

Addis Ababa University
School of Graduate Studies
Institute of Biotechnology



**QTL Analysis, Molecular Genetics Diversity, and Agro-physiological Characterizations of
Heat Tolerance in Chickpea (*Cicer arietinum* L.)**

PhD Dissertation
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December, 2021
Addis Ababa, Ethiopia

QTL Analysis, Molecular Genetics Diversity, and Agro-physiological Characterizations of Heat Tolerance in Chickpea (*Cicer arietinum* L.)

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

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Declaration

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

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Acknowledgments

First and foremost, I would like to express my deepest gratitude to the Almighty GOD for His indefatigable help, blessings and mercy, without which this work would never have been possible. GOD has been always with me to strengthen every step of the way. I extend my sincere gratitude and honor, and heartfelt thanks to my major supervisor Dr. Kassahun Tesfaye, Director General for Ethiopian Biotechnology Institute and an Associate Professor at Institute of Biotechnology, for his kind understanding, excellent guidance and dedications, wise decisions and flexibility, constant and invaluable support throughout the long journey of my PhD research work. I also extend my indebted and sincere gratitude to my supervisor Professor Douglas R. Cook, University of California Davis, USA for admitting me as a PhD student in his project (Feed the Future chickpea Innovation lab, USAID project), valuable comments and suggestions, constant follow up, guidance, and fast responses during the project periods. In addition, I am very grateful to the United States Agency for International Development (USAID); Feed the Future Chickpea Innovation Lab at the University of California Davis for the full financial support of phenotyping as well as genotyping works (generating sequence data) that were done in Ethiopia and India, and at the University of California Davis, USA.

My heartfelt thanks and gratitude go to my co-hoster and co-supervisor Dr. Pooran M. Gaur, Principal Scientist, Chickpea Breeding and Director for Crop Improvement, Asia Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India for his kind gesture, guidance, endless support and constant words of encouragement, without his support, I would not have been successful. It gives me immense pleasure to render my profound thanks to Mr. Krishna (Field Research technical assistance) and all the staff members of

Chickpea Department of ICRISAT for their friendly attitude, invaluable support that helped me a lot to accomplish the difficult work easily and effectively.

I am very fortunate and grateful to honor my co-supervisor Dr. Asnake Fikre for his gracious suggestions, consistent encouragements, all possible help and guidance during my doctoral program. Besides, he provides useful comments and guidance to my PhD thesis. My appreciations and thanks go to the staff members of Debre Zeit Agricultural Research Centers, Pulses research case team, namely Dr. Mekasha Chichaybelu, Mr. Nigusie Girma, Mrs. Konjiet Ayalew, and Mr. Tesfaye Gebre for their kind support and help in managing all the field activities conducted at Debre Zeit and Melaka Werer. I am so grateful to Sirnka Agricultural Research Center for their support and permissions in conducting field experiments in Kobo Sub-center and my special thanks go to Mr. Sisay for data collections and management of field activities in the experimental site in my absence.

My indepted gratitude, honor and special thanks go to Dr. Eric von Wettberg, University of Vermont, USA for his unreserved guidance and help, critical look and advices, technical support, and encouragements throughout my PhD study. I am thankful for the provision of germplasm accessions by the Ethiopian Biodiversity Institute (EBI), Melkasa and Debre Zeit Agricultural Research Centers, and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. I acknowledge Debre Zeit, Melka Werer and Hyderabad (ICRISAT) research centers for the provision of experimental sites. I also acknowledge Ethiopian Meterological Agency and ICRISAT, Hyderabad, India for the provision of meterological data.

It gratifies me in expressing my heartfelt thanks and gratitude to Dr. Rajeev K. Varshney, Principal Scientist and Director, Center of Excellence in Genomics and Systems Biology, ICRISAT for his kindness, cooperation and humble handling in a difficult time. He gave me an excellent opportunity to work in one of the best Genomics laboratory of International standards without a fund. I have acquired all molecular skills and got ample experiences in his laboratory within a short period. I express my sincere gratitude and appreciations to Dr. Mahender Thudi, Senior Scientist, Center of Excellence in Genomics and Systems Biology, ICRISAT, Patancheru, for his critical guidance in accomplishing my genotyping work, and taught me real science and many valuable skills during my stay. I am grateful to Mr. Eshwar Garu for his proper guidance and supreme help in the Genomics laboratories. I am thankful to Madam Anu Chitikineni, all laboratory research technicians and staff members of the department of Genomics who treated me as their staff.

I am very much pleased and honored Addis Ababa University, Institute of Biotechnology for hosting me as a PhD student, cover my tuition fee, and giving me a study leave to get ample skills and experiences during my PhD study. My special thanks go to Prof. Tesafye Sisay, Director of Institute of Biotechnology, for his wonderful taught, kindness, support, encouragement and follow up through out my PhD study. I am also thankful to Dr. Addis Simachew, Deputy Director of Institute of Biotechnology, for his kind support and encouragement. I am thankful to all friends, colleagues and staff members of Institute of Biotechnology for their support, love, teaching, guidance, mentoring, cooperations and friendship.

This PhD thesis is dedicated to my beloved wife Mrs. Birtukan Ademas, my lovely children Benias Tsegaye, Kebron Tsegaye and Yohanna Tsegaye who lost my affections, care and support for eight years as I am doing my PhD research in Addis Ababa University and abroad. Dear Birti without your patience, great sacrifices, care, endless love, critical support, prayer and encouragements, I would never have been able to complete my PhD study. I love you all and I appreciate everything that you have done for me.

Last but not least, I am thankful to my dedicated primary school teachers in Powe (Mender 28), Benshagul Gomez region who had already predicted where I am now. I also wish to mention my deep sense of gratitude to a number of high school teachers in Kutaber (Desie), Amhara region and my University teachers in Addis Ababa University who have shaped me in good behavior and academics, and their unwavering belief that I can achieve so much. Thanks GOD all the nights were over and the day has come. My special thanks extended to my mother Mrs. Alemitu Tefera, my father Mr. Getahun Bogale and my brothers Samuel Getahun, Moges Getahun, Solomon Getahun, and Abreham Getahun for their affections, cares, support, visions, guidance, pray and advices to my successful journey. Thanks mom, I will never forget your challenges and scarifications to shape and grow up your children to this level. Thanks my father, you taught me the value of education starting from my childhood. Besides, I am thankful to my uncle Mr. Getye Tefera for sharing my responsibilities when I was in a difficult time.

List of Abbreviations

A = allelic frequency

ABA = abscisic acid

ADD = additive and dominant

AE = *C. arietinum* x *C.echinospermum*

AFLP = amplified fragment length polymorphism

AMOVA = analysis of molecular variance

ANOVA = analysis of variance

APX = ascorbate peroxidase

AR = *C. arietinum* x *C. reticulatum*

ATP = adenosine triphosphate

BC = backcross

BIN = Binning

BIP = biparental QTL mapping function

BWA = Burrows-Wheeler Alignment Tool

CA = common allele

CAT = catalase

CDS = coding sequence

Chr = chromosome

CI = continuity index

CSA = Central statistics Authority

DArT =diversity array technology

DFF = days to first flowering

D50F = days to 50% flowering

DFP = days to first podding

DH = double haploid

DPM = days to physiological maturity

DZ = Debre Zeit

EBI = Ethiopian Biodiversity Institute

EPI = Epistatic interaction

EV = environmental variance

FA = frequent allele

FAOSTAT = Food and Agriculture Organization Corporate Statistical Database

FDR = False discovery rate

F_{IS} = inbreeding index

F_{IT} = total fixation index

FPP = filled pods per plant

FT = flowering time

F_{ST} = fixation index

F_v/F_m = chlorophyll fluorescence

GA = genetic advance

GAM = genetic advance as percentage of the mean

GATK = Genome Analysis Tool Kit

GBS = genotyping by sequencing

GCV = coefficient of genotypic variance

GECV = coefficient of genotype by environmental variance

GEV = genotype by environmental variance

GFP = grain filling period

GH = growth habit

GL = genetic linkage group

GLM = General Linear Model

GR = glutathione reductase

GST = enzymes glutathione S-transferase

GV = genotypic variance

GWAS = genome wide association studies

GY = grain yield

Hb² = broad sense heritability

He = expected heterozygosity

Ho = observed heterozygosity

HIS = heat susceptibility index

Hsfs = heat stress (shock) transcriptional factors

Hsps = heat shock proteins

HSR = heat stress response

HSW = hundred seed weight

HVS = heat visual score

I = Shannon diversity index

ICIM = Inclusive composite interval mapping

ICRISAT = International Crops Research Institute for Semi-Arid Tropics

KASP = competitive allele specific PCR

LA = leaf angle

LD = linkage disequilibrium

LEF = linear electron flow

LOD = Logarithmism of Odds

LSD = least significance difference

LT = leaf thickness

MAGIC = multi-parent advanced generation inter-cross

MAP = mapping function

MAF = major allele frequency

MAS = marker assisted selection

Mbp = mega base pair

MCMC = Monte Carlo Markov Chain

MS = mean square

MQ = Mapping Quality

Na = allelic number per locus

NAM = nested association mapping

Ne = effective allelic number per locus

NGS = next generation sequencing

NILs = near isogenic lines

Nm = gene flow

OEC = oxygen evolving complex

P = probability

%P = percentage of polymorphism

PAR = photosynthetically active radiation

PB = plant biomass

PCA = principal component analysis

PCoA = principal coordinates analysis

PCR = Polymerase chain reaction

Phi2 = effective quantum yield of photosystem II

PhiNO = the portion of light lost as non-regulated way

PHT = plant height

PhiNPQ = the portion of light lost as regulated way

NPQ = non-photochemical quenching

PIC = polymorphic information content

PIN = a probability in stepwise regression

POD = peroxidase

Pop = population

%PS = percentage of pod set

%PG = percentage of pollen germination

PC/F = pollen cells per flower

PVE% = percentage of explained phenotypic variance

%PV = percentage of pollen viability

PV = phenotypic variance

PCV = coefficient of phenotypic variance

PC = personal computer

PT = pod type

QD = quality depth

qL = coefficient of photochemical quenching using lake model

qP = coefficient of photochemical quenching using puddle model

QTL = quantitative trait loci

R^2 = coefficient of determination

RA = rare allele

RAD-seq = Restricted Site-Associated DNA Sequencing

RFd = chlorophyll fluorescence decline ratio

RILs = recombinant inbred lines

RAPD = randomly amplified polymorphic DNA

RFLP = restriction fragment length polymorphisms

ROS = reactive oxygen species

SA = salicylic acid

SD = standard deviation

SE = semi-erect/standard error

sHsp = small heat shock protein

SNP = single nucleotide polymorphism

SNNP = Southern Nations, Nationalities and People

SPAD = The soil plant chlorophyll meter

SS = sum of square/semi-spreading

SSR = simple sequence repeat

SOD = superoxide dismutase

TPP = total pods per plant

TSP = total seeds per plant

uHe = unbiased expected heterozygosity

UPP = unfilled pods per plant

USAID = United States Agency for International Development

UTRs = un-translated region

WPGMA = weighted pair-group method with arithmetic averages

σ^2_e = environmental variance

σ^2_g = genotypic variance

σ^2_{ge} = genotype-by-environment interaction variance

σ^2_p = phenotypic variance

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QTL Analysis, Molecular Genetics Diversity, and Agro-physiological Characterizations of Heat Tolerance in Chickpea (*Cicer arietinum* L.)

PhD Dissertation

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Addis Ababa University, 2021

Abstract

Chickpea is a cheap source of protein and rich in minerals for people living in developing countries. A major agronomic challenge for chickpea production is temperatures above 35°C, which reduced growth, fertility, development, production and productivity of cultivated chickpea. Besides, domesticated chickpea changes in the nature and intensity of selection during domestication and breeding that constrain the ability to expand the cultivation of crops into arid and semi-arid environments. Therefore, the objectives of the present study were to introgress wild genes into the cultivated gene pool via genomics assisted breeding, assess the molecular genetic diversity and determine the population structures of large collections of chickpea germplasm; screen heat tolerant genotypes and assess phenotypic variations from all sources of chickpea germplasm accessions under heat stress environments; and understand the mechanisms of heat tolerance.

A total of 480 chickpea accessions that comprised of 440 F₃ lines of *C. arietinum* x *C. reticulatum* (17 AR families) and 33 F₃ lines of *C. arietinum* x *C. echinospermum* (3 AE families), and seven standard heat tolerant and sensitive checks which were grown in two replications at ICRISAT, Hyderabad, India under heat stress conditions. GBS SNPs of 1445 and

1541 were used in AR and AE families, respectively for linkage map construction and QTL mapping. Among the 20 families, 9xKalkan_064 was consistently found to be the most heat tolerant pre-breeding family, while 9xKarabache_081A and 9xDerei_072 were in the ranges of heat sensitive to moderately heat tolerant families. A total of 194 QTLs were detected for 16 traits out of which 38 QTLs had pleiotropic effects. The associated SNP markers with pleiotropic loci are important for simultaneous selection of genotypes having more than two related traits for heat stress breeding in chickpea.

In order to assess the existing molecular genetic diversity and to determine population structure in selected Ethiopian chickpea germplasm accessions (118), a set of 46 simple sequence repeat (SSR) markers equally distributed on chickpea genome were genotyped. A total of 572 alleles were detected from 46 SSR markers and the number of alleles per locus varied from 2 (ICCM0289) to 28 (TA22). The average numbers of alleles per locus, polymorphism information content and expected heterozygosity were 12, 0.684 and 0.699, respectively. Phylogenetic analysis grouped the 118 chickpea genotypes from diverse sources into three evolutionary and/or biological groups (improved desi, improved kabuli and landraces). Population structure analysis revealed six sub-populations from 118 chickpea genotypes used in this study. AMOVA revealed that 57%, 29% and 14% of the total genetic variations were observed among individuals, within populations and among populations.

A total of 121 chickpea genotypes were assessed at three field sites in Ethiopia, under heat stressed and non-stressed conditions and 57 genotypes were assessed under high heat stressed conditions at ICRISAT, Hyderabad, India. The results showed that the chickpea genotypes were

severely impacted by excessive heat at Hyderabad as compared to those planted under non-heat stressed conditions in Debre Zeit, Ethiopia. At extremely high temperatures, chickpea plants exhibited reduced floral initiation, arrested seed and pod development, shortened life cycles, and reduced plant height, seed size, grain yield and yield related traits. Across stressed and non-stressed environments, there were highly significant differences among the genotypes for most of the traits (ANOVA, $P \leq 0.001$). Under heat stressed environments, DZ-Cr-0034 was found to be a highly tolerant, while DZ-Cr-0026 was found to be a highly sensitive genotype. Genetic diversity among the genotypes were determined using 5,722 SNPs, revealing a single group of Ethiopian genotypes with small number of cultivars showing introgression from Middle Eastern germplasm.

At the reproductive developmental stages, a total of 13 physiological parameters were measured for 47 chickpea accessions that were grown from February to May, 2019 under a heat stressed environment, Werer (Ethiopia), using a high throughput phenotyping tool (MultispeQ). Based on the physiological responses of chickpea accessions under heat stressed environment, it was confirmed that DZ-2012-ck-0034 and JG 14 were found to be the most heat tolerant, while DZ-2012-ck-0026 and ICC 4567 were the most heat sensitive genotypes. Unlike heat sensitive genotypes, heat tolerant genotypes exhibited high mean values for effective quantum yield of photosystem II (Φ_2), chlorophyll fluorescence decline ratio (RFd), coefficients for photochemical quenching (qP and qL), chlorophyll fluorescence (F_v/F_m), pod set and low mean values for regulated (Φ_{NPQ}) and non-regulated heat dissipations (Φ_{NO}).

Reproductive traits were studied under heat stressed conditions with 17 and 47 chickpea accessions that were grown at ICRISAT (Hyderabad, India) and Werer (Ethiopia), respectively. The pollen viability study was carried out by using 2% acetocarmine stain. The quantity of pollens per flower was counted with haemocytometer. *In vitro* pollen germination was determined by using a modified Brewbaker and Kwack liquid medium. Under heat stressed condition, at ICRISAT (Hyderabad, India), the F₃ individuals of 9xCudiB_022C family had the highest average percentage of pollen viability (94%) which was associated with high pod set (80%). However, 9xKarabche_081A (9%) and ICC 4567 (10%) had low average percentage of pollen viability which was also associated with low pod set (0-10%). The mean values of the pollen cells per flower, *in vitro* pollen germination and pod set of the segregating progenies were higher than the landraces, lines and varieties which implied that the segregating wild progenies resisted the heat stress i.e. linked traits can be introgressed into cultivars in order to enhance thermotolerance.

The present study revealed that the Ethiopian germplasm accessions have genetic variability for heat tolerance that can be harnessed to meet expected shifts towards warmer climate. The physiological and reproductive traits might enhance the efficiency and accuracy of breeding in chickpea for screening and identifying of tolerant genotypes and help to understand the mechanisms of heat tolerance under heat stress conditions and contribute to climate resilient agriculture.

Keywords: GBS SNPs, Mechanisms of heat tolerance, Molecular genetic diversity, Population structure, QTL analysis, RAD-seq.

CHAPTER 1

1. Introduction

1.1. Background

Chickpea (*Cicer arietinum* L.) is a cool season, self-pollinated, annual grain legume and originated in southeastern Turkey and the adjoining part of Syria along with its wild progenitor (Ladizinsky and Alder, 1976; Singh, 1987). Chickpea belongs to the genus *Cicer* and family Leguminosae (Fabaceae) and sub-family Papilionoideae (Singh, 1987). It is a diploid ($2n = 16$) and the only cultivated *Cicer* species with a genome size of 740 Mb (Varshney *et al.*, 2013).

Chickpea is the second most important pulse crop in the world after faba bean (FAOSTAT, 2019) that has grown under rain fed conditions in arid and semi-arid areas around the world. Chickpea is growing in a wide range of environments in more than 57 countries all over the world. The major producers are India, Turkey, Pakistan, Iran, Myanmar, Mexico, and Ethiopia (FAOSTAT, 2019). India is the largest producer of chickpea and Ethiopia ranks sixth which accounts 65% and 3% of the world chickpea production, respectively. Ethiopia is the leading producer and exporter (59,768.4 tons) of chickpea in Africa, which accounts 82.4% of the area coverage and 77.5% of the production (FAOSTAT, 2019; Erana Kebede, 2020).

Chickpea is a rich source of proteins, carbohydrate, fat, fiber, essential amino acids (lysine, methionine, threonine, valine, isoleucine and leucine) and vitamins such as riboflavin, niacin, thiamin, ascorbic acid, folate and the vitamin A precursor, β -carotene (Jukanti *et al.*, 2012). It is a good source of income to farmers and generates foreign currency for many countries.

Moreover, chickpea improves soil fertility by symbiotic nitrogen fixation (Kumar *et al.*, 2012) and enhancing yields of the succeeding cereal crop.

Although the chickpea production potential is high, it is not fully realized in the tropics and the sub-tropics due to several abiotic and biotic stresses (Gaur *et al.*, 2012). Abiotic stresses namely drought, salinity, water logging, high temperature, cold and chilling frequently limit growth, production and productivity of chickpea. Among the abiotic stresses, terminal drought and heat stresses are the major limitations to chickpea grain yield (Summerfield *et al.*, 1990). Global warming, coupled with increased temperatures in arid and semiarid regions has necessitated development of crop varieties that can sustain and yield high in harsh climate. Heat stress has become a major constraint of chickpea production at the reproductive stage in those regions (Thudi *et al.*, 2014). Further, the quantitative inheritance of heat and its interaction with environment have been posing challenges to our understanding of the genetic basis of the trait. As evident by massive yield losses in various food crops, the escalating adverse impacts of heat stress are putting the global food as well as nutritional security at great risk (Jha *et al.*, 2014). Unlike drought and other abiotic stresses, until recently, the importance of breeding for heat stress in chickpea has not been realized (Gaur *et al.*, 2014). Hence, breeding for chickpea cultivars that can sustain high temperatures without significantly affecting its yield potential can address the issue of food and nutritional security in the climate change scenario.

1.2.Statement of the problem

High temperatures have become a global concern that decreases chickpea production in its current area of cultivation. The increasing threat of very high temperatures might results in wide

spread famine (Thudi *et al.*, 2014; Driedonks *et al.*, 2015). The capability of crop plants to survive and produce economic grain yield under heat stress is regarded as heat stress tolerant genotypes (Kaur *et al.*, 2016). However, there are limited studies elucidating the impact of elevated temperature on development, growth and yield of chickpea (Jumrani and Bhatia, 2014; Paul *et al.*, 2018). Heat stress exhibits an array of morpho-anatomical, physiological, biochemical and molecular changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield (Fig 1.1) (Wahid *et al.*, 2007; Hasanuzzaman *et al.*, 2013; Trivedi, 2015).

Temperature requirements for chickpea growth vary with developmental stages; however, the optimum temperatures range for normal flowering, fertilization and seed set varies from 21°C to 29°C maximum and 15°C to 21°C minimum (Imtiaz *et al.*, 2011). High temperatures (>30°C) at flowering and pod development drastically decrease chickpea potential seed yield (Krishnamurthy *et al.*, 2011; Summerfield *et al.*, 1984). Heat stress during the reproductive phase is more harmful than during the vegetative phase due to direct effect on pod and seed numbers, grain yield and dry weight (Hasanuzzaman *et al.*, 2013; Trivedi, 2015). A few days of exposure to high temperatures ($\geq 35^{\circ}\text{C}$) during reproductive phase can cause heavy yield losses (upto 100%) through flower and pod abortion. Recent studies indicate that high temperatures reduced pod set in chickpea by reducing pollen viability and pollen production per flower (Devasirvatham *et al.*, 2012; Devasirvatham *et al.*, 2013; Tsegaye Getahun *et al.*, 2021b).

Heat stress adversely affects plant biomass, pollen viability, pollen tube growth, pollen germination, stigma receptivity, fertilization, pod formation, the number of pods and seeds per

plant, grain yield, seed development and harvest index (Krishnamurthy *et al.*, 2011; Devasirvatham, 2012; Paul *et al.*, 2018; Tsegaye Getahun *et al.*, 2021b). Different authors reported that it decreased chickpea yield from 7 to 16% (Upadhyaya *et al.*, 2011), 8%-30% (Devasirvatham *et al.*, 2012) and 6-24% (Waha *et al.*, 2012). Severe reduction in yield (64-78%) was also reported (Jumrani and Bhatia, 2014) at 34/20°C.



Fig.1.1.Effect of heat stress on plants (Hasanuzzaman *et al.*, 2013; Trivedi, 2015).

Heat stress also significantly reduced physiological processes such as canopy temperature, photosynthetic rate, evapotranspiration, water use efficiency, stomata conductance, leaf water

content, chlorophyll content, membrane integrity, senescence of leaf tissue and photochemical efficiency with a larger effect on heat-sensitive genotypes (Jain, 2014). In India, from a large core-collection of chickpea against heat stress only two heat-tolerant genotypes were screened (Upadhyaya *et al.*, 2011) which showed higher pod set and yield. Similarly, Devasirvatham (2012) reported that among 167 chickpea genotypes, only two were tolerant at $\geq 38^{\circ}\text{C}$.

The Ethiopian chickpea-breeding program is aimed at development of varieties with higher yield potential, enhanced resistance/tolerance to key abiotic and biotic stresses and improved adaptation to different agro-ecologies and cropping systems. However, nothing has been done on heat stress tolerance breeding (Asnake Fikre, 2014; Mekasha Chichaybelu *et al.*, 2021) except a single report by Tesfashbamlak Molla *et al.* (2018) with 18 early flowering chickpea genotypes. In addition, the majority of agricultural systems in Ethiopia are suffered by unpredictable rainfall and temperature variability, which result in crop failure and reduction in agricultural yield. Furthermore, chickpeas have been growing in the lowland areas of Ethiopia such as Melka Werer, Humera, Jinka, Kobo and others. They also started growing at high temperature intercropping with rice and cottons (Yayeh Bitew and Fikremariam Asargew, 2015).

Rani *et al.* (2020) suggested that incorporation of various landraces and a range of crop gene pool harboring “adaptive traits” could enhance the resilience of chickpea genotypes under extreme climates. Exploration and use of large collections of chickpea germplasm comprising landraces and wild *Cicer* species have been conserved in various worldwide gene banks that can increase useful genetic diversity and become resilient to biotic and abiotic stresses in current crops. Wild species and more primitive landraces can be sources of variants useful for improving

elite varieties (von Wettberg *et al.*, 2018). Cultivars that can tolerate high temperatures without reduction in yield are needed for sustainable chickpea production. Promising lines having resistance genes and good agronomic performance have been developed from crosses involving cultivated and wild *Cicer* species (von Wettberg *et al.*, 2018). Understanding the agro-morphological, physiological and genetic basis of heat tolerance is a pre-requisite for improving existing chickpea cultivars and introgressions of wild genes into elite cultigen (von Wettberg *et al.*, 2018).

Besides, a better understanding of heat tolerance mechanisms and description of existing heat tolerance traits is urgently needed (von Wettberg *et al.*, 2018). An effective and simple screening method with well-defined traits for selecting heat-tolerant genotypes in chickpea under field conditions is necessary for practical and economically viable breeding program for heat tolerance (Devasirvatham *et al.*, 2012; Gaur *et al.*, 2014). Selection for heat tolerance can be effectively made by planting the crop at heat stress hot spot conditions and selecting the plants/segregating progenies based on number of pod set, filled pods per plant, grain yield and other yield related traits (Gaur *et al.*, 2014; Paul *et al.*, 2018; Tsegaye Getahun *et al.*, 2021b).

The extensively explored chickpea germplasm are used to assess the molecular genetic diversity and understand the knowledge gap involving the morpho-physiological, biochemical and molecular complex network of abiotic stresses mechanism (Aggarwal *et al.*, 2013). The agro-morphological, phenological and physiological characterizations of heat tolerance would aid the design of strategies to screen germplasm for heat tolerance traits. Moreover, understanding of biochemical and molecular basis of thermo-tolerance in combination with genetic approaches

and expression patterns of genes will assist conventional breeders to develop heat tolerant cultivars (Kaur *et al.*, 2016). Alleles/genes/QTLs for adaptive traits from wild relatives will be introgressed into elite lines through conventional breeding and modern biotechnological tools (Kaur *et al.*, 2016; von Wettberg *et al.*, 2018).

QTLs/genes governing tolerance to heat stress traits are rarely available in chickpea and the DNA-based markers were not yet discovered and further enrichment of genomic resources for marker assisted selection is required so that adequately dense genetic maps should be developed to map all the possible traits and the QTL boundaries should be narrowed down for these traits. Unlike heat stress of chickpea, in drought stress, a “QTL-hotspot” harboring various drought related trait has been introgressed into elite chickpea genotype (Varshney *et al.*, 2016). Thus, genomic resources such as markers linked to phenotypic traits and QTLs/genes governing several traits under heat stress environments remain an assignment in arid and semi-arid chickpea breeding programs.

Therefore, dissection of genomic regions (QTLs/genes), determination of molecular genetic and genomic diversity/relationships and population structure, as well as characterizations of agromorpho-physiological traits were conducted to improve Ethiopian chickpea breeding for an extremely heat stress environments.

1.3.Objective of the study

1.3.1. General objective

To dissect genomic regions (QTLs), assess genetic and genomic diversity, population structure, and agromorpho-physiological characteristics of chickpea for heat tolerance.

1.3.2. Specific objectives

- To screen intercrossed segregating F₃ progenies for heat tolerance.
- To dissect genomic regions (QTLs/genes) for heat visual score, agro-morphological, phenological and growth habit traits using 20 F₃ biparental populations.
- To assess the molecular genetic diversity and population structure of 118 Ethiopian chickpea accessions selected for heat tolerance studies.
- To screen Ethiopian chickpea accessions for heat tolerance using phenological and agro-morphological traits and genomic relationships.
- To assess the physiological mechanisms of heat tolerance in chickpea.
- To determine the effect of high temperatures on the reproductive biology of chickpea.

1.4.Significance of the study

The significances of this study were to deliver heat tolerant wild introgressed pre-breeding lines, advanced breeding lines, landraces, and varieties of chickpea to the national chickpea-breeding program that might increase the production and productivity in dry land areas of the country. The study helps to introgress resilient wild genes into elite cultivars for heat tolerance, which is a new approach for Ethiopian chickpea breeding programs. Besides, it helped to identify genomic tools such as QTL/genes and associated molecular markers, which might accelerate conventional

chickpea breeding program for releasing climate resilient chickpea genotypes. The study equipped the PhD fellow with newskills of advanced molecular and genomics techniques. As a whole, the study benefits smallholder farmers, consumers, merchants, food processors, research institutes, government policy makers and non-governmental organizations.

CHAPTER 2

2. Literature Review

2.1. The biology of chickpea

Chickpea (*Cicer arietinum* L.) is a self-pollinated species with $2n=2x = 16$ chromosomes and a genome size of ~740 Mbp (Varsheny *et al.*, 2013). The English word, chickpea, was actually derived from chichpea, referring to Cicer-pea. Chickpea has several names garbanzo and garabanzo (Spanish speaking countries and US), pois chiche, garvanche, garvance and garavane (French), garabanze (German), nakhut, nohut or nut, naut or nohot in Turkey, Romania, Bulgaria, Iran, Afghanistan and in parts of Soviet Union, and chana in India (van der Maesen, 1987). In Ethiopia, chickpea is namedas shimbira in Amharic, shimbura in Oromigna, Tigrigna and Wolaita. This section covers the origin, taxonomy, domestication, distribution, type, consumption, production and productivity of chickpea in detail.

2.1.1. Origin, taxonomy and domestication

Chickpea (*Cicer arietinum* L.) is originated in the Fertile Crescent around the modern-day southeastern Turkey and adjoining Syria along with the closely related wild annual species of chickpea, *C. bijugum*, *C. echinospermum* and *C. reticulatum* (van der Maesen, 1987). von Wettberg *et al.* (2018) revealed that *C. reticulatum* and *C. echinospermum* are confined to southeastern Turkey, near the origin of the Tigris and Euphrates rivers in a region historically known as Mesopotamia.

The genus *Cicer* belongs to family Leguminosae, sub-family Papilionaceae and tribe Cicereae that contains 44 species namely *Cicer arietinum*, eight annual, and 35 perennial wild *Cicer* species. Of the nine annual species, chickpea (*Cicer arietinum* L.) is the only cultivated species (van der Maesen, 1987). van der Maesen (1987) classified the *Cicer* species into four sections based on their morphological characteristics, life cycle and geographical distribution. Eight annual species namely *C. arietinum*, *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. bijugum*, *C. judaicum*, *C. yamashitae* and *C. cuneatum* were placed in section Monocicer, *C. chorassanicum* and *C. incisum* (perennial species) in section Chamaecicer, 23 perennial species in section Polycicer and seven woody perennial species in section Acanthocicer. Based on crossability results involving the annual *Cicer* species, *C. arietinum* and *C. reticulatum* belong to the primary gene pool; *C. echinospermum* belongs to the secondary gene pool; and the remaining species belong to tertiary gene pool.

Chickpea was found Near East archaeological remains between 8-9 millennia B.C. However, it was only well established in the Bronze Age, spread quickly in the Mediterranean area and chickpea appeared in the Iron Age in Ethiopia (Cubero, 1975). Domesticated plants differ from their wild progenitors in several morpho-physiological traits (Abbo *et al.*, 2003). Large differences between the cultivated and the annual wild taxa were found, especially for leaf area, growth habit, plant height, first pod height, pod dehiscence and 100-seed weight. These findings suggest that these traits underwent major changes during domestication.

The performance of the wild annual *Cicer* species for these morphological traits was modest to poor compared with that of the cultigen. Average leaf area per plant in the wild accessions was

never greater than 30 cm², whereas most cultigens showed leaf areas larger than this. The tallest wild accessions were shorter than the shortest *C. arietinum* accessions. The 100-seed weight of wild accessions was generally below 16 g, whereas most *C. arietinum* accessions had heavier seeds (11-38 g). Moreover, *C. arietinum* was the only species, which showed accessions with a semi-erect growth habit and no pod dehiscence. The differences between the cultigen and the wild annual taxa for the above descriptors are a clear indication that they are among the most anthropocentric chickpea traits, which have been deliberately modified by man during domestication of chickpea (Abbo *et al.*, 2003). For millennia, agriculturalists and breeders have focused on selecting organisms with the desirable phenotypes. Strong selection on a few traits during domestication caused a reduction in adaptive variation due to genetic bottlenecks, the strength of which vary depending on the crop, but are particularly pronounced in chickpea. Domesticated chickpea changes in the nature and intensity of selection during domestication and breeding that impart genetic drift, reduce diversity, and increase the frequency of deleterious alleles that constrain the ability to expand the cultivation of crops into arid and semi-arid environments (von Wettberg *et al.*, 2018).

2.1.2. Wild species of Cicer

Chickpea has 43 wild species of which *C. reticulatum*, *C. echinospermum* and *C. bijugum* had the largest variability and were morphologically closest to the cultigen (Robertson *et al.*, 1997). Archeological evidence suggested that a divergence time between *C. arietinum* and *C. reticulatum* was at 9-12 thousand and the preceding divergence between *C. reticulatum* and *C. echinospermum* was at 95-127 thousand years (von Wettberg *et al.*, 2018).

Wild species have been exploited for the transfer of useful genes in chickpea to produce climate resilient chickpea. As chickpea is an annual crop, researchers are usually exploiting variability present in eight annual wild *Cicer* species. The knowledge of genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and to determine the close relatives, which can be exploited for introgression of useful traits into the cultigen in plant breeding programs. Systematic collection and evaluation of wild species for useful traits has revealed presence of a diverse gene pool for tolerance to biotic and abiotic stresses. Wild species have been exploited for the transfer of useful genes in most of the major crops, but little has been done in chickpea improvement (von Wettberg *et al.*, 2018).

The morphological characteristics, genetic crossability, free gene exchange, and cytogenetic studies revealed *C. reticulatum* as the progenitor of the cultigen, *Cicer arietinum*. Most of the introgressions of the cultigen have been from *C. reticulatum*, which is fully cross compatible with the cultigen. However, *C. echinospermum* is partially compatible with the cultigen. It is a distinct and more distant species from the primary gene pool (Kahraman *et al.*, 2017). The crosses of *C. echinospermum* with cultivated chickpea are low, with sterility being encountered in F₁ hybrids or in early segregating generations (Kahraman *et al.*, 2017). The F₂ and subsequent progenies of these two species exhibited varying degrees of sterility (0-100%) and low pod set (von Wetteberg *et al.*, 2018).

The 20 wild accessions of *Cicer reticulatum* and *echinospermum* reported by Shin *et al.* (2019) representing the genetic and environmental breadth of the wild collections that were crossed into cultivated accessions as the first step towards trait introgression. Wild populations offer unique

opportunities to reduce the burden of minor effect deleterious alleles that are often elevated in domesticated species by identification and introgression of wild genome backgrounds that lack undesirable pleiotropies on crop phenotypes. Wild x cultivated hybrids potentially have multiple uses in crop improvement: identifying desirable traits of wild origin for introgression, reducing the load of deleterious alleles, and re-cycling useful cultivated traits previously selected during domestication.

Geographical distribution of the two wild *Cicer* species: *C. echinospermum* and *C. reticulatum* accessions adapted rainfall and altitude ranges of (405-700 mm, 740-1264 m) and (700-1060 mm, 915-1695 m), respectively, both have grown in vertisol, in an altitudinal ranges of 700-1900 mas and at temperature ranges of 28-35°C at summer season in Anatoli, Turkey. Temperatures are higher and rainfall is lower on average in *C. echinospermum* sites compared to *C. reticulatum* sites. *C. echinospermum* typically occurs on more basaltic substrates at lower elevations in open pastures and disturbed meadows with lower tree cover than for *C. reticulatum*, which occurs more frequently on sandstone or granitic substrates in mixed pastures and some disturbed habitats (von Wettberg *et al.*, 2018).

Cultivars of chickpea, *Cicer arietinum*, are currently being improved by introgression of wild *C. reticulatum* and *C. echinospermum* biodiversity with very different flowering time requirements. More understanding is required for how flowering time will depend on environmental conditions in these cultivars developed by introgression of wild alleles (Kozlov *et al.*, 2019). Chickpea phenology may be strongly predicted by accession geographic origin and local environmental conditions at the site of growth. The site of origin-by-growth environment interaction accounts

for about 14.7% of variation in time from sowing to flowering. Secondly, as the adaptation to specific environments is blueprinted in genomes, the effects of genes on flowering time may be conditioned by environmental factors. Genotype-by-environment interaction accounts for about 17.2% of overall variation in flowering time (Kozlov *et al.*, 2019).

Kozlov *et al.* (2019) reported that day length-by-collection site interaction is important for locations Baristepe3, Cudi1, Cudi2, Destek, Gunasan, Karabache, and both day length and humidity by-collection site interaction are important for Baristepe1, Beslever, Dereici, Kayatepe, Kesentas, Oyali, Siv-Diyar, Sarikaya, and Sirnak1 sampling sites. There were no interactions between climatic factors and collection sites in Baristepe2, Cermik, Egil, Kalkan, Ortanca and Savur. There are dependencies between climatic factors, collection sites, genotypes and phenotype. Increasing temperature led to earlier flowering in some locations (e.g. Ortanca, Savur) and later flowering in others (e.g. Kesentas) (Kozlov *et al.*, 2019).

2.1.3. Botany

The cultivated chickpea is a short annual herb that grows as an autumn or winter crop in the tropics and as a summer or spring crop in the temperate environments and possesses an approximately 20-100 cm height (Cubero, 1975). In Ethiopia, chickpea is sown in rotation with cereal crops such as teff (*Eragrostis tef*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) on the heavy black/clay soils (Vertisols). On-farm studies in major growing areas indicated that mid-August to early September is the ideal period for chickpea planting under rain fed condition. Most often, chickpea grows from September to December (Asnake Fikre *et al.*, 2020; Lijalem Korbu *et al.*, 2020).

Depending on the angle of the primary branches and the soil surface, the plant assumes erect, semi-erect, spreading, semi-spreading and prostrate growth habit (Cubero, 1975; Gaur *et al.*, 2010; Chaudhury *et al.*, 2020); however, most accessions have a semi-erect growth habit. Erect is 0-15°; semi erect is 15-25°; semi spreading is 25-60°; spreading is 60-80° from the vertical; and prostrate branches flat on the ground (Singh and Diwakar, 1995). The entire surface of the plant shoot, except the corolla, is densely covered with fine hairs known as trichomes that secrete a highly acidic substance containing malic, oxalic and citric acids which play an important role in protecting the plant against insect-pests. Chickpea has indeterminate growth habit, if conditions remain conducive, chickpea plants would continue to flower and set pod (Gaur *et al.*, 2010).

A chickpea seed has a seed coat, two cotyledons, and an embryo. The seed coat consists of two layers, the outer testa and the inner tegmen, and a hilum. The hilum is the point of attachment of the seed to the pod. The major developmental stages of chickpea are germination, seedling, vegetative, reproductive and senescence stages. Chickpea seeds have hypogeal germination (emergence); i.e. their cotyledons remain underground, while providing energy to the rapidly growing roots and shoots. A depth of 5-8 cm seems to be ideal for the emergence of chickpea. Emergence occurs in 7-15 days after sowing, depending on soil temperature and sowing depth. The embryo consists of an axis and two fleshy cotyledons. The radicle emerges first followed by the plumule. The portion of the axis above the cotyledon called the epicotyl, elongates and pushes the plumule upward. The growth of the plumule produces an erect shoot and leaves, and the radicle grows to produce the roots. The first true leaf has 3-4 pairs of leaflets. The plumular shoot and lateral branches grow continuously to develop into a plant. The number of leaflets per leaf varies from 5 to 17 (Cubero, 1987).

Primary branches emerge from the main shoot, often at the base of the plant (Cubero, 1987). The angle at which primary branches emerge determines the growth habit of the plant. Secondary branches emerge from primary branches, and bear most of the plant's leaves and pods. Tertiary branches may also be formed. The chickpea has a taproot system that is deep and robust; with roots growing to a depth of 1-2 m. Lateral roots extend from a primary taproot. Close to the soil, surface roots develop nodules through symbiosis with *Rhizobium* bacteria. These fix atmospheric nitrogen, which becomes available to the plant. The majority of nodules occur within the top 15cm of soil. The duration of vegetative growth before flowering generally ranges from 40 to 80 days depending on the variety, location, availability of soil moisture and weather conditions (Gaur *et al.*, 2010).

Chickpea flowers are small pea-like flowers, complete and bisexual, and have papilionaceous corolla. Temperature, day length and availability of moisture are the three major abiotic factors affecting flowering. Each flower has five petals, ten diadelphous stamens (nine fused and one free), a style with a slightly broadened stigma, and an ovary with 1-4 ovules. Most cultivars produce a single flower at each flowering node. A few cultivars with two flowers per node (twin or double flowered) are also found (Gaur *et al.*, 2010). In nature, chickpea plants reproduce sexually. Vegetative propagation of chickpea plants has been reported in the laboratory or greenhouse. The chickpea flower goes through five stages of development: closed bud, hooded bud, half-open flower, fully open flower and fading flower. Chickpea is predominantly self-pollinating, as pollination occurs 1-2 days before flower buds open. Outcrossing rates in chickpea are very low that ranges from 0 to 1.9% (Gaur *et al.*, 2010).

Pods appear around 5-6 days after fertilization. The pod wall expands rapidly in the first two weeks after pod initiation. Seed filling proceeds at a slower pace, over 3-4 weeks, reaching maturity after about 6 weeks. Chickpea pods contain between one to three seeds (Cubero, 1987). Cubero (1987) reported average ranges of 30-150 pods per plant and 20-240 seeds per plant. Chickpea biomass and yield are reduced when crops are sown outside the ideal sowing date range. Chickpea is ready to harvest when 90% of the stems and pods lose their green color and turn light golden yellow.

Eight stages of chickpea pollen development, from pollen mother cell development to mature pollen can be distinguished. The development of the pollen takes place inside the anthers. In early stages, the anthers have several sporogenous layers. These primary layers lead to the development of meiocytes, which occur inside the loculus within the anthers. The loculus is surrounded by the tapetum that provides nutrition, metabolites and enzymes required for the development and the protection of the pollen before it degenerates during mitosis (Paupière *et al.*, 2014).

Meiocytes undergo meiotic division to produce haploid tetrads. The four microspores of the tetrad are released by the action of a glucanase, which is produced by the tapetum. The pollen wall consists of two layers; an outer layer named the exine, which is composed of sporopollenin and shows apertures where the pollen can germinate; the internal layer is the intine, composed of pectin and cellulose. The pollen coat plays a protective role during pollen dispersion and can play a role in the attachment to a pollinator. Microspores undergo mitotic divisions to produce the mature pollen, which are composed of a vegetative nucleus and two sperm cells (Paupière *et*

al., 2014). Soluble sugars can be delivered to the pollen by (i) hydrolysis of starch (ii) transport of sucrose from photosynthetic tissues.

2.1.4. Types of chickpea

Based on seed shape, size and color (Cubero, 1975), cultivated chickpea is broadly categorized into two groups, “Kabuli” (macrosperma) and “Desi” (microsperma). They differed in their diverse gene pools-based geographical distribution and different plant type. Desi chickpeas are characterized by angular seed shape, small seeds with a high percentage of fiber, pink flowers, rough seed surface and either semi-erect or semi-spreading growth habit. The common seed colors include various shades and combinations of brown, yellow, green and black. The seeds are generally small (around 0.2 g per seed). Desi types are generally shorter, possessing small leaflets, pods, and widely grown. The stem and leaves may contain anthocyanin pigmentation (Gaur *et al.*, 2010). Desi type constitutes 85% of global chickpea production.

The seeds of kabuli chickpeas are generally large (around 0.3-0.5 g per seed) to extra-large (more than 0.5 g per seed). The kabuli chickpeas are characterized by cream, white or beige-colored seed with ram’s (owl) head shape, thin seed coat, smooth seed surface, white flowers, and lack of anthocyanin pigmentation on the stem and leaves, having semi-spreading growth habit. As compared to desi types, the kabuli types have higher levels of sucrose and lower levels of fiber. The kabuli types receive higher market price than desi types. The price premium in kabuli types generally increases as the seed size increases (Gaur *et al.*, 2010). Kabuli accounts for about 15% of the world chickpea production. A third type of chickpea is designated as

intermediate or pea-shaped, is characterized by medium to small size and round, pea-shaped seeds (Gaur *et al.*, 2010).

2.1.5. Geographical distribution

Chickpea is one of the world's most important legume crops, grown in five continents (Imatiz *et al.*, 2011). Chickpea originated in the Middle East and spread to European countries in the west to Myanmar in the east. It is also found in West Asia and North Africa covering Turkey in the north to Ethiopia in the south, and Pakistan in the east to Morocco in the west. Vavilov (1926) designated two primary centers of origin (now centers of diversity), south-west Asia and the Mediterranean, and a secondary one, Ethiopia. The kabuli types are grown in the Mediterranean region including Southern Europe, Western Asia, and Northern Africa; while the desi types are grown mainly in Ethiopia and the Indian subcontinent (Vavilov, 1926).

Ethiopia had diverse agro-ecologies that render it suitable as one of the highly productive geographies for chickpea cultivation in the world (Asnake Fikre *et al.*, 2018). Chickpea can grow well in an elevation range of 1500 and 2700 m; however, economic yield is obtained within an altitude range of 1700-2600 m. The crop largely grows in highlands that become potential niches. Chickpea grows over 67 districts in 18 zones of the four major chickpea growing regions Oromia, Amhara, Southern Nations Nationalities and Peoples' (SNNP) and Tigray regional states. It is mainly grown in the Central, Northern and Eastern high land area of the country. The major chickpea growing zones of the country are North Gonder, South Gonder, East Shewa, West Shewa, North Shewa, East Gojam, South Wello, North Wello and West Gojam (Sisay Argaye *et al.*, 2021; Mekasha Chichayebelu *et al.*, 2018). Asnake Fikre (2016) pointed out that

there has been a shift towards chickpea production in Ethiopia, from a simple precursor to a principal crop by itself, has resulted in a dramatic change over the last two decades as a result of the increasing socio-economic and climate-responsive role demonstrated by the crop. The market value of chickpea was among the leading factors for considering chickpea among the priority crops for improving smallholder farmers' livelihoods in Ethiopia.

2.1.6. Productivity and production

Chickpea is the second most important pulse crop in the world next to common bean (FAOSTAT, 2018). It is the largest food legume produced in South Asia (Gaur *et al.*, 2014) and the third important food legume both in area and production after common beans and faba beans in Ethiopia (Mekasha Chichaybelu *et al.*, 2021). Globally, chickpea was cultivated in more than 12.7 million ha of land and approximately 12 million tons was produced. In Ethiopia, 237,182 ha of land were cultivated and 470,000 tons was produced (FAOSTAT, 2019; Erana Kebede, 2020; Mekasha Chichayebelu *et al.*, 2021). Chickpea is the second major export commodity next to white pea beans generating nearly 25% of the total legumes export earnings (Lijalem Korbu *et al.*, 2020). Currently, the average national yield is 2,137.7 kg/ha, which is much higher than the global average of about 1,800 kg/ha (FAOSTAT, 2019; Asnake Fikre *et al.*, 2020); nevertheless, it does not reach the attainable yields of 3,500 kg/ha. The integration of improved agronomical practices and high yielding genotypes with appropriate phenology and increased resilience might help to reach the attainable yield potential (Lijalem Korbu *et al.*, 2020).

2.1.7. Consumption

Chickpea is an important source of protein for millions of people in developing countries in Asia, Africa and other peoples who live in arid and semiarid environments who cannot afford animal products and/or consume little animal proteins. Chickpeas are nutrient dense, provide more than 20% daily value of protein, lipids, dietary fiber, folate, and minerals like calcium, magnesium, zinc, iron and phosphorous and vitamin like riboflavin, niacin, thiamin, β -carotene, and β -sitosterol and beneficial phytochemicals (Gaur *et al.*, 2010; Jukanti *et al.*, 2012; Chaudhury *et al.*, 2020).

Chickpea is prepared in numerous different ways around the world. In India, the seeds of desi varieties are ground into flour or split to produce dhal and green leaves. Green seeds are used as a vegetable in cooking; immature green pods and seeds, and sprouted seeds are also eaten as breakfast, and used as animal feed. In Turkey, completely mature chickpeas are used in cooking or eaten roasted as a snack (Yadav *et al.*, 2007; Gaur *et al.*, 2010). The Middle Eastern foods hummus and falafel, usually prepared from kabuli chickpeas, are also widely consumed in Australia. Chickpea is an important source of diet and consumed in Ethiopia. Chickpea seed can be eaten as green vegetable (eshet), roasted (kollo), boiled (nifro) and dry vegetable. The flour of chickpea is also used to make, 'shimbira asa', popular dish during fasting time. Flour of roasted chickpea seeds (shiro) are used to make shiro wot (sauce) and taken with injera (bread). The germinated seeds are served as snacks, curry, green pea, and salad (Asnake Fikre, 2014; Mekasha Chichaybelu *et al.*, 2018).

2.2.Constraints of chickpea production

Major abiotic and biotic stresses that adversely affect chickpea production and productivity are drought, heat, cold, salt, acidity, fusarium wilt (*Fusarium oxysporum* f.sp.ciceris), dry root rot (*Rhizoctonia bataticola*), ascochyta blight (*Ascochyta rabiei*), collar rot (*Sclerotium rolfsi*), botrytis grey mold (*Botrytis cineria* Pres), pod borer(*Helicoverpa armigera*),root nematodes (*Meloidogyne* sp.), Phytophthora root rot (*Phytophthora medicaginis*), and cutworm (*Agrotis* sp.) (Gaur *et al.*, 2012; Gaur *et al.*, 2014; Asnake Fikre, 2014; Rani *et al.*, 2020; Mekasha Chichaybelu *et al.*, 2021). Besides, cultivated chickpea pool has been suffering from low level of genetic diversity, low productivity of landraces, and poor farming practices (Rani *et al.*, 2020; Mekasha Chichaybelu *et al.*, 2021).

Among the abiotic stresses, terminal drought and heat stresses are the most critical limiting factors for chickpea production. Current trends of unpredictable global climate change have resulted in periodic spells of drought stress and frequent episodes of extreme temperature, thus challenging plant growth, development and yield in several crops, including chickpea. Drought is the most important constraint to yield in chickpea, accounting for about 50% yield reduction globally that generally occurs at the terminal stage as the crop is mostly raised on conserved soil moisture under rain-fed conditions. Heat stress at the reproductive stage has become a major constraint to chickpea production because of a large shift in chickpea area from cooler long-season environments to warmer short-season environments; increased area under late sown conditions due to increased cropping intensity; and an expected overall increase in temperatures due to climate change (Gaur *et al.*, 2012).

2.2.1. High temperatures

Plants are exposed to heat stress either using stressed field environments (arid or semi-arid environments) or controlled system such as growth chamber and greenhouse. The temperature for the induction of heat stress responses coincides with 5-10°C above normal conditions and crucial for various molecular defenses before the appearance of heat damage. Plants endure thermo-tolerance either by basal or acquired means. Acclimation of plants to high temperature before exposing to lethal or sub-lethal heat stress helps them to acquire thermo-tolerance. Temperature acclimation changed plants resistance to photo-inhibition in a manner similar to those toward heat stress (Tsai and Hsu, 2009).

Rising global temperatures due to climate change are affecting crop performance in several regions of the world. Constantly high temperatures cause an array of morpho-anatomical, physiological, and biochemical changes in plants, which affect their growth and development, thereby leading to drastic reductions in economic yield (Kaushal *et al.*, 2016). Heat stress is the rise in temperature beyond a threshold level for a period sufficient to cause irreversible damage to plant growth and development (Wahid *et al.*, 2007). Plant survival under heat stress depends on the ability to perceive the heat stress stimulus, generate and transmit the signal, and initiate appropriate plant responses. Plant responses to heat stress vary with the degree and duration of high temperatures, the plant type and genotypes, and developmental stages (Hemantaranjan *et al.*, 2014). Plants detect heat stress through perturbations in metabolism, RNA species, membrane fluidity, assembly of the cytoskeleton, inactivation of enzymes, inhibition of protein synthesis, change in protein conformation and loss of membrane integrity (Hemantaranjan *et al.*, 2014; Driedonks *et al.*, 2015).

Heat stress affects plants at various organizational levels, primarily accelerating phenology to limit biomass production and shortening reproductive phase to curtail flower and fruit numbers, thus resulting in severe yield losses. Besides, heat stress also disrupts normal growth, development, cellular metabolism, and gene expression, which alters shoot and root structures, branching patterns, leaf surface and orientation, and anatomical, structural, and functional aspects of leaves and flowers. The susceptibility to high temperatures in plants varies with the stage of plant development, heat stress affecting both vegetative and reproductive stages. Morphological changes of heat stress at the vegetative stage include fruit discoloration and damage, scorching and sunburn on leaves, leaf senescence and abscission, shoot growth inhibition, reduction in first internode length, and inhibited root growth (Wahid *et al.*, 2007; Kaushal *et al.*, 2016). Siddique *et al.* (1999) revealed that a few days of heat stress (30-35°C) during seed filling accelerates senescence, decreases seed set and seed weight, and reduces yield in legumes. High-temperature stress reduces seed size due to the insufficient accumulation of photosynthates during seed filling (Kumar *et al.*, 2016). In chickpea, Summerfield *et al.* (1984) suggested that the longer the exposure during reproductive development to a high day temperature of 35°C, the lower the yield.

2.2.1.1. Effect of heat stress at reproductive stages

Heat stress has a significant negative impact on both the reproductive structures and functions compared to other developmental stages. Effect of heat stress on reproductive structures include meiosis disruption, tapetal hypertrophy, anther protein degradation and indehiscence, pollen tube deformation, delayed/accelerated flowering, and flower abortion. The number of flower and pollen, and size of flower, ovary, pollen and style are being reduced. There will be deformity of

floral organ, reduced stigmatic receptivity and callose deposition in the style. The damage to embryo sac component, impaired fertilization and post fertilization. Small endosperm, embryo abortion, poor seed set, and reduced seed filing rate, seed number, seed weight, yield and remobilization of photosynthates to seeds were observed in chickpea, tomato, wheat, maize and field pea (Devasirvatham *et al.*, 2012; Devasirvatham *et al.*, 2013; Kaushal *et al.*, 2016; Sita *et al.*, 2017; Chaudhary *et al.*, 2020). The effects of heat stress on reproductive functions include the reduction of pollen number, pollen viability, pollen germination, and pollen tube growth. In addition, it causes abnormal pollen development, reduced stigma receptivity, pollen-pistil interaction, ovule viability, proembryo and fertilized embryo, small endosperm, embryo abortion, pollen sterility and poor pod set (Devasirvatham *et al.*, 2013; Kaushal *et al.*, 2016).

Pollen is one of the most sensitive organs to heat stress that causes pollen sterility and leads to reduction of fruit sets in tomato (Paupière *et al.*, 2014). The tapetum is the key organ that provides metabolites to the pollen; its development is very sensitive to heat stress. It is clear that the development of the tapetum is critically sensitive to heat stress and can lead to abnormal anther development. Reduction of pollen germination under heat stress can be due to earlier pollen development failure. The authors also found that during normal pollen development, starch accumulated in pollen reaching a maximum content three days before anthesis and from this moment, onwards starch was converted into soluble sugars. However, under hot conditions, starch concentration did not increase and led to a lack of sugar conversion, explaining the decrease of soluble sugars in mature pollen grains (Paupière *et al.*, 2014). The correct functioning and timely degradation of the tapetum is important. Impaired sucrose metabolism in

leaves and anthers was associated with heat stress induced reproductive failure (Firno *et al.*, 2006; Sita *et al.*, 2017).

2.2.1.2. Traits associated with heat tolerancescreening

Pollen-based screening methods can be used for evaluating thermo-tolerance in crop plants. Heat stress during the reproductive phase affects pollen viability, fertilization, pod set and seed development leading to abscission of flowers and pods and substantial losses in grain yield (Gaur *et al.*, 2014). The yield related traits most affected by high temperature were pod number and harvest index. Reduced pod number was mainly because of reduction in reproductive efficiency (pod to flower ratio) indicating that under high temperatures reproductive development was impaired. It is concluded that for heat tolerance in chickpea, breeding efforts need to be focused on improving the reproductive efficiency (Jumrani and Bhatia, 2014). Several phenological and agronomical traits were considered for heat tolerance under field and control conditions to assess the genetic variability under heat stress in chickpea (Devasirvatham, 2012; Paul *et al.*, 2018; Tsegaye Getahun *et al.*, 2021b).

Canci and Toker (2009) identified several heat tolerant genotypes including wild *Cicer* spp and suggested harvest index, grain yield and pods per plant as the traits to be considered for selection. In addition, Krishnamurthy *et al.* (2011), Paul *et al.* (2018) and Tsegaye Getahun *et al.* (2021b) used traits namely number of filled pods and seeds, harvest index, %pod set, and grain yield as traits to be used in order to identify heat tolerant chickpea genotypes. Moreover, Paul *et al.* (2018) and Tsegaye Getahun *et al.* (2021b) used heat visual score as a trait for selection of heat tolerant genotypes. Singh *et al.* (2008) noticed that biological and seed yields had a

significantly positive correlation with total chlorophyll content and relative water content and negatively correlated with membrane injury index. Pollen viability and seed-set were a measure of reproductive success under heat stressed conditions in tomato breeding programs. Positive correlations were found between physiological and yield related traits. For instance, pollen viability was positively correlated with filled pods/plant in chickpea. There is a strong correlation between pollen production and viability, anther dehiscence, and seed-set in tomato. Yield attributes under high temperatures were utilized for correlation analysis with physiological traits to identify the most reliable traits for heat tolerance breeding of rice genotypes (Firon *et al.*, 2006; Singh *et al.*, 2008; Devasirvatham *et al.*, 2013).

2.3. Heat stress tolerance mechanisms in chickpea

Wahid *et al.* (2007) reviewed the mechanisms of plastic responses to heat stress. Plants implement various adaptation mechanisms such as morphological, anatomical, biochemical and molecular mechanisms. Some of them are escaping, change morphological and anatomical structures, maintenance of membrane stability, scavenging of reactive oxygen species, production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated and calcium-dependent protein kinase cascades, and chaperone signaling and transcriptional activation. Based on heat stress responses, they can be classified as tolerance, escape, or avoidance, which is a vital first step towards developing stress-tolerant crops for the future hotter climate (Fig.2.1)(Wahid *et al.*, 2007; Kaur *et al.*, 2016; Rani *et al.*, 2020).

1. Heat escape, escape usually refers to the ability of plants to flower and perform the majority of the whole crop cycle before stress arrival. Plants can escape heat stress with early phenology

(early flowering, podding and maturity) which might reduce total plant biomass and other yield related traits. It might mitigate the effects of terminal drought and heat stresses in breeding for earliness in both chickpea and pigeonpea. Escaping is an adaptation mechanism of chickpea to short-season environments. Flowering time and crop duration are the most important traits for adaptation of chickpea to different agro-climatic conditions including the arid and the semi-arid environments (Gaur *et al.*, 2014; Mallikarjuna *et al.*, 2017).

2. Heat avoidance is associated with change in leaf reflectance and orientation, reduction of non-photosynthetic energy intercepted by the canopy, transpiration rate, stomata conductance and alteration of membrane lipid composition.

3. Heat tolerance is linked to membrane stability, signal cascade and transcriptional control. It is also associated with antioxidant defense, chlorophyll contents, chlorophyll fluorescence, photochemical efficiency, photosynthetic rate, accumulation of heat shock proteins and osmo-protectants (Firno *et al.*, 2006; Devasirvatham *et al.*, 2012; Hasanuzzaman *et al.*, 2013; Kaur *et al.*, 2016).

Few heat tolerant (ICC1205, ICC15614, ICCV92944 (JG 14), DZ-2012-ck-0034) and sensitive (ICC10685, ICC5912, ICC 4567, DZ-2012-ck-0026) chickpea genotypes were identified by exposing plants to high temperatures ($\geq 35^{\circ}\text{C}$) (Devasirvatham *et al.*, 2012; Devasirvatham *et al.*, 2013; Tsegaye Getahun *et al.*, 2021b). Such genotypes are important for understanding the morphological, physiological, biochemical and molecular mechanisms of heat tolerance (Wahid *et al.*, 2007; Kaushal *et al.*, 2016). Heat-tolerant genotypes are characterized by higher photosynthetic rates and chlorophyll contents, increased membrane thermo-stability, relative

water content than sensitive genotypes (Meena *et al.*, 2015). In wheat, the tolerant genotypes maintained high rate of photosynthesis, chlorophyll content, and stomata conductance, high seed set and grain weight, and extended grain filling duration under heat stressed conditions (Yang *et al.*, 2002).

2.3.1. Physiological mechanism

Heat stress reduces the functions of physiological traits such as canopy temperature depressions, photosynthetic rate, evapotranspiration, stomata conductance, photochemical efficiency, leaf relative water content, chlorophyll content, membrane thermo-stability, linear electron flow, chlorophyll fluorescence, closure of stomata, decline in photosystem II activity, and vapor pressure deficit. It also causes the loss of grana stacking and swelling of grana, and alters structural organization of the thylakoids (Wahid *et al.*, 2007; Farooq *et al.*, 2011; Savić *et al.*, 2012; Devasirvatham *et al.*, 2013; Hemantaranjan *et al.*, 2014; Paupière *et al.*, 2014; Meena *et al.*, 2015). Membrane integrity, chlorophyll content, photochemical efficiency and cellular oxidizing ability were inhibited by the increase in temperature, with greater impacts on the sensitive genotypes (Kumar *et al.*, 2012). In chickpea, pollen grains were more sensitive to high temperature than stigmas in both controlled and field conditions (Devasirvatham *et al.*, 2013). Photosynthesis is the most sensitive physiological process to high temperature that is responsible for yield of a crop. Photochemical reactions on the level of thylakoids and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of injury at high temperatures (Wahid *et al.*, 2007).

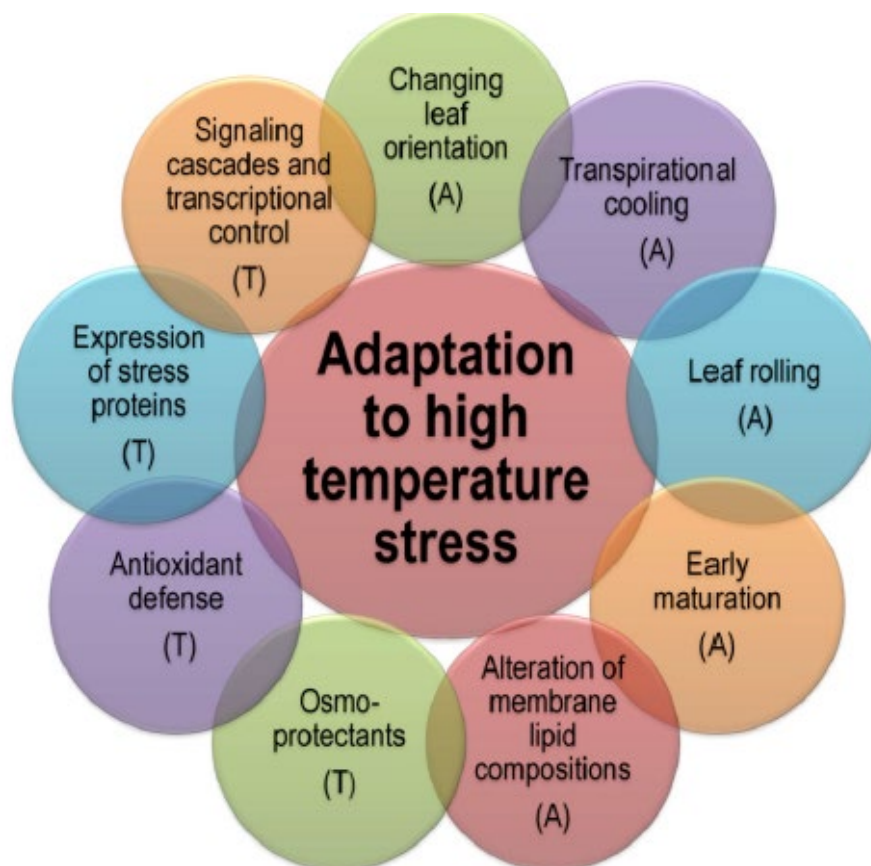


Fig. 2.1. Different adaptation mechanisms of plants to high temperature, A: means avoidance, T: means tolerance (Hasanuzzaman *et al.*, 2013).

Many physiological traits have been used for thermo-tolerance studies in wheat, tomato, soybean, mungbean, field pea, lentil and chickpea to screen large numbers of genotypes tolerant to water and heat stresses, which complement conventional breeding methods and could increase selection efficiency. Drought stress tolerant genotypes possessed higher biomass, chlorophyll fluorescence, stomata conductance, net photosynthesis, and relative water content in comparison to sensitive genotypes (Kadir and von Weihe, 2007). The carbohydrate content of developing and mature pollen grains could be an important factor in determining pollen quality, as heat-tolerant

tomato cultivars appear to have a mechanism for maintaining the appropriate carbohydrate content under heat stress (Firon *et al.*, 2006).

2.3.2. Biochemical mechanisms

Elevated temperature affects the metabolic pathways mainly through oxidative damage to cells, thereby affecting the levels of both primary and secondary metabolites. Reactive oxygen species (ROS) have been recognized as key players in the complex signaling network of plants stress responses. The main injuries of ROS under high temperatures include protein denaturation, increased fluidity of membrane lipids, inactivation of enzymes, reduced synthesis and degradation of proteins, and decrease membrane integrity (Driedonks *et al.*, 2015). The antioxidant defense system in plants involves both enzymatic and non-enzymatic antioxidant systems such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes namely superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX). Non-enzymatic antioxidants cooperate to maintain the integrity of the photosynthetic membranes under oxidative stress. The enzymatic components may directly scavenge ROS or may act by producing a non-enzymatic antioxidant.

Under heat stress, upregulation of various enzymatic and non-enzymatic antioxidants induce thermo-tolerance (Asthir, 2015). It was observed that the activities of SOD, APX, CAT, GR and POD increased significantly at all stages of growth in heat tolerant wheat cultivars in response to heat stress (Almeselmani *et al.*, 2009). Balla *et al.* (2009) demonstrated that the activity of the enzymes glutathione *S*-transferase (GST), APX and CAT was more enhanced in the wheat cultivar showed better tolerance to heat stress and projection against ROS production. They

reported that the tolerant wheat varieties appeared to be correlated with the antioxidant level, though changes in activity were observed for different antioxidant enzymes. Meena *et al.* (2015) studied the effect of high temperature stress on various biochemical parameters of chickpea. In all the chickpea genotypes high temperature stress also showed increased activity of antioxidant enzymes such as POD, GR, and SOD.

Early synthesis of primary metabolites such as free proline, glycine betaine and soluble sugars are immediate response to heat stress (Wahid, 2007). Proline may differentially affect stress tolerance, increase the activity of many enzymes, and stabilize protein integrity, maintain redox balance, regulate development and control mitochondrial functions (Lee and Suh, 2013). The production of glycine betaine in tobacco reduced heat stress induced photo-oxidation by enhancing the protection of photosystem II, correlated with elevated levels of antioxidants, and increased thermo-tolerance (Yang *et al.*, 2007). The accumulation of phenolic compounds such as flavonoids, anthocyanins and lignins increases due to heat stress (Wahid, 2007) thereby reduces the oxidation effect of ROS. In addition, sucrose or starch synthesis is adversely affected as the activity of sucrose phosphate synthase; ADP glucose pyrophosphorylase and invertase are found to be decreased (Trivedi, 2015). Several key phytohormones including abscisic acid (ABA), salicylic acid (SA), and ethylene (ET) also increase their levels under heat stress (Bita and Gerats, 2013).

2.3.3. Molecular mechanisms

Molecular mechanisms of heat stress tolerance involve change in gene expressions (Wahid *et al.*, 2007). Transcriptome analysis of arabidopsis has revealed Hsfs, other transcription factors and

multiple signaling pathways for thermo-tolerance (Kotak *et al.*, 2007). HSR genes have been identified using genetic screens and genome wide expression studies such as transcriptome, proteome and metabolome profiling (Bita and Gerats, 2013; Hemantaranjan *et al.*, 2014). The HSR is a transient reprogramming of gene expression and conserved biological reaction of cells and organisms to elevated temperatures (Hemantaranjan *et al.*, 2014). It is characterized by inhibition of normal transcription and translation, an enhanced expression of heat shock proteins (Hsps) and induction of thermo-tolerance (Trivedi, 2015). Transcriptional profiling has been performed during the onset and recovery of heat stress, between heat-tolerant and heat susceptible variants (Driedonks *et al.*, 2016).

Expression of Hsps, antioxidants, Hsfs and other transcriptional factor genes are adaptive HSRs for heat tolerance in crop plants (Wahid *et al.*, 2007; Farooq *et al.*, 2011; Trivedi, 2015). These genes are classified into regulatory and functional genes (Qu *et al.*, 2013) which have been reported in a variety of plants including rice and arabidopsis. Functional genes are antioxidant and Hsp genes (Qu *et al.*, 2013). Plant gene regulations in response to heat stress are regulated by the diversity of plant heat stress (shock) transcriptional factors (Hsfs) (Koskull-Doring *et al.*, 2007). The expression of Hspgenes is genetically controlled at the transcriptional level by the binding of Hsfs to heat shock elements, located in the promoter regions of Hspgenes (Koskull-Doring *et al.*, 2007). Hsfs are the terminal components of the signal transduction chain mediating the activation of genes responsive to heat stresses (hs).

The up-regulation of “heat shock genes” which encode Hsps is necessary for plant’s survival under fatal high temperature (Chang *et al.*, 2007). Hsps are involved in signaling, translation,

host-defense mechanisms, folding and unfolding of cellular proteins, lipid, carbohydrate and amino acid metabolisms (Wahid *et al.*, 2007; Farooq *et al.*, 2011; Usman *et al.*, 2014; Asthir, 2015). The Hsps comprise five principal classes of proteins, designated by their approximate molecular masses (kD), amino acid sequence homologies and activates as molecular chaperones: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small Hsp with molecular masses 15-30 kD (sHSPs). The most prominent and diverse group of heat induced proteins in plants are sHsps. Hsps are specific to nucleus, cytosol, plastid, mitochondria and endoplasmic reticulum (Wahid *et al.*, 2007; Usman *et al.*, 2014). Each class has specific function in different crops such as rice, wheat, arabidopsis, maize, soybean, sorghum, pearl millet, tomato and barely (Usman *et al.*, 2014). The accumulation of Hsp 18 and Hsp 21 in the tolerant genotypes of potato cultivars is 3 fold and 1.7-2.3 folds higher compared to the sensitive cultivars, respectively (Savić *et al.*, 2012).

Proteomics allows the characterization of heat-responsive proteins in different plant tissues and reveals molecular basis of heat tolerance mechanisms (Huang and Xu, 2008). Transcriptomic and proteomic studies on rice responses to high temperature have obtained valuable results. Genes and proteins responsive to high temperature during rice grain development were identified. The two most abundant groups were photosynthesis/photorespiration- related proteins and heat shock proteins. Fourteen protein spots were identified as Hsps (Peverelli and Rogers, 2013). Dong *et al.* (2015) profiled gene expression in two Chinese cabbage inbred lines with different thermo-tolerances. Many genes exhibited >2-fold changes in expression upon exposure to 0.5-4 h at 45°C. Four sorghum (*Sorghum bicolor* L.) genotypes were exposed to a temperature at 47°C (10

to 180 min). The highest level of Hsp90 gene expression was realized upon exposure to heat for either 60 or 30 min and their expression levels differed among the genotypes (Oi *et al.*, 2011).

2.4.Introgression breeding

Most recent varieties have been developed through hybridization. Two individuals with desirable and complementary phenotypes are crossed; F₁ progeny are self-pollinated to fix new, improved genotype combinations. Hybridization between crops and wild relatives is the current research focus to generate phenotypic novelty that confers adaptive traits, provide powerful tools for trait dissection and appropriate vehicle for genomic basis of agronomic traits. von Wettberg *et al.* (2018) reported a subset of 284 F₃ phenology-normalized lineages obtained from crosses with a single early flowering cultivated lineage (ICCV 96029). Crop wild relatives are a reservoir of phenotypic variation and contain a much wider spectrum of diversity in their genomes absent in the germplasm of cultivated species (Warschefsky *et al.*, 2014).

The major steps to develop wild introgressed pre-breeding lines are evaluation of wild relative to identify promising accessions as donors; hybridization using diverse accessions of wild species as donors and well adapted cultivars as recipients; development of large sized pre-breeding populations having desired agronomic background of cultivated types through backcrossing; precise evaluation of pre-breeding populations across multi-locations; identification of promising pre-breeding populations (introgression lines) in good agronomic background; and enrich variability (Sharma, 2017).

Kahraman *et al.* (2017) reported that zygotic stages were observed in cross pollinated flowers with all crop wild relatives of chickpea, only those from *C. reticulatum* and *C. echinospermum* gene pool species continued to progress to the subsequent embryo stage. Wild relatives such as *C. reticulatum* and *C. echinospermum* are crossable to the cultigen that can be exploited to introgress abiotic stress tolerance to the cultigen. Kahraman *et al.* (2017) revealed that populations of *C. echinospermum* had variable degree of compatibility with cultivated chickpea. In some populations of echinospermum crosses, hybrid sterility occurred in all individuals (F_1 s failed to yield F_2 seed). A portion of F_2 individuals in each population exhibited normal, high rates of pod setting and seed maturation (Kahraman *et al.*, 2017).

Kahraman *et al.* (2017) reported that hybrids from crosses of some accessions of echinospermum; Gunasan and S2Drd exhibited high fertility, while crosses from the Karabache genotype were of variable fertility. They segregated for sterility (hybrid breakdown) in later generations. The number of F_2 seeds from Gunasan and S2Drd varied (<200->400 F_2 seeds per F_1 plant) depending on the cultivation conditions. These yields are comparable with the range of F_2 seeds per F_1 plant obtained from the interspecific hybrids of *C. reticulatum*. Most individuals from Karabache and some individuals from Gunasan and S2Drd crosses, pod development initiated but failed to be sustained beyond 1 to 2 weeks, yielding small empty pods that fell away. Pod development continued longer to sizes of normal seed-filled pods, but at maturity, such pods contained only incompletely developed shriveled seeds; sterile plants tended to grow vigorously, with dense branching and a long flowering period (Kahraman *et al.*, 2017).

Development of a multi-parent advanced generation inter-cross (MAGIC) population and nested associating mapping (NAM) population are the most recent breeding schemes. Now days, multi-parent advanced generation inter-cross (MAGIC) populations were suggested as a way to introduce more genetic variation and determine the genes involved in thermo-tolerance (Ye *et al.*, 2015). The large number of accumulated recombination events in MAGIC populations increase novel rearrangements of alleles and bring about greater genetic diversity. Nested association mapping (NAM) population is derived from a multiple-cross mating design sharing one common parent, which may provide high power and resolution through joint linkage and association analysis, and a broader genetic resource for quantitative trait analysis (Meng *et al.*, 2015).

2.5.Molecular markers

Assessing the existing genetic diversity of chickpea accessions and determining the genetic basis of heat resilience using genomic assisted breeding (identify QTL/genes/alleles, associated molecular markers, and marker trait-associations) using molecular markers are crucial to overcome the number one production constraint of chickpea (high temperatures). The genetic markers include morphological, biochemical and DNA markers (molecular markers) tightly linked with the traits of agronomic importance that can be employed in selection. Morphological markers can be scored quickly without specialized laboratory equipment but their expression at specific growth stages and the possibility of analyzing only a limited number of markers in a single population limit their use in breeding programs (Tanksley and Rick, 1980).

Many protein variants of an enzyme, referred to as isozymes, can be resolved by electrophoresis and are very useful genetic markers. The alleles of most isozyme markers segregate in a co-dominant manner and rarely show epistatic interactions, which allows accumulation of many polymorphic isozyme loci in a single F₂ population and increases the efficiency of gene mapping. Once the map locations of isozyme genes are known, they can be used efficiently as biochemical markers to map other genes for morphological and physiological traits (Tanksley and Rick, 1980). However, only a relatively small number of protein variants may exist between the two parents and this limits the total number of protein loci that can actually be scored in a given mapping population.

A molecular marker is a particular segment of DNA that is representative of the difference at the genome level, which may or may not correlate with phenotypic expression of a trait. Molecular markers are not influenced by environment, stable, detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell and do not exhibit epistatic interactions. The major molecular markers include restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSRs), diversity array technology (DArT) and single nucleotide polymorphisms (SNPs). The PCR based markers have been widely used because of their technical simplicity, ease of screening large number of samples in a short period and can be carried out in a moderately equipped laboratory (Singh *et al.*, 2008). Molecular markers have been widely used to: a) identify and discriminate closely related cultivars, b) assess taxonomic and phylogenetic relationships of a crop species, c)

construct high-density genetic linkage maps, d) study genome organization, and e) tag loci affecting qualitative or quantitative traits (Azhaguvel *et al.*, 2006).

Marker assisted selection is a process by which a molecular marker is used for indirect selection of a genetic determinant, a trait of interest, as a result there is a shift from phenotype-based to genotype-based selection and used to introgress those loci. MAS is an approach where genetic markers, usually DNA based markers, are used to monitor the presence of desirable and undesirable genes in plant breeding. Based on the information provided by the genetic markers, breeders will only need to field test those breeding plants that are most likely to have desirable traits (Collard and Mackil, 2008). When a molecular marker is found that co-segregates with a major gene for an important trait, it may be easier and cheaper to screen for the presence of the marker allele linked to the gene, than to evaluate the trait (Mahajan and Gupta, 2012).

Molecular markers linked with quantitative trait loci (QTL)/genes for traits of interest are useful for the improvement of many crops. Recent progress in the development of molecular markers and molecular mapping of QTL/genes of interest has made it possible to initiate genomics-assisted breeding in chickpea (Gaur *et al.*, 2012). Gene introgression from the wild species can be monitored effectively with the use of molecular markers, which will expedite the transfer of agronomically important traits into desirable genetic backgrounds for the development of varieties with durable resistance through marker-assisted selection. Molecular markers offer direct examination of the introgressed gene at any given locus from the donor genome DNA and can analyze the size and location of residual donor genome carried along through gene

introgression and provide the breeder additional information for monitoring the elimination of unwanted donor DNA.

The development of molecular markers would assist germplasm screening for improved heat tolerance and allow for efficient breeding of the complex trait. Molecular markers have been used extensively for identification and mapping of genes/QTLs in response to heat stress for many crop plants such as bread wheat (Sadat *et al.*, 2013), rice (Buu *et al.*, 2014), tomato (Foolad, 2008), and maize (Naveed *et al.*, 2014). However, limited research has been done to identify genetic markers associated with heat tolerance in chickpea. Thus, there is an urgent need to understand genetic factors affecting heat tolerance as well as to identify new diagnostic markers to be deployed in MAS, which will ensure faster yield gains under stress environments (Kaur *et al.*, 2016).

GBS-based SNP markers were used to identify key genomic regions (QTLs) responsible for many agronomic traits and the associated SNP markers may facilitate introgression of these genomic regions into domesticated chickpea which could help in developing high yielder and stress tolerant pre-breeding lines (von Wettberg *et al.*, 2018). SNPs are a marker system that can differentiate individuals based on variation detected at the level of a single nucleotide base in the genome. They are generally more abundant, stable, efficient and cost-effective than other forms of genetic variants and amenable for high- to ultra-high-throughput detection platforms.

2.5.1. Molecular genetic diversity

Phenotypic variation is the occurrence of differences among individual plants due to their genetic makeup, the environment in which they raised and their interactions. Genetic diversity can be measured by quantifying variation in morphological characters and analyzed using genetic makeup. Evaluation of genetic variation in chickpea germplasm is indispensable for effective selection in genetic improvement of agronomic and yield traits for heat stress. Molecular genetic markers can be used to recognize unique tags to identify individuals in a population and can give an exact resolution in assessing the diversity at gene level. Unlike morphological markers, molecular genetic markers are not influenced by the environment, plant ontogeny and can be analyzed at different stages of plant development and help in accelerating the development of new varieties (Samyuktha *et al.*, 2018).

The genetic diversity of chickpea was assessed using various molecular markers namely ISSR, RFLP, RAPD, AFLP, SSR, SNP, DArT and GBS markers (Varshney *et al.*, 2007; Thudi *et al.*, 2014). Deokar *et al.* (2014) identified 1415 SSR loci that revealed extensive genetic diversity among the chickpea accessions. Parida *et al.* (2015) reported 1470 SSR markers from diverse coding and non-coding regions of the chickpea genome. Thudi *et al.* (2014) used 35 SSRs, 15,360 DArT and 651 SNPs markers for comprehensive genetic characterization of 300 chickpea accessions independently. However, SSR, SNP and GBS markers have become the markers of choice for genetic characterizations and breeding applications in legume crops (Varshney *et al.*, 2007).

Among several molecular genetic markers, microsatellite (SSR) markers are still one of the marker of choice for genetic diversity studies due to its co-dominant nature, high reproducibility and abundance, well distributed in the genome, hyper-variability, suitable for high throughput analysis, multi-allelic feature, high discriminatory power, largely associated with non-repetitive DNA, have PCR-based analytical methodology and high polymorphic information content (Thudi *et al.*, 2014). SSR markers are polymorphic DNA loci consisting of short nucleotide motifs (short tandem repeats) of 2-6 bp long. Among which Di-(GT)_n, tri-(AAT)_n and tetra-(GATA)_n nucleotide repeats are widely distributed throughout the genomes of plants and animals (Parida *et al.*, 2015).

Based on the length of repeat motifs, SSRs markers were classified into two namely hyper variable class I (≥ 20 bp) and potentially variable class II (12-20bp) types. They were found in different coding (CDS) and non-coding (5'-UTRs, introns and 3'-UTRs) sequence components of genes, upstream and intergenic regions. Structural annotation of identified SSRs in chickpea genome revealed their highest frequency in intergenic regions followed by introns, CDS, and upstream regions. As SSR repeat-length variations increase, their polymorphism decreased (Parida *et al.*, 2015). Among the polymorphic SSRs obtained, di-nucleotide repeat-motifs were most abundant followed by tri-nucleotide repeat motifs (Parida *et al.*, 2015). All five different classes of SSR repeat-motifs (di-to hexa-nucleotides) were predominant particularly in the intergenic regions as compared to various coding and non-CDS components of genes in chickpea. The development of SSR markers using molecular methods requires a great deal of time, effort and investment in construction and screening of genomic libraries and sequencing of clones containing SSR, primer development and their validation. However, a large number of

EST and genomic DNA sequences available in the public databases provides an alternative method of microsatellite development.

Characterization of natural allelic diversity and understanding the population genetic structure using highly polymorphic SSR markers are required to facilitate chickpea genetic improvement (Saxena *et al.*, 2014). SSR markers are ideal markers for allele mining, molecular genetic diversity pattern, genetic relationships, association genetics, genetic mapping and identification of genes, phylogenetic patterns, population genetic structure studies, cloning gene(s) and marker assisted selection in chickpea accessions (Parida *et al.*, 2015; Thudi *et al.*, 2014). In addition, a molecular genetic diversity analysis of chickpea germplasm can provide useful information for the selection of parental material, crop conservation and management studies, and help breeding programs to plan ahead of time (Parida *et al.*, 2015; Saxena *et al.*, 2014; Samyuktha *et al.*, 2018).

2.5.2. Genomics diversity and relationships

Using genome wide sequence information, determination of genetic diversity and their relationships among individual breeding materials, within and among populations is very crucial in crop improvement strategies to infer the patterns of diversity and important to promote the conservation and sustainable use of crop plants (Tsegaye Getahun *et al.*, 2021b). One can understand the degree of genetic variation in tested materials so that suitable parents can be selected to develop bi-parental populations for trait-locus mapping and molecular breeding (Basu *et al.*, 2019; Kujur *et al.*, 2015). SNPs are sequence based, high-throughput, inexpensive, highly polymorphic and naturally abundant biallelic molecular markers found throughout the genome. Genome wide SNP markers are the markers of choice for determination of genomic diversity and

phylogeny, to study the extent of LD and population structure, identifying marker-trait associations, to construct high density linkage map and employing marker assisted selection in crop plants (Kujur *et al.*, 2015; Cui *et al.*, 2017; Basu *et al.*, 2019).

In contrast to other molecular markers, genome wide SNP data provided unbiased estimates of genetic diversity and a few thousand SNPs are sufficient to estimate genome wide diversity and to distinguish among populations differing in genetic variation (Fischer *et al.*, 2017). Fischer *et al.* (2017) suggested that analysis of genetic diversity within and among populations by using randomly selected SNP data was used to draw conclusions on genome wide diversity patterns. Kujur *et al.* (2015) depicted that the genome-wide discovery and high-throughput genotyping of SNPs in chickpea natural germplasm lines is indispensable to extrapolate their natural allelic diversity, domestication, and LD patterns in chickpea. Marker-trait associations were reported in chickpea using DArT (Devasirvatham, 2012); and SNP markers (Teshome, 2012; Kujur *et al.*, 2015 and Basu *et al.*, 2019) under stressed and non-stressed conditions.

2.5.3. Modern sequencing technologies in Genomics

The advances in sequencing technologies have motivated the development of several low-cost sequence-based genotyping methods for genome-wide genetic characterization of large number of individuals in genetic mapping and population genetic studies. The new high-throughput sequencing technologies greatly facilitate QTL analysis by providing genetic markers at genome-wide resolution in any species without previous knowledge of its genome (Gomez *et al.*, 2011).

RAD and GBS are the most recent Next Generation Sequencing (NGS) genotyping platforms and become the most popular approaches to conduct population genomics studies in model and non-model organisms, which subsample the genome of multiple individuals at homologous location. They have been used to genotype not hundreds but thousands of markers across almost any genome of interest in a single step (Bhatia *et al.*, 2013). They reduce genome complexity coupled with restriction enzymes and multiplex Next Generation Sequencing (NGS) for high-density SNP discovery and genotyping of many individuals simultaneously.

RAD and GBS have been applied for the construction of linkage map, performing phylogenetic studies, construction of Genome Wide Association Studies (GWAS), studying epigenetics, Marker Assisted Selection (MAS), Genomic Selection, genomic diversity, evolution, conservation and seed purity of crop plants such as oilseed rape, wheat, barely, tef, sunflower, ryegrass, soybean, rice, cotton and others. In addition, RAD and GBS can help trace the evolution of alleles, map based cloning of QTLs and a dense set of SNP marker development, identify new useful haplotypes and guide the development of allele-specific markers for use in the identification and functional validation of candidate genes for quantitative traits (Kole *et al.*, 2015). They also help in understanding the functions of individual genes and their networks, for defining evolutionary relationships and processes.

2.5.4. Genomics assisted breeding (marker-trait associations)

In the past few decades, there have been considerable developments in genetics, biochemical, genomics, transcriptomics, proteomics and metabolomics approaches to enhance the understanding of abiotic stress tolerance (Chaudhary *et al.*, 2020). Genomic technologies

are expected to improve the precision and efficiency of chickpea breeding in the development of improved cultivars with enhanced resistance to abiotic and biotic stresses, better adaptation to existing and evolving agro-ecologies (Gaur *et al.*, 2012; Rani *et al.*, 2020). They are required for introgression of useful traits through identification of underlying genes, alleles or quantitative trait loci from the wild *Cicer* species into the cultigen (Singhet *et al.*, 2008). Crop wild progenitors are likely to be of particular value because they possess unexplored variation ('genomic gems') that could accelerate breeding gains beyond what is possible from the domesticated gene pool alone. Identification and utilization of favorable gene loci or alleles from wild or progenitor species can serve as an effective approach to increasing genetic diversity and breaking abiotic and biotic bottlenecks in plant breeding. The application of genomics to identify and transfer valuable agronomic genes from allied gene pools and crop relatives to elite crops will assist in meeting the challenge of global food security (Kole *et al.*, 2015).

To unravel the genetic basis of complex traits that segregate in wild-cultivated hybrid populations will facilitate chickpea improvement by linking genetic and phenotypic variation in a quantitative genetic framework (Shin *et al.*, 2019; Holland, 2007; Karikari *et al.*, 2019). Genomics-assisted breeding includes the combination of QTL mapping with association mapping that provided an abundance of DNA marker-trait associations (Smallwood, 2012). Shin *et al.* (2019) proposed that there might be introgression of adaptive alleles from the wild progenitors of chickpea into cultivated chickpea for high temperature stresses.

2.5.4.1. Genome wide association study

Understanding the genetic basis of heat tolerance traits is needed in chickpea both for genomic-guided crop improvement and to identify underlying genes (Devasirvatham, 2012; Paul *et al.*, 2018). As a prelude to genetic analyses, one must first identify genotypes that differ in heat tolerance traits. Genome-wide association studies have the potential to identify causal genetic intervals but require large and properly structured study populations, detailed and accurate phenotyping, and significant resources for genomic analysis (von Wettberg *et al.*, 2018; Tsegaye Getahun *et al.*, 2021b).

The essence of Genome-wide association study (GWAS) is a marker-based study of associations between a genome-wide set of genetic variants in a population and a trait. Association mapping is used to identify QTLs/genes responsible for quantitative variation of complex traits like heat, cold and drought by tracing mutational and recombinational history of natural populations, which will enable the identification of agronomically valuable alleles with great precision, and initiate their deployment in crop improvement programs. The identification of genes and molecular markers underlying agronomic traits will help accelerate the breeding process (Devasirvatham *et al.*, 2010; Warschefsky *et al.*, 2014). Three hundred twelve highly significant marker-trait associations were identified for 25 agronomically important traits in chickpea (Thudi *et al.*, 2014).

2.5.4.2. QTL mapping

Quantitative traits refer to phenotypes that vary in degree, can be attributed to polygenic effects, and are products of two or more genes, and their environment (Holeyach *et al.*, 2013). Most of

agriculturally important traits in crop plants such as yield, height, grain quality, tiller, panicle, environmental adaptation, leaf morphology, disease resistance, pest resistance and abiotic stress tolerance are quantitative, polygenic, continuous, or complex traits. They do not show classical Mendelian recessive or dominant inheritance attributable to a single gene locus. Instead, they show continuous variation depicted by a bell curve (Holeyach *et al.*, 2013). The genetic variation of a quantitative trait is controlled by the collective effects of many genes that are known as quantitative trait loci (QTLs).

A QTL is a region of the genome that is associated with an effect on a quantitative trait, can be a single gene or a cluster of linked genes that affect a trait. Mapping means placing the markers in order and assigning them to their linkage groups based on all pairwise recombination frequency between molecular markers. QTL mapping is used to identify the region of the genome that affect the trait of interest and to analyze the effect of the QTL on the trait (%PVE). QTL mapping revolutionize conventional plant breeding into molecular plant breeding, which is fast, less costly and efficient approach (Karikari *et al.*, 2019). Thus, finding novel and effective QTLs is a critical step towards understanding the genetic mechanism of heat tolerance.

Noticeable requirement for QTL mapping are mapping populations generated from contrasting parent, a high-density linkage map based on molecular markers, reliable and precise phenotypic data of the mapping population with minimum missing data, and appropriate statistical package that combines phenotypic data with genotypic data (Moose and Mum, 2008). QTL mapping is usually undertaken in segregating mapping populations such as F₂ and F₂ derived populations, recombinant inbred lines (RILs), near isogenic lines (NILs), double haploid lines (DHs) and

backcross populations (Veeresha *et al.*, 2015). Mapping populations can be classified into two: temporary and permanent populations. In temporary populations such as F₂ or BC, individuals in the population may segregate after self-pollination. In contrast, in a permanent population such as DH or RIL, each individual in the population is genetically homozygous, and the genetic structure will not change through self-pollination. In permanent populations, the phenotypic value of complex quantitative traits can be repeatedly measured through a replicated experimental design, and the same genotype can be tested under different environments, allowing more precise phenotyping and the study of genotype (QTL) by environment interaction.

The process of QTL mapping involves four major steps namely development of mapping population, genotyping, phenotyping and QTL analysis (Veeresha *et al.*, 2015). The discovery of abundant molecular markers, advances in rapid and cost-effective genotyping methods and the development of statistical methods for QTL mapping have revolutionized the field of mapping quantitative traits (Holeyach *et al.*, 2013). The standard mapping populations for linkage maps are between 200-300 segregating individuals, which are derived from crosses between two parents having contrasting characters of a trait under investigation. Expression of QTLs depends upon environmental factors such as drought, heat, salinity, different nutrient concentration and geographical location. A significant P value obtained for the differences between the marker and QTL is due to recombination. The closer a marker is from a QTL, the lower the chance of recombination occurring between marker and QTL. Therefore, the QTL and marker will be usually being inherited together in the progeny, and the mean of the group with the tightly linked marker will be significantly different ($P < 0.05$) to the mean of the group without the marker (Veeresha *et al.*, 2015).

Buu *et al.* (2014) carried out crossing of high yielding and heat susceptible rice genotype with heat tolerant genotypes. As a result, heat tolerant genes/QTLs were introgressed into the elite rice (heat susceptible) genotypes. The identified QTLs explained 10.6% to 31.0% of the total phenotypic variations. Heat tolerant QTLs have been identified in different populations of heat-tolerant rice varieties (Ye *et al.*, 2015). Sadat *et al.* (2013) also used markers linked to some important traits in bread wheat including grain-filling duration, HSI (Heat Susceptibility Index), HSI_single kernel weight of main spike and HSI kernel weight under heat stress. Improvement for stress tolerance can also be achieved by the introduction of heat related genes and QTLs from the wild to modern wheat cultivars (Kaur *et al.*, 2016). Frey *et al.* (2016) elucidated the genetic mechanisms of heat tolerance under field conditions in maize and the genomic regions contributing to natural variation. QTL for heat tolerance with respect to grain yield at field conditions were identified for European maize (Dents and Flents). The authors identified 11 QTLs including two loci for heat tolerance with respect to grain yield. Six heat-tolerance and 112 heat-responsive candidate genes were found. Their findings indicated that Dent genotypes were more heat tolerant during adult stage than Flint genotypes. Rahman *et al.* (2013) tested 25 maize hybrids in the field and three-heat tolerant maize hybrids were screened that produced higher grain yield per unit area as compared to other maize hybrids.

An ultra-high resolution intra-specific genetic map (ICC 12299 x ICC 8261) was constructed by integrating 3625 SNPs on eight LGs of chickpea. Molecular mapping of six major genomic regions underlying robust plant height QTLs on six desi LGs were located on chromosomes. The identified six major genomic regions harboring six robust QTLs (11.5-21.3% PVE), associated with plant height, were mapped within <0.5 cM average marker intervals on six chromosomes

(Kujur *et al.*, 2016). Segregation of hybrid populations of *Cicerechinospermum* accessions with an elite cultigen for sterility in the latter generations (F₃) might coincides with single recessive allele; however, the underlying genetic mechanisms may be more complex i.e. additional genetic loci (QTLs) might contribute to hybrid breakdown of the wild populations (Kahraman *et al.*, 2017).

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CHAPTER 3: QTLs mapping for Heat Tolerance in Chickpea using populations derived from the Cross of *Cicer arietinum* L. x Wild Cicer species (*C. reticulatum* and *C. echinospermum*)

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Abstract

Heat stress ($\geq 35^{\circ}\text{C}$) limits growth, development, production and productivity of cultivated chickpea. Introgressions of wild genes into the cultivated chickpea gene pool provide phenotypic novelties that can improve genetic diversity and withstand the effects of extreme heat stresses in the semi-arid environments. The objective of the present study was to uncover the genomic basis of heat tolerance in chickpea derived from the crosses of an early flowering elite cultivar with twenty diverse wild founders. A total of 480 chickpea accessions that comprised of phenology normalized 440 F_3 lines of *C. arietinum* x *reticulatum* (17 AR families) and 33 F_3 lines of *C. arietinum* x *echinospermum* (3 AE families), and seven standard heat tolerant and sensitive checks. They were grown in two replications at ICRISAT, Hyderabad, India under heat stress conditions. A total of 1445 and 1541 SNPs were used in AR and AE families, respectively for linkage map construction and marker-trait associations. Based on the average values of five-grain yield and yield related traits, 9xKalkan_064 is consistently found to be the most heat tolerant pre-breeding family, while 9xKarabache_081A and 9xDerei_072 are in the ranges of moderately heat tolerant to heat sensitive families. One hundred sixty and 34 QTLs were detected for 16 traits from 17 AR and 2 AE families. Thirty-eight QTLs having pleotropic effects were found from all families, out of which 5 QTLs were associated with three phenological traits and 17 QTLs were associated with more than two-grain yield and yield related traits, respectively. The associated SNP markers with pleotropic loci are important for simultaneous selection of genotypes having more than two related traits for heat stress breeding in chickpea.

Keywords: Heat stress, Genome wide QTL detections, Introgression breeding, Pleotropic effects, GBS SNP markers.

3.1. Introduction

Chickpea (*Cicer arietinum*) is the second food grain legume (FAO, 2018) and rich in protein, fiber, vitamin, minerals, beta-carotene, and unsaturated fatty acids (Jukanti *et al.*, 2012). The major abiotic stresses that limit production and productivity of chickpea are drought, cold, and salinity (Gaur *et al.*, 2007). Recently, global warming has become a worldwide concern due to its adverse effects on agricultural output. Thus, heat stress limits growth, development, production and productivity of chickpea. Chickpea is increasingly affected by heat-stress at reproductive stage. The exposure to temperature ($\geq 35^{\circ}\text{C}$) during reproductive phase has a negative impact on optimum yield in chickpea (Devasirvatham *et al.*, 2012; Paul *et al.*, 2018).

Narrow genetic bases of cultivated chickpea have hindered the speed in realizing high genetic gains and enhanced vulnerability to multiple stresses in chickpea breeding programs. von Wettberg *et al.* (2018) confirmed that domestication and subsequent breeding have eroded genetic diversity in the modern chickpea crop by approximately 100-fold. The causes of narrow genetic bases in cultivated chickpea are monophyletic origin; founder effect related with domestication; substitution of heterogeneous landraces by modern cultivars and their repetitive use; restricted distribution of wild progenitor species; and a shift in agriculture from a winter to a summer crop. These bottlenecks have resulted in the loss of adaptive alleles and fixation of deleterious alleles that can reduce fitness of modern cultivars across wide range of environments (Warschefsky *et al.*, 2014; Kahraman *et al.*, 2017; Cao *et al.*, 2018; von Wettberg *et al.*, 2018).

Introgression of wild germplasm into the cultivated gene pool provides an opportunity to overcome obvious limitations in selective breeding of cultivated plants caused by a narrow

genetic basis (Guo *et al.*, 2017; Shin *et al.*, 2019). Crop wild relatives are a reservoir of phenotypic variation that could accelerate genetic gains and contain a much wider spectrum of diversity in their genomes absent in the germplasm of cultivated species. The annual wild *Cicer* species have great potential for chickpea improvement through base broadening and by providing adaptive traits lost in the cultigens (Dempewolf *et al.*, 2014; Warschefsky *et al.*, 2014). Hybridization between crops and wild relatives is the current research focus to generate phenotypic novelty, provide powerful tools for trait dissection and an appropriate means to understand the genomic basis of agronomic traits.

Reproductive isolation, embryo breakdown, hybrid sterility, ploidy level differences between cultivated and wild species, limited genetic recombination, lack of well characterization of novel traits in the wild species, and linkage drag are major barriers in the interspecific wild hybrids of chickpea (Muehlbauer *et al.*, 1994; Chen *et al.*, 2016; Warschefsky *et al.*, 2014; Kahraman *et al.*, 2017; Sharma, 2017). Linkage drag is the most common problem associated with the utilization of wild species of chickpea in breeding programs such as pod shattering, poor pod and seed features, late maturity, undesirable growth habit, and photoperiod insensitivity (Jaiswal *et al.*, 1987; Kahraman *et al.*, 2017; Sharma, 2017; Mugabe *et al.*, 2019).

Modern genomics and quantitative genetics can resolve the burden of clarifying traits and reduce the barrier to the use of wild species in crop improvement (Shin *et al.*, 2019; von Wettberg *et al.*, 2018). Systematic pre-breeding efforts minimize the challenges of wild species. Wild species of *Cicer* and popular cultivars of chickpea were used as donor and recipient parents, respectively that led to the development of new gene pools having good agronomic performance and higher

frequency of useful genes and alleles` introgressed from wild species. Genomics tools are essential for precise introgression of genes and alleles with minimum linkage drag. They increase the efficiency of introgression of useful genes for important traits from wild species, to shorten the timeframe of introducing novel alleles into breeding pipelines, and to avoid photoperiod insensitivity problem in crop-wild crosses (von Wettberg *et al.*, 2018). Discovery of SNP molecular markers based on plant sequence data is a key step in several genetic studies including linkage mapping, QTL analysis, mapping genes, population genetic analysis, comparative genomics and association mapping (Xu *et al.*, 2017; Deokar *et al.*, 2019).

Mugabe *et al.* (2019) reported that *C. reticulatum* introgressed cold tolerant genes into cultivated chickpea. *C. echinospermum* accessions had several fold pod set than *C. arietinum* genotypes under cold stresses (Berger *et al.*, 2012). Most accessions of *C. bijugum*, *C. echinospermum* and *C. reticulatum* were found to be more cold tolerant than the cultigen (Singh *et al.*, 1991). Likewise, all accessions of *C. echinospermum* and some lines of *C. reticulatum* showed high freezing stress tolerance (Saeed *et al.*, 2010). Four accessions of *C. reticulatum* and one accession of *C. pinnatifidum* were found to be as resistant to drought and heat stress (up to 41.8°C) as the best checks. All accessions of *C. echinospermum* were found to be susceptible to drought and heat stresses (Canci and Toker, 2009). Canci and Toker (2009) and Warschefsky *et al.* (2014) reported that wild progenies of chickpea (*C. reticulatum* accessions) are tolerant to heat and drought stresses. Singh and Ocampo (1997) reported numerous F₂ transgressive segregates from the crosses of *C. reticulatum* and *echinospermum* with the cultigen.

Detail understanding of the genetic basis underlying the trait of interest is required to develop improved cultivars for stresses (Karikari *et al.*, 2019). QTL mapping has been used to dissect the genetic architecture of complex traits and important for fine gene mapping, map-based cloning, and efficient use of genetic information (Cao *et al.*, 2018). QTL mapping is linking genetic variation to phenotypic variation through quantitative genetic and genomic approaches. A genotype and phenotype association for crops will identify QTLs from wild species that can serve as an effective approach to enhance genetic diversity, improve yield, reduce the load of deleterious alleles, and mitigate stresses (Warschefsky *et al.*, 2014; Shin *et al.*, 2019). QTLs were detected from a biparental population in chickpea associated with drought (Kole *et al.*, 2015), salt (Vadez *et al.*, 2011), cold (Mugabe *et al.*, 2019) and heat (Paul *et al.*, 2018) tolerance.

Many QTL mapping studies in crop plants have been limited to a biparental population. Nested associated mapping (NAM) populations are derived from a multiple-cross mating design sharing one common parent, which may provide high power and high resolution through joint linkage and association analysis (Wang *et al.*, 2016; Yu *et al.*, 2008). NAM population has the potential for greater power of QTL detection, more accurate estimation of QTL effects, better resolution of QTL positions and more direct insight about the distribution of functional allelic variation across multiple families (Ogut *et al.*, 2015). Heat-stress due to rise in temperatures remains a challenge in developing crop varieties that are adaptive to changing climate. QTLs for heat tolerance from multiple families have not been so far reported in chickpea and identifying such, QTLs magnificently improve breeding for heat tolerant varieties. Therefore, the objective of the present study was to investigate the molecular Mapping of QTLs for heat tolerance in chickpea derived

from the cross of *Cicer arietinum* L. x wild *Cicer* species (*C. reticulatum* and *C. echinospermum*).

3.2. Materials and Methods

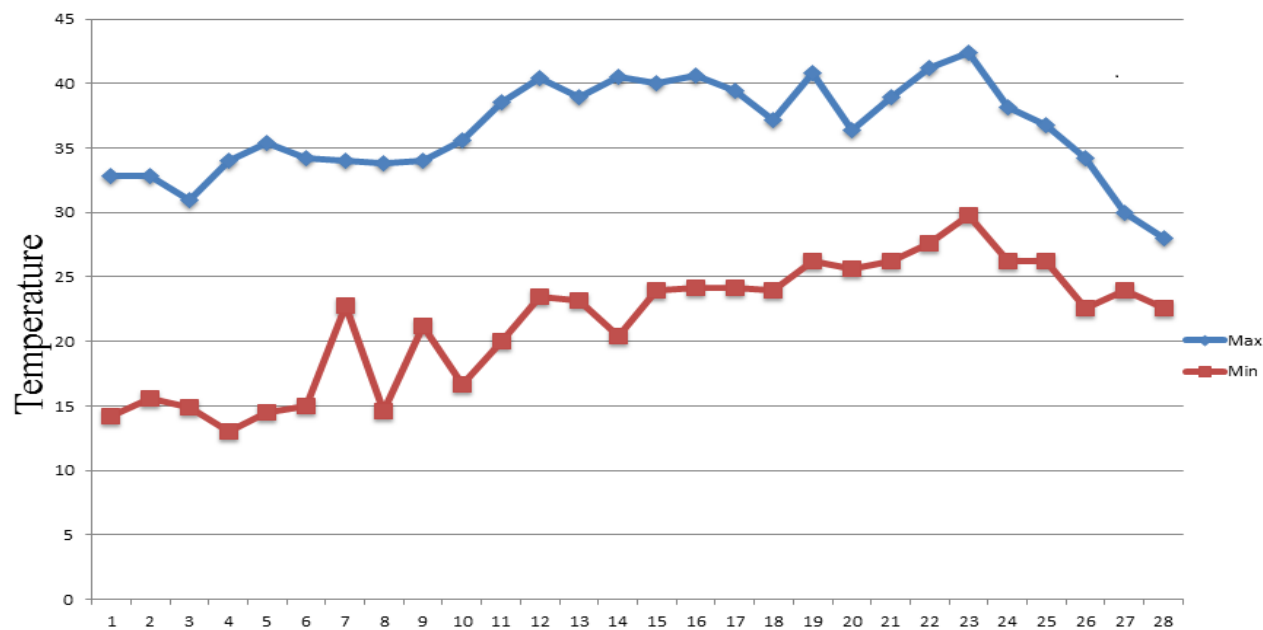
3.2.1. Mapping population development

To uncover the genetic basis of variation for heat tolerance in chickpea, twenty diverse wild founders from two wild *Cicer* species, *C. reticulatum* and *C. echinospermum*, were crossed to an elite, early flowering *C. arietinum* cultivar, ICCV96029, in the greenhouse of University of California Davis, USA to generate the mapping population used in the present study. All F₁ hybrid seeds were planted and self-pollinated to generate F₂ seeds. A total of 2139 segregating F₂ individual plants were grown in the same greenhouse and self-pollinated to produce F₃ seeds.

Based on a competitive allele specific PCR (KASP) genotyping of F₂ plants with flowering time linked molecular marker (chromosome 3 early flowering FT-linked loci), a subset of 473 F₂-derived phenology normalized F₃ families were selected and used as a mapping population in this study. The 473 F₃ families composed of 440 F₃ families of *C. arietinum* x *C. reticulatum* (AR) derived from 17 diverse wild parents and 33 F₃ families of *C. arietinum* x *C. echinospermum* (AE) from 3 wild parents. Each family of F_{2:3} progenies was derived from a single F₂ individual. All F₃ individuals derived from the same F₂ plant belong to the same F_{2:3} family, denoted by F_{2:3}. If the size of each F_{2:3} family is sufficiently large, the average value of the family will represent the genotypic value of the F₂ plant, and thus the power of QTL mapping may be significantly increased (Zhang and Xu, 2003). A total of 480 chickpea accessions (473 F₃ families and seven standard heat tolerant and sensitive checks) were grown in two replications at field site in India (Hyderabad, ICRISAT) under heat stress conditions during February to May, 2017.

3.2.2. Experimental site, design and field management

The experiment was conducted under an extremely heat stressed environment ($\sim 40^{\circ}\text{C}$) at ICRISAT, Hyderabad, India from February to May in 2017. ICRISAT is located at an altitude of 545 mas, latitude $17^{\circ} 30' \text{ N}$, longitude $78^{\circ} 16' \text{ E}$, Patancheru, Hyderabad, India. The daily average minimum and maximum temperatures of the experimental site ranged from 15°C to 29.7°C and 31°C to 42.6°C , respectively (Fig 3.1). The experiment was conducted using an alpha lattice design with two replications. A total of 10 seeds per each of the 473 $F_{2:3}$ families and 7 checks were grown in a single-row plot per replication for evaluation of phenological, growth habit and agronomic traits. Each replication was divided into 20 blocks and in each block ridge and furrow system of sowing was employed and an inter- and intra- row spacing of $60 \times 10 \text{ cm}$, row width and length of 40 cm and 100 cm was used on a vertisol soil. Plants were supported with adequate irrigation to avoid any kind of drought stress. Manual weeding and spraying of insecticide were done to control weeds and pod borer. The reproductive phase of these families was exposed to high temperatures ($\geq 35^{\circ}\text{C}$) (Fig 3.1).



Every 5 days from planting to harvesting

Fig. 3.1. Daily average minimum (Min) and maximum (Max) temperatures during plant growth period (ICRISAT, Patancheru, Hyderabad, India).

3.2.3. Traits evaluated under an extremely heat stress environment (~40°C)

Trait segregation in these wild x cultivated hybrid populations was observed to delineate the genetic underpinnings of domestication traits. Twenty plants in each family under F_3 were evaluated per a single plant basis. A total of 16 agronomic, growth habit and phenological traits were evaluated and analysed.

Growth habit (GH) trait was measured based on four criteria: prostrate ($P = 4$), spreading ($S = 3$), semi-spreading ($SS = 2$), and semi-erect ($SE = 1$), data were recorded at the grain-filling period per a single plant basis. Days to first flowering (DFF) was recorded when the first bud reached full anthesis, days to first podding (DFP) was recorded when the first bud set pod for each

individual plants of each family, and days to physiological maturity (DPM) was recorded when 90% of the pods of each individual plants of each family reached physiological maturity. All the three phenological traits were evaluated per single plant basis.

The agro-morphological traits on individual plant basis considered under this study were plant height per plant (PHT), plant biomass per plant (PBP), filled pods per plant (FPP), unfilled pods per plant (UFP), total pods per plant (TPP), percentage of pod set per plant (%PS), total seeds per plant (TSP), hundred seed weight per plant (HSW), grain yield per plant (GYP), and percentage of harvest index per plant (PHI). HSW and PHI were calculated as GYP divided by TSP; and GYP divided by PBP and multiplied by 100, respectively. In addition, heat visual score (HVS) was made using 1-5 scales per plant (1, highly heat tolerant, 5, highly heat sensitive) and pod type per plant (PT, 1 to 3 scales per plant; 1, single podding, 2, double podding, 3 both single and double podding) was recorded. PHT was measured in centimeter, while PBP, GYP, and HSW were measured in gram.

3.2.4. DNA Extraction, Library Construction, Sequencing and SNP genotyping

In order to perform trait-marker discovery, wild x cultivated crosses were genotyped in addition to phenotyping of traits. Genomic DNA from the parents, ICCV96029 and 20 diverse wild accessions of *C. reticulatum* and *C. echinospermum*, and 2139 F₂ segregating individual lines were extracted using Qiagen DNeasy 96 format Plant Mini Kit (Valencia, CA, USA) and used to construct GBS libraries. To correct for the confounding effect of segregating phenology, F₂ plants were genotyped for the FT locus using KASP genotyping. The common parent (ICCV96029) has an early flowering habit, which is explained by tightly linked FT locus.

Marker-based selection for the cultivated haplotype was sufficient to normalize the majority of phenological variation among the respective progeny (von Wettberg *et al.*, 2018).

DNA was digested with restriction enzymes, HindIII and NlaIII, and ligated with adapters. The ‘barcode’ adapter ligates to HindIII allowing sample pooling. Ninety-six different barcode sequences were used for tagging the samples and placed in a single lane. The ‘common’ adapter ligates to NlaIII. Products were selected for size and amplified through 14 rounds of PCR. The GBS libraries were pooled and sequenced using Illumina. A size of 100 base paired-end reads was generated on an Illumina HiSeq4000 at the University of California at Davis Genome Center DNA Technologies Core. Illumina reads were aligned to the already available draft genome sequence of the desi chickpea *C. arietinum* ICC49582 using Burrows-Wheeler alignment tool (BWA) under default mapping parameters. Single nucleotide polymorphisms (SNPs) were called using the GATK pipeline, which considers indel realignment and base quality score recalibration, and calls variants across all samples simultaneously through the HaplotypeCaller program in GATK.

Sequences from raw data were filtered by checking for the perfectly matched barcode with the expected four base remnant of the enzyme cut site. Variants were filtered using standard hard filtering parameters according to GATK Best Practices recommendation. More precisely, GBS data were filtered to only retain SNP calls with Mapping Quality (MQ)>37 and Quality by Depth (QD)>24. Both metrics take into consideration the quality of the mapping and genotype calls to ensure that only those with highest confidence were used. The SNPs were also filtered to retain those with MQRankSum<|2.0|, which ensures that there is no difference in the Mapping Quality

scores for alleles at a given locus. This filtering removed nearly 60% of variant sites reported by GATK and only retained those that passed all three criteria. The resulting SNPs were filtered using a minor allele frequency (MAF) threshold of >5% and genotype call-rate >90%. Finally, 1445 and 1541 SNPs were filtered in AR and AE families, respectively that were used for linkage map construction and marker-trait discovery.

3.2.5. Binning (BIN) of redundant markers

Binning can help to delete redundant markers, markers with high missing rate (>5%) and high segregation distortion ($P \leq 0.0001$), and generate the input file which can be immediately used for genetic map construction. Redundant markers are markers that are completely correlated in a population, which cannot provide additional information, even if more than one of them is considered. BinID can have values -1, 0, or 1. A value of -1 indicates the marker is either non-polymorphic, or has a missing rate greater than a specified value in the parameter window. A value of 1 indicates that markers are redundant and were classified into one bin. A value of 0 indicates the marker is not redundant, a representative marker of the bin with the low missing data and segregation distortion, and retained for the second step of genetic analysis. The bin grouping of the redundant SNP markers was performed using BIN functionality employed in QTL IciMapping software version 4.1 and a single marker representing each bin was used for linkage map construction using the same software.

3.2.6. Interspecific Linkage Map Construction

Linkage map construction and QTL detection were carried out using QTL IciMapping software version 4.1. All genotype scores at each locus were converted into ABH format as A: ICCV

96029 genotype (mother), B: 20 diverse wild species of chickpea (*C. reticulatum* and *C. echinospermum*, fathers), H: heterozygotes, and X: as missing genotype. The interspecific linkage map construction and QTL analysis were carried out separately for each family of AR and AE using 1445 and 1541 SNP markers, respectively. There are three general steps involved in linkage map construction namely grouping, ordering and rippling in their respective orders. A Chi-squared test ($P < 0.0001$) was used to assess Mendelian segregation distortion of all polymorphic loci data in F₂ population before linkage analysis. LOD threshold has to be specified to declare the linkage relationship between two markers. Any two BIN markers with LOD higher than the threshold will be grouped together; all BIN markers were grouped into eight linkage groups with LOD threshold of 3.0. One unanchored marker will be tested if it can be grouped into any existing anchor groups. If one unanchored marker cannot be grouped into any existing anchor group, a new group will be made.

Following grouping of markers, they were ordered using nnTwo Opt algorithm. nnTwoOpt: nearest neighbor was used for tour construction, and two-opt was used for tour improvement. For each order, the continuity index (CI) was calculated and the best order was considered for the one that gave the smallest CI value. After ordering, each marker sequence needs to be rippled for fine-tuning of the ordered chromosomes based on Sum of Adjacent Recombination Frequencies. Ripple command was used after adding each marker locus to confirm the final marker order with a window size of 5 markers. The shorter the chromosome length, the better of the rippling results. Kosambi map function was used for the conversion of recombination frequency to genetic map distance (Kosambi, 1944) in linkage map construction. The markers on a

chromosome or linkage group are defined by positions and/or marker intervals using a marker space unit, centi-Morgan (cM). Finally, genetic linkage map was constructed for QTL analysis.

3.2.7. QTL identification and determination of its phenotypic effect

Mapping populations can be used to create novel combinations of parental alleles, fix parental alleles, break apart haplotypes, and to test additive or dominance effects. QTL mapping statistics assume using the genotype data representing F₂ individuals and phenotype data representing an average of 20 F₃ individuals per each F₂ individual line. Thus, marker-trait association analyses were conducted to investigate QTLs that are associated with the 16 traits for the 471 F₃ families grown under an extremely heat stress environment. Inclusive composite interval mapping (ICIM) method was introduced to detect QTLs and estimate their phenotypic effects. QTLs with a positive or negative additive effect for a specified trait imply that the increase in the phenotypic value of the trait is contributed by the alleles from diverse wild founders of chickpea or ICCV 96029, respectively. A positive additive effect indicates that wild founders' allele contributed to increase heat tolerance, and a negative value indicated that ICCV 96029 allele was favorable for a trait.

The amount of variation explained by a marker and/or a QTL was calculated using partial coefficient of determination (R^2) value and expressed as percentage of phenotypic variance explained (PVE %). QTLs with $\geq 10\%$ phenotypic variation explained (PVE) were considered as major QTLs otherwise, they were considered as minor QTLs. In additive and epistasis QTL mappings, the total phenotypic variation explained by all additive QTLs and all QTL interactions were determined, respectively.

3.2.8. Data analysis

Phenotypic data analyses of a single environment were made by using QTL IciMapping software version 4.1 (Wang *et al.*, 2016). Based on the phenotypic data of each of the 20 biparental populations of AR and AE, mean, range, minimum, maximum, standard deviation, kurtosis, skewness, ANOVA, heritability, variance, correlations and frequency distributions were computed using AOV functionality of the software. Correlation refers to the degree and direction of the association between two or more variables. The correlation coefficient measures the mutual relationship between various plants characters in which selection of genotypes can be made based on these correlated traits. Its values vary between -1 (perfect negative correlation) and +1 (perfect positive correlation). The family means based heritability can be significantly increased by increasing the number of F₃ progeny.

Linkage map constructions and QTL identifications were made using high density SNP data of AR and AE families. Binning of redundant markers was made before constructions of linkage maps using BIN functionality of the software in order to filter and remove redundant markers. Linkage maps of all the 20 families were constructed using MAP functionality of the software. Mapping of additive and digenic epistatic QTLs of all families were identified based on algorithm BIP functionality of the software. In this study, F_{2:3} designs are genotyped in F₂ plants and phenotyped in the F_{2:3} progeny in order to increase the precision in the detection of QTLs. This is because that residual variance based on family-mean observations has been significantly decreased by increasing the number of F_{2:3} progeny.

In this study, the QTL ICIMapping software version 4.1 uses a statistical method known as inclusive composite interval mapping (ICIM), for QTL mapping. ICIM applies a regression model. Marker variables were considered in a linear model in ICIM for additive mapping. Marker variables and marker-pair multiplications were simultaneously considered for epistasis mapping. Two steps were included in ICIM. In the first step, stepwise regression was applied to identify the most significant regression variables in both cases but with different probability levels of entering and removing variables. In the second step, a one-dimensional scanning or interval mapping was conducted for mapping additive.

Inclusive composite interval mapping (ICIM) was proved more efficient statistical method for background control via a two-step mapping strategy. This statistical method is an efficient mapping method with higher detection power, lower false discovery rate (FDR), less-biased estimation of QTL effects and positions in populations than other mapping methods (Li *et al.*, 2007). Under the assumption of additives of QTL effects on the phenotype of a trait in interest, the additive effect of a QTL can be completely absorbed by the two flanking marker variables, and the epistasis effect between two QTLs can be completely absorbed by the four marker-pair multiplication variables between the two pairs of flanking markers.

ICIM has identified two categories of QTLs; additive and epistasis QTLs. The threshold LOD scores of both additive and epistasis analyses was determined by performing a 10,000 permutation test at a significance level of $P < 0.05$. QTL mapping was done using inclusive composite interval mapping of additive and dominant QTL (ICIM-ADD) module of the software. For the additive analysis, a mapping step of 1.0 cM with a probability in stepwise

regression (PIN) value of 0.001 was used in order to obtain QTLs that are more precise. For the epistasis analysis, a mapping step of 1.0 cM with a PIN value of 0.0001 and a window size of 10 cM was used.

LOD score peaks were used to estimate the most likely position of a QTL on a linkage map. In the present study, the threshold LOD value determining significant QTL was ≥ 3.0 . In the same software, inclusive composite interval mapping for epistasis interaction (ICIM-EPI) model was used for epistasis interactions between QTLs. The LOD cutoff of 5.0 was used to declare the presence of epistasis interactions between QTLs.

3.3. Results

3.3.1. Phenotypic responses of F₃ families under heat stress environment

3.3.1.1. Mean, minimum, maximum and range values of traits

Based on the mean values of days to first flowering (DFF) and days to physiological maturity (DPM) among the 20 families, 9xSavur_063 demonstrated the earliest flowering and maturing, while 9xSirna_060 and 9xKesen_075 were found to be the latest flowering and maturing. Days to first podding (DFP) appeared within the range of 5 to 7 days after DFF. Out of the 20 families, 9xKalkan_064 had the highest average values for percentage of pod set (%PS), filled pods per plant (FPP), and total seeds per plant (TSP), and had the least heat visual score (HVS). 9xKalkan_064, 9xOyali_084, and 9xS2DrD had high average grain yield per plant (GYP) in their respective order. 9xKarabache_081A and 9xDerei_072 had low average FPP, TSP and GYP (Table 3.1 and Appendix 3.1).

All families of AR and AE except 9xCudiA_152 and 9xBari3_072C showed wide ranges of segregations for DFF and DPM traits between an early flowering and maturing mother (ICCV96029) and very late flowering and maturing wild *Cicer* species (*Cicer reticulatum* and *Cicer echinospermum*). Wide ranges of segregations for phenological traits were found within 9xBari3_100D and 9xSavur_063 families. Out of the 20 families, 9xS2DrD and 9xSirna_060 showed wide ranges of segregations for FPP, while 9xKarabache_081A and 9xDerei_072 families showed narrow ranges of segregation for FPP. UPP have shown wide range value within 9xS2DrD and 9xGunas_063 families. TSP and GYP have shown wide range values within 9xS2DrD family. Our results clearly revealed that AE families showed high degree of segregations for HVS, %PS, FPP, and TSP traits. They possessed up right growth habit, narrow

ranges of segregations for phenological traits, wide ranges of segregations for UPP, FPP, TSP and GYP and had high average PHT, HSW and PBP compared to AR families (Table 3.1 and Appendix 3.1).

3.3.1.2. Selections of heat tolerant pre-breeding lines

With the intent of identifying heat tolerant pre-breeding lines under heat stressed environments (Fig 3.2), heat visual score and four yield related traits namely FPP, %PS, TSP, and GYP were often used for selections of heat tolerant lines. Based on an overall performance of these five traits, out of 20 AR and AE families, F₃ lines of 9xKalkan_064 (Fig 3.2e and Table 3.1) were consistently found to be the most heat tolerant pre-breeding lines followed by 9xOyali_084, 9xBari1_092, 9xSavur_063 (Fig 3.2d) and 9xCudiB_22C (Fig 3.2g). Nevertheless, F₃ lines of 9xKarabache_81A (Fig 3.2c (e2)) and 9xDerei_072 were found to be less heat tolerant genotypes (Table 3.1 and Appendix 3.1). In AE families, wide gradients of F₃ individuals (extremely heat tolerant and sensitive individuals) were found. Based on HVS and four yield related traits, 9xS2DrD and 9xGunas_063 contained highly heat tolerant F₃ individuals; however, wide ranges of segregations for fertility were found in these families. Most F₃ individuals of 9xKarabache_081A were heat sensitive (Table 3.1, Appendix 3.1 and Fig. 3.2c (e2)).

Based on an overall performances of five traits (HVS and 4 yield related traits), the tolerant standard check (JG 14) was found to be the most heat tolerant genotype as that of 9xKalkan_064; however, the other tolerant standard check (ICCV 07102) had less performance than the performances of all AR and AE families. Compared to their common parent (ICCV96029, mother), all the 20 families were found to be relatively heat tolerant except F₃ individuals of

9xKarabache_081A. JAKI 9218 and ICC 4567 were heat sensitive and very heat sensitive standard checks. However, any individuals of the 20 families of the present study were not found to be as sensitive as these heat sensitive standard checks (Table 3.1 and Appendix 3.1).

3.3.1.3. Frequency distribution of traits

Under a heat stress environment (ICRISAT, Hyderabad, India), an absolute value of skewness and kurtosis for most traits of the 471 F₃ lines of AR and AE families were less than one and very closer to zero and zero for few traits that might indicate normal frequency distribution, bell shaped curve and continuous variations(Fig 3.3). These values are useful for quantitative genetic analysis. For some families, grain yield and yield related traits were positively skewed from their respective mean values, which showed introgression of these traits from their wild parents, but absent in their common cultivated parent (Table 3.2 and Appendix 3.1). This feature might assure that most wild x cultivated progenies are resilient to an extremely heat(~ 40°C) stress environment (Table 3.2 and Fig 3.2).



Fig. 3.2. Variation in agronomically important traits in wild Cicer (*C. reticulatum* and *C. echinospermum* accessions). a. Flower drying and drop; b. pod abortion; c. F₃ families segregate at high frequency for heat stress tolerance in phenology-normalized lines; typical phenotypes of tolerance (e1, 9xCudiA_152) (seed set, blue asterisk) and sensitivity (e2, 9xKarab_081A) (flower abortion, red arrows) when grown under high temperatures during February to May in Hyderabad, India (von Wettberg *et al.*, 2018); d-f. high pod setting of late flowering individuals from 9xSavur_063, 9xKalkan_064 and 9xBari2_072n2; g. 9xCudiB_22C individuals which were early flowering, having high pod set and semi-erect growth habit; h. The characteristics of

F₃ segregating individuals in a single plot (some individuals physiologically mature, some are flowering and some set pods).

Table 3.1. The mean, standard deviation and range values for each of the 20 Families of AR and AE.

	9xBari1_092		9xBari2_072n2		9xBari3_100		9xBari3_100D		9xBeseve_079		9xCudiA_152		9xCudiB_22C		9xDerei_070		9xDerei_072		9xEgill_065		9xEgill_073	
Trait	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R
DFF	46.1±8.1	34.0	42.4±9.0	36.0	48.5±9.3	35.5	45.8±12.3	46.0	44.9±9.5	35.5	48.2±6.5	22.8	45.1±10.8	33.0	46.1±11	35.0	47.8±10.4	41	48.7±8.7	34	47.6±8	25.5
DFP	51.8±7.8	32.0	49.3±8.2	33.0	54.2±9.6	34.5	52.6±12.8	45.0	50.9±9.9	34.0	53.8±7.2	29.1	52.9±13.2	53.5	53.1±12.6	43.5	54.6±10.5	38	55.2±9.2	37	54.1±8	29.8
DPM	88.7±11.8	50.0	82.7±12.1	51.0	90.8±14.2	54	88.5±17.1	59.5	86.1±14.0	56.0	92.0±9.7	32.8	87.1±16.4	56.5	87.3±15.8	52.5	90.3±15.1	53.5	92.3±13.5	57	89.5±13	50.0
PT	1.7±0.7	2.0	1.4±0.6	2.0	1.6±0.8	2	1.4±0.6	2.0	1.6±0.6	2.0	1.4±0.6	2.0	1.4±0.7	2.0	1.4±0.6	2.0	1.3±0.5	2	1.6±0.7	2	1.7±1	2.0
HVS	2.6±1.1	4.0	2.9±0.9	3.0	3.2±1.0	3	2.6±0.9	3.0	3.0±0.9	3.0	2.8±1.1	4.0	2.5±1.0	4.0	2.6±1	3.5	3.5±1	3	2.9±1	4	2.6±1	4.0
GH	2.9±0.9	4.0	2.5±0.7	2.0	3.2±0.7	3	2.4±0.8	3.0	3.2±0.8	3.0	2.8±1.0	4.0	2.4±0.9	4.0	2.8±0.7	3.0	2.3±0.7	3	2.8±0.7	2	2.5±1	3.0
%PS	49.9±15	60.9	44.2±12.5	40.4	39.7±14.6	60	45.9±13.5	57.5	41.6±14.7	57.5	46.4±17.5	67.5	47.1±13.6	55.0	49.2±15.2	55.0	34.8±15	50	44.6±13.5	52	47.1±15	62.5
PHT	23.3±3.7	14.2	25.9±5.2	18.1	21.8±5.0	26	24.3±4.7	18.8	20.0±4.1	15.3	24.3±4.3	18.6	24.4±3.8	15.4	23.3±5	18.4	23.8±4.7	20.85	22.6±4.9	18	24.3±4	16.4
FPP	31.2±14.9	64.9	25.0±13.8	46.4	21.0±13.1	59.7	23.8±12.1	46.5	22.5±12.7	42.7	22.7±13.4	56.7	28.7±15.8	60.5	24.2±13.7	48.2	14.5±9.6	35.5	23.0±11.6	43	25.1±14	61.6
UPP	3.0±1.8	8.0	1.7±0.7	2.7	2.0±1.7	7.8	2.0±1.0	3.8	1.9±1.0	3.9	1.8±1.6	7.6	1.8±0.8	3.3	1.6±0.7	3.9	1.5±0.7	2.7	2.2±1.4	7	2.5±1	6.7
TPP	34.3±15.7	67.4	26.5±13.8	45.4	22.8±13.7	62.2	25.5±12.9	49.3	24.4±13.8	52.2	24.4±13.9	57.7	30.6±16.3	63.4	25.8±13.8	49.2	16.1±10.2	38.2	25.1±11.5	39	28.3±15	63.4
TSP	31.8±15.2	61.9	26.5±16.3	61.6	21.6±15.3	73.4	25.2±14.8	63.5	25.8±16.9	64.2	21.5±11.5	43.0	28.3±15.7	63.7	23.0±11.7	46.7	14.4±10.3	38.2	22.4±10.9	38	24.5±14	61.6
HSW	9.9±1.2	4.4	10.8±1.3	5.1	9.8±2.4	12.8	9.9±1.8	7.6	9.0±1.5	7.0	10.9±1.9	8.9	11.8±1.9	7.1	9.8±1.9	9.6	11.6±1.6	6.35	10.8±1.5	7	10.5±2.4	7.1
PBP	11.1±4.3	16.5	11.9±5.8	19.6	9.6±4.4	18.8	12.0±6.2	28.7	8.5±3.6	13.5	12.0±5.4	20.2	12.8±4.9	18.6	10.5±4.6	22.0	9.7±4.1	18.65	11.5±4.5	19	9.8±5	23.7
GYP	3.3±1.6	6.1	2.9±1.7	6.1	2.3±1.6	7.15	2.6±1.6	6.9	3.6±5.7	29.9	2.7±1.5	6.1	3.4±3.2	7.0	2.4±1.3	5.4	1.7±1.1	3.9	2.5±1.3	5	2.8±2	7.2
PHI	28.5±9.3	36.4	24.0±11.6	40.5	22.0±10.6	48.6	21.4±8.6	30.5	25.8±10.1	37.7	24.5±10.9	44.4	25.6±8.6	29.3	22.5±8.9	34.0	16.6±9.5	29.35	21.3±8.1	29	24.8±9	30.8
	9xKalkan_064		9xKesen_075		9xOyali_084		9xSavur_063		9xSina_060		9xBari3_072C		9xGunas_063		9xKarabache_81A		9xS2DrD					
	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R	Mean±SD	R				
DFF	45.0±13.4	40	49.6±11.1	40.5	46.5±11.2	38.5	41.4±10.5	45.5	50.8±9.6	45.5	49.6±8.2	24.5	44.6±3.5	11.9	43.5±10.7	27.3	47.3±8.5	31.7				
DFP	50±13.5	43	55.7±11.8	45	52.7±11.1	38	47.1±10.5	45.5	57.7±10.4	45.5	56.4±12.0	39.0	50.6±4.1	15.4	51.9±12.7	31.5	55.3±9.2	33				
DPM	86.4±16.8	49	92.4±15	55	89.0±15.6	54.5	81.3±14.2	59	96.7±14.8	59	95.5±11.5	40.8	86.3±5.1	18.0	87.9±17.7	46.3	92.8±12.2	47.5				
PT	2±0.9	2	1.5±0.6	2	1.6±0.6	2	1.6±0.6	2	1.5±0.7	2	1.4±0.7	2.0	1.2±0.6	2.0	1.2±0.4	1	1.3±0.5	1				
HVS	2±0.9	3	2.7±0.9	4	2.4±0.7	2	2.1±0.8	3.5	2.9±0.9	3.5	2.7±0.8	2.5	3.7±1.1	4.0	4.4±0.8	2	3.8±1.0	3				
GH	2.0±0.6	2	2.7±0.8	3.5	2.4±0.6	2.5	2.6±0.8	3	2.8±0.7	3	3.5±0.7	2.0	3.2±0.9	3.0	1.9±1.0	2.5	2.5±1.2	4				
%PS	66±17.7	65	46.1±15.2	77.5	52.2±13.5	45	58.0±15.5	67.5	42.5±13.8	67.5	46.4±11.4	41.3	31.7±13.3	45.9	22.7±11.4	30	29.9±14.2	41.3				
PHT	26±6.7	25.4	21.8±4.3	17.8	24.1±5.3	16.5	24.9±4.7	17.1	22.0±3.4	17.1	19.7±3.3	10.5	27.1±3.7	10.8	28.4±5.5	14.5	30.3±7.4	29.6				
FPP	42±14.9	50.7	22.2±13.3	67.9	29.9±13.3	46.5	30.2±12.4	43.5	25.7±16.8	43.5	20.4±13.2	44.2	29.3±15.8	50.7	8.5±5.9	15.1	27.9±29.1	117.5				
UPP	2±1.3	4.2	1.4±1	5.3	1.7±1.1	3.8	2.4±1.1	3.8	2.0±1.2	3.8	2.2±0.9	2.6	3.6±3.4	9.7	2.5±2.6	6.2	2.1±3.5	14.5				

TPP	44±16.1	51.5	23.7±14	72.05	31.4±13.7	48.8	32.4±13.1	45.9	27.7±17.7	45.9	22.2±13.7	46.5	33.0±18.4	60.9	13.3±7.0	16.8	30.0±32.1	131
TSP	40±16	49.1	22.7±12.6	49.8	30.8±13.6	57.2	29.0±12.9	50.2	27.1±18.9	50.2	20.5±13.3	43.3	28.4±15.8	57.0	9.0±5.8	14.6	27.6±30.9	123.3
HSW	10±1.5	5.4	10.8±1.6	7.8	11.2±1.5	6.1	9.4±1.7	6.7	10.0±1.2	6.7	9.3±2	5.7	10.8±0.9	2.6	11.7±2.8	7.0	11.8±3.1	12.9
PBP	13.2±5.1	15.7	9.7±3.1	16.4	12.5±6.4	24.3	9.6±3.9	18.8	11.6±7.8	18.8	8.9±3.7	11.2	16.8±7.5	25.7	11.3±5.8	13.3	15.1±9.9	35.5
GYP	4.0±1.8	6.1	2.5±1.3	5.4	3.9±1.5	5.5	2.8±1.3	5.6	2.6±1.8	5.6	2.2±1.3	4.2	3.1±1.7	5.9	1.4±0.7	1.9	3.8±5.9	23.7
PHI	29.7±10	39.8	23.5±9.2	40.15	31.9±12.4	43.2	30.1±11.6	53.1	21.4±9.1	53.1	23.0±9.4	31.2	18.2±5.3	15.3	16.7±13.2	33.6	21.4±15.6	63.2

AE = *Cicer arietinum* and *C. reticulatum*, AR = *Cicer arietinum* and *C. reticulatum*, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, R = range, SD = standard deviation, TPP = total pod per plant, TSP = Total seed per plant, PT = pod type, UPP = unfilled pod per plant.

Table 3.2. ANOVA, phenotypic variance, kurtosis, skewness, heritability, and least significant difference values for each of the 20 families of AR and AE.

	9xBari1_092						9xBari2_072n2						9xBari3_100						9xBari3_100D					
Trait	MS	V	Sk	KU	H ² b	LSD	MS	V	Sk	KU	H ² b	LSD	MS	V	Sk	KU	H ² b	LSD	MS	V	Sk	KU	H ² b	LSD
DFF	130.5***	65.2	-0.5	0.2	87.3	11.2	164.3***	80.3	0.1	0.4	89.7	11.7	166.1***	86.5	-0.4	-0.4	83.7	14.7	229.3***	152.3	0.5	-0.5	87	17.7
DFF	121.7***	60.8	-0.6	0.2	86.6	11.1	143.4***	68.0	0.3	0.4	81.5	14.6	169.4***	91.6	-0.5	-0.5	80	16.4	314.4***	163.0	0.6	-0.6	88.4	17.2
DPM	279.7***	139.8	-0.3	-0.1	86.2	17.1	277.3***	146.8	0.5	0.9	87.2	16.9	388.0***	203.0	-0.4	-0.3	74.1	28.3	556.8***	291.7	0.4	-1.1	82.8	27.7
PT	1.0 ^{ns}	0.5	0.6	-0.8	22.7	2.5	0.68 ^{ns}	0.4	1.2	0.5	15.4	2.1	0.9 ^{ns}	0.6	0.7	-0.9	33	2.3	0.7 ^{ns}	0.4	1.0	-0.2	10.2	2.3
HVS	2.4***	1.3	0.6	-0.5	86.9	1.6	1.4 ^{ns}	0.7	0.2	-1.0	40.1	2.6	1.6***	1.0	0.5	-0.8	74.7	1.9	1.7*	0.9	0.7	-0.7	50.8	2.6
GH	1.4***	0.7	0.6	0.9	87.7	1.2	0.98**	0.5	1.3	0.5	71.4	1.5	0.8 ^{ns}	0.5	-0.7	1.0	44.2	2	1.3*	0.7	0.3	-0.5	73	1.7
%PS	242.9***	225.2	0.0	-0.4	72.2	30.9	297.8*	156.3	-0.4	-1.1	53.6	33.3	374.4***	213.1	-0.7	0.1	75.9	28.1	362.6 ^{ns}	181.1	0.0	-0.3	44.6	40.2
PHT	27.9 ^{ns}	14.0	0.5	-0.3	28.2	12.5	52.1*	27.0	0.5	-0.7	64.4	12.4	31 ^{ns}	24.7	1.2	3.4	49.8	12	44.6*	22.4	0.5	-0.3	68.2	10.7
FPP	445.8 ^{ns}	222.6	0.7	0.3	26.5	50.4	610.1 ^{ns}	190.3	0.3	-0.8	9.1	67.8	294.6 ^{ns}	171.9	1.0	1.1	0	66	267.8 ^{ns}	146.7	0.4	-0.4	0	50.4
UPP	6.6 ^{ns}	3.3	0.9	0.8	26.6	6.1	2.2 ^{ns}	0.5	0.5	-0.3	0	5.8	5.1 ^{ns}	2.8	1.7	3.2	0	6.9	2 ^{ns}	1.1	0.3	-0.8	0	4.6
TPP	492.7 ^{ns}	246.1	0.5	0.0	18.2	56	651.8 ^{ns}	190.3	0.4	-0.9	6.2	71.2	319.8 ^{ns}	188.1	0.9	0.9	0	68.4	304.8 ^{ns}	167.4	0.3	-0.4	0	53.4
TSP	465.2 ^{ns}	232.5	0.4	-0.3	20.3	53.7	735 ^{ns}	264.2	0.7	0.0	19.6	70	405.8 ^{ns}	235.0	1.5	2.8	0	76.9	350.2 ^{ns}	220.2	1.0	1.3	10.2	50.3
HSW	2.6*	1.3	0.3	-0.4	51	3.1	4.7 ^{ns}	1.8	-0.9	0.5	30.2	5.2	7.3 ^{ns}	6.0	-2.4	7.1	46.7	6	5.8 ^{ns}	3.1	-0.5	0.4	29.5	5.7
PBP	36.7 ^{ns}	18.8	0.3	-0.8	43.2	12.7	61.1 ^{ns}	33.9	0.8	-0.4	0	24.4	32.7 ^{ns}	19.2	0.5	-0.1	0	21.5	56.4 ^{ns}	37.9	1.6	3.3	0	21.8
GYP	5.2 ^{ns}	2.6	0.4	-0.5	21	11.9	7.4 ^{ns}	3.0	0.5	-0.4	11.5	7.4	4.4 ^{ns}	2.5	1.4	1.8	0	7.2	4.3 ^{ns}	2.7	1.1	1.1	40.4	4.5
PHI	158 ^{ns}	85.9	0.3	-0.3	6.2	33.9	239 ^{ns}	134.3	0.3	-0.5	39.5	34.6	149.7 ^{ns}	113.3	0.4	0.1	4	36.5	149 ^{ns}	74.6	0.5	-0.6	33.4	28.2
	9xBeseve_079						9xCudiA_152						9xCudiB_22C						9xDerei_070					
DFF	182.2 ^{ns}	91.1	0.0	-0.6	96.3	7.2	85**	42.5	0.5	-0.9	62	15.8	223.5***	115.9	0.0	-1.2	75.2	21.4	239.9 ^{ns}	119.9	0.1	-0.9	91.2	16.3
DFF	195.8 ^{ns}	97.9	0.0	-0.9	94.7	9	103.9**	52.0	1.2	0.9	63.8	17.1	339.8***	174.2	0.6	0.3	84.4	20.6	317.7 ^{ns}	158.9	0.4	-1.0	89.1	16.3
DPM	394.6***	197.3	0.1	-0.4	88	19.1	189.9 ^{ns}	94.9	0.5	-0.9	46.5	28.1	549.9**	270.1	0.0	-1.0	72.5	34.7	500.3 ^{ns}	250.2	0.3	-0.9	89.4	20.1
PT	0.7 ^{ns}	0.4	0.6	-0.6	0	2.6	0.6 ^{ns}	0.3	1.1	0.3	0	2.4	0.7 ^{ns}	0.4	1.5	1.1	42.6	1.9	0.6 ^{ns}	0.3	1.2	0.3	11	2.1
HVS	1.6**	0.8	0.3	-1.2	68.5	2	2.2**	1.2	0.2	-0.9	66.7	2.4	1.5**	0.9	0.6	0.3	75.9	1.8	1.9***	1.0	1.0	0.2	71.8	2
GH	1.1*	0.6	0.1	-0.3	61.2	1.8	1.8 ^{ns}	1.0	0.5	-0.2	51	2.7	1.5**	0.8	1.2	1.5	1.5	2	1.0***	0.5	0.2	-0.1	71.5	1.5
%PS	423.8***	217.2	0.1	-0.5	72.8	30.6	525.8***	308.0	0.3	-0.5	76.4	31.9	351.1**	185.2	0.3	-0.3	73.1	28.8	431.9*	230.8	-0.2	-0.5	58.1	37.9
PHT	816.9**	16.8	0.5	-0.5	67.6	9.2	31.7 ^{ns}	18.1	0.1	0.2	0	17.1	31.1*	14.2	0.5	0.2	64.8	9.7	46.7***	24.5	0.2	-0.6	75.7	9.5
FPP	297.4 ^{ns}	160.2	0.8	-0.2	17.9	45.1	350.7*	180.8	1.0	1.0	61.2	33.9	490.3*	249.8	1.0	0.6	55.3	43.4	365.4 ^{ns}	186.7	0.6	-0.6	10	51.1
UPP	1.9 ^{ns}	1.0	0.5	-0.7	30	3.3	2.5 ^{ns}	2.6	2.0	4.6	0	5.9	1.3 ^{ns}	0.7	0.8	0.2	0	3.6	1.0 ^{ns}	0.6	0.3	1.9	0	4.1
TPP	354.2 ^{ns}	189.8	1.0	0.2	12.9	50.7	366.4*	194.3	0.8	0.6	54.7	37.4	520.9*	266.5	1.0	0.6	57.2	43.7	370.8 ^{ns}	190.0	0.6	-0.6	5.1	52.9
TSP	532 ^{ns}	284.1	1.2	1.2	11.6	62.7	264.6 ^{ns}	132.0	0.5	-0.5	47	34.4	520.4*	247.0	1.2	1.1	57.5	43.5	268.5 ^{ns}	136.8	0.4	-0.4	0	46.3

HSW	117.2 ^{ns}	2.3	-1.1	1.9	0	7.2	7.5 ^{ns}	3.6	1.2	2.3	21.7	7	6.6**	3.5	0.1	-0.3	66.3	4.4	7.2 ^{ns}	3.7	-0.2	0.9	31.8	6.3
PBP	23.1 ^{ns}	13.2	0.4	-0.4	16.7	12.7	54.7 ^{ns}	29.6	0.2	-0.7	31.9	17.7	48.8 ^{ns}	24.3	0.4	-0.5	44	15.3	42.4 ^{ns}	21.5	1.2	1.8	23.8	16
GYP	32.9***	32.2	4.2	17.3	82.5	6.9	4.6 ^{ns}	2.4	0.8	0.1	5.8	6.1	6.9 ^{ns}	3.2	0.8	0.4	41.7	4.3	3.3 ^{ns}	1.7	0.7	0.0	10.6	4.9
PHI	197.2 ^{ns}	102.8	0.2	-0.6	24.7	35.2	249.7 ^{ns}	118.3	0.3	-0.4	8.6	43.9	145.7 ^{ns}	74.7	-0.2	-1.2	29.1	29.8	151.2 ^{ns}	78.4	0.2	-0.6	11.7	32.6
9xDerei_072				9xEgill_065				9xEgill_073				9xKalkan_064												
DFE	218.3***	109.2	-0.1	-0.1	86	15.4	151.2***	75.6	0.2	-0.2	75.9	16.7	117.6***	58.8	0	-1	73.9	15.4	292.3***	180	-0.1	-1.1	94	12.2
DFP	221.2***	110.6	0.0	-0.6	79.8	18.6	170.1**	85.0	0.5	-0.1	67.1	20.7	143.4***	71.7	0	-1	71.3	17.8	331.8***	182	0.2	-0.8	94.2	12.8
DPM	455***	227.5	-0.3	-0.6	85.7	22.5	263.8***	181.9	0.4	-0.2	77.6	25	329.1**	164.5	0	-1	67.5	28.7	488.4***	281	-0.2	-1.3	92.1	18.1
PT	0.6 ^{ns}	0.3	1.5	1.5	14.6	2	0.9 ^{ns}	0.5	0.8	-0.8	25.5	2.3	1.1 ^{ns}	0.6	1	-1	43.2	2.3	1.2 ^{ns}	1	0.5	-1.4	45.4	2.4
HVS	2.0**	1.1	0.2	-1.5	66.4	2.4	1.9***	1.0	0.6	-0.4	70.7	2.1	1.9***	1.0	0	0	79.9	1.8	2.7***	1	1.4	0.8	80.7	2.1
GH	1.0*	0.5	0.1	0.1	60.8	1.8	0.9 ^{ns}	0.4	0.4	-0.8	32.3	2.2	1.4***	0.7	0	0	77.2	1.6	0.7**	0	1.4	1.1	72.5	1.3
PPS	414.3***	223.6	-0.2	-1.3	75.4	28.9	360.6**	182.6	-0.4	-0.6	67.2	30.9	425.6***	213.9	0	0	83.3	24.3	813*	314	-0.5	0.0	66.7	49.2
PHT	37.7 ^{ns}	22.3	-0.2	-0.1	25.1	15.4	48.1***	24.2	0.9	0.1	72	10.4	34.2*	19.6	0	-1	54.6	11.4	76.6**	45	0.3	0.1	74.3	13.3
FPP	178.3 ^{ns}	93.1	0.5	-0.8	10.3	36.7	239.2 ^{ns}	135.1	0.5	-0.7	10.7	41.5	393.6 ^{ns}	201.1	1	1	42.2	43.6	559.8 ^{ns}	222	-1.2	0.5	0	78.6
UPP	1.0 ^{ns}	0.6	-0.1	-0.8	0	4.4	2.8**	1.9	1.1	2.1	63.6	2.9	4.3 ^{ns}	2.2	1	2	38.6	4.7	2.6 ^{ns}	2	0.4	-0.7	45.5	3.6
TPP	199.2 ^{ns}	104.3	0.5	-0.8	20.4	36.6	250.3 ^{ns}	133.2	0.3	-1.0	5.3	43.7	44.5 ^{ns}	226.0	1	1	41.8	46.5	644.5 ^{ns}	259	-1.2	0.3	0	79
TSP	202.9 ^{ns}	105.6	0.6	-0.6	9.4	39.4	211.9 ^{ns}	118.0	0.3	-0.9	11.2	38.9	389.6 ^{ns}	202.1	1	1	41.1	43.8	537.6 ^{ns}	256	-0.9	-0.3	0	76.3
HSW	4.9 ^{ns}	2.5	0.6	-0.1	0	11	4.5 ^{ns}	2.4	0.2	-0.2	26.4	5.1	4.7 ^{ns}	2.4	0	0	0	9.4	4.7 ^{ns}	2	-0.7	-0.2	28	5.5
PBP	31.7 ^{ns}	17.1	0.6	0.4	0	23.5	38.3 ^{ns}	20.5	0.3	-0.1	0	19.2	45.6 ^{ns}	24.8	1	3	28.7	16.5	43.3 ^{ns}	26	-0.3	-1.2	0	20.6
GYP	2.4 ^{ns}	1.3	0.4	-0.9	0	5	2.9 ^{ns}	1.7	0.4	-0.7	14.6	4.5	4.7 ^{ns}	2.5	1	1	24.9	5.4	6.4 ^{ns}	3	-0.3	-0.8	0.5	7.5
PHI	171 ^{ns}	89.6	0.2	-1.3	43.5	28.5	128 ^{ns}	66.2	-0.2	-0.8	45.9	23.6	148.7 ^{ns}	75.5	0	-1	8.5	33.7	220.7*	100	-0.7	0.7	64.7	26.4
9xKesen_075				9xOyali_084				9xSavur_063				9xSirna_060												
DFE	245.7***	122.9	-0.1	-0.7	86.1	16.3	252.6***	126.3	-0.2	-0.8	94	11.2	221.5***	111	0.7	0.9	85	16	179.6***	92.2	-0.2	-0.2	83.7	15.1
DFP	276.3 ^{ns}	138.1	0.1	-0.7	91.1	13.8	244.8***	122.4	0.0	-1.0	87.5	15.9	219.5***	110	0.8	1.0	84.4	16.2	214.7***	109.1	0.4	0.0	80	18.3
DPM	451.8***	225.9	-0.2	-0.6	87.7	20.7	484.5***	242.2	-0.1	-0.9	90.6	19.4	402.3***	201	0.3	0.0	81.8	23.8	429.9***	218.0	0.0	-0.2	81	25.2
PT	8.1 ^{ns}	0.4	0.8	-0.4	10.7	2.4	0.6 ^{ns}	0.4	0.3	-0.7	0	3.5	0.8 ^{ns}	0.4	0.6	-0.6	0	2.7	0.9 ^{ns}	0.5	1.1	0.0	22.4	2.4
HVS	1.7***	0.9	0.4	0.3	75	1.9	0.9 ^{ns}	0.5	0.1	-1.2	38.4	2.3	1.2*	0.6	1.1	2.0	50.6	2.1	1.7*	0.9	0.3	-1.1	52.2	2.6
GH	1.3***	0.7	0.8	0.4	83.9	1.3	0.7 ^{ns}	0.4	-0.1	-0.4	0	2.9	1.1***	0.6	1.6	2.2	81.8	1.3	51.4 ^{ns}	0.5	0.1	-1.2	0	21.5
PPS	450.1***	230.8	0.9	2.1	82.1	25.6	347.5 ^{ns}	183.3	0.7	-0.6	48.9	40.9	478.1*	239	-0.3	-0.2	48.9	43.5	321.7 ^{ns}	189.5	0.0	-0.5	40.2	40.7
PHT	36 ^{ns}	18.3	0.4	-0.2	9.1	16.3	53.8*	28.1	0.3	-1.2	62.7	13.8	45*	22.5	0.3	-0.8	52	13.1	57.3 ^{ns}	11.4	-0.2	-0.4	15.7	20.7
FPP	249.8 ^{ns}	176.7	1.7	4.0	20.1	40.3	331.4 ^{ns}	175.8	0.3	-0.7	0	62.4	308 ^{ns}	154	0.5	-0.9	26.8	42.3	343.2 ^{ns}	283.3	1.2	1.8	0	60.6
UPP	1.4 ^{ns}	1.1	2.4	7.0	16.9	3.1	2.4 ^{ns}	1.3	0.4	-0.8	23.9	4.2	2.3 ^{ns}	1.1	0.3	-1.0	30.9	3.5	84 ^{ns}	1.5	0.9	0.6	3.5	26.8
TPP	274.1 ^{ns}	197.3	1.8	4.6	18.6	42.6	351.3 ^{ns}	187.5	0.3	-0.7	0	64.3	342.4 ^{ns}	171	0.5	-0.8	29.5	43.7	606.6 ^{ns}	313.0	1.2	1.7	50.6	51.3

TSP	272.8 ^{ns}	159.2	1.0	0.6	0	51.2	399.2 ^{ns}	183.6	0.5	0.2	0	67.4	334 ^{ns}	167	0.6	-0.5	14.9	47.4	426.6 ^{ns}	358.3	1.7	3.9	0	68.3
HSW	5.0 ^{ns}	2.6	-0.8	1.3	12.7	6	3.4 ^{ns}	2.3	1.1	0.9	17.7	5.1	5.3 ^{***}	2.7	0.0	-0.5	76	3.2	5.1 ^{ns}	1.5	1.2	1.1	0	7.5
PBP	13.7 ^{ns}	9.5	1.2	3.5	0	13.5	78.9 ^{ns}	40.7	1.0	0.6	3.8	26.7	30.8 ^{ns}	15.6	1.3	2.3	44.5	11.6	72.1*	61.2	2.5	7.4	54.9	17
GYP	3.0 ^{ns}	1.6	0.7	0.1	0	5.9	3.9 ^{ns}	2.2	0.4	-0.5	0	7.5	3.6 ^{ns}	1.8	1.1	0.7	0	5.4	31.2 ^{ns}	3.3	1.9	5.2	50.2	11.7
PHI	168.4 ^{ns}	84.0	0.3	0.1	14.2	34.3	250.2 ^{ns}	153.1	0.8	0.0	0	48.9	259.8 ^{ns}	135.0	0.9	0.9	63.4	53.1	126.4 ^{ns}	82.4	0.1	-0.7	0	34.6
9xBari3_072C						9xGunas_063						9xKarabache_081A						9xS2DrD						
DFE	150.8**	67.7	0.6	-0.7	76.4	17.5	24.4 ^{ns}	12.2	0.0	-0.8	42.8	11.6	227.2 ^{ns}	113.6	1.0	-0.4	78.5	32.2	145.3 ^{ns}	72.7	-0.5	-0.1	55.4	24
DFP	261.8***	143.4	1.2	0.5	83.2	19.5	34 ^{ns}	17.0	0.3	0.0	61.8	11.2	323.4*	161.7	0.9	-0.6	87	29.9	168.2 ^{ns}	84.1	-0.1	-0.5	25.9	33.2
DPM	334.7**	131.8	0.5	0.0	70.1	29.4	52.9 ^{ns}	26.4	-0.5	-0.2	59.9	14.3	627.5*	313.8	0.8	-0.6	91.4	33.9	299 ^{ns}	149.5	-0.5	0.3	29.3	43.3
PT	0.6 ^{ns}	0.5	1.4	0.6	10.4	2.4	0.7 ^{ns}	0.3	3.0	7.1	1	0	0.4 ^{ns}	0.2	1.5	0.3	0	4.5	0.4 ^{ns}	0.2	1.1	-0.9	0	2.5
HVS	1.4 ^{ns}	0.6	0.1	-0.8	53.5	2.5	2.5**	1.2	-1.1	1.0	80.1	2.2	1.3 ^{ns}	0.7	-1.2	-0.2	30.7	5.8	1.7 ^{ns}	1.0	-0.5	-1.2	33.7	3.3
GH	0.8 ^{ns}	0.6	1.1	-0.4	45.9	2.1	1.8 ^{ns}	0.9	0.1	-0.7	1.7	4.1	1.9 ^{ns}	1.1	0.7	-0.8	36	6.9	2.5*	1.4	0.7	-0.6	71.7	2.7
%PS	332.4*	129.3	0.0	0.0	67	32.9	355.7**	177.9	0.6	-0.1	79.9	26.2	257.2 ^{ns}	128.9	1.0	-0.3	65.1	58.3	365.0 ^{ns}	200.7	0.5	-1.2	27.5	51.4
PHT	13.7 ^{ns}	10.7	-0.4	-0.5	0	14.7	26.7 ^{ns}	13.3	0.0	-1.1	58.3	10.4	57.8 ^{ns}	29.9	0.6	-0.7	0	63.9	67.6 ^{ns}	54.6	0.9	0.9	43.7	20.2
FPP	267.6 ^{ns}	175.0	1.2	1.0	22.7	45.3	496.9 ^{ns}	248.4	0.7	-0.5	0	81.2	65.7 ^{ns}	34.5	0.1	-1.2	31.8	41.2	914.2 ^{ns}	845.7	2.4	5.3	52.7	68.1
UPP	1.7 ^{ns}	0.9	0.1	-1.4	3.6	4	22.8 ^{ns}	11.4	1.2	-0.2	31.1	12.3	12.2 ^{ns}	6.8	1.4	0.1	68.3	12.1	12.2***	12.4	3.2	8.9	88.2	3.9
TPP	295.4 ^{ns}	187.4	1.3	1.1	20.2	48.3	679.8 ^{ns}	339.9	0.9	-0.1	0	81.9	73.8 ^{ns}	49.5	0.1	-1.5	0	58.6	1083.6 ^{ns}	1032	2.6	6.2	57.8	70
TSP	382.6 ^{ns}	176.3	1.1	0.3	36.2	49.2	498.1 ^{ns}	249.1	1.4	1.3	27.2	59.2	67.3 ^{ns}	33.7	0.2	-1.2	0	66.4	990.0 ^{ns}	957.0	2.4	5.6	58.7	66.2
HSW	5.0 ^{ns}	3.9	-1.0	-0.6	0	8.4	1.7 ^{ns}	0.8	-0.1	-1.4	0	5.1	7.2 ^{ns}	7.9	1.4	0.1	0	19.6	14.5 ^{ns}	9.4	0.1	0.9	0	14.5
PBP	30.3 ^{ns}	13.6	0.5	-0.9	0	19.9	101.6 ^{ns}	55.8	0.8	-0.1	60.2	20.7	61.2 ^{ns}	33.2	0.0	-1.6	49.3	34.3	111.2 ^{ns}	97.1	1.2	0.7	51.5	24
GYP	3.8 ^{ns}	1.7	1.0	0.0	16.4	5.6	5.5 ^{ns}	2.7	1.3	1.0	29.1	6.1	1.0 ^{ns}	0.5	-0.9	-0.5	0	10.6	33.4**	34.5	3.1	8.3	81.6	8.1
PHI	150.9 ^{ns}	88.0	-0.2	-0.5	15.7	35.5	56.5 ^{ns}	28.2	0.2	-1.4	0	27.2	351.8 ^{ns}	173.5	0.7	-0.8	61.4	71.7	292.8 ^{ns}	242.4	1.9	3.7	37.4	44.3

ANOVA = analysis of variance, KU = kurtosis, LSD = least significant difference, H²b = broad senese heritability, AR = *Cicer arietinum* and *C. reticulatum*, AE = *Cicer arietinum* and *C. reticulatum*, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, MS = mean square, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type, TPP = total pod per plant, TSP = Total seed per plant, SK = skweness, UPP = unfilled pod per plant, V = variance, ns = non-significant, * significant at P≤0.05, ** Significant at P≤0.01, *** Significant at P≤0.001.

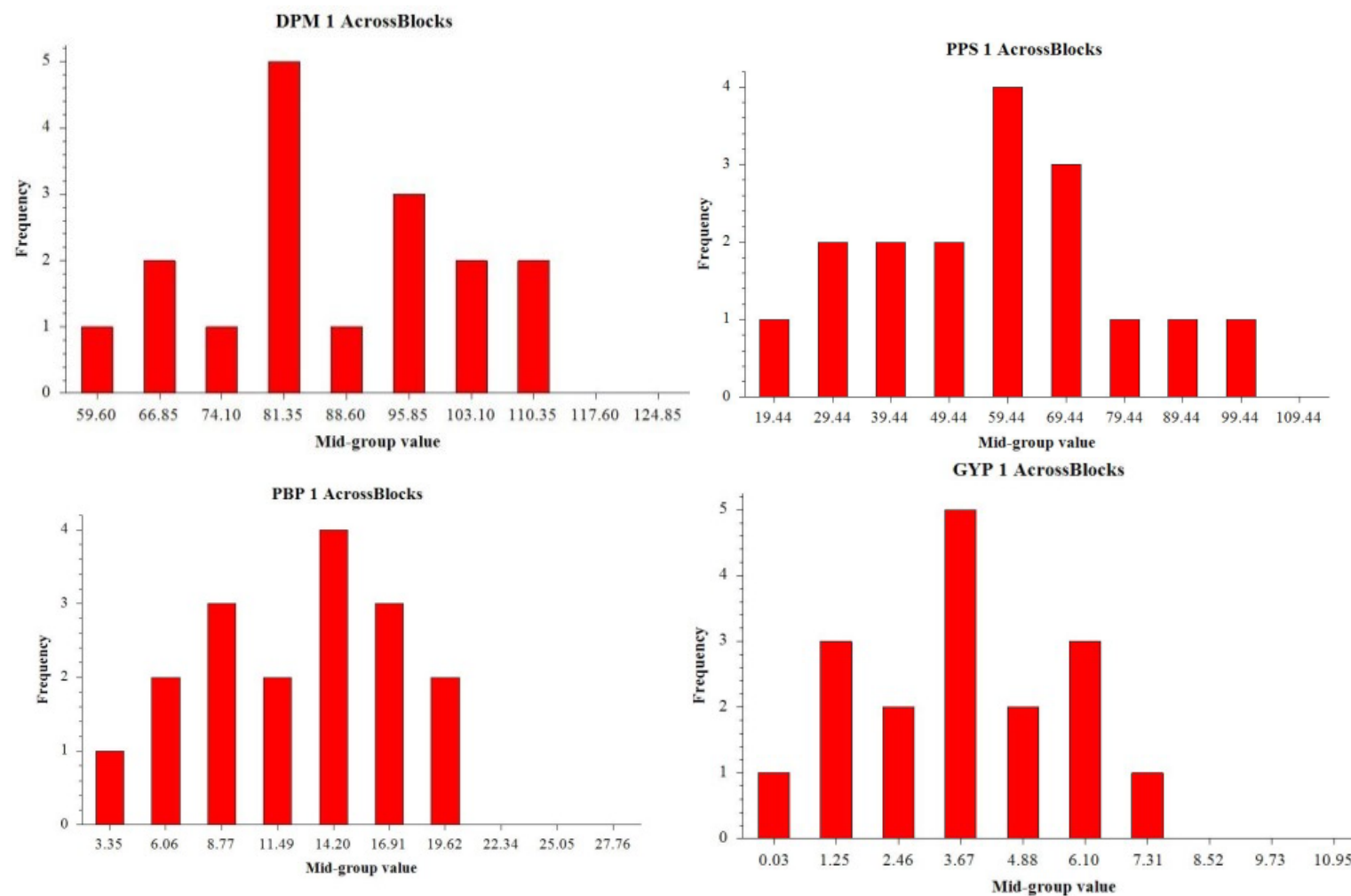


Fig 3.3. Frequency distributions of four traits of F₃ lines of 9xKalkan_064. a. DPM, b. %PS, c. PBP, d. GYP. DPM = days to physiological maturity, %PS = percentage of pod set, GYP = grain yield per plant, PBP = plant biomass per plant

Analysis of variance among traits

There were highly significant differences ($P < 0.001$) among F_3 lines of 13 AR families for three phenological traits and significant differences were not found among F_3 lines of 9xDerei_070 for these traits. TPP and TSP were significantly different among F_3 lines of 9xCudiB_22C. Highly significant differences were found among F_3 lines of 9xBari1_092 for %PS, HVS and growth habit. There were no significant differences among F_3 lines of most AR families for the majority of the traits used under this study. Least significant difference (LSD at $P = 0.05$) values among lines were high for TSP, TPP, FPP, %PS and PHI in most families (Table 3.2).

3.3.3. Variance and heritability of traits

All families had high phenotypic variances among F_3 lines for yield related traits (TSP, TPP, FPP, and %PS) and DPM. Out of 16 traits, very high phenotypic variability was obtained for FPP and TSP among F_3 lines of 9xS2DrD. Less phenotypic variances were found among F_3 lines of all families for HSW, GYP, and UPP. 9xBari3_100D and 9xOyali_084 had the highest phenotypic variability for phenological traits, while 9xGunas_063 had the least phenotypic variability for phenological traits. The highest and least phenotypic variance was found among F_3 lines of 9xBari1_092 and 9xOyali_084 for HVS, respectively. There was no phenotypic variability among F_3 lines of 9xKalkan_064 for growth habit, while high phenotypic variability was found among AE families for growth habit. Comparatively, F_3 lines of 9xS2DrD had the highest phenotypic variability for PBP, GYP and PHI, while F_3 lines of 9xKesen_075 had the least phenotypic variability for PBP and GYP. Phenological traits in most families had very high heritability (62-95%) than other traits. Most families had high heritability for HVS, GH, %PS

and PHT. In all families, GYP and yield related traits (PBP, TSP, FPP, PHI and HSW) had low to high heritability (Table 3.2).

3.3.4. Correlations among traits

DFP was positively and highly significantly correlated with DFP and DPM in all families. DFF and DPM were positively correlated with HVS in all families except 9xS2DrD and negatively correlated with %PS in all families except 9xGunas_063. These traits were negatively correlated with GYP and yield related traits (FPP, TPP, TSP and PHI) in most families. HVS was negatively and highly significantly correlated with GYP and yield related traits in all families. GH was negatively correlated with PHT in most families, while it was positively correlated with grain yield and yield related traits in all families except 9xCudiB_22C and 9xKalkan_064. PHT was positively correlated with GYP and yield related traits in all families except 9xKarabache_081A. %PS was positively and significantly correlated with GYP and yield related traits in all families except 9xOyali_084 and 9xKarabache_081A. FPP was positively and highly significantly correlated with TSP and GYP in all families. It was also positively and highly significantly correlated with PBP and PHI in most families. UPP was a common trait within 9xKarabache_081A family and it was negatively correlated with TSP, GYP, PBP and PHI. TSP was positively and significantly correlated with GYP, PBP and PHI in all families. PBP was positively and significantly correlated with GYP in all families and negatively correlated with PHI in most families. GYP was positively and significantly correlated with PHI in all families (Appendix 3.2).

3.3.5. Interspecific genetic linkage maps

A total of 19 interspecific genetic linkage maps were constructed for each of the 19 families of AR (17) and AE (2). In AR families, 1445 SNPs were used to form a range of recombination bins, 565 to 88, which were used for the construction of interspecific genetic linkage maps. Out of 17 interspecific genetic linkage maps of the AR families, the genetic linkage map of ICCV96029xDerei_072 family consists of the largest number of bin SNPs (565), while the family ICCV96029xBari3_072C (88) consists of small number of bin SNPs. The total genetic map distance covered by these recombination bins were 660 cM and 259.6 cM of the chickpea genome, respectively that were distributed in eight chromosomes. An overall average marker density of AR families ranges from 0.34 to 1.44 SNPs/cM and genetic linkage maps covered within the ranges of 140.4 cM to 660 cM of the chickpea genome. The family ICCV96029xKesen_075 (1.44 SNPs/cM) had the highest marker density, while the family ICCV96029xBari3_072C (0.34 SNP/cM) had the least marker density (Table 3.3).

In AE families, 1541 SNPs were used to form 161 and 154 recombination bin SNPs that were used for the construction of genome wide interspecific genetic linkage maps for ICCV96029xS2DrD and ICCV96029xGunas_063 families, respectively. The linkage maps spanned a total genetic map distance of 588.6 cM and 475.1 cM of the chickpea genome in eight chromosomes and had marker density of 0.27 and 0.32 SNP/cM (Table 3.3).

3.3.6. Detection of QTLs for traits under heat stress

At LOD threshold ≥ 3.0 (Figs 3.4 and 3.5), 160 QTLs were detected for 16 phenological, agronomic and growth habit traits in 17 AR families under an extremely heat stress environment.

A range of 1 to 16 QTLs were detected and localized into two to seven linkage groups per each AR family (Figs 3.5 and 3.6). Maximum numbers of QTLs were detected from 9xKalkan_064 (16) family, while minimum numbers of QTLs were detected from 9xEgill_073 family. In AR families, out of 16 traits, large numbers of QTLs were detected for phenological traits: DFF (20), DPM (16), and DFP (15), while small numbers of QTLs were detected for GYP (4). An average of 9 and 10 QTLs were detected per family and per trait, respectively (Fig 3.6). The QTLs for all traits were localized into 3 to 7 linkage groups of the chickpea genome (Fig 3.6). Large number of QTLs were detected in linkage groups 4 (33), 3 (31) and 6 (28). Small numbers of QTLs (3) were detected in linkage group 8 (Table 3.4).

A total of 31 QTLs having pleiotropic effects were found and distributed in all linkage groups (Fig 3.7). Most of these pleiotropic loci were localized in linkage groups 3 and 4 (Figs 3.6 and 3.7c). Out of 31 QTLs, 11 QTLs are responsible for either two or three of the phenological traits (DFF, DFP and DPM) in which most of them were distributed in linkage groups 3, 5 and 6 (Figs 3.6 and 3.7). Among 11 QTLs, the four QTLs are responsible for the three phenological traits (Figs 3.6 and 3.7). A total of 10 QTLs are responsible for grain yield and yield related traits. Out of 10 QTLs, a single QTL obtained from 9xSirna_060 family is responsible for five-grain yield and yield related traits (FPP, TPP, TSP, GYP and PBP), located in linkage group 4 (Fig 3.6). Two QTLs, each located in linkage groups 6 (HVS and %PS) and 8 (HVS and TSP) had pleiotropic effect for these pair of traits (Fig 3.6). A maximum of 4 pleiotropic loci have found from each of 9xKesen_075, 9xBari3_100, and 9xDerei_070 families (Table 3.4).

A maximum LOD value for AR families was 21.5, which was associated with a single QTL for DFF, located in linkage group 4, explained 59.3% of the total phenotypic variations, and detected from 9xDerei_072. The total phenotypic variations explained by 160 QTLs detected from AR families ranges from 3% to 93%. Out of 160 QTLs, 154 (96.3%) QTLs were major and 6 QTLs were minor which explained the range of phenotypic variations from 10.5% to 93% and 3% to 9.3%, respectively. Out of 160 QTLs, 59 QTLs were introgressed from 17 diverse wild parents of *C. reticulatum*. Large numbers of QTLs were introgressed from Kesen_075 (7), Kalkan_064 (6), and Bari3_100 (6) accessions of *C. reticulatum* (Table 3.4).

Our results revealed that one or more QTLs were introgressed into the genetic background of the elite cultivar, ICCV96029, for all traits from any of the 17 diverse wild accessions of *C. reticulatum* under an extremely heat stress condition. Large numbers of QTLs were introgressed for the traits of PHT (10), PHI (7) and DFF (5), while small numbers of QTLs were introgressed for the traits of GH (2) and GYP (1). Five QTLs were introgressed for filled pod per plant from Sirna_060, Kesen_075 and Bari3_100.

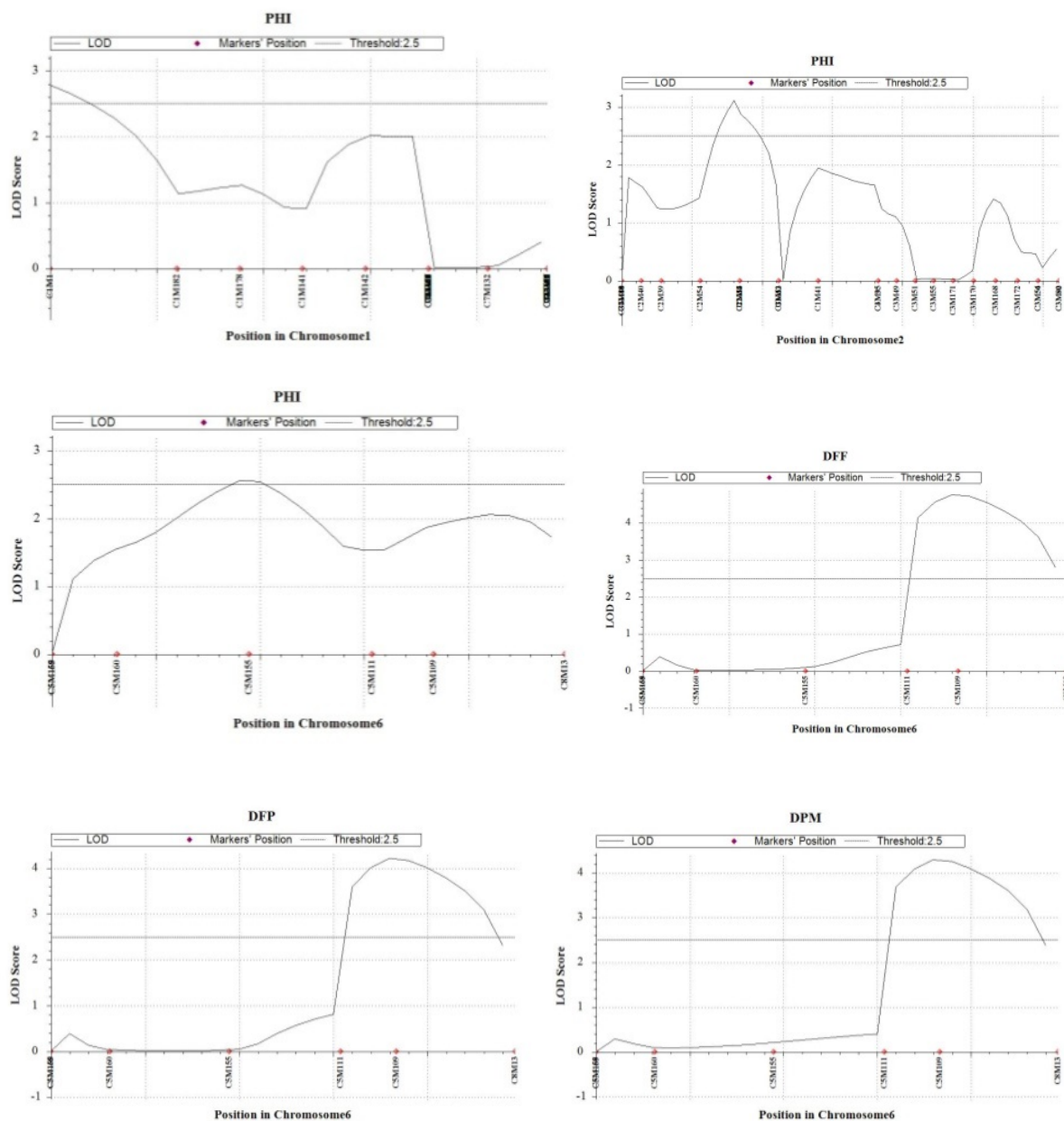
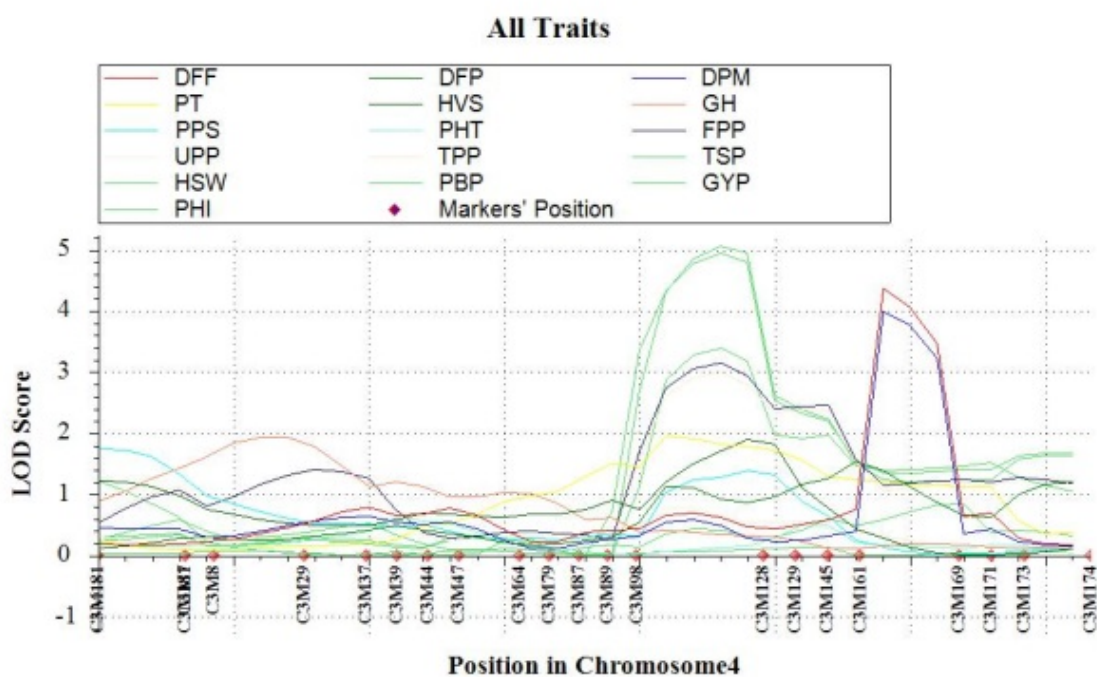
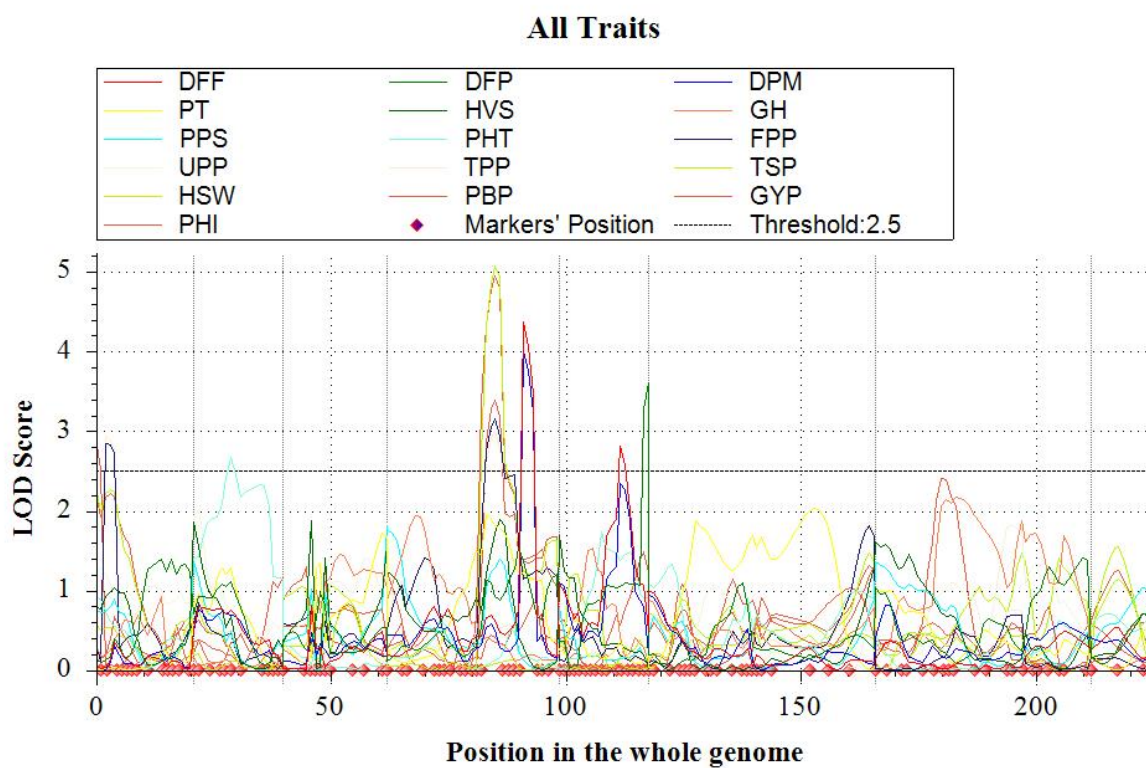


Fig. 3.4. The LOD scores of different traits of 9xKalkan_064 across the genome. a-c, different LOD scores of the same trait (PHI) in a different genome positions (Chrs 1, 2 and 6). d-f, the same LOD score and genome position (Chr 6) for three phenological traits (DFF, DFP and DPM). DFF, days to first flowering, DFP, days to first podding, DPM, days to physiological maturity, LOD = Logarithm of the odds, PHI = percentage of harvest index.



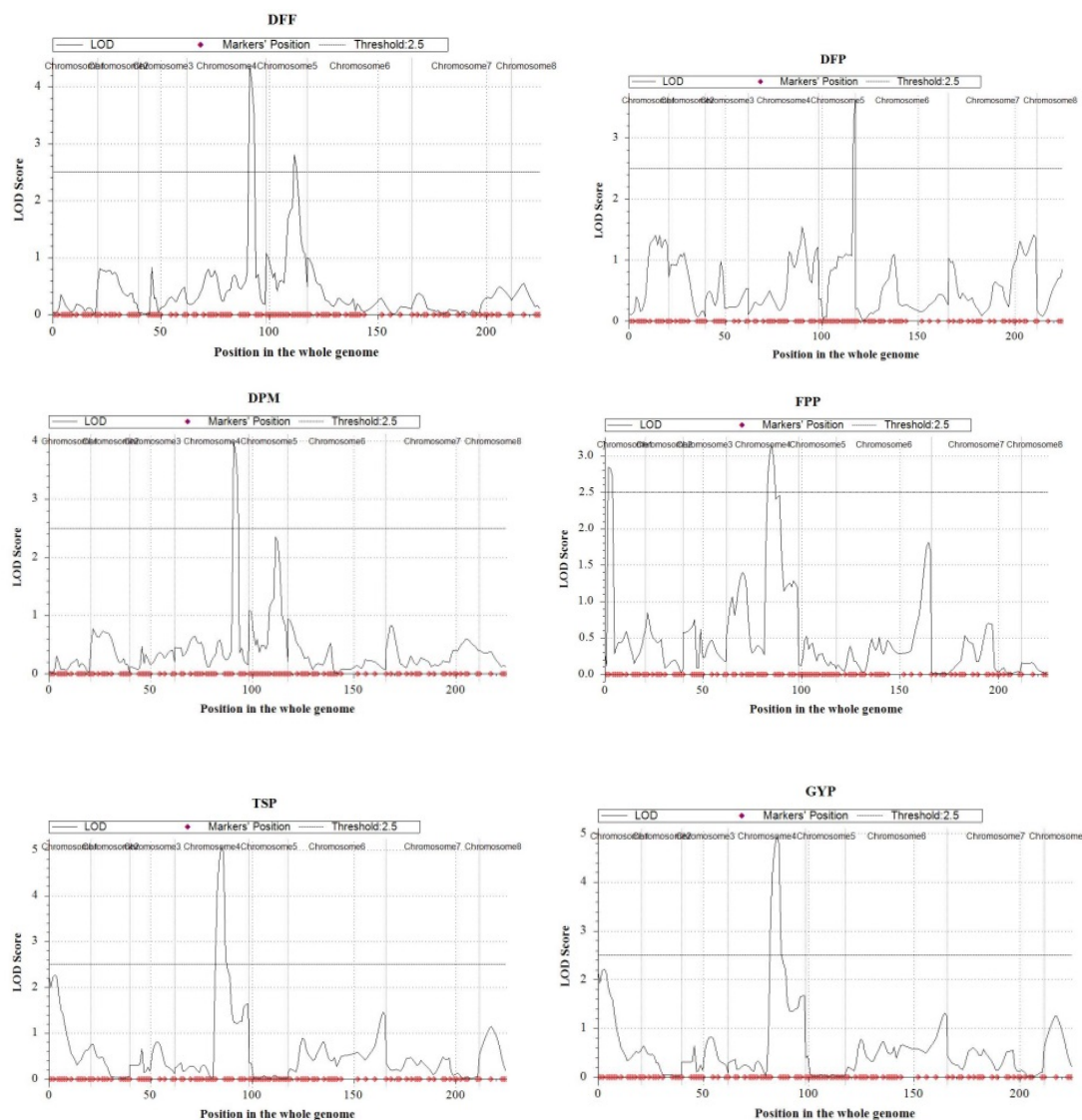


Fig. 3.5. The positions and LOD values of single traits and all traits from a biparental population of ICCV96029 crossed with a *Cicer reticulatum* accession Sirna_060. a, the position of the 16 traits on the whole genome position. b, the position of all traits located at chromosome 4. c, the LOD values and positions of DFF in the whole genome positions. d, the LOD values and positions of DFP in the whole genome positions. e, the LOD values and positions of DPM in the whole genome positions, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed

weight, HVS = heat visual score, GYP = grain yield per plant, LOD = Logarithm of the odds, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, PPS = percentage of pod set, PT = pod type per plant, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant.

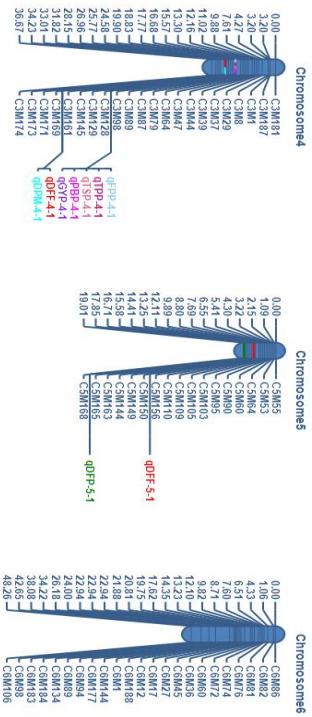
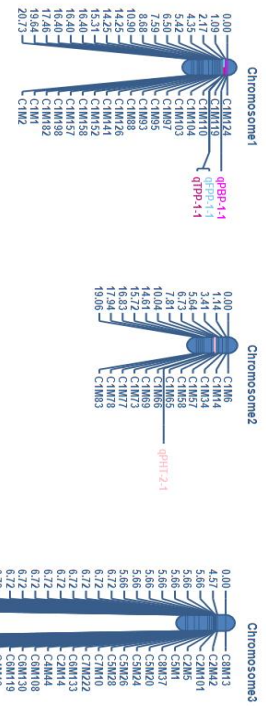


Fig. 3.6. Linkage and QTL mapping of 16 traits in all linkage groups from the biparental population of ICCV96029 crossed with a *Cicer reticulatum* accession Sirna_060. A QTL responsible for the expressions of five yield related traits(FPP, TSP, TPP, GYP, PBP) were located on Chr 4 and another QTL on the same chromosome was responsible for the phenotypic expressions of DFF and DPM. Markers are on the right, marker positions are on the left, QTLs (genomic regions) are indicated by different colors, which are flanked by different markers. Chr = chromosome, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GYP = grain yield per plant, PBP = plant biomass per plant, PHT = plant height, TPP = total pod per plant, TSP = Total seed per plant.

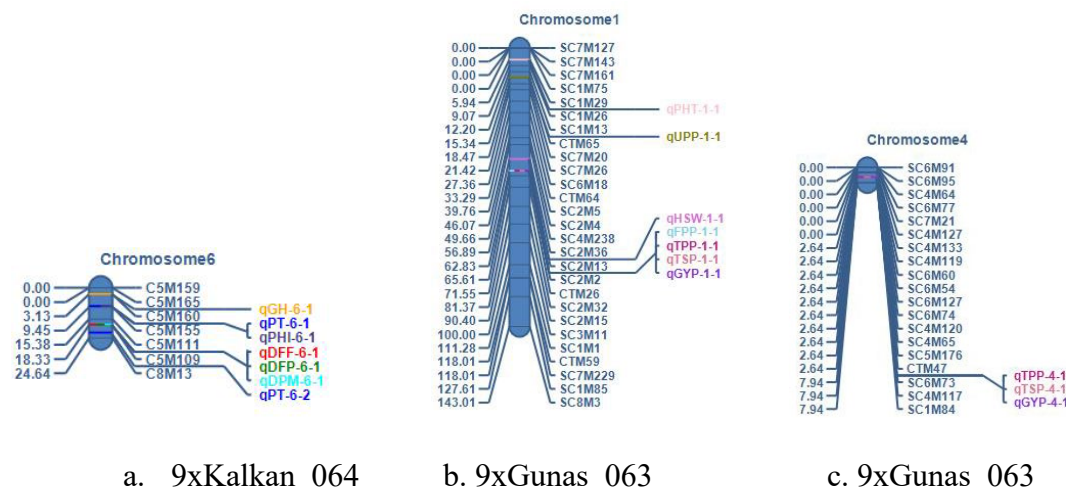


Fig. 3.7. Pleiotropic effects of a QTL for more than three phenological and agronomic traits. a, a QTL located on Chr 6 of 9xKalkan_064 family was responsible for three phenological traits (DFF, DFP, and DPM). b, a single QTL located on chr1 of 9xGunas_063 was responsible for phenotypic expressions of four closely related yield related traits such as FPP, TPP, TSP, and GYP. c, a single QTL located on chromosome 4 was responsible for the phenotypic expressions of three closely related yield related traits such as TPP, TSP and GYP. DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, GYP = grain yield per plant, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, PT = pod type per plant, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant.

Table 3.3. Interspecific linkage map for each of the 19 families (biparental populations) of AR and AE.

Family	Total Number of SNPs, genetic distance (cM) and marker density (SNP/cM), in their respective order								
	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Whole genome
9xBari1_092	116, 21.82, 5.3	26, 43.55, 0.6	6, 8.91, 0.7	15, 29.47, 0.5	4, 6.58, 0.6	13, 16.18, 0.8	10, 13.92, 0.72	1, 0.0	191, 140.43, 1.4
9xBari2_72n2	16, 45.46, 0.4	28, 49.15, 0.6	41, 42.6, 1	16, 40.78, 0.4	11, 27.52, 0.4	6, 18.43, 0.3	1, 0.0	1, 0.0	120, 223.94, 0.5
9xBari3_100	7, 13.3, 0.5	2, 10.82, 0.2	33, 92.85, 0.4	5, 5.1, 1	14, 21.39, 0.7	4, 6.79, 0.6	24, 55.82, 0.43	12, 30.56, 0.39	101, 237.1, 0.43
9xBari3_072C	26, 0.0	6, 0.0	13, 67, 0.2	16, 50.3, 0.3	10, 55.67, 0.2	12, 74.36, 0.16	4, 12.2, 0.33	1, 0.0	88, 259.57, 0.34
9xBari3_100D	7, 20.33, 0.34	53, 53.3, 1	16, 60.73, 0.26	3, 5.9, 0.51	8, 28.49, 0.28	14, 42.72, 0.33	1, 0.0	1, 0.0	103, 211.47, 0.49
9xBeseve_079	105, 24.48, 4.3	27, 25.94, 1	4, 11, 0.36	44, 28.34, 1.6	10, 16.72, 0.6	58, 60.69, 1	19, 11.74, 1.6	25, 39.31, 0.64	292, 218.22, 1.34
9xCudiA_152	43, 59.56, 1.4	75, 2.44, 30.7	55, 72.26, 0.76	3, 2.47, 1.2	33, 56, 0.6	35, 63.32, 0.55	2, 1.22, 1.6	34, 41.76, 0.81	280, 299.1, 0.94
9xCudiB_22C	42, 49.59, 0.85	8, 11.93, 0.67	16, 48.13, 0.33	36, 43.91, 0.82	15, 32.22, 0.47	18, 48.46, 0.37	8, 17.33, 0.46	12, 45.61, 0.26	155, 297.2, 0.52
9xDerei_070	66, 88.61, 0.74	35, 34.44, 1	12, 45.43, 0.26	22, 30.17, 0.73	30, 51.11, 0.6	19, 32.55, 0.58	14, 22.87, 0.61	1, 0.0	198, 305.18, 0.65
9xDerei_072	51, 64.76, 0.79	92, 60.78, 1.5	101, 43.74, 2.3	99, 155.79, 0.64	68, 86.69, 0.78	74, 131.49, 0.56	65, 92.68, 0.7	15, 24.1, 0.62	565, 659.99, 0.86
9xEgill_065	15, 27.76, 0.54	16, 27.62, 0.6	25, 25.29, 0.99	18, 57, 0.32	56, 50.53, 1.1	30, 67.21, 0.45	19, 23.26, 0.82	21, 42.13, 0.5	200, 320.83, 0.62
9xEgill_073	21, 33.27	74, 21.81, 3.4	6, 14, 0.43	15, 24.99, 0.60	22, 46.24, 0.48	12, 15.77, 0.76	8, 13, 0.62	22, 59, 0.37	180, 228.13, 0.79
9xKalkan_064	53, 23.3, 2.27	27, 62.31, 0.43	17, 48, 0.35	7, 25.18, 0.277	18, 27.73, 0.65	7, 24.64, 0.28	15, 58.83, 0.25	2, 2.78, 0.72	146, 272.78, 0.54
9xKesen_075	103, 37.83, 2.7	11, 22.8, 0.48	76, 30.87, 2.5	97, 108.41, 0.89	65, 34.9, 1.9	90, 39.22, 2.3	25, 36.7, 0.68	14, 22.43, 0.62	481, 333.17, 1.44
9xOyali_084	19, 40.99, 0.46	7, 13.31, 0.53	57, 53.35, 1.1	22, 49.59, 0.44	21, 64.87, 0.32	22, 62.87, 0.35	12, 38.67, 0.31	1, 0.0	160, 323.65, 0.49
9xSavur_063	41, 75.31, 0.54	51, 83.36, 0.61	11, 28.78, 0.38	27, 40.7, 0.66	18, 46.91, 0.38	25, 48.1, 0.52	5, 7.51, 0.67	1, 0.0	178, 330.66, 0.54
9xSirna_060	18, 20.73, 0.87	12, 19.1, 0.63	81, 22.6, 3.6	22, 36.67, 0.6	17, 19, 0.89	23, 48.26, 0.48	23, 45.77, 0.5	5, 13.34, 0.37	201, 225, 0.89
9xGunas_063	27, 143, 0.19	33, 120.2, 0.27	38, 5.27, 7.2	19, 7.94, 2.4	9, 42.69, 0.21	14, 62.18, 0.23	12, 87.55, 0.14	2, 6.31, 0.32	154, 475.1, 0.32
9xS2DrD	73, 194.57, 0.38	4, 12.83, 0.31	32, 95.39, 0.34	22, 118.65, 0.19	6, 21.94, 0.27	2, 19, 0.11	22, 126.18, 0.17	1, 0.0	161, 588.58, 0.27

AR = *Cicer arietinum* and *reticulatum*, AE = *Cicer arietinum* and *echinospermum*, chr = chromosome, SNP = single nucleotide polymorphism, cM = CentiMorgan

They were located in linkage groups of 1, 3, 5, and 7 which explained 20.8 to 43.1% of the total phenotypic variance (Table 3.4).

Ten QTLs were introgressed for PHI from wild *C. reticulatum* parents, out of which 3 and 2 QTLs were located in linkage groups of 3 and 6. A single QTL was located in each of linkage groups 1, 2, 4, 7, and 8. The major wild parents of *C. reticulatum* which had positive additive values and positive contributions to the introgressed QTLs for this trait under a heat stress condition were Kalakan_064, Bari3_100, Oyali_084, Beseve_079, and CudiB_022C. The total phenotypic variances explained by introgressed QTLs from these wild parents varied from 25.1% to 56.8%. The wild parents of *C. reticulatum* namely Kesen_075, Sirna_060, and Bari1_092 contributed 4 QTLs for TPP. Two of the QTLs were located in linkage group 3 and one QTL was located in each of linkage groups 1 and 5 that explained 20.1 to 32.8% of the total phenotypic variances (Table 3.4).

At LOD threshold ≥ 3 (Table 3.4), 40 QTLs were detected for 16 phenological, agronomic and growth habit traits in 2 AE families under a heat-stress environment. Twenty-one and 13 QTLs were detected from 9xS2DrD and 9xGunas_063 families, respectively. Seven QTLs had pleiotropic effects. All the pleiotropic loci identified from 9xGunas_063 were responsible for the expression of yield related traits (FPP, TPP, TSP and GYP) (Fig 3.7b, c). Each QTL explained the total phenotypic variations ranges from 3.5% to 86.6%. The same QTL was identified from both 9xGunas_063 and 9xS2DrD that was responsible for the expression of four-grain yield and yield related traits (FPP, TPP, TSP and GYP). Out of 34 QTLs, 33 QTLs were major QTLs ($>10\%$) and only a single QTL was a minor QTL ($<10\%$). The QTLs responsible for %PS

(85.8%) and HVS (77%) were introgressed from S2DrD. QTLs responsible for FPP (79.4%), TPP (94.6%), TSP (66.1%) and GYP (66.4%) were introgressed from Gunas_063 (Table 3.4).

3.3.7. Positioning QTLs on the physical and genetic maps of the genome

The sequences of the regions flanking the SNPs were used to anchor the QTLs to the chickpea physical map of the genome. The physical locations of all 230 QTLs found from 19 families were confirmed by each pair of flanking SNP markers located to an identical pseudochromosome of chickpea (Figs 3.6 and 3.7). A physical map of a QTL corresponds to its genetic map. For example, in Bari1_092, the genetic map distance of all QTLs varied from 0.5 to 6.5 cM which corresponded to a physical map distances of 0.48 to 20.5 Mb (Appendix 3.3).

In AR families, narrow range of genetic and physical map distances of QTLs flanked by a pair of SNP markers were revealed in 9xCudiA_152 (1-5 cM, 0.065-2.95 Mb). A total of 137 QTLs were located in the physical map distance of chickpea pseudochromosomes varied from 0-5 Mb, while 23 QTLs were mapped on the physical map distances of 5.5 to 49.3 Mb. The genetic map distances of 153 QTLs were varied from 0-10 cM, while 7 QTLs were mapped on the genetic map distances varied from 11 to 17 cM. In AE families, the genetic map distance of all QTLs from 9xGunas_063 varied from 1 to 8.5 cM which corresponded to the physical map distances varied from 0.3 to 23.5 Mb, while in 9xS2DrD, the genetic map distances of all QTLs ranges from 2 to 14 cM which corresponded to the physical map distances varied from 1 to 64.5 Mb (Appendix 3.3).

3.3.8. Epistatic interactions among digenic QTLs

In AR families, less pairs of epistatic interactions were revealed in 9xBari1_092 (5), while high pairs of epistatic interactions were found in 9xKalkan_064 (183) (Fig 3.8 and Table 3.5), 9xSavur_063 (101), 9xOyali_084 (71) and 9xKesen_075 (71) (Table 3.5). In each AR family, epistatic interactions were observed within the ranges of 4 to 16 traits. In all families, epistatic interactions (1-29) were observed for PT trait. In most families, yield related traits (FPP, GYP, TPP, TSP, PBP, PHI, HSW) possessed a pair of epistatic interactions varied from 1-22. Epistatic pairs of interactions were observed within the ranges of one linkage group to seven linkage groups. Phenotypic variations explained by most of the digenic QTL interactions of all families were less than 10%. In 9xSirna_060 family, out of 26 pairs of digenic epistatic interactions, 22 pairs of digenic epistatic interactions were for yield related traits and the phenotypic variations explained by each pair of epistatic interactions was greater than 10% (Table 3.5 and Fig 3.8).

In AE families, 242-306 epistatic pairs of interactions were observed (Table 3.5).

Table 3.4. Trait, QTL, Chromosome, LOD, PVE (%), and additive effect from each of the 19 biparental populations of *C. arietinum* crosses with *C. reticulatum* and *C. echinospermum*

9xBari1_092						9xBari2_072n2						9xBari3-072C					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
DFE	2	qDFF-2-1	3.0	14.1	0.3	DFE	4	qDFF-4-1	3.8	28.5	-3.6	DFE	5	qDFF-5-1	4.2	61.4	-10.1
DPM	1	qDPM-1-1	6.7	47.3	-15.6		4	qDFF-4-2	3.0	24.5	-3.7		6	qDFF-6-1	3.6	13.3	-2.3
	2	qDPM-2-1	3.5	20.8	1.0		7	qDFF-7-1	3.6	23.6	-3.4		6	qDFF-6-2	3.0	12.9	-2.4
%PS	1	q%PS-1-1	3.0	40.9	-2.6	DPM	6	qDPM-6-1	3.0	27.3	-2.6	DPM	5	qDPM-5-1	3.0	39.7	-13.5
PBP	3	qPBP-3-1	3.2	38.6	0.9	HVS	4	qHVS-4-1	3.6	60.9	-0.2		5	qDPM-5-2	3.0	37.7	-12.2
						HSW	3	qHSW-3-2	3.2	34.6	1.3	PT	4	qPT-4-1	3.0	73.5	0.6
												HVS	6	qHVS-6-1	3.4	15.2	-0.2
												TSP	4	qTSP-4-1	4.5	14.5	1.3
												HSW	3	qHSW-3-1	3.0	6.7	0.1
													5	qHSW-5-1	5.5	93.0	-0.3
												PBP	4	qPBP-4-1	3.7	82.2	-0.8
												PBP	6	qPBP-6-1	3.0	14.8	0.9
9xBari3_100						9xBari3-100D						9xBeseve_079					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
DFE	3	qDFF-3-1	3.5	28.8	-1.5	TSP	2	qTSP-2-1	3.6	39.7	-18.4	DPM	6	qDPM-6-1	4.17	51.06	-15.61
	6	qDFF-6-1	5.1	46.9	-10.4	GYP	2	qGYP-2-1	3.4	38.7	-2.0	PT	2	qPT-2-1	3.00	10.51	0.45
DFP	6	qDFP-6-1	3.9	46.3	-12.8								4	qPT-4-1	3.84	37.84	-0.45
DPM	3	qDPM-3-1	3.2	34.2	-0.4								4	qPT-4-2	3.28	24.49	-0.60
	6	qDPM-6-1	3.8	35.7	-14.9							GH	6	qGH-6-1	5.55	38.51	-0.81
FPP	5	qFPP-5-1	3.7	20.8	9.8							%PS	5	q%PS-5-1	3.95	33.51	8.59
	7	qFPP-7-1	5.5	43.1	0.1							PHT	1	qPHT-1-1	3.43	45.59	2.52
UPP	3	qUPP-3-1	3.0	37.1	0.9							PHI	7	qPHI-7-1	3.00	46.07	8.42
TPP	5	qTPP-5-1	3.6	20.1	9.7												
	7	qTPP-7-1	5.6	42.5	-1.1												
TSP	3	qTSP-3-1	3.4	42.4	-4.6												

PBP	3	qPBP-3-1	3.1	38.6	-2.0												
GYP	3	qGYP-3-1	4.1	39.4	-0.4												
PHI	3	qPHI-3-1	3.2	25.1	6.4												
	3	qPHI-3-2	3.9	35.7	10.7												
	8	qPHI-8-1	3.0	15.9	-6.1												
9xCudiA_152						9xCudiB_22C						9xDerei_070					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
PT	3	qPT-3-1	3	41.9497	0.2637	DFF	4	qDFF-4-1	3.6	54.9	-12.6	DFF	1	qDFF-1-1	11.7	60.0	-13.1499
GH	5	qGH-5-1	3	26.6242	-0.614	DFP	4	qDFP-4-1	3.4	51.9	-15.3	DFP	1	qDFP-1-1	10.1	60.0	-18.5086
	5	qGH-5-2	3.644	31.0659	-1.008	DPM	4	qDPM-4-1	5.2	51.1	-17.8	DPM	1	qDPM-1-1	9.7	60.6	-21.9142
PHT	1	qPHT-1-1	3	38.2615	3.6512		6	qDPM-6-1	3.4	26.3	-4.2	GH	2	qGH-2-1	3.0	43.3	-0.6774
PBP	3	qPBP-3-1	3	40.5167	0.2782	GH	4	qGH-4-1	3.7	55.5	-0.9	GH	5	qGH-5-1	3.0	26.6	0.4248
						FPP	3	qFPP-3-1	3.3	27.1	-17.4	%PS	1	q%PS-1-1	3.8	29.2	-2.5767
							3	qFPP-3-2	3.0	26.3	-16.3		3	q%PS-3-1	4.6	37.8	-14.7159
						UPP	1	qUPP-1-1	3.0	34.6	-0.7	PHT	1	qPHT-1-1	2.8	24.1	2.3737
							6	qUPP-6-1	3.5	39.6	0.9	PHT	2	qPHT-2-1	3.8	29.5	3.7092
						TPP	3	qTPP-3-1	3.4	27.3	-18.3	FPP	1	qFPP-1-1	3.7	25.5	-11.4492
							3	qTPP-3-2	2.6	26.3	-16.9		2	qFPP-2-1	3.1	21.8	-12.182
							1	qTSP-1-2	3.0	26.7	-25.8	TPP	1	qTPP-1-1	3.5	25.0	-11.4474
							3	qTSP-3-1	3.0	24.8	-16.0		2	qTPP-2-1	3.0	21.2	-12.2721
						PBP	2	qPBP-2-1	3.9203	25.2	4.0	GYP	4	qGYP-4-1	3.0	20.1	-0.7985
							6	qPBP-6-1	6.9547	59.3	-1.3						
						PHI	6	qPHI-6-1	3.0432	45.0	7.6						
9xDerei_072						9xEgill_065						9xSavur_063					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
DFF	1	qDFF-1-1	6.4	3.0	0.1	DFP	2	qDFP-2-1	4.2	40.9	0.0414	HVS	6	qHVS-6-1	3.0	49.1	-0.5
	4	qDFF-4-1	21.5	59.3	-13.4		5	qDFP-5-1	3.0	24.5	7.0371	GH	4	qGH-4-1	3.0	49.5	-0.6
	4	qDFF-4-2	17.4	29.4	-9.7	PT	4	qPT-4-1	3.1	39.8	0.5689	PPS	6	qPPS-6-1	3.2	53.3	12.9
DFP	2	qDFP-2-1	3.2	8.3	-8.0		9xEgill_073					PHT	1	qPHT-1-1	4.3	52.6	4.1
	2	qDFP-2-2	3.0	7.8	-7.8	Trait	Chr	QTL	LOD	PVE(%)	Add	HSW	3	qHSW-3-1	5.2	60.5	1.9
	4	qDFP-4-1	8.2	65.3	-12.5	PHT	4	qPHT-4-1	3.0	37.9	1.0237	PBP	4	qPBP-4-1	5.3	62.4	-5.6

DPM	2	qDPM-2-1	6.7	29.9	-13.0												
	4	qDPM-4-1	9.7	59.8	-17.0												
PPS	5	qPPS-5-1	4.0	22.9	-6.9												
	7	qPPS-7-1	3.1	18.8	-3.2												
PHT	4	qPHT-4-1	3.0	45.9	5.4												
UPP	3	qUPP-3-1	5.4	68.3	-0.1												
TSP	4	qTSP-4-1	3.3	29.9	6.0												
PBP	5	qPBP-5-1	3.0	25.5	2.5												
9xKalkan_064						9xKesen_075						9xOyali_084					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
	6	qDFF-6-1	4.8	74.7	-15.6	HVS	8	qHVS-8-1	3.0	40.5	-0.8	DFF	3	qDFF-3-1	3.6	5.3	-0.2
	7	qDFF-7-1	3.7	13.5	2.2	GH	4	qGH-4-1	3.7	47.8	0.1		3	qDFF-3-2	7.8	23.5	0.9
DFP	2	qDFP-2-1	3.0	14.0	-4.2	PPS	3	qPPS-3-1	3.0	39.7	13.2		6	qDFF-6-1	12.6	65.9	-13.0
	6	qDFP-6-1	4.2	81.3	-15.3	PHT	4	qPHT-4-1	3.5	46.4	-0.3	DFP	3	qDFP-3-1	3.0	16.9	-3.7
	6	qDPM-6-1	4.3	81.7	-19.1	FPP	3	qFPP-3-1	3.7	32.7	12.0		5	qDFP-5-1	3.0	15.1	-3.8
PT	2	qPT-2-1	3.1	26.3	-0.4		3	qFPP-3-2	2.7	26.1	9.3		6	qDFP-6-1	4.4	61.1	-13.9
	6	qPT-6-1	3.6	28.2	-0.3	TPP	3	qTPP-3-1	3.7	32.8	12.7	DPM	6	qDPM-6-1	11.0	67.1	-19.0
	6	qPT-6-2	7.9	37.0	-0.5		3	qTPP-3-2	3.0	26.5	9.8	HVS	6	qHVS-6-1	3.0	60.2	-0.6
GH	6	qGH-6-1	4.1	83.0	-0.7	TSP	8	qTSP-8-1	3.2	38.9	12.5	PPS	6	qPPS-6-1	3.1	53.2	13.3
PHT	1	qPHT-1-1	3.7	79.2	7.8							PHT	5	qPHT-5-1	3.4	49.8	-0.3
	5	qPHT-5-1	3.0	16.1	4.7							UPP	1	qUPP-1-1	4.8	68.3	-0.9
UPP	1	qUPP-1-1	4.8	53.9	-0.1							HSW	3	qHSW-3-1	3.6	25.2	-0.2
	2	qUPP-2-1	4.1	37.5	1.0								6	qHSW-6-1	6.3	59.3	-1.8
	4	qUPP-4-1	3.0	6.8	-0.2							PHI	3	qPHI-3-1	3.2	56.8	12.8
PHI	1	qPHI-1-1	3.0	30.7	6.6												
	2	qPHI-2-1	3.1	33.2	7.2												
9xSirna_060						9xGunas_063						9xS2DrD					
Trait	Chr	QTL	LOD	PVE(%)	Add	Trait	Chromosome	QTL	LOD	PVE(%)	Add	Trait	Chr	QTL	LOD	PVE(%)	Add
DFP	4	qDFF-4-1	4.4	39.0	3.1	PHT	1	qPHT-1-1	3.1	70.2	-2.2	DFF	1	qDFF-1-1	3.6	69.5	-11.3
	5	qDFF-5-1	3.0	14.8	-3.6	FPP	1	qFPP-1-1	3.2	79.4	14.8		7	qDFF-7-1	3.1	22.2	-3.7
DFP	5	qDFP-5-1	3.6	45.9	-5.4	UPP	1	qUPP-1-1	7.1	67.6	-0.2	DFP	1	qDFP-1-1	3.3	38.9	6.8

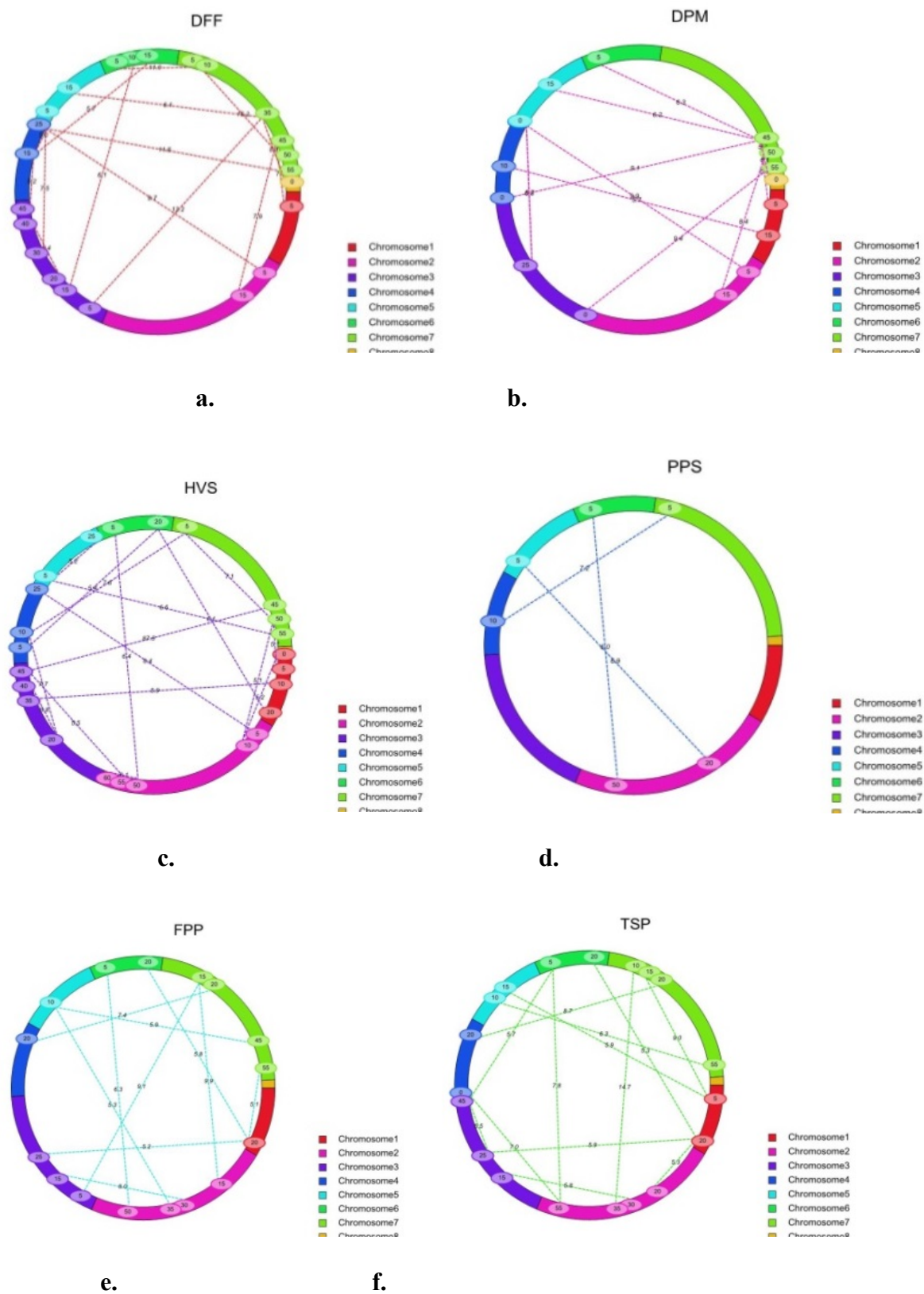
DPM	4	qDPM-4-1	4.0	27.9	3.4		3	qUPP-1-1	3.5	3.5	0.9	DPM	1	qDPM-1-1	3.7	38.8	12.0
PHT	2	qPHT-2-1	3.0	34.4	2.8	TPP	1	qTPP-1-1	3.0	27.4	17.1		7	qDPM-7-1	3.0	31.2	-6.0
FPP	1	qFPP-1-1	3.0	30.1	2.6		4	qTPP-4-1	3.0	30.3	21.6	PT	1	qPT-1-1	4.4	19.7	-0.4
FPP	4	qFPP-4-1	3.2	25.0	-18.5	TSP	1	qTSP-1-1	3.0	32.1	13.5		7	qPT-7-1	5.2	20.5	0.4
TPP	1	qTPP-1-1	3.0	31.3	2.7		4	qTSP-4-1	3.0	34.0	17.9		7	qPT-7-2	4.3	19.7	0.4
TPP	4	qTPP-4-1	3.0	24.2	-19.0		8	qTSP-8-1	3.0	30.7	-20.2	HVS	4	qHVS-4-1	7.6	77.0	0.0
TSP	4	qTSP-4-1	5.1	35.9	-24.7	HSW	1	qHSW-1-1	4.9	86.6	-0.3	GH	4	qGH-4-1	3.0	30.1	-1.0
PBP	1	qPBP-1-1	3.0	29.4	-0.2	GYP	1	qGYP-1-1	3.0	32.2	1.3		4	qGH-4-2	3.4	29.7	-1.0
PBP	4	qPBP-4-1	3.4	24.9	-6.5		4	qGYP-4-1	3.0	34.2	1.8	PPS	1	qPPS-1-1	3.0	10.7	-5.0
GYP	4	qGYP-4-1	4.9	33.8	-2.4	PHI	2	qPHI-2-1	3.0	70.4	-2.1		4	qPPS-4-1	6.2	85.8	3.4
												PHT	7	qPHT-7-1	3.0	60.2	-7.8
												FPP	1	qFPP-1-1	3.0	46.8	-13.8
												TSP	1	qTSP-1-1	3.0	32.1	-12.1
													3	qTSP-3-1	3.0	28.3	-14.6
												PBP	1	qPBP-6-1	3.0	32.3	-2.0
													7	qPBP-7-2	4.9	31.8	-11.5
												GYP	1	qGYP-1-1	3.2	44.0	-1.5
												PHI	1	qPHI-1-1	3.7	55.0	-8.0

DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type per plant, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant, LOD = Logarithms of the odds, q =QTL, QTL = quantitative trait loci, PVE% = explained phenotypic variance, Add =additive effect, Chr = chromosome.

Table 3.5. The total epistatic pairs of interactions for each trait and for 16 traits per family.

Family name	Epistatic pairs of interactions for each trait																Total epistatic pairs of interactions
	DFF	DFP	DPM	GH	PT	HVS	%PS	PHT	FPP	UPP	TPP	TSP	HSW	PBP	GYP	PHI	
9xBari1_092					1			1	1		1				1		5
9XBari2_072n2	5	9	10	10	3	2	4		3	2	3		6	4		2	63
9xBari3_100				6	6			2	2	2		3	17	2	9	1	50
9xBari3_100D					5	4								5		1	15
9xBari3_072C	8	9	7	9	10	14	9	11	10	7	10	9	11	8	10	11	153
9xBeseve_079	8	6		3	19			2					5		18	1	62
9xCudiA_152	1		3	1	1	1	2	1	1	13	1		2			1	28
9xCudiB_22C	1	2	2	5	17	1	2		7	5	5	6	4		2	4	63
9xDerei_070	4	10	9	3	1	6	1	1	1	1	2	3	1	6		3	52
9xDerei_072	4	10	1	8	12	4	6	4	2	9	2		3		1	2	68
9xEgill_065					4	4	4	3	2	4	1	6	1	4	1	2	36
9xEgill_073	1				10			1	2	1		2	1	4	2	1	25
9xKalkan_064	15	12	12	15	29	17	3	9	10	7	7	13	6	14	9	5	183
9xKesen_075		2	1	2			4		11	22	12	5	3	3	5	1	71
9xOyali_084	4	7	2	4	15	4	4	3	2	6	2	2	5	5	2	4	71
9xSavur_063	12	13	10	7	8	2	1	3	3	1	3	5	8	12	10	3	101
9xSirna_060			1	1	2				2	1	2	5		5	7		26
9xS2DrD	18	11	16	21	25	20	17	21	19	15	19	22	24	23	19	16	306
9xGunas_063	10	13	18	20		20	13	16	12	15	13	18	19	15	19	21	242

DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type per plant, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant.



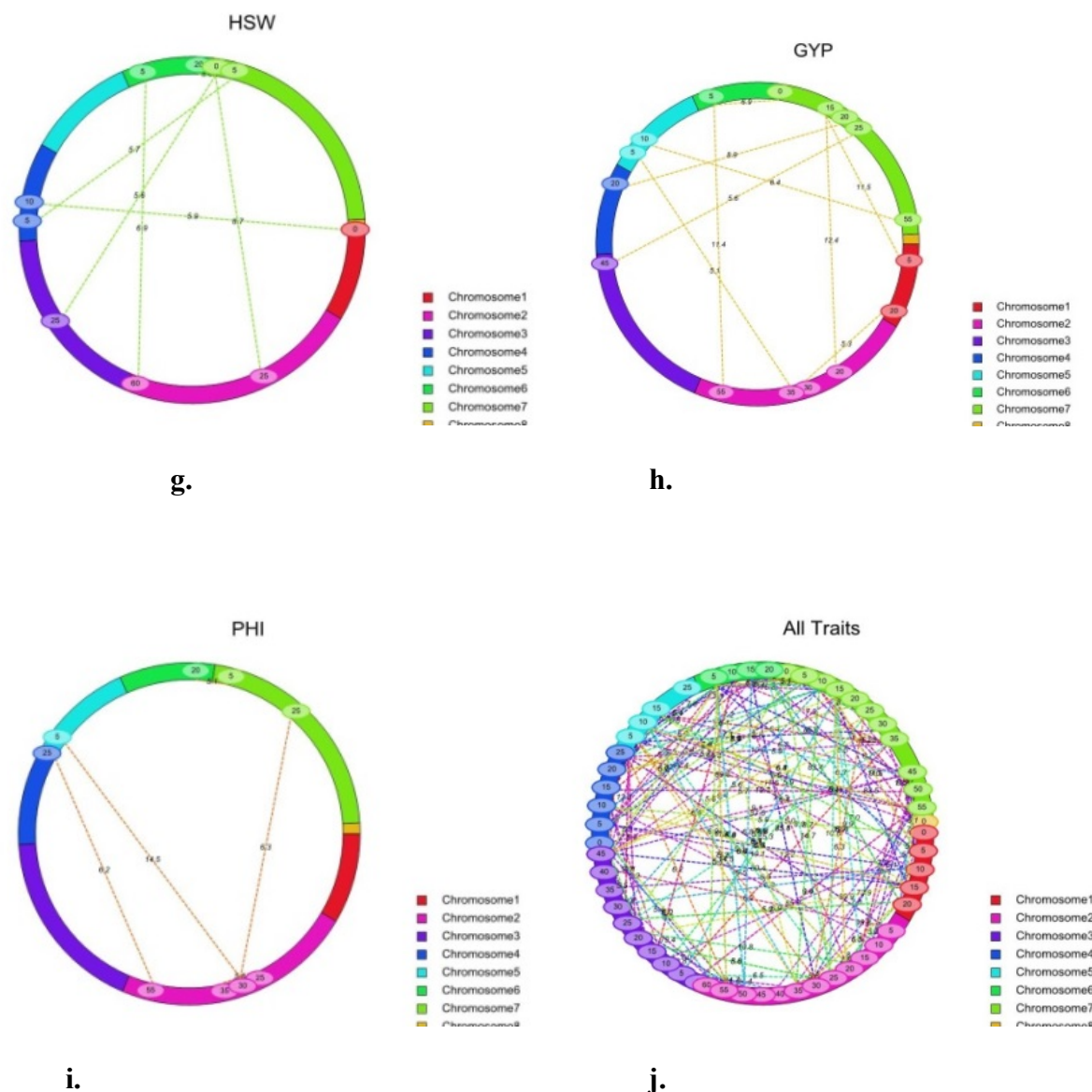


Fig. 3.8. Epistatic interactions of a single trait and all traits from the biparental population ICCV96029 crossed with a *Cicer reticulatum* accession Kalkan_064. a. DFF, b. DPM, c. HVS, d. %PS, e. FPP, f. TSP, g. HSW, h. GYP, i. PHI, j. all traits. DFF = days to first flowering, DPM = days to physiological maturity, FPP = filled pod per plant, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, PHI = percentage of harvest index, PHT = plant height, PPS (%PS) = percentage of pod set, TSP = Total seed per plant.

3.4. Discussion

3.4.1. Phenotypic responses of the 20 interspecific families under heat stress environment

It is important to explore genetic variation in the wild *Cicer* species from the primary (*C. reticulatum*) and the secondary (*C. echinospermum*) gene pool and introgress highly valuable genomic regions (QTLs) into the cultigen for enhancing genetic diversity and avoiding the effect of an extreme heat stress (Shin *et al.*, 2019). Identification and understanding of parameters that result in improved heat tolerance are important in the selection of lines under heat stress conditions. Several authors demonstrated that planting of chickpea accessions in the month of February under field conditions in arid and semi-arid tropics and using key agronomic traits such as grain yield, total filled pods, percentage of pod set, and total seeds are important parameters as heat tolerance indicators in breeding of tolerance in chickpea (Divasirvatham, 2012; Gaur *et al.*, 2014; Tesfashbamlak Molla *et al.*, 2018; Paul *et al.*, 2018; Tsegaye Getahun *et al.*, 2021b).

Under a heat stress environment, the highest phenotypic variability was found among F₃ lines of 9xKalkan_064 and 9xS2DrD families. All families had high phenotypic variances among F₃ lines for yield related traits (TSP, TPP, FPP, and %PS) and DPM. F₃ lines of 9xKalkan_064 had the highest phenotypic variability for %PS. Wide ranges of segregations for DFF and DPM between an early flowering and maturing mother (ICCV96029) and very late flowering and maturing wild *Cicer* species (*C. reticulatum* and *C. echinospermum* accessions) is an indication of high genetic variability in F₃ segregating progenies with regard to phenological traits that is indicative of effects of introgressive alleles from the wild parents. Highly significant differences ($P < 0.001$) were found among F₃ lines of most AR families for three phenological traits (DFF, DFP, and DPM).

The lifecycle of chickpea is strongly determined by environmental factors and different genotypes, which may react differently to climatic factors. Effects of day length and temperature on flowering time are location-dependent. Increasing temperature led to earlier flowering in some genotypes (Savur) and later flowering in others (Kesentas) (Kozlov *et al.*, 2019) which is in line with the present study. The phenology of adaptive traits, like flowering time, may be strongly predicted by plant geographic origin and local environmental factors. The range for time to flowering of the 21 wild accessions varied from 64 to 221 day (Kozlov *et al.*, 2019). As the adaptation to specific environments is blueprinted in genomes, the effects of genes on flowering time may be conditioned by environmental factors. Even after correcting for the early flowering time locus, DPM of the 20 families differed by 60-65 days between the earliest and latest maturing populations in the ICCV96029 background that demonstrated wild-parent specific influences on phenology.

Pre-breeding is useful to enrich the primary gene pool for cultivar improvement. Thus, hybridization and introgression have a potential to generate phenotypic novelty (Warschefsky *et al.*, 2014). In the present study, based on the mean values of heat visual score (HVS), high rates (249 lines among 473 lines) of segregation for heat stress tolerance (~40 °C) was observed, a phenotype not present in the cultivated parent that provides an immediate target for introgression breeding (von Wettberg *et al.*, 2018). By using the mean values of HVS, GYP and three yield related traits (FPP, PPS, TSP), F₃ lines of 9xKalkan_064 were consistently found to be the most heat tolerant pre-breeding family followed by 9xOyali_084, 9xBari1_092, 9xSavur_063, 9xCudiB_22C, 9xKesen_075 and 9xSirna_060 among the 20 families. Likewise, from the result of cooling degree days from February to May, von Wettberg *et al.* (2018) indicated that Kalkan

had the highest cooling degree days i.e. implies that the plants had high transpiration rate as a consequence they become cool and this is one of an adaptation mechanism of a heat tolerant plant. Under non-stress condition, von Wettberg *et al.* (2018) have found 200 FPP from 9xGunas_063. However, in this study, we have found a maximum of 60.1 FPP from the same family, which clearly showed that heat stress decreased FPP by fourfold.

The presence of transgressive segregants suggested that the two parental lines each had desirable and undesirable alleles in various proportions for loci governing this trait (Vengadessan *et al.*, 2013). Transgressive segregation of traits implies their mean values for a segregating progeny were outside the ranges defined by the two contrasting parental population means (Xu *et al.*, 2017). In the present study, most traits exhibited high degrees of transgressive segregation within the 20 families. Similarly, Paul *et al.* (2018) in RILs of chickpea, Upadhyaya *et al.* (2015) in F₆ population of chickpea, Paliwal *et al.* (2012) in RILs of wheat, Buu *et al.* (2014) in BC₂F₂ population of rice and Xu *et al.* (2017) in F_{2:3} population of tomato reported several transgressive segregants for heat tolerance. Shin *et al.* (2019) observed differences between AR and AE families similar to the present study. AE families have greatest difference in time to seed maturity; have semi-erect growth habit; greater plant height; higher 1000 seed weight; and darker seeds color than AR families.

In this study, ANOVA revealed that highly significant phenotypic variations were observed for some traits in all families and significant phenotypic variations were observed in some other traits, which might show the presence of several folds of genetic diversity in the selected wild parents under heat-stress condition. According to the reports of Karikari *et al.* (2019), ANOVA

showed highly significant differences among RILs of mapping population in soybean for two traits ($P < 0.01$). The combined ANOVA for both the stress environments revealed that significant variation existed in RILs of chickpea for all the traits measured, except PB (Paul *et al.*, 2018). ANOVA indicated significant differences among the RILs for days to flowering, plant height, and ascochyta blight resistance (Daba *et al.*, 2018). A wide range of variation was exhibited by the RILs for days to flower, days to maturity, node of first flowering, plant height, and ascochyta blight resistance. von Wettberg *et al.* (2018) have also identified variation in seed nutrient density, phenology, resistance to pod borer, heat tolerance, and water deficit response from interspecific progenies of chickpea. The present study may have significant values for introgression of desirable genes from wild chickpeas into an elite variety for selection of heat tolerant pre-breeding lines.

Heritability information helps to know the extent of genetic expression under the given environment. Traits with higher broad sense heritability across different growing environments and/or seasons can be reliably selected in breeding and controlled by genetic factors. Traits having moderate and low heritability are affected by environmental changes. The high heritability coupled with high genetic advance indicates the influence of additive gene effects in these traits. In the present study, phenological traits in most families had high heritability (62-95%). Most families had high heritability for HVS, GH, %PS and PHT. In all families, GYP and yield related traits (PBP, TSP, FPP, PHI and HSW) had low to high heritability. Similarly, the heritability of all the traits was high (72.0-90.7%), except PB (Paul *et al.*, 2018). Broad sense heritability of plant height (75-78%) and days to flowering (45-87%) was obtained (Daba *et al.*, 2018). Broad sense heritability for days to flowering in chickpea was 80%. Vadez *et al.* (2011)

reported that heritability was usually higher for seed yield components than for seed yield. The four traits namely filled pods, total seeds, HVS and percentage of pod set were strongly and positively correlated (50-90%) with grain yield and detecting QTLs for these traits under heat stress environments would be helpful in breeding for heat tolerance in chickpea (Paul *et al.*, 2018).

An approximately normal distribution was suggested based on the absolute skewness and kurtosis value of traits that are close to 0, indicating that the population was suitable for map construction and QTL identification (Xu *et al.*, 2017; Zhang *et al.*, 2017). In this study, the frequency distribution of measured traits showed the characteristics of continuous variation (Fig 3.3 and Table 3.2) i.e. imply that the traits are controlled by polygenes and suitable for QTL mapping. Likewise, frequency distribution of average days to flowering trait variation measured in 190 individuals and parental accessions of F₆ mapping population (ICC 16374 × ICC 762) and showed normal distribution (Upadhyaya *et al.*, 2015). In the F₂ population of tomato, phenotypic data for all reproductive traits scattered over wide continuous ranges under mild heat stress conditions (Xu *et al.*, 2017).

3.4.2. Marker-trait associations under heat stress environment in chickpea

In the present study, one or more genomic regions were introgressed from the wild accessions of *C. reticulatum* and *C. echinospermum* into the genetic background of an elite cultivar (ICCV 96029) for 15 traits among 16 traits that explained phenotypic variances of 3-62.2%. Since quantitative traits are regulated by complex genetic networks, knowledge of the genomic basis of traits that segregate in wild-cultivated hybrid populations will facilitate chickpea to identify

genomic regions (QTLs) for several traits under heat stress environment by linking genetic and phenotypic variation in a quantitative genetic framework (Shin *et al.*, 2019; von Wettberg *et al.*, 2018). Thus, the present study applied genomics-assisted breeding, a marker-trait association, for quantitative dissection of 16 phenological, agronomic and growth habit traits in a set of chickpea x wild hybrid populations under a heat stress environment. The interspecific genetic linkage map consists of 1445-1541 SNP markers in 471 lines (20 families) that were distributed across eight chickpea genome in order to capture the majority of the recombination events in each family, which result in increased precision in QTL mapping. We found several markers linked to the major QTLs, which can facilitate marker-assisted breeding for heat tolerance in chickpea.

Likewise, several authors reported a high-density sequence-based genetic map of legumes which facilitated anchoring QTLs to physical map and enabled identification of QTLs in a narrow physical interval, closely linked flanking markers, provided better understanding of genetic architecture and candidate gene information, and facilitate marker assisted selection (Deokar *et al.*, 2019; Karikari *et al.*, 2019; Paul *et al.*, 2018). Many authors constructed a single high density genetic linkage maps in chickpea consist of 3430 SNPs (429 recombination bins) (Deokar *et al.*, 2019), 271 SNPs (Paul *et al.*, 2018), 410 SNPs and SSR (Upadhyaya *et al.*, 2015), 1,007 SSR and SNPs (Jaganathan *et al.*, 2015), 747 SNPs (Mugabe *et al.*, 2019), 349 SNPs (Daba *et al.*, 2018), 135 SSR (Singh *et al.*, 2016) and 241 SSR (Varshney *et al.*, 2014a) markers. The maps cover a total map length of 868, 529.1, 971.5, 727.3, 393.7, 653, 310.2 and 482 cM of the chickpea genome, respectively. The corresponding average marker density of the maps was 0.2, 0.51, 0.42, 1.3, 1.8, 0.53, 0.43 and 0.5 markers/cM.

In this study, large numbers of SNP markers (1445-1541) were incorporated into the existing 19 interspecific genetic maps (140.4-660 cM) that can further narrow down the genomic regions (QTLs) identified and a relatively high marker density (3.44 SNPs/cM) was found compared to the above reported genetic linkage maps. These results laid a foundation in understanding heat tolerance and increase the confidence of breeders to proceed with early generation selection for heat tolerance through marker-assisted breeding. Our findings might be of great usefulness for marker-assisted breeding, and could provide detailed information for accurate QTL localization and candidate gene discovery. Estimated genetic effects were positive if the male parent allele contributed positively to the trait of interest and negative if the female parent allele contributed positively towards the trait of interest. In line with the present study, Gonzalez-Segovia *et al.* (2019) reported that the spread of maize cultivation to the highlands of central Mexico was accompanied by substantial introgression of local adaptive genes from the endemic wild teosinte *Zea mays* that differentiate highland and lowland Mexican maize.

LOD threshold value of the present study was 2.5 for detecting significant QTLs. Analysis of F_{2:3} progeny in replicated manner could be also another approach to enhance the power of QTL detection and estimation of its phenotypic effect. Similarly, Paul *et al.* (2018) used the threshold LOD value of 2.5 for detecting significant QTLs of a trait associated with a marker in an intraspecific map of chickpea. The results from the 20 families of AR and AE of the present study revealed that phenological traits such as DFF, DFP and DPM were confirmed as complex quantitative traits because QTLs for each trait were detected in seven linkage groups of the chickpea genome.

Similarly, many authors reported that more than two QTLs were responsible for phenological traits. Upadhyaya *et al.* (2015) reported five flowering time-associated robust QTLs mapped on a high-resolution intraspecific genetic linkage map in which each QTL explains a total phenotypic variance ranges from 11.6-27.3%. Daba *et al.* (2018) reported seven QTLs for days to flowering and located on chromosomes 3, 4, 5, and 8 that individually explained 9-44% of the phenotypic variation. Two QTL for days to maturity were identified on chromosomes 3 (19%, PVE) and 8 (15%, PVE). Three and two QTLs were identified for DFF and DPM, respectively (Jaganathan *et al.*, 2015). Varshney *et al.* (2014a) reported 2 and 3 QTLs which were responsible for DFF and DPM, respectively that explained phenotypic variation ranges from 10.5-26.9%, and 12.1-19.7%, respectively. Several other studies also reported QTLs for flowering time in LG1 (Rehman *et al.*, 2011), LG2 (Karami *et al.*, 2015), LG3 (Upadhyaya *et al.*, 2015), LG4 (Varshney *et al.*, 2014; Upadhyaya *et al.*, 2015; Mallikarjuna *et al.*, 2017), LG5 (Upadhyaya *et al.*, 2015), LG6 (Mallikarjuna *et al.*, 2017) and LG8 (Varshney *et al.*, 2014; Mallikarjuna *et al.*, 2017) using different parental lines in chickpea. Identification of QTLs on different linkage groups suggested that there might be several genes for flowering time in chickpea. The report of previous authors and the present study confirmed that flowering time is a complex quantitative trait governed by multiple genes.

In the present study, 190 and 40 QTLs in 16 traits were identified from 17 AR and 2 AE families (biparental populations), respectively under an extremely heat stress environment (ICRISAT, Hyderabad, India). In line with this, Paul *et al.* (2018) identified 17 QTLs for six traits in chickpea from a single biparental population under the same extreme heat stress environment. Kole *et al.* (2015) identified 134 QTLs for 19 drought related traits in chickpea. Shin *et al.*

(2019) reported 50 QTLs in 11 traits from 284 F₃ lines of AR. Sixty-four main-effect QTLs were identified for 20 traits in chickpea (Jaganathan *et al.*, 2015).

3.4.3. Identification of heat tolerant QTLs for marker assisted breeding in chickpea

A total of 108 QTLs were identified for GYP and yield related traits from 19 F₃ families of AR and AE which are useful genomics tools for screening of heat tolerant pre-breeding lines, fine mapping, future marker assisted selection and genetic transformation in molecular breeding of chickpea. Most of these QTLs were introgressed from S2DrD, Gunas_063, CudiB_022C, Bari3_100, Derei_070, Sirna_060, Kesen_075, Oyali_084, and Kalkan_064 wild parents. Similarly, Paul *et al.* (2018) identified 17 QTLs for six yield related traits and explained phenotypic variations from 3.92-16.56% under an extremely heat stress environment. The authors explained that the heat tolerant parent is contributing the desirable alleles for all the QTLs. In addition, Mugabe *et al.* (2019) found 3 QTLs for cold tolerance from the interspecific crosses of a sensitive desi chickpea with a cold tolerant accession of *C. reticulatum* that explained a phenotypic variance of 7.2 to 44.8%. As expected, all large-effect QTLs were associated with cold tolerance and derived from *C. reticulatum* i.e. introgress positive alleles for cold tolerance from wild progenitor into cultivated chickpea. Genomic regions (QTLs) were identified for 50% flowering, seed number, seed yield, 100-seed weight, and shoot dry weight under saline conditions and explained phenotypic variance varied from 19 to 37.7% (Vadez *et al.*, 2011). Under normal conditions, 16 and 11 QTLs were identified for yield and yield-related traits by Jaganathan *et al.* (2015) and Varshney *et al.* (2014a), respectively.

Co-localization of QTLs for some traits indicates trait dependency or pleiotropic-effect loci and demonstrated possibly the existence of genes or gene clusters with major effects that control significant proportions of the phenotypic variation in several quantitatively inherited traits. Hence, no single gene can improve yield and other desirable agro-morphological traits both under normal and stress environmental conditions. Simultaneous improvement of yield related traits often requires pyramiding of multiple genes that might possess associated pleiotropic effects (Varshney *et al.*, 2014a; Jaganathan *et al.*, 2015; Vadez *et al.*, 2011; Paul *et al.*, 2018; Mugabe *et al.*, 2019; Shin *et al.*, 2019). Likewise, in this study, 42 QTLs having pleiotropic effects were found from the 20 families out of which 17 were responsible for GYP and yield related traits. Some of the SNP markers namely CTM47, C3M98, C7M70, C4M227 and C2M13 are closely linked to more than three yield related traits, which are essential for simultaneous improvement and selection of the traits in chickpea breeding. Moreover, five pleiotropic loci were identified from all families, which are essential for simultaneous selections of the three phenological traits (DFF, DFP, DPM) which are important for breeding of early flowering and maturing chickpea germplasm. C6M55, C5M111, C6M19, C7M173 and C5M155 are linked SNP markers to these pleiotropic QTLs. All the closely linked SNP markers are another genomics tools important to implement marker assisted breeding in chickpea and other legumes. In chickpea, limited information is available on genetic parameters for heat tolerance. This is the first ever reported genomic tools (QTLs, genes, SNP markers) for heat tolerance in chickpea from interspecific crossing of *Cicer* species (*C. reticulatum* and *C. echinospermum*) with the cultigen.

Similarly, according to the report of Shin *et al.* (2019), phenological traits were controlled by two pleiotropic QTLs. DPM and GH traits shared a common QTL located on chromosome 1

(Shin *et al.*, 2019). Paul *et al.* (2018) have found four major co-localized QTLs for four yield related traits, which explained the phenomenon of pleiotropy. Multiple loci controlling different traits are co-localized in four intervals in four linkage groups that contain desirable alleles and can be introgressed into commercial petunia germplasm to expand the genetic base and improve plant performance and flower characteristics in petunia (Cao *et al.*, 2018).

3.4.4. Epistatic interactions for selection of traits in chickpea breeding

Interestingly, absence of epistatic interaction in main-effect QTLs increases the heritability of the trait and makes selection easier (Paul *et al.*, 2018; Karikari *et al.*, 2019). Compared to additive and dominance gene effects, minimum effects were observed for epistasis ($\leq 10\%$). Epistatic interaction analysis revealed that 19 QTL pairs involved significant epistatic interactions covering seven LGs (Paul *et al.*, 2018). In the present study, large numbers of epistatic interactions were observed in each family. This might be the reason why heritability of GYP and yield related traits in most families were varied from low to high.

3.5. Conclusions

High genetic variability and large numbers of an extremely heat stress ($>40^{\circ}\text{C}$) tolerant pre-breeding lines were found which are absent in the cultigen. This might be due to the introgressions of genes from 20 wild founders into the genetic background of an early flowering and elite cultivar, ICCV 96029. Mining and deployment of missing genes from a wild is a feasible option and approach in crop improvement. Higher agronomic performances of the progenies were observed compared to their common parent. Wide ranges of segregations were observed for DFF and DPM even after correcting for the early flowering time locus that

demonstrated wild-parent specific influences on phenology. Under this heat stress condition, flower and pod abortion occur in almost all families; however, the degree of abortion differs from 9xKalkan_064 to 9xKarabache_081A. Knowledge of the genomic basis of traits that segregate in wild-cultivated hybrid populations will facilitate chickpea to identify genomic regions (QTLs) for several traits under heat stress environment by linking genetic and phenotypic variation in a quantitative genetic framework. A total of 230 QTLs were detected for 16 traits in 19 families which explained a phenotypic variance of 3-93%. Out of which, 220 and 8 QTLs were major and minor, respectively and 108 QTLs were identified for grain yield and yield related traits. Simultaneous improvement of yield related traits often requires pyramiding of multiple genes that might possess associated pleiotropic effects. All QTLs, pleiotropic QTLs, associated SNP markers, and candidate genes we have found are important genomics tools for screening of heat tolerant pre-breeding lines, fine mapping, future marker assisted selection and genetic transformation in molecular breeding of chickpea.

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CHAPTER 4: Molecular Genetic Diversity and Population Structure in Ethiopian Chickpea Germplasm Accessions

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Abstract

Chickpea (*Cicer arietinum* L.) is a cheap source of protein and rich in minerals for people living in developing countries. The existing molecular genetic diversity of Ethiopian chickpea accessions were seldomly studied. In order to assess the existing molecular genetic diversity and determine population structures in selected Ethiopian chickpea germplasm accessions (118) were genotyped with a set of 46 simple sequence repeat (SSR) markers equally distributed on chickpea genome. A total of 572 alleles were detected from 46 SSR markers and the number of alleles per locus varied from 2 (ICCM0289) to 28 (TA22). The average number of alleles per locus, polymorphism information content and expected heterozygosity were 12, 0.684 and 0.699, respectively. Phylogenetic analysis grouped the 118 chickpea genotypes from diverse sources into three evolutionary and/or biological groups (improved desi, improved kabuli and landraces). Population structure analysis revealed six sub-populations from 118 chickpea genotypes studied. AMOVA revealed that 57%, 29% and 14% of the total genetic variations were observed among individuals, within populations and among populations, respectively. The insights into the genetic diversity at molecular levels in the Ethiopian germplasm lines can be used for designing conservation strategies as well as the diverse germplasm lines identified in this study can be used for trait dissection and trait improvement.

Keywords: Chickpea; Microsatellite markers; Genetic diversity; Population structure

4.1. Introduction

Chickpea (*Cicer arietinum* L.) is a diploid crop plant ($2n = 2x = 16$) with a haploid genome size of approximately 740 Mb (Varshney *et al.*, 2013). Southeastern Turkey and adjoining Syria are the primary Vavilovian centers of origin, and Ethiopia is the secondary center of diversity (Vavilov, 1926; van der Maesen, 1987). In Ethiopia, chickpea is one of the most economically important legumes produced on production of 470,000 tons on an area of 258,486 ha (CSA, 2016; Asnake Fikre *et al.*, 2020; Erana Kebede, 2020). Ethiopia is one among the top ten chickpea growing countries across the world and is the leading producer, consumer and exporter of chickpea in Africa (Mekasha Chichaybelu *et al.*, 2021). Chickpea can be consumed as green vegetable (eshet), roasted (kollo), boiled (nifro), dry vegetable, ‘shimbira asa’, shiro wot (sauce) and snacks which are cheap and healthy diets that are rich in protein, vitamins and minerals for the poor farmers who cannot afford animal products. Moreover, chickpea generates income for the poor farmers and foreign currency to the country, improves food and nutritional security and soil fertility, provides livestock feed, and requires low production cost (Erana Kebede, 2020; Mekasha Chichaybelu *et al.*, 2021). The major chickpea growing zones of Ethiopia are South Gondar, North Gondar, East Gojam, West Gojam, North Shewa, East Shewa, West Shewa, South Wollo, North Wollo and Tigray (Bejiga and Eshete, 1996). In Ethiopia, there are about 1173 chickpea accessions collected from different agro-ecologies and geographical origins and stored at the Institute of Biodiversity and Conservation (Chandora *et al.*, 2020). Although Ethiopia is bestowed with diverse agro-ecologies and especially crop diversity, the productivity of chickpea does not reach the maximum world production (3,500 kg/ha) (Asnake Fikre *et al.*, 2020; Mekasha Chichaybelu *et al.*, 2021), due to several biotic and abiotic stresses.

Deeper insights into genetic diversity will enable the use of appropriate germplasm lines in breeding programs to develop climate resilient varieties. The investigation of the nature and structure of genetic diversity and relatedness within and among the cultivated chickpea and its wild relatives helps to identify new sources of germplasm bearing valuable genes for improving yield, grain quality and enhancing resistance to various biotic and abiotic stresses. Besides, studying genetic diversity is important in management, conservation and selection of diverse plant materials for intraspecific and interspecific crossing (Choudhary *et al.*, 2012). Genomics revolution during the last two decades led to the development of several genomics resources including the genome sequence (Varshney *et al.*, 2013), molecular markers (Nayak *et al.*, 2010; Thudi *et al.*, 2011) and technologies for assessing the genetic diversity of germplasm lines at genome level (Thudi *et al.*, 2016a; Thudi *et al.*, 2016b; Varshney *et al.*, 2019).

Earlier efforts were made to understand the genetic structure using highly polymorphic simple sequence repeat (SSR) markers that are required to facilitate chickpea genetic improvement (Saxena *et al.*, 2014). SSR markers are robust and quite cheap markers for allele mining, molecular genetic diversity pattern, genetic relationships, association genetics, genetic mapping and identification of genes, phylogenetic patterns, population genetic structure studies, cloning gene(s) and marker assisted selection in chickpea accessions (Upadhyaya *et al.*, 2008; Gemechu Keneni *et al.*, 2011; Tadesse Sefera *et al.*, 2011; Thudi *et al.*, 2014; Parida *et al.*, 2015). However, most of these studies assessed the genetic diversity among germplasm lines from Southeast Asia (Upadhyaya *et al.*, 2008; Tadesse Sefera *et al.*, 2011; Thudi *et al.*, 2014; Parida *et al.*, 2015; Samyuktha *et al.*, 2018) and Mediterranean regions (Saeed *et al.*, 2011; Naghavi *et al.*, 2012; Hajibarat *et al.*, 2014; Torutaeva *et al.*, 2014; Giovanni *et al.*, 2016). The genetic diversity

among the Ethiopian germplasm lines was seldom studied (GemechuKeneni *et al.*, 2011; Tadesse Sefera *et al.*, 2011) using SSR markers and most of the genetic diversity studies of chickpea so far focused on morphological characterizations. Ethiopia being the secondary center of origin, assessing the molecular genetic diversity and determining the population structures among Ethiopian germplasm lines will help in designing breeding programs as well as germplasm conservation and management strategies. In this study, we report assessment of molecular genetic diversity and population structures of 118 chickpea genotypes (115 Ethiopian chickpea landraces, breeding lines and cultivars, and 3 Indian elite varieties) by using 46 genome- wide SSR markers.

4.2. Materials and methods

4.2.1. Plant materials and DNA extraction

A total of 118 chickpea genotypes (115 Ethiopian landraces, lines and varieties, and 3 Indian elite varieties) were grown at Debre Zeit Lath house, Ethiopia (Table 4.1). DNA was extracted from 22 days old seedlings (leaves from ten plants per genotype were pooled) using a modified CTAB protocol (Borsch *et al.*, 2003) at Institute of Biotechnology, Addis Ababa University, Ethiopia. At ICRISAT, Patancheru, India, the DNA samples was treated with RNase A (by adding 15 µl of RNase A (10mg/ml) to each DNA sample) for three hours at 37°C. The RNase A treatment was further purified following NucleoSpin 96 Plant II kit purification protocol (Angel, 2008). The quality check, quantification and normalization to 5 ng/µl of DNA samples were made using 1% agarose by using lambda DNA (50 ng) as a standard and 8000 Nano Drop Spectrophotometer (Thermo Fisher Scientific, USA).

4.2.2. Polymerase chain reaction, Capillary gel electrophoresis, Allele calling and Sizing

A genome-wide distributed and polymorphic 46 SSR markers (Table 4.2) distributed equally on the genome were used to genotype 118 chickpea genotypes. The 46 SSR markers were selected based on quality criteria, genome coverage and locus-specific information content of published data (Gemechu Keneni *et al.*, 2011; Tadesse Sefera *et al.*, 2011, Upadhyaya *et al.*, 2008; Varshney *et al.*, 2014) and unpublished data. All chickpea accessions were genotyped with 46 SSR markers as described earlier (Nayak *et al.*, 2010; Thudi *et al.*, 2011). PCR products were separated based on the size of amplicons by automated DNA fragment analyzer and Sanger sequencer (Capillary gel electrophoresis, ABI Prism 3730 XL, Applied Biosystems, Foster City,

CA, USA) by using an internal size standard, GeneScan-500 LIZ. The GeneMapper4.0 (Applied Biosystems, Foster City, CA, USA) software was used for allele calling.

4.2.3. Diversity analysis

Based on SSR allelic data, molecular genetic relationships (dendrogram) among 118 chickpea genotypes were determined by using neighbour-joining weighted pair-group method with arithmetic averages (WPGMA), i.e. clustering of simple matching dissimilarity indices with the help of DARwin-6.0 program (Perrier *et al.*, 2010). PowerMarker version 3.51 (Liu *et al.*, 2005) was used to determine major allele frequency, the number of alleles per locus, polymorphic information content (PIC), gene diversity (expected heterozygosity) and observed heterozygosity.

4.2.4. Population Structure

The STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) software was used to estimate the number of natural genetic groups (K), the distribution of individuals among these groups, and to assign individual genotypes to a specified number of groups “K” based on membership coefficients calculated from the genotype data. Genetic structure of 118 chickpea genotypes was explored using the Admixture model implemented in the STRUCTURE software. A range of population numbers (K= 2 to K= 10) was assessed using a burn-in period of 10,000 steps followed by 100,000 Monte Carlo Markov Chain (MCMC) replicates with 5X iterations. The rate of change of Napierian logarithm probability relative to the standard deviation (ΔK) was determined by using Evanno *et al.* (2005) method. The output of this population structure was taken from STRUCTURE HARVESTER program online (Earl and VonHoldt, 2012) which showed the

highest peak at $K = 6$, indicating the presence of six major distinct populations. The 46 SSR loci were analyzed based on populations (geographical distributions) of the chickpea genotypes using GenALEX 6.5 software (Peakall and Smouse, 2012). Population specific genetic diversity, allelic frequency, observed, expected and unbiased expected heterozygosity, Shannon information index, total number of alleles, average number of alleles and genotype specific alleles per locus, percentage of polymorphism, gene flow, Nei's pairwise genetic distance between populations (Nei, 1972) and genetic differentiation were computed. Based on pair-wise genetic distance between genotypes, principal coordinate analysis (PCoA) was also analyzed in GenALEX 6.5 to compliment the analyses of STRUCTURE and dendrogram.

The statistical significances of molecular genetic variance components (AMOVA) for each hierarchical comparison (among populations, among individuals, individuals within populations) were tested using 999 permutations. Moreover, F statistics among individuals, within and among populations were determined using GenALEX 6.5 (Peakall and Smouse, 2012).

Table 4.1. Passport information of chickpea genotypes used in present study.

S.N	Acc.no	Altitude	Country (Region)	Zone	Biological Status	Seed type	S.N	Acc.no	Altitude	Country (Region)	Zone	Biological Status	Seed type
1	41037	1490	SNNP	South Omo	Landrace	Desi	31	41282	1890	Amhara	Bahir Dar	Landrace	Desi
2	41052	1510	Oromiya	West Harerge	Landrace	Desi	32	41286	1855	Amhara	South Gondar	Landrace	Desi
3	41055	1500	Oromiya	West Harerge	Landrace	Desi	33	41288	1855	Amhara	South Gondar	Landrace	Desi
4	41107	1820	Amhara	North Shewa	Landrace	Desi	34	41289	1855	Amhara	South Gondar	Landrace	Desi
5	41108	1450	Amhara	North Shewa	Landrace	Desi	35	41295	1820	-	-	Landrace	Desi
6	41110	1220	Amhara	North Shewa	Landrace	Desi	36	207651	1820	Amhara	North Shewa	Landrace	Desi
7	41111	1400	Amhara	North Shewa	Landrace	Desi	37	207652	1820	Amhara	North Shewa	Landrace	Desi
8	41113	1650	Amhara	South Wollo	Landrace	Desi	38	207654	1450	Amhara	North Shewa	Landrace	Desi
9	41114	1560	Amhara	South Wollo	Landrace	Desi	39	207655	1220	Amhara	North Shewa	Landrace	Desi
10	41117	1850	Oromiya	East Shewa	Landrace	Desi	40	207656	1220	Amhara	North Shewa	Landrace	Desi
11	41119	1860	Oromiya	East Shewa	Landrace	Desi	41	207657	1400	Amhara	North Shewa	Landrace	Desi
12	41120	1880	Oromiya	East Shewa	Landrace	Desi	42	207658	1400	Amhara	North Shewa	Landrace	Desi
13	41122	1860	-	-	Landrace	Desi	43	207661	1850	Oromiya	East Shewa	Landrace	Desi
14	41136	1870	Oromiya	East Shewa	Landrace	Desi	44	207673	1860	Oromiya	East Shewa	Landrace	Desi
15	41145	1830	Amhara	North Shewa	Landrace	Desi	45	207739	1790	Amhara	East Shewa	Landrace	Desi
16	41146	1830	Amhara	North Shewa	Landrace	Desi	46	208900	1700	SNNP	North Omo	Landrace	Desi
17	41147	1830	Amhara	North Shewa	Landrace	Desi	47	208991	1670	Oromiya	West Shewa	Landrace	Desi
18	41148	1820	Amhara	North Shewa	Landrace	Desi	48	208992	1610	Oromiya	West Shewa	Landrace	Desi
19	41149	1820	Amhara	North Shewa	Landrace	Desi	49	208993	1500	Oromiya	West Shewa	Landrace	Desi
20	41150	1810	Amhara	North Shewa	Landrace	Desi	50	208994	1510	Oromiya	West Shewa	Landrace	Desi
21	41153	1860	Oromiya	East Shewa	Landrace	Desi	51	208995	1670	Oromiya	West Shewa	Landrace	Desi
22	41155	1860	Oromiya	East Shewa	Landrace	Desi	52	208996	1700	Oromiya	West Shewa	Landrace	Desi
23	41156	1880	Oromiya	East Shewa	Landrace	Desi	53	208997	1700	Oromiya	West Shewa	Landrace	Desi
24	41157	1880	Oromiya	East Shewa	Landrace	Desi	54	208999	1490	SNNP	North Omo	Landrace	Desi
25	41189	1870	SNNP	Gurage	Landrace	Desi	55	209000	1540	SNNP	North Omo	Landrace	Desi
26	41267	1790	Amhara	East Gojam	Landrace	Desi	56	209002	1580	SNNP	North Omo	Landrace	Desi
27	41268	1770	Amhara	East Gojam	Landrace	Desi	57	209003	1640	SNNP	North Omo	Landrace	Desi
28	41269	1820	Amhara	East Gojam	Landrace	Desi	58	209004	1360	SNNP	North Omo	Landrace	Desi

29	41271	1880	Amhara	West Gojam	Landrace	Desi	59	209007	1330	SNNP	North Omo	Landrace	Desi
30	41279	1890	Amhara	Bahir Dar	Landrace	Desi	60	209008	1850	SNNP	Hadiya	Landrace	Desi
61	209017	1830	SNNP	Gurage	Landrace	Desi	91	DZ-2012-ck-0206		-	-	Kabuli line	Kabuli
62	209093	1710	-	-	Landrace	Desi	92	DZ-2012-ck-0074		-	-	Kabuli line	Kabuli
63	209096	1850	Oromiya	Arsi	Landrace	Desi	93	DZ-2012-ck-0198		-	-	Kabuli line	Kabuli
64	209102	1800	Oromiya	Bale	Landrace	Desi	94	Chefe		-	-	Kabuli variety	Kabuli
65	209109	1870	Oromiya	West Shewa	Landrace	Desi	95	DZ-2012-ck-0036		-	-	Desi line	Desi
66	209110	1880	Oromiya	West Shewa	Landrace	Desi	96	DZ-2012-ck-0031		-	-	Desi line	Desi
67	212477	1610	Amhara	North Shewa	Landrace	Desi	97	DZ-2012-ck-0040		-	-	Desi line	Desi
68	212478	1620	Amhara	North Shewa	Landrace	Desi	98	DZ-2012-ck-0038		-	-	Desi line	Desi
69	212589	1600	Amhara	South Omo	Landrace	Desi	99	DZ-2012-ck-0027	-	-		Desi line	Desi
70	212914	1580	-	-	Landrace	Desi	100	DZ-2012-ck-0029	-	-		Desi line	Desi
71	235394	1640	Tigray	CentralTigray	Landrace	Desi	101	DZ-2012-ck-0034	-	-		Desi line	Desi
72	41209	2600	Amhara	North Shewa	Landrace	Desi	102	Minjar	-	-		Desi variety	Desi
73	207721	2650	Amhara	North Shewa	Landrace	Desi	103	DZ-2012-ck-0228	-	-		Desi line	Desi
74	207134	2850	Amhara	North Shewa	Landrace	Desi	104	DZ-2012-ck-0232	-	-		Desi line	Desi
75	208984	2680	Oromiya	North Shewa	Landrace	Desi	105	DZ-2012-ck-0236	-	-		Desi line	desi
76	225876	2540	Amhara	South Wollo	Landrace	Desi	106	DZ-2012-ck-0240	-	-		Desi line	Desi
77	DZ-2012-ck-0024		-	-	Kabuli line	Kabuli	107	DZ-2012-ck-0229	-	-		Desi line	Desi
78	DZ-2012-ck-0025		-	-	Kabuli line	Kabuli	108	DZ-2012-ck-2011S0041	-	-		Desi line	Desi
79	DZ-2012-ck-0017		-	-	Kabuli line	Kabuli	109	Teketay	-	-		Desi line	Desi
80	DZ-2012-ck-0026		-	-	Kabuli line	Kabuli	110	DZ-2012-ck-0235	-	-		Desi line	Desi
81	DZ-2012-ck-0019		-	-	Kabuli line	Kabuli	111	DZ-2012-ck-0231	-	-		Desi line	Desi
82	DZ-2012-ck-0016				Kabuli line	Kabuli	112	DZ-2012-ck-0230	-	-		Desi line	Desi
83	Habru		-	-	Kabuli	Kabuli	113	DZ-2012-ck-0234	-	-		Desi line	Desi
					variety								
84	DZ-2012-ck-0209		-	-	Kabuli line	Kabuli	114	Dalota	-	-		Desi line	Desi
85	DZ-2012-ck-0208		-	-	Kabuli line	Kabuli	115	Local check		Amhara	North Wollo	Desi line	Desi
												Landrace	Desi
86	DZ-2012-ck-0210		-	-	Kabuli line	Kabuli	116	ICC 4567		India	ICRISAT	Desi line	Desi

87	DZ-2012-ck-0199	-	-	Kabuli line	Kabuli	117	JG 14	India	ICRISAT	Desi line	Desi
88	Ejere	-	-	Kabuli variety	Kabuli	118	ICCV96029	India	ICRISAT	Desi line	Desi
89	DZ-2012-ck-0196	-	-	Kabuli line	Kabuli						
90	DZ-2012-ck-0201	-	-	Kabuli line	Kabuli						

SNNP = Southern Nations, Nationalities, and Peoples.

4.3. Results

A total of 572 alleles were detected from 46 genome wide polymorphic SSR markers across 118 chickpea genotypes out of which 113 were considered rare, 435 common and 34 most frequent alleles. Large numbers of rare allelic composition was found in TS72, CaM1101 and TA14. The number of alleles per locus varied from 2 (ICCM0289) to 28 (TA22) and an average of 12 alleles per locus was perceived (Table 4.2). A total of 126 genotype specific alleles were also found (data not shown). Polymorphic information content (PIC), expected heterozygosity (H_e) or genetic diversity and observed heterozygosity (H_o) varied from 0.05 to 0.94, 0.05 to 0.95 and 0 to 0.52, respectively. An average value of PIC, H_e , H_o and major allele frequency (MAF) were 0.684, 0.69, 0.22 and 0.41 in their respective order. Allele sizes of 46 polymorphic markers varied from 115 (TA194) to 345 bp (CaM1101) (Table 4.2).

Large number of alleles and high PIC values were obtained (15-28, >0.8) with 23 SSR markers namely TA22, CaM1101, TS72, TA130, ICCM249, TA118, TA96, TA176, CaM1158, CaM0443, TA14, TA37, CaM0436, H1G16, TR29, CaM1402, TA78, TA113, STMS11, TA71, TA122, TA194 and TA103 in their respective order which might have great potential to discriminate 118 chickpea genotypes into different genetic groups. Nevertheless, SSR markers such as GAA47, ICCM0289, CaM0610 and H1D24 had low polymorphic power ($PIC \leq 0.1$) to discriminate 118 chickpea genotypes into different genetic groups. The numbers of alleles per locus were directly proportional to variations of allele size, PIC and H_e , and inversely proportional to major allele frequency (MAF). There was parallelism between PIC and H_e , both might reflect the discriminating powers (genetic diversity) of the SSR markers across 118 chickpea genotypes (Table 4.2).

4.3.1. Phylogenetic relationships and principal coordinate analysis

Molecular genetic relationships among 118 chickpea genotypes were revealed by phylogenetic relationships (dendrograms) and principal coordinate analysis (PCoA) (Figs 4.1 and 4.2). The phylogenetic relationships of 118 chickpea genotypes were determined based on pairs of simple matching dissimilarity matrix of hierarchical neighbor-joining weighted pair group arithmetic average (WPGMA) that resolved the 118 chickpea genotypes into three clusters: landraces, improved kabuli and desi (Fig 4.1). In addition, principal coordinate analysis (PCoA) of the molecular data was analyzed based on pairwise distance matrix across 118 chickpea genotypes by using GenAlex software. Hence, the PCoA analysis confirmed the same result as WPGMA dendrogram, 118 chickpea genotypes were grouped into three clusters: landraces, improved desi and kabuli (Fig 4.2).

4.3.2. Population structure, genetic differentiation and geographic distribution

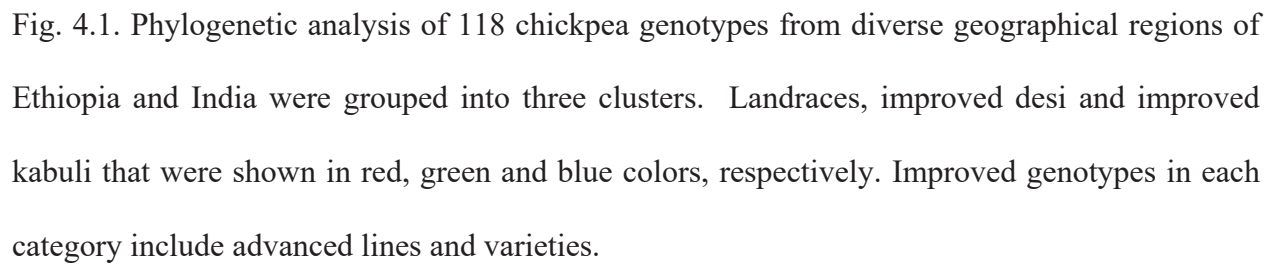
According to an online computation of population structure (Earl and VonHoldt, 2012) by using an Evano *et al.* (2005) method, from the varying probable numbers of populations ($K = 2$ to $K = 10$), maximum delta K (ΔK) value clearly obtained at $K = 6$ (Fig 4.3a). Therefore, the admixture model in STRUCTURE 2.3.4 software analysis clearly revealed that 118 chickpea genotypes (115 Ethiopian and 3 Indian genotypes) were categorized into six groups of populations (desi, kabuli, and 4 distinct groups of landraces, Fig 4.3b). Populations 1, 4, 5 and 6 were comprised of landraces collected from North Shewa, SNNP (Southern Nations, Nationalities and Peoples), West Shewa, South Wollo and Tigray; East Shewa, Arsi, North Shewa, and South Wollo; North Shewa and West Harerge; and South Gondar, East Gojam and West Gojam, respectively. Pop2 and Pop3 comprised of improved kabuli and desi (Fig 4.3b).

Table 4.2. Major allele frequency, polymorphic information content, heterozygosity, allele size, number of alleles per locus and allelic composition of 46 SSR markers across 118 chickpea genotypes.

Marker	Linkage group	Major allele frequency	Number of alleles	He	Ho	PIC	Size range (bp)	Allelic composition		
								RA	CA	FA
GAA47	CaLG03	0.95	3	0.10	0.00	0.10	152-168	0	2	1
ICCM0289	CaLG01	0.95	2	0.10	0.01	0.09	215-222	0	1	1
CaM1101	CaLG06	0.16	24	0.92	0.28	0.91	258-345	7	17	0
TA103	CaLG02	0.22	12	0.85	0.18	0.84	150-186	4	7	1
CaM0600	CaLG03	0.89	6	0.21	0.02	0.20	228-241	2	3	1
CaM0610	CaLG03	0.98	3	0.05	0.00	0.05	267-276	1	1	1
CaM0436	CaLG04	0.34	15	0.83	0.25	0.82	214-255	4	9	1
H1D24	CaLG08	0.96	5	0.07	0.01	0.07	251-276	3	1	1
GA26	CaLG06	0.54	8	0.67	0.17	0.64	229-253	2	5	1
TA14	CaLG06	0.16	16	0.89	0.24	0.88	243-286	6	10	0
TS72	CaLG04	0.16	23	0.90	0.15	0.89	227-310	10	13	0
TA135	CaLG03	0.31	11	0.79	0.26	0.77	183-224	3	6	2
CaM0336	CaLG02	0.93	4	0.14	0.00	0.13	227-269	1	2	1
TA22	CaLG04	0.10	28	0.95	0.33	0.94	200-288	3	25	0
H1G16	CaLG01	0.23	15	0.87	0.34	0.85	270-316	2	12	1
TR29	CaLG05	0.20	15	0.88	0.22	0.87	199-247	1	14	0
CaM0519	CaLG03	0.93	4	0.14	0.02	0.13	298-306	1	2	1
TA116	CaLG07	0.37	11	0.79	0.31	0.76	173-206	2	8	1
CaM1402	CaLG06	0.20	14	0.86	0.26	0.85	219-260	4	10	0
TA76s	CaLG04	0.45	11	0.73	0.20	0.70	193-220	3	6	2
TA96	CaLG02	0.17	17	0.90	0.35	0.89	249-298	3	14	0
CaM0787	CaLG08	0.93	4	0.14	0.01	0.13	135-157	2	1	1
TA194	CaLG02	0.26	14	0.85	0.27	0.84	115-163	3	10	1
CaM0317	CaLG07	0.35	10	0.79	0.52	0.77	258-292	2	7	1
CaM0443	CaLG07	0.17	16	0.89	0.32	0.89	234-288	2	14	0
CaM0622	CaLG07	0.53	11	0.69	0.17	0.67	201-223	0	10	1
CaM1125	CaLG06	0.61	3	0.49	0.02	0.39	299-303	0	1	2
TA78	CaLG07	0.18	15	0.89	0.29	0.88	189-238	1	14	0
TR56	CaLG04	0.35	13	0.80	0.33	0.78	226-264	1	11	1

TA113	CaLG01	0.18	14	0.88	0.36	0.87	168-232	2	12	0
CaM1158	CaLG04	0.16	16	0.90	0.21	0.89	226-263	3	13	0
TA130	CaLG01	0.15	22	0.93	0.43	0.92	224-289	4	18	0
TA3	CaLG08	0.38	7	0.77	0.29	0.73	285-303	1	5	1
NCPGR21	CaLG04	0.36	10	0.79	0.26	0.77	132-159	2	7	1
ICCM249	CaLG04	0.15	21	0.91	0.37	0.91	155-223	5	16	0
STMS11	CaLG04	0.36	15	0.82	0.31	0.80	192-232	4	10	1
NCPGR127	CaLG04	0.46	12	0.74	0.30	0.72	229-262	1	10	1
TAA170	CaLG04	0.55	13	0.66	0.18	0.64	208-276	3	9	1
TA176	CaLG06	0.20	16	0.89	0.11	0.88	233-280	3	12	1
TA37	CaLG02	0.26	17	0.88	0.33	0.87	285-334	2	14	1
TA05	CaLG05	0.52	7	0.68	0.00	0.65	260-289	0	6	1
TA71	CaLG05	0.18	15	0.88	0.41	0.86	171-225	4	11	0
TA122	CaLG01	0.25	14	0.87	0.31	0.85	166-204	2	11	1
NCPGR27	CaLG04	0.51	10	0.69	0.34	0.67	266-286	0	13	1
TS45	CaLG03	0.36	12	0.79	0.21	0.77	226-256	2	9	1
TA118	CaLG01	0.18	18	0.91	0.27	0.90	175-238	3	15	0
Mean		0.41	12	0.70	0.22	0.68				

He = expected heterozygosity, Ho = observed heterozygosity, PIC = polymorphic information content, CA = common allele, FA = frequent allele, RA = rare allele



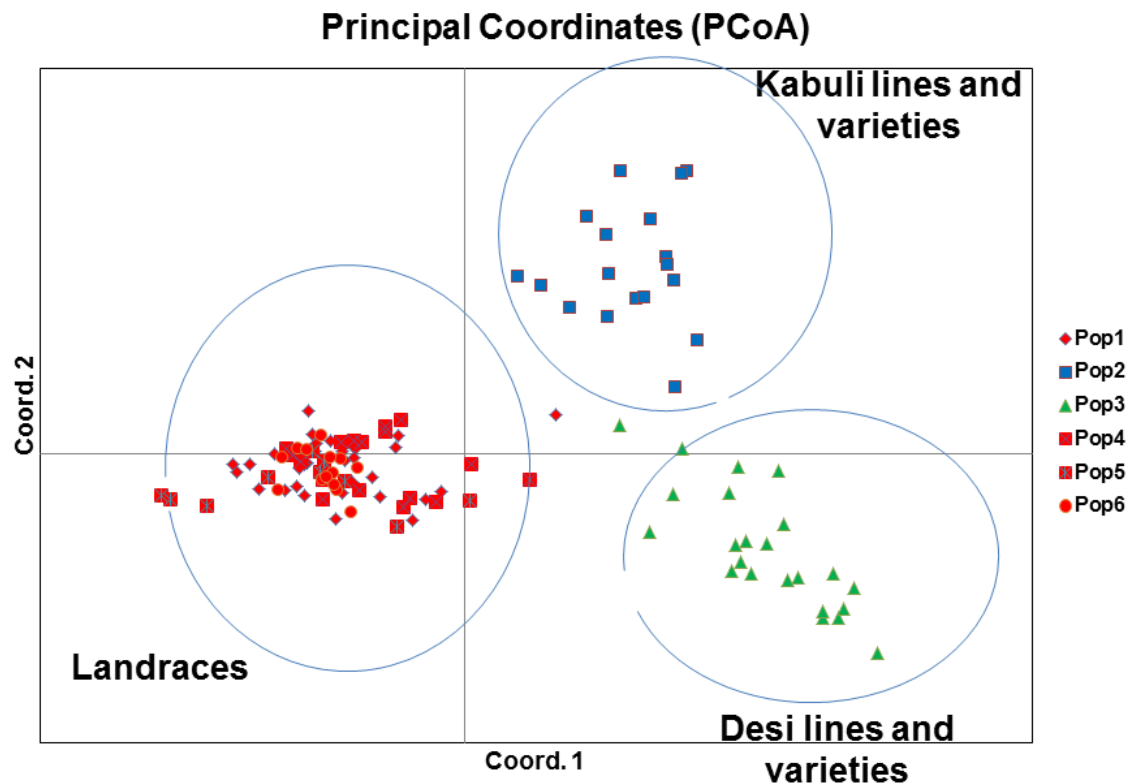


Fig. 4.2. Principal coordinates analysis (PCoA) grouped 118 chickpea genotypes into three clusters: landraces, desi lines and varieties (improved desi), and kabuli lines and varieties (improved kabuli).

In this study, 60% of membership coefficient was used as a threshold value of individual genotypes to be clustered into their own genetic groups. However, an average membership coefficient value of individual genotypes per population group was greater than 80%. Based on this threshold membership coefficient 32, 18, 22, 15, 7 and 13 individual chickpea genotypes belong to populations 1-6 in their respective order; nevertheless, 11 genotypes possessed a membership coefficient of less than 60% that have admixture ancestry among the 6 populations.

Pop1 possessed the largest number of alleles (390) and genotype specific alleles (47) per population, maximum average number of alleles (8.5) and average genotype specific alleles per locus (1.1), Shannon information index (1.49), and observed heterozygosity (0.3), and high percentage of polymorphism (93.48%), genetic diversity (0.62) and unbiased expected heterozygosity (0.63). Pops 3 and 2 had high percentage of polymorphism (93.48%-95.65%), the highest F value (0.71-0.77), less observed heterozygosity (0.13-0.17), and Shannon information index (1.21-1.22). Pop6 had the least average genotype specific alleles per locus (0.17), Shannon information index (1.18), and percentage of polymorphism (84.78%) (Table 4.3).

Table 4.3. Allelic richness and genetic diversity among six populations of chickpea.

Populations	N	Number of allele/ population	Number Unique of allele/ locus	% P	He	uHe	Ho	I	F	
Pop 1	32	390	8.5	1.1	93.48	0.62	0.63	0.30	1.49	0.56
Pop2	18	241	5.2	0.65	93.48	0.59	0.60	0.16	1.21	0.71
Pop3	22	268	5.8	0.76	95.65	0.57	0.58	0.13	1.21	0.77
Pop4	15	296	6.4	0.54	95.65	0.64	0.65	0.22	1.40	0.68
Pop 5	7	190	4.1	0.17	91.3	0.50	0.53	0.18	0.98	0.61
Pop6	13	237	5.2	0.17	84.78	0.56	0.58	0.26	1.17	0.51
Mean	19.7	270.3	5.86	0.565	92.39	0.58	0.59	0.21	1.24	0.46

N = population size, %P = percentage of polymorphism, He = expected heterozygosity, uHe = unbiased expected heterozygosity, Ho = observed heterozygosity, I = Shannon information index, F = f-statistics index.

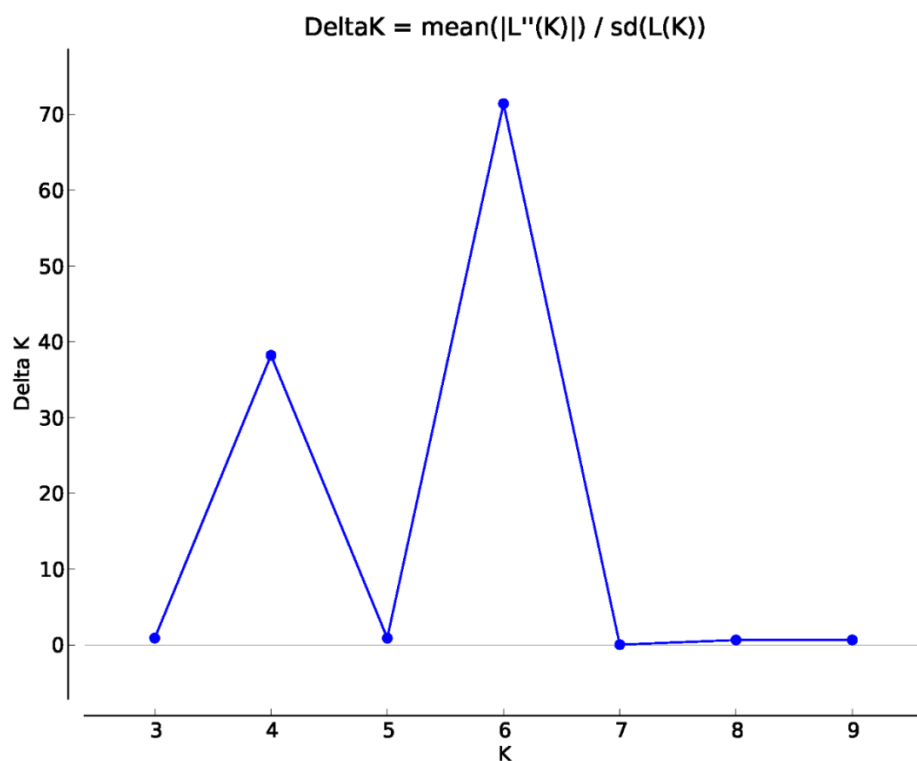


Fig.4.3a. According to Evano et al. (2005), the probable number populations was detected from $K = 2$ to $K = 10$ and the highest pick was depicted at $K = 6$

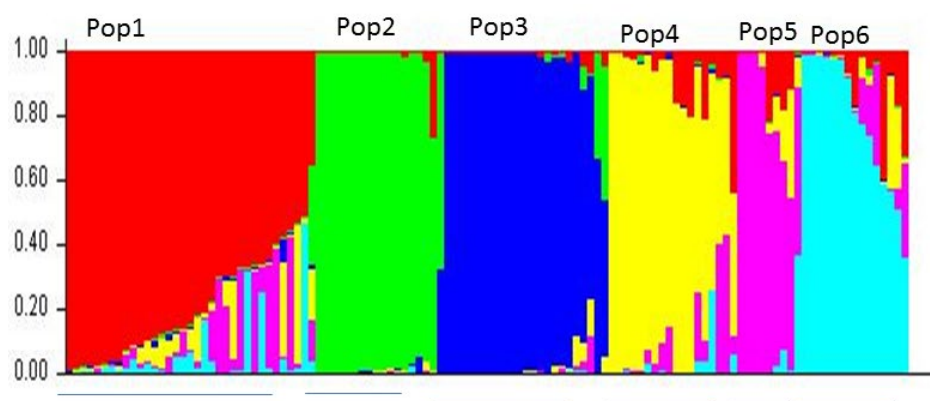


Fig.4.3b. Six sub-population were identified among 118 chickpea genotypes using STRUCTURE 2.3.4 software analysis. Six separate bar plots were shown and each bar plot represented distinct population.

Nei's pairwise genetic distances among populations varied from 0.15 to 0.52. Pop1 was distant from Pop3 (0.52) and Pop2 (0.46) while Pop1 was closer to Pop6 (0.15) and Pop4 (0.16). Pops 2 and 3 were close (0.44) to each other than to either landrace groups and distant from Pop5 (0.66 and 0.67) and Pop6 (0.55 and 0.58). Among 118 chickpea genotypes, the most distant and closest chickpea genotypes to all other genotypes were an Indian elite desi variety, ICCV96029 (Pop3) and an Ethiopian landrace, 207654 (Pop1) collected from North Shewa. Landraces collected from different regions of Ethiopia were very close to each other than lines and varieties of desi and kabuli taken from Ethiopia and India (ICRISAT) (Table 4.4).

Pairwise genetic divergence (F_{ST}) among populations varied from 0.04 to 0.12. Pops 1 and 3 had the highest genetic divergence (0.12) and Pops 1 and 4 had the least genetic divergence (0.04). The inbreeding coefficient value of each population varied from 0.08 (Pop 1) to 0.42 (Pop5) (Table 4.4). The trends of genetic differentiation detected by F_{ST} among six populations was consistent with that of Nei's pairwise genetic distance. Population structure analysis of this study confirmed the same result as WPGMA dendrogram and PCoA except further clustering of landraces into four distinct populations. This is partially due to geographical proximity of collected landraces; nevertheless, in some case, landraces collected from geographically distant zones like North Shewa, West Shewa, South Wollo, Tigray and SNNP were clustered into the same population. Landraces collected from North Shewa and West Harerge was also grouped under the same population. Moreover, even landraces collected from the same geographical zone (for example in North Shewa) was clustered into 3 distinct groups of populations (Pops 1, 4 and 5).

Table 4.4. Pairwise population matrix of Nei genetic distance, pairwise population divergence values, and mean genetic distance and mean genetic divergence among individuals within populations.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Genetic distance	Fixation index
								0.0809
Pop1	0.000	0.458	0.516	0.163	0.177	0.153	0.6469	0.2539
Pop2	0.103	0.000	0.435	0.525	0.659	0.554	0.6046	0.2586
Pop3	0.118	0.110	0.000	0.557	0.670	0.582	0.5691	0.1274
Pop4	0.041	0.110	0.119	0.000	0.260	0.246	0.6626	0.4188
Pop5	0.057	0.157	0.165	0.077	0.000	0.301	0.4469	0.2195
Pop6	0.045	0.135	0.138	0.068	0.098	0.000	0.5795	

4.3.3. Analysis of molecular variance

Genetic diversity among 118 chickpea genotypes were depicted by Shannon information index, genetic diversity parameters, analysis of molecular variance, F statistics and gene flow. Shannon information index revealed that the genetic diversity among population was 28% while, within populations was 72%. Population differentiation based on analysis of molecular variance (AMOVA) has shown that 14%, 57% and 29% of the total genetic variation was obtained among populations, among individuals and within populations, respectively (Table 4.5 and Fig 4.4) and all the genetic variance components were highly significantly different ($P < 0.001$). F statistics (fixation index or inbreeding coefficient) explained that the genetic variation among individuals (FIS, 0.71) and within subpopulations (FIS, 0.67) were higher than among populations (FST, 0.14). The mean gene flow (Nm) among the six populations was 1.57 (Table 4.5).

Table 4.5. Analysis of molecular variance (AMOVA), gene flow and genetic divergences within individuals, among populations and individuals.

Source	df	SS	MS	Est. Var.	%	F-Stat	Value	P
Among Populations	5	569.93	113.98	2.34	14%	Fst	0.13	0.001
Among individuals	112	2755.62	24.60	9.83	57%	Fis	0.66	0.001
Within populations	118	581.50	4.92	4.92	29%	Fit	0.71	0.001
Total	235	3907.06		17.11	1	Nm	1.57	

df = degree of freedom, SS = sum of square, MS = mean square, Est. Var. = estimated variance, % = percentage of variation, Fst, Fis and Fit = genetic divergence among populations, within populations and among individuals, respectively, Nm = gene flow.

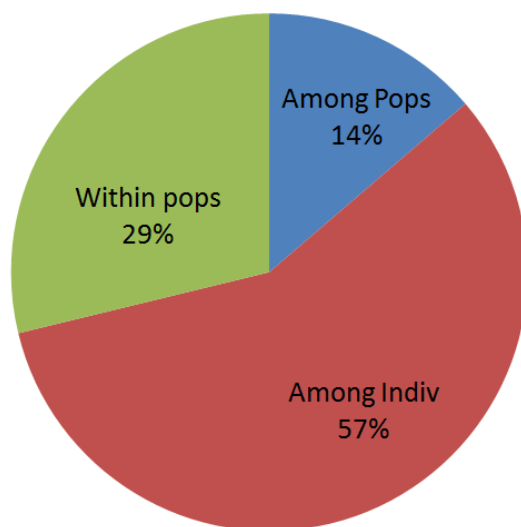
Percentages of Molecular Variance

Fig.4.4. The percentage of molecular genetic variations of 118 chickpea genotypes within populations , among individuals and populations revealed by SSR markers.

4.4. Discussion

4.4.1. Allelic variation and molecular genetic diversity

The number of alleles per locus, number of genotype specific, rare, common, frequent and group specific alleles, variability of allele sizes, PIC values and H_e were allelic variation and genetic diversity parameters that can infer the discrimination power of an SSR marker for measuring phylogenetic relationships, molecular genetic diversity, geographical relationship and genetic differentiation patterns of chickpea accessions (Upadhyaya *et al.*, 2008; Gemechu Keneni *et al.*, 2011; Saeed *et al.*, 2011; Sefera *et al.*, 2011; Naghavi *et al.*, 2012; Saxena *et al.*, 2014; Torutaeva *et al.*, 2014; Parida *et al.*, 2015; Giovanni *et al.*, 2016; Samyuktha *et al.*, 2018). Number of alleles per locus showed a positive correlation with the ranges of allele sizes, PIC and genetic diversity (Upadhyaya *et al.*, 2008; Tadesse Sefera *et al.*, 2011; Choudhary *et al.*, 2012; Torutaeva *et al.*, 2014) which were in agreement with the present study.

In the present study, a total of 572 alleles were detected from 46 SSR markers, number of alleles and allele sizes varied from 2 to 28 alleles per locus and 115 to 345 bp, an average number of alleles per locus, PIC and genetic diversity were 12, 0.68 and 0.69, respectively. Similar results were reported in previous studies (Upadhyaya *et al.*, 2008; Tadesse Sefera *et al.*, 2011; Naghavi *et al.*, 2012; Giovanni *et al.*, 2016). In these studies, 218, 122, 504 and 309 alleles were detected with 22, 9, 48 and 16 SSR markers, respectively. Number of alleles per locus varied from 2 to 26 (mean 9.9), 9 to 20 (mean 13.5), 3 to 22 (mean 10.5) and 8 to 29 (mean 19.3) in their respective order. Their average genetic diversity and PIC ranges from 0.7-0.8. The allele sizes were in a similar range (131 to 344 bp) as reported earlier (Giovanni *et al.*, 2016).

In the contrary, Upadhyaya *et al.* (2008) and Thudi *et al.* (2014) reported relatively large number of total numbers of alleles (917 and 1683) from 35 and 48 SSR markers, average number of alleles per locus (26.2 and 35), and genetic diversity (0.48-0.96 and 0.87). On the other hand, others detected small number of alleles per locus and average alleles per locus, less average PIC and genetic diversity values (Gemechu Keneni *et al.*, 2011; Saeed *et al.*, 2011; Choudhary *et al.*, 2012; Hajibarat *et al.*, 2014; Cevik *et al.*, 2015). These authors detected 58, 119, 480, 117, and 111 alleles from 10, 19, 100, 38 and 33 SSR markers in their respective order. According to the reports of these authors, number of alleles, average alleles per locus, PIC and genetic diversity varied from 2-13, 3-6.25, 0.41-0.68, respectively. The number, type, genome wide distribution and polymorphic nature of the SSR markers and the number, biological status, type, geographical distributions and agro-ecology of the germplasm accessions might account for these differences.

The presence of genotype specific alleles are important for genetic characterizations of agronomic and quality traits, and marker assisted selection (gene tagging) (Lahiri *et al.*, 2015). A total of 106 genotype specific alleles were identified from 307 Iranian chickpea accessions (Upadhyaya *et al.*, 2008; Naghavi *et al.*, 2012) and reported 470 unique alleles across 2915 composite chickpea collections as revealed by SSR markers. Moreover, Upadhyaya *et al.* (2008) found higher percentage (55.5%) of rare alleles than common (42.8%) and most frequent (1.7%) alleles. In the present study, a comparatively large numbers of genotype specific alleles (126) were found. Common alleles (74%) were found to be extremely higher than the rare alleles (19.9%). Upadhyaya *et al.* (2008) confirmed that accessions from East Africa regions are highly polymorphic and genetically diverse with beneficial traits.

In the present study, from the total molecular genetic variations, 72% and 28% of the genetic variation was found within and among populations. In agreement with this study, analysis of molecular variance (AMOVA) revealed that 73% and 27% (Gemechu Keneni *et al.*, 2011), 59% and 41% (Saeed *et al.*, 2011), and 62 % and 38% (Torutaeva *et al.*, 2014) of the variation observed in the chickpea accessions was found within and among populations. This might be due to presence of high genetic variation within landraces collected from the primary and second centers of chickpea (Giovanni *et al.*, 2016). The study of genetic diversity and relationships among chickpea genotypes used under this study might be important on the management, conservation and selection of diverse plant materials for intraspecific crossing of distantly related chickpea genotypes (e.g. Ethiopian landraces with Indian elite varieties, ICCV96029 and JG 14) to improve yield and mitigate abiotic stresses such as drought, heat, cold and salinity in the national chickpea breeding programs. Phenotypic data confirmed that few of the chickpea accessions proven to be heat resilient under heat stress environments (Tsegaye Getahun *et al.*, 2021b). Similarly, Gemechu Keneni *et al.* (2011) emphasized that crossing between genetically distant parents and those from diverse local sources may produce higher heterosis, better genetic recombination and segregation in their progenies and result in varieties with broad genetic base.

4.4.2. Population structure and patterns of population genetic divergence

Chickpea accessions might form clusters based on evolutionary (phylogenetic) relationships, market classes and geographical distributions (Upadhyaya *et al.*, 2008; Saeed *et al.*, 2011; Tadesse Sefera *et al.*, 2011; Naghavi *et al.*, 2012). In the present study, 118 chickpea genotypes were taken from diverse geographical regions of Ethiopia, Debre Zeit Agricultural Research Center (Ethiopia) and ICRISAT (India). They were grouped into three clusters and six distinct

genetic populations based on phylogenetic and structure analyses, respectively. Similarly, phylogenetic analysis of 48 (Hajibarat *et al.*, 2014) and 103 (Giovanni *et al.*, 2016) chickpea entries were clustered into five and three distinct populations. Saeed *et al.* (2011) and Tadesse Sefera *et al.* (2011) reported that by using UPGMA clustering method, 44 and 48 chickpea accessions were clustered into eight and two populations, respectively. In addition, population structure analysis revealed that 46 (Parida *et al.*, 2015), 155 (Gemechu Keneni *et al.*, 2011) and 94 (Saxena *et al.*, 2104) chickpea genotypes were divided into five, five and six distinct populations, respectively.

In this study, chickpea landraces collected from a single origin or two/more geographical origins clustered into a single population and landraces collected from a single geographical origin can be clustered into more than one cluster. For instance, all landraces collected from SNNP grouped into a single cluster; however, landraces collected from North Shewa were failing into four of the landrace clusters with different proportions of individuals. The number of entries varied from cluster to cluster and individual chickpea genotypes within a cluster were definitely more closely related with each other (possessed a membership coefficient of $\geq 60\%$) than with those individual members in different clusters. Intra and inter regional similarities were observed between adjoining geographical origins due to exchange of germplasm. Especially, there might be massive seed accumulation and movement from North Shewa (central part of the country) into different geographical origins of Ethiopia through market or other mechanisms. This could be due to: North Shewa is an adjoining geographical area to the national chickpea-breeding program that might have received significant benefit out of it. In agreement to the present study, Choudhary *et al.* (2012) reported that the overall clustering pattern did not strictly

follow the grouping of accessions according to their geographic origins. Moreover, Gemechu Keneni *et al.* (2011) explained that chickpea genotypes taken from different sources might be evolved from different lines of ancestry and/or derived from independent events of evolutionary forces namely genetic drift, mutation, migration, selection and in flux/out flux of genes in the form of germplasm exchange that differentiated chickpea genotypes into different gene pools. Three elite desi varieties taken from ICRISAT (Hyderabad, India) were clustered together with Ethiopian improved desi genotypes. This clearly indicated that Ethiopia and India have had common breeding lines and varieties, which have been exchanging chickpea genetic material often. This is in line with the report of Tadesse Sefera *et al.* (2011) there was correspondence between the grouping of cultivars released in Ethiopia and India.

4.5. Conclusions

A total of 572 natural alleles and 126 genotype specific alleles were detected from 46 SSR loci across 118 chickpea genotypes. The number of alleles per locus varied from 2 (ICCM0289) to 28 (TA22) and an average of 12 alleles per locus was found. TA22 and CaM0610 markers had the highest and least polymorphic information content, genetic diversity, allele sizes, and total number of alleles per locus, respectively. AMOVA revealed that there was high genetic diversity (59%) among individuals than among populations (14%). The presence of high genetic diversity within populations, wide range of allele sizes, large numbers of alleles per locus and unique alleles per population, high polymorphic information content and expected heterozygosity have breeding implications to the national chickpea improvement program: help to enhance yield and improve specific traits such as heat, cold and drought through crossing of genetically distant genotypes.

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CHAPTER 5: Screening of heat-tolerant Ethiopian chickpea accessions: assessment of phenological and agro-morphological traits and genomic relationships

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Abstract

A major agronomic challenge for chickpea production is temperatures above 35°C, which cause reduced fertility and seed development. This study was aimed at assessing the phenotypic variation of chickpea genotypes under variable heat stress conditions. Chickpea genotypes were grown under heat stressed locations both in Ethiopia and in India to assess phenotypic variation for heat tolerance. In addition, genomic relationships among the genotypes were assessed using genome-wide single nucleotide polymorphism (SNP) markers. A total of 121 genotypes were assessed at three field sites in Ethiopia, under heat stress and non-stress conditions and 57 genotypes were assessed under high heat stress conditions at ICRISAT, Hyderabad, India. Data for 5 phenological and 7 agro-morphological traits were recorded. The results showed that the chickpea genotypes were severely impacted by excessive heat at Hyderabad as compared to those planted under non-heat stress conditions in Debre Zeit, Ethiopia. At extremely high temperatures, chickpea plants exhibited reduced floral initiation, arrested seed and pod development, shortened life cycles, and reduced plant height, seed size, grain yield and yield related traits. Across stressed and non-stressed environments, there were highly significant differences among the genotypes for most of the traits (ANOVA, $P \leq 0.001$). Under heat stressed environments, DZ-Cr-0034 was found to be a highly tolerant, while DZ-Cr-0026 was found to be a highly sensitive genotype. Genetic relationships among the genotypes were determined using 5,722 SNPs, revealing a single group of Ethiopian genotypes with small number of cultivars showing introgression from Middle Eastern germplasm. This study clearly demonstrated that there is genetic variability in chickpea for heat tolerance that can be harnessed to meet expected shifts towards warmer climate.

Keywords: ANOVA, Chickpea, Genomic relationships, Heat stress, Multi location, SNP.

5.1. Introduction

In sub-Saharan Africa (SSA), chickpea (*Cicer arietinum* L.) is predominately cultivated in Ethiopia, Sudan, Eritrea, Kenya, Tanzania and Malawi (Bejiga and Van der Maesen, 2006). Chickpea was likely introduced into sub-Saharan Africa (SSA) through Ethiopia, and indeed Vavilov designated Ethiopia as a secondary center of diversity for chickpea (Sokolkova *et al.*, 2020). Ethiopian production accounts for 90% (Verkaar *et al.*, 2017) of the total production in SSA and 3% of the world's production (FAOSAT, 2018) which makes Ethiopia sixth among the world's leading chickpea producers. Chickpea is a source of dietary protein, a legume that improves soil fertility, and an important cash crop for Ethiopia's small farm holders, while also generating foreign currency through export markets (Bejiga and Eshete, 1996).

High temperatures (30-35°C) limit growth and yield, with primary impact on flower development, fertility, and pod development, and overall impact on plant biomass and nitrogen fixation of chickpea (Devasirvatham, 2012; Krishnamurthy *et al.*, 2011; Summerfield *et al.*, 1984; Summerfield *et al.*, 1990). Devasirvatham (2012) reported that high temperatures impact several chickpea physiological and agronomical traits. Several reports describe reduced yields when altered planting times expose the crop to high temperatures, although this experimental approach confounds several different factors such as day length and temperature. Kalra *et al.* (2008) reported a yield reduction from 53-330 kg/ha among early maturing genotypes and Upadhyaya *et al.* (2011) calculated a 10 to 15% grain yield loss for each 1°C rise above optimum temperatures in India. Only two heat-tolerant genotypes were identified in the international mini-core collection of 300 genotypes that represents the global diversity of chickpea (Upadhyaya *et al.*, 2011). Similarly, Devasirvatham (2012) reported that among 167 chickpea genotypes, only

two were heat tolerant at $\geq 38^{\circ}\text{C}$. In Ethiopia, although 22 improved chickpea varieties were released for drought tolerance (Asnake Fikre, 2014); no variety has been released as yet for heat stress tolerance. Chickpeas are increasingly grown in Ethiopia's lowland regions, including Melka Werer, Humera, Kobo and South Omo, which exposes the crop to higher temperatures (Yayeh Bitew and Fikremariam Asargew, 2015).

Understanding the genetic basis of heat tolerance traits is needed in chickpea both for genomic-guided crop improvement and to identify underlying genes (Devasirvatham, 2012; Paul *et al.*, 2018). As a prelude to genetic analyses, one must first identify genotypes that differ in heat tolerance traits. Genome-wide association studies have the potential to identify causal genetic intervals but require large and properly structured study populations, detailed and accurate phenotyping, and significant resources for genomic analysis. Alternatively, one can understand the degree of genetic variation in tested materials so that suitable parents can be selected to develop bi-parental populations for trait-locus mapping and molecular breeding (Basu *et al.*, 2019; Kujur *et al.*, 2015).

There are few reports on heat tolerance in chickpea and a single report only considers heat tolerance in Ethiopia using 18 early-maturing Ethiopian and Indian genotypes (Teshfashamlak Molla *et al.*, 2018). While Teshfashamlak Molla *et al.* (2018) demonstrated the feasibility of discovering heat tolerant genotypes, there remains an urgent need to identify additional sources for heat tolerance in breeding material. The objectives of the present study were to assess the phenotypic variation in a multi-location field trial for heat tolerance and to determine genomic relationships among the chickpea genotypes.

5.2. Materials and Methods

5.2.1. Plant materials

A total of 121 chickpea genotypes were used at three experimental sites in Ethiopia (Kobo, Werer, and Debre Zeit) and 57 were used at one field site in India (ICRISAT, Hyderabad), respectively (Table 5.1). The chickpea genotypes used in Ethiopia were 81 landraces including a local check, 34 improved lines and 6 commercial cultivars. Among 81 landraces, 76 originated from low and mid altitudes (<1900 mas) and 5 genotypes were from high altitudes (>2400 mas). The 57 genotypes used in India (Hyderabad) include 10 landraces, 40 improved lines and commercial cultivars, and seven standard heat sensitive and tolerant checks (Table 5.1). Plants were grown at three experimental locations in Ethiopia: Kobo (2017), Werer (2018) and Debre Zeit (2016) and at one location in India: Hyderabad, ICRISAT (2017).

Debre Zeit is located at 1900 masl, latitude of 08° 44' N and longitude of 38° 58' E. The soil type is black (Vertisols) having pH values ranging from 6.0 to 7.5. Kobo is located at 1490 masl, latitude of 12° 09' 67" N and longitude of 39° 37' 48" E. It is dominated by Eutric-Vertisol and Eutric-Fuvisol soils and their pH values range from 7.4 to 8.5. Werer is located at 750 masl, latitude of 9° 16' N and longitude of 40° 9' E. The soil types are Salic-Fluvisols, Eutric- Fluvisols and Eutric-Vertisols. The pH of the soils ranges from 7.4 to 8.4. Hyderabad, India is located at 545 masl, latitude of 17.53° N and longitude of 78.27° E. The soil type is black (Vertisol). The pH of the soil is alkaline that ranges from 8.35-8.85.

5.2.2. Experimental design and field management

An alpha lattice design with two replications was used at Hyderabad, while three replications were used at three locations in Ethiopia. Plants were grown from March to June in 2016 at Debre Zeit, March to June and February to May at Hyderabad and Kobo, respectively in 2017, and February to May in 2018 at Werer. Individual plots with two rows were 1 m by 0.6 m and contained 20 seeds of a single genotype planted at 0.1 m, 0.3 m and 1 m spacing between plants, rows and replications, respectively. Due to a shortage of space, only a single row with 10 seeds was planted at Hyderabad and spacing between plants and replications were the same as above.

Table 5.1. List of chickpea genotypes used at experimental field sites in India (Hyderabad, ICRISAT) and Ethiopia (Kobo, Werer, Debre Zeit)

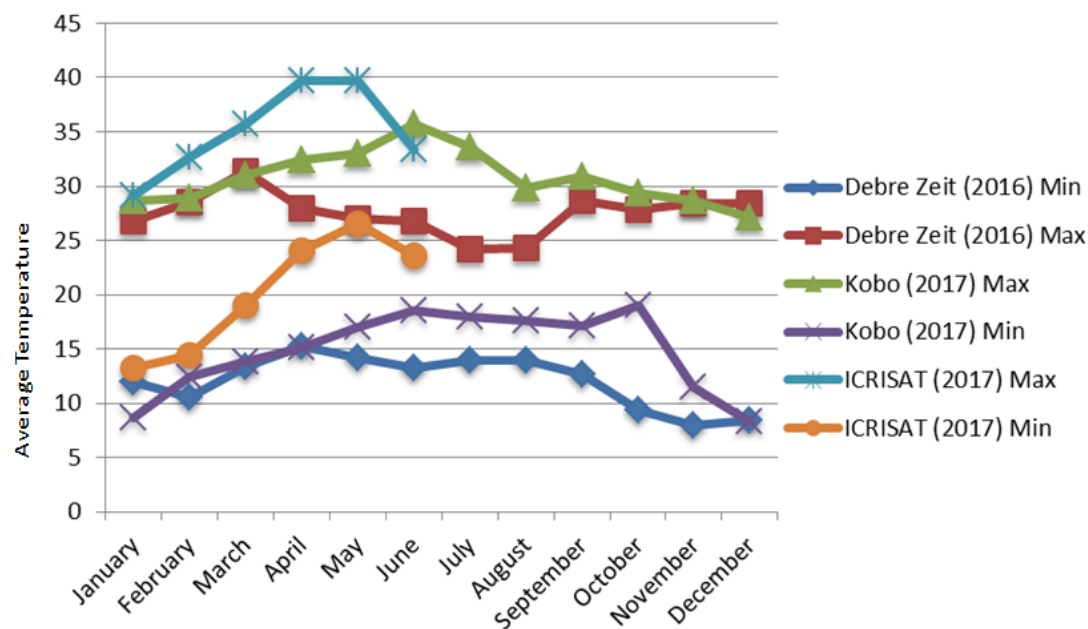
Hyderabad				Experimental Sites in Ethiopia					
SN	Genotype/Accessions	SN	Genotype/Accessions	SN	Genotype/ Accessions	SN	Genotypes/ Accessions	SN	Genotype/ Accessions
1	DZ-2012-ck-0016	44	ICCV 7109	1	DZ-2012-ck-0016	44	41055	87	207658
2	DZ-2012-ck-0017	45	JAKI 9218	2	DZ-2012-ck-0017	45	41107	88	207661
3	DZ-2012-ck-0019	46	41110	3	DZ-2012-ck-0019	46	41108	89	207673
4	DZ-2012-ck-0023	47	41144	4	DZ-2012-ck-0023	47	41109	90	207721
5	DZ-2012-ck-0024	48	41147	5	DZ-2012-ck-0024	48	41110	91	207739
6	DZ-2012-ck-0025	49	41268	6	DZ-2012-ck-0025	49	41111	92	208829
7	DZ-2012-ck-0026	50	41289	7	DZ-2012-ck-0026	50	41113	93	208900
8	DZ-2012-ck-0027	51	ICCV 92944	8	DZ-2012-ck-0027	51	41114	94	208984
9	DZ-2012-ck-0029	52	ICCV 96029	9	DZ-2012-ck-0029	52	41117	95	208991
10	DZ-2012-ck-0031	53	209109	10	DZ-2012-ck-0031	53	41119	96	208992
11	DZ-2012-ck-0034	54	212589	11	DZ-2012-ck-0034	54	41120	97	208993
12	DZ-2012-ck-0036	55	235720	12	DZ-2012-ck-0036	55	41122	98	208994
13	DZ-2012-ck-0038	56	235721	13	DZ-2012-ck-0038	56	41136	99	208995
14	DZ-2012-ck-0040	57	235963	14	DZ-2012-ck-0040	57	41144	100	208996
15	DZ-2012-ck-2011S 0041			15	DZ-2012-ck-2011S-1-	58	41145	101	208997
16	DZ-2012-ck-0074			16	0041	59	41146	102	208999
17	Habru			17	DZ-2012-ck-0074	60	41147	103	209000
18	Ejere			18	Habru	61	41148	104	209002
19	Chefe			19	Ejere	62	41149	105	209003
20	Minjar			20	Chefe	63	41150	106	209004
21	Teketay			21	Minjar	64	41153	107	209007
22	Dalota			22	Teketay	65	41155	108	209008
23	JG 130			23	Dalota	66	41156	109	209016
24	DZ-2012-ck-0196			24	Local check	67	41157	110	209017
25	DZ-2012-ck-0198			25	DZ-2012-ck-0196	68	41189	111	209093
26	DZ-2012-ck-0199			26	DZ-2012-ck-0198	69	41209	112	209096

27	DZ-2012-ck-0201	27	DZ-2012-ck-0199	70	41267	113	209102
28	DZ-2012-ck-0202	28	DZ-2012-ck-0201	71	41268	114	209109
29	DZ-2012-ck-0206	29	DZ-2012-ck-0202	72	41269	115	209110
30	DZ-2012-ck-0208	30	DZ-2012-ck-0206	73	41271	116	212477
31	DZ-2012-ck-0209	31	DZ-2012-ck-0208	74	41279	117	212478
32	DZ-2012-ck-0210	32	DZ-2012-ck-0209	75	41282	118	212589
33	DZ-2012-ck-0228	33	DZ-2012-ck-0210	76	41286	119	212914
34	DZ-2012-ck-0229	34	DZ-2012-ck-0228	77	41288	120	225876
35	DZ-2012-ck-0230	35	DZ-2012-ck-0229	78	41289	121	235394
36	DZ-2012-ck-0231	36	DZ-2012-ck-0230	79	41295		
37	DZ-2012-ck-0232	37	DZ-2012-ck-0231	80	207234		
38	DZ-2012-ck-0234	38	DZ-2012-ck-0232	81	207651		
39	DZ-2012-ck-0235	39	DZ-2012-ck-0234	82	207652		
40	DZ-2012-ck-0236	40	DZ-2012-ck-0235	83	207654		
41	DZ-2012-ck-0240	41	DZ-2012-ck-0236	84	207655		
42	ICC 4567	42	DZ-2012-ck-0240	85	207656		
43	ICCV 7102	43	41037	86	207657		
			41052				

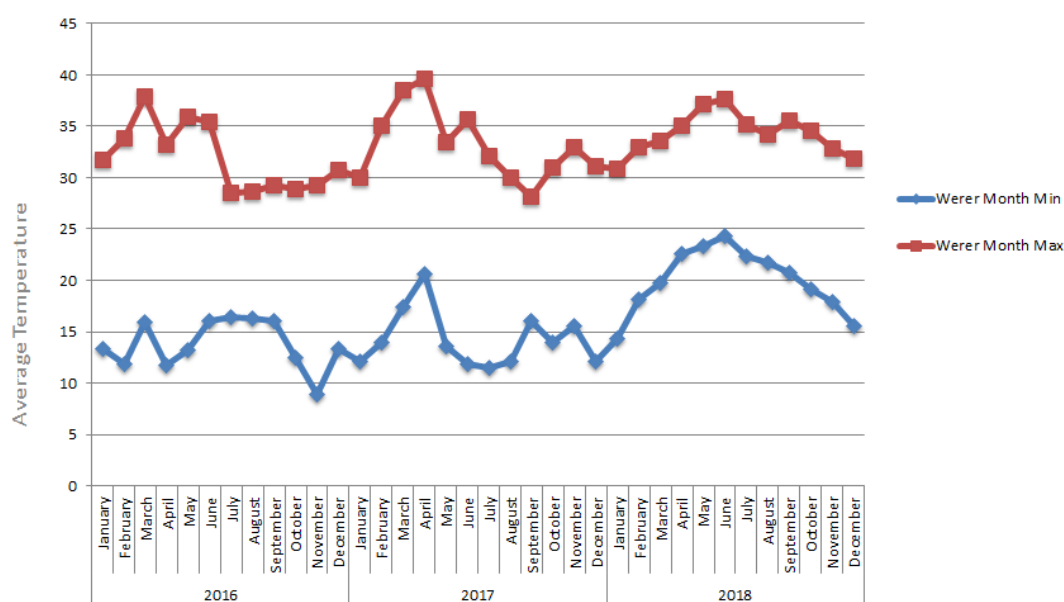
In all locations, plants were grown using supplemental irrigation to avoid drought stress. Plots were managed by frequent manual weeding. Chemical sprayings were applied for pod borer (*Helicoverpa armigera*), Ascochyta blight (*Ascochyta rabiei*) and Fusarium wilt (*Fusarium oxysporum*). Root rot (*Rhizoctonia solani*) was the most frequent disease in Hyderabad; and we controlled it by soil solarization and regular removal of infected individuals.

5.2.3. Temperatures of the experimental field sites

Debre Zeit (DZ) was used as a control (non-heat stressed environment), while the remaining field sites were heat stressed environments. The monthly average minimum and maximum temperatures at DZ varied from 13.3°C to 13.8°C and 26.8°C to 31.4°C, respectively. ICRISAT, Hyderabad, India had the most extreme monthly average minimum and maximum temperatures, which ranged from 19°C to 26.5°C and 35.7°C to 39.7°C, respectively. The monthly average minimum and maximum temperatures at Kobo were 12.4°C to 17°C and 28.9°C to 33°C, respectively (Fig 5.1a). The monthly average minimum and maximum temperatures at Werer were 18.1°C to 23.3°C and 33°C to 37.1°C, respectively (Fig 5.1b). Maximum daily temperatures at Kobo, Werer, and India (Hyderabad) were above 36°C, 40°C, and 45°C, respectively. At Hyderabad, India, the length of the day (14 hours) was longer than the night (10 hours) during the field experiment. All Ethiopian field sites, Kobo, DZ, and Werer, had similar day and night lengths that were 13 and 11 hours, respectively.



a. Kobo and ICRISAT (Hyderabad, India)



b. Werer

Fig. 5.1. Temperature data, monthly average minimum (Min) and maximum (Max) values for heat stressed and non-stressed locations in Ethiopia and India, a. Temperature data at Kobo (Ethiopia) and ICRISAT, Hyderabad (India) in 2017, and Debre Zeit (Ethiopia) in 2016, b. Temperature data at Werer (Ethiopia) from 2016-2018.

5.2.4. Phenotyping

Twelve phenological and agronomic traits were recorded, namely days to first flowering (DFF), days to 50% flowering (D50F), days to first podding (DFP), grain filling period (GFP), days to physiological maturity (DPM), plant height (PHT), grain yield (GY), 100 seeds weight (HSW), total number of pods per plant (TPP), total number of seeds per plant (TSP), plant biomass (PB), and percentage of harvest index (PHI). Two additional traits, heat visual scores (1-5 tolerant to sensitive) and percentages of pod sets data were also scored only at the Werer field site. Five individual plants were randomly selected for most traits and values for each trait were calculated as an average. Data for GY, PB and PHI were taken on a per plot basis.

5.2.5. DNA extraction

Leaf samples from 15 days old seedlings of 54 genotypes of chickpea were collected from the greenhouse of University of California Davis. Seventy to hundred milligram leaf samples were placed in 12 × 8 well strip tube with strip cap (Marsh Biomarket, New York, USA) together with two 4-mm stainless steel grinding balls (Spex CertiPrep, USA) for DNA extraction using a Retsch Mixer Mill and Qiagen 96-well PlantDNeasy kits. DNA quality and quantity were assessed using a NanoDrop 1000 spectrophotometer (Wilmington, DE 19810 USA).

5.2.6. RAD library development and sequencing

Individual genome samples were prepared for genotyping using a restriction site associated DNA RAD-seq approach, as described in von Wettberg *et al.* (2018). Briefly, DNA was digested with HindIII and NlaIII adaptors added as barcodes and to facilitate en masse PCR amplification. Library quality was assessed using an Agilent 2100 Bio-Analyzer. Pooled samples were

sequenced as 100 bp reads on an Illumina HiSeq4000 at the University of California at Davis Genomics Facility. Calling of SNPs was performed with the Genome Analysis Tool Kit pipeline, following Genome Analysis Tool Kit best practices (McKenna et al., 2010), with filtering thresholds described in von Wettberg et al. (2018) and Sani et al. (2018). Raw sequencing data can be found in the NCBI Umbrella BioProject PRJNA353637, which includes Illumina data from RAD sequencing of over 500 Ethiopian landrace accessions (PRJNA507628).

5.2.7. Data Analysis

All phenological and agronomic traits are considered as dependent variables; however, genotype, block, replication and location (field site) were taken as independent variables (Gomez and Gomez, 1984). For all traits, raw data means were calculated. For the combined analysis of variance (ANOVA), locations and genotypes were considered as random and fixed variables, respectively. Thus, ANOVA was computed based on a mixed linear additive model, using the GLM (General Linear Model) procedure of the SAS 9.0 software for single and combined location analyses of phenotypic data (Gomez and Gomez, 1984).

The linear additive model for a single location is given by P_{ijk} is equal to $\mu + \tau_i + \beta_k(j) + \pi_j + \xi_{ijk}$ where, P_{ijk} is phenotypic value of i th genotype under j th replication and k th incomplete block, μ is Grand mean, τ_i is the effect of i th genotype, $\beta_k(j)$ is the effect of incomplete block k within replication, π_j is the effect of replication j , ξ_{ijk} is experimental or pooled error. The linear additive model for combined location analysis is given by P_{ijks} is equal to $\mu + \tau_i + \beta_k(j)(s) + \pi_j(s) + L_s + (\tau \times l) + \xi_{ijks}$ where, P_{ijks} is phenotypic value of i th genotypes under j th replication at s th location and k th incomplete block within replication j and location s ; μ is Grand mean; τ_i is

the effect of i th genotype; $\beta_{k(j)(s)}$ is the effect of incomplete block k within replication j and location s ; $\pi_{j(s)}$ is the effect of replication j within location s ; L_s is the effect of location; (τ_{xl}) is the interaction effects between genotype and location; and ξ_{ijks} is pooled error. For the combined analysis of variance, locations and genotypes were considered as random and fixed variables, respectively (Gomez and Gomez, 1984).

The total variation (σ^2_p) for each trait was partitioned into variance components: genotypic (σ^2_g), environmental (σ^2_e) and genotype-by-environmental interaction (σ^2_{ge}) variance using the VARCOMP procedure of SAS. These variance components, coefficients of variation and genetic advance (GA) per location and combined over locations were estimated based on the methods of Singh and Chaudhary (1985). Broad-sense heritability (H^2) was estimated as suggested by Ekebil et al. (1977). In addition, cluster and principal component analyses were done using JMP15 (2019) software.

We assessed genetic distance and principal coordinates analysis (PCoA) to visualize the relationship among genotypes. We examined percentage of polymorphism and heterozygosity (genetic diversity) to assess the extent of molecular variability in the landraces, lines and improved varieties. All the molecular data were analyzed using GenALEX 6.5 (Peakall and Smouse, 2012).

5.3. Results

5.3.1. Genotypic response to heat stress

The mean, minimum and maximum number of days to physiological maturity (DPM) of genotypes under heat stressed environments was 7, 8 and 10 days shorter than non-heat stressed environments, respectively (Table 5.2). At Hyderabad, the daily average temperatures were $\geq 40^{\circ}\text{C}$ at the reproductive stage. Consequently, two genotypes (DZ-2012-ck-0026 and ICC 4567) did not initiate flowers and pods at all. For instance, accessions 235721, 41268 and 41289, Habru, and DZ-2012-ck-235 attained days to first flowering (DFF) but did not manifest days to first podding (DFP) and days to 50% flowering (D50F). Delays of DFP responses were also observed in ICCV 7102, DZ-2012-ck-231, DZ-2012-ck-208, DZ-2012-ck-229 and Minjar, possibly due to the severity of heat stress. The minimum and maximum plant height (PHT) of genotypes at Kobo and Hyderabad were shorter by 12.4 cm and 16.2 cm; and 16.9 cm and 19.2 cm than Debre Zeit (DZ), respectively. The mean, minimum and maximum grain yield (GY) of genotypes at Kobo was 2.5, 10, and 2-fold lower than DZ. At Hyderabad, the GY values were 39, 15, and 16-fold lower than DZ (Table 5.2).

Similar to other heat stressed environments, the maximum temperatures, which reached 39°C at Werer (Fig 5.2b), had no effect on the germination of the genotypes. Standard heat sensitive and tolerant checks of ICRISAT were included along with Ethiopian accessions (Fig 5.2f, g). We successfully collected data for four heat traits (mean $\pm$ SD); heat visual scores (HVS, 3.3 ± 1.2), percentage of pod set (%PS, 33.5 ± 21.1), TPP (10.1 ± 10) and TSP (10.9 ± 11). The mean value of TSP at this field site was 2 and 4 times less than Kobo and DZ, respectively. Some heat tolerant genotypes and checks survived, and had reasonable pod sets (40-80%) (Fig 5.2). Both HVS and

%PS data revealed that only 5% of the accessions used at Werer were highly heat tolerant. A heat sensitive check from ICRISAT, ICC 4567 did not set pod but initiated flowers (Fig 5.2f), as it did in Hyderabad. Highly heat sensitive (DZ-2012-ck-0026) and tolerant (DZ-2012-ck-0034) (Fig 5.2i) lines were identified at Werer experimental site, similar to other heat stressed experimental sites. The sensitive accession did not initiate flower throughout the growth period (Fig 5.2j). Similar to Hyderabad, at Werer kabuli genotypes (Fig 5.2b) were highly affected by heat stress.

Table 5.2. Mean, Standard Error, Minimum, Maximum and Range values of traits for 2 heat stressed (Kobo, Hyderabad) and non-heat stressed (Debre Zeit) environments.

Trait	Kobo				Debre Zeit				Hyderabad			
	Mean±SE	Min	Max	R	Mean±SE	Min	Max	R	Mean±SE	Min	Max	R
DFE	42.1±0.27	37.3	48	10.7	47.0±0.32	40.3	53.7	13.4	41.6±0.6	35	55	20
D50F	45.0 ±0.28	39.7	50.7	11	51.7±0.29	46.7	64.7	18	44.5±0.9	39	66	27
DFP	47.8±0.3	43	53.7	10.7	57.6±0.32	50.3	68.7	18.4	37.1±2.9	46.5	61.5	15
GFP	40.9±0.34	37	46.3	9.3	41.7±0.46	34.3	56.3	22	34.6±1.1	24.5	49.5	25
DPM	85.9±0.47	79.3	97	17.7	93.4±4.7	88.3	109.7	21.4	85.5±0.74	80.5	101.5	21.5
PHT(cm)	24.3±0.4	18.6	34.8	16.2	40.2±0.43	31	51.7	20.7	21.9±0.44	14.8	32.5	17.7
TPP	15.2±0.99	5.3	31.7	26.4	32.0±1.8	10	115	105	0.5±0.09	0	2.1	2.1
TSP	19.07±1.4	5.7	45.3	39.6	38.2±2.1	13.3	129.7	116.4	0.6±0.13	0	2.8	2.8
HSW(g)	11.3±0.35	6	26.6	20.6	13.3±0.35	8.2	23.3	15.1	7.2±1.0	0	21.5	21.5
PB(g)	146.3±9.7	27.7	446	418.3	228.3±7.3	98	659	561	30.7±3.0	0	87.0	87
GY(g)	28.8±2.6	1.5	152.3	150.8	71.0±2.9	14.6	255	240.4	1.8±0.46	0	15.7	15.7
PHI	19.3±1.1	2.2	52.6	50.4	31.8±1.0	7.8	61.9	54.1	6.6±1.3	0	38.8	38.8

DFE = days to first flowering, D50F = days to 50% flowering, DFP = days to first podding, GFP= grain filling period, DPM = days to physiological maturity, PHT = plant height, TPP = total number of pods per plant, TSP = total number of seeds per plant, HSW = hundred seeds weight, PB = plant biomass, GY = Grain yield, PHI = percentages of harvest index, SE = standard error, Min = minimum, Max= maximum, R= range.

5.3.2. Analysis of Variance (ANOVA)

ANOVA revealed that at Hyderabad, there were highly significant ($P \leq 0.001$) differences among genotypes for nine agronomic and phenological traits. Similar significant differences were found among genotypes for all traits at Kobo. ANOVA revealed significant difference among sites ($P \leq 0.01$) except DPM and TPP. Significant genotypes by environment interactions ($P \leq 0.05$)

were found for all traits except TPP, TSP and PB. Moreover, at Debre Zeit, ANOVA revealed that genotypes differed significantly ($P \leq 0.001$) for all traits except PHT (Table 5.3). The environmental, genotypic and phenotypic variances of genotypes at Kobo were very high for PB, GY and other yield related traits. The environmental variance at Hyderabad for phenological traits was relatively higher. Similarly, PB had the highest genotypic and phenotypic variances. In the pooled data of stressed environments, PB had the highest environmental, genotypic, genotype by environmental interaction and phenotypic variances. All traits had higher coefficient of phenotypic variances compared to the corresponding coefficient of genotypic variances. At Hyderabad, coefficient of genotypic and phenotypic variances for GY and yield related traits were extremely higher than the phenological traits (Table 5.4).

Table 5.3. ANOVA for 12 traits under heat stressed, pool of stressed and non-stressed environments.

Hyderabad				Kobo		
Variables	Rep(1)	Block(Rep = 2)	Gen(56)	Rep(2)	Block(Rep)(30)	Gen(120)
DFE	87.3***	20.9*	33.3***	10.5*	2.7	19.1***
D50F	286.4***	46.9	61.0***	121.3***	5.6**	17.0***
DFP	419.2	288.7	608.0***	190.3***	11.7***	17.8***
GFP	327.6**	40.1	73.2*	185.8***	19.7***	16.3***
DPM	99.2	76.3	114.2	223.7***	26.7***	45.4***
PHT	65.7*	75.3	31.4*	576.6***	27.0***	18.9***
TPP	3.1***	0.001*	0.8***	4478.2***	223.5***	121.1***
TSP	3.7***	0.01	1.6***	7964***	500.0***	236.4***
HSW	135.2**	33.4	97.2***	43.6***	8.3***	15.5***
PB	3896.8***	1713.1**	788.5***	149115.9***	12866.9***	13678.2***
GY	41.9**	0.5	18.1***	13175.5***	1011.8***	1098.7***
PHI	135.5*	6.6	168.5***	872.3***	67.9*	255.3***

Debre Zeit				Pool of two heat stressed environments (Kobo and Hyderabad)			
Variables	Rep(2)	Block(Rep)(30)	Gen(120)	Loc(1)	Rep (Loc =3)	Gen(47)	GxE(45)
DFE	60.1**	26.9***	45.5***	161.7**	13.8**	34.7***	20.720.7***
D50F	19.2*	11.2*	16.9***	1294.6***	72.4***	58.0***	36.3***
DFP	8.7	16.7***	17.1***	3154.3***	51.8	383.9***	455.7***
GFP	60.1**	26.9***	45.5***	930.2***	172.8***	51.5***	62.8***
DPM	11.5	9.7*	37.8***	26.6	51.2**	46.5*	33.0***
PHT	427.0***	49.2**	31.2*	521.6***	226.4***	28.2*	29.5*
TPP	68.05***	22.9**	869.5***	8880.7	393.5***	38.5	34.2
TSP	164.5***	26.1**	1170.2***	11152.8***	451.0***	75.5	67.8
HSW	1.06*	0.37	19.4***	1689.8***	54.8*	85.1***	74.9***
PB	561.8*	97.0	16532.2***	788641.2***	12486.6	7264.6	8452
GY	204.9**	50.0	2390.8***	44808.0***	1752.8***	1075.6***	891.1***
PHI	16.3	8.3	293.2***	7259.1***	182.0***	293.7***	105.9***

DFE = days to first flowering, D50F = days to 50% flowering, DFP = days to first podding, GFP = grain filling period, DPM = days to physiological maturity, PHT = plant height, TPP = total number of pods per plant, TSP = total number of seeds per plant, HSW = hundred seeds weight, PB = plant biomass, GY = grain yield, PHI = percentages of harvest index, MS = mean square, Rep = replication, Loc = location, numbers in bracket (df) = degrees of freedom, Gen = genotype, GxE = genotype by environment interaction, ANOVA = analysis of variance.

Table 5.4. Variance components, heritability, genetic advance and genetic advance as percentage of the mean of traits under heat stressed, pool of stressed and non-stressed environments.

Trait	EV	GV	PV	GCV%	PCV%	H^2	GAM
Hyderabad							
DFF	100.4	24	124.4	11.8	26.8	19.3	10.6
D50F	216.7	36.9	253.6	13.7	35.8	14.6	10.7
DFP	285.6	286.8	572.4	45.6	64.5	50.1	66.4
GFP	210.9	33.3	244.2	16.7	45.2	13.6	12.6
DPM	100.8	7.5	108.3	3.2	12.2	6.9	1.7
PHT	29.2	2.9	32.1	7.8	25.9	9	4.8
TPP	0.3	0.2	0.5	89.4	141.4	40	116.3
TSP	0.7	0.2	0.9	74.5	158.1	22.2	72.2
HSW	76.2	29.4	105.6	75.3	142.7	27.8	81.6
PB	194.7	385.6	580.3	64	78.5	66.4	107.1
GY	7.4	1.7	9.1	72.4	167.6	18.7	64.4
PHI	21.6	76.1	97.7	132.2	149.8	77.9	240.3
Kobo							
DFF	2.6	6.5	9.1	6.1	5.9	71.4	10.5
D50F	3.2	5.6	8.8	5.3	5.1	63.6	8.6
DFP	4.1	5.7	9.8	5	4.7	58.2	7.8
GFP	9.7	2.6	12.3	3.9	4.8	21.1	3.7
DPM	13.3	13	26.3	4.2	2.4	49.4	6.1
PHT	13.4	4.1	17.5	8.3	11.9	23.4	8.3
TPP	85.1	10	95.1	20.8	30	10.5	13.9
TSP	174.9	24	198.9	25.7	26.6	12.1	18.4
HSW	3.7	10.8	14.5	29.1	47.7	74.5	51.6
PB	6,527.4	3,911.5	10,438.9	42.7	4.5	37.4	53.7
GY	403.1	316.6	719.7	61.8	27.3	44	84.3
PHI	37.6	96.8	134.4	51	37	72	88.9
Debre Zeit							
DFF	7.9	4.1	12	4.3	4.4	34.2	5.2
D50F	6.8	3.6	10.4	3.7	3.7	34.6	4.4
DFP	8.2	3.7	11.9	3.3	3.2	31.1	3.8
GFP	10.2	14.1	24.3	9	7.2	58	14.1
DPM	5.6	15.4	21	4.2	2.2	73.3	7.4

PHT	22.9	3.9	26.8	4.9	5.5	14.6	3.9
TPP	10	386.6	396.6	61.4	24.5	97.5	124.8
TSP	11.1	518.5	529.6	59.6	20.2	97.9	121.3
HSW	0.5	15.1	15.6	29.2	40.6	96.8	59.1
PB	124.9	6,347.3	6,472.2	34.9	2.6	98.1	71.1
GY	41	997.2	1,038.2	44.5	9.4	96.1	89.7
PHI	7	114.6	121.6	33.7	18.3	94.2	67.2

Pooled (Kobo and Hyderabad)

Trait	EV	GV	GEV	PV	GCV%	PCV%	GECV	H ²	GAM
DFE	30	7.8	32.9	70.7	6.7	20.1	13.7	11	4.6
D50F	95.1	13.8	26.6	135.5	8.3	26.2	11.6	10.1	5.4
DFP	102.1	53.9	200.9	356.9	17	43.7	32.8	15.1	13.6
GFP	72.2	7.4	38.6	118.2	7.1	28.2	16.1	6.3	3.7
DPM	31.1	2.4	20.9	54.4	1.8	8.6	5.3	4.4	0.8
PHT	16.4	1.4	5.6	23.4	4.8	19.8	9.7	6	2.5
TPP	37.5	3.7	1.1	42.3	22.9	77.4	12.5	8.7	13.8
TSP	45.4	6	14.1	65.5	26.1	86.1	39.9	9.2	16.3
HSW	10.9	0.8	25.6	37.3	8.1	55.5	46	2.1	2.4
PB	4,622.2	384.9	816.2	5,823.3	18.4	71.7	26.8	6.6	9.7
GY	318.1	62.8	166.4	547.3	40.6	120	66.2	11.5	28.4
PHI									

DFE = days to first flowering, D50F = days to 50% flowering, DFP = days to first podding, GFP = grain filling period, DPM = days to physiological maturity, PHT = plant height, TPP = total number of pods per plant, TSP = total number of seeds per plant, HSW = hundred seeds weight, PB = plant biomass, GY = grain yield, PHI = percentages of harvest index, EV = environmental variance, GV = genotypic variance, PV = phenotypic variance, GEV = genotype by environment interaction variance, GCV% = percentage of coefficient of genotypic variance, PCV% = percentage of coefficient of phenotypic variance, GECV% = percentage of coefficient of genotype by environment interaction variance, H² = percentage of broad sense heritability, GA = genetic advance, GAM = Genetic advance as percentage of the mean.



Fig. 5.2. Field pictures of chickpea genotypes under heat stressed field sites in Ethiopia and India (ICRISAT, Hyderabad). a-d and e-j were pictures at ICRISAT, Hyderabad (India) and Werer

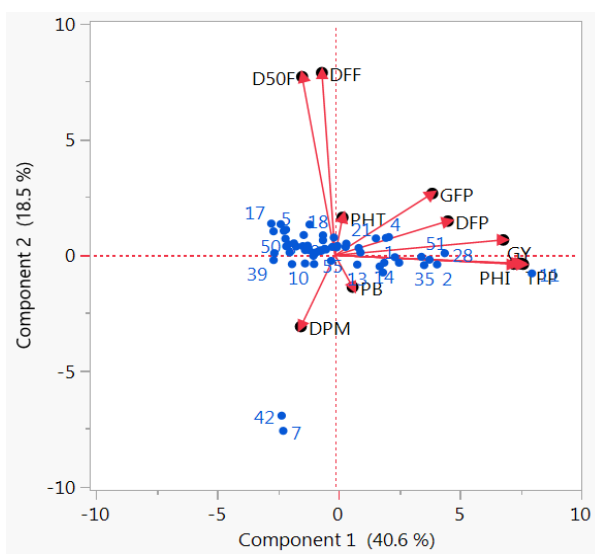
(Ethiopia) field sites, respectively. a. Flower drying and dropping, b. Yellowing of leaves and pod abortions of kabuli genotype, c. The view of vegetative stage of Ethiopian chickpea genotypes at Hyderabad (India) field site, d. Majority of the genotypes was burnt during maturity (heat stress caused forced maturity) but, DZ-2012-ck-0034 had 60% pod sets, e. Pod of a heat tolerant genotype, f. Heat sensitive check (ICC4567), g. Some heat tolerant genotypes, h. Standard heat tolerant genotype (JG 14 or ICCV 92944), i and j were the identified highly heat tolerant (DZ-2012-ck-0034) and heat sensitive (DZ-2012-ck-0026) genotypes, respectively. Similar to Hyderabad, at Werer kabuli genotypes (Fig 5.2b) were highly affected by heat stress.

5.3.3. Correlation of traits

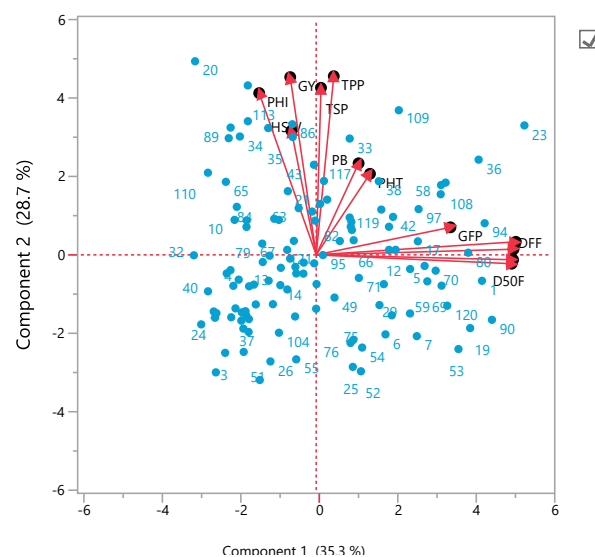
Under all conditions, the genotypic correlations of all quantitative traits were greater than their respective phenotypic correlations. Under heat stressed and pool of stressed environments, GFP was positively correlated with GY and yield related traits except HSW and PB. Under stressed, pool of stressed and non-stressed environments, DFF and DPM were negatively correlated with GY and yield related traits. GY was positively and significantly ($P \leq 0.001$) correlated with yield related traits and PHT (Appendix 5.1).

5.3.4. Principal component and cluster analyses

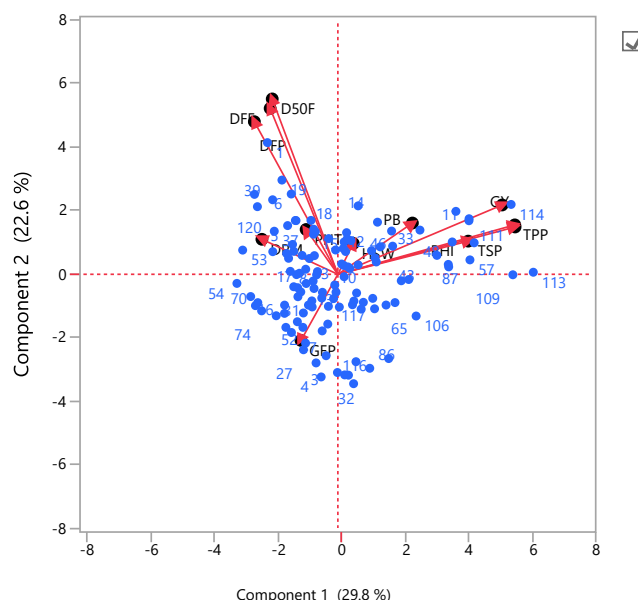
At Hyderabad, PCA biplot revealed that the associations of GY, TPP, TSP, and PHI traits were strongly positive and contributed the most to the phenotypic variability among the genotypes. These traits were negatively associated with DPM and D50F (Fig 5.3a and Appendix 5.1). At Kobo, strong and positive associations were found among the four phenological traits (DFF, D50F, DFP, DPM) as revealed by PCA biplot. GY, TPP, TSP and PHI were positively associated traits. GY and PHI were negatively associated with D50F (Fig 5.3b and Appendix 5.1). At Debre Zeit, positive and strong associations were found among GY, PHI, TSP and TPP traits as revealed by PCA biplot. In addition, DFP, DFF, and D50F were positively and strongly associated traits (Fig 5.3c and Appendix 5.1).



a. ICRISAT(Hyderabad, India)



b. Kobo



c. Debre Zeit

Fig 5.3. PCA biplots of twelve agronomic and phenological traits for two heat stressed (a, Hyderabad and b, Kobo) and a non-stressed environments (c, Debre Zeit). PCA, principal component analysis, DFF, days to first flowering, D50F, days to 50% flowering, DFP, days to first podding, GFP, grain filling period, DPM, days to physiological maturity, PHT, plant height, TPP, total number of pods per plant, TSP, total number of seeds per plant, HSW, hundred seed weight, PB, plant biomass, GY, grain yield, PHI, percentage of harvest index.

Under heat stressed environments, GY, PHI, TSP and TPP vectors had high weighting towards heat tolerant genotypes, i.e. important agronomic traits for the selection of genotypes for heat tolerance. Based on the two principal components, we observed more variability in the heat-stressed environments of Kobo (64%) and Hyderabad (59.1%), and less in the non-heat stressed environment of DZ (52.4%). The PCA biplots revealed the presence of genetic variability among the genotypes that scattered between highly heat tolerant and sensitive categories under heat stressed environments (Fig 5.3a, b).

Cluster analysis of 12 phenotypic traits at Hyderabad showed that 57 chickpea genotypes were categorized into 5 clusters. Cluster 5 contained only a highly heat tolerant line, DZ-2012-ck-0034 (Figs 5.4, 5.2g and Table 5.1), while cluster 1 contained heat tolerant genotypes including the standard heat tolerant check, ICCV 92944 and ICCV 7102 (Fig 5.4). Cluster 4 contained highly heat sensitive genotypes, DZ-2012-ck-0026 and ICC 4567, while genotypes categorized in cluster 3 were heat sensitive, which contained high plant biomass but did not set pod at all. Cluster 2 contained moderately heat tolerant to sensitive genotypes (Fig 5.4).

5.3.5. Genomic diversity and relationships

Based on sequencing of restriction site-associated DNA tags, a total of 5,722 SNPs were called from the sequence data of 54 chickpea genotypes which were used to determine LD, population structure and marker-trait associations. In addition, they were used to assess genomic diversity and relationships within populations, among populations and among individuals by using the genetic parameters namely Shannon diversity index (I), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), gene flow (Nm), F-statistics

(fixation index), principal coordinates analysis (PCoA), allelic frequency (A), allele number (Na) per locus, effective allele number (Ne) per locus, percentage of polymorphism (%P) and analysis of molecular variance (AMOVA).

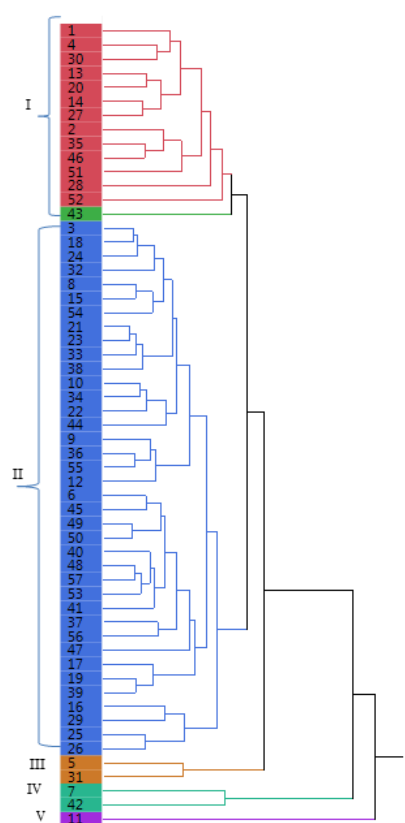


Fig. 5.4. Cluster analysis of 57 chickpea genotypes at ICRISAT, Hyderabad, India based on their phenotypic data. Clusters 5, 4, 3, 2 and 1 were identified by purple (V), light green (IV), orange (III), blue (II), green and red (I) colors in their respective order.

5.3.5.1. PCOA

By using 5,722 SNPs data, principal coordinate analysis (PCoA) revealed that the Ethiopian chickpea genotypes grouped into two populations: one big population with five clusters and a

smaller population that contained commercial cultivars. The percentage of genetic variation explained by coordinates 1 (16.03%) and 2 (4.95%) of the PCoA was 20.98% (Fig 5.5). The genomic relationship results from PCoA analysis were consistent with the results of population genetic structure analysis (Fig 5.6). The presence of high percentage of polymorphism (82%) within landraces might infer the existence of high expected heterozygosity ($H_e = 0.045-0.1$) (Appendix 5.2) among the landraces for heat tolerance. The second group, containing commercial cultivars, is interpreted as those with introgression from breeding lines outside Ethiopia.

Furthermore, to confirm the genomic relationships among the genotypes, two landraces (accessions 41110 and 212589) were chosen as an example. Accession 41110 was collected from an extremely lowland area, Amhara region, North Shewa zone, and grown at an altitude of 1220 mas. Accession 212589 was collected from Amhara region, South Wello zone, and grown at an altitude of 1600 mas. Cluster analysis based on phenotypic data (Fig 5.4) revealed that accessions 41110 and 212589 were categorized under a heat tolerant and a moderately heat tolerant genotypes, respectively (Fig 5.4). Similarly, based on the analysis of genomic relationships (PCoA), the 2 accessions were also grouped within separate clusters. Accessions 212589 and 41110 were grouped separately from most other accessions, as they are indicated by two arrows (Fig 5.5), indicating they are not closely related to each other. Besides, PCoA revealed that each pair of genotypes; EBI 209093 and EBI 209096, EBI 209109 and EBI 209017, and EBI 212589 and EBI 212478 was close to each other in their respective orders. The accession EBI 212478 was the most distant accession within population one and among

accessions followed by accessions EBI 209096, EBI 209093, EBI 212589, EBI 209109 and EBI 209017. EBI41108 was the closest accession to most of the genotypes.

5.3.5.2. Population structure

The population structure was investigated using 5,722 SNPs. The numbers of population groups were identified based on maximum likelihood values. The minimum and maximum values of K tested were $K = 2$ and $K = 10$, respectively. The number of runs and replicates were 53 and 6, respectively. The Evanno criterion gave the strongest signal for $K = 2$ and the weakest signal for $K = 9$. Based on the given sequence data, the optimal numbers of population in the 54 chickpea genotypes were estimated and grouped into two distinct populations. The group with green color in the figure consisted of 100% landrace accessions collected from different geographical regions and ecotypes. The group with red color in the figure consisted of released varieties. Out of the 54 genotypes, 52 (96.3%) of the genotypes were grouped into population 1 and the remaining parts (3.7%) were grouped into population 2 (Fig 5.6).

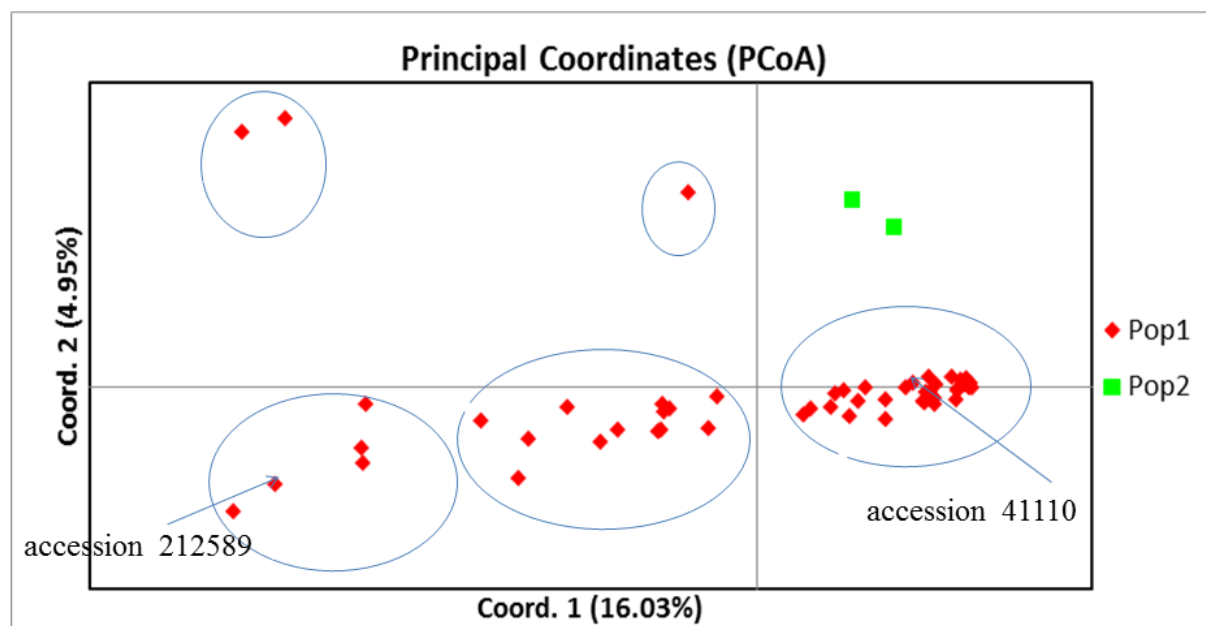


Fig. 5.5. Principal coordinates analysis (PCoA) of 54 chickpea genotypes based on pair wise genetic distance. The red color depicted pop1 (landraces) which was further divided into five clusters based on their genomic relationships and the green color depicted pop 2 (commercial cultivars). The two arrows confirmed genomic distinctions of the two landraces (212589 and 41110) as they were distinct in geographical origin and altitude.



Fig. 5.6. Population genetic structure diagram for 54 chickpea (*Cicer arietinum*) germplasm. The genetic clusters represented by vertical bar along the horizontal axis were categorized into two color segments (green, population 1 and red, population 2). The vertical axis indicated ancestry coefficient between the two genetic clusters.

5.3.5.3. Allelic number and frequency

Average allele frequency and major allele frequency for pop 1 were 0.499 and 0.97. Average allele frequency and major allele frequency for pop 2 were 0.50 and 0.86. Among 5,722 SNP loci, the percentage of polymorphic loci for population 1 (82.02%) was four fold greater than the polymorphic loci for population 2 (22.65%). For populations 1 and 2, allelic frequency varied from 0 to 1. Number of alleles per locus ranged from 1 to 3 and 0 to 2 for populations 1 and 2, respectively. Mean number of alleles per locus were 1.82 and 1.2 for populations 1 and 2, respectively. For population 1, out of 5,722 SNP loci; 81.9% of the loci had two alleles, 18% of the loci had single allele and 0.1% (572 SNPs) of the loci had three alleles per locus. The average number of effective allele per locus for population 1 (1.06) was less than population 2 (1.19) (Appendix 5.2).

5.3.5.4. Heterozygosity and Shannon diversity index

Observed heterozygosity (0.047), expected heterozygosity (0.103) and unbiased expected heterozygosity (0.138) of population 2 were greater than population 1 (H_o (0.03), H_e (0.045) and uH_e (0.045)). The expected and unbiased expected heterozygosity of population 1 and 2 varied from 0 to 0.5, respectively. The genetic divergence (F_{ST}) of individual accessions within population 1 (0.139) was less than population 2 (0.46). The genetic divergence between populations was 0.171. Shannon diversity index for populations 1 and 2 varied from 0 to 0.692 and 0 to 0.693, respectively. The mean Shannon diversity index for populations 1 and 2 were 0.091 and 0.146, respectively (Appendix 5.2).

5.3.5.5. Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) revealed that the percentages of molecular variance among individual samples (70%) was 6x and 4x greater than within population (13%) and among populations (17%) variances, respectively. There were very significant variations within populations and among individuals at $P \leq 0.001$. Significant difference was observed among populations ($P \leq 0.05$). Very high genetic variations were observed among individual genotypes than within and among populations (Fig 5.7 and Appendix 5.3).

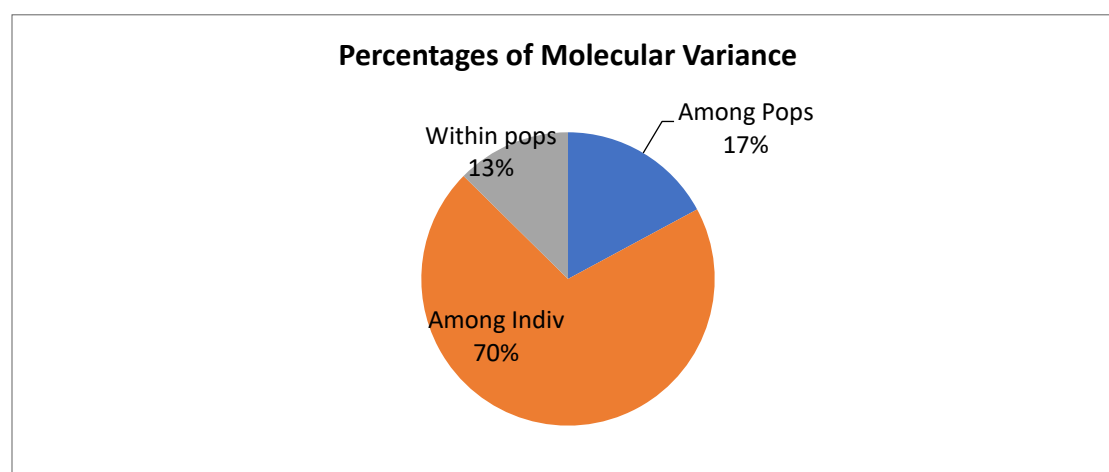


Fig. 5.7. Percentage of molecular variances among and within populations, and among individuals.

5.4. Discussion

5.4.1. Phenotypic responses of genotypes under heat-stress environments

Global warming assumed to be aggravated by human interference, is becoming a recurrent political agenda since recently and poses significant risks for and in many cases is already affecting a broad range of human and natural systems. Ambient temperatures are predicted to rise in the future owing to several reasons associated with global climate changes which cause a large shift in chickpea area from cooler long-season environments to warmer short-season environments. These temperature increases can result in heat stress that imposes challenges for chickpea and has deleterious effects on the morphology, physiology, and reproductive growth of the plants. It's effect has become severe at the reproductive stage. Therefore, global warming, coupled with increased temperatures in arid and semiarid regions has urged development of chickpea varieties that can sustain and yield high in harsh climate (Devasirvatham, 2012; Thudi *et al.*, 2014; Sita *et al.*, 2017).

Assessment of whole plant growth, flowering, pod sets and grain filling periods under warmer environments in the field are useful preliminary heat resilient screening parameters (Devasirvatham *et al.*, 2013; Gaur *et al.*, 2014; Jumrani and Bhatia, 2014; Krishnamurthy *et al.*, 2011; Summerfield *et al.*, 1984). In addition, planting time is a useful strategy to detect heat and drought tolerances in chickpea (Devasirvatham *et al.*, 2013; Gaur *et al.*, 2014; Tesfashbamlak Molla *et al.*, 2018). In order to adapt and produce economic yield under heat stress environments, chickpea plants might have been qualified with some genetic adjustment mechanisms besides adjusting the planting date that can be used as an important adaptation strategy to climate change (Wahid *et al.*, 2007).

At Hyderabad and Werer, our two high-temperature environments, we have found more than 20 heat sensitive genotypes that cannot flower at all and others that can flower but could not set pods. This is likely due to stress caused by high daily average temperature ($\geq 40^{\circ}\text{C}$) that could hinder flower initiations and induce sterility (Krishnamurthy *et al.*, 2011; Summerfield *et al.*, 1984; Upadhyaya *et al.*, 2011). Moreover, heat sensitive genotypes have shown delayed physiological maturity (Fig 5.2f) compared to heat tolerant genotypes under heat stressed environments. This indicates that the transition from vegetative to reproductive phase of the plants might have been affected by the heat stress resulting in poor allocation of assimilates from the leaves into pods and seeds (Krishnamurthy *et al.*, 2011; Upadhyaya *et al.*, 2011).

Grain yield (GY) and yield related traits were highly affected under all heat stressed environments compared to non-heat stressed environments. Particularly, at Hyderabad, there were high monthly average maximum/minimum temperatures all the way from germination ($35.7/24.1^{\circ}\text{C}$) to maturity ($39.7/26.5^{\circ}\text{C}$) (Fig 5.1a), and consequently the majority of the genotypes yielded very little and performed poorly in yield related traits (Table 5.2). Consistently, at Werer, most genotypes had poor pod sets, with reduced total pods and seeds per plant compared to non-stressed settings (data not shown). In agreement with the present study, Summerfield *et al.* (1984) found that the greater the proportion of the reproductive period occurs in hot days ($\geq 35^{\circ}\text{C}$), the lower the yields. Plants transferred to temperatures above 35°C at 50% flowering did not produce pods. The negative effects of heat stress on these traits might also be associated with the reduction in reproductive efficiency, with low levels of carbohydrates and growth regulators released in plant sink tissues and induced early maturity (Jumrani and Bhatia, 2014).

At Hyderabad, both the vegetative and reproductive stages were exposed to heat stress (monthly average temperatures $\geq 35.7^{\circ}\text{C}$) (Fig 5.1a). The effects of heat stress on the vegetative stages of chickpea were drying of leaves, shoots, branches, stems and whole plants, exhibited by stunted growth, delayed reproductive stages, yellowing of leaves, branches, flowers and pods, and decrease in the number of plants per plot (Fig 5.2c, d). This is because high temperatures might affect some of the physiological processes and might re-allocate some of the resources of chickpea genotypes to withstand the heat stress. A single highly heat tolerant genotype (DZ-2012-ck-0034) which possessed high pod set, TPP, TSP and GY was found under all heat stressed environments both in Ethiopia and India compared to the standard and local checks (Figs 5.2i, 5.3a,b, 5.4). In agreement with these findings, Devasirvatham (2012), Tesfashbamlak Molla *et al.* (2018) and Upadhyaya *et al.* (2011) have found very few heat tolerant chickpea genotypes under high heat stressed conditions.

In the present study, ANOVA revealed highly significant differences ($P \leq 0.001$) among the genotypes for most traits under stressed and non-stressed environments. In line with this, Devasirvatham (2012), Kanouni *et al.* (2009), Krishnamurthy *et al.* (2011), Saeed and Darvishzadeh (2017) and Upadhyaya *et al.* (2011) reported significant differences ($P \leq 0.01$) among chickpea genotypes for most of the traits they studied under stressed and non-stressed conditions. Our results suggest that there are heat tolerant accessions available in Ethiopian germplasm that can be harnessed in breeding efforts, addressing this increasing abiotic challenge.

5.4.2. Performance of variance components under heat stressed environments

In the current study, environmental, genotypic and phenotypic variances became higher for GY and yield related traits among genotypes at Kobo than at Hyderabad. Geographic location, altitude, the extent of heat stress, and the composition and number of genotypes might account for this difference. In agreement with this study, wide phenotypic variations were detected for GY and yield related traits among 291 chickpea accessions (Basu *et al.*, 2019). Various authors have observed phenotypic, genotypic, and GxE interactions for traits studied in chickpea genotypes (Krishnamurthy *et al.*, 2011; Saeed and Darvishzadeh, 2017; Paul *et al.*, 2018; Upadhyaya *et al.*, 2011). This study also found large coefficients of phenotypic and genotypic variances for GY and yield related traits in all heat stressed environments than non-stressed environment; this is because the trait value of individual genotype had large deviations from the mean value of the trait as depicted by PCA biplot analysis (Fig 5.3a, b) than the non-stressed environment (Fig 5.3c). These results were in agreement with the reports of Paul *et al.* (2018), where they found a high genotypic coefficient of variation and phenotypic coefficient of variation for traits assessed under heat stressed environments. Like other stresses, high temperature conditions may cause greater phenotypic variation than less stressful environments that has been noted in natural populations (e.g., Stanton *et al.*, 2000). Further investigation of the impacts of multiple stresses on phenotypic variation in chickpea germplasm is needed.

5.4.3. Genomic relationship and diversity and their patterns of distribution

The present study used 5,722 genome wide SNPs data to determine genome-wide relationships and patterns of distribution among 54 chickpea genotypes. In the landrace group (population 1), out of 5,722 SNP loci; 572 SNPs (0.1%) of the loci had three alleles per locus.

The landraces of chickpea in Ethiopia had higher genetic variation ($H_e = 0.045-0.1$) compared to the genetic diversity ($H_e = 0.02-0.04$) of landraces of chickpea in India, Iran and Pakistan as reported by Sani *et al.* (2018). This might be important for agro-climatic adaptation in their respective agro-ecological zones. In line with this, Sani *et al.* (2018) reported that with this low amount of genetic diversity (0.02) in Pakistan, landraces of chickpea have longstanding cultivation histories under heat and drought environmental conditions. Penmetsa *et al.* (2016) reported that cultivated chickpea accessions had low genetic diversity ($H_e = 0.065$) compared to the wild species, *Cicer reticulatum* ($H_e = 0.332$) and *Cicer echinospermum* ($H_e = 0.301$), using a set of SNPs from a GoldenGate assay. Similarly, in the present study, the genetic variation among 52 chickpea genotypes ($H_e = 0.1$) is approximately 3x less than the genetic diversity of these wild species ($H_e = 0.30-0.33$).

Sani *et al.* (2018) revealed low genome wide differentiation among Pakistan chickpea landraces even though significant relationships were found between genetic, geographic locations and climatic factors. Similarly, in this study, our PCoA analysis categorized 54 chickpea genotypes into two groups, desi landraces (52) and commercial cultivars (2, Habru and Minjar) (Fig 5.5). In line with this idea, in the present study, PCoA (Fig 5.5) confirmed the analysis of population genetic structure (5.6). In the same scenario, 66 (Kebede Teshome, 2012) and 93 (Kujur *et al.*, 2015) chickpea accessions were categorized into three distinct populations: wild, kabuli and desi; 291 chickpea accessions were categorized into two major populations, desi and kabuli (Basu *et al.*, 2019). Likewise, Penmetsa *et al.* (2016) reported that 247 chickpea accessions were clustered into three populations ($K=3$) namely desi, kabuli and wild.

Importantly, we found that the few heat tolerant genotypes in our phenotypic study did not cluster closely together, suggesting that they are not closely related. This means that this critical trait likely segregates across the Ethiopian genepool. It also indicates that different tolerant accessions may do so by different genetic mechanisms. If this proves to be the case, crossing these accessions could create new varieties with superior heat tolerance. Stacking heat tolerance genes will help increase the climatic resilience of this important crop.

Fischer *et al.* (2017) suggested that analysis of genetic diversity within and among populations by using randomly selected SNP data was used to draw conclusions on genome wide diversity patterns. Similarly, the present study used 5,722 representative and high quality genome wide SNPs data to determine AMOVA that can estimate genome wide diversity within and among chickpea subpopulations. AMOVA among individual samples (70%) was greater than within (13%) and among (17%) populations. Similar results were reported in chickpea (Saeed and Darvishzadeh, 2017), cowpea (Fatokun *et al.*, 2018) and sesame (Cui *et al.*, 2017). A contrary result was reported from sorghum accessions; AMOVA revealed that 51.6%, 25.1% and 23.3% of the variation was attributed to within population, among individuals and populations (Chen *et al.*, 2017). Breeding system might account for the similarities and differences of AMOVA in crop plants.

In the present study, the FIS and FIT values of chickpea accessions were 0.848 and 0.874, respectively. The inbreeding nature of chickpea accessions and the very low levels of diversity in the Ethiopian genepools account for the higher values of FIS and FIT. A similar report was found in cowpea lines, the FIS and FIT values were 0.78 and 0.82, respectively indicating that

the cowpea lines making up the groups are inbred lines (Fatokun *et al.*, 2018). In this study, the genetic divergence (FST) of individual accessions within population 1 (0.139) was less than population 2 (0.46), because the later composed of kabuli and desi varieties. A pair wise genetic divergence among the two chickpea populations was 0.171. This low value of genetic differentiation agreed with other findings. FST value among groups of cowpea lines (Fatokun *et al.*, 2018), between clusters in peanut accessions (Zheng *et al.*, 2018) and among sesame subgroups (Cui *et al.*, 2017) were 0.16, 0.284 and 0.15, respectively. FST values among chickpea accessions (Kujur *et al.*, 2015), in prunus (Campoy *et al.*, 2016), *Arabidopsis* (Fischer *et al.*, 2017) and grape cultivars and its wild relatives (Emanuelli *et al.*, 2013) ranged from 0.30 to 0.62, 0.043 to 0.142, 0.02 to 0.09 and 0.07 to 0.12, respectively. In breeding populations shared such low values of FST.

5.5. Conclusions and implications to improve chickpea for heat tolerance environments

Choosing appropriate experimental field sites and planting dates under heat stressed environments and characterizations of phenological and agronomic traits are crucial to select and identify heat tolerant genotypes. Understanding genome-wide relationships among genotypes via SNP markers helps to assess the clustering patterns of genotypes and their relationships. Heat stress is a function of plant genotypes, high temperatures, plant phenology and soil types. The experimental materials we have examined demonstrate that the production range of chickpea can be stretched in environmental heat gradients and using existing trait variability. Our field observations revealed that heat stress ($\geq 35^{\circ}\text{C}$) mainly affects the reproductive stages. If plant germination occurs at high temperatures ($\geq 35^{\circ}\text{C}$), heat stress has negative effects on vegetative stages of chickpea genotypes. Grain yield, total number of pods and seeds per plant, and pod set

are the best traits for screening of heat tolerant and sensitive genotypes under heat stressed environments. A total of 5,722 genome-wide SNPs were used to determine genome-wide relationships among 54 chickpea genotypes. PCoA has found five different clusters within the landrace group. Each genomic cluster might possess specific adaptive genes to their respective geographical locations. The genotypes that showed better performance in this experiment can be used in crossing blocks to improve the productivity of chickpea in high temperature agro-ecological regimes.

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CHAPTER 6: Physiological mechanisms of heat tolerance in chickpea through high throughput phenotyping

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Abstract

Changes in physiological responses under heat stressed conditions are important to understand the thermo-tolerance mechanisms of chickpea (*Cicer arietinum* L). The objective of the present study is to understand the physiological mechanisms of heat tolerance in chickpea. At the reproductive developmental stages, a total of 13 physiological parameters were measured for 47 chickpea accessions that were grown from February to May, 2019 under a heat stressed environment, Werer (Ethiopia), using a high throughput phenotyping tool (MultispeQ). Based on the physiological responses of chickpea accessions under heat stressed environment, it was confirmed that DZ-2012-ck-0034 and JG 14 were found to be the most heat tolerant, while DZ-2012-ck-0026 and ICC 4567 were the most heat sensitive genotypes. Heat tolerant genotypes exhibited high mean values for effective quantum yield of photosystem II (Phi2), chlorophyll fluorescence decline ratio (RFd), coefficients for photochemical quenching (qP and qL), chlorophyll fluorescence (Fv/Fm), pod set and low mean values for regulated (PhiNPQ) and non-regulated heat dissipations (PhiNO). On the contrary, the sensitive genotypes had low mean values for Phi2, RFd, qP, qL, and high mean values for PhiNPQ and PhiNO. This clearly indicated that the absorbed photons, which were dissipated as heat energy by the sensitive genotypes (70%), were two fold greater than the tolerant genotypes (32-40%). Consequently, heat stress severely damages the photosynthetic systems of sensitive genotypes thereby the pod set and other yield related traits decreases. In both reproductive stages, SPAD (chlorophyll reading values), Phi2, qP and qL were significantly different ($P \leq 0.05$) among genotypes. Phi2 was negatively correlated with PhiNO and PhiNPQ; however, it was positively correlated with Fv/Fm, SPAD, LEF, RFd, qP and qL traits. Out of 13 physiological traits, 7 traits such as Phi2, NPQt (estimate of photochemical quenching), RFd, PhiNO, PhiNPQ, qL, and qP were highly

correlated traits and had high broad sense heritability and genetic gain. The present study revealed that the aforementioned seven physiological traits might enhance the efficiency and accuracy of physiological breeding in chickpea for screening and identifying of tolerant genotypes under drought and heat stresses.

Keywords: Chickpea, Heat stress environment, MultispeQ, physiological responses,
Physiological traits.

6.1. Introduction

Chickpea (*Cicer arietinum*) is one of the most economically important food legumes, and a significant source of proteins. It is cultivated in more than 50 countries across Asia, Africa, Europe, Australia, North America, and South America. Chickpea production is limited by various abiotic stresses namely cold, heat, drought, salt, acidity and others. In late-sown environments, chickpea faces high-temperature stress ($\geq 35^{\circ}\text{C}$) during reproductive and pod filling stages, causing considerable yield losses (Rani *et al.*, 2020). Heat stress is a temperature that causes irreversible damage to plant function or development (Hemantaranjan *et al.*, 2014). It is a function of plant genotype, high temperature, water status and soil type. A predicted increase of 1-3 degrees in the twenty first century urges breeders to develop thermo-tolerant crops. Physiological breeding for thermo-tolerance is a challenge due to the low heritability of physiological traits (Paupière *et al.*, 2014).

Changes in various physiological parameters under heat stressed conditions are good indicators of thermo-tolerance of plants. Photosynthesis is the most sensitive physiological process to high temperature that is responsible for yield of a crop (Wahid *et al.*, 2007) and survival of a crop. Heat stress disrupts the structure and function of chloroplasts and reduces chlorophyll contents. The three major heat-sensitive sites in photosynthetic machinery are the photosystems, mainly photosystem II with its oxygen-evolving complex (OEC), the ATP generating processes in thylakoid lamellae, and carbon assimilation processes in stroma of chloroplast (Wise *et al.*, 2004; Srinivasan *et al.*, 1996). According to the bipartite model for photosystem presented by Butler (1978), when a pigment absorbs a photon three things can happen. The photon's energy can be dissipated as heat (non-photochemical quenching); released as fluorescence; or channeled

through photosynthesis (photochemical quenching, electrons moved into an excited state) (Maxwell and Johnson, 2000). Photosystem II is involved firstly, leads to an accumulation of protons within the thylakoid space coming from partly the hydrolyze of water molecules (H_2O), and oxygen molecules are released. An ATP synthase uses the gradient of protons to synthesize ATP molecules released within the stroma. Electron transferred through the photosystem I is finally caught by the $NADP^+$ molecules that reduced into NADPH (Baker *et al.*, 2007).

The MultispeQ instrument is an open-design plant phenotyping tool designed to work in combination with the open science PhotosynQ platform (<https://photosynq.org>) as a step towards overcoming current limitations such as cost, usability, adaptability, integration of multiple sensors, data sharing and accessing. Connecting the MultispeQ instrument using smart-phone applications via Bluetooth provide a user-friendly interface allowing even novice users to collect high-quality plant phenotyping data (Fig 6.1) (Kuhlgert *et al.*, 2016). MultispeQ is an instrument in Photosynq that is used to measure photosynthetic functions such as net photosynthesis, chlorophyll concentration, photosynthetic efficiency, photosynthetically active radiation, photochemical quenching, linear electron flow, non-photochemical quenching, coefficients of photochemical quenching, and chlorophyll fluorescence (Kuhlgert *et al.*, 2016; Fernández-Calleja *et al.*, 2020).

This instrument is ideal for the study of different stresses, drought and heat stresses, under controlled and field conditions. Drought stress had an important effect on photosynthetic efficiency, energy allocation, and crop status as indicated by the measurements derived from the MultispeQ device (Fernández-Calleja *et al.*, 2020). The authors revealed that the energy allocation ratios depend up on environmental conditions, type of genotypes, colored lights,

photosynthetic pathways, the day from morning to late afternoon, and the color of the leaves. The MultispeQ focuses on seven main measurements such as Phi2, PhiNPQ, PhiNO, qP, qL, LEF, and NPQ.

Phi2 (effective quantum yield of photosystem II or photochemical quenching) measures the amount of incoming light (excited electrons) that the plant actually uses for photosynthesis. Photosystem II is where most light energy is converted into food. PhiNPQ (non-photochemical quenching) can be thought of as “sunblock” that measures the amount of incoming light that is dissipated as heat or other energy in a number of regulated ways so that the plant is not damaged. PhiNO (non-regulated heat dissipation) can be thought of as “sunburn” that measures the amount of incoming light that is used for neither photosynthesis nor regulated heat dissipation, effectively causing potential damage to the leaf. It is the combination of a number of unregulated processes whose by-products can inhibit photosynthesis or be harmful to the plant. LEF (linear electron flow) can be considered approximate measurement of photosynthesis; how much energy is being moved around in the chloroplasts following exposure to light. SPAD measures relative chlorophyll content and can be used as an indicator of plant nitrogen as well as an indicator of plant stress (Kuhlgert *et al.*, 2016).

Singh *et al.* (2020) reported that the tolerant maize genotype maintained high chlorophyll contents than heat sensitive maize genotype. Similarly, when chickpea plants exposed to high temperatures (27/18°C-42/25°C) under control conditions, sensitive chickpea genotypes (ICC16374, ICC10685, and ICC5912) had lower leaf chlorophyll contents than tolerant genotypes (JG14 and ICC15614) (Kaushal *et al.*, 2013; Parankusam *et al.*, 2017). Under heat

stressed conditions ($\geq 35^{\circ}\text{C}$), higher level of total chlorophyll content and SPAD value were observed in heat tolerant sugarcane compared to heat susceptible genotypes (Kohila and Gomathi, 2018). Heat tolerant mungbean had significantly higher SPAD readings compared to susceptible genotypes (Patriyawaty *et al.*, 2018). In lentil, at $38/28^{\circ}\text{C}$ - $40/30^{\circ}\text{C}$, the heat sensitive genotypes exhibited 40-50% more chlorophyll loss than heat tolerant ones (Sita *et al.*, 2017).

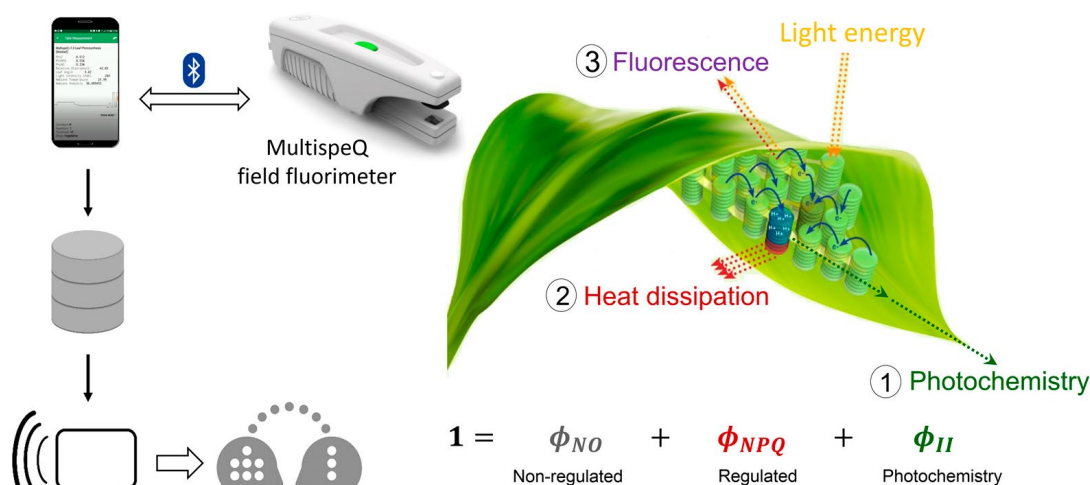


Fig 6.1. A picture that depicts how the MultispeQ (PhotosynQ) works in crop plants (Fernández-Calleja *et al.*, 2020).

Chlorophyll fluorescence can give insights into the ability of a plant to tolerate environmental stresses (Fernández-Calleja *et al.*, 2020). Correlation among different physiological traits, and between yield related traits and physiological traits were reported by several authors (Fernández-Calleja *et al.*, 2020; Kohila and Gomathi, 2018; Kadir and von Weihe, 2007; Kaushal *et al.*, 2013). A strong negative association of effective quantum yield of PSII was observed with grain yield under heat stress. Higher values of effective quantum yield (Φ_2) have been correlated

with a higher proportion of open reaction centers. The higher values of qP and qL found under water stressed condition could be a response to lower light intensity during the sampling (Fernández-Calleja *et al.*, 2020).

Chlorophyll fluorescence is used to determine the effects of environmental stresses on photosynthesis in plants exposed to adverse conditions. The tolerant genotypes had higher photosynthetic rates and grain yield than the sensitive genotypes at the warmer site. There was high photosynthetic efficiency in heat tolerant (F_v/F_m , 0.57) than sensitive genotype (F_v/F_m , 0.46) (Kaushal *et al.*, 2013). Drought tolerant genotypes had higher LEF, which correlates with the larger qP under water stressed conditions (Fernández-Calleja *et al.*, 2020). Changes in the fluorescence yield reflect changes in photochemical efficiency and heat dissipation. Low F_v/F_m values in plants under stress indicate damage to the PSII reaction center, a low in effective quantum efficiency and a higher non-photochemical quenching of PSII (Kadir and von Weihe, 2007).

Physiological mechanisms of heat tolerance using this high throughput phenotyping were well studied in tomato, maize, and wheat globally. Although traditionally, leaf photosynthetic properties have been measured using slow and laborious gas exchange methods, they are unfit for testing the large populations managed in chickpea breeding programs. An accurate high-throughput phenotyping, especially under field conditions, is still a limiting factor for breeding of water and heat stress-tolerant varieties. Therefore, the objectives of the present study were to understand the physiological mechanisms of heat tolerance in chickpea and to identify the most

important physiological parameters, which are useful for physiological screening of heat tolerant genotypes in chickpea breeding.

6.2. Materials and methods

6.2.1. Plant materials, experimental design and field management

A total of 23 landraces, lines, cultivars and standard checks and 24 F₅ interspecific lines derived from the crosses of cultivated chickpea with *C. reticulatum* and *C. echinospermum* were used in this study. All the germplasm accessions were grown at Werer experimental site in 2019 using an alpha lattice design with two replications. Irrigation and weeding were manually managed. Intra and inter row spacing was 10 cm and 30 cm, respectively. The plot size was 1m x 0.6m and 20 seeds per accession were planted into two rows. PhotosynQ connects to a sophisticated handheld instrument (MultispeQ v1.0) to a web based platform (www.photosynq.org) to store, visualize, map, and share plant health data. Almost the developmental stages of the chickpea accessions have exposed to heat stress at daily maximum and minimum temperatures that range from 34.2 to 44°C and 13.6 to 26.6°C, respectively. The daily maximum and minimum temperatures of the reproductive stages range from 35.4 to 40°C and 17.2 to 24.4°C, respectively, which was higher, compared to the vegetative stage (Fig 6.2).

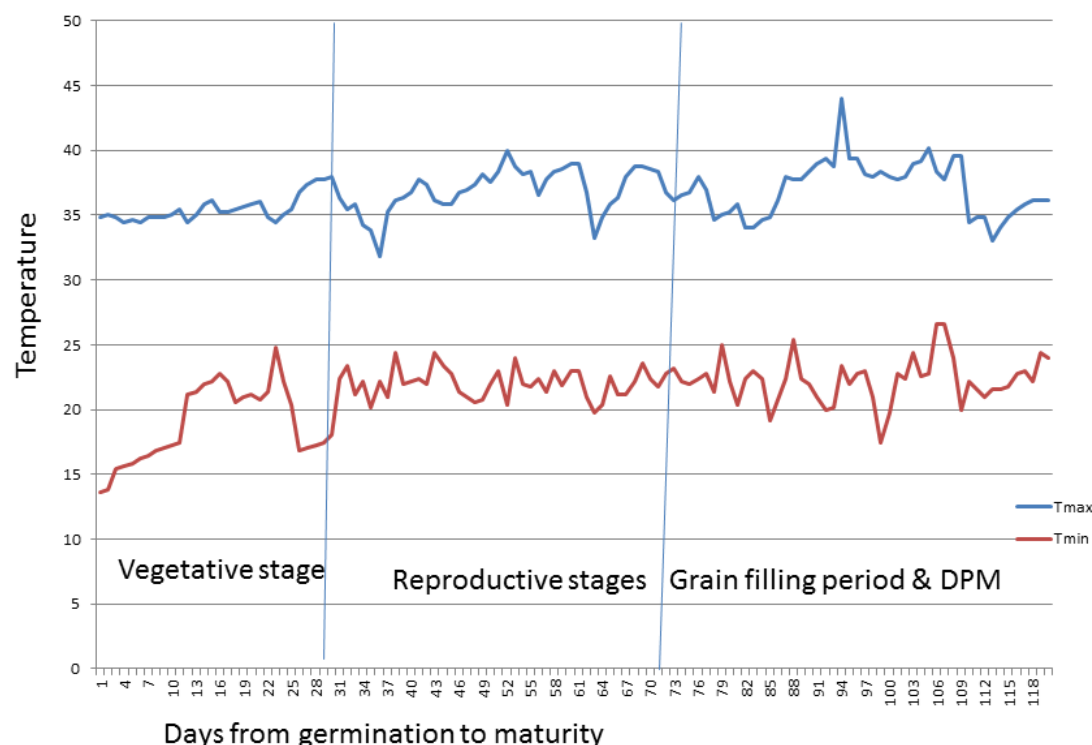


Figure 6.2. The daily minimum and maximum temperatures of Werer field site (February to May, 2019) from germination to maturity. TMax, maximum temperature, Tmin, minimum temperature, DPM, days to physiological maturity.

6.2.2. Measurement of physiological parameters

Physiological parameters were measured from 9:00 to 11:00 hr with MultispeQ. This instrument combines a fluorimeter, a chlorophyll meter, and a spectrometer into one. The MultispeQ device was connected via Bluetooth with the Photosynq application installed on a smartphone (Fig 6.1). The data collected in the field was sent to the cloud, and then the users can use analysis tools, discuss and share the data in the web interface. The PhotosynQ project connects the MultispeQ instrument to a cloud-based database for global sharing of plant health data including drought. The MultispeQ is a sophisticated, inexpensive, and non-destructive plant photosynthesis meter that measures key chlorophyll fluorescence, absorbance and absorbance kinetics parameters. The

resulting data from smartphones via Bluetooth connection to MultispeQ were connected to the PhotosynQ platform (www.photosynq.org) facilitating the generation of large data sets useful to collaborators worldwide. These big data sets can be further analyzed for quantifying additional responses by plants.

The “MultispeQ v1.0, leaf photosynthesis (Masked)” protocol (<https://photosynq.org/>) was run to measure environmental variables, chlorophyll fluorescence yield changes, and light-induced absorbance changes. The associated macro was applied to calculate photosynthetic efficiency and crop status-related parameters based on the measurements taken. Using the “MultispeQ v1.0”, the measurement automatically started once the leaf was clamped. A single leaf measurement took about 8 seconds, and twice that time to complete the whole operation until clamping the next plant (Fernández-Calleja *et al.*, 2020). Physiological parameters measured by MultispeQ protocol were ambient humidity and temperature, leaf temperature differential, linear electron flow ($LEF = \Phi_2 \times PAR \times 0.45$), light Intensity (PAR), NPQt, Φ_2 , Φ_{NO} , Φ_{NPQ} , leaf thickness, leaf angle, chlorophyll content (SPAD value), RFd (chlorophyll fluorescence decline ratio), chlorophyll fluorescence (F_v/F_m), and coefficient of photochemical quenching (q_L and q_P) (Kuhlgert *et al.*, 2016). These data were taken twice, at first days of flowering (DFF) and days to 50% flowering (D50F) to podding (DFP) stages.

6.2.3. Data analysis

All physiological traits are considered as dependent variables; however, genotype, block, and replication were taken as independent variables. ANOVA was computed based on a mixed linear additive model, using the GLM (General Linear Model) procedure of the SAS 9.0 software for a

single location (Gomez and Gomez, 1984). The total variation (σ^2_p) for each trait was partitioned into variance components: genotypic (σ^2_g) and environmental (σ^2_e) variances. These variance components, coefficients of variation and genetic advance (GA) per location were determined according to Singh and Chaudhary (1985). Broad sense heritability (H_b^2) was estimated as suggested by Ekebil *et al.* (1977). Physiological data were subjected to statistical analysis such as raw data means, ANOVA, variance components (univariate), and bivariate analysis (correlation) using SAS 9.0 software (SAS Institute, 2002).

6.3. Results

6.3.1. Physiological responses under heat stressed environment

Both at days to first flowering (DFF) and days to 50% flowering (D50F) to days to first podding (DFP) stages, heat tolerant genotypes such as JG14, DZ-2012-ck-0034, 41110, 209110, 9xCudiB_022C, 9xOyali_084, 9xBesev_079, 9xKalkan_064, 9xKesen_075 and 9xSavur_063 contained higher mean values for effective quantum yield of photosystem II (Φ_2), q_L (coefficient of photochemical quenching based on lake model), q_P (coefficient of photochemical quenching based on puddle model), chlorophyll fluorescence (F_v/F_m), and RFd (chlorophyll fluorescence decline ratio) traits than sensitive genotypes such as ICC 4567, 41114, 41117 and DZ-2012-ck-0026. Heat tolerant and sensitive genotypes showed higher and lower mean values for the above mentioned physiological traits at the D50F to DFP stage compared to their corresponding mean values at the DFF stage, respectively (Table 6.1).

The heat tolerant genotypes contained lower mean values for both heat dissipation traits, Φ_{NPQ} (regulated heat dissipation) and Φ_{NO} (non-regulated heat dissipation), than sensitive genotypes. For example, in tolerant genotypes (JG14 and DZ-2012-ck-0034), 60-68% of the absorbed photons were used to carry out photochemical reactions (photosynthesis) and 32-40% were lost as regulated and non-regulated heat at D50F to DFP stage. In sensitive genotypes (ICC 4567 and DZ-2012-ck-0026), approximately 30% and 70% of the absorbed photons were used to carry out photochemical reactions and lost as heat, respectively. Heat tolerant genotypes contained very low leaf thickness than sensitive genotypes. Out of 13 physiological traits, seven were the most important physiological traits further used for screening of heat tolerant and sensitive chickpea genotypes (Table 6.1).

The mean values of SPAD reading, linear electron flow (LEF) and photosynthetically active radiation (PAR) of most genotypes at D50F to DFP stage were lower than their corresponding mean values at the DFF stage. The minimum mean physiological efficiency of wild introgressed segregating populations (0.23) was twice greater than the minimum mean values of fixed genotypes (0.12) at the reproductive stage. The mean values of each genotype for seven physiological traits (Table 6.1) confirmed the results found by agro-morphological screening approach.

Table 6.1. Mean values of 13 physiological traits of each genotype at days to first flowering and days to 50% flowering to days to first podding stages.

Days to first flowering stage for landraces, lines and varieties

Genotype	SPAD	Phi2	PhiNPQ	PhiNO	NPQt	qL	Fv/Fm	qP	LEF	RFd	PAR	LA	LT
41110	106.1	0.36	0.36	0.28	1.29	0.26	0.68	0.53	296.5	0.56	1844.5	14.0	0.45
41037	101.3	0.25	0.49	0.25	1.96	0.21	0.62	0.41	218.8	0.34	1913	10.5	0.12
41052	108.9	0.27	0.33	0.41	0.97	0.16	0.72	0.38	197.3	0.37	1661.5	14.7	0.55
41114	103.1	0.49	0.26	0.25	1.17	0.39	0.70	0.67	154.7	1.19	994	10.3	0.29
41117	110.4	0.42	0.27	0.31	0.89	0.28	0.72	0.58	225.0	0.73	1217.5	21.0	0.24
41144	108.8	0.29	0.47	0.24	1.91	0.24	0.63	0.46	230.7	0.41	1766	6.5	0.25
207673	104.9	0.41	0.26	0.33	0.79	0.25	0.73	0.56	133.6	0.69	727	7.1	0.42
207657	100.0	0.32	0.39	0.3	1.63	0.23	0.66	0.47	197.8	0.47	1431	23.9	0.19
209102	109.3	0.42	0.33	0.25	1.33	0.35	0.68	0.62	247.6	0.74	1313.5	16.8	0.15
209109	125.8	0.31	0.41	0.28	1.49	0.23	0.66	0.47	217.5	0.45	1561	16.4	0.14
209016	91.1	0.53	0.21	0.26	0.79	0.42	0.73	0.73	241.5	1.14	1009	15.7	0.21
209110	112.6	0.36	0.40	0.24	1.69	0.32	0.64	0.56	232.2	0.58	1482	13.8	0.14
212589	102.9	0.39	0.35	0.26	1.36	0.32	0.67	0.58	250.9	0.66	1465.5	10.1	0.18
235394	99.2	0.375	0.36	0.27	1.37	0.29	0.67	0.56	262.9	0.60	1559.5	12.5	0.21
ICC 4567	105.5	0.379	0.29	0.32	0.96	0.25	0.71	0.53	271.9	0.62	1611.3	16.7	0.91
JG 14	117.2	0.44	0.33	0.24	1.39	0.39	0.67	0.65	148.3	0.80	847	10.4	0.095
ICCV96029	113.8	0.17	0.36	0.47	0.76	0.08	0.74	0.24	138.2	0.21	1782	10.3	0.24
DZ-2012-ck-0026	95.5	0.38	0.40	0.22	1.81	0.36	0.64	0.61	186.7	0.63	1079	5.1	0.135
DZ-2012-ck-0034	103.4	0.43	0.29	0.29	1.01	0.3	0.71	0.59	218.9	0.78	1265	9.7	0.18
DZ-2012-ck-0074	104.8	0.44	0.26	0.3	0.87	0.32	0.72	0.60	167.5	0.88	1072	3.6	0.28
DZ-2012-ck-0230	130.7	0.51	0.21	0.28	0.74	0.37	0.74	0.69	203.4	1.04	885	8.36	0.15
Habru	134.3	0.39	0.37	0.24	1.56	0.33	0.659	0.59	235.8	0.67	1424.5	14.9	0.21
Minjar	114.3	0.34	0.41	0.26	1.57	0.27	0.655	0.51	281.9	0.50	1870.5	6.7	0.17

Days to 50% flowering to days to first podding stage for landraces, lines and varieties

41110	112.9	0.46	0.27	0.28	0.94	0.35	0.72	0.62	102.7	1.1	594.5	27.9	0.45
41037	96.1	0.36	0.33	0.31	1.09	0.24	0.70	0.52	286.2	0.56	1766	3.5	0.35
41052	103.7	0.50	0.23	0.27	0.85	0.38	0.73	0.69	170.7	1.01	763	10.9	0.37
41114	106.7	0.16	0.58	0.267	0.59	0.44	0.76	0.77	118.7	1.4	457	5.5	0.3
41117	57.2	0.12	0.40	0.48	0.87	0.05	0.72	0.16	97.2	0.13	1017	4.2	0.15
41144	85.5	0.25	0.53	0.22	3.44	0.24	0.56	0.43	197.3	0.36	1742	12.8	0.18
207673	94.4	0.17	0.51	0.33	1.55	0.10	0.66	0.26	145.2	0.20	749	9.5	0.1
207657	91.2	0.33	0.43	0.24	1.81	0.29	0.63	0.526	287.6	0.5	1212	5.5	0.09
209102	106.1	0.34	0.41	0.25	1.66	0.29	0.65	0.528	199.6	0.53	1346	19.3	0.21
209109	118.5	0.17	0.74	0.09	7.99	0.38	0.35	0.49	144.5	0.21	1870	8.9	0.08
209016	100.4	0.41	0.33	0.265	1.23	0.33	0.69	0.58	202.5	0.898	1318.8	22.9	0.19
209110	88.7	0.73	0.03	0.241	0.11	0.62	0.81	0.899	65.6	2.74	199	6.5	0.13
212589	103.3	0.33	0.42	0.24	1.75	0.30	0.64	0.51	136.0	0.56	1365.5	10.3	0.3
235394	108.5	0.43	0.38	0.19	2.68	0.42	0.62	0.64	105.0	1.14	1203.3	12.3	0.37
ICC 4567	101.2	0.29	0.47	0.23	2.59	0.23	0.61	0.43	137.7	0.57	1290	11.9	0.58
JG 14	109.7	0.68	0.08	0.25	0.29	0.56	0.79	0.85	62.98	2.19	329.5	14.9	0.29
DZ-2012-ck-0034	107.5	0.60	0.09	0.30	0.31	0.33	0.73	0.61	185.5	1.03	711	16.7	0.18

DZ-2012-ck-0074	111.3	0.45	0.24	0.31	0.79	0.41	0.79	0.77	163.8	1.5	734.3	9.6	0.35
DZ-2012-ck-0230	122.2	0.49	0.2	0.31	0.64	0.33	0.75	0.66	214.1	0.97	967	16.4	0.86
Habru	105.5	0.50	0.15	0.35	0.44	0.30	0.77	0.65	148.9	1.02	656	13.7	0.44
Minjar	108.2	0.35	0.27	0.39	0.69	0.19	0.74	0.467	174.5	0.53	1120	12.6	0.33

Days to 50% flowering to days to first podding stage for a segregating family (F₅)

9xBari1_092	123.9	0.37	0.37	0.26	1.4	0.29	0.67	0.55	258.6	0.59	1556	43.4	0.1
9xBari2_072n2	115.9	0.39	0.28	0.32	0.89	0.26	0.72	0.55	183.9	0.67	1067.8	13.9	0.20
9xBari3_106D	120.9	0.34	0.37	0.27	1.4	0.25	0.68	0.50	171.8	0.58	1188	16.7	0.319
9xBesev_079	103.5	0.51	0.14	0.34	0.42	0.31	0.78	0.66	219.7	1.07	964.5	6.1	0.315
9xCudiA_152	80.8	0.23	0.33	0.45	0.73	0.10	0.74	0.31	209.9	0.29	2069	3.1	0.07
9xCudiB_022C	100.6	0.58	0.15	0.27	0.56	0.44	0.76	0.76	94.2	1.61	403.5	44.5	0.16
9xDerei_070	120.9	0.377	0.35	0.28	1.25	0.28	0.68	0.55	221.5	0.60	1307	19.6	0.31
9xEgill_065	110.7	0.399	0.15	0.33	0.45	0.18	0.79	0.51	99.2	0.66	552	4.03	0.44
9xEgill_073	88.1	0.33	0.35	0.32	1.12	0.21	0.7	0.47	187.6	0.52	1392	5.4	0.14
9xKalkan_064	114.0	0.41	0.32	0.29	1.18	0.26	0.69	0.53	153.7	0.62	942.3	10.9	0.31
9xKesen_075	101.8	0.40	0.31	0.28	1.14	0.29	0.698	0.56	249.7	0.76	1571.1	16.9	0.18
9xOyali_084	102.8	0.52	0.28	0.21	1.31	0.50	0.679	0.76	47.7	1.06	206	27.3	0.16
9xSavur_063	103.7	0.43	0.29	0.29	1.02	0.32	0.71	0.59	165.1	0.89	963.9	12.9	0.17

RFd, chlorophyll fluorescence decline ratio; Fv/Fm, chlorophyll fluorescence; Phi2, effective quantum yield of photosystem II;

PhiNO, the portion of light lost as non-regulated way; NPQ, non-photochemical quenching; PhiNPQ, the portion of light lost as

regulated way; PAR, photosynthetically active radiation; LA, leaf angle; LT, leaf thickness; qP, coefficient of photochemical

quenching using puddle model; qL, coefficient of photochemical quenching using lake model; SPAD, The soil plant chlorophyll

meter; %PS, percentage of pod set.

Based on the mean values of all genotypes, photochemical quenching (Φ_2) were higher than non-regulated heat dissipation (Φ_{NO}) and non-photochemical quenching (Φ_{NPQ}) in both reproductive stages. For landraces, lines and varieties, the mean Φ_2 value at the D50F to DFP stage was greater than at the DFF stage. At the former reproductive stage, the ranges (Max-Min) of all traits except F_v/F_m were higher than their corresponding values at the DFF stage for landraces, lines and varieties, which implies that the genetic variability of 12 physiological traits was higher at the D50F to DFP stage than their corresponding values at the DFF stage.

6.3.2. Analysis of variance (ANOVA)

Under heat stressed environment, ANOVA revealed highly significant differences ($P \leq 0.001$) for SPAD values irrespective of the reproductive stages and the types of genotypes used in this study. At the DFF stage, Φ_2 , qP and qL differed significantly ($P \leq 0.05$) among genotypes. At the D50F to DFP stage, all physiological traits except leaf thickness were significantly different ($P \leq 0.05$) among genotypes. For segregating populations, all physiological traits except Rf_d were significantly different ($P \leq 0.05$) among genotypes. Highly significant differences ($P \leq 0.001$) were observed for Φ_{NO} , LEF and PAR among F_5 wild families of chickpea (Table 6.2).

6.3.3. Variance components and heritability

Under heat stressed environment, irrespective of the reproductive stages and types of genotypes, SPAD, PAR and LEF had high environmental, genotypic and phenotypic variances. Moreover, coefficients of phenotypic variances were higher than their respective coefficients of genotypic variances. All physiological traits in both reproductive stages had higher coefficient of phenotypic variances than their corresponding coefficient of genotypic variances. Leaf angle has

no genotypic variance, heritability and genetic advance among landraces, lines and varieties. At the DFF stage, SPAD, Phi2, PhiNO, qL, and qP had high heritability ($>30\%$) and the remaining traits had low to medium heritability. SPAD (21%) and qL (22.6%) had the highest genetic advance as percentages of the mean (Table 6.3).

Table 6.2. Analysis of variance (ANOVA), Mean, Standard deviation, Minimum and maximum values of physiological traits at reproductive stages.

Days to first flowering stage for landraces, lines and varieties								Days to 50% flowering to days to first podding stage for landraces, lines and varieties						
Trait	Gen MS	Rep MS	Bl.(Rep) MS	Mean	SD	Min	Max	Gen MS	Rep MS	Bl.(Rep) MS	Mean	SD	Min	Max
SPAD	423.7***	1.7 ^{ns}	94.9 ^{ns}	112.3	15.4	91.05	165.5	376.5***	248.1*	21.2 ^{ns}	100.7	13.79	57.2	122.2
Phi2	0.014*	6x10 ^{-5ns}	0.036 ^{ns}	0.376	0.08	0.17	0.53	0.043**	0.037 ^{ns}	0.0027 ^{ns}	0.39	0.167	0.1167	0.733
PhiNPQ	0.0114 ^{ns}	9x10 ^{-5ns}	0.029 ^{ns}	0.339	0.074	0.206	0.494	0.05*	0.06 ^{ns}	0.004 ^{ns}	0.337	0.178	0.027	0.736
PhiNO	0.0069 ^{ns}	3x10 ^{-5ns}	0.00039 ^{ns}	0.284	0.057	0.219	0.47	0.012***	0.003 ^{ns}	0.0001 ^{ns}	0.276	0.08	0.092	0.483
NPQt	0.309 ^{ns}	0.045 ^{ns}	0.534 ^{ns}	1.275	0.39	0.74	1.96	5.6**	4.6 ^{ns}	0.053 ^{ns}	1.5388	1.7	0.112	7.99
qL	0.013*	0.0016 ^{ns}	0.016 ^{ns}	0.288	0.079	0.075	0.416	0.0123***	0.003 ^{ns}	0.00013 ^{ns}	0.33	0.133	0.05	0.624
Fv/Fm	0.0028 ^{ns}	0.0003 ^{ns}	0.0046 ^{ns}	0.6856	0.037	0.622	0.737	0.033**	0.0036 ^{ns}	0.0015 ^{ns}	0.6868	0.1	0.35	0.814
qP	0.025*	0.001 ^{ns}	0.043*	0.5474	0.109	0.235	0.727	0.0189*	0.022 ^{ns}	0.00042 ^{ns}	0.573	0.178	0.162	0.9
LEF	4306.3 ^{ns}	290.3 ^{ns}	1926 ^{ns}	215.65	45.34	133.6	296.5	0.055**	0.032 ^{ns}	0.0056 ^{ns}	159.4	60.3	62.98	287.6
RFd	0.1262 ^{ns}	0.0002 ^{ns}	0.36 ^{ns}	0.655	0.246	0.208	1.19	7174.68**	4525 ^{ns}	30.57 ^{ns}	0.911	0.65	0.132	2.74
PAR	259,302 ^{ns}	42631 ^{ns}	651230.5 ^{ns}	1381.8	353.1	727	1913	0.6855**	0.34 ^{ns}	0.013 ^{ns}	1024	0.911	329.5	1870
LA	42.6 ^{ns}	5.97 ^{ns}	52.6 ^{ns}	12.13	5.03	3.57	23.9	75.6 ^{ns}	34.3 ^{ns}	54.4 ^{ns}	12.17	6.15	3.5	27.9
LT	0.0685 ^{ns}	0.0028 ^{ns}	0.147 ^{ns}	0.255	0.18	0.095	0.91	0.062**	0.098 ^{ns}	0.0029 ^{ns}	0.2987	0.185	0.08	0.86
Days to 50% flowering to days to first podding stage for F ₅ segregating families														
SPAD	714.7***	242.2 ^{ns}	25.6 ^{ns}	106.7	12.78	80.83	123.87							
Phi2	0.015*	0.003 ^{ns}	0.002 ^{ns}	0.403	0.09	0.225	0.583							
PhiNPQ	0.017*	0.008 ^{ns}	0.006 ^{ns}	0.28	0.084	0.144	0.368							
PhiNO	0.0055***	0.0003 ^{ns}	0.0002 ^{ns}	0.3	0.056	0.21	0.449							
NPQt	0.35*	0.1795 ^{ns}	0.299 ^{ns}	0.99	0.35	0.146	1.415							
qL	0.015*	0	0	0.283	0.1	0.103	0.502							
Fv/Fm	0.0038*	0.002 ^{ns}	0.002 ^{ns}	0.715	0.039	0.67	0.786							
qP	0.02**	0.012 ^{ns}	0.0006 ^{ns}	0.56	0.12	0.305	0.759							
LEF	7923***	2638 ^{ns}	2397 ^{ns}	174	62.72	47.72	258.57							
RFd	0.24 ^{ns}	0.04 ^{ns}	0.11 ^{ns}	0.763	0.333	0.291	1.613							
PAR	435,572.4***	156844.9 ^{ns}	77187.74 ^{ns}	1091	512.44	206	2069							
LA	127.5**	3.8 ^{ns}	129.3 ^{ns}	17.26	13.7	3.1	44.46							
LT	0.03*	0.022 ^{ns}	0.0017 ^{ns}	0.22	0.107	0.07	0.44							

Gen, genotype, MS, mean square, Rep, replication, SD, standard deviation, Min, minimum, Max, maximum, Bl, block, RFd, chlorophyll fluorescence decline ratio; Fv/Fm, chlorophyll fluorescence; Phi2, effective quantum yield of photosystem II; PhiNO, the portion of light lost as non-regulated way; NPQ, non-photochemical quenching; PhiNPQ, the portion of light lost as regulated way; PAR, photosynthetically active radiation; LA, leaf angle; LT, leaf thickness; qP, coefficient of photochemical quenching using puddle model; qL, coefficient of photochemical quenching using lake model; SPAD, The soil plant chlorophyll meter; %PS, percentage of pod set; ANOVA, analysis of variance; ns, not significant; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$.

Table 6.3. Variance components of 13 physiological traits during reproductive stages.

Days to first flowering stage for Landraces, lines and varieties

Trait	EV	GV	PV	GCV%	PCV%	H ² b%	GA	GAM%
SPAD	60.2	175.6	235.8	11.8	13.7	74.5	2356.7	21.0
Phi2	0.007	0.003	0.0105	15	27.3	30.5	6.4	17.1
PhiNPQ	0.009	0.0008	0.00987	8.2	29.3	7.8	1.6	4.7
PhiNO	0.003	0.002	0.0047	14.1	24.1	34	4.8	16.9
NPQt	0.244	0.022	0.26644	11.7	40.4	8.4	8.9	7.0
qL	0.006	0.003	0.009	19	32.9	33.3	6.5	22.6
Fv/Fm	0.002	0.0002	0.00239	2	7.1	7.95	0.8	1.2
qP	0.0099	0.007	0.0164	14.7	23.4	39.6	10.4	19.1
LEF	3134.6	394.8	3529.4	9.2	27.5	11.2	1370.7	6.4
RFd	0.087	0.015	0.1015	18.4	48.6	14.3	9.4	14.3
PAR	217570.9	10459.8	228030.7	7.4	34.6	4.6	4525.0	3.3
LA	56.9	0	56.9	0	62.2	0	0	0
LT	0.059	0.005	0.0636	27.5	98.9	7.7	4.0	15.7
Days to 50% flowering to days to first podding stage for Landraces, lines and varieties								
SPAD	45	165.5	210.5	12.8	14.4	78.6	2349.2	23.3
Phi2	0.013	0.015	0.0278	29.6	41	52.2	17.9	44.1
PhiNPQ	0.020	0.014	0.0345	37.5	58.7	40.9	15.6	49.4
PhiNO	0.003	0.004	0.0069	24	30.1	63.8	10.9	39.6
NPQt	1.3	2.2	3.5	96.4	121.5	62.9	242.4	157.5
qL	0.003	0.004	0.0069	24	30.1	63.8	10.9	39.6
Fv/Fm	0.008	0.013	0.02002	35	44.2	62.5	18.2	56.9
qP	0.006	0.006	0.0125	11.6	16.3	50.4	11.6	16.9
LEF	0.016	0.0199	0.0354	24.6	32.8	56.2	21.9	38.0
RFd	2114.6	2380.4	4495	30.6	42.1	53	7319.9	45.9
PAR	0.17	0.257	0.422	55.6	71.3	60.9	81.5	89.5
LA	56.9	0	56.9	0	0.61982	0	0	0
LT	0.059	0.0049	0.0636	23.4	84.4	7.8	4.1	13.6
Days to 50% flowering to days to first podding stage for F ₅ segregating families								
SPAD	69.3	350.9	420.2	17.2	19.3	83.5	3525.9	33.2
Phi2	0.007	0.004	0.0112	1	27.1	39.3	8.6	22.0
PhiNPQ	0.006	0.007	0.0125	27.1	37.3	52.8	12.2	40.5
PhiNO	0.001	0.002	0.00304	14.5	18.6	60.5	6.9	23.2
NPQt	0.103	0.125	0.228	35.7	48.2	54.8	53.9	54.4
qL	0.006	0.004	0.0102	23.3	36.3	41.2	8.6	30.8
Fv/Fm	0.0012	0.0014	0.00253	5.2	7	54.5	5.6	7.9
qP	0.0078	0.007	0.0143	14.7	21.7	45.5	11.2	20.4
LEF	1672.9	3497.5	5170.4	32.4	39.4	67.6	10013.3	54.9
RFd	0.18	0.032	0.212	24.4	62.7	15.1	14.3	19.5
PAR	85975	212422.6	298397.6	39.8	47.2	71.2	80120.7	69.2
LA	77.2	85.4	162.6	60.4	83.4	52.5	1379.1	90.2
LT	0.009	0.013	0.0218	53.3	69	59.6	18.1	84.7

RFd, chlorophyll fluorescence decline ratio; Fv/Fm, chlorophyll fluorescence; Phi2, effective quantum yield of photosystem II; PhiNO, the portion of light lost as non-regulated way; NPQ, non-photochemical quenching; PhiNPQ, the portion of light lost as regulated way; PAR, photosynthetically active radiation; LA, leaf angle; LT, leaf thickness; qP, coefficient of photochemical quenching using puddle model; qL, coefficient of photochemical quenching using lake model; SPAD, The soil plant chlorophyll meter. EV, environmental variance; GV, genotypic variance; %GCV, coefficient of genotypic variance; % PCV, coefficient of phenotypic variance; H²b, broad sense heritability; GA, genetic advance; GAM, genetic advance as percentage of the mean.

At the D50F to DFP stage, all physiological traits except leaf thickness and angle of landraces, lines and varieties had high heritability (40.9%-78.6%). Most of the physiological traits had high genetic advance (>30%) at the D50F to DFP stage. The heritability and genetic advance of all physiological traits except leaf thickness and angle of landraces, lines and varieties at the D50F to DFP stage were higher than their respective values at the DFF stage. For F₅ segregating progenies of chickpea, all physiological traits except RFd had high heritability (39.3%-83.6%). Among 13 physiological traits of F₅ segregating progenies, 8 of them had high genetic advance (>30%) (Table 6.3).

6.3.4. Correlations among physiological traits

Under heat stressed environment, SPAD reading value for landraces was positively correlated with Phi2, qL, qP, and %PS. Phi2 was negatively correlated with PhiNO, NPQ, LA, and LT. It was negatively and strongly ($P \leq 0.001$) correlated with PhiNPQ and PAR. Phi2 was strongly

($P \leq 0.001$) and positively correlated with RFd and qL traits. Negative correlation was observed between PhiNPQ and PhiNO traits, which were negatively correlated with most physiological traits. Both traits were strongly and negatively correlated with Fv/Fm. qL was positively correlated with Phi2, LEF, and %PS. It was strongly ($P \leq 0.001$) and positively correlated with RFd and qP. Fv/Fm was positively correlated with Phi2, qP, RFd, LA and LT. LEF was positively correlated with PAR, LA, LT, %PS, qP, Fv/Fm, qL, and Phi2. RFd was positively correlated with PAR, LA, LT, %PS, qP, Fv/Fm, qL, and Phi2. RFd was positively correlated with %PS and LEF. PAR was negatively correlated with RFd, qP, qL, NPQt, PhiNPQ, Phi2, and SPAD. %PS was positively correlated with SPAD, Phi2, qL, qP, LEF, RFd, and LA. It was negatively correlated with LT, PhiNO, PhiNPQ, and Fv/Fm (Table 6.4).

Table 6.4. Correlations of 13 physiological traits at reproductive stages.

SPAD	Phi2	PhiNPQ	PhiNO	NPQt	qL	Fv/Fm	qP	LEF	RFd	PAR	LA	LT	% PS
SPAD	0.026 ^{ns}	-0.175 ^{ns}	-0.19 ^{ns}	-0.248 ^{ns}	0.13 ^{ns}	-0.265 ^{ns}	0.062 ^{ns}	0.12 ^{ns}	-0.086 ^{ns}	-0.14 ^{ns}	-0.24 ^{ns}	-0.13 ^{ns}	0.088 ^{ns}
Phi2		-0.75***	-0.5*	-0.37 ^{ns}	0.909***	0.368 ^{ns}	0.062 ^{ns}	0.0128 ^{ns}	0.965***	-0.78***	-0.06 ^{ns}	-0.089 ^{ns}	0.323 ^{ns}
PhiNPQ			-0.20 ^{ns}	0.88***	-0.446*	-0.89***	-0.58**	-0.243 ^{ns}	-0.8***	-0.72***	-0.02 ^{ns}	-0.24 ^{ns}	-0.195 ^{ns}
PhiNO				-0.6**	-0.76***	-0.613**	-0.67***	-0.33 ^{ns}	-0.404 ^{ns}	0.212 ^{ns}	0.124 ^{ns}	0.444*	-0.253 ^{ns}
NPQt					-0.002 ^{ns}	-0.99***	-0.16 ^{ns}	-0.28 ^{ns}	-0.248*	0.44*	-0.02 ^{ns}	-0.39 ^{ns}	-0.0045 ^{ns}
qL						-0.004 ^{ns}	0.972***	0.079 ^{ns}	0.86***	-0.67***	-0.13 ^{ns}	-0.297 ^{ns}	0.222 ^{ns}
Fv/Fm							0.154 ^{ns}		0.44*	-0.45*	0.045 ^{ns}	0.389 ^{ns}	-0.08 ^{ns}
qP								0.0944 ^{ns}	0.91***	-0.73***	-0.08 ^{ns}	-0.018 ^{ns}	0.17 ^{ns}
LEF									-0.12***	0.56**	0.3 ^{ns}	0.16 ^{ns}	0.222 ^{ns}
RFd										-0.77***	-0.10 ^{ns}	-0.09 ^{ns}	0.06 ^{ns}
PAR											0.167 ^{ns}	0.163 ^{ns}	0.02 ^{ns}
LA												0.1566 ^{ns}	0.31 ^{ns}
LT													-0.18 ^{ns}
%PS													

RFd, chlorophyll fluorescence decline ratio; Fv/Fm, chlorophyll fluorescence; Phi2, effective quantum yield of photosystem II; PhiNO, the portion of light lost as non-regulated way; NPQt, non-photochemical quenching; PhiNPQ, the portion of light lost as regulated way; PAR, photosynthetically active radiation; LA, leaf angle; LT, leaf thickness; qP, coefficient of photochemical quenching using puddle model; qL, coefficient of photochemical quenching using lake model; SPAD, The soil plant chlorophyll meter; %PS, percentage of pod set; ns, not significant; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$.

6.4. Discussion

Large-scale high-throughput plant phenotyping (phenomics) is becoming increasingly important in agriculture for precise measuring of the plant's photosynthetic ability under drought and heat stresses which can be conducted in the laboratory and/or field. MultispeQ is one of the high-throughput plant phenotyping devices that linked through the PhotosynQ platform to larger communities of plant researchers and growers. PhotosynQ platform can be used to provide deeper insights into the environmental, physiological and genotypic factors that govern complex plant responses (Kuhlgert *et al.*, 2016). In line with several authors (Kuhlgert *et al.*, 2016; Dutbayev *et al.*, 2020; Epaku *et al.*, 2021; Kamanga *et al.*, 2018; Mwale *et al.*, 2017; Sooriyapathirana *et al.*, 2021) MultispeQ device was used in the present study to collect data for 13 most useful physiological traits in chickpea. Our results showed the potential of MultispeQ for physiological research and breeding in chickpea under an extremely heat stress environment.

When a chickpea leaf is illuminated, the leaf's chlorophyll absorbs the light, which can serve as three functions. It can be used for the light dependent processes of photosynthesis in the thylakoids (Photochemistry, Φ_2), excess energy can be dissipated as heat (Φ_{NPQ} and Φ_{NO}) or it can be re-emitted as light (chlorophyll fluorescence, F_v/F_m). The summation of Φ_2 , Φ_{NPQ} , and Φ_{NO} is equal to one (Kuhlgert *et al.*, 2016); however, as confirmed in this study, the proportion of each of the three traits varied depending upon the extent of the heat stress, the plant growth stages and the type of genotypes. In the present study, heat tolerant genotypes exhibited high photosynthesis (Φ_2), R_Fd , qP , qL , and low $NPQt$, Φ_{NPQ} and Φ_{NO} , while the sensitive genotypes had low Φ_2 , R_Fd , qP , qL , and high $NPQt$, Φ_{NPQ} and Φ_{NO} . Φ_2 is an effective quantum yield of photosystem II where most light energy is converted into food.

This implied that the net CO₂ assimilation rate of the heat tolerant genotypes were higher than the sensitive genotypes. Although both PhiNPQ and PhiNO decreased an effective quantum yield of photosystem (Phi2), PhiNPQ is the amount of light dissipated as heat or other energy forms, so that the leaves do not get photo-damaged. However, PhiNO is the amount of received light that has not been used by the leaf for photosynthesis or dissipated out, thereby; PhiNO represents potential damage (Mwale *et al.*, 2017; Kamanga *et al.*, 2018; Sooriyapathirana *et al.*, 2021). Similar to the present study, Prinzenberg *et al.* (2018) reported that at 45/40°C, genotypes with the highest PSII also tended to have the lowest NPQt.

Similarly, Epaku *et al.* (2021) reported that drought tolerant maize inbred lines had high Phi2 and LEF than sensitive maize inbred lines. Mwale *et al.* (2017) explained that cowpea genotypes that maintained a higher chlorophyll content and efficiency of photosystem II had higher chances of recovering from drought. Baker (2008) reported that the high photosystem II at high temperatures indicated that most of the light energy absorbed was being partitioned towards fixing carbon. Sooriyapathirana *et al.* (2021) also reported that the quantum efficiency for photosystem II photochemistry (Phi2) represents the amount of light received by the leaf that goes to the photosystem II and ultimately is converted to carbohydrates. As revealed in the present study, LEF can be considered an indicator of photosynthesis as it reflects the amount of energy that is moving around the chloroplast upon exposure to the light (Sooriyapathirana *et al.*, 2021). At 38/28°C, the photosynthetic efficiency of heat tolerant lentil genotypes were higher than sensitive genotypes (Sita *et al.*, 2017). Similar to the present study, Srinivasan *et al.* (1996) reported that photosynthetic rates were higher during 50% flowering to pod formation than the vegetative stage in chickpea. In line with the present study, RFd was also an indicator of the

potential photosynthetic activity and, when measured at saturation irradiance, has been directly correlated to the net CO₂ assimilation rate of leaves (Fernández-Calleja *et al.*, 2020).

In the present study, heat tolerant genotypes had high SPAD readings than sensitive genotypes. In line with this, under heat stressed conditions, increment in total chlorophyll contents has shown association with thermo-tolerance (Camejo *et al.*, 2005). More reduction in total chlorophyll contents were observed in all cultivars during reproductive (42%) than vegetative phases (14.9%) (Sailaja *et al.*, 2015).

Chlorophyll fluorescence (Fv/Fm) is a fast, non-destructive method and has been suggested as quantitative measures of the photochemical efficiency of the photosystem II (PSII) complex under different environmental stresses (Adams *et al.*, 1990). Chlorophyll fluorescence is defined as photons that chlorophyll molecules re-emit upon return from excited to non-excited states. Chlorophyll fluorescence serves as an important tool to screen for genotypes that can maintain photochemistry, protect photosystem II (PSII) reaction centers and dissipate excess energy while maintaining productivity under drought and heat stresses (Kamanga *et al.*, 2018). In the present study, tolerant genotypes had high chlorophyll fluorescence (0.67-0.79) than sensitive genotypes (0.35-0.71). Likewise, Shefazadeh *et al.* (2012) found that heat-tolerant wheat lines had greater Fv/Fm ratios than heat-sensitive lines at the grain-filling period in warmer irrigated environments, which were linked to higher grain yield. Under heat stressed condition, the highest Fv/Fm ratio was observed in tolerant than sensitive genotypes (Kohila and Gomathi, 2018). In chickpea, heat stress (>30°C) in the field during the reproductive stage reduced Fv/Fm more

(0.48, 0.41) in heat-sensitive genotypes (ICC10685 and ICC5912) than in two heat-tolerant genotypes (ICC15614 and ICCV92944) (0.64, 0.60) (Kaushal *et al.*, 2013).

In the present study, the extent of heat stress was higher at the D50F to DFP stage than DFF. Thus, the sensitive genotypes contained low Phi2, Fv/Fm, %PS, and high NPQt. Similarly, Kamanga *et al.* (2018) reported that as a response of an excess water stress, in sensitive genotypes, ground fluorescence (Fo) and maximal fluorescence (Fm) increase, while variable fluorescence (Fv) decreases. The synthesis of ATP and NADPH₂ decreases. Maximal quantum yields of PSII photochemistry or chlorophyll fluorescence (Fv/Fm), effective quantum yields of PSII photochemistry (Phi2), and coefficient of photochemical quenching (qP) decrease, and increases in non-photochemical quenching (NPQt). Overall, this decreases assimilation of carbon dioxide and electron transport rate. A decline in Fv/Fm and PSII indicates photo-inhibitory damage of water stress to PSII photosystems i.e. inactivates PSII reaction centers. Drought stress consistently and significantly reduced the efficiency of photosystem II, chlorophyll content of the tested genotypes though the effect varied in both improved and local genotypes (Mwale *et al.*, 2017).

In the present study among 13 physiological traits, 7 highly correlated traits such as Phi2, NPQt, RFd, PhiNO, PhiNPQ, qL, and qP had high broad sense heritability and genetic gain that were used for differentiations between heat tolerant and sensitive chickpea genotypes. These traits confirmed the report of Tsegaye Getahun *et al.* (2021b). In line with this study, Mwale *et al.* (2017) reported that chlorophyll content and efficiency of photosystem II could be used as efficient reference indicators in the selection of drought tolerant genotypes. In addition, Epaku *et*

al. (2021) revealed that selection of drought tolerant maize inbred lines were made under excessive water stress conditions using three physiological parameters such as Phi2, LEF and NPQt that had significant correlations among the traits.

In the present study, at D50F to DFP stage most traits were significantly different among the chickpea accessions. This clearly indicated the presence of genetic variations for these physiological traits, which are useful for selections of heat tolerant chickpea genotypes under heat stress environment. In agreement to the present study, significant differences ($P < 0.01$) were found among 76 maize inbred lines for Phi2, NPQt, LEF, and relative chlorophyll content traits under drought stress (Epaku *et al.*, 2021). Likewise, Mwale *et al.* (2017) reported that cowpea genotypes were significantly different for chlorophyll content ($P \leq 0.01$), efficiency of photosystem II ($P \leq 0.05$), and non-photochemical quenching ($P \leq 0.05$) under drought stress. The results from analysis of variance for chlorophyll content, efficiency of photosystem II, and non-photochemical quenching showed that genotypes were significantly different, indicating the presence of genetic variation among the cowpea and maize genotypes for these traits that is essential for improvement of the crop for drought tolerance (Mwale *et al.*, 2017; Epaku *et al.*, 2021).

In the present study, under heat stressed environment, Phi2, Fv/Fm, SPAD, and LEF were positively correlated traits that were negatively correlated with PhiNPQ and PhiNO. These two traits were also negatively correlated with each other which implied that as the non-regulated heat dissipation increases and the regulated heat dissipation decreases i.e. photo damage to the plant increases thereby the pod set decreases. Similarly, when different genotypes of crop plants

were subjected to drought and flooding stresses, Phi2, SPAD and LEF were negatively correlated to NPQt, but Phi2 and SPAD were positively correlated with LEF (Epaku *et al.*, 2021; Mwale *et al.*, 2017; Sooriyapathirana *et al.*, 2021). LEF and PhiNO, and SPAD and PhiNPQ were strongly ($P \leq 0.001$) and negatively correlated with each other separately. Phi2 and SPAD were negatively correlated with PhiNPQ and PhiNO. The positive correlations between Phi2 and SPAD indicate that the utilizable amount of light is dependent on the chlorophyll content. The strong negative correlation between SPAD and PhiNPQ reflects that when the leaf contains a higher amount of chlorophyll, the amount of light dissipated as heat/energy is less, implying that the plants utilize a higher proportion of light for biomass assimilation. Strong negative correlation between LEF and PhiNO indicates that when chloroplasts receive more energy, the amount of light that can cause photo-damage gets minimized (Sooriyapathirana *et al.*, 2021). Mwale *et al.* (2017) revealed that the reduction in efficiency of photosystem II was supported by an increase in non-photochemical quenching (NPQt) as shown by a strong negative correlation, suggesting that a greater portion of the energy was thermally dissipated.

6.5. Conclusions

This study applies a rapid, low-cost, and reliable high-throughput phenotyping tool to explore genotypic variation for photosynthetic performance of 47 chickpea accessions under heat stressed field conditions. MultispeQ is a high throughput phenotyping tool that combines a fluorimeter, a chlorophyll meter, and a spectrometer into one which is used to measure several physiological traits simultaneously which has enabled large-scale, rapid and accurate data collection under controlled and field conditions and boosted the power of trait-based crop breeding “physiological breeding”. The energy allocation ratios of MultispeQ instrument depend

up on environmental conditions, type of genotypes, colored lights, photosynthetic pathways, the day from morning to late afternoon, and the color of the leaves. Under heat stressed environment, the physiological responses of heat tolerant and sensitive genotypes were the same as the agromorphological responses. In heat tolerant genotypes 60-68% of the absorbed photons were used to carryout photochemical reactions (photosynthesis) and 32-40% were dissipated as heat, while in sensitive genotypes only 30% of the absorbed photons were used for photosynthesis and 70% were dissipated as heat. The summation of Φ_2 , Φ_{NPQ} , and Φ_{NO} is equal to one; however, the dynamic competing fates of these three traits varied depending upon the extent of the heat stress, the plant growth stages and the type of genotypes. Φ_2 is an effective quantum yield of photosystem II where most light energy is converted into food. This implied that the net CO_2 assimilation rate of the heat tolerant genotypes were higher than the sensitive genotypes. Φ_{NPQ} and Φ_{NO} were also negatively correlated with each other which implied that as the non-regulated heat dissipation increases and the regulated heat dissipation decreases i.e. photo damage to the plant increases thereby the pod set decreases. Most of the physiological traits were positively correlated with pod set and other yield related traits.

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CHAPTER 7: Reproductive responses of diverse genetic background of chickpeas to heat stress and associated with pod setting and yield related traits

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Abstract

Reproductive responses of chickpea (*Cicer arietinum* L) are negatively impacted by high temperatures ($\geq 35^{\circ}\text{C}$) as a consequence pod set and yield related traits are reduced. The objectives of the study were to assess the effect of heat stress on the number of pollen cells per flower, pollen viability, *in vitro* pollen germination, and demonstrate the association of these traits with pod set and yield related traits. The study was conducted under heat stressed conditions with 17 and 47 chickpea accessions that were grown at ICRISAT (Hyderabad, India) and Werer (Ethiopia), respectively. The pollen viability study was carried out using 2% acetocarmine stain. The quantity of pollens per flower was counted with haemocytometer. *In vitro* pollen germination was determined by using a modified Brewbaker and Kwack liquid medium. Under heat stressed condition, at ICRISAT (Hyderabad, India), the F₃ individuals of 9xCudiB_022C family had the highest average percentage of pollen viability (94%) which was exhibited through high pod set (80%). However, 9xKarab_081A (9%) and ICC 4567 (10%) had low average percentage of pollen viability which was also associated with low pod set (0-10%). Our finding revealed that F₃ wild progenies of *Cicer echinospermum* had low pollen viability and pod set compared to heat tolerant check and *Cicer reticulatum* accessions. The lines, landraces and varieties of chickpea have shown wide range of variations for all reproductive traits and pod set compared to F₅ segregation of wild progenies, which implied that high genetic variability was observed within the landraces, lines and varieties. However, the mean values of the pollen cells per flower (23,298.7), *in vitro* pollen germination (30.0%) and pod set (60%) of the segregating progenies were higher than the landraces, lines and varieties (17,343.5, 23.5%, and 43.7%) which implied that the segregating wild progenies resisted the heat stress i.e. linked traits can be introgressed into cultivars in order to enhance heat-tolerance. ANOVA revealed highly

significant differences ($P \leq 0.001$) for all reproductive traits among chickpea accessions. Pollen viability was positively associated with pollen cells per flower, *in vitro* pollen germination, and pod set. Reproductive traits are useful traits for screening contrasting genotypes under heat stressed field environments in chickpea breeding program.

Keywords: Associated traits, Chickpea, High temperatures, Reproductive traits.

7.1. Introduction

Reproductive processes such as pollen numbers, pollen viability, pollen tube growth, ovule viability, stigma receptivity, stigmatic and style positions, fertilization and post-fertilization processes, growth of the endosperm, pro-embryo, fertilized embryo, plant biomass, pod formation, the number of pods and seeds per plant, grain yield, and harvest index are adversely affected by high temperatures ($\geq 35^{\circ}\text{C}$) (Foolad, 2005; Krishnamurthy *et al.*, 2011; Devasirvatham, 2012). Heat stress reduces pollen number, pollen tube growth, pollen germination, cause abnormal pollen development, anther indehiscence, delayed flowering, flower abortion, reduce number and size of flower and pollen, deformity of floral organ, poor fruit set, impaired fertilization and post fertilization, small endosperm, and aborted seeds (Kaushal *et al.*, 2016).

In addition, high temperature can induce anther and pollen structural aberrations that are key factors in reducing chickpea yield under high temperature (Devasirvatham *et al.*, 2013). While the male reproductive organs are more sensitive to heat stress than the female counterpart is, the complete reproductive process from gamete formation to fertilization and seed maturation are highly vulnerable to raised temperatures. High temperatures reduced pod set in chickpea by reducing pollen viability and pollen production per flower. Micro-sporogenesis is disrupted at high temperatures due to damage caused by the tapetal layer and nutrient imbalance in developing pollen, resulting in sterility (Devasirvatham *et al.*, 2013). Fertilization is impaired due to reduced pollen viability, stigma receptivity, and pollen tube growth (Sita *et al.*, 2017).

The responses of reproductive traits under heat stress conditions were dependent upon the extent and duration of a stress, types of genotypes and reproductive stages. As reported by several authors (Firno *et al.*, 2006; Devasirvatham *et al.*, 2012; Devasirvatham *et al.*, 2013; Kaushal *et al.*, 2013; Sita *et al.*, 2017; Patriyawaty *et al.*, 2018), heat tolerant genotypes were characterized by high pollen number, *in vitro* pollen germination, and pollen viability per flower, normal pollen size, deeply stained pollen, less damage to pollen functions, normal pollen tube growth, high pod set, numbers of seeds and grain yield per plant. For example, Firno *et al.* (2006) revealed that the number and quality of pollen grains, the number of fruits and seeds per fruit in tomato were less affected by high temperatures for the heat-tolerant cultivars. These results indicated that the carbohydrate content of developing and mature tomato pollen grains might be an important factor in determining pollen quality, and suggest that heat-tolerant cultivars have a mechanism for maintaining the appropriate carbohydrate content under heat stress (Firno *et al.*, 2006). In addition, the anthers of heat-tolerant rice cultivars dehisce more easily than those of the susceptible cultivars under high temperature conditions (Prasad *et al.*, 2006; Jagadish *et al.*, 2010).

On the contrary, heat sensitive genotypes were characterized by reduction in the number of pollen grains, impaired pollen viability and germination, reduced pollen size and bursting of pollen cells, a reduced or non in pollen function, weakly stained pollen, abnormal pollen tube growth (zigzag), reduced fruit set and the numbers of seeds per fruit. Heat stress significantly inhibited the sucrose metabolism in leaves, anthers and seeds leading to decreased pollen function, seed filling and size, and pod set (Firno *et al.*, 2006; Devasirvatham *et al.*, 2012; Devasirvatham *et al.*, 2013; Kaushal *et al.*, 2013; Sita *et al.*, 2017; Patriyawaty *et al.*, 2018).

Under heat stress condition, Patriyawaty *et al.* (2018) showed that reduction of pod set in mungbean genotypes is primarily a consequence of reduction in pollen viability as evidenced by a positive relationship between viable pollen number, seed set and grain yield.

Reproductive traits have been used to understand the mechanisms of heat tolerance in many crop plants such as field pea, maize, tomato, rice, wheat, soybean, mungbean, chickpea and others. In order to confirm the field screening experiments and understand the mechanisms of heat tolerance in chickpea at reproductive stage, reproductive functions such as number of pollen grains, percentage of pollen germination, pollen tube growth and pod set can be employed. Moreover, as reported by Devasirvatham *et al.* (2012) and Kumar *et al.* (2012), the study of pollen may help to predict genetic variation among genotypes for reproductive phase heat tolerance. Pollen viability and seed-set as a measure of reproductive success under heat stress are also candidate traits for selection in breeding programs. Reproductive traits are used as an accurate screening technique for assessing heat tolerance in field pea (Jiang *et al.*, 2017). Naveed *et al.* (2014) reported percentage pollen viability, pollen production, percentage pollen germination and pollen tube length traits among maize accessions, which provided wide range of option for selection and opportunity to improve tolerance level against heat stress.

Pollen viability was determined by three methods such as *in vitro* pollen germination, staining with acetocarmine and absolute pollen viability. The trait was well studied in tomato, maize and wheat; however, it was not well studied in chickpea as the other crop plants and there was no report for Ethiopian chickpea accessions including their wild segregating progenies. Therefore, the objectives of the present study were to assess the effect of heat stress on total number of

pollens per flower, pollen viability, *in vitro* pollen germination of chickpea accessions and association of these traits with pod set and yield related traits.

7.2. Materials and Methods

7.2.1. Plant materials, field management and experimental design

Fifteen chickpea accessions that include 13 interspecific F₃ lines (7 F₃ families of AR and AE) and 2 standard checks were used for pollen viability study. They were grown at ICRISAT field station, Hyderabad, India from February to May 2017 using an alpha lattice design with two replications. Irrigation and weeding were manually managed. Intra and inter row spacing was 10 cm and 30 cm, respectively. The plot size was 1mx0.3m and 10 seeds per accession were planted into a single row. The average daily minimum and maximum temperatures of chickpea genotypes from germination to maturation range from 13 to 30.6°C and 30.4 to 42.6°C, respectively. The reproductive stages of chickpea genotypes exposed to daily average maximum and minimum temperatures that range from 36.6 to 40.4°C and 14.3 to 26°C, respectively (Fig 7.1).

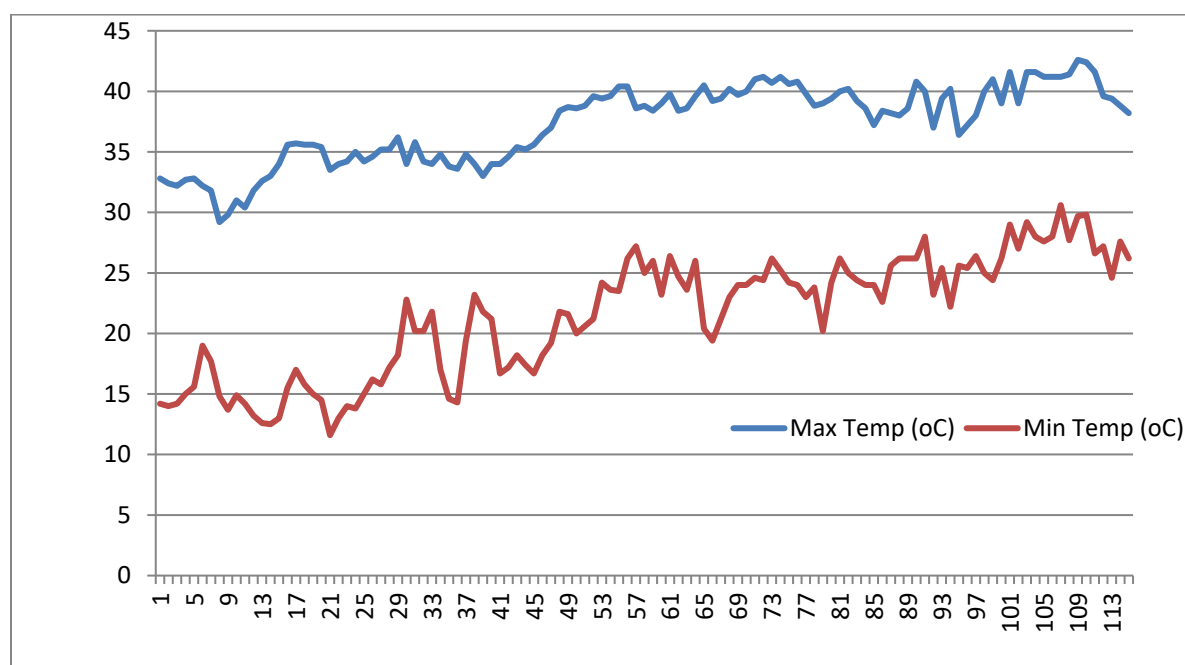


Fig.7.1. Minimum and maximum daily average temperatures at ICRISAT, Hyderabad (India) from germination to maturation (February to May 2017).

7.2.1.1. Pollen viability test using acetocarmine

Half-opened flowers were collected from field at 8:00-9:00 am to determine pollen viability test using acetocarmine stain. Five flowers per genotype were used for this study. Each flower was used as a replication. Fifteen field views per replication were used for each genotype and the average was recorded per genotype. The experiment was repeated three times. Steps of pollen viability study: half-opened flower buds per genotype were picked before anthesis and they were put in the fridge before use. Slides were prepared and all anthers of a single flower were opened on a slide; drops of 2% acetocarmine stain was added; then anthers were properly crashed to get out many pollens as much as possible; the debris from the slides were removed; the slides were covered with cover slips; The resulting pollen suspension was incubated for 20 min at 37°C; the pollens were observed under a camera fitted Olympus compound microscope with 10x magnification; pictures were taken up by a software installed in a PC; the sterile and fertile pollen cells were identified (the sterile pollens were anticipated to be white or weakly stained, while the fertile pollens absorb the stain deeply and become red). Percentage of pollen viability per field view was calculated as $[\text{number of stained pollen cells} / \text{total number of pollen cells}] * 100$.

Acetocarmine stains the pollen grain nuclei and weakly stains the cytoplasm, and gives good contrast between the grain and surrounding medium. Viable pollen was determined on pollen size, pollen shape (triangular or spherical) and intensity of stain taken up by pollen (Kaushal *et al.*, 2013). The stained and normal size of pollen grains was recorded as fertile, whereas, unstained and smaller were counted as sterile.

7.2.2. Determination of pollen numbers per flower and *in vitro* pollen germination

7.2.2.1. Plant materials, Field management and experimental design

Fourty seven chickpea germplasm that include 23 lines, landraces, cultivars and 24 F₅ interspecific lines were used in this study. They were grown at Werer experimental sites, Ethiopia usingan alpha lattice design from February to May 2019. Irrigation and weeding were manually managed. Intra and inter row spacing was 10 cm and 30 cm, respectively. The plot size was 1mx0.6m and 20 seeds per accession were planted into two rows.

Almost the whole developmental stages of the materials have been exposed to heat stress at daily maximum and minimum temperatures that range from 34.2 to 44°C and 13.6 to 26.6°C, respectively. The daily maximum and minimum temperatures of the reproductive stages range from 35.4 to 40°C and 17.2 to 24.4°C, respectively, which was higher compared to the vegetative stage (Fig 7.2).

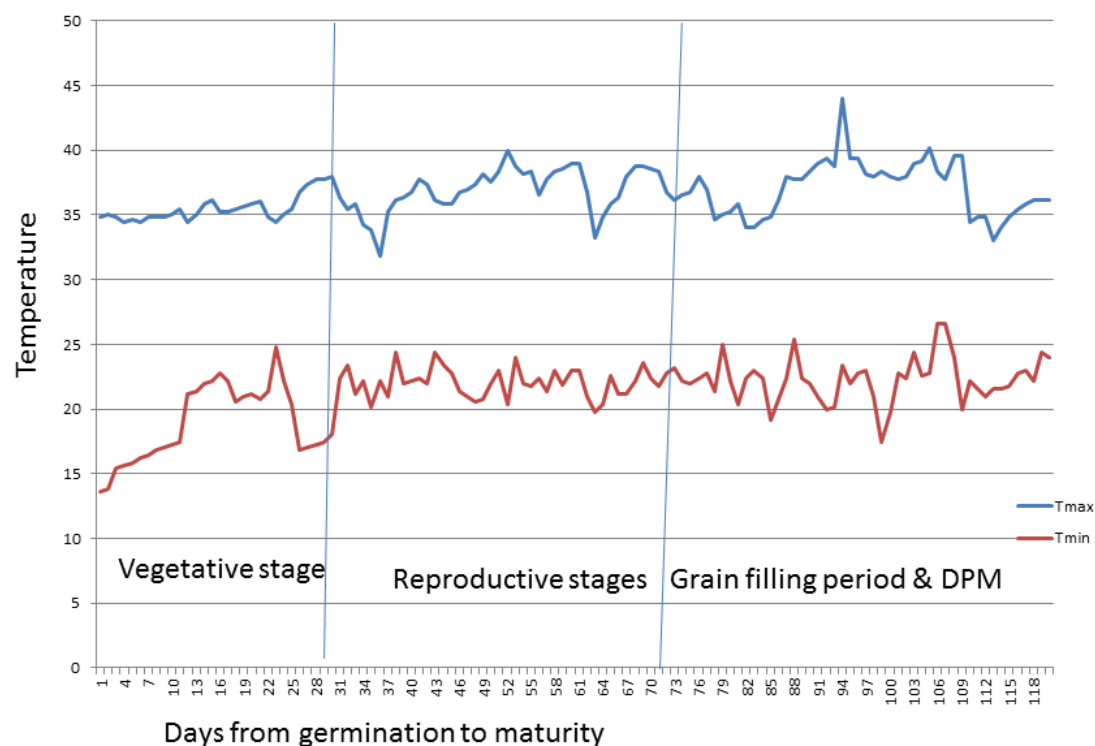


Fig.7.2. The daily minimum and maximum temperatures of Werer field site (February to May 2019) from germination to maturity. Tmax, maximum temperature, Tmin, minimum temperature, DPM, days to physiological maturity.

7.2.2.2.Counting pollen cells

Each flower was diluted in a 2 ml Eppendorf tube containing 100 ml distilled water. A total of 10ul solution of pollen cells per flower was placed on a two-counting area containing Thoma (haemocytometric) slide (0.1mm in depth) to where a special cover slip was placed. The pollens were distributed randomly on five large squares. The number of pollens at the central large square (containing 25 small squares, 0.1 μ L) of the haemocytometer was counted and converted

into pollen number (PN) based on total re-suspension volume. Total numbers of pollen grain per flower was calculated as $\text{Pollen count} \times 100 \text{ ul} / 0.1 \text{ ul}$.

7.2.2.3. *In vitro* pollen germination

To test *in vitro* pollen germination, nine half-opened flowers per genotype were collected regularly from a heat stressed environment. Three flowers were pooled in an eppendorf tube and stored at a 4°C refrigerator. Each eppendorf tube was used as a replication. Anthers from each flower were gently squeezed with forceps to release the pollens into a drop of germination medium on a microscopic slide. Three slides per genotype were then transferred into 90-mm-diameter Petri dishes with moistened filter paper. Two slides per dish were placed simultaneously. The dishes contain a piece of filter paper that lined the dish lid's inner surface absorbed any condensation. Petri dishes were then sealed with one layer of Para film® (Bemis NA, Neenah, WI) to maintain high humidity and placed at room temperature.

Pollen grains germinate and grow in a modified Brewbaker and Kwack (1963) liquid medium which composed of 2.5% sucrose, 10% polyethylene glycol (PEG), 100 mg/L Boric Acid, 300 mg/L $\text{Ca}(\text{NO}_3)_2$, 200 mg/L MgSO_4 and 100 mg/L KNO_3 . The pH of the medium was adjusted into 7.0. Polyethylene glycol has been used to prevent the grains bursting. The medium was sterilized in an autoclave at 121°C and 15 psi for 20 minutes. Following sterilization, medium was dropped onto slides and pollens were transferred into each slide under the laminar airflow cabinet. After 24 h incubation of the cultures, slides were examined using a dissection microscope. Pollen grains in three slides per genotype were counted for determination of percentage of germination using microscopic observation at 10× magnification. Each slide was

considered as a replication. Observations of pollen viability recorded in each microscopic field per genotype, ten times in each replication and the average was taken. Pollen grains were scored as germinated if pollen tube length exceeded the pollen-grain diameter. The percentage of germination was determined from at least 100 pollen grains per replicate. *In vitro* pollen germination (%) was calculated as (number of germinated pollen grains/number of counted pollen grains) x 100.

7.2.3. Data analysis

All reproductive and pod set traits are considered as dependent variables; however, genotype, block, and replication were taken as independent variables. ANOVA was computed based on a mixed linear additive model, using the GLM (General Linear Model) procedure of the SAS 9.0 software for a single location (Gomez and Gomez, 1984). The total variation (σ^2_p) for each trait was partitioned into variance components: genotypic (σ^2_g) and environmental (σ^2_e) variances. These variance components, coefficients of variation and genetic advance (GA) per location were determined according to Singh and Chaudhary (1985). Broad sense heritability (H_b^2) was estimated as suggested by Ekebil *et al.* (1977). All physiological data were subjected to statistical analysis such as raw data means, ANOVA, variance components (univariate), and bivariate analysis (correlation) using SAS 9.0 software (SAS Institute, 2002).

7.3. Results

7.3.1. Pollen viability associated with yield related traits

F₃ wild progenies of *Cicer arietinum* by *C. reticulatum* and *C. echinospermum* along with heat tolerant and sensitive checks were used for pollen viability test by using acetocarmine solution. Under an extremely heat stress environment (ICRISAT, Hyderabad, India), 9xCudiB_022C (94%) and 9xBari3_72C (94%) have shown the highest average percentage of pollen viability followed by Bari1_092 (89%) and JG14 (81%), while 9xKarabache_081A (9%) and ICC 4567 (10%) had low average percentage of pollen viability. This confirmed that the interspecific F₃ hybrid progenies from *C. arietinum* by *C. reticulatum* showed the highest pollen viability compared to the heat tolerant standard check (JG 14). However, the F₃ wild progenies of *Cicer echinospermum* had low pollen viability compared to heat tolerant check and *Cicer reticulatum* accessions (Fig 7.3).

JG14 and 9xCudiB_022C contained the maximum average percentage of pod set (80%) followed by Bari3_072C (66.7%) and Bari1_092 (60%), while 9xKarabache_081A (8.3%) and ICC4567 (10%) contained low average percentage of pod set. Similar to their average percentage of pollen viability, the other two accessions of *Cicer echinospermum* had still low average percentage of pod set in correspondence with the average value of pollen viability. Based on average filled pods (FFP) and total seeds (TSP) per plant, 9xCudiB_022C and 9xBari3_72C had the highest average values. Unlike the average values of pollen viability and pod set, 9xGunas_063 had a comparable FFP and TSP as JG 14 (Fig 7.3).

Our microscopic observations with 10x magnifications revealed that the heat tolerant genotypes had large number of pollen cells per field view, the pollen cells were deeply stained with acetocramine, had cylindrical shape and bigger in size. The pollen cells were densely populated. However, the sensitive genotypes had scattered pollen cells, small number of pollen cells per field view, had small sized and weakly stained or white pollen cells. Bursting of pollen cells was observed, example in ICC 4567. Based on the five yield related traits, 9xCudiB_022C, 9xBari3_072C and Bari1_092 were found to be the most heat tolerant F₃ wild progenies of *Cicer reticulatum* (Figs 7.3 and 7.4).

The pod set status of accessions of F₃ wild progenies in figures 7.3-7.5 indicated that individuals of 9xCudiB_22C, 9xSavur_063 and 9xOyali_084 had high pod set. Oyali_084 possessed the double podding features of the common mother (ICCV96029). Most of the F₃ wild progenies of chickpea had high genetic variability for heat tolerance unlike the landraces, lines and varieties. F₃ wild progenies of *Cicer echinospermum* accessions showed high degree of variability for heat tolerance compared to F₃ wild progenies of *Cicer reticulatum*. Thus, some F₃ individuals of 9xGunas_063 and 9xS2DrD_107B had low pollen viability, pod set, and other yield related traits (Figs 7.3-7.5).

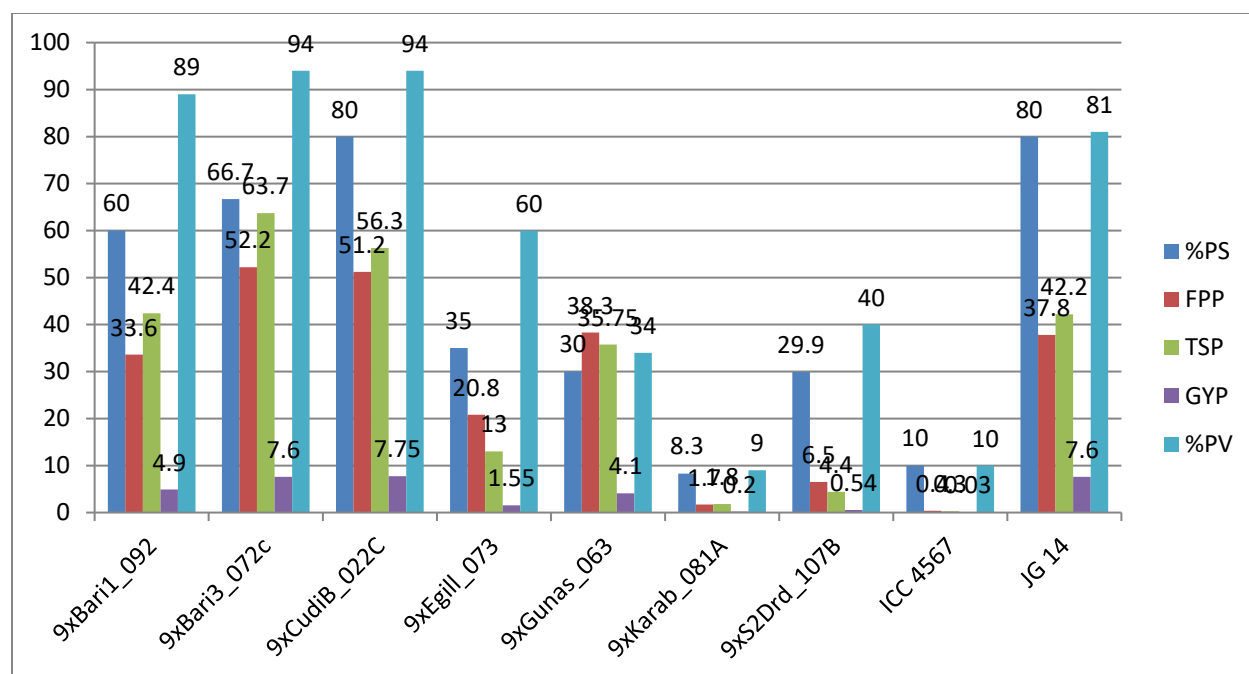


Fig.7.3. The average percentage of pollen viability of chickpea accessions associated with four yield related traits. %PS, percentage of pod set; FPP, filled pod per plant; TSP, total seed per plant; GYP, grain yield per plant; %PV, percentage of pollen viability; GYP, grain yield per plant.

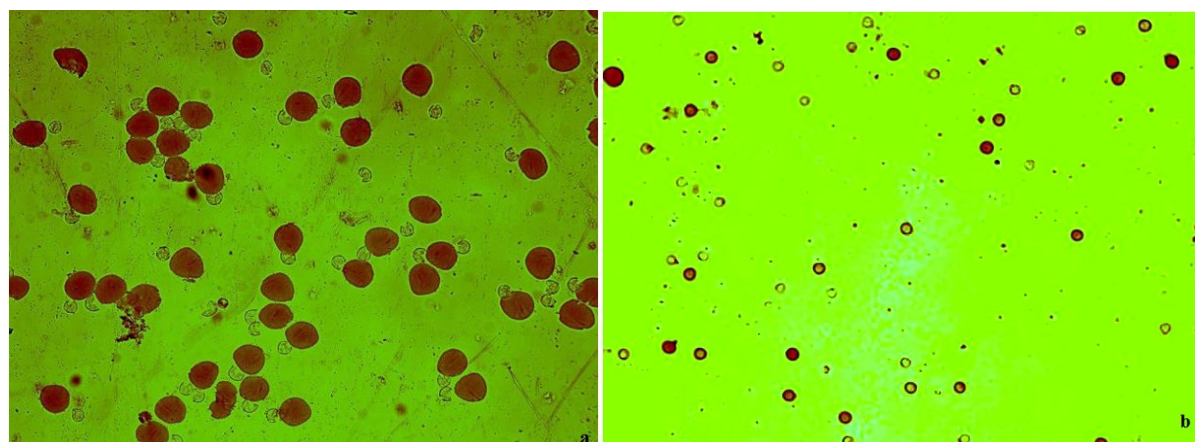


Fig. 7.4. Pollen viability of heat tolerant and sensitive chickpea genotypes: a, Heat tolerant wild progeny (9xCudiB_022C) and b, Heat sensitive wild progeny (9xGunas_063).



Fig.7.5. Flower and pod abortions and pod set of heat tolerant and sensitive F₃ wild progenies from the crosses of *Cicer arietinum* with *Cicer reticulatum* and *Cicer echinospermum* accessions. a. The most heat sensitive wild progeny that has zero pod set and all flowers were dried (9xS2Drd_107B), b. Moderately heat tolerant genotype with pod abortion, c. Dried and dropped flowers, d. A dropped pod that showed color changes (becomes yellow), e. Heat tolerant genotype (9xSavur_063) with high percentage of pod set, f. Heat tolerant genotype (9xOyali_084) having double podding feature and pod set, g. High pod set of 9xCudiB_22C.

7.3.2. Quantification of pollen cells per flower

By using haemocytometer, the total numbers of pollen cells per flower were determined. The lines, landraces and varieties of chickpea have shown wide range of variations for the numbers of pollen cells per flower (6,800-40,000) compared to F₅ segregations of wild progenies of chickpea (12,000-34,400). Among the 23 lines, landraces and varieties of chickpea, heat tolerant

genotypes namely JG14 (standard tolerant check, 40,000), 209102 (25,700), DZ-2012-ck-0034 (24,200), and Minjar (21,000) contained large number of pollen cells per flower, while the sensitive genotypes such as 41114 (6,800), DZ-2012-ck-0026 (11,300), Habru (7,400) and ICC 4567 (12,700) contained low numbers of pollen cells per flower (Table 7.1). Among F₅ segregating wild progenies of chickpea 9xCudiA_152A, 9xBesev_079, 9xBari2_072n2, 9xSavur_063, 9xKalkan_064 and 9xKesen_075 contained comparable quantity of pollen cells per flower as the tolerant lines, landraces and varieties. The mean values of the pollen cells per flower of the segregating genotypes (23,298.7) were higher than the landraces, lines and varieties (17, 343.5) (Table 7.1). ANOVA revealed highly significant difference ($P \leq 0.001$) among the lines, landraces and varieties for the trait (Table 7.2).

7.3.3. *In vitro* pollen germination

In vitro pollen germination was one way of assessing pollen viability of chickpea genotypes under heat stress environment. The mean values of *in vitro* pollen germination for segregating wild progenies (30.0%) were higher than the lines, landraces and varieties (23.5%). Among 23 lines, landraces and varieties, the heat tolerant genotypes namely DZ-2012-ck-0034 (42.9%) and JG 14 (41.1%) contained high *in vitro* pollen germination rate, while the sensitive genotypes such as DZ-2012-ck-0026, ICC4567, 41114, 41117, and Habru contained low *in vitro* pollen germination rate. Lines, landraces and varieties had wide ranges of *in vitro* pollen germination (6.7%-42.9%) than F₅ wild progenies of chickpea (21.6%-36.3%). The maximum *in vitro* pollen germination of segregating progenies was 36.3%, which was less than the tolerant lines, landraces and varieties. The minimum *in vitro* pollen germination of F₅ wild progenies of chickpea were 3-fold greater than the minimum *in vitro* pollen germination of lines, landraces

and varieties (Table 7.1). ANOVA revealed that there was highly significant difference among lines, landraces and varieties for *in vitro* pollen germination (Table 7.2).

All the *in vitro* pollen germinations were conducted in a liquid media containing 10% sucrose; however, pollen cells that have grown at 15% sucrose concentration became shrinking and bursting. As depicted in Fig 7.6b, the normal shape of pollen cells was round or triangular. The heat tolerant genotype, 9xSavur_063 had long pollen tube growth than 9xBesev_079, 9xEgill_073 and sensitive genotypes, which correspond to the percentage of pod set and yield related traits in heat stressed field environments (ICRISAT and Werer) (Fig 7.6).

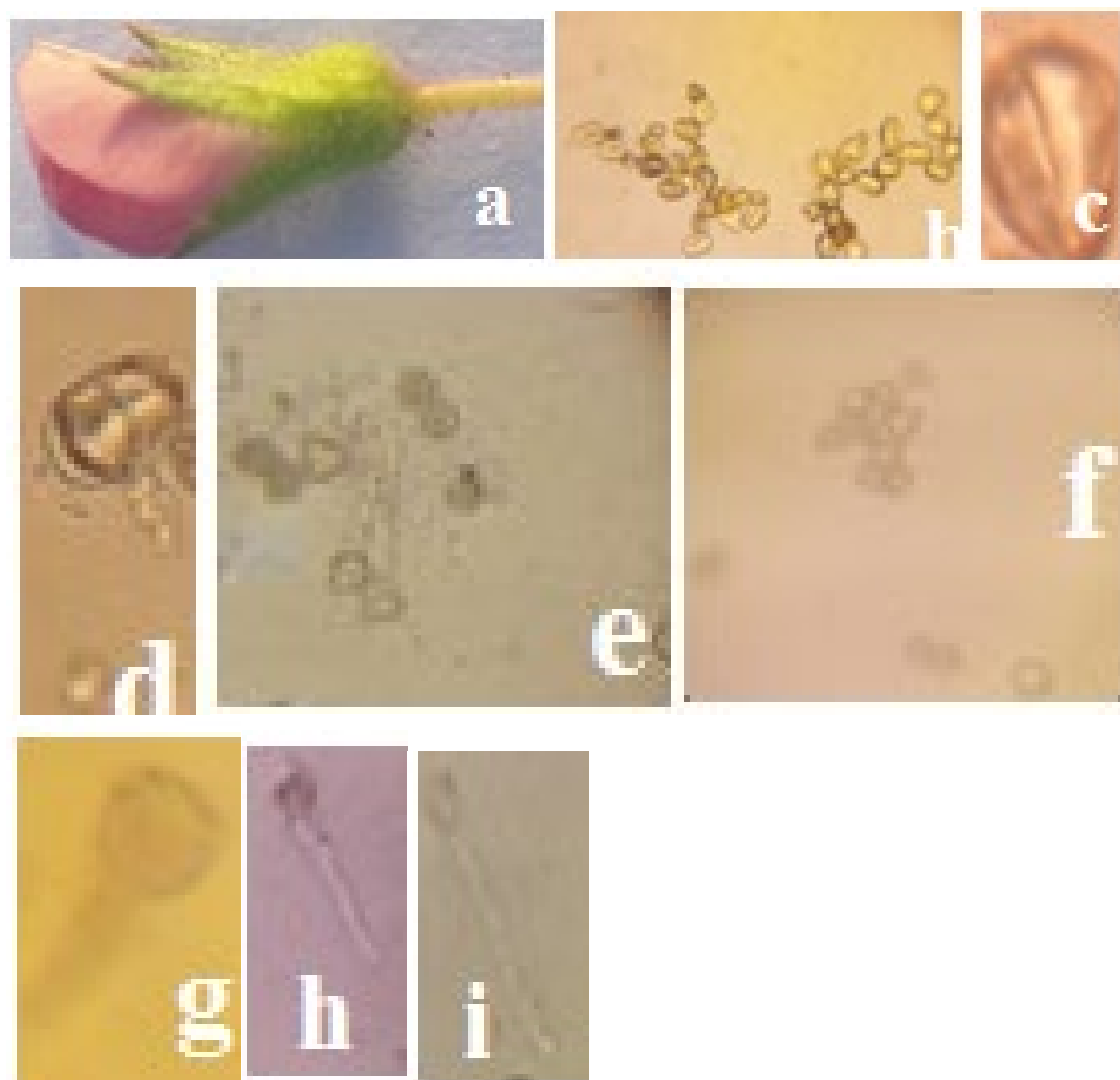


Fig.7.6. *In vitro* pollen germinations of chickpea accessions using 10% sucrose. a. A half opened flower used for the study of reproductive traits; b. Actual shape of pollen cells (round or triangular); c. Ungerminated pollen cell of 9xBesev_079_F5; d. Germinated pollen cell of 9xBesev_079_F5; e. Heat tolerant pollen cells of 9xSavur_063; f. Pollen cells of a heat sensitive genotype; g. Pollen tube growth of a landrace 212589; h. Pollen tube growth of 9xEgill_073_F5; i. Pollen tube growth of 9xSavur_063_F5.

7.3.4. Percentage of pod set

The average pod set of F₅ wild progenies (60%) were greater than the corresponding values of lines, varieties and landraces (43.7%). The later genotypes had wide range of pod set (0-90%) than F₅ wild progenies of chickpea (30-80%), implied that the landraces, lines and varieties had variable level of tolerance (highly sensitive to highly tolerant genotypes). The heat tolerant genotypes such as 209102 (90%), JG 14 (80%), DZ-2012-ck-0034 (80%), 9xBesev_079 (80%), 9xKalkan_064 (70%), and 9xKesen_075 (70%) had high pod set, while sensitive genotypes such as DZ-2012-ck-0026 (0%) and ICC 4567 (10%) had low pod set. Most of the segregating wild progenies resist the extreme heat stress at Werer experimental sites than the lines, landraces and varieties of chickpea (Table 7.1). ANOVA revealed that there was highly significant difference ($P \leq 0.001$) among lines, landraces and varieties of chickpea for the %PS (Table 7.2).

Table 7.1. The performance of chickpea accessions for three reproductive traits under heat stressed environment (Werer).

Genotype	PC/F	%PG	%PS	Parentage	PC/F	%PG	%PS
41110	17250.0	30.0	40.0	9xBari1_092	16955.5	25.21	50
41037	17000.0	19.5	40.0	9xBari2_072n2	28550	21.6	30
41052	17000.0	17.4	40	9xBari3_106D	20116.7	36.3	55
41114	6800.0	14.3	30.0	9xBesev_079	29800	30	80
41117	16650.0	14.2	25.0	9xCudiA_152A	34400	31.3	60
41144	15700.0	25.1	30.0	9xDerei_070	27950	29	40
207673	16000.0	22.5	30.0	9xEgill_073	13220	35	50
207657	14000.0	24.6	65.0	9xKalka_064	23350	26.8	70
209102	25700.0	29.7	90.0	9xKesen_075	23087.5	34.4	70
209109	14800.0	16.7	50.0	9xOyali_084	12000	31.2	50
209016	11600.0	17.7	40.0	9xSavur_063	26856.7	29.68	60
209110	15600.0	18.9	40.0	Mean	23298.7	30.0	60
212589	7200.0	40.0	50.0	Min	12000	21.6	30

235394	16300.0	31.7	40.0	Max	34400	36.3	80
ICC 4567	12700.0	15.5	10.0				
JG 14	40000	41.1	80.0				
ICCV96029	32200.0	15.0	65.0				
DZ-2012-ck-0026	11300.0	6.7	0.0				
DZ-2012-ck-0034	24200.0	42.9	80.0				
DZ-2012-ck-0074	11600.0	20.0	10.0				
DZ-2012-ck-0230	12800.0	25.8	60.0				
Habru	7400.0	14.6	50.0				
Minjar	21000.0	32.5	40.0				

%PS, percentage of pod set, PC/F, pollen cells per flower and %PG, percentage of *in vitro* pollen germination.

7.3.5. Correlation of traits

There was significant ($P < 0.05$) and positive correlation between pollen cells per flower and *in vitro* pollen germination. Pod set and *in vitro* pollen germination were strongly ($P < 0.001$) and positively correlated traits (Table 7.2). In addition, the *in vitro* pollen tube growth was positively correlated with pod set (Fig 7.6).

7.3.6. Variance, heritability and genetic gain

Irrespective of the type of chickpea accessions used in this study, the three reproductive traits had high phenotypic variance, coefficient of genotypic and phenotypic variances, broad sense heritability and genetic advance as percentage of the mean. These implied that the three traits are useful for the identification and selection of heat tolerant and sensitive chickpea genotypes (Table 7.3).

Table 7.2. Analysis of variance, mean, minimum, maximum and Pearson correlations of reproductive traits for lines, landraces and varieties under heat stress environment (Werer).

ANOVA				Mean, Maximum, Minimum and SD				Pearson correlations		
Trait	Gen MS	Rep MS	Block(Rep) MS	Trait mean	SD	Min	Max	PC/F	%PG	%PS
PC/F	142, 403, 939***	9, 438, 794 ^{ns}	10,210, 968 ^{ns}	17,343.50	8261	6800	40,000	PC/F	0.44*	0.6262**
%PG	183.3***	7.9 ^{ns}	7.6 ^{ns}	23.5	9.67	6.7	42.9	%PG		0.654***
%PS	1068.4***	2.4 ^{ns}	2.4 ^{ns}	43.7	22.6	0	90	%PS		

Gen, genotype, Rep, replication, MS, mean square error, SD, standard deviation, PC/F, pollen cells per flower, %PG, percentage of *in vitropollen* germination, %PS, percentage of pod set, ns, not significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$.

Table 7.3. Variance components, heritability and genetic gain of genotypes for reproductive traits.

Lines, landraces and varieties at reproductive stage								
Trait	EV	GV	PV	%GCV	%PCV	H ² b%	GA	GAM%
PC/F	20, 141,311.5	58,960,862.40	79,102,173.90	44.3	51.3	74.5	1364953	78.7
%PV	8.2	89.4	97.6	40.2	42	91.6	1864.2	79.3
%PS	6.5	508.9	515.4	51.6	52	98.7	4615.9	105.6
F ₅ Segregating populations								
Trait	EV	GV	PV	%GCV	%PCV	Hb ² %	GA	GAM%
PC/F	31,294,250.5	42,801,958.3	74,096,208.8	28.1	37	57.8	1,024,927	44.0
%PV	0.19	20.8	20.99	15.1	15.2	99.1	935.3	30.96
%PS	0.82	69.7	70.52	15	15	98.8	1,709.2	30.6

PC/F, pollen cells per flower, %PG, percentage of *in vitropollen* germination, %PS, percentage of pod set; EV, environmental variance, GV, genotypic variance, %GCV, coefficient of genotypic variance, % PCV, coefficient of phenotypic variance, H²b, broad sense heritability, GA, genetic advance, GAM, genetic advance as percentage of the mean.

7.4. Discussion

Number of pollen cells per flower, pollen viability, *in vitro* pollen germination, and pod set are reproductive traits, which are essential for accurate screening and identifying of heat and drought tolerant genotypes in many crop plants in the field and/or under control conditions. Heat stress negatively affects these reproductive functional traits (Devasirvatham *et al.*, 2013; Bagha, 2014; Naveed *et al.*, 2014; Jiang *et al.*, 2017; Patriyawaty *et al.*, 2017). In the present study, highly significant difference ($P < 0.001$) was found among the chickpea genotypes under heat stressed environment for total numbers of pollen cells per flower, pollen viability, *in vitro* pollen germination, and pod set traits. Similar to the report by Tsegaye Getahun *et al.* (2021b), unlike in non-heat stress environments, there was significant reduction in the percentage of pod set under heat stressed environment which was associated with reduced pollen viability and pollen germination.

Similarly, Patriyawaty *et al.* (2018) found highly significant difference ($P < 0.001$) among mungbean genotypes for pollen viability trait. Devasirvatham *et al.* (2013) reported that under high temperatures ($\geq 35^{\circ}\text{C}$), significant variation was observed among chickpea genotypes for pollen viability. Besides, Naveed *et al.* (2014) reported significant differences for percentage of pollen viability, pollen production, and percentage of *in vitro* pollen germination traits among maize accessions. Jiang *et al.* (2017) reported that cultivars differed significantly for *in vitro* germination of their pollen at high temperatures but not for pollen tube length of germinated grains at 36°C . In line with the present study, Patriyawaty *et al.* (2018) and Naveed *et al.* (2014) explained that these highly significant reproductive functional traits are important to understand

the underlying mechanism for heat tolerance and provided wide range of option for selection and opportunity to improve tolerance level against heat stress.

Under non-stressed conditions, in *Senna pendula* (Fabaceae family), a flower produced an average of 53,187.5 pollen cells per flower which ranged from 33, 928.6 to 72,446.4 (Pinheiro-Costa *et al.*, 2018). The average pollen cells per flower in *Collinsia heterophylla* and *Brassica napus* were found to be 56,461 and 34,905, respectively (Mudd and Arath, 2012). Nevertheless, drought and heat stress conditions decreased the numbers of pollen cells per flower in plants (Devasirvatham *et al.*, 2013; Naveed *et al.*, 2014; Nayyar *et al.*, 2107). In the present study, the average numbers pollen cells per flower for heat tolerant and sensitive chickpea genotypes were 40,000 and 6, 800, respectively under heat stress condition. Likewise, based on the genetic pattern and/or environmental growing conditions of the quince cultivars, Dalkiliç and Mestav (2011) revealed that the pollen cells per flower ranged from 20,063 to 11,906 pollens/flower with haemocytometer.

Heat tolerant genotypes had higher pollen functionality since these were able to minimize their cellular damage unlike in heat-sensitive genotypes, consequently the heat tolerant genotypes showed significantly high pod set (Devasirvatham *et al.*, 2012; Kumar *et al.*, 2012). In the present study, at an extremely heat stress environment (Hyderabad), the pollen cells of heat tolerant chickpea genotypes deeply stained with acetocarmine than sensitive genotypes which were weakly stained and/or white. At Werer experimental site, heat tolerant genotypes had high *in vitro* pollen germination (42.9%) and pod set (80%) than sensitive genotypes (6.8%, 0-10%) (Fig 7.2). Similarly, Devasirvatham *et al.* (2012) revealed that pollen grains of heat tolerant

genotype of chickpea were deeply stained with acetocarmine at $\geq 35^{\circ}\text{C}$; however, the sensitive genotypes became weakly stained or white (sterile). Pollen sterility in the heat-sensitive genotypes is a function of lower sucrose levels, resulting in poor pollen function and pod set (Kaushal *et al.*, 2013).

In addition, Devasirvatham *et al.* (2012, 2013) reported that pollens of heat tolerant genotypes contained 41% of pollen fertility and 41-65% pod set, while pollens of sensitive genotypes were almost sterile with near zero pollen fertility and contained 1-4% pod set at the same temperature. In field pea, the highest reduction of pollen viability and *in vitro* pollen germination was found in heat sensitive genotypes (31.1-87.8%). The small reduction in pollen viability and *in vitro* pollen germination values was observed in tolerant genotypes (21.0%-52.2%). The highest (76.1-82.0%) and the lowest (27.9%-35.7%) reduction of fruit set were found in sensitive and tolerant genotypes, respectively (Jiang *et al.*, 2017). High temperatures reduced pod set by reducing pollen viability, *in vitro* pollen production per flower and pollen tube growth under heat stressed condition at Hyderabad (ICRISAT) (Devasirvatham *et al.*, 2013). The percentage of *in vitro* pollen germination and tube growth exactly reflects the percentage of pod set under high temperature (Devasirvatham *et al.*, 2013) which was in line with the present study. Heat stress significantly inhibited the sucrose metabolism in leaves, anthers and seeds leading to decreased pollen function, seed filling and size, and fruit set (Frion *et al.*, 2006; Devasirvatham *et al.*, 2012).

In the present study, the number of pollen cells per flower, *in vitro* pollen germination, and pollen viability was positively correlated with pod set and other yield related traits. Likewise, at

high temperature (35/18°C), *in vitro* pollen germination in field pea was positively associated with seed retention, number of seeds per pod, and seed to ovule ratio (Jiang *et al.*, 2017). There is a strong correlation between pollen production and viability, anther dehiscence, and seed-set in tomato (Firon *et al.*, 2006).

The sterility of the pollen grains in heat sensitive chickpea genotypes might be due to the effect of heat stress at meiosis or early pollen development stage. Likewise, Bagha (2014) described that high temperatures ($\geq 32^{\circ}\text{C}$) impaired anther dehiscence in heat sensitive rice cultivars either by preventing septum cell wall degradation, which is essential for pollen dispersal and induced abortion in pollen development during meiosis primarily, or at the uninucleate stage. Abortion during meiosis was associated with autophagic-programmed cell death, whereas failure at the uninucleate stage of pollen development was associated with features of necrosis such as cytoplasmic shrinkage and cell wall collapse (Bagha, 2014).

7.5. Conclusions

Half-opened flower is the best stage to study reproductive traits. The present study has revealed that heat stress reduces number of pollen cells per flower, *in vitro* pollen germination, and pollen size, induces pollen sterility, causes abnormal pollen development, anther indehiscence, flower abortions, pod drops and poor pod set, formation of false (small) pods and incompletely developed seeds. As a consequence, fertilization impaired and post fertilization processes disrupted. The responses of reproductive traits under heat stress conditions were dependent upon the extent and duration of a stress, types of genotypes and reproductive stages. At heat stressed environments, unlike heat stress genotypes, heat tolerant genotypes were characterized by high

pollen number, *in vitro* pollen germination, normal pollen size, deeply stained pollen with acetocarmine, less damage to pollen functions, normal and, normal seed size, high pod set, large numbers of seeds and grain yield per plant. This is justified as the heat tolerant genotypes possessed high carbohydrate contents during early to mature pollen developments, and proper anther dehiscence and tapetal layer development to withstand heat stress.

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CHAPTER EIGHT: Overall Conclusions and Recommendations

8.1. Overall conclusions

High genetic variability and large numbers of an extremely heat stress ($>40^{\circ}\text{C}$) tolerant pre-breeding lines were found from wild-cultivated hybrids which are absent in the landraces and cultivated varieties of chickpea. This might be due to the introgressions of wild genes from 20 wild founders into the genetic background of an early flowering elite cultivar (ICCV 96029). Knowledge of the genomic basis of traits that segregate in wild-cultivated hybrid populations will facilitate chickpea to identify genomic regions (QTLs) for several traits under heat stress environment by linking genetic and phenotypic variation in a quantitative genetic framework. A total of 230 QTLs were detected for 16 traits in 19 families which explained a phenotypic variance of 3-93%.

A total of 572 natural alleles were detected from 46 SSR loci across 118 chickpea genotypes. The presence of high genetic diversity within populations, wide range of allele sizes, large numbers of alleles per locus and unique alleles per population, high polymorphic information content and expected heterozygosity have breeding implications to the national chickpea improvement program: help to enhance yield and improve specific traits such as heat, cold and drought through crossing of genetically distant genotypes.

Choosing an appropriate experimental field sites and planting dates under heat stressed environments and characterizations of phenological and agronomic traits are crucial to select and identify heat tolerant genotypes. Understanding genome-wide relationships among genotypes via SNP markers helps to assess the clustering patterns of genotypes and their relationships. Heat stress is a function of plant genotypes, high temperatures, plant phenology and soil types. Grain

yield, total number of pods and seeds per plant, and pod set are the best traits for screening of heat tolerant and sensitive genotypes under heat stressed environments. PCoA analysis using 5,722 SNPs has found five different clusters within the landrace group. Each genomic cluster might possess specific adaptive genes to their respective geographical locations.

MultispeQ is a high throughput phenotyping tool that combines a fluorimeter, a chlorophyll meter, and a spectrometer into one which is used to measure several physiological traits simultaneously which has enabled large-scale, rapid and accurate data collection under controlled and field conditions and boosted the power of trait-based crop breeding “physiological breeding”. The energy allocation ratios of MultispeQ instrument depend upon environmental conditions, type of genotypes, colored lights, photosynthetic pathways, the day from morning to late afternoon, and the color of the leaves. Under heat stressed environment, the physiological responses of heat tolerant and sensitive genotypes were the same as the agro-morphological responses. In heat tolerant genotypes 60-68% of the absorbed photons were used to carryout photochemical reactions (photosynthesis) and 32-40% were dissipated as heat, while in sensitive genotypes only 30% of the absorbed photons were used for photosynthesis and 70% were dissipated as heat.

Half-opened flower is the best stage to study reproductive traits. Heat stress reduces the number of pollen cells per flower, *in vitro* pollen germination and pollen size, induces pollen sterility, causes abnormal pollen development, anther indehiscence, flower abortions, pod drops and poor pod set, formation of false (small) pods and incompletely developed seeds. As a whole, it impaired fertilization and post fertilization processes. The responses of reproductive traits under

heat stress conditions were dependent upon the extent and duration of a stress, types of genotypes and reproductive stages. At heat stressed environments, unlike heat stress genotypes, heat tolerant genotypes were characterized by high pollen number (40,000), *in vitro* pollen germination (42.9%), normal pollen size, deeply stained pollen with acetocarmine, less damage to pollen functions, normal and long pollen tube growth, normal seed size, high pod set (60-90%), large numbers of seeds (40-60) and grain yield (4g) per plant. This is justified as the heat tolerant genotypes possessed high carbohydrate contents during early to mature pollen developments, and proper anther dehiscence and tapetal layer development to withstand heat stress.

In heat stressed environments both in Ethiopia and in India, we have observed several phenotypic plasticity responses from chickpea plants grown under heat stressed environments unlike under non-heat stressed environment. These might be due to the cumulative effects of morphological, anatomical, physiological, biochemical and molecular changes under high temperature stresses ($\geq 35^{\circ}\text{C}$). Based on the above findings the following recommendations have been suggested.

8.2. Recommendations

- We recommend ICRISAT/USAID feed the future chickpea innovation lab to further screen the F_4 segregating populations using a single plant selection method until F_6 - F_8 generations to get homozygous heat tolerant genotypes. We also recommend conducting backcrossing of some of the pre-breeding lines with the elite cultivar (ICC 96029) to fix important agronomic characters.

- We recommend Debre Zeit Agricultural Research Center to include the identified Ethiopian heat tolerant advanced line, DZ-Cr-0034, in a national variety trial for heat stress environments.
- We recommend crossing of the contrasting Ethiopian advanced lines (parents) (DZ-Cr-0034 and DZ-Cr-0026) for QTL mapping, identification of candidate genes and markers for marker assisted breeding.
- We also recommend crossing of the heat tolerant genotype, DZ-Cr-0034, with a high yielding elite genotypes to introgress heat resilient genes.
- We have submitted 50 F₆ wild-cultivated hybridlines to Debre Zeit Agricultural Research Center and we recommend it to include them in its breeding schemes as a source of enriched gene pool to overcome climate resiliences such as drought, cold, heat, water logging and salinity in the country.
- From the chickpea passport data found in EBI, we have received less than 100 chickpea landraces collected from lowland and mid altitudes that were used in the present study. We recommend EBI to collect large number of landraces from lowland areas, which are ideal for climate resilience in the Ethiopian lowland farming system.
- Selected chickpea accessions should have been screened under greenhouse and growth chamber to compare their performance under heat stressed fields.
- In order to understand the molecular mechanisms of heat tolerance, transcriptomics, proteomics, and metabolomics should have been conducted by using contrasting genotypes.

- For getting very reliable genomic resources such as QTLs, molecular markers and candidate genes, recombinant inbred lines (RILs) and/or homozygous NAM populations should be used instead of F₂ and F₃ segregating populations.
- Validations of markers and QTLs (genomic regions) for some heat traits should have been done using KASP.
- Developing countries should have well protected and managed field environments to conduct heat experiments.
- There should be conducive environments to conduct some physiological traits (Pollen tube growth, stigma receptivity, etc) and biochemical traits to further understand the mechanisms of heat resilience.

Appendices

Appendix 3.1. Summary statistics for phenotypic data analyses of the 20 biparental populations.

9xBari1_092										9xBari2_072n2										
Trait	Mean±SD	V	Sk	KU	Min	Max	R	MS	H²	LSD	Mean±SD	V	SK	KU	Min	Max	R	MS	H²	LSD
DFF	46.1±8.1	65.2	-0.5	0.2	26.0	60.0	34.0	130.5***	87.3	11.2	42.4±9.0	80.3	0.1	0.4	27.0	63.0	36.0	164.3***	89.7	11.7
DFF	51.8±7.8	60.8	-0.6	0.2	32.5	64.5	32.0	121.7***	86.6	11.1	49.3±8.2	68.0	0.3	0.4	35.0	68.0	33.0	143.4***	81.5	14.6
DPM	88.7±11.8	139.8	-0.3	-0.1	61.5	111.5	50.0	279.7***	86.2	17.1	82.7±12.1	146.8	0.5	0.9	62.0	113.0	51.0	277.3***	87.2	16.9
PT	1.7±0.7	0.5	0.6	-0.8	1.0	3.0	2.0	1.0 ^{ns}	22.7	2.5	1.4±0.6	0.4	1.2	0.5	1.0	3.0	2.0	0.68 ^{ns}	15.4	2.1
HVS	2.6±1.1	1.3	0.6	-0.5	1.0	5.0	4.0	2.4***	86.9	1.6	2.9±0.9	0.7	0.2	-1.0	1.5	4.5	3.0	1.4 ^{ns}	40.1	2.6
GH	2.9±0.9	0.7	0.6	0.9	1.0	5.0	4.0	1.4***	87.7	1.2	2.5±0.7	0.5	1.3	0.5	2.0	4.0	2.0	0.98**	71.4	1.5
%PS	49.9±15	225.2	0.0	-0.4	19.2	80.0	60.9	242.9***	72.2	30.9	44.2±12.5	156.3	-0.4	-1.1	22.5	62.9	40.4	297.8*	53.6	33.3
PHT	23.3±3.7	14.0	0.5	-0.3	17.3	31.4	14.2	27.9 ^{ns}	28.2	12.5	25.9±5.2	27.0	0.5	-0.7	18.5	36.6	18.1	52.1*	64.4	12.4
FPP	31.2±14.9	222.6	0.7	0.3	7.4	72.3	64.9	445.8 ^{ns}	26.5	50.4	25.0±13.8	190.3	0.3	-0.8	5.5	51.9	46.4	610.1 ^{ns}	9.1	67.8
UPP	3.0±1.8	3.3	0.9	0.8	0.3	8.3	8.0	6.6 ^{ns}	26.6	6.1	1.7±0.7	0.5	0.5	-0.3	0.5	3.2	2.7	2.2 ^{ns}	0	5.8
TPP	34.3±15.7	246.1	0.5	0.0	7.7	75.1	67.4	492.7 ^{ns}	18.2	56	26.5±13.8	190.3	0.4	-0.9	7.5	52.9	45.4	651.8 ^{ns}	6.2	71.2
TSP	31.8±15.2	232.5	0.4	-0.3	6.4	68.2	61.9	465.2 ^{ns}	20.3	53.7	26.5±16.3	264.2	0.7	0.0	3.2	64.8	61.6	735 ^{ns}	19.6	70
HSW	9.9±1.2	1.3	0.3	-0.4	8.1	12.5	4.4	2.6*	51	3.1	10.8±1.3	1.8	-0.9	0.5	7.4	12.5	5.1	4.7 ^{ns}	30.2	5.2
PBP	11.1±4.3	18.8	0.3	-0.8	4.3	20.8	16.5	36.7 ^{ns}	43.2	12.7	11.9±5.8	33.9	0.8	-0.4	3.7	23.3	19.6	61.1 ^{ns}	0	24.4
GYP	3.3±1.6	2.6	0.4	-0.5	0.6	6.7	6.1	5.2 ^{ns}	21	11.9	2.9±1.7	3.0	0.5	-0.4	0.4	6.5	6.1	7.4 ^{ns}	11.5	7.4
PHI	28.5±9.3	85.9	0.3	-0.3	9.1	45.5	36.4	158 ^{ns}	6.2	33.9	24.0±11.6	134.3	0.3	-0.5	5.9	46.4	40.5	239 ^{ns}	39.5	34.6
9xBari3_100										9xBari3_100D										
DFF	48.5±9.3	86.5	-0.4	-0.4	29.0	64.5	35.5	166.1***	83.7	14.7	45.8±12.3	152.3	0.5	-0.5	27.5	73.5	46.0	229.3***	87	17.7
DFF	54.2±9.6	91.6	-0.5	-0.5	35.0	69.5	34.5	169.4***	80	16.4	52.6±12.8	163.0	0.6	-0.6	34.0	79.0	45.0	314.4***	88.4	17.2
DPM	90.8±14.2	203.0	-0.4	-0.3	61.0	115	54	388.0***	74.1	28.3	88.5±17.1	291.7	0.4	-1.1	60.5	120.0	59.5	556.8***	82.8	27.7
PT	1.6±0.8	0.6	0.7	-0.9	1.0	3	2	0.9 ^{ns}	33	2.3	1.4±0.6	0.4	1.0	-0.2	1.0	3	2.0	0.7 ^{ns}	10.2	2.3
HVS	3.2±1.0	1.0	0.5	-0.8	2.0	5	3	1.6***	74.7	1.9	2.6±0.9	0.9	0.7	-0.7	1.5	4.5	3.0	1.7*	50.8	2.6
GH	3.2±0.7	0.5	-0.7	1.0	1.0	4	3	0.8 ^{ns}	44.2	2	2.4±0.8	0.7	0.3	-0.5	1.0	4.0	3.0	1.3*	73	1.7
%PS	39.7±14.6	213.1	-0.7	0.1	0.0	60	60	374.4***	75.9	28.1	45.9±13.5	181.1	0.0	-0.3	17.5	75.0	57.5	362.6 ^{ns}	44.6	40.2
PHT	21.8±5.0	24.7	1.2	3.4	13.3	39.3	26	31 ^{ns}	49.8	12	24.3±4.7	22.4	0.5	-0.3	16.5	35.2	18.8	44.6*	68.2	10.7
FPP	21.0±13.1	171.9	1.0	1.1	0.0	59.7	59.7	294.6 ^{ns}	0	66	23.8±12.1	146.7	0.4	-0.4	3.5	50.0	46.5	267.8 ^{ns}	0	50.4
UPP	2.0±1.7	2.8	1.7	3.2	0.0	7.8	7.8	5.1 ^{ns}	0	6.9	2.0±1.0	1.1	0.3	-0.8	0.3	4.0	3.8	2 ^{ns}	0	4.6
TPP	22.8±13.7	188.1	0.9	0.9	0.0	62.2	62.2	319.8 ^{ns}	0	68.4	25.5±12.9	167.4	0.3	-0.4	3.9	53.1	49.3	304.8 ^{ns}	0	53.4
TSP	21.6±15.3	235.0	1.5	2.8	0	73.4	73.4	405.8 ^{ns}	0	76.9	25.2±14.8	220.2	1.0	1.3	3.9	67.3	63.5	350.2 ^{ns}	10.2	50.3
HSW	9.8±2.4	6.0	-2.4	7.1	0	12.8	12.8	7.3 ^{ns}	46.7	6	9.9±1.8	3.1	-0.5	0.4	5.4	12.9	7.6	5.8 ^{ns}	29.5	5.7
PBP	9.6±4.4	19.2	0.5	-0.1	1	19.8	18.8	32.7 ^{ns}	0	21.5	12.0±6.2	37.9	1.6	3.3	4.0	32.7	28.7	56.4 ^{ns}	0	21.8
GYP	2.3±1.6	2.5	1.4	1.8	0	7.15	7.15	4.4 ^{ns}	0	7.2	2.6±1.6	2.7	1.1	1.1	0.4	7.3	6.9	4.3 ^{ns}	40.4	4.5
PHI	22.0±10.6	113.3	0.4	0.1	0	48.6	48.6	149.7 ^{ns}	4	36.5	21.4±8.6	74.6	0.5	-0.6	8.2	38.7	30.5	149 ^{ns}	33.4	28.2
9xBeseve-079										9xCudia_152										
DFF	44.9±9.5	91.1	0.0	-0.6	28.0	63.5	35.5	182.2 ^{ns}	96.3	7.2	48.2±6.5	42.5	0.5	-0.9	39.3	62.0	22.8	85**	62	15.8
DFF	50.9±9.9	97.9	0.0	-0.9	33.5	67.5	34.0	195.8 ^{ns}	94.7	9	53.8±7.2	52.0	1.2	0.9	45.4	74.5	29.1	103.9**	63.8	17.1
DPM	86.1±14.0	197.3	0.1	-0.4	61.5	117.5	56.0	394.6***	88	19.1	92.0±9.7	94.9	0.5	-0.9	78.3	111.0	32.8	189.9 ^{ns}	46.5	28.1

PT	1.6±0.6	0.4	0.6	-0.6	1.0	3.0	2.0	0.7 ^{ns}	0	2.6	1.4±0.6	0.3	1.1	0.3	1.0	3.0	2.0	0.6 ^{ns}	0	2.4
HVS	3.0±0.9	0.8	0.3	-1.2	1.5	4.5	3.0	1.6**	68.5	2	2.8±1.1	1.2	0.2	-0.9	1.0	5.0	4.0	2.2**	66.7	2.4
GH	3.2±0.8	0.6	0.1	-0.3	2.0	5.0	3.0	1.1*	61.2	1.8	2.8±1.0	1.0	0.5	-0.2	1.0	5.0	4.0	1.8 ^{ns}	51	2.7
%PS	41.6±14.7	217.2	0.1	-0.5	15.0	72.5	57.5	423.8***	72.8	30.6	46.4±17.5	308.0	0.3	-0.5	17.5	85.0	67.5	525.8***	76.4	31.9
PHT	20.0±4.1	16.8	0.5	-0.5	13.0	28.3	15.3	816.9***	67.6	9.2	24.3±4.3	18.1	0.1	0.2	14.4	33.0	18.6	31.7 ^{ns}	0	17.1
FPP	22.5±12.7	160.2	0.8	-0.2	6.2	48.9	42.7	297.4 ^{ns}	17.9	45.1	22.7±13.4	180.8	1.0	1.0	5.4	62.1	56.7	350.7*	61.2	33.9
UPP	1.9±1.0	1.0	0.5	-0.7	0.1	4.0	3.9	1.9 ^{ns}	30	3.3	1.8±1.6	2.6	2.0	4.6	0.0	7.6	7.6	2.5 ^{ns}	0	5.9
TPP	24.4±13.8	189.8	1.0	0.2	6.8	59.0	52.2	354.2 ^{ns}	12.9	50.7	24.4±13.9	194.3	0.8	0.6	5.8	63.5	57.7	366.4*	54.7	37.4
TSP	25.8±16.9	284.1	1.2	1.2	6.7	70.8	64.2	532 ^{ns}	11.6	62.7	21.5±11.5	132.0	0.5	-0.5	5.5	48.5	43.0	264.6 ^{ns}	47	34.4
HSW	9.0±1.5	2.3	-1.1	1.9	4.5	11.5	7.0	117.2 ^{ns}	0	7.2	10.9±1.9	3.6	1.2	2.3	8.1	17.0	8.9	7.5 ^{ns}	21.7	7
PBP	8.5±3.6	13.2	0.4	-0.4	2.7	16.2	13.5	23.1 ^{ns}	16.7	12.7	12.0±5.4	29.6	0.2	-0.7	2.7	22.8	20.2	54.7 ^{ns}	31.9	17.7
GYP	3.6±5.7	32.2	4.2	17.3	0.6	30.5	29.9	32.9***	82.5	6.9	2.7±1.5	2.4	0.8	0.1	0.7	6.8	6.1	4.6 ^{ns}	5.8	6.1
PHI	25.8±10.1	102.8	0.2	-0.6	8.9	46.6	37.7	197.2 ^{ns}	24.7	35.2	24.5±10.9	118.3	0.3	-0.4	6.2	50.6	44.4	249.7 ^{ns}	8.6	43.9
9xCudiB_22C										9xDerei_070										
DFF	45.1±10.8	115.9	0.0	-1.2	28.5	61.5	33.0	223.5***	75.2	21.4	46.1±11	119.9	0.1	-0.9	28.5	63.5	35.0	239.9 ^{ns}	91.2	16.3
DFP	52.9±13.2	174.2	0.6	0.3	34.0	87.5	53.5	339.8***	84.4	20.6	53.1±12.6	158.9	0.4	-1.0	33.0	76.5	43.5	317.7 ^{ns}	89.1	16.3
DPM	87.1±16.4	270.1	0.0	-1.0	60.5	117.0	56.5	549.9**	72.5	34.7	87.3±15.8	250.2	0.3	-0.9	63.0	115.5	52.5	500.3 ^{ns}	89.4	20.1
PT	1.4±0.7	0.4	1.5	1.1	1.0	3.0	2.0	0.7 ^{ns}	42.6	1.9	1.4±0.6	0.3	1.2	0.3	1.0	3.0	2.0	0.6 ^{ns}	11	2.1
HVS	2.5±1.0	0.9	0.6	0.3	1.0	5.0	4.0	1.5**	75.9	1.8	2.6±1	1.0	1.0	0.2	1.5	5.0	3.5	1.9***	71.8	2
GH	2.4±0.9	0.8	1.2	1.5	1.0	5.0	4.0	1.5**	1.5	2	2.8±0.7	0.5	0.2	-0.1	1.5	4.5	3.0	1.0***	71.5	1.5
%PS	47.1±13.6	185.2	0.3	-0.3	20.0	75.0	55.0	351.1**	73.1	28.8	49.2±15.2	230.8	-0.2	-0.5	20.0	75.0	55.0	431.9*	58.1	37.9
PHT	24.4±3.8	14.2	0.5	0.2	18.2	33.6	15.4	31.1*	64.8	9.7	23.3±5	24.5	0.2	-0.6	14.3	32.7	18.4	46.7***	75.7	9.5
FPP	28.7±15.8	249.8	1.0	0.6	9.4	69.9	60.5	490.3*	55.3	43.4	24.2±13.7	186.7	0.6	-0.6	3.8	52.0	48.2	365.4 ^{ns}	10	51.1
UPP	1.8±0.8	0.7	0.8	0.2	0.6	3.8	3.3	1.3 ^{ns}	0	3.6	1.6±0.7	0.6	0.3	1.9	0.0	3.9	3.9	1.0 ^{ns}	0	4.1
TPP	30.6±16.3	266.5	1.0	0.6	10.5	73.9	63.4	520.9*	57.2	43.7	25.8±13.8	190.0	0.6	-0.6	4.4	53.6	49.2	370.8 ^{ns}	5.1	52.9
TSP	28.3±15.7	247.0	1.2	1.1	8.9	72.5	63.7	520.4*	57.5	43.5	23.0±11.7	136.8	0.4	-0.4	3.9	50.5	46.7	268.5 ^{ns}	0	46.3
HSW	11.8±1.9	3.5	0.1	-0.3	8.1	15.2	7.1	6.6**	66.3	4.4	9.8±1.9	3.7	-0.2	0.9	4.9	14.5	9.6	7.2 ^{ns}	31.8	6.3
PBP	12.8±4.9	24.3	0.4	-0.5	4.9	23.5	18.6	48.8 ^{ns}	44	15.3	10.5±4.6	21.5	1.2	1.8	3.2	25.2	22.0	42.4 ^{ns}	23.8	16
GYP	3.4±3.2	3.2	0.8	0.4	1.1	8.1	7.0	6.9 ^{ns}	41.7	4.3	2.4±1.3	1.7	0.7	0.0	0.4	5.7	5.4	3.3 ^{ns}	10.6	4.9
PHI	25.6±8.6	74.7	-0.2	-1.2	10.8	40.0	29.3	145.7 ^{ns}	29.1	29.8	22.5±8.9	78.4	0.2323	-0.6	5.4	39.4	34.0	151.2 ^{ns}	11.7	32.6
9xDerei_072										9xEgill_065										
DFF	47.8±10.4	109.2	-0.1	-0.1	26	67	41	218.3***	86	15.4	48.7±8.7	75.6	0.2	-0.2	32	65	34	151.2***	75.9	16.7
DFP	54.6±10.5	110.6	0.0	-0.6	34	72	38	221.2***	79.8	18.6	55.2±9.2	85.0	0.5	-0.1	38	75	37	170.1**	67.1	20.7
DPM	90.3±15.1	227.5	-0.3	-0.6	59	112.5	53.5	455***	85.7	22.5	92.3±13.5	181.9	0.4	-0.2	65	122	57	263.8***	77.6	25
PT	1.3±0.5	0.3	1.5	1.5	1	3	2	0.6 ^{ns}	14.6	2	1.6±0.7	0.5	0.8	-0.8	1	3	2	0.9 ^{ns}	25.5	2.3
HVS	3.5±1	1.1	0.2	-1.5	2	5	3	2.0**	66.4	2.4	2.9±1	1.0	0.6	-0.4	2	5	4	1.9***	70.7	2.1
GH	2.3±0.7	0.5	0.1	0.1	1	4	3	1.0*	60.8	1.8	2.8±0.7	0.4	0.4	-0.8	2	4	2	0.9 ^{ns}	32.3	2.2
%PS	34.8±15	223.6	-0.2	-1.3	10	60	50	414.3***	75.4	28.9	44.6±13.5	182.6	-0.4	-0.6	17	68	52	360.6**	67.2	30.9
PHT	23.8±4.7	22.3	-0.2	-0.1	12.5	33.35	20.85	37.7 ^{ns}	25.1	15.4	22.6±4.9	24.2	0.9	0.1	16	34	18	48.1***	72	10.4
FPP	14.5±9.6	93.1	0.5	-0.8	1.2	36.7	35.5	178.3 ^{ns}	10.3	36.7	23.0±11.6	135.1	0.5	-0.7	6	49	43	239.2 ^{ns}	10.7	41.5
UPP	1.5±0.7	0.6	-0.1	-0.8	0.2	2.9	2.7	1.0 ^{ns}	0	4.4	2.2±1.4	1.9	1.1	2.1	0	7	7	2.8**	63.6	2.9
TPP	16.1±10.2	104.3	0.5	-0.8	1.4	39.6	38.2	199.2 ^{ns}	20.4	36.6	25.1±11.5	133.2	0.3	-1.0	6	45	39	250.3 ^{ns}	5.3	43.7
TSP	14.4±10.3	105.6	0.6	-0.6	0.7	38.9	38.2	202.9 ^{ns}	9.4	39.4	22.4±10.9	118.0	0.3	-0.9	6	44	38	211.9 ^{ns}	11.2	38.9
HSW	11.6±1.6	2.5	0.6	-0.1	9.2	15.55	6.35	4.9 ^{ns}	0	11	10.8±1.5	2.4	0.2	-0.2	8	15	7	4.5 ^{ns}	26.4	5.1

PBP	9.7±4.1	17.1	0.6	0.4	2.1	20.75	18.65	31.7 ^{ns}	0	23.5	11.5±4.5	20.5	0.3	-0.1	4	23	19	38.3 ^{ns}	0	19.2
GYP	1.7±1.1	1.3	0.4	-0.9	0.1	4	3.9	2.4 ^{ns}	0	5	2.5±1.3	1.7	0.4	-0.7	1	6	5	2.9 ^{ns}	14.6	4.5
PHI	16.6±9.5	89.6	0.2	-1.3	2.8	32.15	29.35	171 ^{ns}	43.5	28.5	21.3±8.1	66.2	-0.2	-0.8	7	36	29	128 ^{ns}	45.9	23.6
9xEgill_073										9xKalkan_064										
Trait	Mean±SD	V	SK	KU	Min	Max	R	MS	H ²	LSD	Mean±SD	V	SK	KU	Min	Max	R	MS	H ²	LSD
DFP	47.6±8	58.8	0	-1	33.0	58.5	25.5	117.6***	73.9	15.4	45.0±13.4	180	-0.1	-1.1	27.0	67	40	292.3***	94	12.2
DFP	54.1±8	71.7	0	-1	37.8	67.5	29.8	143.4***	71.3	17.8	50±13.5	182	0.2	-0.8	32.0	75	43	331.8***	94.2	12.8
DPM	89.5±13	164.5	0	-1	57.8	107.8	50.0	329.1**	67.5	28.7	86.4±16.8	281	-0.2	-1.3	60.0	109	49	488.4***	92.1	18.1
PT	1.7±1	0.6	1	-1	1.0	3.0	2.0	1.1 ^{ns}	43.2	2.3	2±0.9	1	0.5	-1.4	1.0	3	2	1.2 ^{ns}	45.4	2.4
HVS	2.6±1	1.0	0	0	1.0	5.0	4.0	1.9***	79.9	1.8	2±0.9	1	1.4	0.8	1.0	4	3	2.7***	80.7	2.1
GH	2.5±1	0.7	0	0	1.0	4.0	3.0	1.4***	77.2	1.6	2.0±0.6	0	1.4	1.1	2.0	4	2	0.7**	72.5	1.3
%PS	47.1±15	213.9	0	0	15.0	77.5	62.5	425.6***	83.3	24.3	66±17.7	314	-0.5	0.0	30.0	95	65	813*	66.7	49.2
PHT	24.3±4	19.6	0	-1	17.5	33.8	16.4	34.2*	54.6	11.4	26±6.7	45	0.3	0.1	14.8	40.1	25.4	76.6**	74.3	13.3
FPP	25.1±14	201.1	1	1	1.6	63.1	61.6	393.6 ^{ns}	42.2	43.6	42±14.9	222	-1.2	0.5	7.9	58.6	50.7	559.8 ^{ns}	0	78.6
UPP	2.5±1	2.2	1	2	0.5	7.2	6.7	4.3 ^{ns}	38.6	4.7	2±1.3	2	0.4	-0.7	0.6	4.8	4.2	2.6 ^{ns}	45.5	3.6
TPP	28.3±15	226.0	1	1	3.7	67.1	63.4	445 ^{ns}	41.8	46.5	44±16.1	259	-1.2	0.3	8.5	60.0	51.5	644.5 ^{ns}	0	79
TSP	24.5±14	202.1	1	1	1.9	63.5	61.6	389.6 ^{ns}	41.1	43.8	40±16	256	-0.9	-0.3	6.1	55.2	49.1	537.6 ^{ns}	0	76.3
HSW	10.5±2.4	2.4	0	0	7.5	14.6	7.1	4.7 ^{ns}	0	9.4	10±1.5	2	-0.7	-0.2	6.7	12.1	5.4	4.7 ^{ns}	28	5.5
PBP	9.8±5	24.8	1	3	3.2	26.8	23.7	45.6 ^{ns}	28.7	16.5	13.2±5.1	26	-0.3	-1.2	4.5	20.2	15.7	43.3 ^{ns}	0	20.6
GYP	2.8±2	2.5	1	1	0.4	7.6	7.2	4.7 ^{ns}	24.9	5.4	4.0±1.8	3	-0.3	-0.8	0.7	6.8	6.1	6.4 ^{ns}	0.5	7.5
PHI	24.8±9	75.5	0	-1	7.6	38.4	30.8	148.7 ^{ns}	8.5	33.7	29.7±10	100	-0.7	0.7	7.4	47.2	39.8	220.7*	64.7	26.4
9xKesen_075										9xOyali_084										
DFP	49.6±11.1	122.9	-0.1	-0.7	28.0	68.5	40.5	245.7***	86.1	16.3	46.5±11.2	126.3	-0.2	-0.8	26.0	64.5	38.5	252.6***	94	11.2
DFP	55.7±11.8	138.1	0.1	-0.7	33.0	78	45	276.3 ^{ns}	91.1	13.8	52.7±11.1	122.4	0.0	-1.0	33.5	71.5	38	244.8***	87.5	15.9
DPM	92.4±15	225.9	-0.2	-0.6	62.0	117	55	451.8***	87.7	20.7	89.0±15.6	242.2	-0.1	-0.9	61.5	116	54.5	484.5***	90.6	19.4
PT	1.5±0.6	0.4	0.8	-0.4	1.0	3	2	8.1 ^{ns}	10.7	2.4	1.6±0.6	0.4	0.3	-0.7	1.0	3	2	0.6 ^{ns}	0	3.5
HVS	2.7±0.9	0.9	0.4	0.3	1.0	5	4	1.7***	75	1.9	2.4±0.7	0.5	0.1	-1.2	1.5	3.5	2	0.9 ^{ns}	38.4	2.3
GH	2.7±0.8	0.7	0.8	0.4	1.5	5	3.5	1.3***	83.9	1.3	2.4±0.6	0.4	-0.1	-0.4	1.0	3.5	2.5	0.7 ^{ns}	0	2.9
%PS	46.1±15.2	230.8	0.9	2.1	15.0	92.5	77.5	450.1***	82.1	25.6	52.2±13.5	183.3	0.7	-0.6	35.0	80	45	347.5 ^{ns}	48.9	40.9
PHT	21.8±4.3	18.3	0.4	-0.2	14.5	32.3	17.8	36 ^{ns}	9.1	16.3	24.1±5.3	28.1	0.3	-1.2	17.0	33.45	16.45	53.8*	62.7	13.8
FPP	22.2±13.3	176.7	1.7	4.0	1.5	69.4	67.9	249.8 ^{ns}	20.1	40.3	29.9±13.3	175.8	0.3	-0.7	8.4	54.9	46.5	331.4 ^{ns}	0	62.4
UPP	1.4±1	1.1	2.4	7.0	0.1	5.4	5.3	1.4 ^{ns}	16.9	3.1	1.7±1.1	1.3	0.4	-0.8	0.0	3.8	3.8	2.4 ^{ns}	23.9	4.2
TPP	23.7±14	197.3	1.8	4.6	2.8	74.8	72.05	274.1 ^{ns}	18.6	42.6	31.4±13.7	187.5	0.3	-0.7	9.0	57.75	48.75	351.3 ^{ns}	0	64.3
TSP	22.7±12.6	159.2	1.0	0.6	2.9	52.65	49.8	272.8 ^{ns}	0	51.2	30.8±13.6	183.6	0.5	0.2	6.3	63.5	57.2	399.2 ^{ns}	0	67.4
HSW	10.8±1.6	2.6	-0.8	1.3	5.9	13.7	7.8	5.0 ^{ns}	12.7	6	11.2±1.5	2.3	1.1	0.9	9.3	15.3	6.05	3.4 ^{ns}	17.7	5.1
PBP	9.7±3.1	9.5	1.2	3.5	3.9	20.3	16.4	13.7 ^{ns}	0	13.5	12.5±6.4	40.7	1.0	0.6	5.0	29.25	24.25	78.9 ^{ns}	3.8	26.7
GYP	2.5±1.3	1.6	0.7	0.1	0.3	5.65	5.4	3.0 ^{ns}	0	5.9	3.9±1.5	2.2	0.4	-0.5	1.3	6.8	5.5	3.9 ^{ns}	0	7.5
PHI	23.5±9.2	84.0	0.3	0.1	3.2	43.3	40.15	168.4 ^{ns}	14.2	34.3	31.9±12.4	153.1	0.8	0.0	16.0	59.1	43.15	250.2 ^{ns}	0	48.9
9xSavur_063										9xSirna_060										
DFP	41.4±10.5	110.8	0.7	0.9	26.5	72	45.5	221.5***	85	16	50.8±9.6	92.2	-0.2	-0.2	27.0	69.5	42.5	179.6***	83.7	15.1
DFP	47.1±10.5	109.7	0.8	1.0	32.5	78	45.5	219.5***	84.4	16.2	57.7±10.4	109.1	0.4	0.0	35.5	83.25	47.75	214.7***	80	18.3
DPM	81.3±14.2	201.2	0.3	0.0	59.5	118.5	59	402.3***	81.8	23.8	96.7±14.8	218.0	0.0	-0.2	62.0	125	63	429.9***	81	25.2
PT	1.6±0.6	0.4	0.6	-0.6	1	3	2	0.8 ^{ns}	0	2.7	1.5±0.7	0.5	1.1	0.0	1.0	3	2	0.9 ^{ns}	22.4	2.4
HVS	2.1±0.8	0.6	1.1	2.0	1	4.5	3.5	1.2*	50.6	2.1	2.9±0.9	0.9	0.3	-1.1	1.5	4.5	3	1.7*	52.2	2.6

GH	2.6±0.8	0.6	1.6	2.2	2	5	3	1.1***	81.8	1.3	2.8±0.7	0.5	0.1	-1.2	2.0	4	2	51.4 ^{ns}	0	21.5
%PS	58.0±15.5	239.1	-0.3	-0.2	20	87.5	67.5	478.1*	48.9	43.5	42.5±13.8	189.5	0.0	-0.5	20.0	71.25	51.25	321.7 ^{ns}	40.2	40.7
PHT	24.9±4.7	22.5	0.3	-0.8	17.5	34.55	17.05	45*	52	13.1	22.0±3.4	11.4	-0.2	-0.4	14.7	28.45	13.8	57.3 ^{ns}	15.7	20.7
FPP	30.2±12.4	154.1	0.5	-0.9	12.65	56.1	43.45	308 ^{ns}	26.8	42.3	25.7±16.8	283.3	1.2	1.8	3.1	79.95	76.85	343.2 ^{ns}	0	60.6
UPP	2.4±1.1	1.1	0.3	-1.0	0.8	4.6	3.8	2.3 ^{ns}	30.9	3.5	2.0±1.2	1.5	0.9	0.6	0.0	5.35	5.35	84 ^{ns}	3.5	26.8
TPP	32.4±13.1	171.3	0.5	-0.8	14.95	60.8	45.85	342.4 ^{ns}	29.5	43.7	27.7±17.7	313.0	1.2	1.7	4.1	84.05	79.95	606.6 ^{ns}	50.6	51.3
TSP	29.0±12.9	167.0	0.6	-0.5	8.35	58.5	50.15	334 ^{ns}	14.9	47.4	27.1±18.9	358.3	1.7	3.9	3.9	95.9	92	426.6 ^{ns}	0	68.3
HSW	9.4±1.7	2.7	0.0	-0.5	6.35	13	6.65	5.3***	76	3.2	10.0±1.2	1.5	1.2	1.1	8.3	13.35	5.1	5.1 ^{ns}	0	7.5
PBP	9.6±3.9	15.6	1.3	2.3	3.1	21.85	18.75	30.8 ^{ns}	44.5	11.6	11.6±7.8	61.2	2.5	7.4	4.0	43.5	39.5	72.1*	54.9	17
GYP	2.8±1.3	1.8	1.1	0.7	0.5	6.1	5.6	3.6 ^{ns}	0	5.4	2.6±1.8	3.3	1.9	5.2	0.4	9.6	9.3	31.2 ^{ns}	50.2	11.7
PHI	30.1±11.6	135.0	0.9	0.9	10.35	63.4	53.05	259.8 ^{ns}	24.5	39.4	21.4±9.1	82.4	0.1	-0.7	5.4	39.4	34.0	126.4 ^{ns}	0	34.6
9xGunas-063									9xKarabache_081A											
DFF	44.6±3.5	12.2	0.0	-0.8	38.5	50.4	11.9	24.4 ^{ns}	42.8	11.6	43.5±10.7	113.6	1.0	-0.4	33.8	61	27.25	227.2 ^{ns}	78.5	32.2
DFP	50.6±4.1	17.0	0.3	0.0	43.8	59.2	15.4	34 ^{ns}	61.8	11.2	51.9±12.7	161.7	0.9	-0.6	41.0	72.5	31.5	323.4*	87	29.9
DPM	86.3±5.1	26.4	-0.5	-0.2	76.5	94.5	18.0	52.9 ^{ns}	59.9	14.3	87.9±17.7	313.8	0.8	-0.6	69.8	116	46.25	627.5*	91.4	33.9
PT	1.2±0.6	0.3	3.0	7.1	1.0	3.0	2.0	0.7 ^{ns}	1	0	1.2±0.4	0.2	1.5	0.3	1.0	2	1	0.4 ^{ns}	0	4.5
HVS	3.7±1.1	1.2	-1.1	1.0	1.0	5.0	4.0	2.5**	80.1	2.2	4.4±0.8	0.7	-1.2	-0.2	3.0	5	2	1.3 ^{ns}	30.7	5.8
GH	3.2±0.9	0.9	0.1	-0.7	2.0	5.0	3.0	1.8 ^{ns}	1.7	4.1	1.9±1.0	1.1	0.7	-0.8	1.0	3.5	2.5	1.9 ^{ns}	36	6.9
%PS	31.7±13.3	177.9	0.6	-0.1	15.0	60.9	45.9	355.7**	79.9	26.2	22.7±11.4	128.9	1.0	-0.3	11.7	41.65	30	257.2 ^{ns}	65.1	58.3
PHT	27.1±3.7	13.3	0.0	-1.1	21.5	32.3	10.8	26.7 ^{ns}	58.3	10.4	28.4±5.5	29.9	0.6	-0.7	22.4	36.8	14.45	57.8 ^{ns}	0	63.9
FPP	29.3±15.8	248.4	0.7	-0.5	9.4	60.1	50.7	496.9 ^{ns}	0	81.2	8.5±5.9	34.5	0.1	-1.2	1.5	16.5	15.05	65.7 ^{ns}	31.8	41.2
UPP	3.6±3.4	11.4	1.2	-0.2	0.5	10.2	9.7	22.8 ^{ns}	31.1	12.3	2.5±2.6	6.8	1.4	0.1	0.9	7.05	6.2	12.2 ^{ns}	68.3	12.1
TPP	33.0±18.4	339.9	0.9	-0.1	9.9	70.8	60.9	679.8 ^{ns}	0	81.9	13.3±7.0	49.5	0.1	-1.5	5.2	22.0	16.8	73.8 ^{ns}	0	58.6
TSP	28.4±15.8	249.1	1.4	1.3	10.9	67.9	57.0	498.1 ^{ns}	27.2	59.2	9.0±5.8	33.7	0.2	-1.2	2.5	17.0	14.6	67.3 ^{ns}	0	66.4
HSW	10.8±0.9	0.8	-0.1	-1.4	9.5	12.1	2.6	1.7 ^{ns}	0	5.1	11.7±2.8	7.9	1.4	0.1	9.8	16.7	7.0	7.2 ^{ns}	0	19.6
PBP	16.8±7.5	55.8	0.8	-0.1	7.3	33.0	25.7	101.6 ^{ns}	60.2	20.7	11.3±5.8	33.2	0.0	-1.6	4.6	17.9	13.3	61.2 ^{ns}	49.3	34.3
GYP	3.1±1.7	2.7	1.3	1.0	1.3	7.2	5.9	5.5 ^{ns}	29.1	6.1	1.4±0.7	0.5	-0.9	-0.5	0.3	2.2	1.9	1.0 ^{ns}	0	10.6
PHI	18.2±5.3	28.2	0.2	-1.4	11.85	27.1	15.3	56.5 ^{ns}	0	27.2	16.7±13.2	173.5	0.7	-0.8	3.7	37.3	33.6	351.8 ^{ns}	61.4	71.7
9xS2DrD									9xBari3_072C											
DFF	47.3±8.5	72.7	-0.5	-0.1	28.0	59.7	31.7	145.3 ^{ns}	55.4	24	49.6±8.2	67.7	0.6	-0.7	40.0	64.5	24.5	150.8**	76.4	17.5
DFP	55.3±9.2	84.1	-0.1	-0.5	37.0	70	33	168.2 ^{ns}	25.9	33.2	56.4±12.0	143.4	1.2	0.5	44.0	83.0	39.0	261.8***	83.2	19.5
DPM	92.8±12.2	149.5	-0.5	0.3	64.0	111.5	47.5	299 ^{ns}	29.3	43.3	95.5±11.5	131.8	0.5	0.0	77.8	118.5	40.8	334.7**	70.1	29.4
PT	1.3±0.5	0.2	1.1	-0.9	1.0	2	1	0.4 ^{ns}	0	2.5	1.4±0.7	0.5	1.4	0.6	1.0	3.0	2.0	0.6 ^{ns}	10.4	2.4
HVS	3.8±1.0	1.0	-0.5	-1.2	2.0	5	3	1.7 ^{ns}	33.7	3.3	2.7±0.8	0.6	0.1	-0.8	1.5	4.0	2.5	1.4 ^{ns}	53.5	2.5
GH	2.5±1.2	1.4	0.7	-0.6	1.0	5	4	2.5*	71.7	2.7	3.5±0.7	0.6	1.1	-0.4	3.0	5.0	2.0	0.8 ^{ns}	45.9	2.1
%PS	29.9±14.2	200.7	0.5	-1.2	11.3	52.5	41.25	365.0 ^{ns}	27.5	51.4	46.4±11.4	129.3	0.0	0.0	25.0	66.3	41.3	332.4*	67	32.9
PHT	30.3±7.4	54.6	0.9	0.9	19.4	49	29.6	67.6 ^{ns}	43.7	20.2	19.7±3.3	10.7	-0.4	-0.5	14.5	25.0	10.5	13.7 ^{ns}	0	14.7
FPP	27.9±29.1	845.7	2.4	5.3	4.0	121.5	117.5	914.2 ^{ns}	52.7	68.1	20.4±13.2	175.0	1.2	1.0	7.2	51.4	44.2	267.6 ^{ns}	22.7	45.3
UPP	2.1±3.5	12.4	3.2	8.9	0.0	14.5	14.5	12.2***	88.2	3.9	2.2±0.9	0.9	0.1	-1.4	0.9	3.5	2.6	1.7 ^{ns}	3.6	4
TPP	30.0±32.1	1031.7	2.6	6.2	5.0	136	131	1083.6 ^{ns}	57.8	70	22.2±13.7	187.4	1.3	1.1	8.1	54.5	46.5	295.4 ^{ns}	20.2	48.3
TSP	27.6±30.9	957.0	2.4	5.6	4.8	128	123.3	990.0 ^{ns}	58.7	66.2	20.5±13.3	176.3	1.1	0.3	6.2	49.5	43.3	382.6 ^{ns}	36.2	49.2
HSW	11.8±3.1	9.4	0.1	0.9	6.0	18.9	12.9	14.3 ^{ns}	0	14.5	9.3±2	3.9	-1.0	-0.6	5.4	11.1	5.7	5.0 ^{ns}	0	8.4
PBP	15.1±9.9	97.1	1.2	0.7	1.5	37	35.5	111.2 ^{ns}	51.5	24	8.9±3.7	13.6	0.5	-0.9	4.4	15.6	11.2	30.3 ^{ns}	0	19.9
GYP	3.8±5.9	34.5	3.1	8.3	0.6	24.3	23.7	33.4**	81.6	8.1	2.2±1.3	1.7	1.0	0.0	0.7	4.9	4.2	3.8 ^{ns}	16.4	5.6

PHI	21.4±15.6	242.4	1.9	3.7	5.4	68.5	63.2	292.8 ^{ns}	37.4	44.3	23.0±9.4	88.0	-0.2	-0.5	5.3	36.5	31.2	150.9 ^{ns}	15.7	35.5

ANOVA = analysis of variance, KU = kurtosis, LSD = least significant difference, H²b = broad sense heritability, AR = *Cicer arietinum* and *reticulatum*, AE = *Cicer arietinum* and *reticulatum*, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, MS = mean square, Max = maximum, Min = minimum, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type, R = range, SD = standard deviation, SK = skewness, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant, V = variance, ns = non-significant, * significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, *** Significant at $P \leq 0.001$.

Appendix 3.2. Correlation among 16 traits for each of the 20 families of AR and AE under an extremely heat stress environment (Hyderabad, ICRISAT), in each section below and above diagonals is the first and the second families, respectively.

Bari2_72n2 and Bari1_092																
Trait	DFP	DFP	DPM	PT	HVS	GH	%PS	PHT	FPP	UPP	TPP	TSP	HSW	PBP	GYP	PHI
DFP		0.9682***	0.9636***	-0.035ns	0.3401ns	-0.0395ns	-0.3934ns	-0.3177ns	-0.3158ns	0.0729ns	-0.2917ns	-0.2612ns	0.1245ns	-0.0283ns	-0.2505ns	-0.4882*
DFP	0.9801***		0.9522***	-0.023ns	0.4088ns	-0.0422ns	-0.4472*	-0.3443ns	-0.3570ns	0.0225ns	-0.3335ns	-0.2983ns	0.1236ns	-0.0065ns	-0.2770ns	-0.5369**
DPM	0.9838***	0.9695***		-0.095ns	0.3626ns	0.0230ns	-0.3899ns	-0.2806ns	-0.2142ns	0.2030ns	-0.1824ns	-0.1954ns	0.0696ns	0.0378ns	-0.2119ns	-0.5383**
PT	-0.3510ns	-0.3266ns	-0.2971ns		-0.3099ns	-0.0850ns	0.3489ns	0.0770ns	0.1589ns	0.0203ns	0.1367ns	0.2560ns	0.0695ns	0.1130ns	0.2840ns	0.2797ns
HVS	0.4174*	0.4259*	0.3771*	-0.4467*		-0.2459ns	-0.929***	-0.2516ns	-0.5896**	-0.1173ns	-0.5645**	-0.6429**	0.0089ns	-0.2569ns	-0.6107**	-0.6279**
GH	-0.0052ns	-0.0134ns	-0.0684ns	0.0000ns	-0.0301ns		0.1877ns	-0.3108ns	0.1105ns	0.1916ns	0.1217ns	0.1155ns	-0.3783ns	0.1955ns	0.0776ns	-0.1865ns
%PS	-0.4905**	-0.5008**	-0.4495*	0.4337*	-0.921***	0.0566ns		0.3454ns	0.5229*	0.0402ns	0.4984*	0.5782**	0.0490ns	0.1994ns	0.5368*	0.5916**
PHT	0.0405ns	0.0172ns	0.0748ns	-0.008ns	-0.3882*	-0.4583*	0.3498ns		0.3606ns	-0.0219ns	0.3529ns	0.3084ns	0.3823ns	0.2695ns	0.3875ns	0.3124ns
FPP	0.0207ns	0.0147ns	0.0513ns	0.4013*	-0.643***	0.1779ns	0.6266***	0.3264ns		0.5589**	0.9984***	0.9763***	-0.1018ns	0.7045***	0.9182***	0.5178*
UPP	-0.2272ns	-0.2739ns	-0.2228ns	0.2190ns	-0.5596**	0.2504ns	0.6388***	0.1006ns	0.5117**		0.5922**	0.4832*	-0.1735ns	0.3578ns	0.3383ns	-0.0304ns
TPP	0.0083ns	-0.0009ns	0.0419ns	0.4199*	-0.665***	0.1932ns	0.6526***	0.3127ns	0.9939***	0.5623**		0.9695***	-0.1055ns	0.7081***	0.9072***	0.4879*
TSP	0.0164ns	0.0229ns	0.0491ns	0.4348*	-0.661***	0.1918ns	0.7014***	0.2139ns	0.9253***	0.6202***	0.9344***		-0.0465ns	0.7022***	0.9583***	0.5635**
HSW	0.2275ns	0.2294ns	0.2291ns	-0.011ns	-0.0618ns	-0.3376ns	0.0348ns	0.5007**	-0.0542ns	-0.2331ns	-0.0822ns	-0.0816ns		0.0499ns	0.1381ns	0.1946ns
PBP	0.0624ns	0.1009ns	0.1152ns	0.4487*	-0.589***	0.1326ns	0.5254**	0.3581ns	0.8533***	0.3608ns	0.8536***	0.8028***	0.0911ns		0.7312***	0.0175ns
GYP	0.0492ns	0.0634ns	0.0818ns	0.3726*	-0.669***	0.2163ns	0.6826***	0.3195ns	0.8798***	0.5209**	0.8786***	0.9425***	0.0981ns	0.7789***		0.6143**
PHI	0.0412ns	-0.0143ns	0.0363ns	0.0324ns	-0.3038ns	0.0023ns	0.4135*	0.1927ns	0.2962ns	0.3227ns	0.2970ns	0.4167*	0.1647ns	-0.0577ns	0.5096**	
Bari3_072C and Bari3_100																
DFP		0.9574***	0.9363***	0.1065ns	0.2502ns	-0.0716ns	-0.2213ns	-0.2311ns	0.1023ns	-0.1237ns	0.1030ns	0.3042ns	0.2167ns	0.6692**	0.3012ns	-0.3230ns
DFP	0.9346***		0.9488***	0.1039ns	0.3445ns	-0.1796ns	-0.3415ns	-0.3081ns	-0.0311ns	-0.2173ns	-0.0321ns	0.1567ns	0.1813ns	0.5883**	0.1407ns	-0.4723*
DPM	0.9808***	0.9359***		0.0216ns	0.4126ns	-0.1878ns	-0.3670ns	-0.2445ns	-0.0170ns	-0.1705ns	-0.0126ns	0.1795ns	0.1132ns	0.5009*	0.1637ns	-0.4237ns
PT	0.4645**	0.4612*	0.4973**		-0.1746ns	0.1364ns	0.0486ns	-0.0229ns	0.0147ns	-0.0737ns	0.0147ns	-0.0090ns	0.0468ns	0.1971ns	-0.0098ns	-0.0011ns
HVS	0.4541*	0.3842*	0.3877*	-0.169ns		-0.4263ns	-0.918***	-0.2060ns	-0.747***	-0.6614**	0.7486***	-0.6811**	0.0005ns	-0.3030ns	-0.6697**	-0.772***
GH	0.1661ns	0.1656ns	0.1470ns	0.1367ns	0.0498ns		0.3880ns	-0.0995ns	0.5090*	0.3697ns	0.5145*	0.3863ns	0.2244ns	-0.0110ns	0.3581ns	0.6056**
%PS	-0.3756*	-0.3297ns	-0.3175ns	0.1853ns	-0.956***	-0.0834ns		0.2570ns	0.7602***	0.6784**	0.7499***	0.7242***	-0.0143ns	0.3355ns	0.7386***	0.8293***
PHT	-0.583***	-0.662***	-0.5859***	-0.529**	-0.0590ns	-0.5104**	0.0659ns		0.2562ns	0.1475ns	0.2338ns	0.2748ns	0.2510ns	0.0266ns	0.2513ns	0.4814*
FPP	-0.1119ns	-0.1033ns	-0.0591ns	0.2426ns	-0.649***	0.1037ns	0.7225***	-0.0290ns		0.7381***	0.9964***	0.9219***	0.1271ns	0.5360*	0.8857***	0.7846***
UPP	-0.3198ns	-0.2798ns	-0.2979ns	0.0187ns	-0.4736**	0.3072ns	0.4751**	-0.0319ns	0.5179**		0.7765***	0.6904**	-0.0336ns	0.3051ns	0.6510**	0.6097**
TPP	-0.1489ns	-0.1404ns	-0.0977ns	0.2090ns	-0.664***	0.1266ns	0.7343***	-0.0228ns	0.9942***	0.5933***		0.9230***	0.1234ns	0.5391*	0.8874***	0.7671***
TSP	-0.1046ns	-0.0947ns	-0.0621ns	0.1302ns	-0.5221**	0.1241ns	0.5939***	0.0412ns	0.9554***	0.5208**	0.9519***		0.1568ns	0.6905**	0.9772***	0.6867**
HSW	0.1423ns	0.1547ns	0.1293ns	0.1985ns	-0.2211ns	0.1364ns	0.3525ns	-0.1873ns	0.0968ns	0.1618ns	0.1146ns	0.0654ns		0.2996ns	0.2330ns	0.1112ns
PBP	-0.1086ns	-0.0444ns	-0.0450ns	0.2134ns	-0.5199**	0.0659ns	0.6333***	0.0019ns	0.7669***	0.4257*	0.7575***	0.7744***	0.2437ns		0.7181***	0.0937ns
GYP	-0.0597ns	-0.0436ns	-0.0246ns	0.2215ns	-0.4951**	0.1698ns	0.5667**	0.0210ns	0.9211***	0.5693**	0.9170***	0.9595***	0.1158ns	0.7817***		0.6587**
PHI	-0.1702ns	-0.2431ns	-0.1980ns	0.0487ns	-0.3353ns	0.2761ns	0.3206ns	0.1584ns	0.4732**	0.4439*	0.4980**	0.4982**	0.2566ns	0.1111ns	0.5629**	
Bari3_100D and Besave_079																
DFP		0.9816***	0.9673***	-0.252ns	0.0932ns	-0.3834ns	-0.2706ns	-0.2956ns	0.0424ns	0.0986ns	0.0434ns	0.0405ns	0.3013ns	0.4260*	0.1535ns	-0.2988ns
DFP	0.9767***		0.9689***	-0.189ns	0.0012ns	-0.3674ns	-0.2141ns	-0.2995ns	0.0833ns	0.1601ns	0.0890ns	0.0998ns	0.2714ns	0.4495*	0.2028ns	-0.2730ns
DPM	0.9835***	0.9759***		-0.223ns	0.0705ns	-0.3697ns	-0.2673ns	-0.2927ns	0.0651ns	0.0909ns	0.0637ns	0.0876ns	0.3762ns	0.4385*	0.1860ns	-0.3030ns

PT	0.1353ns	0.1253ns	0.2067ns		-0.3853ns	0.2985ns	0.3999*	0.3050ns	0.1551ns	0.0397ns	0.1628ns	0.1527ns	0.0078ns	-0.1322ns	0.1255ns	0.3856ns
HVS	0.6739***	0.6647***	0.6340***	-0.072ns		-0.1432ns	-0.878***	-0.2247ns	-0.636***	-0.6170**	-0.652***	-0.636***	-0.0147ns	-0.3335ns	-0.6074**	-0.618***
GH	-0.1140ns	-0.1330ns	-0.1338ns	-0.085ns	-0.3885*		0.1794ns	-0.1751ns	0.2484ns	0.0453ns	0.2609ns	0.3893ns	-0.2762ns	0.1401ns	0.2679ns	0.1574ns
%PS	-0.735***	-0.726***	-0.6982***	0.0482ns	-0.93***	0.2282ns		0.3807ns	0.6356***	0.5280**	0.6391***	0.5720**	-0.0246ns	0.1538ns	0.5320**	0.7326***
PHT	-0.5765**	-0.5450**	-0.5210**	-0.107ns	-0.1458ns	-0.3719ns	0.3036ns		0.2640ns	0.2203ns	0.2572ns	0.1388ns	0.3645ns	-0.0631ns	0.2109ns	0.5758**
FPP	-0.4279*	-0.3761ns	-0.4054*	-0.076ns	-0.663***	0.1841ns	0.7661***	0.2772ns		0.6836***	0.9965***	0.9264***	0.3868ns	0.6854***	0.9178***	0.6550***
UPP	-0.2409ns	-0.2135ns	-0.2056ns	0.3262ns	-0.5942**	0.0659ns	0.6140***	0.0974ns	0.5986***		0.7092***	0.7085***	0.1339ns	0.4860*	0.6752***	0.4829*
TPP	-0.4089*	-0.3531ns	-0.3847*	-0.022ns	-0.676***	0.1715ns	0.7746***	0.2542ns	0.9927***	0.6597***		0.9249***	0.3639ns	0.6883***	0.9158***	0.6488***
TSP	-0.4543*	-0.4215*	-0.4499*	-0.001ns	-0.621***	0.0397ns	0.7516***	0.3064ns	0.9125***	0.5458**	0.9254***		0.3147ns	0.7561***	0.9681***	0.5898**
HSW	-0.0909ns	-0.0693ns	-0.0465ns	0.1424ns	-0.2523ns	-0.1228ns	0.3094ns	0.2629ns	0.3581ns	0.3584ns	0.3807ns	0.3030ns		0.4526*	0.4479*	0.1853ns
PBP	-0.4578*	-0.3923*	-0.4146*	-0.008ns	-0.706***	0.2221ns	0.7037***	0.3057ns	0.8565***	0.6100***	0.8588***	0.7475***	0.3558ns		0.8178***	0.0838ns
GYP	0.2748ns	0.1990ns	0.2083ns	-0.185ns	0.0148ns	-0.0501ns	0.0671ns	-0.2401ns	0.3161ns	0.0627ns	0.3101ns	0.3736ns	0.0354ns	-0.0423ns		0.5925**
PHI	-0.5691**	-0.5095**	-0.5451**	0.0842ns	-0.4750*	-0.0191ns	0.6146***	0.4719*	0.6412***	0.3435ns	0.6331***	0.7068***	0.4463*	0.5087**	-0.1049ns	
CudiA_152 and CudiB_22C																
DFE		0.9293***	0.9353***	-0.115ns	0.4269*	0.0179ns	-0.3473ns	-0.2077ns	-0.3426ns	-0.2064ns	-0.3443ns	-0.4661*	0.2138ns	-0.0947ns	-0.2366ns	-0.3027ns
DFP	0.9481***		0.9065***	-0.168ns	0.4747*	-0.1078ns	-0.4017*	-0.0837ns	-0.3520ns	-0.2781ns	-0.3614ns	-0.4584*	0.1055ns	-0.0738ns	-0.2781ns	-0.3952*
DPM	0.9828***	0.9568***		-0.049ns	0.3906*	0.0334ns	-0.2941ns	-0.1529ns	-0.3344ns	-0.0939ns	-0.3264ns	-0.4299*	0.0541ns	-0.0978ns	-0.2627ns	-0.3438ns
PT	0.0621ns	-0.0238ns	0.0055ns		-0.2376ns	-0.1454ns	0.2933ns	0.0762ns	0.4617*	-0.1168ns	0.4408*	0.3517ns	-0.0697ns	0.3255ns	0.3228ns	0.1351ns
HVS	0.2289ns	0.4241*	0.2948ns	-0.309ns		-0.3178ns	-0.941***	0.0131ns	-0.780***	-0.4846*	-0.795***	-0.777***	-0.1139ns	-0.4573*	-0.5442**	-0.2049ns
GH	0.2465ns	0.2426ns	0.2190ns	-0.024ns	0.3415ns		0.2741ns	-0.0626ns	0.1364ns	0.2953ns	0.1609ns	0.1429ns	0.2839ns	0.1070ns	0.2225ns	0.0852ns
%PS	-0.3166ns	-0.4500*	-0.3377ns	0.3753ns	-0.901***	-0.3434ns		-0.0221ns	0.8161***	0.5581**	0.8359***	0.7531***	0.1367ns	0.5165**	0.5515**	0.1156ns
PHT	-0.2919ns	-0.3023ns	-0.1939ns	-0.080ns	-0.4248*	-0.3197ns	0.5538**		0.0747ns	-0.2788ns	0.0532ns	0.0430ns	0.1366ns	0.2628ns	0.1249ns	-0.1157ns
FPP	0.1101ns	0.0294ns	0.1315ns	0.0550ns	-0.5985**	-0.0864ns	0.5624**	0.2912ns		0.3185ns	0.9969***	0.9314***	0.2392ns	0.6394***	0.7547***	0.2578ns
UPP	0.0206ns	-0.0207ns	0.0026ns	0.4881*	-0.5084*	0.0046ns	0.4147ns	0.1463ns	0.4589*		0.3879ns	0.2884ns	-0.0123ns	0.3137ns	0.2305ns	-0.2086ns
TPP	0.1014ns	0.0222ns	0.1204ns	0.0675ns	-0.6037**	-0.0761ns	0.5636**	0.2818ns	0.9983***	0.4968*		0.9247***	0.2308ns	0.6396***	0.7502***	0.2384ns
TSP	0.1447ns	0.0667ns	0.1572ns	0.0636ns	-0.686***	-0.0965ns	0.6478**	0.3335ns	0.9688***	0.4663*	0.9694***		0.1112ns	0.5642**	0.7606***	0.3527ns
HSW	0.1136ns	0.1287ns	0.1580ns	0.0023ns	-0.2868ns	-0.4015ns	0.2462ns	0.3967ns	-0.1794ns	-0.0897ns	-0.1822ns	-0.0645ns		0.3520ns	0.4381*	0.3307ns
PBP	0.4300*	0.4069ns	0.4732*	0.0026ns	-0.4427*	-0.0931ns	0.3822ns	0.2323ns	0.7453***	0.5249*	0.7557***	0.7429***	0.1577ns		0.6854***	-0.1971ns
GYP	0.2161ns	0.1453ns	0.2416ns	0.0745ns	-0.750***	-0.1764ns	0.6982***	0.4546*	0.8861***	0.4762*	0.8879***	0.9488***	0.2095ns	0.8047***		0.4017*
PHI	-0.4264*	-0.5065*	-0.4114ns	0.0711ns	-0.673***	-0.3148ns	0.7213***	0.5566**	0.5754**	0.1352ns	0.5678**	0.6231**	0.0824ns	0.1178ns	0.6178**	
Derei_070 and Derei_072																
DFE		0.9824***	0.9831***	0.1081ns	0.4669**	-0.3642*	-0.630***	-0.574***	-0.576***	-0.1551ns	-0.5791***	-0.5439**	0.0809ns	-0.0842ns	-0.4787**	-0.5188**
DFP	0.9826***		0.9882***	0.1380ns	0.4300*	-0.3863*	-0.582***	-0.5665**	-0.5430**	-0.1879ns	-0.5486**	-0.5133**	0.0588ns	-0.0686ns	-0.4671**	-0.5126**
DPM	0.9647***	0.9507***		0.0789ns	0.4461*	-0.3686*	-0.598***	-0.5172**	-0.5344**	-0.2119ns	-0.5415**	-0.5035**	0.1030ns	-0.0501ns	-0.4447*	-0.5071**
PT	-0.2193ns	-0.2779ns	-0.2200ns		-0.2007ns	0.0442ns	0.0917ns	-0.3532ns	-0.0972ns	-0.0355ns	-0.0986ns	-0.1222ns	-0.2225ns	-0.2685ns	-0.2546ns	0.0664ns
HVS	0.5889**	0.6334***	0.5540**	-0.4556*		-0.2512ns	-0.926***	-0.2554ns	-0.697***	-0.2144ns	-0.702***	-0.705***	0.0007ns	-0.4145*	-0.5731***	-0.4457*
GH	0.1272ns	0.1110ns	0.1198ns	-0.047ns	-0.0533ns		0.2838ns	-0.0146ns	0.2621ns	-0.0404ns	0.2604ns	0.3202ns	-0.1925ns	-0.0443ns	0.2700ns	0.1826ns
%PS	-0.622***	-0.671***	-0.5957**	0.4183*	-0.945***	0.2078ns		0.4106*	0.8295***	0.1692ns	0.8308***	0.8260***	0.0106ns	0.4207*	0.6945***	0.5422**
PHT	-0.4127*	-0.4658*	-0.4033*	0.2618ns	-0.5858**	-0.3225ns	0.5600**		0.4811**	0.1083ns	0.4820**	0.4694**	0.2893ns	0.2693ns	0.4926**	0.4477*
FPP	-0.5199**	-0.5808**	-0.4843*	0.4611*	-0.765***	0.1069ns	0.7847***	0.5075**		0.1599ns	0.9986***	0.9507***	0.2202ns	0.5790***	0.8794***	0.6160***
UPP	-0.3420ns	-0.3524ns	-0.2885ns	0.3737ns	-0.3299ns	0.0672ns	0.3763ns	0.2235ns	0.3711ns		0.2115ns	0.0844ns	-0.0977ns	0.1772ns	0.0861ns	0.0568ns
TPP	-0.5190**	-0.5785**	-0.4834*	0.4965**	-0.764***	0.0823ns	0.7766***	0.5055**	0.9957***	0.4301*		0.9462***	0.2126ns	0.5829***	0.8755***	0.6126***
TSP	-0.625***	-0.679***	-0.5967**	0.4627*	-0.785***	0.0748ns	0.7954***	0.5104**	0.9680***	0.3989*	0.9700***		0.2479ns	0.5406**	0.9370***	0.7167***
HSW	0.2076ns	0.1418ns	0.1829ns	0.2463ns	-0.0362ns	-0.0612ns	0.0249ns	-0.0321ns	-0.0540ns	-0.1115ns	-0.0420ns	-0.0757ns		0.5425**	0.4518*	0.2343ns

PBP	0.2831ns	0.2042ns	0.2318ns	0.0444ns	-0.1510ns	0.0642ns	0.1745ns	0.1783ns	0.2915ns	-0.0508ns	0.2896ns	0.2344ns	0.3626ns		0.6642***	0.0194ns
GYP	-0.5738**	-0.635***	-0.5388**	0.5527**	-0.802***	0.0013ns	0.7929***	0.5406**	0.9366***	0.4082*	0.9483***	0.9651***	0.0761ns	0.3195ns		0.6532***
PHI	-0.632***	-0.649***	-0.6156***	0.5847**	-0.741***	0.0654ns	0.7313***	0.3275ns	0.8323***	0.4400*	0.8446***	0.8235***	-0.0934ns	-0.0270ns	0.7944***	
	Egill_065 and Egill_073															
DFP		0.9872***	0.9660***	0.0533ns	0.1187ns	0.0483ns	-0.1602ns	-0.3852*	0.0285ns	-0.0046ns	-0.0174ns	0.1522ns	0.3402ns	0.1822ns	0.1930ns	-0.2633ns
DFP	0.9758***		0.9529***	0.0646ns	0.1603ns	-0.0059ns	-0.2061ns	-0.3823*	0.0059ns	0.0150ns	-0.0519ns	0.1074ns	0.3877*	0.1880ns	0.1581ns	-0.3008ns
DPM	0.9441***	0.9459***		0.0870ns	0.0221ns	0.0318ns	-0.0775ns	-0.3268ns	0.0534ns	-0.0013ns	0.0113ns	0.2063ns	0.3675ns	0.1892ns	0.2401ns	-0.2045ns
PT	0.3132ns	0.2763ns	0.2248ns		-0.3590ns	0.0190ns	0.4000*	-0.0812ns	0.2525ns	0.4752*	0.2183ns	0.3498ns	-0.0026ns	0.0032ns	0.3102ns	0.4841**
HVS	0.2059ns	0.2380ns	0.1915ns	-0.182ns		0.0142ns	-0.957***	-0.3926*	-0.751***	-0.2095ns	-0.752***	-0.768***	-0.1190ns	-0.6244***	-0.7347***	-0.4012*
GH	-0.4309*	-0.4849*	-0.5129**	-0.157ns	-0.4247*		-0.1126ns	-0.5043**	0.2115ns	-0.0715ns	0.1819ns	0.2785ns	-0.4682*	-0.0299ns	0.1881ns	0.3020ns
%PS	-0.2441ns	-0.2588ns	-0.1945ns	-0.009ns	-0.919***	0.5053**		0.4301*	0.7517***	0.2706ns	0.7698***	0.7653***	0.1703ns	0.5737**	0.7543***	0.4744*
PHT	-0.0001ns	-0.0130ns	0.0201ns	0.3111ns	0.0151ns	-0.4903**	-0.0171ns		0.1444ns	-0.1551ns	0.1431ns	0.1301ns	0.2717ns	0.3516ns	0.1604ns	0.0214ns
FPP	-0.0853ns	-0.1411ns	-0.0874ns	0.1039ns	-0.785***	0.3911*	0.8153***	0.1453ns		0.2803ns	0.9787***	0.8846***	0.1817ns	0.5837**	0.8869***	0.5821**
UPP	-0.1523ns	-0.1617ns	-0.2402ns	-0.033ns	-0.3665ns	0.3045ns	0.4202*	0.0918ns	0.3483ns		0.3693ns	0.2912ns	0.0750ns	0.0487ns	0.2885ns	0.3440ns
TPP	-0.1274ns	-0.1780ns	-0.1419ns	0.0779ns	-0.817***	0.4131*	0.8470***	0.1583ns	0.9714***	0.5312**		0.8550***	0.1818ns	0.5473**	0.8510***	0.5749**
TSP	-0.0125ns	-0.0648ns	0.0022ns	0.0616ns	-0.754***	0.3467ns	0.7884***	0.1071ns	0.9667***	0.3079ns	0.9438***		0.1620ns	0.5606**	0.9664***	0.6478***
HSW	0.3724ns	0.3443ns	0.4564*	0.0042ns	0.2808ns	-0.3641ns	-0.2287ns	0.2020ns	-0.2459ns	-0.2611ns	-0.2607ns	-0.2147ns		0.4490*	0.3205ns	-0.1101ns
PBP	0.3149ns	0.3106ns	0.3684ns	0.1769ns	-0.5467**	-0.0741ns	0.5259**	0.2948ns	0.7225***	0.1191ns	0.6776***	0.7306***	0.1110ns		0.6364***	-0.0774ns
GYP	0.0787ns	0.0480ns	0.1340ns	0.0206ns	-0.732***	0.2069ns	0.7421***	0.1261ns	0.8770***	0.1919ns	0.8447***	0.9361***	-0.0246ns	0.8190***		0.5905***
PHI	-0.3189ns	-0.3513ns	-0.3091ns	-0.162ns	-0.4632*	0.7252***	0.5497**	-0.2375ns	0.4225*	0.2895ns	0.4563*	0.4186*	-0.2742ns	-0.0078ns	0.3288ns	
	Kalkan_064 and Kesen_075															
Trait	DFP	DFP	DPM	PT	HVS	GH	PPS	PHT	FPP	UPP	TPP	TSP	HSW	PBP	GYP	PHI
DFP		0.9855***	0.9542***	-0.207ns	0.4079ns	0.3658ns	-0.5451*	-0.5372*	-0.1584ns	-0.3327ns	-0.1517ns	-0.0699ns	0.0891ns	0.1798ns	-0.0687ns	-0.5902*
DFP	0.9852***		0.9150***	-0.23ns	0.4783ns	0.3386ns	-0.5890*	-0.5581*	-0.2344ns	-0.3743ns	-0.2229ns	-0.1655ns	0.0948ns	0.0740ns	-0.1631ns	-0.6167**
DPM	0.9843***	0.9822***		-0.194ns	0.2274ns	0.2074ns	-0.3925ns	-0.3734ns	-0.0006ns	-0.1982ns	0.0053ns	0.0864ns	0.0707ns	0.3348ns	0.0835ns	-0.5076*
PT	-0.0518ns	-0.1280ns	-0.1028ns		-0.4066ns	0.2891ns	0.4531ns	-0.0785ns	0.3465ns	0.2483ns	0.3461ns	0.2810ns	-0.1542ns	0.0031ns	0.1895ns	0.3552ns
HVS	0.4686*	0.5088**	0.4910**	-0.504**		0.4702ns	-0.933***	-0.6986**	-0.878***	-0.5090*	-0.868***	-0.771***	0.0336ns	-0.4904*	-0.7151**	-0.6805**
GH	-0.0874ns	-0.0488ns	-0.0439ns	-0.099ns	-0.0662ns		-0.4026ns	-0.6185**	-0.3029ns	-0.3175ns	-0.3116ns	-0.1935ns	0.1868ns	-0.0233ns	-0.1376ns	-0.3667ns
%PS	-0.4735*	-0.4938**	-0.4961**	0.4621*	-0.931***	0.0732ns		0.6877**	0.8192***	0.4298ns	0.8106***	0.6964**	-0.0709ns	0.3604ns	0.6322**	0.7507***
PHT	-0.3720ns	-0.3948*	-0.3620ns	0.1500ns	-0.3678ns	-0.4424*	0.4526*		0.5941*	0.6356**	0.5992*	0.4806ns	0.1543ns	0.4570ns	0.5528*	0.4307ns
FPP	-0.0543ns	-0.0420ns	-0.0567ns	0.0986ns	-0.623***	0.2023ns	0.6315***	0.2280ns		0.4967*	0.9955***	0.9534***	-0.0490ns	0.6702**	0.8982***	0.7027**
UPP	0.3573ns	0.3677ns	0.3490ns	-0.092ns	-0.2177ns	0.1459ns	0.2136ns	0.0308ns	0.7237***		0.5455*	0.5016*	0.0829ns	0.5821*	0.5279*	0.2311ns
TPP	-0.0327ns	-0.0207ns	-0.0362ns	0.0910ns	-0.613***	0.2063ns	0.6174***	0.2167ns	0.9984***	0.7567***		0.9558***	-0.0159ns	0.6976**	0.9060***	0.6820**
TSP	-0.0952ns	-0.0948ns	-0.1086ns	0.1684ns	-0.653***	0.0448ns	0.6731***	0.2834ns	0.9227***	0.6382***	0.9179***		-0.0371ns	0.7845***	0.9500***	0.6350**
HSW	0.1514ns	0.1583ns	0.1942ns	-0.16ns	0.2938ns	-0.2118ns	-0.2830ns	0.4108*	-0.1764ns	-0.0646ns	-0.1728ns	-0.1494ns		0.3278ns	0.2352ns	-0.1436ns
PBP	0.2619ns	0.2903ns	0.2681ns	-0.084ns	-0.1400ns	0.1946ns	0.1961ns	0.1043ns	0.7465***	0.7538***	0.7572***	0.6879***	-0.0281ns		0.8756***	0.1314ns
GYP	-0.0883ns	-0.0941ns	-0.0852ns	0.1601ns	-0.607***	-0.0226ns	0.6095***	0.3422ns	0.8496***	0.5610**	0.8430***	0.9675***	-0.0219ns	0.6318***		0.5698*
PHI	-0.5004**	-0.5116**	-0.4951**	0.2700ns	-0.649***	-0.0720ns	0.6156***	0.4079*	0.4976**	0.0929ns	0.4800*	0.5880**	0.0600ns	-0.0270ns	0.6384***	
	Oyali_084 and Savur_063															
DFP		0.9795***	0.9787***	-0.206ns	0.5241*	0.1109ns	-0.6774**	-0.4186ns	-0.1670ns	-0.1586ns	-0.1865ns	-0.1999ns	0.6870**	0.4005ns	0.1978ns	-0.2017ns
DFP	0.9936***		0.9765***	-0.225ns	0.5354*	0.0744ns	-0.6617**	-0.4255ns	-0.1552ns	-0.0965ns	-0.1685ns	-0.1948ns	0.6264**	0.4221ns	0.1813ns	-0.2298ns
DPM	0.9791***	0.9782***		-0.215ns	0.5004*	0.1312ns	-0.6452**	-0.4291ns	-0.1382ns	-0.1269ns	-0.1591ns	-0.1776ns	0.7289***	0.3827ns	0.1318ns	-0.2377ns
PT	-0.2894ns	-0.3217ns	-0.2944ns		-0.1253ns	0.1156ns	0.1952ns	-0.2502ns	-0.0873ns	-0.0472ns	-0.0819ns	-0.0149ns	0.1517ns	-0.5188*	-0.2961ns	0.6172**
HVS	0.4545*	0.4759*	0.4292*	-0.348ns		-0.1340ns	-0.910***	-0.6198**	-0.4087ns	-0.0739ns	-0.4075ns	-0.5026*	0.2907ns	0.0231ns	-0.3027ns	-0.2078ns

GH	0.0063ns	0.0352ns	0.0259ns	0.0179ns	-0.1117ns		0.0575ns	-0.3890ns	0.2700ns	-0.0114ns	0.2477ns	0.2829ns	0.0675ns	0.1639ns	0.2270ns	-0.1713ns
%PS	-0.609***	-0.6404***	-0.5943**	0.3838*	-0.895***	-0.0514ns		0.6104**	0.4487ns	0.0406ns	0.4487ns	0.4984*	-0.4115ns	-0.0984ns	0.2613ns	0.3650ns
PHT	-0.1915ns	-0.2028ns	-0.1138ns	0.2414ns	-0.3936*	0.1039ns	0.4756*		0.3684ns	0.0743ns	0.3727ns	0.3770ns	-0.2906ns	0.1778ns	0.1980ns	-0.0066ns
FPP	-0.3501ns	-0.3770ns	-0.3028ns	0.0425ns	-0.624***	0.3543ns	0.5751**	0.3814*		0.3597ns	0.9971***	0.9548***	-0.1717ns	0.6842**	0.5788**	-0.1141ns
UPP	-0.1952ns	-0.2232ns	-0.1148ns	0.0202ns	-0.2934ns	0.2197ns	0.2985ns	0.4417*	0.7073***		0.4189ns	0.3961ns	-0.2362ns	0.3653ns	0.1267ns	-0.3124ns
TPP	-0.3481ns	-0.3767ns	-0.3001ns	0.0304ns	-0.605***	0.3263ns	0.5647**	0.3646ns	0.9977***	0.7306***		0.9579***	-0.1925ns	0.6855**	0.5732*	-0.1269ns
TSP	-0.3001ns	-0.3213ns	-0.2671ns	-0.002ns	-0.656***	0.4302*	0.5889**	0.3413ns	0.9504***	0.6098***	0.9402***		-0.1809ns	0.6111**	0.5416*	-0.1429ns
HSW	0.3045ns	0.2919ns	0.2636ns	0.0945ns	-0.0876ns	0.1484ns	0.0564ns	0.2317ns	-0.2357ns	-0.2871ns	-0.2731ns	-0.0686ns		0.0184ns	-0.1995ns	-0.0385ns
PBP	0.2405ns	0.2476ns	0.2855ns	0.0921ns	-0.3406ns	0.6376***	0.2183ns	0.4315*	0.3534ns	0.3880*	0.3271ns	0.4105*	0.3952*		0.6800**	-0.4968*
GYP	-0.1979ns	-0.2252ns	-0.1727ns	0.0192ns	-0.662***	0.3575ns	0.6131***	0.4863*	0.8310***	0.5093**	0.8130***	0.8969***	0.1923ns	0.5295**		0.0830ns
PHI	-0.3267ns	-0.3720ns	-0.3475ns	-0.032ns	-0.4797*	-0.1365ns	0.5386**	0.1419ns	0.6143***	0.1996ns	0.6145***	0.6544***	-0.0046ns	-0.1839ns	0.6680***	
Sirna_060 and Gunas_063																
DFP		0.9396***	0.9640***	-0.036ns	0.1602ns	-0.0607ns	-0.2839ns	-0.3859*	0.0515ns	-0.0796ns	0.0165ns	-0.0557ns	-0.0316ns	0.3309ns	-0.0959ns	-0.5104**
DPM	0.9334***		0.9450***	-0.151ns	0.2654ns	-0.0514ns	-0.3667ns	-0.3395ns	0.1137ns	-0.0913ns	0.0671ns	0.0144ns	-0.0354ns	0.4745**	-0.0617ns	-0.5784**
PT	0.9500***	0.9458***		-0.105ns	0.2078ns	-0.0599ns	-0.3349ns	-0.3179ns	0.0426ns	-0.0765ns	0.0089ns	-0.0628ns	-0.0109ns	0.3782*	-0.0892ns	-0.5688**
HVS	-0.0695ns	-0.1877ns	0.0049ns		-0.5382**	0.0206ns	0.4672*	-0.0157ns	0.2677ns	0.0196ns	0.2165ns	0.2473ns	0.1116ns	0.0179ns	0.1042ns	0.2715ns
GH	0.1502ns	0.1737ns	0.0213ns	-0.763**		-0.1972ns	-0.959***	-0.2946ns	-0.581***	-0.2529ns	-0.5471**	-0.606***	-0.1460ns	-0.2292ns	-0.3702*	-0.4719**
%PS	0.0650ns	0.1331ns	0.1015ns	0.0977ns	-0.2181ns		0.1550ns	0.7626***	0.3444ns	0.9768***	0.6403***	0.4918**	0.6544***	0.1457ns	0.9453***	0.0583ns
PHT	-0.1069ns	-0.1875ns	0.0196ns	0.6875*	-0.919***	0.3418ns		0.3007ns	0.5575**	0.2282ns	0.5193**	0.5879***	0.0767ns	0.1860ns	0.3333ns	0.5341**
FPP	-0.6984*	-0.6264*	-0.6577*	0.2935ns	-0.4898ns	0.0479ns	0.2856ns		0.3319ns	0.7993***	0.5574**	0.4535*	0.6005***	0.1594ns	0.7854***	0.1160ns
UPP	-0.4709ns	-0.4539ns	-0.2690ns	0.6143*	-0.7607**	-0.1466ns	0.6999*	0.5667ns		0.3898*	0.9390***	0.9617***	0.2489ns	0.7023***	0.5974***	0.3364ns
TPP	-0.2857ns	-0.2679ns	-0.2031ns	0.6190*	-0.843***	-0.1011ns	0.6201*	0.7182**	0.7413**		0.6803***	0.5093**	0.6862***	0.1240ns	0.9471***	0.1029ns
TSP	-0.4626ns	-0.4424ns	-0.2725ns	0.6454*	-0.8047**	-0.1418ns	0.7113**	0.6146*	0.9922***	0.8168**		0.9570***	0.4487*	0.6185***	0.8297***	0.2957ns
HSW	-0.4545ns	-0.4746ns	-0.2913ns	0.7869**	-0.8003**	-0.1247ns	0.7078*	0.5431ns	0.9528***	0.7567**	0.9580***		0.3104ns	0.6806***	0.7255***	0.3735*
PBP	0.2262ns	0.2712ns	0.2670ns	-0.096ns	0.1909ns	-0.1760ns	-0.2021ns	-0.3796ns	0.0074ns	-0.1700ns	-0.0335ns	0.0026ns		0.1793ns	0.6663***	-0.0471ns
GYP	-0.2227ns	-0.1226ns	0.0245ns	0.6826*	-0.7824**	0.0864ns	0.6852*	0.3968ns	0.8858***	0.6942*	0.8902***	0.8783***	0.1562ns		0.3606ns	-0.2041ns
PHI	-0.4666ns	-0.4745ns	-0.3165ns	0.7690**	-0.7656**	-0.1714ns	0.6535*	0.5352ns	0.9321***	0.7649**	0.9425***	0.9909***	0.0617ns	0.8674***		0.1816ns
Karabache_081 and S2DrD																
DFP		0.9970***	0.9897**	-0.037ns	0.1456ns	-0.2609ns	0.1729ns	-0.0160ns	0.0798ns	-0.4795ns	0.4904ns	-0.1100ns	0.9358*	0.5006ns	0.1597ns	-0.4090ns
DPM	0.9431***		0.9858**	-0.008ns	0.1002ns	-0.2046ns	0.2191ns	-0.0890ns	0.1241ns	-0.5455ns	0.4998ns	-0.0611ns	0.9318*	0.4841ns	0.2299ns	-0.3551ns
PT	0.9277***	0.9393***		0.0915ns	0.1941ns	-0.1749ns	0.1307ns	0.0310ns	-0.0235ns	-0.4698ns	0.3775ns	-0.2194ns	0.8986*	0.6094ns	0.1811ns	-0.4887ns
HVS	0.3394ns	0.2224ns	0.2216ns		0.0680ns	0.8729ns	-0.0079ns	-0.1196ns	-0.3980ns	-0.3484ns	-0.6450ns	-0.4229ns	-0.2147ns	0.6371ns	0.5616ns	-0.2147ns
GH	-0.3558ns	-0.1811ns	-0.3903ns	-0.5579*		-0.3118ns	-0.9467*	0.7825ns	-0.1832ns	0.3612ns	0.0869ns	-0.4001ns	0.3046ns	0.6579ns	-0.2199ns	-0.3984ns
%PS	-0.2261ns	-0.1133ns	-0.0335ns	-0.018ns	0.1293ns		0.2886ns	-0.5272ns	-0.0690ns	-0.5200ns	-0.5335ns	-0.0023ns	-0.4000ns	0.2101ns	0.7453ns	0.2349ns
	0.2510ns	0.1170ns	0.2825ns	0.5713*	-	0.0572ns		-0.7913ns	0.1744ns	-0.5354ns	0.0206ns	0.3287ns	-0.0207ns	-0.449ns	0.3065ns	0.2460ns
0.9232***																
PHT	0.2527ns	0.4138ns	0.3979ns	-0.311ns	0.0078ns	0.0063ns	0.0381ns		-0.6120ns	0.8022ns	-0.2320ns	-0.7131ns	0.0151ns	0.4392ns	-0.7417ns	-0.7055ns
FPP	0.3446ns	0.3374ns	0.5283*	0.0602ns	-0.6630**	0.0150ns	0.6672**	0.4695ns		-0.5548ns	0.8178ns	0.9606**	0.3313ns	-0.447ns	0.5151ns	0.8512ns
UPP	0.1810ns	0.1445ns	0.3600ns	-0.196ns	-0.5512*	-0.1351ns	0.4426ns	0.2706ns	0.8707***		-0.3665ns	-0.4905ns	-0.4530ns	-0.129ns	-0.9076*	-0.4033ns
TPP	0.3321ns	0.3212ns	0.5173*	0.0344ns	-0.6590**	-0.0010ns	0.6496**	0.4533ns	0.9984***	0.8967***		0.6870ns	0.7283ns	-0.224ns	0.1577ns	0.4170ns
TSP	0.3665ns	0.3825ns	0.5593*	0.0132ns	-0.6145*	0.0270ns	0.6106*	0.5131ns	0.9925***	0.8755***	0.9925***		0.1062ns	-0.655ns	0.4638ns	0.9353*
HSW	0.2133ns	0.1570ns	0.2671ns	-0.227ns	-0.4100ns	-0.0954ns	0.5250*	0.4072ns	0.7541**	0.6740**	0.7510**	0.7289**		0.4138ns	0.1751ns	-0.2041ns
PBP	0.4363ns	0.5369*	0.6285*	-0.168ns	-0.3012ns	0.1903ns	0.3563ns	0.8103***	0.7602**	0.5355*	0.7439**	0.8015***	0.6297*		0.1807ns	-0.6578ns

GYP	0.3500ns	0.3501ns	0.5339*	-0.070ns	-0.5649*	-0.0512ns	0.5230*	0.4672ns	0.9695***	0.9372***	0.9792***	0.9786***	0.7261**	0.7327**	0.5172ns
PHI	0.1714ns	0.1357ns	0.2503ns	0.1135ns	-0.5337*	-0.1336ns	0.5344*	0.1997ns	0.7835***	0.8088***	0.7994***	0.7798***	0.5724*	0.3571ns	0.8313***

AR = *Cicer arietinum* and *reticulatum*, AE = *Cicer arietinum* and *reticulatum*, DFF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant, ns = non-significant, * significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$.

Appendix 3.3. Trait, QTL, Chromosome, Closest marker, Position (cM), Interval (cM and Mb), LOD, PVE (%), Additive from each of the 19 biparental populations of *C. arietinum* crosses with *C. reticulatum* and *C. echinospermum*.

9xBari1_092															
Trait	Chr	QTL	Position (cM)	LeftMarke r	RightMarke r	LOD	PVE(%)	Add	Contributing parent	LeftCI (cM)	RightCI (cM)	Closest marker	Interval(cM)	Physical distance(Mb)	Genet dist/Physical dist(cM/Mb)
DFF	2	qDFF-2-1	22.0	C7M173	C7M154	3.0	14.1	0.3	Bari1_092	21.5	23.5	C7M173	2	2.6	0.77
DPM	1	qDPM-1-1	5.0	C5M157	C5M155	6.7	47.3	-15.6	ICCV96029	3.5	5.5	C5M155	2	0.48	4.2
	2	qDPM-2-1	22.0	C7M173	C7M154	3.5	20.8	1.0	Bari1_092	21.5	23.5	C7M173	2	2.6	0.77
%PS	1	q%PS-1-1	18.0	C3M146	C3M141	3.0	40.9	-2.6	ICCV96029	16.5	19.5	C3M141	3	0.67	4.5
PBP	3	qPBP-3-1	4.0	C1M33	C1M48	3.2	38.6	0.9	Bari1_092	3.5	6.5	C1M48	3	1.2	2.5
9xBari2_072n2															
DFF	4	qDFF-4-1	12	C8M33	C5M19	3.8	28.5	-3.6	ICCV96029	5.5	15.5	C5M19	10	15.7	0.64
	4	qDFF-4-2	38	C5M90	C5M134	3.0	24.5	-3.7	ICCV96029	37.5	40	C5M90	2.5	4.8	0.52
	7	qDFF-7-1	0	C5M169	C5M169	3.6	23.6	-3.4	ICCV96029	0	0	C5M169	0	0	0
DPM	6	qDPM-6-1	8	C7M78	C7M88	3.0	27.3	-2.6	ICCV96029	4.5	10.5	C7M88	6	1.1	5.5
HVS	4	qHVS-4-1	16	C5M19	C5M59	3.6	60.9	-0.2	ICCV96029	12.5	18.5	C5M59	6	5.5	1.1
HSW	3	qHSW-3-2	42	C4M1	C4M26	3.2	34.6	1.3	Bari2_72n2	37.5	42	C4M26	4.5	8.8	0.5
9xBari3_072C															
DFF	5	qDFF-5-1	29.0	C4M148	C4M29	4.2	61.4	-10.1	ICCV96029	24.5	36.5	C4M148	12	18	0.67
	6	qDFF-6-1	41.0	C7M180	C7M132	3.6	13.3	-2.3	ICCV96029	40.5	46.5	C7M180	6	9	0.67
	6	qDFF-6-2	58.0	C7M132	C7M196	3.0	12.9	-2.4	ICCV96029	50.5	65.5	C7M132 & C7M132	15	12.3	1.2
DPM	5	qDPM-5-1	20.0	C4M193	C4M198	3.0	39.7	-13.5	ICCV96029	15.5	23.5	C4M198	8	1.98	4
	5	qDPM-5-2	32.0	C4M148	C4M29	3.0	37.7	-12.2	ICCV96029	24.5	39.5	C4M148 & C4M29	15.0	18	0.83
PT	4	qPT-4-1	40.0	C6M1	C6M89	3.0	73.5	0.6	Bari3_072C	23.5	40.5	C6M89	17	26.7	0.64
HVS	6	qHVS-6-1	37.0	C8M34	C8M38	3.4	15.2	-0.2	ICCV96029	33.5	39.5	C8M38	6	0.034	176.5
TSP	4	qTSP-4-1	40.0	C6M1	C6M89	4.5	14.5	1.3	Bari3_072C	36.5	40.5	C6M89	4	26.7	0.15

HSW	3	qHSW-3-1	56.0	C6M36	C6M54	3.0	6.7	0.1	Bari3_072C	52.5	60.5	C6M36 &C6M54		8.0	3.3	2.4
	5	qHSW-5-1	13.0	C4M193	C4M198	5.5	93.0	-0.3	ICCV96029	6.5	18.5	C4M198	12	0.9	13.3	
PBP	4	qPBP-4-1	0.0	C3M224	C3M182	3.7	82.2	-0.8	ICCV96029	0	3.5	C3M224	3.5	9.96	0.35	
PBP	6	qPBP-6-1	14.0	C7M76	C7M67	3.0	14.8	0.9	Bari3_072C	6.5	23.5	C7M76	17	1.4	12.1	
9xBari3_100																
DFF	3	qDFF-3-1	79.0	C4M3	C4M19	3.5	28.8	-1.5	ICCV96029	76.5	84.5	C4M3	8.0	4.8	1.7	
	6	qDFF-6-1	0.0	C5M155	C5M157	5.1	46.9	-10.4	ICCV96029	0.0	1.5	C5M155	1.5	0.5	2.8	
DFP	6	qDFP-6-1	0.0	C5M155	C5M157	3.9	46.3	-12.8	ICCV96029	0.0	1.5	C5M155	1.5	0.5	2.8	
DPM	3	qDPM-3-1	80.0	C4M3	C4M19	3.2	34.2	-0.4	ICCV96029	76.5	85.5	C4M3	9.0	4.8	1.9	
	6	qDPM-6-1	0.0	C5M155	C5M157	3.8	35.7	-14.9	ICCV96029	0.0	1.5	C5M155	1.5	0.5	2.8	
FPP	5	qFPP-5-1	19.0	C5M25	C5M53	3.7	20.8	9.8	Bari3_100	14.5	21.0	C5M53	6.5	3.6	1.8	
	7	qFPP-7-1	49.0	C6M56	C6M69	5.5	43.1	0.1	Bari3_100	47.5	50.5	C6M56 &C6M69		3.0	4.2	0.7
UPP	3	qUPP-3-1	9.0	C2M61	C2M8	3.0	37.1	0.9	Bari3_100	6.5	13.5	C2M61	7.0	1.7	4	
TPP	5	qTPP-5-1	11.0	C5M19	C8M33	3.6	20.1	9.7	Bari3_100	8.5	12.5	C8M33	4.0	15.7	0.26	
	7	qTPP-7-1	49.0	C6M56	C6M69	5.6	42.5	-1.1	ICCV96029	47.5	50.5	C6M56 &C6M69		3.0	4.2	0.72
TSP	3	qTSP-3-1	7.0	C2M61	C2M8	3.4	42.4	-4.6	ICCV96029	2.5	14.5	C2M61	12.0	17.4	0.7	
PBP	3	qPBP-3-1	13.0	C2M61	C2M8	3.1	38.6	-2.0	ICCV96029	3.5	18.5	C2M8	15.0	17.4	0.86	
GYP	3	qGYP-3-1	5.0	C8M13	C2M61	4.1	39.4	-0.4	ICCV96029	1.5	14.5	C8M13	13.0	15.5	0.84	
PHI	3	qPHI-3-1	29.0	C2M1	C4M44	3.2	25.1	6.4	Bari3_100	24.5	30.5	C4M44	6.0	10.2	0.59	
	3	qPHI-3-2	57	C4M109	C4M131	3.9	35.7	10.7	Bari3_100	55.5	57.5	C4M131	2.0	2.9	0.69	
	8	qPHI-8-1	27	C7M44	C7M76	3.0	15.9	-6.1	ICCV96029	25.5	30.0	C7M44	4.5	4.8	0.94	
9xBari3_100D																
TSP	2	qTSP-2-1	2.0	C1M20	C1M50	3.6	39.7	-18.4	ICCV96029	0.0	4.5	C1M20	4.5	2.5	1.8293	
GYP	2	qGYP-2-1	2.0	C1M20	C1M50	3.4	38.7	-2.0	ICCV96029	0.0	4.5	C1M20	4.5	2.5	1.8293	
9xBeseve_079																
DPM	6	qDPM-6-1	11	C5M91	C5M90	4.17	51.06	-15.61	ICCV96029	10.5	11.5	C5M91&C5M90		1	0.14	7.1
PT	2	qPT-2-1	9	C7M104	C7M107	3.00	10.51	0.45	Beseve_079	6.5	11.5	C7M104	5	0.3	16.7	
												&C7M107				
	4	qPT-4-1	10	C3M104	C3M92	3.84	37.84	-0.45	ICCV96029	9.5	10.5	C3M104	&	1	5.6	0.18
												C3M92				
	4	qPT-4-2	15	C3M52	C3M45	3.28	24.49	-0.60	ICCV96029	13.5	15.5	C3M45	2	1.14	1.8	

GH	6	qGH-6-1	49	C5M187	C8M20	5.55	38.51	-0.81	ICCV96029	47.5	49.5	C8M20	2	3.19	0.63
%PS	5	q%PS-5-1	14	C2M22	C2M117	3.95	33.51	8.59	Beseve_079	13.5	16	C2M22	3.5	9.34	0.37
PHT	1	qPHT-1-1	7	C1M151	C1M170	3.43	45.59	2.52	Beseve_079	5.5	9.5	C1M151 & C1M170	4	2.38	1.7
PHI	7	qPHI-7-1	9	C5M155	C5M145	3.00	46.07	8.42	Beseve_079	6.5	9.5	C5M145	3	1.23	2.4
		9xCudiA_152													
PT	3	qPT-3-1	52	C7M128	C7M126	3	41.9497	0.263	CudiA_152	51.5	55.5	C7M128	4	0.065	61.5
							7								
GH	5	qGH-5-1	20	C3M120	C3M119	3	26.6242	-0.614	ICCV96029	19.5	20.5	C3M120 & C3M119	1	0.089	11.2
	5	qGH-5-2	29	C3M91	C3M76	3.64	31.0659	-1.008	ICCV96029	27.5	32.5	C3M91	5	2.1	2.4
						4									
PHT	1	qPHT-1-1	42	C1M162	C1M159	3	38.2615	3.651	CudiA_152	40.5	42.5	C1M162	2	0.44	4.5
							2								
PBP	3	qPBP-3-1	51	C2M1	C2M17	3	40.5167	0.278	CudiA_152	49.5	51.5	C2M17	2	2.95	0.68
							2								
		9xCudiB_22C													
DFP	4	qDFP-4-1	39.0	C5M168	C5M164	3.6	54.9	-12.6	ICCV96029	34.5	40.5	C5M164	6.0	1.4	4.3
DFP	4	qDFP-4-1	39.0	C5M168	C5M164	3.4	51.9	-15.3	ICCV96029	34.5	40.5	C5M165	6.0	1.4	4.3
DPM	4	qDPM-4-1	41.0	C5M163	C5M160	5.2	51.1	-17.8	ICCV96029	40.5	43.0	C5M163	2.5	0.2	14.7
	6	qDPM-6-1	1.0	C6M133	C6M129	3.4	26.3	-4.2	ICCV96029	0.0	3.5	C6M133	3.5	0.8	4.4
GH	4	qGH-4-1	11.0	C4M34	C4M31	3.7	55.5	-0.9	ICCV96029	10.5	14.5	C4M34	4.0	0.5	7.5
FPP	3	qFPP-3-1	1.0	C2M50	C2M48	3.3	27.1	-17.4	ICCV96029	0.0	7.5	C2M50	7.5	0.8	9
	3	qFPP-3-2	24.0	C8M13	C2M28	3.0	26.3	-16.3	ICCV96029	22.5	27.5	C8M13	5.0	5.0	1
UPP	1	qUPP-1-1	0.0	C1M124	C1M117	3.0	34.6	-0.7	ICCV96029	0.0	2.5	C1M124	2.5	0.6	3.9
	6	qUPP-6-1	45.0	C6M34	C6M36	3.5	39.6	0.9	CudiB_22C	40.5	48.0	C6M36	7.5	0.5	15
TPP	3	qTPP-3-1	1.0	C2M50	C2M48	3.4	27.3	-18.3	ICCV96029	0.0	7.5	C2M50	7.5	0.8	9
	3	qTPP-3-2	24.0	C8M13	C2M28	2.6	26.3	-16.9	ICCV96029	22.5	26.5	C8M13	4.0	5.0	0.8
	1	qTSP-1-2	13.0	C1M128	C1M129	3.0	26.7	-25.8	ICCV96029	10.5	14.5	C1M128 & C1M129	4.0	0.2	17.4
	3	qTSP-3-1	1.0	C2M50	C2M48	3.0	24.8	-16.0	ICCV96029	0.0	7.5	C2M50	7.5	0.8	9
PBP	2	qPBP-2-1	0.0	C1M52	C1M57	3.92	25.2	4.0	CudiB_22C	0.0	1.5	C1M52	1.5	0.3	5.6

	6	qPBP-6-1	35	C6M137	C6M17	6.95	59.3	-1.3	ICCV96029	32.5	36.5	C6M17	4.0	28.1	0.14
						5									
PHI	6	qPHI-6-1	1	C6M133	C6M129	3.04	45.0	7.6	CudiB_22C	0.0	1.5	C6M129	1.5	0.8	1.9
						3									
		9xDerei_070													
DFF	1	qDFF-1-1	12.0	C5M144	C5M150	11.7	60.0	-13.1	ICCV96029	9.5	12.5	C5M150	3.0	0.7	4.6
DFP	1	qDFP-1-1	10.0	C5M155	C5M144	10.1	60.0	-18.5	ICCV96029	9.5	12.5	C5M155	3.0	1.3	2.3
DPM	1	qDPM-1-1	11.0	C5M155	C5M144	9.7	60.6	-21.9	ICCV96029	9.5	12.5	C5M156	3.0	1.3	2.3
GH	2	qGH-2-1	25.0	C7M150	C7M159	3.0	43.3	-0.68	ICCV96029	24.5	28.5	C7M150	4.0	1.4	2.9
GH	5	qGH-5-1	24.0	C4M217	C4M11	3.0	26.6	0.425	Derei_070	21.5	26.5	C4M217 & C4M11	4.0	30.7	0.13
%PS	1	q%PS-1-1	49.0	C7M70	C1M82	3.8	29.2	-2.58	ICCV96029	48.5	55.5	C7M70	7.0	2.4	2.9
	3	q%PS-3-1	21.0	C2M29	C2M18	4.6	37.8	-14.7	ICCV96029	19.5	25.5	C2M29	6.0	4.9	1.2
PHT	1	qPHT-1-1	49.0	C7M70	C1M82	2.8	24.1	2.374	Derei_070	48.5	54.5	C7M70	6.0	2.4	2.5
PHT	2	qPHT-2-1	22.0	C7M77	C7M73	3.8	29.5	3.709	Derei_070	20.5	22.5	C7M73	2.0	0.5	4.3
FPP	1	qFPP-1-1	58.0	C7M70	C1M82	3.7	25.5	-11.4	ICCV96029	54.5	61.5	C7M70	7.0	2.4	2.9
	2	qFPP-2-1	9.0	C7M67	C1M78	3.1	21.8	-12.2	ICCV96029	4.5	11.5	C1M78	7.0	2.3	3
TPP	1	qTPP-1-1	58.0	C7M70	C1M82	3.5	25.0	-11.4	ICCV96029	54.5	61.5	C7M70	6.0	2.4	2.5
	2	qTPP-2-1	9.0	C7M67	C1M78	3.0	21.2	-12.3	ICCV96029	4.5	11.5	C1M78	7.0	2.3	3
GYP	4	qGYP-4-1	0.0	C8M35	C3M1	3.0	20.1	-0.8	ICCV96029	0	3.5	C3M1	3.5	10.3	0.34
		9xDerei_072													
DFF	1	qDFF-1-1	55.0	C1M41	C1M32	6.4	3.0	0.1	Derei_072	53.5	56.5	C1M41&C1M32	3.0	1.0	3
	4	qDFF-4-1	3.0	C5M134	C5M136	21.5	59.3	-13.4	ICCV96029	0	3.5	C5M136	3.5	0.1	50
	4	qDFF-4-2	132.0	C1M114	C1M106	17.4	29.4	-9.7	ICCV96029	130.5	132.5	C1M114 & C1M106	1.0	0.6	1.6
DFP	2	qDFP-2-1	32.0	C3M47	C3M46	3.2	8.3	-8.0	ICCV96029	31.5	37.5	C3M47	6.0	0.2	35.3
	2	qDFP-2-2	48.0	C3M80	C3M82	3.0	7.8	-7.8	ICCV96029	45.5	50.5	C3M80 & C3M82	5.0	0.1	50
	4	qDFP-4-1	3.0	C5M134	C5M136	8.2	65.3	-12.5	ICCV96029	0	3.5	C5M136	3.5	0.1	50
DPM	2	qDPM-2-1	28.0	C3M67	C3M49	6.7	29.9	-13.0	ICCV96029	25.5	28.5	C3M49	3.0	2.3	1.3
	4	qDPM-4-1	2.0	C5M155	C5M134	9.7	59.8	-17.0	ICCV96029	0	3.5	C5M134	3.5	2.4	1.5
PPS	5	qPPS-5-1	40.0	C2M44	C2M45	4.0	22.9	-6.9	ICCV96029	37.5	43.5	C2M44	6.0	0.1	120
	7	qPPS-7-1	61.0	C7M130	C7M129	3.1	18.8	-3.2	ICCV96029	58.5	62.5	C7M129	4.0	0.4	9.3
PHT	4	qPHT-4-1	134.0	C1M87	C1M128	3.0	45.9	5.4	Derei_072	132.5	135.5	C1M87&C1M128	3.0	3.9	0.8

UPP	3	qUPP-3-1	4.0	C4M86	C4M92	5.4	68.3	-0.1	ICCV96029	1.5	5.5	C4M92	4.0	1.2	3.3
TSP	4	qTSP-4-1	26.0	C5M60	C5M70	3.3	29.9	6.0	Derei_072	24.5	27.5	C5M60&C5M70	3.0	0.9	3.2
PBP	5	qPBP-5-1	53.0	C2M91	C2M90	3.0	25.5	2.5	Derei_072	51.5	53.5	C2M90	2.0	0.6	3.3
		9xEgill_065													
DFP	2	qDFP-2-1	21.0	C1M143	C1M144	4.2	40.9	0.041	Egill_065	20.5	22.5	C1M143	2.0	0.1	15.4
	5	qDFP-5-1	10.0	C5M15	C8M33	3.0	24.5	7.037	Egill_065	6.5	12.5	C8M33	6.0	15.1	0.4
PT	4	qPT-4-1	27.0	C8M13	C2M42	3.1	39.8	0.569	Egill_065	18.5	33.5	C2M42	15.0	11.8	1.3
		9xEgill_073													
PHT	4	qPHT-4-1	20.0	C2M61	C2M56	3.0	37.9	1.024	Egill_073	17.5	22.5	C2M61&C2M56	5.0	2.0	2.5
		9xKalkan_064													
	6	qDFF-6-1	18	C5M111	C5M109	4.8	74.7	-15.6	ICCV96029	15.5	22.5	C5M111	7	0.4	18.9
	7	qDFF-7-1	36	C6M59	C6M72	3.7	13.5	2.2	Kalkan_064	34.5	36.5	C6M72	2	4.1	0.5
DFP	2	qDFP-2-1	1	C2M48	C2M40	3.0	14.0	-4.2	ICCV96029	0	2.5	C2M48	2.5	1.6	1.6
	6	qDFP-6-1	18	C5M111	C5M109	4.2	81.3	-15.3	ICCV96029	15.5	22.5	C5M111	7	0.4	18.9
	6	qDPM-6-1	18	C5M111	C5M109	4.3	81.7	-19.1	ICCV96029	15.5	22.5	C5M111	7	0.4	18.9
PT	2	qPT-2-1	53	C3M170	C3M168	3.1	26.3	-0.4	ICCV96029	50.5	56.5	C3M170	6	0.1	42.9
	6	qPT-6-1	9	C5M160	C5M155	3.6	28.2	-0.3	ICCV96029	0	13.5	C5M155	13.5	0.9	15.2
	6	qPT-6-2	22	C5M109	C8M13	7.9	37.0	-0.5	ICCV96029	20.5	22.5	C8M13	2	14.0	0.14
GH	6	qGH-6-1	3	C5M165	C5M160	4.1	83.0	-0.7	ICCV96029	0	9.5	C5M165	9.5	1.0	9.5
PHT	1	qPHT-1-1	15	C1M142	C1M88	3.7	79.2	7.8	Kalkan_064	12.5	17.5	C1M142&C1M188	5.0	5.6	0.89
	5	qPHT-5-1	0	C4M30	C4M41	3.0	16.1	4.7	Kalkan_064	0	2.5	C4M30	2.5	1.5	1.7
UPP	1	qUPP-1-1	23	C7M132	C7M167	4.8	53.9	-0.1	ICCV96029	20.5	23	C7M167	2.5	5.9	0.42
	2	qUPP-2-1	37	C3M35	C3M49	4.1	37.5	1.0	Kalkan_064	36.5	38.5	C3M35&C3M49	2.0	3.3	0.6
	4	qUPP-4-1	12	C4M4	C4M3	3.0	6.8	-0.2	ICCV96029	9.5	18.5	C4M4	9	0.02	500
PHI	1	qPHI-1-1	0	C1M1	C1M182	3.0	30.7	6.6	Kalkan_064	0	4.5	C1M1	4.5	2.0	2.3
	2	qPHI-2-1	16	C2M54	C2M3	3.1	33.2	7.2	Kalkan_064	12.5	21.5	C2M54	9	16.3	0.55
		9xKesen_075													
HVS	8	qHVS-8-1	14.0	C6M98	C6M89	3.0	40.5	-0.8	ICCV96029	12.5	18.5	C6M98	6.0	2.00	3
GH	4	qGH-4-1	48.0	C7M67	C7M69	3.7	47.8	0.1	Kesen_075	47.5	49.5	C7M67	2.0	0.02	100
PPS	3	qPPS-3-1	22.0	C5M165	C5M164	3.0	39.7	13.2	Kesen_075	20.5	22.5	C5M164	2.0	0.08	23.8

PHT	4	qPHT-4-1	46.0	C7M48	C7M50	3.5	46.4	-0.3	ICCV96029	44.5	47.5	C7M48&C7M50	3.0	0.27	11.1
FPP	3	qFPP-3-1	3.0	C5M100	C5M111	3.7	32.7	12.0	Kesen_075	0	8.5	C5M100	8.5	1.7	5
	3	qFPP-3-2	21.0	C5M165	C5M164	2.7	26.1	9.3	Kesen_075	20.5	22.5	C5M165	2.0	0.08	23.8
TPP	3	qTPP-3-1	3.0	C5M100	C5M111	3.7	32.8	12.7	Kesen_075	0	8.5	C5M100	8.5	1.7	5
	3	qTPP-3-2	21.0	C5M165	C5M164	3.0	26.5	9.8	Kesen_075	20.5	22.5	C5M165	2.0	0.08	23.8
TSP	8	qTSP-8-1	16.0	C6M98	C6M89	3.2	38.9	12.5	Kesen_075	14.5	19.5	C6M98	5.0	2.0	2.5
	9xOyali_084														
DFF	3	qDFF-3-1	2.0	C7M132	C7M143	3.6	5.3	-0.2	ICCV96029	0	5.5	C7M132	5.5	1.7	3.2
	3	qDFF-3-2	32.0	C3M153	C3M161	7.8	23.5	0.9	Oyali_084	30.5	33.5	C3M153&C3M161	3.0	1.0	3
	6	qDFF-6-1	13.0	C5M155	C5M132	12.6	65.9	-13.0	ICCV96029	10.5	15.5	C5M155&C5M132	5.0	2.8	1.8
DFP	3	qDFP-3-1	32.0	C3M153	C3M161	3.0	16.9	-3.7	ICCV96029	30.5	33.5	C3M153&C3M161	3.0	1.0	3
	5	qDFP-5-1	6.0	C6M69	C6M54	3.0	15.1	-3.8	ICCV96029	4.5	7.5	C6M69&C6M54	3.0	5.0	0.6
	6	qDFP-6-1	13.0	C5M155	C5M132	4.4	61.1	-13.9	ICCV96029	10.5	16.5	C5M155	6.0	2.8	2.1
DPM	6	qDPM-6-1	18.0	C5M129	C5M109	11.0	67.1	-19.0	ICCV96029	16.5	19.5	C5M129&C5M109	3.0	1.4	2.1
HVS	6	qHVS-6-1	35.0	C5M64	C5M66	3.0	60.2	-0.6	ICCV96029	32.5	36.5	C5M66	4.0	0.2	19
PPS	6	qPPS-6-1	27.0	C5M90	C5M89	3.1	53.2	13.3	Oyali_084	23.5	29.5	C5M89	6.0	0.9	6.6
PHT	5	qPHT-5-1	49.0	C4M54	C4M61	3.4	49.8	-0.3	ICCV96029	47.5	50.5	C4M54&C4M61	3.0	1.0	3
UPP	1	qUPP-1-1	3.0	C1M75	C1M73	4.8	68.3	-0.9	ICCV96029	0	5.5	C1M73	5.5	0.1	29.3
HSW	3	qHSW-3-1	30.0	C2M1	C2M4	3.6	25.2	-0.2	ICCV96029	28.5	30.5	C2M1&C2M4	2.0	0.7	2.8
	6	qHSW-6-1	20.0	C5M109	C5M90	6.3	59.3	-1.8	ICCV96029	18.5	22.5	C5M109	4.0	2.6	1.5
PHI	3	qPHI-3-1	41.0	C7M105	C7M104	3.2	56.8	12.8	Oyali_084	39.5	45.5	C7M105	6.0	0.1	60
	9xSavur_063														
HVS	6	qHVS-6-1	5.0	C5M164	C5M145	3.0	49.1	-0.5	ICCV96029	1.5	10.5	C5M164	9.0	15.5	0.6
GH	4	qGH-4-1	22.0	C3M82	C3M79	3.0	49.5	-0.6	ICCV96029	20.5	23.5	C3M82&C3M79	3	0.3	10
PPS	6	qPPS-6-1	5.0	C5M164	C5M145	3.2	53.3	12.9	Savur_063	2.5	7.5	C5M164&C5M145	5	15.5	0.3
PHT	1	qPHT-1-1	57.0	C1M95	C1M87	4.3	52.6	4.1	Savur_063	52.5	59.5	C1M87	7	1.0	6.7
HSW	3	qHSW-3-1	28.0	C2M108	C8M13	5.2	60.5	1.9	Savur_063	25.5	28	C8M13	2.5	30.7	0.08
PBP	4	qPBP-4-1	22.0	C3M82	C3M79	5.3	62.4	-5.6	ICCV96029	20.5	23.5	C3M82	3	0.3	10
	9xSirma_060														
DFF	4	qDFF-4-1	29.0	C3M161	C3M169	4.4	39.0	3.1	Sirma_060	28.5	31.5	C3M161	3	0.63	4.8

	5	qDFF-5-1	13.0	C5M156	C5M150	3.0	14.8	-3.6	ICCV96029	10.5	15.5	C5M156&C5M150	5	0.66	7.6
DFP	5	qDFF-5-1	19.0	C5M165	C5M168	3.6	45.9	-5.4	ICCV96029	17.5	19	C5M165	1.5	1.40	1.1
DPM	4	qDPM-4-1	29.0	C3M161	C3M169	4.0	27.9	3.4	Sirna_060	28.5	31.5	C3M161	3	0.63	4.8
PHT	2	qPHT-2-1	8.0	C1M65	C1M66	3.0	34.4	2.8	Sirna_060	2.5	9.5	C1M66	6.5	0.13	50
FPP	1	qFPP-1-1	2.0	C1M119	C1M110	3.0	30.1	2.6	Sirna_060	1.5	4.5	C1M119	3	0.58	5.2
FPP	4	qFPP-4-1	23.0	C3M98	C3M128	3.2	25.0	-18.5	ICCV96029	20.5	24.5	C3M128	4	3.60	1.1
TPP	1	qTPP-1-1	2.0	C1M119	C1M110	3.0	31.3	2.7	Sirna_060	1.5	4.5	C1M119	3	0.58	5.2
TPP	4	qTPP-4-1	23.0	C3M98	C3M128	3.0	24.2	-19.0	ICCV96029	20.5	24.5	C3M128	4	3.6	1.1
TSP	4	qTSP-4-1	23.0	C3M98	C3M128	5.1	35.9	-24.7	ICCV96029	20.5	24.5	C3M129	5	3.6	1.4
PBP	1	qPBP-1-1	0.0	C1M124	C1M119	3.0	29.4	-0.2	ICCV96029	0	1.5	C1M124	1.5	0.4	3.8
PBP	4	qPBP-4-1	23.0	C3M98	C3M128	3.4	24.9	-6.5	ICCV96029	20.5	24.5	C3M128	4	3.6	1.1
GYP	4	qGYP-4-1	23.0	C3M98	C3M128	4.9	33.8	-2.4	ICCV96029	20.5	24.5	C3M128	4	3.6	1.1
		9xGunas_063													
PHT	1	qPHT-1-1	6.0	C1M29	C1M26	3.1	70.2	-2.2	ICCV96029	0	8.5	C1M26	8.5	0.97	8.8
FPP	1	qFPP-1-1	63.0	C2M13	C2M2	3.2	79.4	14.8	Gunas_063	58.5	65.5	C2M2	7	6.44	1.1
UPP	1	qUPP-1-1	15.0	C1M13	CTM65	7.1	67.6	-0.2	ICCV96029	12.5	18.5	C1M13	6	11.06	0.54
	3	qUPP-1-1	5.0	C5M70	C5M94	3.5	3.5	0.9	ICCV96029	2.5	5	C5M94	2.5	1.97	1.3
TPP	1	qTPP-1-1	63.0	C2M13	C2M2	3.0	27.4	17.1	Gunas_063	58.5	65.5	C2M2	7	6.44	1.1
	4	qTPP-4-1	5.0	CTM47	C6M73	3.0	30.3	21.6	Gunas_063	2.5	7	C6M73	4.5	23.50	0.19
TSP	1	qTSP-1-1	63.0	C2M13	C2M2	3.0	32.1	13.5	Gunas_063	58.5	65.5	C2M2	7	6.44	1.1
	4	qTSP-4-1	5.0	CTM47	C6M73	3.0	34.0	17.9	Gunas_063	2.5	7	C6M73	4.5	23.5	0.19
	8	qTSP-8-1	0.0	C6M133	C6M154	3.0	30.7	-20.2	ICCV96029	0	6	C6M133	6	1.98	3
HSW	1	qHSW-1-1	57.0	C2M36	C2M13	4.9	86.6	-0.3	ICCV96029	54.5	61.5	C2M36&C2M13	7.0	8.1	0.9
GYP	1	qGYP-1-1	63.0	C2M13	C2M2	3.0	32.2	1.3	Gunas_063	58.5	65.5	C2M2	7	6.44	1.1
	4	qGYP-4-1	5.0	CTM47	C6M73	3.0	34.2	1.8	Gunas_063	2.5	7	C6M73	4.5	23.5	0.19
PHI	2	qPHI-2-1	34.0	C3M65	C3M63	3.0	70.4	-2.1	ICCV96029	32.5	34.5	C3M63	2	0.3	6.7
		9xS2DrD													
DFF	1	qDFF-1-1	159.0	C5M144	CTM61	3.6	69.5	-11.3	ICCV96029	158.5	162.5	C5M144	4	29.6	0.14
	7	qDFF-7-1	99.0	C6M19	C6M1	3.1	22.2	-3.7	ICCV96029	95.5	102.5	C6M19&C6M1	7.0	8.8	0.8
DFP	1	qDFP-1-1	134.0	CTM48	C1M219	3.3	38.9	6.8	S2DrD	129.5	137.5	C1M219	8	64.5	0.12

DPM	1	qDPM-1-1	134.0	CTM48	C1M219	3.7	38.8	12.0	S2DrD	132.5	137.5	C1M219	5	64.5	0.08
	7	qDPM-7-1	98.0	C6M19	C6M1	3.0	31.2	-6.0	ICCV96029	95.5	102.5	C6M19	7	8.8	0.8
PT	1	qPT-1-1	38.0	C7M56	C1M131	4.4	19.7	-0.4	ICCV96029	35.5	40.5	C7M56 & C1M131	5.0	5.0	1
	7	qPT-7-1	51.0	CTM49	C6M114	5.2	20.5	0.4	S2DrD	47.5	51.5	C6M114	4	34.4	0.1
	7	qPT-7-2	78.0	C6M98	C6M95	4.3	19.7	0.4	S2DrD	76.5	81.5	C6M98	5	0.5	9.6
HVS	4	qHVS-4-1	106.0	C6M211	C4M55	7.6	77.0	0.0	S2DrD	101.5	109.5	C4M55	8	35.4	0.23
GH	4	qGH-4-1	8.0	C4M1	CTM43	3.0	30.1	-1.0	ICCV96029	1.5	15.5	C4M1 & CTM43	14.0	1.4	10
	4	qGH-4-2	48.0	C4M152	C4M219	3.4	29.7	-1.0	ICCV96029	43.5	50.5	C4M219	7	19.9	0.4
PPS	1	qPPS-1-1	71.0	C7M144	C7M149	3.0	10.7	-5.0	ICCV96029	68.5	73.5	C7M144 & C7M149	5.0	1.0	5
	4	qPPS-4-1	106.0	C6M211	C4M55	6.2	85.8	3.4	S2DrD	101.5	109.5	C4M55	8	35.4	0.23
PHT	7	qPHT-7-1	98.0	C6M19	C6M1	3.0	60.2	-7.8	ICCV96029	91.5	102.5	C6M1	11.0	8.8	1.3
FPP	1	qFPP-1-1	5	C4M227	CTM42	3.0	46.8	-13.8	ICCV96029	0	11.5	C4M227	11.5	55.9	0.2
TSP	1	qTSP-1-1	4	C4M227	CTM42	3.0	32.1	-12.1	ICCV96029	0	11.5	C4M227	11.5	55.9	0.2
	3	qTSP-3-1	48	C2M64	C2M74	3.0	28.3	-14.6	ICCV96029	46.5	48.5	C2M74	2.0	3.5	0.6
PBP	1	qPBP-6-1	6	C4M227	CTM42	3.0	32.3	-2.0	ICCV96029	1.5	10.5	C4M227 & CTM42	9	55.9	0.16
	7	qPBP-7-2	98	C6M19	C6M1	4.9	31.8	-11.5	ICCV96029	95.5	102.5	C6M19	7	8.8	0.8
GYP	1	qGYP-1-1	6	C4M227	CTM42	3.2	44.0	-1.5	ICCV96029	0	12.5	C4M227	12.5	55.9	0.2
PHI	1	qPHI-1-1	100	C2M4	C1M217	3.7	55.0	-8.0	ICCV96029	95.5	104.5	C2M4 & C1M217	9	43.8	0.2

DDF = days to first flowering, DFP = days to first podding, DPM = days to physiological maturity, FPP = filled pod per plant, GH = growth habit, HSW = hundred seed weight, HVS = heat visual score, GYP = grain yield per plant, MS = mean square, PBP = plant biomass per plant, PHI = percentage of harvest index, PHT = plant height, %PS = percentage of pod set, PT = pod type, TPP = total pod per plant, TSP = Total seed per plant, UPP = unfilled pod per plant, LOD = Logarithms odds, q = QTL, QTL = quantitative trait loci, PVE% = explained phenotypic variance, Add = additive effect, Chr = chromosome, cM = Centi Morgan, Mb = Mega base pair, CI = continuity index.

Appendix 5.1. Above and below diagonals are the genotypic and phenotypic correlation coefficients of 12 traits, respectively.

Kobo, heat stressed environment

	DFE	D50F	DFP	GFP	DPM	PHT	TPP	TSP	HSW	PB	GY	PHI
DFE	1	0.96***	0.93***	0.44***	0.85***	0.3***	0.07	0.01	-0.03	0.2*	-0.08	-0.23
D50F	0.86***	1	0.97***	0.39***	0.84***	0.22*	0.05	0.003	-0.13	0.14	-0.13	-0.22
DFP	0.80***	0.93***	1	0.42***	0.84***	0.17	0.05	0.01	-0.17	0.1	-0.15	-0.2
GFP	0.33***	0.2**	0.28***	1	0.82***	0.15	0.2	0.13	-0.03	0.17	0.03	0.07
DPM	0.73***	0.73***	0.75***	0.82***	1	0.22*	0.13	0.08	-0.1	0.19*	-0.1	-0.18
PHT	0.2***	0.25***	0.23***	0.06	0.19***	1	0.24**	0.09	0.36***	0.2*	0.23*	0.05
TPP	0.05	0.16**	0.18***	0.1	0.16**	0.46***	1	0.94***	0.23*	0.21*	0.62***	0.65***
TSP	0.02	0.14**	0.17**	0.09	0.14**	0.35***	0.95***	1	0.1	0.16	0.56***	0.66***
HSW	-0.02	-0.06	-0.07	-0.01	-0.04	0.34***	0.31***	0.23***	1	0.4***	0.72***	0.43***
PB	0.15	0.18***	0.16**	0.1	0.17	0.28***	0.41***	0.39***	0.42***	1	0.5***	-0.05
GY	-0.05	0.015	0.01	0.01	0.02	0.35***	0.67***	0.65***	0.65***	0.6***	1	0.75***
PHI	-0.2	-0.1	-0.08	0.005	-0.07	0.21***	0.58***	0.58***	0.44***	0.05	0.7***	1

ICRISAT, heat stressed environment

	DFE	D50F	DFP	GFP	DPM	PHT	TPP	TSP	HSW	PB	GY	PHI
DFE	1	0.7***	0.09	0.5***	-0.2	0.3**	-0.06	-0.03	0.06	-0.2	-0.02	-0.03
D50F	0.61***	1	0.4**	0.5***	-0.2	0.2	0.05	0.05	0.2	-0.12	0.05	0.05
DFP	0.12	0.29**	1	0.5***	-0.01	0.05	0.45	0.4	0.6	-0.04	0.37**	0.4**
GFP	0.4***	0.25**	0.46***	1	-0.02	0.07	0.3*	0.3*	0.3*	-0.26	0.26*	0.29*
DPM	-0.12	-0.12	0.003	0.1	1	0.25	-0.25	-0.2	-0.09	0.25	-0.15	-0.16
PHT	0.23	0.14	0.08	0.2	0.29**	1	0.04	0.06	0.13	0.5***	0.04	-0.05
TPP	-0.05	0.01	0.36***	0.24*	-0.21	0.08	1	0.96***	0.73***	0.07	0.9***	0.85***
TSP	-0.04	0.01	0.33***	0.24*	-0.17	0.09	0.94***	1	0.73***	0.11	0.94***	0.89***
HSW	0.04	0.12	0.54***	0.3**	-0.08	0.15	0.67***	0.69***	1	0.05	0.75***	0.71***
PB	-0.06	-0.13	0.06	-0.04	0.22*	0.53***	0.14	0.17	0.15	1	0.14	-0.05
GY	-0.03	0.01	0.29**	0.21	-0.1	0.07	0.87***	0.94***	0.7***	0.21*	1	0.89***
PHI	-0.02	0.02	0.34***	0.22	-0.13	-0.04	0.79***	0.87***	0.66***	-0.02	0.85***	

DZ, non-stressed environment

	DFE	D50F	DFP	GFP	DPM	PHT	TPP	TSP	HSW	PB	GY	PHI
DFE	1	0.92***	0.7***	-0.28**	0.25**	0.1	-0.07	-0.07	0.074	0.06	-0.04	-0.1
D50F	0.88***	1	0.78***	-0.3***	0.27**	0.16	-0.05	-0.06	-0.02	0.07	-0.006	-0.064
DFP	0.6***	0.72***	1	-0.13	0.32***	0.23*	-0.18	-0.2*	0.19*	0.07	-0.06	-0.15
GFP	-0.35***	-0.44***	-0.26***	1	0.83***	0.17	-0.19*	-0.19*	0.12	-0.05	-0.07	0.03
DPM	0.22***	0.22***	0.22***	0.78***	1	0.27**	-0.22*	-0.22	0.11	-0.014	-0.08	-0.01
PHT	0.03	0.08	0.16**	0.11*	0.17**	1	-0.09	-0.09	0.18	0.08	0.5	-0.01
TPP	-0.06	-0.04	-0.14**	-0.16**	-0.2***	-0.05	1	0.99***	-0.07	0.33***	0.71***	0.58***
TSP	-0.06	-0.04	-0.15**	-0.16**	-0.2***	-0.05	0.99***	1	-0.1	0.32	0.71***	0.59***
HSW	-0.06	-0.02	0.13**	0.11*	0.11*	0.12	-0.07	-0.09	1	0.11	0.39***	0.31***

PB	0.04	0.05	0.04	-0.05	-0.01	0.04	0.33***	0.32***	0.11*	1	0.52***	-0.2
GY	-0.04	-0.01	0.04	-0.06	-0.08	0.03	0.7***	0.7***	0.39***	0.52***	1	0.68***
PHI	-0.08	-0.06	-0.06	-0.12*	-0.03	-0.01	0.57***	0.58***	0.3***	-0.19***	0.69***	1

DFF = days to first flowering, D50F = days to 50% flowering, DFP = days to first podding, GFP = grain filling period, DPM = days to physiological maturity, PHT = Plant height, TPP = Total number of pods per plant, TSP = Total number of seeds per plant, HSW = hundred seeds weight, PB = plant biomass, GY = Grain yield, PHI = Percentages of harvest index, * = Significant at $P \leq 0.05$, ** = Significant at $P \leq 0.01$, *** = Significant at $P \leq 0.001$, MS = Mean Square, R^2 = R-square (coefficient of determination), CV= coefficient of variation, Rep = replication, Loc = Location, df= degree of freedom, Gen = genotype, MSE = mean square error, GxE = Genotype by environment interaction.

Appendix 5.2. Mean and standard error (SE) of genetic diversity parameters over Loci for each Pop.

Pop		N	Na	Ne	I	Ho	He	uHe	F
Pop1	Mean	47.244	1.821	1.061	0.091	0.030	0.045	0.045	0.139
	SE	0.065	0.005	0.002	0.002	0.001	0.001	0.001	0.004
Pop2	Mean	1.976	1.224	1.191	0.146	0.047	0.103	0.138	0.461
	SE	0.002	0.006	0.005	0.004	0.002	0.003	0.003	0.009
Grand Mean and SE over Loci and Pops									
		N	Na	Ne	I	Ho	He	uHe	F
Total	Mean	24.610	1.523	1.126	0.119	0.039	0.074	0.091	0.209
	SE	0.214	0.005	0.003	0.002	0.001	0.001	0.002	0.004
F-Statistics and estimates of Nm over all Pops for each locus									
		Fis	Fit	Fst		Nm			
	Mean	0.202	0.246	0.102		33.089			
	SE	0.006	0.006	0.003		0.930			

Nm, gene flow, Fit, total fixation index, Fis, inbreeding coefficient, Fst, fixation index, N, population size, Na, allele number per locus, effective allele number per locus, I, Shannon diversity index, Ho, observed heterozygosity, He, expected heterozygosity, uHe, unbiased expected heterozygosity, F, fixation index, SE, standard error.

Appendix 5.3. Summary of Analysis of Molecular Variance (AMOVA) within pops, among individuals and pops.

SoV	df	SS	MS	Est. Var.	%	F-Value	P
Among Pops	1	1856.553	1856.553	111.578	17%	0.171	0.012
Among Indivs	52	51843.327	996.987	457.424	70%	0.848	0.001
Within pop	54	4435.500	82.139	82.139	13%	0.874	0.001
Total	107	58135.380		651.141	100%	0.810	

Df = degree of freedom, SS =sum of squares, Mean sum of squares, Est.var = estimated variance, % = percentage of molecular variance within and among populations, and among individuals, F-value = F-statistics value, P = probability, AMOVA = analysis of variance, Pops= populations, Indivs = Individuals.

List of Publications

1. von Wettberg, E., Chang, P. L., Başdemir, F., Carrasquilla-Garcia, N., Korbu, B. L., Moenga, M. S., Bedada, G., Greenlon, A., Moriuchi, S. K., Singh, V., Cordeiro, A. M., Noujdina, V. N., Dinegde, N. K., Sani, A. G. S., **Getahun, T.**, Vance, L., Bergmann, E., Lindsay, D., Mamo, E. B., ... Cook, D. R. (2018). Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. *Nature Communications* 9: 649. <https://doi.org/10.1038/s41467-018-02867>.
2. **Getahun, T.**; Tesfaye, K.; Fikre, A.; Hailelassie, T.; Chitikineni, A.; Thudi, M.; Varshney, R.K. Molecular Genetic Diversity and Population Structure in Ethiopian Chickpea Germplasm Accessions. *Diversity* 2021, 13, 247. <https://doi.org/10.3390/d13060247>.
3. **Getahun T.**, Negash K., Chang P. L., von Wettberg E., Carrasquilla-Garcia N., Gaur P. M., Fikre A., Hailelassie T., Cook D., and Tesfaye K. (2021). Screening of heat-tolerant Ethiopian chickpea accessions: Assessment of phenological and agromorphological traits and genomic relationships. *Agrosyst Geosci Environ*. e20211. <https://doi.org/10.1002/agg2.20211>

Poster Presentations

1. Phenotyping of chickpea (*Cicer arietinum* L.) germplasm using agro-morphological and phenological traits to screen heat stress tolerant genotypes. USAID Feed the Future, Chickpea Innovation lab annual meeting, Izmir, Turkey, 2016.

2. Phenotyping of chickpea (*Cicer arietinum* L.) F₃ Interspecific segregating populations, Landraces and Lines for heat stress. USAID Feed the Future, Chickpea Innovation lab annual meeting, Hyderabad, India, December 2017.

Certificates being Awarded

1. Research Fellow on topics of “Phenotyping of Chickpea Interspecific Segregating Material for Heat Stress”. February 13 to July 2, 2017. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India.
2. Research Scholar on topic of “Molecular genetic diversity analysis and marker assisted breeding for selected Ethiopian chickpea (*Cicer arietinum* L.) accessions for heat tolerance”. February 10 to May 9 2020. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India.

Additional Manuscripts Prepared from the Thesis

1. **Getahun, T.**, Korbu, L., Negash, Carrasquilla-Garcia, N., Chang, L.P., Gaur, P.M., Fikre, A., von Wettberg, E., Haileselassie, T., Tesfaye, K., Cook, D.R. **QTL Mapping of Heat Tolerance in Chickpea using populations derived from the Cross of *Cicer arietinum* L. x Wild Cicer species (*C. reticulatum* and *C. echinospermum*).**
2. **Getahun, T.***, Cook, D.R., Fikre, A., Haileselassie, T., Tesfaye, K. **Physiological mechanisms of heat tolerance in chickpea through high throughput phenotyping.**
3. **Getahun, T.***, Cook, D.R., Fikre, A., Haileselassie, T., Tesfaye, K. **Reproductive responses of diverse genetic background of chickpeas to heat stress associated with pod setting and yield related traits.**

International Conferences being Attended

1. International Drought V Confernce Held at Hyedrabad International Convention Center (HICC), Hyderabad, India, February 21 to 25, 2017.