPP	0.020	-0.095	0.373	-0.371	-0.112	0.036	0.407	0.344
PHT	0.025	0.275	0.566	0.292	0.015	0.036	0.360	0.586
HSW	0.041	-0.385	0.249	-0.063	-0.178	-0.197	-0.512	-0.159
BMY	0.346	-0.277	-0.284	0.141	0.497	-0.013	-0.131	0.077
HI	0.381	0.077	0.248	0.233	0.500	-0.016	-0.134	0.057
GYLD	0.508	-0.140	-0.028	0.232	0.507	-0.014	-0.132	0.066

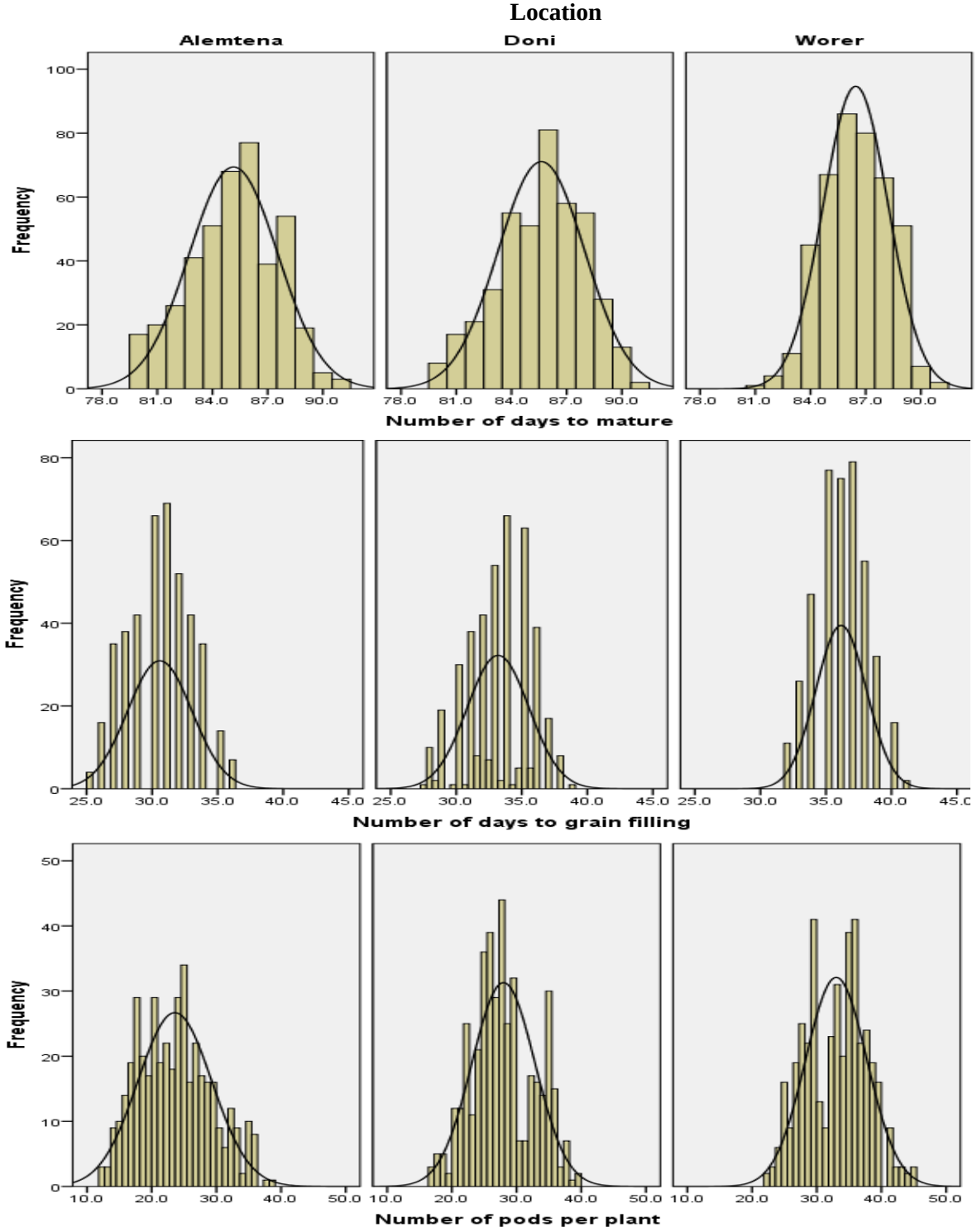
Appendix 1b. Principal Component analysis for 10 traits of 210 genotypes tested under water-stress and full-water conditions at Doni in 2014 cropping season.

	Under water-stressed condition				Under full-watered condition			
PC	1	2	3	4	1	2	3	4
Eigen Value	3.762	1.534	1.068	1.006	3.396	2.082	1.127	1.082
Difference	2.228	0.467	0.061	0.105	1.314	0.955	0.045	0.168
Proportion	0.376	0.153	0.107	0.101	0.34	0.208	0.113	0.108
Cumulative	0.376	0.53	0.636	0.737	0.34	0.548	0.661	0.769
Eigen vectors								
Trait	Prin1	Prin2	Prin3	Prin4	Prin1	Prin2	Prin3	Prin4
DTF	-0.108	0.714	0.145	-0.078	0.034	0.231	0.143	-0.780
DTM	0.322	0.450	-0.064	0.420	-0.134	0.646	-0.141	0.010
DGGF	0.391	-0.142	-0.171	0.467	-0.156	0.584	-0.210	0.342
PPP	0.418	-0.063	0.003	-0.300	0.354	0.074	0.086	-0.057
SPP	0.078	-0.145	0.755	-0.274	0.022	-0.115	0.634	0.353
PHT	0.199	0.247	0.359	0.156	-0.056	0.264	0.462	0.252
HSW	-0.023	-0.341	0.423	0.573	-0.072	-0.275	-0.527	0.253
BMY	0.379	-0.192	-0.196	-0.200	0.519	0.094	-0.082	0.078
HI	0.366	0.164	0.172	-0.087	0.525	0.085	-0.066	0.085
GYLD	0.483	-0.038	-0.014	-0.185	0.527	0.090	-0.068	0.077

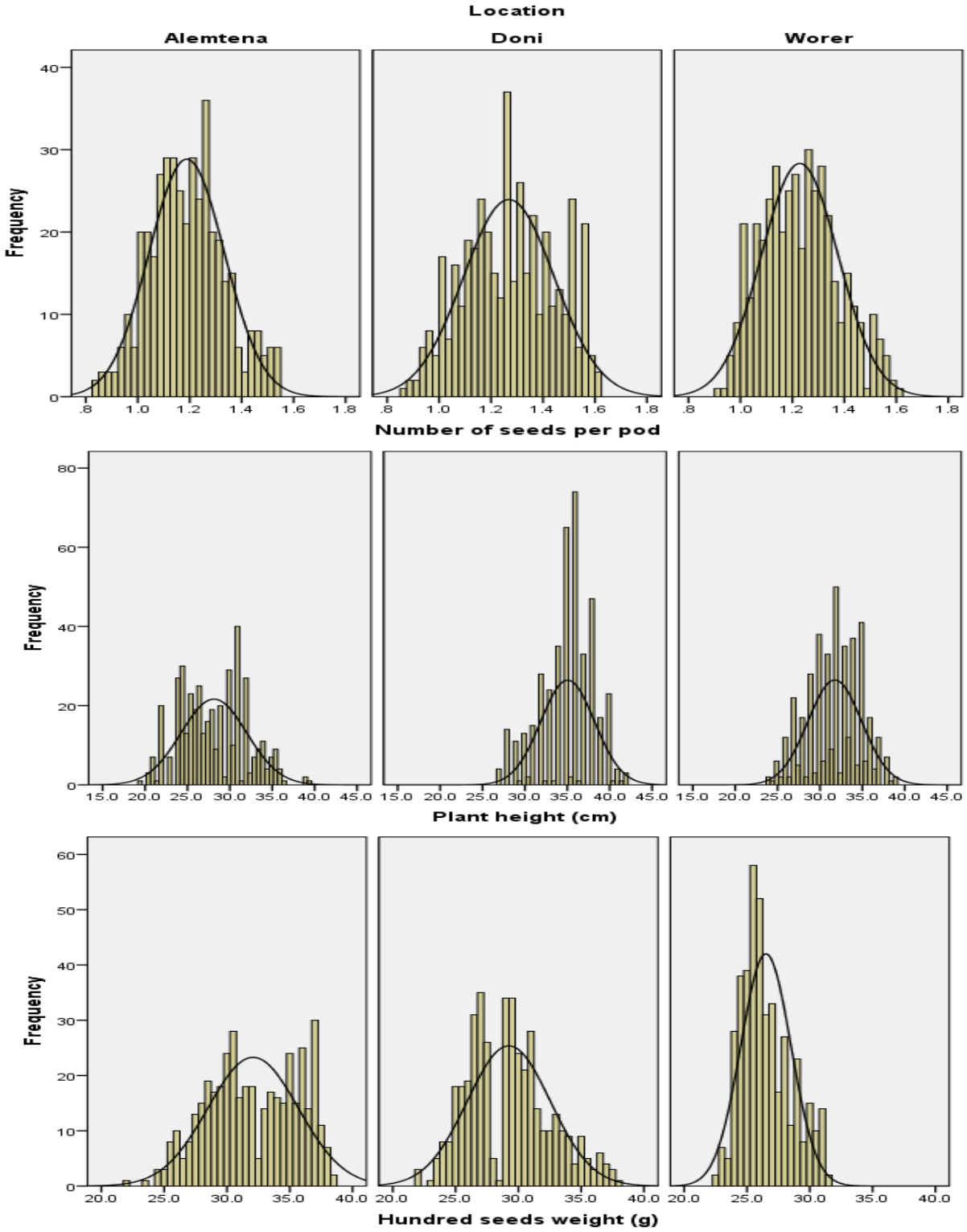
Appendix 1c. Principal Component analysis for 10 traits of 210 genotypes tested under water-stress and full-water conditions at Werer in 2014 cropping season.

	Under water-stressed condition				Under full-watered condition			
PC	1	2	3	4	1	2	3	4
Eigen Value	4.045	1.493	1.022	0.938	3.39	2.016	1.392	1.222
Difference	2.551	0.472	0.083	0.023	1.374	0.623	0.27	0.272
Proportion	0.405	0.149	0.102	0.094	0.339	0.202	0.139	0.112
Cumulative	0.405	0.554	0.656	0.75	0.339	0.541	0.68	0.792
Eigen vectors								
Trait	Prin1	Prin2	Prin3	Prin4	Prin1	Prin2	Prin3	Prin4
DTF	-0.266	0.560	0.138	0.127	0.089	0.184	-0.557	0.548
DTM	0.207	0.614	-0.137	0.421	-0.026	0.6587	0.160	0.171
DTGF	0.398	0.035	-0.231	0.242	-0.080	0.554	0.489	-0.149
PPP	0.405	0.068	0.001	-0.197	0.352	0.032	-0.004	-0.173
SPP	0.070	-0.093	0.874	0.407	-0.057	0.008	-0.279	-0.695
PHT	0.141	0.399	0.010	-0.137	0.082	0.366	-0.406	-0.025
HSW	0.095	-0.336	-0.331	0.693	-0.105	-0.300	0.393	0.368
BMY	0.420	-0.118	0.143	-0.069	0.528	-0.021	0.092	0.006
HI	0.369	0.084	0.030	-0.156	0.529	0.016	0.103	0.004
GYLD	0.467	-0.042	0.117	-0.125	0.530	-0.015	0.095	0.000

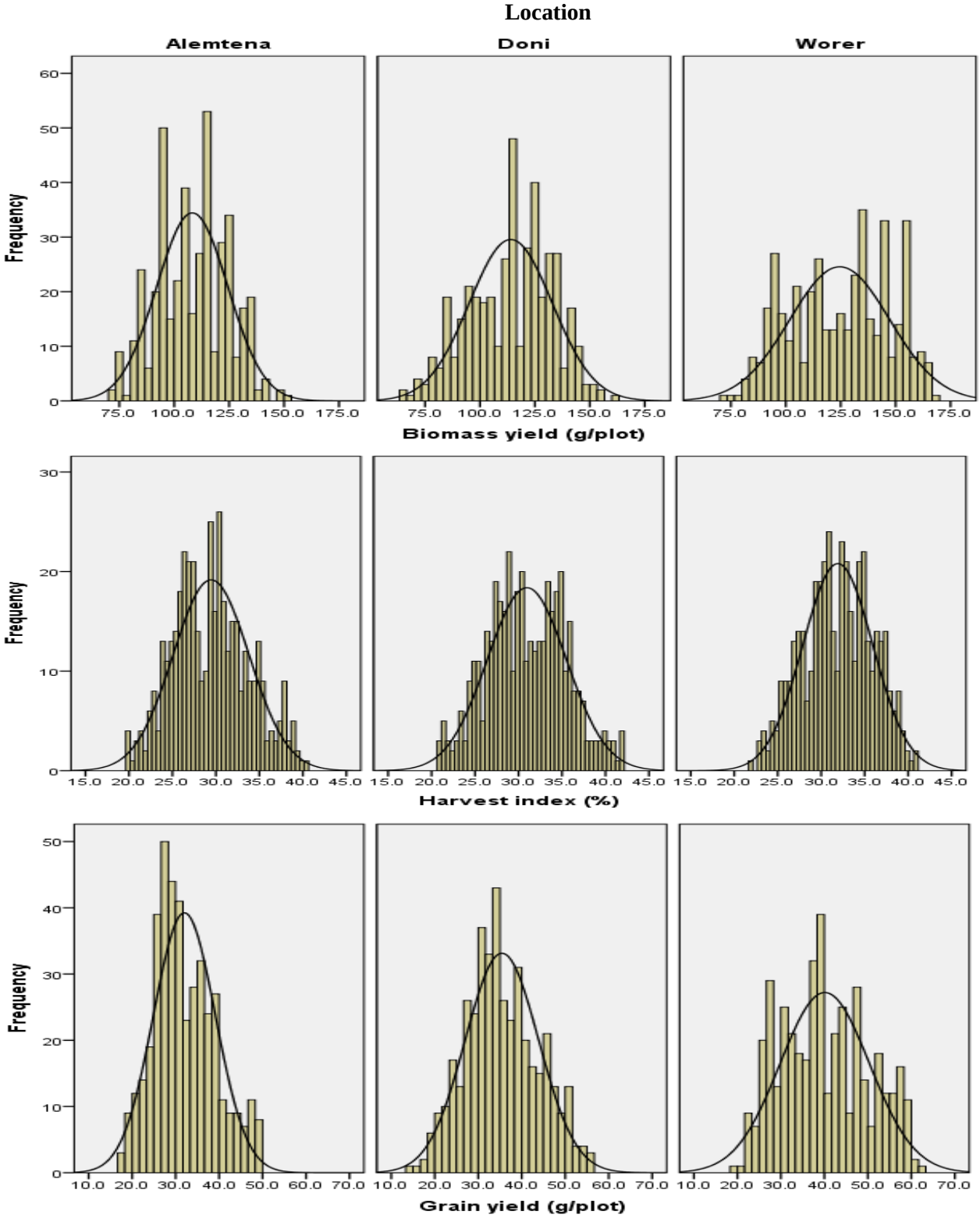
Appendix 2a. Frequency distribution of ten traits of chickpea genotypes tested under full-water condition at three locations (Alem Tena, Doni and Werer) in 2014 cropping season



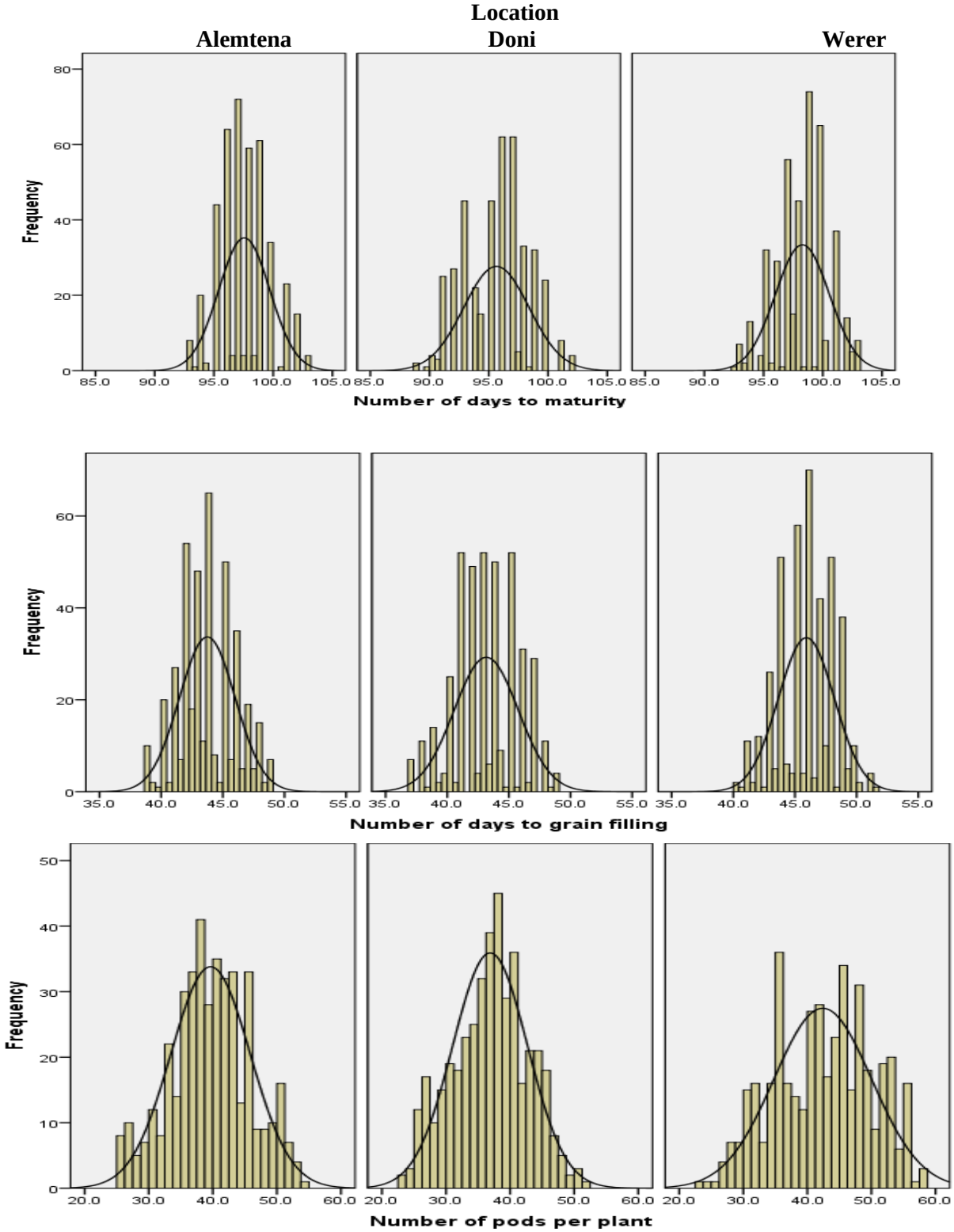
Appendix 2a. Continued.



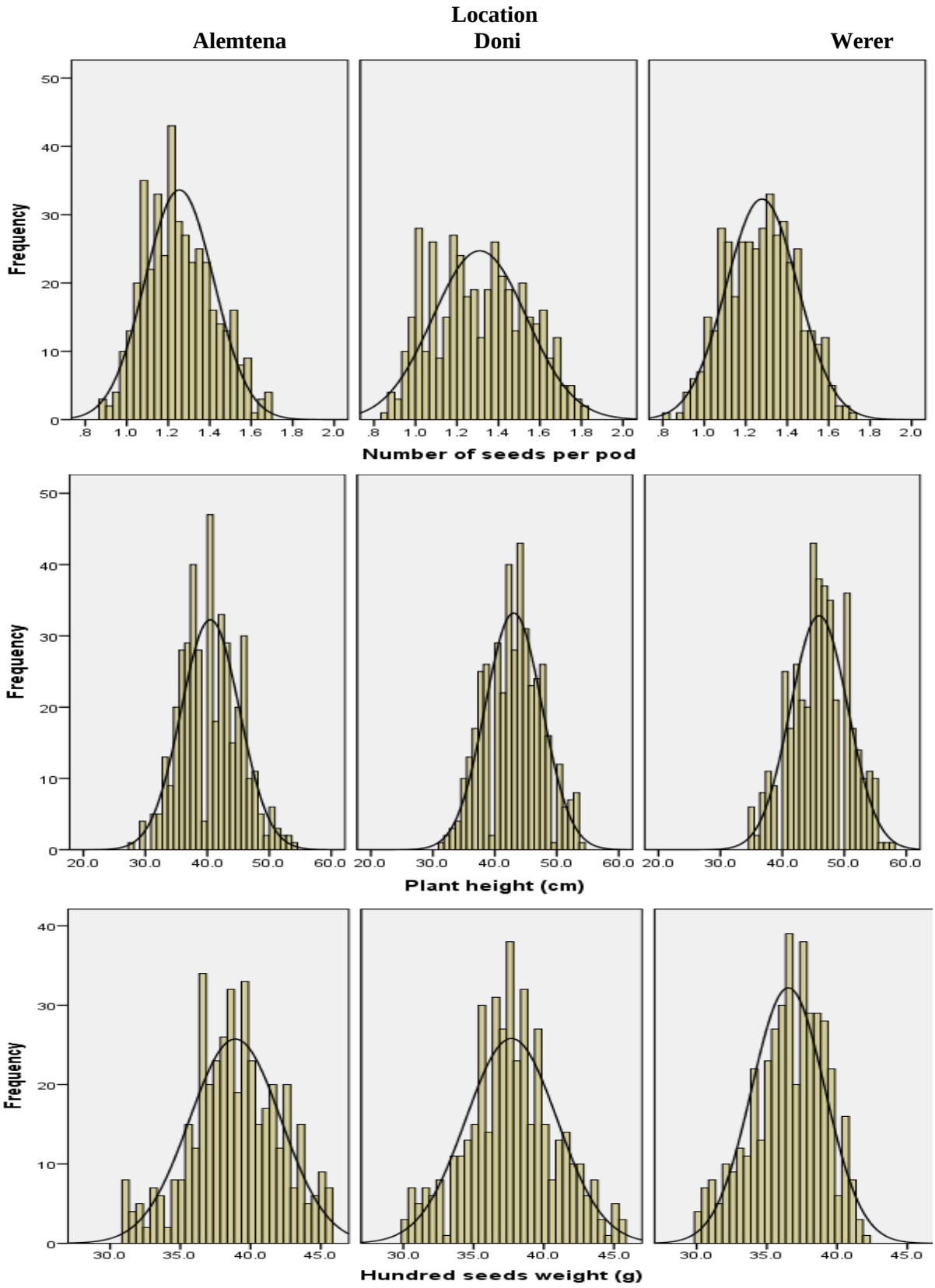
Appendix 2a. Continued.



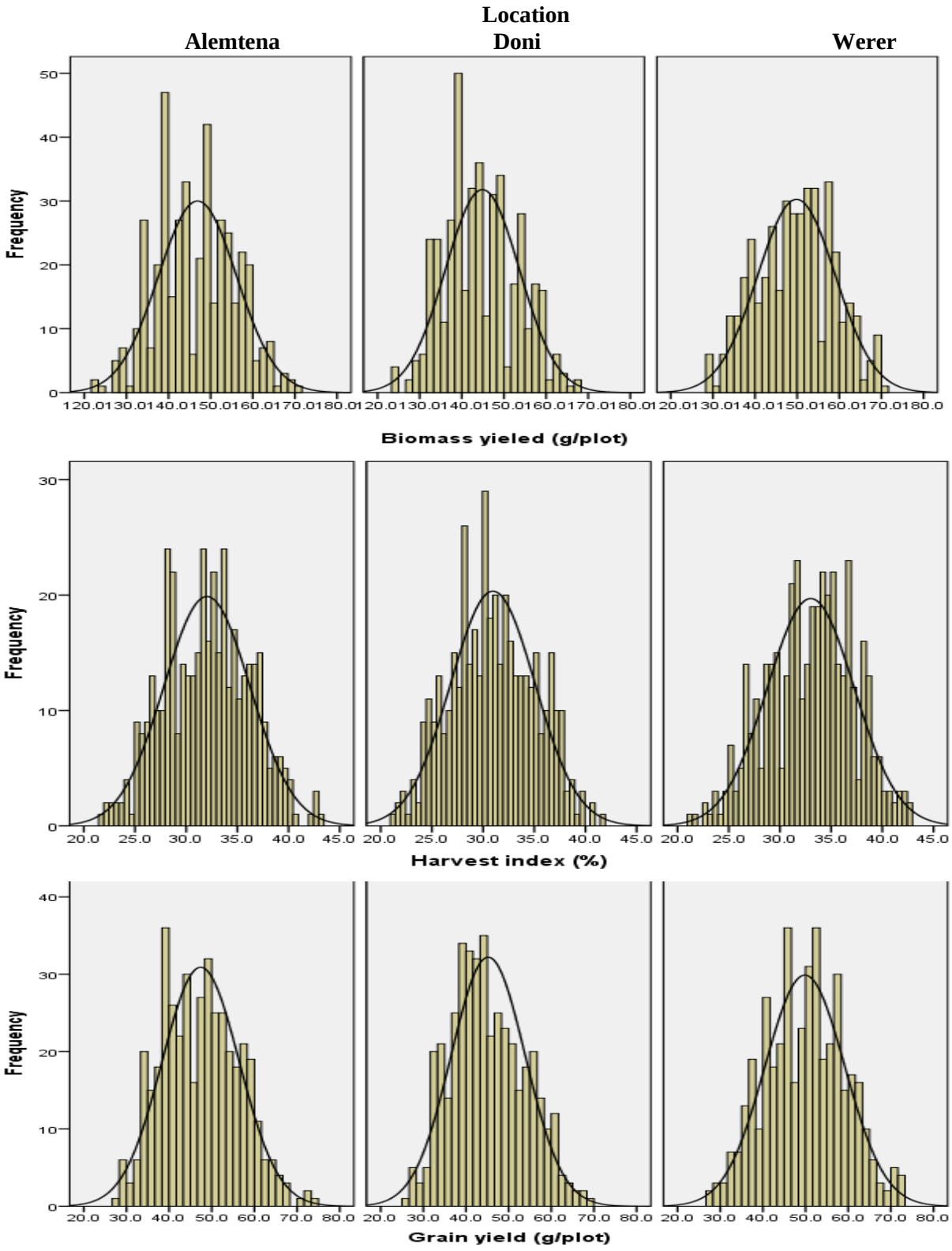
Appendix 2b. Frequency distribution for 10 traits of chickpea genotypes tested at the three test location (Alem Tena, Doni and Werer) under water-stress condition in 2014 cropping season



Appendix 2b. Continued.



Appendix 2b. Continued.



Appendix 3a. Genetic (V_G), environmental (V_E), and phenotypic (V_P) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability (H^2_b), genetic gain from selection (GA) and genetic gain as a percentage of mean (GAM) of 10 agro-morphological traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Alem Tena in 2014 cropping season

	Alem Tena under full water condition							
Trait	V_G	V_E	V_P	PCV	GCV	H^2_b	GA	GAM
DTF	1.095	0.368	1.279	2.1	1.9	85.6	2.6	4.7
DTM	2.991	1.033	3.508	1.9	1.8	85.3	4.3	4.4
DTGF	3.039	1.253	3.666	4.5	4.1	82.9	4.2	10.0
AB	0.209	0.165	0.292	24.0	20.3	71.7	1.0	45.9
PPP	30.540	3.985	32.533	14.3	13.8	93.9	14.3	35.7
SPP	0.021	0.005	0.024	12.6	11.9	89.0	0.4	29.8
PHT	15.980	4.611	18.286	10.5	9.8	87.4	10.0	24.5
HSW	8.300	1.690	9.145	7.9	7.6	90.8	7.3	19.2
BMY	61.762	18.269	70.897	5.9	5.5	87.1	19.6	13.6
HI	13.199	3.508	14.953	11.7	11.0	88.3	9.1	27.6
GYLD	59.353	18.460	68.583	17.5	16.3	86.5	19.1	40.3
	Alem Tena under water-stressed condition							
DTF	2.763	1.223	3.375	3.4	3.0	81.9	4.0	7.3
DTM	3.065	1.481	3.806	2.3	2.1	80.5	4.2	4.9
DTGF	3.697	1.747	4.571	7.0	6.3	80.9	4.6	15.2
AB	0.436	0.114	0.493	28.1	26.4	88.4	1.7	66.2
PPP	24.645	7.207	28.249	16.4	15.3	87.2	12.4	38.1
SPP	0.012	0.009	0.017	10.8	9.3	73.7	0.3	21.3
PHT	10.445	4.267	12.579	12.6	11.5	83.0	7.9	27.9
HSW	8.324	3.641	10.145	9.8	8.9	82.1	7.0	21.4
BMY	185.532	67.427	219.246	13.7	12.6	84.6	33.4	30.9
HI	10.514	5.828	13.428	12.4	11.0	78.3	7.7	25.8
GYLD	38.490	14.040	45.510	21.1	19.4	84.6	15.2	47.5

Appendix 3b. Genetic (V_G), environmental (V_E), and phenotypic (V_P) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability (H^2_b), genetic gain from selection (GA) and genetic gain as a percentage of mean (GAM) of 10 agro-morphological traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Doni in 2014 cropping season

	Doni under full water condition							
Trait	V_G	V_E	V_P	PCV	GCV	H^2_b	GA	GAM
DTF	1.033	0.326	1.196	2.1	1.9	86.4	2.5	4.8
DTM	5.382	0.877	5.821	2.5	2.4	92.5	6.0	6.1
DTGF	4.782	0.842	5.203	5.2	4.9	91.9	6.0	12.6
PPP	22.105	5.490	24.850	13.6	12.8	89.0	11.8	32.3
SPP	0.044	0.008	0.047	16.6	16.0	92.1	0.5	40.8
PHT	13.752	4.278	15.891	9.5	8.8	86.5	9.2	21.8
HSW	8.138	2.143	9.210	8.1	7.6	88.4	7.2	19.0
BMY	54.367	14.062	61.398	5.6	5.3	88.5	18.5	13.2
HI	11.944	3.508	13.698	11.5	10.7	87.2	8.6	26.6
GYLD	52.849	17.416	61.557	17.3	16.0	85.9	18.0	39.5
	Doni under water-stressed condition							
DTF	3.169	1.127	3.733	3.7	3.4	84.9	4.4	8.3
DTM	2.142	1.559	2.922	2.0	1.7	73.3	3.3	3.9
DTGF	3.214	1.740	4.084	6.2	5.5	78.7	4.2	12.9
PPP	16.962	6.440	20.182	16.0	14.7	84.0	10.1	35.9
SPP	0.023	0.007	0.027	12.8	11.9	86.3	0.4	29.5
PHT	4.907	4.130	6.972	7.5	6.3	70.4	5.0	14.2
HSW	5.990	4.550	8.265	10.0	8.5	72.5	5.6	19.3
BMY	276.248	73.018	312.757	15.5	14.6	88.3	41.6	36.6
HI	10.411	6.096	13.459	11.8	10.4	77.4	7.7	24.4
GYLD	55.532	23.978	67.521	23.2	21.1	82.2	18.0	50.9

Appendix 3c. Genetic (V_G), environmental (V_E), and phenotypic (V_P) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability (H^2_b), genetic gain from selection (GA) and genetic gain as a percentage of mean (GAM) of 10 agro-morphological traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Werer in 2014 cropping season

	Werer under full water condition							
Trait	V_G	V_E	V_P	PCV	GCV	H^2_b	GA	GAM
DTF	1.286	0.450	1.511	2.3	2.2	85.1	2.8	5.3
DTM	2.612	0.907	3.066	1.8	1.6	85.2	4.0	4.0
DTGF	3.051	1.011	3.557	4.1	3.8	85.8	4.3	9.4
PPP	45.190	7.212	48.796	16.6	16.0	92.6	17.2	41.0
SPP	0.025	0.005	0.028	13.2	12.6	91.4	0.4	32.1
PHT	13.009	2.519	14.269	8.2	7.9	91.2	9.2	20.0
HSW	6.332	1.961	7.313	7.3	6.8	86.6	6.2	16.9
BMY	64.536	15.225	72.149	5.8	5.5	89.4	20.3	13.8
HI	13.094	3.811	15.000	11.5	10.8	87.3	9.0	26.8
GYLD	64.442	17.072	72.978	17.2	16.2	88.3	20.1	40.5
	Werer under water-stressed condition							
DTF	2.476	0.913	2.933	3.4	3.1	84.4	3.9	7.6
DTM	1.486	0.907	1.940	1.6	1.4	76.6	2.8	3.3
DTGF	2.363	1.118	2.922	4.8	4.3	80.9	3.7	10.3
AB	14.207	7.901	18.158	12.9	11.4	78.2	8.9	26.9
PPP	0.012	0.010	0.017	10.5	8.7	68.9	0.2	19.3
SPP	4.818	4.229	6.933	8.3	6.9	69.5	4.9	15.4
PHT	1.568	2.222	2.679	6.4	4.9	58.5	2.6	10.1
HSW	427.501	105.574	480.288	17.6	16.6	89.0	52.0	41.7
BMY	10.003	5.546	12.776	11.2	9.9	78.3	7.5	23.3
HI	85.861	30.441	101.082	25.0	23.1	84.9	22.8	56.6
GYLD	2.476	0.913	2.933	3.4	3.1	84.4	3.9	7.6

Appendix 4a. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients of ten morpho-agronomic traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Alem 2014 cropping season

Trait	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
Under full-water condition										
DTF	1	*0.21***	*-0.40***	*0.16**	*-0.03*^{ns}	*-0.02*^{ns}	*-0.11*^{ns}	*0.18**	*0.19***	*0.19***
DTM	0.23**	1	*0.80***	*0.09*^{ns}	*-0.04*^{ns}	*-0.05*^{ns}	*-0.02*^{ns}	*0.03*^{ns}	*0.02*^{ns}	*0.02*^{ns}
DTGF	-0.40**	0.80**	1	*0.00*^{ns}	*-0.02*^{ns}	*-0.03*^{ns}	*0.05*^{ns}	*-0.07*^{ns}	*-0.09*^{ns}	*-0.09*^{ns}
PPP	0.17*	0.09^{ns}	0.00^{ns}	1	*-0.16**	*0.12*	*-0.24***	*0.49***	*0.49***	*0.50***
SPP	-0.04^{ns}	-0.03^{ns}	-0.02^{ns}	-0.18*	1	*0.08*^{ns}	*-0.05*^{ns}	*-0.17***	*-0.20***	*-0.19***
PHT	-0.02^{ns}	-0.08^{ns}	-0.06^{ns}	0.13^{ns}	0.09^{ns}	1	*-0.09*^{ns}	*0.08*^{ns}	*0.07*^{ns}	*0.07*^{ns}
HSW	-0.13^{ns}	-0.03^{ns}	0.06^{ns}	-0.27**	-0.04^{ns}	-0.10^{ns}	1	*-0.18**	*-0.20***	*-0.20***
BMY	0.22**	0.02^{ns}	-0.11^{ns}	0.54**	-0.18^{ns}	0.08^{ns}	-0.20**	1	*0.92***	*0.96***
HI	0.23**	-0.01^{ns}	-0.14*	0.53**	-0.23**	0.06^{ns}	-0.21**	0.93**	1	*0.99***
GYLD	0.23**	0.00^{ns}	-0.13^{ns}	0.54**	-0.21**	0.07^{ns}	-0.22**	0.97**	0.99**	1
Under water-stressed condition										
DTF	1	*0.36***	*-0.53***	*-0.15**	*-0.07*^{ns}	*0.06*^{ns}	*-0.17**	*-0.16**	*-0.28***	*-0.30***
DTM	0.37**	1	*0.60***	*0.26***	*-0.02*^{ns}	*0.06*^{ns}	*-0.06*^{ns}	*0.22***	*0.12*^{ns}	*0.23***
DTGF	-0.53**	0.60**	1	*0.37***	*0.04*^{ns}	*0.00*^{ns}	*0.09*^{ns}	*0.34***	*0.35***	*0.46***
PPP	-0.17*	0.28**	0.40**	1	*-0.02*^{ns}	*-0.02*^{ns}	*-0.02*^{ns}	*0.59***	*0.51***	*0.74***
SPP	-0.09^{ns}	0.01^{ns}	0.09^{ns}	-0.04^{ns}	1	*0.09*^{ns}	*0.04*^{ns}	*0.02*^{ns}	*0.04*^{ns}	*0.03*^{ns}
PHT	0.07^{ns}	0.06^{ns}	-0.01^{ns}	-0.01^{ns}	0.13^{ns}	1	*0.02*^{ns}	*-0.08*^{ns}	*0.13*^{ns}	*0.04*^{ns}
HSW	-0.22**	-0.07^{ns}	0.13^{ns}	-0.02^{ns}	0.05^{ns}	0.03^{ns}	1	*0.07*^{ns}	*0.09*^{ns}	*0.12*^{ns}
BMY	-0.20**	0.25**	0.40**	0.66**	0.04^{ns}	-0.10^{ns}	0.09^{ns}	1	*0.16**	*0.78***
HI	-0.35**	0.12^{ns}	0.41**	0.58**	0.06^{ns}	0.17*	0.12^{ns}	0.21**	1	*0.74***
GYLD	-0.35**	0.25**	0.53**	0.82**	0.05^{ns}	0.05^{ns}	0.15*	0.80**	0.74**	1

* and ** indicate significance (P<0.05 and 0.01, respectively); DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP and SPP=number of pods per plant and seeds per pod, respectively; PHT=plant height (cm); HSW=hundred seeds weight (g); BMY=biomass yield (g/plot); HI= harvest index (%); GYLD=grain yield (g/plot).

Appendix 4b. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients of ten morpho-agronomic traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Doni in 2014 cropping season.

Trait	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
Under full-water condition										
DTF	1	*0.34***	*-0.12*^{ns}	*0.04*^{ns}	*-0.07*^{ns}	*0.02*^{ns}	*-0.16**	*0.08*^{ns}	*0.08*^{ns}	*0.08*^{ns}
DTM	0.34**	1	*0.89***	*0.04*^{ns}	*-0.17**	*0.08*^{ns}	*-0.11*^{ns}	*-0.04*^{ns}	*-0.05*^{ns}	*-0.05*^{ns}
DTGF	-0.10^{ns}	0.90**	1	*0.02*^{ns}	*-0.15**	*0.08*^{ns}	*-0.04*^{ns}	*-0.08*^{ns}	*-0.09*^{ns}	*-0.09*^{ns}
PPP	0.06^{ns}	0.05^{ns}	0.03^{ns}	1	*-0.05*^{ns}	*0.11*^{ns}	*-0.26***	*0.46***	*0.48***	*0.48***
SPP	-0.09^{ns}	-0.18*	-0.15*	-0.05^{ns}	1	*0.05*^{ns}	*-0.05*^{ns}	*0.04*^{ns}	*0.06*^{ns}	*0.05*^{ns}
PHT	0.04^{ns}	0.09^{ns}	0.08^{ns}	0.12^{ns}	0.06^{ns}	1	*-0.08*^{ns}	*0.00*^{ns}	*0.02*^{ns}	*0.02*^{ns}
HSW	-0.19*	-0.12^{ns}	-0.04^{ns}	-0.30**	-0.06^{ns}	-0.11^{ns}	1	*-0.07*^{ns}	*-0.07*^{ns}	*-0.08*^{ns}
BMY	0.10^{ns}	-0.04^{ns}	-0.09^{ns}	0.53**	0.04^{ns}	0.02^{ns}	-0.07^{ns}	1	*0.96***	*0.98***
HI	0.10^{ns}	-0.04^{ns}	-0.09^{ns}	0.56**	0.05^{ns}	0.04^{ns}	-0.07^{ns}	0.98**	1	*0.99***
GYLD	0.11^{ns}	-0.04^{ns}	-0.10^{ns}	0.55**	0.04^{ns}	0.03^{ns}	-0.08^{ns}	0.99**	0.99**	1
Under water-stressed condition										
DTF	1	*0.38***	*-0.63***	*-0.22***	*-0.02*^{ns}	*0.01*^{ns}	*-0.18**	*-0.22***	*-0.17**	*-0.26***
DTM	0.39**	1	*0.48***	*0.22***	*0.00*^{ns}	*0.13*^{ns}	*-0.05*^{ns}	*0.27***	*0.20***	*0.30***
DTGF	-0.67**	0.42**	1	*0.39***	*0.02*^{ns}	*0.10*^{ns}	*0.14**	*0.43***	*0.33***	*0.49***
PPP	-0.27**	0.25**	0.46**	1	*0.13*^{ns}	*0.18**	*-0.04*^{ns}	*0.60***	*0.63***	*0.77***
SPP	-0.03^{ns}	0.01^{ns}	0.04^{ns}	0.16*	1	*0.12*^{ns}	*0.09*^{ns}	*0.09*^{ns}	*0.12*^{ns}	*0.14**
PHT	0.05^{ns}	0.20**	0.11^{ns}	0.20**	0.16*	1	*-0.03*^{ns}	*0.15**	*0.18**	*0.21***
HSW	-0.23**	-0.08^{ns}	0.18*	-0.04^{ns}	0.11^{ns}	0.00^{ns}	1	*0.01*^{ns}	*-0.02*^{ns}	*0.02*^{ns}
BMY	-0.24**	0.33**	0.50**	0.72**	0.10^{ns}	0.17*	0.00^{ns}	1	*0.26***	*0.85***
HI	-0.21**	0.22**	0.38**	0.68**	0.16*	0.22**	-0.04^{ns}	0.36**	1	*0.72***
GYLD	-0.28**	0.34**	0.55**	0.85**	0.16*	0.24**	0.00^{ns}	0.88**	0.75**	1

* and ** indicate significance (P<0.05 and 0.01, respectively); DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP and SPP=number of pods per plant and seeds per pod, respectively; PHT=plant height (cm); HSW=hundred seeds weight (g); BMY=biomass yield (g/plot); HI= harvest index (%); GYLD=grain yield (g/plot).

Appendix 4c. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients of ten morpho-agronomic traits of 210 chickpea genotypes tested under full-water and water-stress conditions at Werer in 2014 cropping season.

Trait	DTF	DTM	DTGF	PPP	SPP	PHT	HSW	BMY	HI	GYLD
Under full-water condition										
DTF	1	*0.23***	*-0.43***	*-0.02*^{ns}	*-0.07*^{ns}	*0.17**	*-0.09*^{ns}	*0.13*^{ns}	*0.12*^{ns}	*0.13*^{ns}
DTM	0.23**	1	*0.78***	*-0.02*^{ns}	*-0.14**	*0.14**	*0.01*^{ns}	*-0.02*^{ns}	*-0.01*^{ns}	*-0.01*^{ns}
DTGF	-0.43**	0.78**	1	*-0.01*^{ns}	*-0.08*^{ns}	*0.02*^{ns}	*0.06*^{ns}	*-0.10*^{ns}	*-0.08*^{ns}	*-0.09*^{ns}
PPP	-0.02^{ns}	-0.04^{ns}	-0.02^{ns}	1	*-0.09*^{ns}	*0.08*^{ns}	*-0.21***	*0.47***	*0.48***	*0.48***
SPP	-0.09^{ns}	-0.16*	-0.09^{ns}	-0.09^{ns}	1	*0.10*^{ns}	*-0.15**	*-0.08*^{ns}	*-0.09*^{ns}	*-0.08*^{ns}
PHT	0.18*	0.15*	0.02^{ns}	0.08^{ns}	0.10^{ns}	1	*-0.15**	*0.18**	*0.18**	*0.19**
HSW	-0.10^{ns}	0.03^{ns}	0.09^{ns}	-0.23**	-0.17*	-0.17*	1	*-0.08*^{ns}	*-0.09*^{ns}	*-0.09*^{ns}
BMY	0.13^{ns}	-0.03^{ns}	-0.11^{ns}	0.50**	-0.09^{ns}	0.21**	-0.09^{ns}	1	*0.98***	*0.99***
HI	0.13^{ns}	-0.02^{ns}	-0.10^{ns}	0.53**	-0.10^{ns}	0.21**	-0.11^{ns}	0.99**	1	*0.99***
GYLD	0.13^{ns}	-0.02^{ns}	-0.11^{ns}	0.52**	-0.09^{ns}	0.21**	-0.11^{ns}	0.99**	0.99**	1
Under water-stressed condition										
DTF	1	*0.41***	*-0.67***	*-0.37***	*-0.03*	*-0.02*	*-0.01*	*-0.44***	*-0.28***	*-0.44***
DTM	0.42**	1	*0.40***	*0.15**	*0.01*	*0.17**	*0.01*	*0.16**	*0.18**	*0.20***
DTGF	-0.69**	0.36**	1	*0.50***	*0.04*	*0.17**	*0.02*	*0.57***	*0.43***	*0.60***
PPP	-0.47**	0.20**	0.64**	1	*0.05*	*0.24***	*0.01*	*0.62***	*0.67***	*0.75***
SPP	-0.04^{ns}	0.02^{ns}	0.06^{ns}	0.08^{ns}	1	*-0.01*	*-0.03*	*0.15**	*0.03*	*0.13*
PHT	-0.03^{ns}	0.22**	0.20**	0.29**	-0.05^{ns}	1	*-0.10*	*0.22***	*0.14**	*0.23***
HSW	0.02^{ns}	0.02^{ns}	0.00^{ns}	0.03^{ns}	-0.08^{ns}	-0.18*	1	*0.03*	*0.02*	*0.03*
BMY	-0.49**	0.19*	0.65**	0.72**	0.21**	0.28**	0.05^{ns}	1	*0.45***	*0.91***
HI	-0.31**	0.26**	0.52**	0.74**	0.02^{ns}	0.17*	0.03^{ns}	0.52**	1	*0.76***
GYLD	-0.48**	0.25**	0.69**	0.83**	0.17*	0.29**	0.06^{ns}	0.93**	0.78**	1

* and ** indicate significance (P<0.05 and 0.01, respectively); DTF, DTM, DTGF=number of days to flowering, maturity, and grain filling, respectively; PPP and SPP=number of pods per plant and seeds per pod, respectively; PHT=plant height (cm); HSW=hundred seeds weight (g); BMY=biomass yield (g/plot); HI= harvest index (%); GYLD=grain yield (g/plot).