# GENOTYPIC AND PHENOTYPIC DIVERSITY IN CHICKPEA (*Cicer arietinum* L.) REFERENCE SET

# THESIS SUBMITTED TO OSMANIA UNIVERSITY FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN GENETICS

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DEPARTMENT OF GENETICS OSMANIA UNIVERSITY, HYDERABAD 2012





# CERTIFICATE

This is to certify that Ms. Lalitha Nanumasa has carried out the research work embodied in the present thesis entitled "Genotypic and Phenotypic Diversity in Chickpea (*Cicer arietinum* L.) Reference set" for the degree of Doctor of Philosophy under the joint-supervision of Dr. H.D. Upadhyaya, Assistant Research Program Director-grain Legumes and Principal Scientist and Head Gene Bank, International Crops Research Institute for the Semi- Arid Tropics (ICRISAT), Patancheru and Prof. P.B. Kavi Kishor, Department of Genetics, Osmania University, Hyderabad.

This is an original work carried out at ICRISAT and is satisfactory for the award of Doctor of Philosophy. Any part of this work has not been submitted for the award of any degree or diploma of any other University or Institute.

Dr. H.D. Upadhyaya Supervisor

Prof. P. B. Kavikishor Co-Supervisor

# **DECLARATION**

I hereby declare that the research work presented in this thesis entitled "Genotypic and Phenotypic Diversity in Chickpea (*Cicer arietinum* L.) Reference set", has been carried out under the supervision of Dr. H.D. Upadhyaya at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru and co-supervision of Prof. P.B. Kavi Kishor, Department of Genetics, Osmania University, Hyderabad.

This is the original and no part of the thesis has been submitted earlier for the award of any degree or diploma of any University.

Date: 19.12.2012 Place: Hyderabad (Lalitha Nanumasa)

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# CONTENTS

Chapter No.	Title	Page No.
Ι	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIALS AND METHODS	
IV	RESULTS	
V	DISCUSSION	
VI	SUMMARY	
VII	LITERATURE	
VIII	APPENDICES	

## **ABBREVIATIONS**

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
BLUPs	Best Linear Unbiased Predictors
bp	base pair
cm	Centimeter
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribo Nucleic Acid
dNTP	deoxy Nucleotide Tri-Phosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EST-SSR	Expressed Sequence Tag-SSR
g	Gram
GCV	genotypic coefficient of variation
GD	genetic distance
Η'	Shannon and Weaver diversity index
h b	Heritability in the broad sense
HCL	hydrochloric acid
Kg ha"1	Kilogram per hectare
LD	linkage disequilibrium
М	Molar
МСМС	Markov Chain Monte Carlo
mg	milligram
MgCL-	Magnesium chloride
ml	millilitre
mm	milli metre
mM	millimolar
MTAs	Marker Trait Associations
NaCl	Sodium chloride
ng	nanogram
PCA	Principal Component Analyses
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PCs	Principal Components

PCV	Phenotypic Coefficient of Variation
PIC	Polymorphic Information Content
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
REML	Residual Maximum Likelihood
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
Rpm	revolutions per minute
SE	Standard Error
SNP	Single-Nucleotide Polymorphism
Ар	Phenotypic standard deviation
SSR	Simple sequence repeats
TASSEL	Trait Analysis by association, Evolution and
	Linkage
TBE	Tris Borate EDTA
TE	Tris EDTA
UPGMA	Unweighed Pair Group Method based on Arithmatic
	Average
UV	Ultraviolet
V	volt
%	per cent
°C	degree Celsius

## LIST OF TABLES

Table No	Title	Page No
1	Major Genebanks holding Chickpea germplasm	
2	Core and mini core collections developed for ICRISAT mandate crops	
3	Genomic resources available for Chickpea	
4	Some Genetic Diversity studies in Chickpea	
5	Geographic distribution of Chickpea germplasm with different seed types from different countries	
6	List of 300 accessions present in Chickpea reference set with five control cultivars used in this study along with seed type and origin	
7	Geographic distribution of Chickpea reference set accessions with different seed types from different countries	
8	Meteorological details of five environments were chickpea reference set was evaluated	
9	List of qualitative characters studied in chickpea reference set	
10	List of quantitative characters studied in chickpea reference set	
11	Details of 91 Chickpea SSR markers used to genotype Chickpea reference set, chromosome location, repeat motif, forward and reverse primer sequences	
12	Frequency distribution of accessions for various qualitative traits in different seed types and geographical regions in the Chickpea reference set	
13	Variance due to genotypes ( $\sigma^2 g$ ) and genotype x environment interaction ( $\sigma^2 ge$ ), and residual, ( $\sigma^2 e$ ) in different environments for the quantitative traits in the chickpea reference set	
14	Mean (± Standard error) and range values for quantitative traits in different environments and pooled over environments in the chickpea reference set	
15	Means and variance for quantitative traits in different geographical regions of chickpea reference evaluated in different environments and overall in pooled analysis	

16	Heritability, genotypic (GCV) and phenotypic coefficient of variance (PCV)	
	in the chickpea reference set evaluated in different environments and overall	
	in pooled analysis	
17	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set evaluated during 2006-2007 postrainy season (E1), at ICRISAT,	
	Patancheru, India.	
18	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set evaluated during 2007-2008 postrainy season (E2), at ICRISAT,	
	Patancheru, India.	
19	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set evaluated during 2008-2009 postrainy season (E3), at ICRISAT,	
	Patancheru, India.	
20	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set evaluated during 2008-2009 postrainy season (E4), at UAS,	
	Dharwad India.	
21	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set evaluated during 2008-2009 spring season (E5), at ICRISAT,	
	Patancheru, India.	
22	Phenotypic correlation coefficients between 17 quantitative traits in chickpea	
	reference set in pooled analysis.	
23	Meaningful correlation (r>0.500) for quantitative traits in the chickpea	
	reference set evaluated in five environments and in pooled analysis	
24	Shannon-weaver diversity (H') for qualitative and quantitative traits in	
24	chickpea reference set evaluated during E1 (2006-07), E2 (2007-08), E3	
	(2008-09) post-rainy season at ICRISAT Centre, E4 (2008-09) post-rainy	
	season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT, Patancheru and	
	pooled analysis	
25	Shannon-weaver diversity (H') observed for qualitative traits in different seed	
23	types and geographical regions in the chickpea reference set.	
26		
26	Shannon-weaver diversity (H') in different seed types observed for	
	quantitative traits in chickpea reference set evaluated during E1 (2006-07), E2	
	(2007-08), E3 (2008-09) post-rainy season at ICRISAT Centre, E4 (2008-	
	09) post-rainy season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT	
	Patancheru and in overall pooled analysis	

27	Shannon-weaver diversity (H') based on geographical origin observed for	
	quantitative traits in chickpea reference set evaluated during E1 (2006-07), E2	
	(2007-08), E3 (2008-09) post-rainy season at ICRISAT Centre, E4 (2008-09)	
	post-rainy season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT,	
	Patancheru and in overall pooled analysis.	
28	Percentage of variation (%) and vector loading explained by first ten Principle	
	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	evaluated during 2006-07 (E1) post-rainy season at ICRISAT Centre,	
	Patancheru, India	
29	Percentage of variation (%) and vector loading explained by first ten Principle	
	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	evaluated during 2007-08 (E2) post rainy, at ICRISAT Centre, Patancheru,	
	India	
30	Percentage of variation (%) and vector loading explained by first ten Principle	
50	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	evaluated during 2008-09 (E3) post rainy, at ICRISAT Centre, Patancheru,	
	India	
31	Percentage of variation (%) and vector loading explained by first ten Principle	
	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	evaluated during 2008-09 (E4) post rainy, at UAS, Dharwad, India	
32	Percentage of variation (%) and vector loading explained by first ten Principle	
	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	evaluated during 2008-09 (E5) spring, at ICRISAT Centre, Patancheru, India	
33	Percentage of variation (%) and vector loading explained by first ten Principle	
55	component (PCs) estimated for 17 quantitative traits in chickpea reference set	
	in overall pooled analysis.	
34	Phenotypic diversity index in chickpea reference set evaluated in different	
	environments at ICRISAT, Patancheru and UAS, Dharwad, India.	
35	Mean (± Standard error), variance component and heritability in Chickpea	
	Reference set evaluated during (E3) 2008-09 post-rainy, (E5) spring season	
	for SPAD Chlorophyll Meter Readings (SCMR) related traits	
36	Expression of drought tolerance related traits in chickpea reference set	
	evaluated in cylinders during (E2) 2007-08, (E3) 2008-09 post-rainy season at	
	ICRISAT Patancheru, India	

37	Expression of drought tolerance related traits in chickpea reference setevaluated in cylinders in overall pooled analysis	
38	Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set during, E2 (2007-08) post rainy season at ICRISAT, Patancheru, India.	
39	Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set during E3 (2008-09) post rainy season at ICRISAT, Patancheru, India	
40	Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set in pooled analysis.	
41	Expression of resistance to <i>H.armigera</i> using detached leaf assay duringflowering stage in Chickpea Reference set evaluated during (E2) 2007-08,(E3) 2008-09 post-rainy season at ICRISAT Patancheru, India.	
42	Expression of resistance to <i>H.armigera</i> using detached leaf assay during flowering stage in Chickpea Reference set evaluated during (E2) 2007-08, (E3) 2008-09 post-rainy season at ICRISAT, Patancheru, India.	
43	List of trait specific germplasm in the chickpea reference set	
44	Allelic richness, major allele frequency, gene diversity, heterozygosity, polymorphic information content (PIC), allele range, rare, common and most frequent alleles of 91 SSR loci in the chickpea reference set (300 accessions)	
45	Allelic richness, major allele frequency, gene diversity, heterozygosity, polymorphic information content (PIC), allele range, rare, common and most frequent alleles of 91 SSR loci of biological races in the chickpea reference set (300 accessions)	
46	Range and average gene diversity of both biological status and geographical regions in the chickpea reference set	
47	Details of the accessions present in four clusters identified by unweighted neighbor joining tree based on 91 SSR markers in the chickpea reference set	
48	Range and average gene diversity of both biological status and geographical regions in the chickpea reference collection	
49	Average logarithm of the probability of data likelihoods ( <i>LnP(D)</i> ) in the         chickpea reference set	

50	Overall proportion of membership of the sample in each of the 13
50	subpopulations in the chickpea reference set
<b>5</b> 1	Summary statistics in chickpea reference set accessions based subpopulations
51	detected by STRUCTURE analysis using 91 SSR markers
52	AMOVA_Subpop-Pairwise Population Fst Values in the chickpea reference set
	AMOVA_Subpop-Pairwise Population Matrix of Nei Genetic Distance
53	Analysis of molecular variance (AMOVA) based on 13 subpopulations (SP1 to
54	SP13) identified by software STRUCTURE in the chickpea reference set
55	Principal Coordinates Analysis (PCoA) of chickpea reference set accessions
	using 91 SSR markers based on estimates of Nei (1973) distance
56	Marker trait associations (MTAs) detected for qualitative traits in the
	Chickpea reference set
57	Marker trait associations (MTAs) detected for different traits in the Chickpea
	reference set in five environments and in overall pooled analysis
58	List of highly significant (P<=0.001) marker trait associations detected in
38	
	2005-06 (E1) post rainy season at ICRISAT, Patancheru, India
59	List of highly significant (P<=0.001) marker trait associations detected in
	2006-07 (E2) post rainy season at ICRISAT, Patancheru, India
60	List of highly significant (P<=0.001) marker trait associations detected in
	2008-09 (E3) post rainy season at ICRISAT, Patancheru, India
61	List of highly significant (P<=0.001) marker trait associations detected in
01	
	2008-09 (E4) post rainy season at UAS, Dharwad, India
62	List of highly significant (P<=0.001) marker trait associations detected in
	2008-09 (E5) spring at ICRISAT, Patancheru, India
63	List of highly significant (P<=0.001) marker trait associations detected in
	overall pooled analysis data
64	List of markers associated more than one trait evaluated in the chicknes
04	List of markers associated more than one trait evaluated in the chickpea
	reference set

#### LIST OF FIGURES

Fig. No	Title	Page No.
1	Geographical distribution of 300 chickpea reference set accessions	
2	Number of accessions in each seed types of the chickpea reference set	
3	Heritability, genotypic (GCV) and phenotypic coefficient of variance	
	(PCV) in the chickpea reference set for 17 quantitative traits based on	
	pooled BLUPs of five environments	
4a	Frequency distribution of accessions for various qualitative traits in the chickpea	
	reference set : Frequency distribution of the chickpea reference set accessions	
	for Growth Habit	
4b	Frequency distribution of the chickpea reference set accessions for Plant	
	pigmentation	
4c	Frequency distribution of the chickpea reference set accessions for Flower color	
4d	Frequency distribution of the chickpea reference set accessions for Seed color	
4e	Frequency distribution of the chickpea reference set accessions for Seed shape	
4f	Frequency distribution of the chickpea reference set accessions for Seed dots	
4g	Frequency distribution of the chickpea reference set accessions for Seed surface	
5a	Scatter plot of first two principal components (PCs) of the chickpea reference	
	set accessions using pooled BLUPs of five environments for yield contributing	
	traits: Days to 50% flowering (DF) vs. plot yield (YKGH)	
5b	Days to maturity (DM) vs. Plot yield (YKGH)	
5c	100 seed weight vs. Plot yield (YKGH)	
6	Ward's clustering of the chickpea reference set accessions for geographic	
	origins based on scores of first three PCs	
7	Dendrogram based on 7 qualitative traits of the chickpea reference set	
	accessions based on different seed types (Desi, Kabuli, Pea Shaped and Wild)	
8	Distribution of number of alleles per locus among 91 SSR markers used for	
	genotyping the chickpea reference set	
9a	Unweighted neighbor-joining tree based on the simple matching dissimilarity	
	matrix of 91 SSR markers genotyped across the chickpea reference set	
9b	Factorial analysis based on the simple matching dissimilarity matrix of 91 SSR	
	markers genotyped across the chickpea reference set	
10	Rate of change in Ln P(D) between successive K (K averaged over the five run)	
	in the chickpea reference set accessions	
11a	Population structure of the chickpea reference set based on 91 SSR markers	

	(k=13) revealed by STRUCTURE analysis (Bar plot in single lines)	
11b	Population structure of the chickpea reference set based on 91 SSR markers	
	(k=13) revealed by STRUCTURE analysis (Bar plot in multiple lines)	
12	Principal coordinates analysis (PCoA) of the chickpea reference set accessions	
	using 91 SSR markers based on Nei (1973) distance estimates.	

#### LIST OF PLATES

Plate.	Title	Page
No		No.
1	Field Evaluation of the Chickpea Reference set at ICRISAT, Patancheru,	
	India	
2	Field Evaluation of the Chickpea Reference set at UAS, Dharwad,	
	India	
3	Diversity in Chickpea Germplasm at ICRISAT, Patancheru, India	
4	Diversity for Foliage Color in Chickpea Reference set	
5	Diversity for Leaf and Stem Type and Shape in Chickpea Reference set	
6	Diversity for Flower Shape and Color in Chickpea Reference set	
7	Diversity for Pod Shape and Color in Chickpea Reference set	
8	Diversity for Pod Number in Chickpea Reference set	
9	Diversity for Seed Shape and Color in Chickpea Reference set	
10	PCR products tested for amplification on 1.2 per cent agarose gel in Chickpea	
	Reference set	
11	Allele sizing of the data obtained from ABI 3730xl genetic analyzer using	
	Genotyper software version 4.0 (Applied Biosystems, USA) in Chickpea	
	Reference set	
12	Pod borer screening of the chickpea reference set accessions- Detached leaf	
	bioassay	
13	Phenotyping of the chickpea reference set for drought tolerance using PVC	
	cylinder technique	
14	Chickpea reference set accessions showing diversity in root lengths	

#### LIST OF APPENDIX

S.No	Title	Page No.
1	Scores of 7 qualitative traits for 300 accessions in chickpea reference set	
2	Mean performance of 300 accessions in chickpea reference set accessions	
	for 17 quantitative traits based on overall pooled analysis	

#### ABSTRACT

Chickpea reference set consisting of 300 accessions was evaluated at five environments for 7 qualitative and 17 quantitative traits to study the phenotypic diversity and to identify trait specific accessions for grain quality traits, resistance to pod borer, for traits related to drought tolerance and also molecularly profiled using 91 SSR markers to study molecular genetic diversity, population structure and to identify SSR markers associated with the agronomic, quality, pod borer and drought tolerance related traits.

In REML analysis variance due to genotypes ( $\sigma^2 g$ ) and genotype x environment ( $\sigma^2 ge$ ) were significant for all the traits except tertiary branches and pods per plant for quantitative traits. On the basis of phenotypic dissimilarity between pair of accessions, ten pairs of most diverse accessions were identified for use in crop improvement program for developing high yielding cultivars with a broad genetic base and for the development of mapping populations. On the basis of pooled BLUPs (Best Linear Unbiased Predictors) of five environments, we have identified trait specific accessions for economically important traits such as yield, pod borer resistant, accessions with high protein content, anthocyanin content, drought tolerance traits and its traits contributing to yield (10 accessions for each trait). These accessions could be used in recombination breeding to develop cultivars with desirable combination of traits.

The SSR markers detected a total of 2411 alleles with an average of 26.45 alleles per locus. Of these, 2299 alleles were detected in cultivated types and 433 alleles in wild types, of which 1980 were unique in cultivated, 114 in wild accessions. In cultivated chickpea, desi accessions contained the largest number of unique alleles (864) followed by kabuli (836) and pea type (52) which were specific to a particular accession and useful for germplasm identification. The genetic diversity of chickpea in this study was correlated well with actual classification of chickpea and showed greater genetic distance among three seed types. Large molecular variation observed in reference set, could be utilized effectively for selection of diverse parents for breeding cultivars and development of mapping populations.

The STRUCTURE analysis provided the evidence for the presence of thirteen

subpopulations. A general linear model was implemented to identify the SSR markers associated with the qualitative, quantitative and grain quality traits, resistance to pod borer and for traits related to drought tolerance in chickpea reference set based on population structure (Q matrix) and relatedness relationship. 64 (P $\leq$ 0.001) significant MTAs were detected involving 49 SSR markers in E1, with maximum phenotypic diversity of 43.4% for anthocyanin content. 86 significant MTAs were detected involving 46 SSR markers in E2 with maximum phenotypic diversity of 42% for tertiary branches whereas in E3, 76 significant MTAs with 50 SSR markers and maximum phenotypic diversity of 42.9% for leaf area, in E4 74 significant MTAs with 52 SSR markers and maximum phenotypic diversity of 34.8% for plant width.

In pooled analysis, the number of significant MTAs ( $P \le 0.001$ ) were 27 for qualitative traits with 21 markers, 76 ( $P \le 0.001$ ) for quantitative trait, two for SCMR, one for protein content, two for pod borer resistance traits and 21 for drought related traits. The major MTAs with <20% phenotypic variation across all the environments were 7 for qualitative, 39 for quantitative, 1 for SPAD and 8 for drought tolerance related traits, as the major associations in chickpea reference set.

Hence, these most significant MTAs were believed to be associated with colocalized/pleiotropic QTLs. In summary, the co-localization of specific genes/QTLs/markers could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response and pod borer resistance traits. The presence of several co-localized/pleiotropic QTLs verified the complex quantitative nature of drought tolerance, pod borer resistance in chickpea and allowed the identification of some important genomic regions for traits related to high yield, high protein content, drought tolerance and resistance to pod borer. The results from this research also demonstrated the use of reference set as association mapping panel to determine marker-trait associations in chickpea for traits that could lead to effective utilization of ex-situ conserved genetic resources.

Introduction

#### **1. INTRODUCTION**

Chickpea (Cicer arietinum L.) commonly known as Bengal gram or garbanzo bean, is one of the oldest (earlier than 9500 BC) and widely cultivated pulse crops in over 50 countries of the world. It is a highly self-pollinating (Auckland and van der Maesen 1980) annual grain legume, ranking second among edible pulses in global markets (Yadav et al., 2007). Chickpea is widely cultivated in the Mediterranean, North Africa, the Middle East, and the Indian subcontinent. It is a member of the family Leguminosae, sub-family Papilionoideae and tribe Vicieae. Chickpea most probably originated in Southeastern Turkey adjoining Syria (Ladizinsky, 1975) and subsequently spread to India and Europe (Singh and Auckland, 1975). Wild annual *Cicer* originated mainly in the Mediterranean regions having a wide ecogeographic range, differing in habitat, topographic and climatic conditions (Abbo et al., 2003; Berger et al., 2003). Chickpea is generally grown across a wide temperature regime ranging from <5 °C in sub-tropics to >30 °C in the arid tropics (Sinha, 1977). Optimum growing conditions include 21-29 °C day and 18-26 °C night temperatures with an annual rainfall of 600-1000 mm (Duke, 1981; Smithson et al., 1985; Muehlbauer et al., 1988).

The world area under chickpea is about 11.98 Mha, with a total production of 10.89 Mt, and an average productivity of 0.91 t ha<sup>-1</sup> (FAO, 2010). Important chickpea producing countries are India (0.91 t ha<sup>-1</sup> in 8.21 Mha), Pakistan (0.55 t ha<sup>-1</sup> in 1.06 Mha), Turkey (1.20 t ha<sup>-1</sup> in 0.44 Mha), Myanmar (1.5 t ha<sup>-1</sup> in 0.27 Mha) and China (2.83 t ha<sup>-1</sup> in 0.003 Mha). Large variations in chickpea yield, from 0.36 t ha<sup>-1</sup> in Kenya to 2.83 t ha<sup>-1</sup> in China are reported. Chickpea productivity records in the last four decades revealed interesting trend: productivity consistently increased in India and Mexico, declined in Turkey, Pakistan, and Iran.

Chickpea is the important grain legume grown for protein-rich seeds for human consumption, restore and maintain the soil fertility by nitrogen fixing capability, and fit very well in various cropping patterns. Over 90% of the chickpea is produced and consumed in Asia (FAO, 2010). Chickpea seeds contain protein, fibre, calcium, potassium, phosphorus, iron, zinc and magnesium along with appreciable quantities of selenium, sodium and copper, which make it one of the nutritionally best composed edible dry legumes, for human consumption (Esha, 2010). Chickpea seeds contain 23% protein, 64% carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble

sugar and 3% ash (FAO, 2010). Chickpea like other beans is a good source of cholesterol lowering fiber (Pittaway *et al.*, 2006). In addition to lowering cholesterol, the high fiber content prevents blood sugar levels from rising, making chickpea a good choice for individuals with diabetes, insulin resistance or hypoglycemia (McIntosh and Miller, 2001). The crop also enhances environmental sustainability due to its nitrogen fixation ability and rotational benefit, all of which facilitate higher cropping intensification (Miller *et al.*, 2002). Hair like structures on the stems, leaves and pods secrete acids that provide the first line defense against pests, reducing the need for chemical sprays (Yadav *et al.*, 2007).

Genetic diversity studies in a crop are important in management of genetic resources, identification of duplicate accessions in the germplasm collection and use of genetic resources in applied breeding programs. A large number of chickpea germplasm accessions (more than 98,000) are conserved in several genebanks (Gowda *et al.*, 2011). Some of important genebanks that conserve large germplasm collection of chickpea are International Crop Research Institute for Semi Arid Tropics (ICRISAT) in India, International Center for Agricultural Research in Dry Areas (ICARDA) in Syria, Vavilov institute in Russia, the USDA-ARS Regional Plant Introduction Station at Pullman in the U.S and NBPGR, New Delhi, India. The genebanks at ICRISAT and ICARDA, the two CGIAR centers have global responsibility for chickpea germplasm. ICRISAT maintains the largest collection of 20,267 accessions from 60 countries which include 18,392 landraces, 98 advanced cultivars, 1293 breeding lines, 288 accessions of wild *Cicer* species and 196 accessions with no information on biological status.

Plant breeders have successfully improved the yield potential of most crops, which has resulted in higher production in last four decades, but further progress is not impressive. One of the main reasons for such a situation is the use of limited genetic diversity by the plant breeders who tend to use their working collection of highly adapted material (Evans, 1983; Upadhyaya *et al.*, 2006b; 2011a) or advanced breeding lines as parents and only a small proportion of the available germplasm has been used in national and international breeding programs. In India, which has a strong chickpea breeding program, 41% of the 126 cultivars released in the past four decades have Pb 7 (desi type) in their pedigree followed by IP 58, F 8, S 26 (all desi) and Rabat (kabuli, 34 g 100 seed  $^{-1}$ ) (Kumar *et al.*, 2004). In the breeding program at

ICRISAT, less than 1% of germplasm has been used in developing more than 3700 breeding lines during 1978-2008 (Upadhyaya *et al.*, 2006b, 2009a). Of the 92 germplasm lines used, only 19 were kabuli types, 6 of which had large seed size (>40g 100 seed <sup>-1</sup>). L 550, a small seeded (20 g 100 seed <sup>-1</sup>) kabuli cultivar was frequently used (983 times) in the breeding program. One of the main reasons for low use of germplasm in breeding programs is the lack of information on traits of economic importance which show high genotype x environment interaction, and require multilocational replicated evaluation to identify parents. Thus, the large variability in the germplasm instead of prompting more use has created a situation of not knowing where to begin (Upadhyaya *et al.*, 2005). The importance of diverse germplasm to generate new variability and to enhance the genetic yield potential and to stabilize it against various biotic and abiotic stresses has been well established (Singh, 1987; Upadhyaya *et al.*, 2009a).

Various methods have been used to assess the genetic diversity in crops, such as analyzing the range of morphological, agronomical and ecogeographical traits and molecular tools, each with its own associated advantages and disadvantages (Gepts, 1995). Most plant traits are quantitative and are influenced by environment and display high genotype-environment interaction. Phenotypic data therefore cannot correctly reflect the genetic diversity among the germplasm accession. If genotypic values can be predicted based on phenotypic data, then genetic distance based on genotypic values among accessions can be measured more accurately (Hu et al., 2000). Understanding the distribution of genetic diversity among individuals, populations and genepools is crucial for efficient management of germplasm collections and its use in crop improvement. Diversity analysis is routinely carried out using sequencing of selected gene(s) or molecular marker technologies. Molecular marker technologies are becoming increasingly important tools for genetic and genomics studies, breeding and diversity research. The major advantage of molecular and a biochemical marker is their genotypic nature which can reflect direct changes at DNA sequence level.

Several DNA-based molecular markers are available for genetic diversity analysis for most of the crops. The smaller core collection accessions have been characterized initially using DNA markers such as random amplified fragment DNA (RAPD) in common bean (*Phaseolus vulgaris L.*) (Skroch *et al.*, 1998), potato (*Solanum*)

*tuberosum* L.) (Ghislain *et al.*, 1999) and isoenzyme markers in Wild barley (*Hordeum vulgare* sp. *spontaneum*) (Liu *et al.*, 2002). The AFLP markers have been used for studying the variation in core subsets of oats (Fu *et al.*, 2005). However, the SSR markers are now the markers of choice in most areas of molecular genetics as they are highly polymorphic even between closely related lines, require low amount of DNA, can be easily automated for high throughput screening, can be exchanged between laboratories and are highly transferable between populations. Microsatellite (SSR) markers were utilized in apple (*Malus* spp.) (Hokanson *et al.*, 1998), common beans (*Phaseolus vulgaris* L.) (Blair *et al.*, 2009) core collections and US peanut mini core collection (Kottapalli *et al.*, 2007) to reveal genetic diversity.

Molecular markers linked to major quantitative trait loci (QTLs) can greatly facilitate breeding for complex traits through marker assisted selection (MAS) in segregating generations. Linkage analysis and association mapping are two most commonly used tools for dissecting complex traits and identifying major QTLs causing variation in the traits of interest. Association mapping does not require a bi-parental cross derived mapping population which is time consuming and expensive to develop. A manageable diverse natural population is sufficient to carryout association mapping and has become a promising approach for the dissection of complex traits in plants (Wilson et al., 2004; Breseghello and Sorrells, 2006). Association mapping, also known as linkage disequilibrium (LD) mapping, has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level (Nordbourg and Tavare, 2002; Risch and Merikangas, 1996). Association mapping identifies QTLs by examining the marker-trait associations that can be attributed to the strength of LD between markers and functional polymorphism across a set of diverse germplasm. Since its introduction to plants (Thornsberry *et al.*, 2001), association mapping has gained popularity in genetic research because of advances in high throughput genomic technologies, interests in identifying novel and superior alleles, and improvements in statistical methods. Information about the extent and genomic distribution of LD within the population under consideration is of fundamental requirement for association mapping (Stich et al., 2005).

The development of gene-based markers based on information derived from a model plant is a key component. Upadhyaya *et al.*, (2006), developed a global composite

collection of 3,000 accessions which included 1956 core collection (Upadhyaya *et al.*, 2001) accessions representing ICRISAT collection, 709 cultivated accessions representing unique accession from ICARDA, 39 advanced breeding lines and released cultivars, 35 distinct morphological variants, 20 wild species accessions and 241 accessions carrying specific traits such as tolerance/resistance to biotic, abiotic stresses and important agronomic characters. Using the genetic structure, diversity and allelic richness in composite collection, a genotype- based reference set of 300 accessions was developed for diverse applications in chickpea genomics and breeding (Upadhyaya *et al.*, 2008b). Further assessment of genetic diversity and dissection of population structure, based on morpho-agronomic characters alone might be biased because distinct morpho-types can result from few mutations and share a common genetic background. Therefore present investigation was carried out with following objectives:

- 1. To assess the phenotypic diversity in chickpea reference set for morphological, agronomic, and grain quality traits, resistance to pod borer and for traits related to drought tolerance.
- 2. To quantify the level of genetic diversity and determine population structure of chickpea reference set using SSR markers.
- 3. To identify allelic variation associated with beneficial traits using association mapping in the reference set of chickpea.
- 4. To identify most diverse accessions with beneficial traits for use in mapping and improvement of chickpea.

Review of Literature

#### **2. REVIEW OF LITERATURE**

Chickpea (*Cicer arietinum* L.) is one of the oldest (earlier than 9500 BC) and widely cultivated pulse crops in over 50 countries of the world. Chickpea is a member of the West Asian Leolithic crop assemblage, associated with the origin of agriculture in the Fertile Crescent, some 10,000 years ago (Lev-Yadun *et al.*, 2000; Zohary and Hopf, 2000). South west Asia and the Mediterranean region are the two primary centres of origin, and Ethiopia the secondary centre of diversity (Vavilov, 1926; 1950). It most probably originated in Southeastern Turkey adjoining Syria. The cultivated species, *C. arietinum* is found only under cultivation and cannot colonize successfully without human intervention. Three wild annual *Cicer* species, *C. bijugum*, *C. echinospermum* and *C. reticulatum*, closely related to cultivated chickpea, cohabit in this area and occur in weedy habitats, these three wild *Cicer* species, eight more wild *Cicer* species occur naturally in Turkey, out of 43 known today in the *Cicer* genus (Van der Maesen, 1987).

On the basis of Harlan and de Wet's (1971) definition, and results obtained from crossability, biochemical or molecular diversity, and karyotypic studies, a revised model of the wild annual *Cicer* gene pools has been proposed (Croser *et al.*, 2003). The primary gene pool of *Cicer* consists of *Cicer arietinum* and only one wild species, the wild annual progenitor *C. reticulatum*. The secondary gene pool thus consists of *C. echinospermum* only. *C. bijugum*, *C. pinnatifidum* and *C. judaicum*, which have been reported to give hybrids readily when crossed with the cultivated species (Verma *et al.*, 1990; Singh *et al.*, 1994; Singh *et al.*, 1999a, b; Croser *et al.*, 2003). Ahmad *et al.* (2005) have proposed that the above three species should be placed in the tertiary gene pool of chickpea, along with the remaining annual species *C. chorassanicum*, *C. yamashitae* and *C. cuneatum*. Thus until proven these perennial *Cicer* spp should be appropriately placed in the tertiary gene pool along with the six other annual wild species.

Chickpea is known by several names, such as Garbanzo bean, Indian pea, Ceci bean, Bengal gram, chana, kadale kaalu, sanagalu, shimbra, kadala. It has been an integral part of agriculture since long time because of its nitrogen fixing ability in the field and diversified uses as food and feed along with its importance in crop diversification. It is a good source of energy, protein, minerals, vitamins, fibre and also contains potentially health-promoting phytochemicals. The nutritional quality of seeds can vary depending on the environment, climate, soil nutrient status, soil biology, agronomic practices and stress factors (biotic and abiotic). Amino acid composition is well balanced; with limited sulphur containing amino acids (methionine and cysteine), and high lysine. Due to high protein content, it is used as a protein rich animal feed and the vegetative biomass is used as a fodder.

#### 2.1.1 Importance of genetic diversity

Diverse gene pools are the foundation for effective crop improvement programmes. The genetic diversity in plant breeding is of paramount importance in developing high yielding cultivars having resistance to biotic and abiotic stresses and with a broad genetic base. The recognition of such diversity, its nature and magnitude are crucial to any breeding program. The genetic variation in crop plants has been narrowed during domestication due to continuous selection pressure for particular traits like high yield or disease resistance. It is therefore important to study the genetic composition of the germplasm and existing cultivars for comparison with their ancestors and related species, to find new and useful genes, and provide information about the phylogenetic relationship and molecular markers are now being widely used to classify the germplasm, to establish genetic linkages between markers and traits of agronomic and economic interest.

#### 2.1.2 Germplasm collection and its uses

Genetic diversity in crop plants is continuously being lost in farmer's field and in nature. In this context, genebanks assume paramount importance as reservoirs of biodiversity and source of alleles that can be easily retrieved for genetic enhancement of crop plants. Increasingly, efforts are being made to collect threatened landraces, obsolete cultivars, genetic stocks and wild relatives of cultivated species (Ortiz *et al.*, 2004). All these materials are important for crop improvement because breeding gains rely largely on access to the genetic variation in the respective gene pool. International germplasm collections play a very important role in securing genetic diversity and promoting its use. This has resulted in assemblage of large collections in national and international genebanks. Some of major genebanks holding chickpea germplasm are presented in Table1.

Country	Institute					
Australia	Australian Temperate Field Crops Collection (ATFCC), Horsham Victoria					
Ethiopia	Institute of Biodiversity Conservation (IBC), Addis Ababa					
Hungary	Institute for Agrobotany, Tápiószele					
India	Indian Agricultural Research Institute (IARI), New Delhi					
	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru					
	National Bureau of Plant Genetic Resources (NBPGR), New Delhi	16881				
Iran	College of Agriculture, Tehran University, Karaj	1200				
	National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Karaj	5700				
Mexico	Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (IA-Iguala ), Iguala	1600				
Pakistan	Plant Genetic Resources Institute (PGRP), Islamabad	2146				
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR), St. Petersburg	2091				
Syria	International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo	13818				
Turkey	Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Izmir					
Ukraine	Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Kharkiv	1021				
USA	Western Regional Plant Introduction Station, USDA-ARS, Pullman	6789				
Uzbekistan	Uzbek Research Institute of Plant Industry (UzRIPI), Botanica	1055				
Total		93977				

 Table: 1 Major Genebanks holding chickpea germplasm (more than 1000 accessions)

The present status of germplasm collections held at ICRISAT genebank are 1,19,739 accessions as on 15.10.2012 from 144 countries which include 1,17,032 cultivated and 2,707 wild species of ICRISAT mandate crops and six small millets. The collection includes 37,949 accessions of sorghum, 22,211 accessions of pearl millet, 20,267 accessions of chickpea, 13,632 accessions of pigeonpea, 15,445 accessions of groundnut and 10,235 accessions of small millets (Upadhyaya *et al.*, 2010a). Gradual loss of variability from cultivated species and their wild forms and wild relatives is due to the advent of advanced breeding lines and replacement of genetically variable landraces by the improved, genetically uniform cultivars. A large number of germplasm lines are distributed by the genebank for use in crop improvement programs. ICRISAT genebank distributed more than 7, 00,000 samples of accessions to scientists in India and 143 other countries. Of the germplasm supplied by the genebank, a very small proportion has been used in crop improvement programs. For example, at ICRISAT, between 1986 and 2008, a total of 10,331 advanced groundnut

breeding lines (ICGV #) were developed from thousands of crosses involving 1,270 unique parents, out of these only 171 were germplasm lines, which includes 10 wild, out of 15,445 accessions (Upadhyaya *et al.*, 2010a). This is mainly due to lack of reliable information on large collections particularly for traits of economic importance which show high genotype x environment interaction and require multilocational replicated evaluation to identify parents for use by breeders (Upadhyaya *et al.*, 2010a).

In crops such as, wheat (Dalrymple, 1986); spring barley (Vellve, 1992); groundnut (Jiang and Duan, 1998, Upadhyaya *et al.*, 2005); chickpea and pigeonpea (Shiv Kumar *et al.*, 2004, Upadhyaya *et al.*, 2006c, Upadhyaya *et al.*, 2007b); only a small proportion of germplasm has been used in breeding programs. For effective utilization of existing genetic resources in research, it is necessary to characterize the germplasm for identification of trait-specific sources for crop improvement. This requires a small sample of germplasm lines, which represent the entire diversity present in the crop species, multi-environmental evaluation data of these subsets, would greatly encourage the breeders to utilize more germplasm lines in to their breeding program. Thus, the concept of core collection was proposed.

#### 2.1.3 Core collection

Frankel (1984) proposed the 'core collection' concept, which would 'represent with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives'. A core collection is a subset, consisting of  $\sim 10\%$  of total accessions, which between them capture most of the available diversity in the entire collection (Brown, 1989a). Core collections are cost-effective means of identifying accessions with desirable agronomic traits as well new sources of disease and pest resistance or abiotic stress tolerance.

Ever since the concept of core collection was developed, a number of core collections have already been established for many crop species including perennial glycine (Brown *et al.*, 1987); perennial medicago species (Diwan *et al.*, 1994; Basigulp *et al.*, 1995); common bean (Tohme *et al.*, 1995); okra (Mahajan *et al.*, 1996); quinoa (Ortiz *et al.*, 1998); alfalfa (Skinner *et al.*, 1999); sweet potato (Huaman *et al.*, 1999); safflower (Diwedi *et al.*, 2005). Core collections developed for ICRISAT mandate crops are listed in Table 2.

Upadhyaya *et al.*, (2001a) developed a chickpea core collection of 1956 accessions that consisted of 1465 desi, 433 kabuli, and 58 intermediate types representing more than 85% variation of the entire collection based on geographical origin of accessions and 13 quantitative traits. This core collection was subjected to multi-environmental evaluation to identify diverse germplasm with beneficial traits.

#### 2.1.4 Minicore collection

The germplasm collections held by most International Agricultural Research Centers (IARCs) genebanks are very large in size. For example the IRRI genebank holds more than 108,000 rice accessions; hence the size of core collection (~10%) will be about 11000 accessions, which again restricts its proper evaluation and use by breeders. To overcome this, Upadhyaya and Ortiz (2001) postulated the minicore concept. A minicore is core of core (10% of core or 1% of entire collection) representing the species diversity. Upadhyaya and Ortiz (2001) developed minicore collection of chickpea consisting of 211 accessions (Table 2). This strategy was followed by scientists in different countries such USA (Holbrook and Dong, 2005), Japan (Ebana *et al.*, 2008), and it has been recognized worldwide as an "International Public Good" (IPG). The reduced size of minicore collections has provided ample opportunities to the breeders for their efficient and economic multi-environment evaluation, which has lead to the identification of several new sources of variation for different traits for utilization in crop improvement programs. Minicore collections developed for ICRISAT mandate crops are listed in Table 2.

			Collection	Accessions in	
Сгор	Accessions	Traits	developed	subset	Reference
	3350		Core	505	Hannan et al., 1994
Chickpea	16,991	13	Core	1,956	Upadhyaya <i>et al.</i> ,2001
	1956	22	Minicore	211	Upadhyaya and Ortiz, 2001
Groundnut	7,432		Core collection	831	Holbrook et al., 1993
		15	Asian core	504	Upadhyaya <i>et al.</i> ,2001b
	14,310	14	Core	1,704	Upadhyaya et al.,2003
			Valencia core	77	Dwivedi et al.,2008
	1704	31	Minicore	184	Upadhyaya et al.,2002
Pigeonpea	12,153	14	Core	1,290	Reddy et al., 2005
0 1	1,290	33	Minicore	146	Upadhyaya et al.,2006c
Sorghum					Prasada Rao and
Sorghum	33,100	7	Core	3,475	Ramanatha Rao, 1995
	22,473	20	Core	2,247	Grenier et al.,2001
	40,000		Core	3,011	Dahlberg et al.,2004

Table 2: Core and mini core collections developed for ICRISAT mandate crops

Сгор	Accessions	Traits	Collection developed	Accessions in subset	Reference
	2,247	21	Minicore	242	Upadhyaya <i>et al.</i> ,2009b
Pearl	16,063	11	Core	1,600	Bhattacharjee et al., 2007
millet	20,766	12	Core (Augmented)	2,094	Upadhyaya <i>et al.</i> ,2009a
	2,094	18	Minicore	238	Upadhyaya <i>et al.</i> ,2010c
Finger millet	5,940	14	Core	622	Upadhyaya <i>et al.</i> ,2006b
			Minicore	80	Upadhyaya <i>et al.</i> ,2010b
Foxtail millet	1,474	23	Core	155	Upadhyaya <i>et al</i> .,2008a

#### 2.2 Genetics of Qualitative and Quantitative traits.

Most of the economically important characters in chickpea including yield are complex and polygenically controlled. The expression of these traits is likely to be affected to a greater extent by environmental factors and genotype x environment interactions. A thorough understanding of genetic diversity for yield and its attributes, extent of genetic variation and its heritability would help in developing strong crop improvement programmes. Investigations on yield and its components made on genetic variability, heritability, genetic advance, character association, direct and indirect effects of component traits on grain yield and genetic diversity has been very useful in plant improvement programmes.

A brief review available on above aspects in chickpea is presented in this section, under the following sub-headings.

2.2.1 Studies on range of variation and variability parameters (Mean, Range, heritability and genetic advance)

2.2.2 Correlation studies

2.2.3 Genetic divergence

#### 2.2.1 Variability Studies

Phenotypic variability expressed by a group of genotypes in any species can be partitioned into genotypic and phenotypic components. The heritable genotypic part of the total variability and its magnitude influence the selection strategies to be adopted by the breeder.

#### 2.2.1.1 Qualitative traits

Chickpea germplasm has abundant genetic variation for all traits.

Plant characters often are referred to as simple morphological or complex agronomic characters, depending on ease of classification, the number of genes that control them and the importance of the environment in their expression. Qualitative characters have phenotypes that can be divided into discrete classes.

Genetics of many qualitative traits have been reported by several investigators.

#### a. Plant pigmentation

Plant pigmentation is an important morphological descriptor, characterized by presence or absence of anthocyanin pigment. It imparts purplish colour to different parts of the plant and was found that low anthocyanin content is dominant over high anthocyanin and light green colour (Rao *et al.*, 1980). Pundir *et al.*, (1985) reported that 67.1% accessions of the ICRISAT germplasm collection are low in anthocyanin, 32.4% had no anthocyanin and the remaining 0.5% had high anthocyanin content and also revealed that ICC 5325 has yellow-green foliage which is a rare occurrence. Sandhu *et al.*, (1993) reported a chickpea line ICC 6071 having anthocyanin pigmentation on all parts of the plant and pigmentation being stable throughout the crop growth period (germination to maturity). ICC 5763 had anthocyanin pigmentation on the parts of the plant exposed to sunlight, the unexposed parts being green (Mathur, 1998). Upadhyaya *et al.*, (2001) evaluated chickpea core collection at ICRISAT and reported that 652 accessions had no anthocyanin (33.40%), 1254 were with low anthocyanin (64.24%), and 50 were with high anthocyanin pigmentation (2.56%).

#### **b.** Flower colour

Flower colour is one of the most important diagnostic characters in chickpea and is widely used as morphological marker in genetic studies and breeding work. Pundir *et al.*, (1985) at ICRISAT recognized three main flower colours in chickpea, pink (71.0%), white (18.9%), light pink (9.4%), and a small proportion of dark pink, blue and light blue. Gill and Cubero (1993) enumerated the dominance of purple flower over white flower and reported that geographically, the pink flower colour dominates in the Indian subcontinent and the white flower colour in the Mediterranean and Andean regions, and Mexico. Pink and white as well as light pink flower colours

occur together in West Asia, Afghanistan and Ethiopia. Pink flower colour, which is characteristic of desi type, was the most predominant, represented by 1329 of 1956 core subset accessions (67.94%), followed by white flower (24.59%), which is characteristic of kabuli type (481 accessions) and light pink (6.03%, 118 accessions). White flower with pink streaks was found in two accessions (0.10%) at ICRISAT (Upadhyaya *et al.*, 2001). Arshad *et al.*, (2008) reported blue flower color in a disease resistant, high yielding chickpea variety "Thal 2006". Chaturvedi *et al.*, (2009) reported that 11 genotypes with white flower, two with purple flower, one with blue flower and rest 74 with pink flowers among 88 chickpea genotypes collected from various parts of India.

#### c. Growth habit

Growth habit is associated with early seedling establishment and maturity, contributing to higher yield under adverse conditions like drought (Gupta, 1985; Singh *et al.*, 1997; Sabaghpour *et al.*, 2003). The growth habit of *Cicer* varies from prostrate to erect. Roberts (1986) and Roberts and Osei-Bonsu, (1988) presented evidence that erect growth habit was dominant to prostrate habit and also reported that prostrate type of growth habit may reduce seed yields. Semi-erect (80.73%) was the most predominant growth habit (1579 accessions) followed by semi-spreading (17.54%, 343 accessions), whereas prostrate growth habit was observed in only one accession (0.05%) in chickpea core collection evaluated at ICRISAT (Upadhyaya *et al.*, 2001). One genotype exhibited prostrate growth habit whereas 24 were erect and other 63 with semi-erect habitat from 88 chickpea genotypes collected from various parts of the country (Chaturvedi *et al.*, 2009).

#### d. Seed shape and Seed type

Seed shape and type are of interest to the breeders attempting to satisfy diverse marketing criteria. There are three different seed shapes angular, owl and pea shaped and three type's desi, kabuli and intermediate in chickpea (Upadhyaya *et al.*, 2002) seed types. Desi and kabuli chickpea differ in nutrition as crude fibre (Jambunathan and Singh 1980 and Singh *et al.*, 1984), acid detergent fibre and neutral detergent fibre (Singh and van Rheenen 1994). The protein and oil (Muhammad *et al.*, 2007) were similar in these two groups (Jambunathan and Singh 1980). Breeders have found it convenient to classify chickpea into two main types, namely desi (characterized by small size, angular shape, and coloured seed with high percentage of fibre) and kabuli

(characterized by large size, ram's head shape and beige coloured seeds with a low percentage of fibre). A third type, designated the intermediate, is characterized by medium to small size, pea shape and cream coloured seeds. The desi type accounts for about 85% of the world production, the remainder being kabuli. Hawtin and Singh (1980) reported that there is a fairly clear distinction between the two types, which is generally based upon seed shape and colour but also takes account of geographical origin. Such round seeded types are generally designed "intermediate" or "pea" type by breeders. Pundir et al., (1985) reported that 78.3% of ICRISAT germplasm accounted angular shape, 15.46% were owl and 6.25% were pea shaped seeds. Desi types account for about 85% of world production and the remainder being kabuli (Singh et al., 1985). Desi seed type was found to be dominant over kabuli, while pea type was dominant to both desi and kabuli types (Knights, 1980). It is commonly accepted that kabuli (macrosperma) chickpea originated from desi (microsperma) (Salimath et al., 1984). Upadhyaya et al., (2001) evaluated chickpea core collection (1956 accessions) and reported that angular seed shape (74.90%), which is characteristic of desi types, was most frequent (1465 accessions) followed by the owl shape (22.14%) of kabuli type (433 accessions) and pea shape (2.97%) of the intermediate type (58 accessions). In chickpea minicore collection (211 accessions), 159 entries were desi (75.4%), 44 were kabuli (20.9%), and 8 were intermediate (3.8%) types, which corresponded very well with the number of desi (12,779, 75.5%), kabuli (3,528, 20.8%) and intermediate (621, 3.7%) types in the entire collection of ICRISAT genebank (Upadhyaya et al., 2001).

#### e. Seed surface

Seed surface can have an overriding importance in determining market classes of chickpea and in acceptance of improved cultivars. Three types of seed surface are classified in chickpea, viz rough, smooth and tuberculated (Pundir *et al.*, 1988). About 79.39% accessions of world germplasm collection of chickpea had rough seed surface, 18.65% were smooth and 1.96% were tuberculated (Pundir *et al.*, 1985). In a core collection evaluated at ICRISAT, 1437 accessions were rough (73.47%), while 473 are smooth (22.34%) and 46 were tuberculated (2.35%) (Upadhyaya *et al.*, 2001).

#### f. Seed colour

The utilization of seed of chickpea largely depends on its seed coat colour. Seed colour is important with regard to consumer preference, which varies from region to region. The variation for seed colour in chickpea is enormous. Seed coat colour is known to change during seed development and ageing. Balasubramanian (1950a, 1950b) described thirteen seed colour classes ranging from yellow to dark brown. Several factors are involved, which interact with each other, and some have pleiotropic effects (Smithson *et al.* 1985). Of the 24 seed colours reported in the chickpea core collection by Upadhyaya *et al.*, (2001), yellow brown (61.06%) was the most commonly represented (690 accessions) followed by beige (38.85%, 439 accessions). Orange was seen in only one accession (0.09%).

#### g. Seed dots

Dots on the seed testa, is a morphological trait which is characterised by the presence or absence of small black dots on the seed surface. Minute black dots were present (66.82%) on the seed testa of 1307 accessions and in the remaining 649 the black dots were absent (33.18%) in chickpea core collection evaluated at ICRISAT (Upadhyaya *et al.*, 2001).

#### 2.2.1.2 Quantitative Traits

In general most agronomic characters display a continuous distribution of phenotypes. The variability is associated with the segregation of multiple minor genes or polygenes, which have small individual effects and are influenced markedly by the environment. Studies on quantitative variation in chickpea depicted that economic traits such as plant height, pod number, number of branches, seed weight and yield are quantitatively inherited. A thorough trait wise understanding of its genetic nature, heritability and relationship with other characters is necessary for choosing appropriate breeding and selection method in the crop improvement.

For the purpose of summarization, the traits studied were grouped into three broad categories based on the life cycle of the chickpea plant (Gowda *et al.*, 2011):

**Vegetative traits:** plant height, plant width, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches and tertiary branches;

**Reproductive traits:** days to 50 percent flowering, flowering duration, days to maturity;

**Yield and yield component traits:** pods per plant, seeds per pod, 100-seed weight, grain yield and productivity per day.

## a. Vegetative traits:

## (i) Plant height and width

Farmers, particularly in the Mediterranean region, desire mechanization of cultural operations in chickpea cultivation. One reason for lack of satisfactory mechanization is low plant height. Tall plants are often mentioned as ideal in chickpea for improving the yield potential (Bahl et al., 1984; Singh et al., 1980). Plant height is receiving attention as several workers (Bhardwaj and Singh, 1980, Kumar et al., 1981, Singh et al., 1990, Misra, 1991, Sandhu et al., 1991, Dasgupta et al., 1992, Panchbhai et al., 1992, Chavan et al., 1994, Bhatia et al., 1993, Rao et al., 1994, Naseem et al., 1995, Singh et al., 1995, Mathur and Mathur 1996, Kumar et al., 2001, Somyasharma and Singh, 2001, Burli et al., 2004) opined that taller stature is necessary for mechanical harvesting and improving yield. Geneticists in the Indian subcontinent and in the Mediterranean region have been devoting some of their resources in breeding plants with taller stature. Arora, (1991), Patil, (1996) and Arora and Jeena, (2000) reported a moderate variability in chickpea genotypes whereas low variability was reported by Singh and Rao, (1991), Pushpa et al., 1993 and Mishra et al., 1994, Subhash et al., (2001) studied variability in 33 chickpea genotypes grown in five environments and confirmed large variability for plant height. Chaturvedi et al., (2009) reported a wide range of variation among 88 genotypes for plant height (31.5cm to 84.5 cm) with an overall mean of 59.7 cm and reported, 48 genotypes having plant height above the overall mean.

Plant width is an average spread of plant and is an important trait in evaluation of chickpea germplasm. Upadhyaya *et al.*, (2001) evaluated chickpea core collection and reported that means of desi, kabuli, and intermediate types were significantly different from each other for plant width and kabuli types have greater plant width than desi and intermediate types. Bhat and Singh, (1980), Mishra *et al.*, (1988) and Chavan *et al.*, (1994) reported that plant width increases yield as it is related with branching pattern and number of pods per plant.

Variable estimates of heritability (h<sup>2</sup>b) have been reported for plant height and plant width. While Samal and Jagdev, (1989), Sharma *et al.*, (1990), Singh and Rao,

(1991), Mishra, (1991), Chavan *et al.*, (1994), Mishra *et al.*, 1994, Rao *et al.*, 1994, Patil, (1996), Mathur and Mathur, (1996), Dubey and Srivastav, (2007) and Gowda *et al.*, (2011) reported high  $h^2b$ , Rastogi and Singh, (1977); Setty *et al.*, (1977), Sharma *et al.*, 1989, Sandhu *et al.*, (1991) and Panchbhai *et al.*, (1992), Arora and Jeena, (2000) and Dubey and Srivastav, (2007) reported moderate and Samal and Jagdev, (1989), Salimath and Patil, (1990), Mishra, (1991), Chavan *et al.*, (1994) and Mishra *et al.*, (1988) reported low estimates of  $h^2b$  for plant height and width.

Similarly, variable genetic advance have been reported for plant height and plant width. It was reported to be low by Sandhu *et al.*, (1991) and Panchbhavi *et al.*, (1992) for plant height and Mishra *et al.*, (1988) for plant width, moderate by Sharma *et al.*, (1990), Chavan *et al.*, (1994), Geletu *et al.*, (1995), Kumar *et al.*, (2000), Dubey and Srivastav, (2007) and high by Mandal and Bahl, (1983), Dumbre *et al.*, (1984), Agarwal, (1986), Rao *et al.*, (1994), Patil, (1996) and Dubey and Srivastav, (2007) for plant width.

#### (ii) Branches

The chickpea plant is a short bush with several major and minor branches. Branching affects growth habit, and strongly influences the number and position of reproductive structures that ultimately determine yield. Pundir et al., (1988) reported five groups of branching patterns namely, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches and tertiary branches. Several workers have reported the importance of number of primary branches. Rang, (1980), Kumar et al., (1981), Singh et al., (1982), Mandal and Bahl, (1983), Rao et al., (1984), Malhotra and Singh, (1989), Singh et al., (1990), Dasgupta et al., (1990), Sandhu et al., (1991), Singh et al., (1993), Singh and Rao, (1991), Chavan et al., (1994), Ghirase and Deshmukh, (2000) and Shaukatali et al., (2002) whereas Mishra et al., (1988), Sharma et al., (1989), Malhotra and Singh, (1989), Arora et al., (1991), Singh and Rao, (1991), Sandhu et al., (1991), Maynez et al., (1993), Jahagirdar et al., (1994), Rao et al., (1994) and Patil, (1996) reported the importance of number of secondary branches and Arora, (1991), Rao et al., (1994), Patil, (1996) reported the importance of number of tertiary branches and reported that large are number of branches are important from the yield point of view. Subhash et al., (2001) studied variability in 33 chickpea genotypes grown in five environments and confirmed large variability for number of primary and secondary branches per plant. Upadhyaya et al., (2001) evaluated chickpea core collection and reported that the variances between chickpea types were homogeneous for number of apical secondary branches, basal secondary branches and tertiary branches. Bhavani *et al.*, (2009) studied role of genetic variability in 27 chickpea accessions and reported wide variations in number of primary branches.

Variable estimates of heritability ( $h^2b$ ) have been reported for number of branches per plant. While Sharma *et al.*, (1990), Mishra *et al.*, (1991), Chavan *et al.*, (1994), Jha *et al.*, (1997), Subhaschandra *et al.*, (2001), Gowda *et al.*, (2011) reported high  $h^2b$ , moderate by Patil, (1996), while Singh and Rao, (1991), Rao *et al.*, (1994) and Rana *et al.*, (1995) reported low estimates of  $h^2b$  for number of primary branches per plant. Yadav *et al.*, (1989), Singh and Rao, (1991), Jahagirdar *et al.*, (1994), Patil, (1996) and Chauhan and Singh, (2000) reported high  $h^2b$ , moderate by Patil, (1996), while Rao *et al.*, (1994) reported low estimates of  $h^2b$  for number of secondary branches per plant Singh and Rao, (1991), Jahagirdar *et al.*, (1994), Patil, (1996), while Rao *et al.*, (2000) reported high  $h^2b$ , moderate by Patil, (1996) and Chauhan and Singh, (2000) reported high  $h^2b$ , moderate by Patil, (1996) and Chauhan and Singh, (2000) reported high  $h^2b$ , moderate by Patil, (1996) and Chauhan and Singh, (2000) reported high  $h^2b$ , moderate by Patil, (1996) and Chauhan and Singh, (2000) reported high  $h^2b$ , moderate by Patil, (1996), while Rao *et al.*, (1994) reported low estimates of  $h^2b$  for number of secondary branches per

Similarly, variable genetic advance have been reported for number of primary and secondary branches per plant. It was reported to be low by Sharma and Maloo, (1988), Sandhu *et al.*, (1991) and Arora and Jeena, (2000), moderate by Kumar *et al.*, (2001) while high by Sharma *et al.*, (1990) Mishra *et al.*, (1991), Chavan *et al.*, (1994), Rao *et al.*, (1994), Patil, (1996) and Subhaschandra *et al.*, (2001) for number of primary branches. It was reported to be high by Sharma *et al.*, (1989), Jahagirdar *et al.*, (1994), Patil, (1996) and Chauhan and Singh, (2000) for number of secondary branches. It was reported to be high by Jahagirdar *et al.*, (1994), Patil, (1996) and Chauhan and Singh, (2000) for number of secondary branches. It was reported to be high by Jahagirdar *et al.*, (1994), Patil, (1996) and Chauhan and Singh, (2000) for number of secondary branches.

### **b. Reproductive traits:**

## (i) Days to 50 percent flowering and maturity

Time of flowering is the major component of crop environmental adaptation, particularly when the growing season is restricted by climatic factors such as drought and high temperatures (Subba Rao *et al.*, 1995). Early flowering will help in minimizing the losses due to biotic (pod borer) and abiotic (terminal moisture and

heat) stresses and in enhancing the per day productivity. So there is a need to develop early maturing chickpea varieties with large biomass (Chaturvedi and Ali, 2004). Early flowering, mediated by photoperiod insensitivity was suggested as a means to increase chickpea adaptability (Sandhu and Hodges, 1971) but, no genetic studies have been reported until recent years (Kumar and van Rheenen, 2000; Or *et al.*, 1999). In semi-arid habitats, the time of flowering is of great adaptive value for both wild and cultivated plants (Or *et al.*, 1999), as early flowering helps the crop to mature before the onset of biotic and abiotic stresses (Subba Rao *et al.*, 1995, Van Rheenen *et al.*, 1997).

In chickpea, the duration of flowering is a major yield determinant (Kumar and Abbo, 2001), phenology of the crop has an immense influence on productivity and stability. Murfet and Reid, (1985) have reported that flowering genes influence maturity and crop yield through their effects on the onset of reproductive phase, number of branches, and number of flowers per node. The flowering time of chickpea genotypes varies with latitude and temperature variations. In the trails conducted by ICRISAT on 25 genotypes at three locations: Patancheru (18°N), Gwalior (26°N) and Hisar (29°N), the range for flowering time did not overlap (80-102 days in Hisar, 71-78 in Gwalior and 40-61 days in Patancheru) and the mean number of days to 50 percent flowering was 51, 76 and 96 for three locations, respectively. Pundir et al., (1988), evaluated the world chickpea germplasm maintained at ICRISAT and listed 43 accessions that flowered in less than 39 days at Patancheru. Kumar and Abbo, (2001) evaluated ICCV 96029 and control Pant G 114 for their flowering time at Patancheru and Hisar. The number of days taken to flower by ICCV 96029 was 29 and 43 at Patancheru and Hisar respectively. This might indicate that mutations for early flowering genes also survived in sub tropical environments. Upadhyaya et al., (2001) evaluated chickpea core collection (1956 accessions) for identification of diverse germplasm lines for use in crop improvement and reported twelve early maturing genotypes and also reported that means of desi, kabuli, and intermediate types were significantly different from each other for days to maturity and kabuli types matured later than desi and intermediate types. Kumar and Abbo, (2001) described the effect of flowering time on chickpea adaptation, seed weight, seed yield and stability under semi-arid Near-East and Indian sub continental environments. Subhash et al., (2001) studied variability in 33 chickpea genotypes grown in five environments and confirmed large variability for days to 50 percent flowering and days to maturity.

Sandhu et al., (2002) evaluated three genotypes (super early ICCV 96029, early ICCV 2 and late flowering control PBG 1) on three different sowing dates, and reported that ICCV 96029 flowered in 28-35 days followed by, ICCV 2 in 31-40 days, while PBG 1 took twice the number of days to flower than ICCV 96029 and ICCV 2 in all three sowing dates. Kumar and Johansen, (2002) reported that the super early genotype ICCV 96029 took 43 days to flower and matured in 128 days at Hisar in early November sown crop. Upadhyaya et al., (2007) identified six most early maturing genotypes by evaluating twenty eight early maturing genotypes selected from core and entire collection of ICRISAT genebank. Chaturvedi et al., (2009) evaluated 88 chickpea lines and reported that days to 50 percent flowering varied from 36 to 103 days with an overall mean of 87 days and confirmed that 44 lines flowered earlier than the control cultivar (96 days). Similarly days to maturity varied from 116 days to 137 days with an overall mean of 130 days and 37 lines took less number of days to mature than the overall mean. Agarwal, (1985), Shaukatali et al., (2002) and Dubey and Srivastav, (2007) reported high variability for days to 50% flowering whereas Dasgupta et al., (1992) Rao et al., (1994) and Rao and Kumar et al., (2000) reported moderate variability for days to 50% flowering.

Variable estimates of heritability ( $h^2b$ ) have been reported for days to 50 percent flowering and maturity. While Chandra, (1968); Joshi, (1972); Agarwal, (1985), Samal and Jagdev, (1989); Sharma *et al.*, (1990); Misra, (1991); Singh and Rao, (1991); Panchbhavi *et al.*, (1992); Chavan *et al.*, (1994); Jahagirdar *et al.*, (1994); Mathur and Mathur, (1996), Arora and Jeena, (2000), Burli *et al.*, (2004); Dubey and Srivastav, (2007), Upadhyaya *et al.*, (2007) and Gowda *et al.*, (2011) reported high  $h^2b$  for days to flowering and maturity.

Similarly, variable genetic advance have been reported for days to flowering. It was reported to be low by Sharma *et al.*, (1990), Misra, (1991) and Rao *et al.*, (1994) and moderate by Arora, (1991), Arora and Jeena, (2000), while high by Agarwal, (1985), Jahagirdar *et al.*, (1994) Burli *et al.*, (2004) and Dubey and Srivastav, (2007), for days to flowering and Mishra *et al.*, (1994) for days to maturity.

#### c. Yield and yield component traits:

The major yield components of chickpea are pod number per plant, seed number per pod and 100-seed weight.

#### (i) Pods per plant and Seeds per pod

In chickpea the number of pods per plant and seeds per pod are directly correlated with seed yield (Zafar and Khan, 1968, Gupta et al., 1974, Katiyar, 1975, Bhat and Singh, 1980, Bhardwaj and Singh, 1980, Kumar et al., 1981, Deshmukh and Bhapkar, 1982a, Singh et al., 1982, Singh and Paroda, 1986, Mishra et al., 1988, Fillipetti, 1990, Arora, 1991, Sandhu et al., 1991, Dasgupta et al., 1992, Bhatia et al., 1993, Chavan et al., 1994, Jahagirdar et al., 1994, Mishra et al., 1994, Rao et al., 1994, Patil, 1996, Jha et al., 1997, Kumar, 2001, Upadhyaya et al., 2002, Burli et al., 2004 and Dubey and Srivastav, 2007 ). Normally single flowers are borne on pedicels suspended by single peduncles in the axils of the leaves, at the rate of one pedicel (one flower) per peduncle which contributes to more stable yield (Smithson et al. 1985). Sheldrake et al., (1978) obtained 6-13% higher yield in double podded plants compared to single podded plants. Singh and van Rheenen, (1994) suggested double poddedness can contribute positively to higher productivity in chickpea. Upadhyaya et al., (2001) evaluated chickpea core collection and reported that means of desi, kabuli, and intermediate types were significantly different from each other for pods per plant and kabuli types have the lowest average number of pods than desi and intermediate types. Bhavani et al., (2009) studied role of genetic variability in 27 chickpea accessions for 12 quantitative traits and reported a wide variation in number of seeds per pod and pods per plant. Chaturvedi et al., (2009) reported varied number of pods per plant from 19 to 64 in six genotypes with overall mean of 37 pods. Twenty genotypes exhibited higher number of pods per plant than the best control cultivar (45). The mean number of seeds per pod varied from 0.9 to 2.2 with overall mean of 1.4 seeds and 4 genotypes had more number of seeds per pod than the overall mean.

Estimates of heritability (h<sup>2</sup>b) for number of pods per plant varied from high (Joshi, 1972, Mishra *et al.*, 1988; Samal and Jagdev, 1989, Mishra, 1991; Kumar *et al.*, 1991; Singh and Rao, 1991, Dasgupta *et al.*, 1992; Chavan *et al.*, 1994, Jahagirdar *et al.*, 1994; Mishra *et al.*, 1994; Mehndi *et al.*, 1994, Rao *et al.*, 1994; Mathur and Mathur, 1996, Patil, 1996, Arunkumar *et al.*, 1998; Kumar, 2001, Narayana and Reddy, 2002,

Sial *et al.*, 2003; Dubey and Srivastava, 2007 and Gowda *et al.*, 2011) to low (Sandhu *et al.*, 1991; Mishra *et al.*, 1994; Rao *et al.*, 1994, Rana *et al.*, 1995 and Arora and Jeena, 2000). While moderate heritability for seeds per plant was reported by Pandey *et al.*, 1989 and low heritability was reported by Pundir *et al.*, (1991) and Panchbhavi *et al.*, (1992). Low to moderately high heritability was reported by Rao *et al.*, 1994, Iqbal *et al.*, 1994 and Arora and Jeena, 2000 low estimates of  $h^2b$  for pods per plant as reported by Sandhu *et al.*, (1991); Mishra *et al.*, (1994); Rao *et al.*, (1994) and Rana *et al.*, (1995). For seeds per pod also varying estimates of  $h^2b$  have been reported. Low to moderately high  $h^2b$  estimates were reported by Iqbal *et al.*, (1994), moderate  $h^2b$  estimates were reported by Iqbal *et al.*, (1994), moderate  $h^2b$  estimates were reported by Iqbal *et al.*, (1994), moderate  $h^2b$ 

The expected genetic gain was reported to be low (Agarwal, 1985, Panchbhavi *et al.*, 1992) for number of seeds per plant and pods per plant, high for pods per plant by Jivani and Yadavendra, (1988); Mishra *et al.*, (1991), Kumar *et al.*, (1991), Chavan *et al.*, (1994), Jahagirdar *et al.*, (1994), Mishra *et al.*, (1994), Rao *et al.*, (1994), Patil, (1996), Arunkumar *et al.*, (1998), Kumar, (2001) and Dubey and Srivastav, (2007).

### (ii) Seed weight and size

Seed size (as measured by 100-seed weight) is not only the most important yield component (Singh and Paroda, 1986), but also an important criterion for consumer preference (Deshmukh and Bhapkar, 1982a, Mandal and Bahl, 1983, Agarwal, 1985, Salimath and Bahl, 1985, Singh, 1987, Malik et al., 1988, Filipetti, 1990, Salimath and Patil, 1990, Sharma et al., 1990, Singh et al., 1990, Bhatia et al., 1993, Maynez et al., 1994, Bhoyta et al., 1994, Rao et al., 1994, Patil, 1996, Shaukatali et al., 2002 ). Tomar et al., (1982) reported that small-seeded cultivars were phenotypically more stable than large-seeded cultivars. Small-seeded cultivars are a major hurdle in the large-scale introduction of winter sowing of chickpea (Malhotra et al., 1997). Therefore improvement in seed size is an important goal in chickpea breeding programmes. Yadav and Sharma, (1999) evaluated 108 kabuli chickpea accessions to study various seed quality characteristics under irrigated conditions and they observed high variation in 100-seed weight. Upadhyaya et al., (2001) evaluated chickpea core collection and reported that means of desi, kabuli, and intermediate types were significantly different from each other for 100-seed weight and kabuli types have the highest 100-seed weight than desi and intermediate types. Bhavani et al., (2009) studied role of genetic variability in 27 chickpea accessions for 12 quantitative traits and reported a wide variation in 100- seed weight. Chaturvedi *et al.*, (2009) reported that the 100-seed weight ranged from 10.2g to 36.6g with the overall mean of 19.2g. Twenty six genotypes were at par with overall mean, whereas 24 genotypes showed larger 100-seed weight than the large seeded control cultivar.

Varying estimates of heritability ( $h^2b$ ) have been reported for 100-seed weight. While Mandal and Bahl, (1983); Agarwal, (1985); Salimath and Bahl, (1985); Salimath and Patil, (1985); Samal and Jagdev, (1989); Sharma *et al.*, (1990); Kumar *et al.*, (1991); Mishra *et al.*, (1991); Sandhu *et al.*, (1991); Singh and Rao, (1991); Dasgupta *et al.*, (1992); Chavan *et al.*, (1994); Jahagirdar *et al.*, (1994); Rao *et al.*, (1994); Patil, (1996); Tripathi, (1998); Subhaschandra *et al.*, (2001); Saleem *et al.*, (2002); Toker, (2004); Burli *et al.*, (2004); Dubey and Srivastav, (2007) and Gowda *et al.*, (2011) reported high  $h^2b$  for 100-seed weight; whereas Sandha and Chandra (1969), Joshi, (1972), Rastogi and Singh, (1977), Sandhu *et al.*, (1991) and Singh *et al.*, (1992) observed moderate heritability for 100-seed weight.

Similarly, variable genetic advance have been reported for 100-seed weight. It was reported to be low (Agarwal,1985; Mishra *et al.*, 1991; Sandhu *et al.*, 1991; Arshad *et al.*, 2003, 2004) and moderate (Agarwal,1985; Mishra *et al.*, 1991) to high (Mandal and Bahl, (1983); Agarwal, (1985); Sharma *et al.*, (1990); Kumar *et al.*, (1991); Jahagirdar *et al.*, (1994); Rao *et al.*, (1994); Patil, (1996); Mathur and Mathur, 1996; Tripathi, (1998); Nimbalkar, 2000; Burli *et al.*, (2004) and Dubey and Srivastav, (2007)).

### (iii) Grain yield and productivity

Grain yield of chickpea is a quantitative character which is influenced by many genetic factors as well as environmental factors (Muehlbauer and Singh, 1987). Grain yield per plant is the major determinant of plot yield (Deshmukh and Bhapkar, (1982), Islam *et al.*, (1984), Malik *et al.*, (1988), Mishra *et al.*, (1988), Reddy and Rao, (1988), Fillipetti, (1990), Patil, (1996), Arora, (1991), Sandhu *et al.*, (1991), Singh and Rao, (1991), Dasgupta *et al.*, (1992), Bhatia *et al.*, (1993), Maynez *et al.*, (1993), Jirali *et al.*, (1994), Rao *et al.*, (1994), Srivastav and Jain, (1994), Wanjari *et al.*, (1996), Rao and Kumar, (2000), Kumar, (2001), Burli *et al.*, (2004) and Dubey and Srivastav, (2007). Although direct selection for grain yield could be misleading, indirect selection via yield related characters with high heritability might be more

effective (Toker, 1998). Raju et al., (1978) reported high genetic variability, heritability, genetic advance and trait correlations with respect to yield and its components in chickpea. Pundir et al., (1991) evaluated twenty-five short and medium duration chickpea germplasm accessions of diverse geographic origin and reported wide variation for physio-morphic and yield traits. Bakhsh et al., (1998) reported a consistent and positive association of biological yield per plant, pods per plant, harvest index and secondary branches per plant with grain yield. Ali et al., (1999) reported that yield was accounted by the plant height, number of secondary branches and pods per plant, under normal field conditions. The findings are consistent with the results obtained by Ghafoor et al., (1990) and Khattak et al., (1995, 1997, and 1999) in mungbean. Upadhyaya et al., (2001) evaluated chickpea core collection and reported that means of desi, kabuli, and intermediate types were significantly different from each other for plot yield and kabuli types have the lowest plot yield than desi and intermediate types. Saleem et al., (2002) observed high coefficient of variability for grain yield and other yield parameters in chickpea. Raval and Dobariya, (2003) estimated genetic variability and interrelationships among thirteen yield components in chickpea. Arshad et al., (2004) reported high range of yield per plant for twenty-four varieties of chickpea. Ali et al., (2002), Kaur et al., (2004), Qureshi et al., (2004), Sharma et al., (2005), Singh, (2007) and Sidramappa et al., (2008) reported that parameters with high genetic variability could be focused for genetic improvement in chickpea. Renukadevi and Subbalakshmi, (2006) reported the positive direct effect of number of branches, pods per plant and 100-seed weight on yield per plant in chickpea genotypes. Bhavani et al., (2009) studied genetic variability in 27 chickpea accessions on 12 quantitative traits and reported a wide range of variation in plot yield. Chaturvedi et al., (2009) evaluated 88 chickpea lines collected from various parts of the country and reported that the mean yield per plant ranged from 3.4g to 14.4g with overall mean of 8.7g.

Variable estimates of  $h^2b$  for yield have been reported. Some workers have reported low  $h^2b$  (Salimath and Patil, 1990, Sharma *et al.*, 1990, Panchbhai *et al.*, 1992, Rao *et al.*, 1994 and Wanjari *et al.*, 1996), whereas others have reported moderate  $h^2b$ estimates (Mandal and Bahl, 1980, Wanjari *et al.*, 1996 and Arora and Jeena, 2000), and still others reported high estimates for seed yield (Patil and Phandnis, 1977, Mishra *et al.*, 1988, Sandhu *et al.*, 1991, Singh and Rao,1991, Singh *et al.*, 1993, Chavan *et al.*, 1994, Jahagirdar *et al.*, 1994, Mehndi *et al.*, 1994, Mishra *et al.*, 1994, Mathur and Mathur, 1996, Patil, 1996, Arunkumar *et al.*, 1998, Sandhu *et al.*, 1999, Nimbalkar, 2000, Kumar, 2000, Singh *et al.*, 2003, Dubey and Srivastav, 2007 and Gowda *et al.*, 2011). While  $h^2b$  for seed yield varied from low, moderate and high (Mehndi *et al.*, 1994, Arshad *et al.*, 2003, 2004, Upadhyaya *et al.*, 2007). Low to moderately  $h^2b$  high estimates reported by Iqbal *et al.*, (1994).

Variable estimates of  $h^2b$  for yield per plant have been reported. Samal and Jagdev, (1989); Jahagirdar *et al.*, (1994); Singh and Rao, (1991); Chavan *et al.*, (1994); Gowda *et al*, (2011) reported high  $h^2b$ . While low, moderate to high estimates where reported by Iqbal *et al.*, (1994).

Similarly, variable genetic advance have been reported for seed yield and yield per plant. It was reported to be high by Mishra *et al.*, (1988), Chavan *et al.*, (1994), Dasgupta *et al.*, (1994), Jahagirdar *et al.*, (1994), Rao *et al.*, (1994), Patil, (1996), Arunkumar *et al.*, (1998), Jeena and Arora, (2000a, b), Subhash *et al.*, (2001) and Dubey and Srivastav, (2007), while moderate by Mandal and Bahl, 1980, Mishra *et al.*, 1991 and Arora and Jeena, 2000 and low by Wanjari *et al.*, 1996. Low for seed yield per plant by Sharma *et al.*, (1990), Misra, (1991) and Panchbhai *et al.*, (1992), Gowda *et al.*, (2011) and for seed yield by Chavan *et al.*, 1994, Rao *et al.*, (1994), Misra *et al.*, (1994) and Mathur and Mathur, (1996), Patil *et al.*, (1996), Gowda *et al.*, (2011).

### 2.2.2 Correlation among traits

The correlation analysis helps to determine the nature and degree of relationship between any two measurable characters. Correlation among traits may result from pleiotropy or physiological associations among characters, which often indicate useful selection indices for two or more traits. Study of correlations is important to know the relationship between traits and co-adapted gene complexes. It also provides information on correlated response.

Yield is the end product of many complex component characters, which singly or jointly influence the yield. Yield does not possess genes for *per se* as such. Therefore, selection of a genotype based on yield alone is likely to be ineffective. The efficiency of selection for yield mainly depends on the direction and magnitude of association between yield and its components (Breese, 1989). The studies on association of various yield components with grain yield in chickpea are reviewed here under:-

Characters	Association	References
Days to 50 percent flowering	Positive	Paliwal <i>et al.</i> , 1987;
		Mishra, 1991;
		Choudary <i>et al.</i> , 1992;
		Chavan <i>et al.</i> , 1994;
		Vijayalakshmi <i>et al.</i> , 2000;
		Upadhyaya <i>et al.</i> , 2001;
	Negeting	Saleem <i>et al.</i> , 2002;
	Negative	Khorgade, 1988;
		Narayana and Reddy, 2002; Sial <i>et al.</i> , 2003 ;
Plant height	Positive	Khan and choudary, 1975;
r lant neight	rostive	Mandal, 1977;
		Sharma <i>et al.</i> , 1989;
		Yadav, 1990;
		Mishra,1991;
		Choudary <i>et al.</i> , 1992;
		Roshanlal <i>et al.</i> , 1993;
		Bhambota <i>et al.</i> , 1994;
		Naseem <i>et al.</i> , 1995;
		Rao, 1998;
		Tripathi, 1998;
		Yucel et al., 2006;
	Negative	Govil, 1980;
	_	Salimath and Patil, 1990;
Number of primary branches	Positive	Katiyar <i>et al.</i> , 1977;
per plant		Jatasra <i>et al.</i> , 1978;
		Mishra et al., 1988;
		Sandhu <i>et al.</i> , 1988;
		Sharma and Maloo.1988;
		Sandhu and Mandal, 1989;
		Uddin <i>et al.</i> , 1990;
		Chavan <i>et al.</i> , 1994;
		Sarvaliya and Goyal, 1994a,1994b;
		Geletu <i>et al.</i> , 1995; Singh <i>et al.</i> , 1995;
		Rana <i>et al.</i> , 1995;
		Patil, 1996;
		Rao, 1998;
		Tripathi, 1998;
		Bakhsh <i>et al.</i> , 1998,
		Vijayalakshmi <i>et al.</i> , 2000;
		Upadhyaya <i>et al.</i> , 2001;
		Saleem <i>et al.</i> , 2002;
		Arshad <i>et al.</i> , 2002;
		Narayana and Reddy, 2002;
		Raval and Dobariya, 2003;
		Sial <i>et al.</i> , 2003;
		Arshad <i>et al.</i> , 2004 ;
		Hassan <i>et al.</i> , 2005;
		Yucel <i>et al.</i> , 2006;
		Babar <i>et al.</i> , 2008;
	NT	Malik <i>et al.</i> , 2010;
	Negative	Singh <i>et al.</i> , 1989;
	D :::	Patil, 1996;
Number of secondary	Positive	Upadhyaya <i>et al.</i> , 2001;
branches per plant		Saleem <i>et al.</i> , 2002,
		Arshad <i>et al.</i> , 2002;
		Narayana and Reddy, 2002; Payal and Dobariya, 2003 :
		Raval and Dobariya, 2003 ;

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		Sial <i>et al.</i> , 2003;
		Arshad <i>et al.</i> , 2004 ;
		Hassan <i>et al.</i> , 2005;
		Yucel <i>et al.</i> , 2006;
		Babar <i>et al.</i> , 2008;
		Malik <i>et al.</i> , 2010;
	Negative	Sandhu and Singh,1970;
Number of tertiary branches	Positive	Uddin et al., 1990;
per plant		Chavan <i>et al.</i> , 1994;
		Upadhyaya <i>et al.</i> , 2001;
		Saleem <i>et al.</i> , 2002,
		Yucel et al., 2006;
		Babar <i>et al.</i> , 2008;
		Malik <i>et al.</i> , 2010;
	Negative	Patil,1996;
Do do non nlont	Positive	
Pods per plant	Positive	Dasgupta <i>et al.</i> , 1992;
		Bhatia <i>et al.</i> , 1993;
		Roshanlal <i>et al.</i> , 1993;
		Bhoyta <i>et al.</i> , 1994;
		Bhambopta <i>et al.</i> , 1994;
		Rao <i>et al.</i> , 1998;
		Berger and Turner, 2000;
		Vijayalaxmi et al., 2000;
		Guler <i>et al.</i> , 2001;
		Narayana and Reddy, 2002;
	Negative	Fillipetti,1990;
	-	Kharat <i>et al.</i> , 1991;
		Dasgupta et al., 1992;
		Singh <i>et al.</i> , 1995;
		Berger and Turner, 2000;
100-Seed weight	Positive	Benjamini, 1981;
100 Seed weight	robitive	Singh, 1982;
		Tomar <i>et al.</i> , 1982;
		Salimath and Bhal,1986;
		Malik <i>et al.</i> , 1988;
		Sandhu and Mandal, 1989;
		Sandhu <i>et al.</i> , 1989;
		Mishra <i>et al.</i> , 1994;
		Jirali <i>et al.</i> , 1994;
		Srivastava <i>et al.</i> , 1994;
		Naseem <i>et al.</i> , 1995;
		Vijayalaxmi et al., 2000;
		Sial <i>et al.</i> , 2003;
		Arshad <i>et al.</i> , 2004;
		Hassan <i>et al.</i> , 2005;
		Babar <i>et al.</i> , 2008;
	Negative	Khan and choudary, 1975;
	-	Singh <i>et al.</i> , 1976;
		Narayana and Macefield, 1976;
		Rostagi and Singh,1977;
		Fillipetti,1990;
		Roashanlal <i>et al.</i> , 1993;
		Chand <i>et al.</i> , 1995;

Reviews on inter-relationship between traits other than grain yield are presented below

Traits	Associated traits	Direction	Author
Dous to 50 moreout	Flowering duration, days to	Positive	Upadhyaya et al., 2001;
	maturity		
Days to 50 percent flowering	Flowering duration, number of	Negative	Upadhyaya et al., 2007;
nowering	primary and secondary		
	branches, pods per plant.		
	Seeds per pod	Negative	Khorgade et al., 1995;
100-seed weight	Plant height	Negative	Mathur and Mathur, 1996;
	Protein content	Negative	Pundir et al., 1991;
Number of branches	Pods per plant	Positive	Upadhyaya et al., 2007;
Flower colour	Seed shape	Positive	Upadhyaya et al., 2001;
Days to maturity	Apical secondary branches	Positive	Upadhyaya et al., 2007;
Seeds per pod	100-seed weight	Negative	Pundir <i>et al.</i> , 1991;
Pods per plant	100-seed weight	Negative	Upadhyaya et al., 2007;

Jivani and Yadavendra (1988) reported that number of branches per plant, pods per plant and seed weight should be given importance in direct selection for increased yield owing to their greater direct effects on yield. Sharma and Maloo (1988) showed that pod per plant was the character to have greatest influence on seed yield followed by number of primary branches. Days to maturity, pods per plant, 100 seed weight, and conventional harvest index had positive direct effects on yield per plant (Uddin *et al.*, 1990).Bhambota (1994) observed that pods per plant and plant height had considerable positive direct effect on seed yield. Number of branches had a negative direct effect on yield but a positive indirect effect via pods per plant. Chavan *et al.* (1994) concluded that branches per plant, pods per plant should be used as selection criteria for yield improvement. Sarvaliya and Goyal (1994a) found that number of pods per plant and 100-seed weight had high direct effect on seed yield.

Bhattacharya *et al.* (1995) concluded that days to 50 percent flowering influence seed yield greatly under moisture stress condition. Arora and Jeena (1999) in a study of path analysis in 43 genotypes indicated that plant height; pods per plant were important characters for seed yield. Khedar and Maloo (1999) in a study of path analysis in 40 genetically diverse chickpea genotypes reported that pods per plant had the highest direct effect on seed yield, followed by seeds per pod, 100-seed weight and number of primary branches per plant.

Rao and Kumar (2000) found that days to 50 percent flowering and duration of reproductive phase had positive direct effect on yield, while plant height, days to maturity and 100-seed weight had negative direct effect. Netrapal Singh (2001) in a study of path analysis in 34 genotypes reveled that biological yield had highest direct

effect on yield followed by number of pods, days to maturity. While 100-seed weight, number of primary branches and days to 50 percent flowering have negative direct effect.

Mishra *et al.* (2002) reported that the number of pods per plant had the highest positive direct effect on seed yield. Narayana and Reddy (2002) conducted path analysis in 31 chickpea genotypes and they reported high direct effects of number of pods per plant, 100-seed weight, number of seeds per pod and harvest index on seed yield. Pratap *et al.* (2002) carried out path analysis in 57 chickpea genotypes and they observed positive direct effect on grain yield by biological yield, number of pods plant and harvest index.

The study of relationships among quantitative traits is important for assessing the feasibility of joint selection of two or more traits and hence for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. A positive genetic correlation between two desirable traits makes the job of the plant breeder easy for improving both traits simultaneously. Even the lack of correlation is useful for the joint improvement of the two traits. On the other hand, a negative correlation between two desirable traits impedes a significant improvement in both traits.

### 2.2.3 Diversity studies

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. Analysis of genetic relationships in crop species is an important component of crop improvement program, since it provides information about genetic diversity of the crop species which is a basic tool for crop improvement. Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purpose (Mohammadi and Prasanna, 2003).

### 2.2.3.1 Importance of Diversity Studies

Diversity is the foundation in which selection is practiced. Diversity studies in a crop are important for various aspects such as management of genetic resources, identification of duplicate accessions in the germplasm and in applied breeding programs. Various data have been used to analyze the genetic diversity in crops, including morphological, agronomical and ecogeographical traits. Most economic traits of the crop varieties are quantitative traits that are affected by the crop environment and also by genotype-environment interaction. Traditionally phenotypic traits (Nozzolillo 1985; De Leonardis *et al.* 1996; Robertson *et al.*, 1997; Hassan 2000; Javedi and Yamaguchi 2004), hybridization success (Ladizinsky and Alder 1976; Pundir and VanderMaesen 1983; Pundir and Mengesha 1995; Badami *et al.*, 1997) analysis of chromosome pairing in hybrids (Ladizinsky and Alder 1976; Ahmad 1988), and the study of chromosomes structure (Ohri and Pal 1991; Tayyar *et al.*, 1994; Ahmad 2000) have been widely used methods for analysis of genomic relationships and the construction of phylogenies among *Cicer* species. Over the past 15 years, electrophoretic data based on seed storage protein (Ladizinsky and Alder 1975a; Vairinhos and Murray 1983; Ahmad and Slinkard 1992) and isozymes (Kazan and Muehlbauer 1991; Ahmad and Slinkard 1992; Labdi *et al.*, 1996; Tayyar and Waines 1996; Gargav and Gaur 2001) have also been applied to systematic studies in *Cicer*.

### 2.2.3.2 Phenotypic diversity studies

Genetic improvement mainly depends upon the amount of genetic variability present in the population. Information on the nature and degree of genetic divergence would help the plant breeder in choosing the right parents for breeding programme. In respect of quantitative characters, a breeder is primarily interested in genetic diversity, because it decides response to selection. Several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives, of which Mahalanobis's generalized distance is by and large widely used by plant breeders. The utility of the Mahalanobis's D<sup>2</sup> analysis to detect divergence in a group of genotypes and to identify genotypes that can effectively be used in crossing programme has been stressed repeatedly (Anilkumar *et al.*, 1993).

Malik *et al.*, (2010) studied twenty chickpea genotypes for various yield parameters and reported clustering based on Euclidean dissimilarity which placed all genotypes in three clusters at 50% linkage distance. Cluster I, II and III possessed 8, 5 and 7 genotypes, respectively.

Farshadfar and Farshadfar, (2008) conducted a study to determine the genetic variability among 360 chickpea lines and reported that 63% variance was explained by five PCs and the genotypes could be classified into four clusters.

Upadhyaya *et al.*, (2007) identified the diverse germplasm lines for agronomic traits in the chickpea core collection at ICRISAT by conducting hierarchical cluster analysis, where the first five principal components accounted for 80.5% variation. The 39 selected accessions and two control cultivars (Annigeri and L 550) were grouped into three clusters. Cluster I represented early maturing large-seeded kabuli types, cluster II early and late maturing desi types and cluster 3 late maturing intermediate and kabuli types. The newly identified lines were diverse than the control cultivar and could be used in crop improvement.

Vural *et al.*, (2007) performed cluster analysis based on principal components (PCs) on eleven varieties grown in Turkey which were separated into two main clusters and three subclusters.

Upadhyaya, (2003) performed principal component analysis on the world chickpea germplasm collection held at ICRISAT, using 13 quantitative traits. The clustering of germplasm accessions based on the first three PC scores delineated two regional clusters consisting Africa, South Asia, and Southeast Asia (all desi types) in the first cluster and the Americas, Europe, West Asia, Mediterranean region and East Asia (all kabuli types) in the second cluster.

Upadhyaya *et al.*, (2007) identified new early-maturing germplasm lines using the core collection approach. The average phenotypic diversity values across traits was higher for plot yield, apical primary branches and number of pods per plant

Prakash, (2006) conducted divergence analysis in 81 kabuli chickpea accessions under irrigated conditions and observed wide variations in plot yield, 100 seed weight and seeds per pod.

Upadhyaya, (2003) determined diversity in different regions of world for seven qualitative traits and 13 quantitative traits in the world collection of chickpea germplasm (16,820 accessions). The Shannon-Weaver diversity index (H<sup>\*</sup>) was variable in different regions, seed colour among qualitative traits and days to 50% flowering among quantitative traits showed the highest pooled diversity index.

Islam *et al.*, (1984) evaluated 140 chickpea varieties to study phenotypic diversity based on 7 quantitative traits during postrainy season and observed maximum diversity in number of pods and plot yield followed by minimum diversity in days to 50 percent flowering and days to maturity.

Dwevedi and Gaibriyal, (2009) reported the magnitude of genetic divergence among 25 genotypes of chickpea, using Mahalanobis's  $D^2$  statistics, which were grouped into six clusters and also identified diverse parents which can be utilized in crop improvement programs.

Durga *et al.*, 2005 assessed the genetic diversity based on seven characters in 132 chickpea genotypes and grouped them into 9 clusters. Cluster I was the largest, comprising of 20 genotypes, followed by clusters V and VII with 16 and 15 genotypes, respectively. Maximum inter cluster distance was noticed between clusters I and VIII (511.4) and suggested that crossing the genotypes between clusters I and VIII may lead to maximum diversity in the segregating populations and development of high yielding cultivars.

Raval and Dobariya, (2004) studied genetic divergence among 52 chickpea genotypes and grouped them into 15 clusters. No parallelism was observed between geographic distribution and genetic diversity.

Jeena and Arora, (2002) evaluated thirty six genetically diverse genotypes of chickpea for 16 quantitative attributes following Tocher's method as described by Rao (1952) based on Mahalanobis's D<sup>2</sup>statistics. Twenty eight genotypes were grouped in cluster I, two genotypes each in cluster II and III and one genotype each in clusters IV, V, VI and VII.

Narendra Singh, (2002) carried out multivariate analysis in 300 kabuli chickpea accessions using  $D^2$  statistic and grouped them into 10 non overlapping clusters with like genotypes within clusters for different attributes and also reported no association between clustering pattern and eco-geographical distribution of the genotype.

Sivakumar and Muthiah, 2001 carried out genetic divergence analysis with 126 chickpea cultivars and were grouped into seven clusters. The highest divergence was observed between clusters IV and VII while the lowest was between clusters IV and V. The intra cluster divergence varied from 0 to 2.99.

Darshanlal et al., (2001) estimated genetic divergence among 33 genotypes of

chickpea using  $D^2$  statistic based on yield related traits, which were grouped into 5 clusters. The grouping pattern did not show any relationship between genetic divergence and geographic diversity.

Jethava *et al.*, (2000) estimated genetic divergence using Mahalanobis's  $D^2$  statistic among 70 chickpea genotypes with different ecogeographical region, which were grouped into 16 clusters indicating that the geographical distribution and genetic diversity were not related. Seed yield per plant, number of pods per plant and 100seed weight contributed maximum to genetic diversity.

Harisatyanarayana and Reddy, (2000) estimated the genetic divergence among the 31 genotypes of chickpea based on ten characters and were grouped into seven clusters based on the mean performance, genetic divergence and clustering pattern.

Chand, (1999) studied 49 genotypes for magnitude of genetic diversity using  $D^2$  analysis by considering seven quantitative characters like days to flowering, days to maturity, plant height, branches per plant, pods per plant, 100 seed weight and seed yield per plant. Forty nine genotypes were grouped into eight clusters.

Pooranchand and Chand, (1999) studied genetic divergence among 49 genotypes of chickpea using  $D^2$  analysis for seven quantitative traits, which were grouped into eight clusters.

Bhattacharya and Ganguly, (1998) carried out genetic diversity analysis in twenty six genotypes of chickpea under normal and late seeding conditions. Genotypes grown under normal seeding were grouped into ten clusters and under late seed condition into seven clusters and geographical origin of genotypes did not show any definite relationship with genetic diversity.

Narendra Kumar, (1997) reported grouping of sixty entries of chickpea into five clusters based on seven characters using Mahalanobis  $D^2$  statistics and the grouping of entries in different clusters was not related to their geographic origin.

Samal and Jagdev, (1996) estimated genetic divergence among 32 cultivars of chickpea using Mahalanobis's  $D^2$  statistics for seven yield related characters and were grouped into six clusters

Dangaria *et al.*, (1994) studied 32 genotypes of chickpea for genetic divergence for nodulation characters like nodule number, nodule weight and nodule size. Thirty two

genotypes were grouped into 5 clusters with inter-cluster distance ranging from 7.93 (between I and III) to 17.53 (between IV and V).

Sarvaliya and Goyal, (1994b) estimated genetic divergence among 76 genotypes of chickpea, which were mostly of Indian origin. There were significant differences among the genotypes for 10 agronomic characters studied and were grouped into 10 clusters. There was no relationship between geographical distribution and genetic diversity.

Anilkumar *et al.*, (1993) estimated genetic divergence in a collection of 52 true breeding advanced generation lines and two check varieties of chickpea on the basis of photosynthetic and yield related traits including nodulation parameters to identify physiologically efficient types. These genotypes fell in nine and Cluster V had the highest number of genotypes.

Lokender Kumar and Arora, (1992) used D<sup>2</sup> statistics to group 40 genotypes of chickpea collected from various geographical regions into 10 clusters based on 18 characters and reported that there was no definite relationship between genetic diversity and geographical distribution.

Khan *et al.*, (1991) classified 132 chickpea lines into eight groups on the basis of physiological and morphological traits using multivariate analysis and reported weak correspondence between D<sup>2</sup> analysis and canonical variate analysis.

Sandhu and Gumber, (1991) studied 59 strains of chickpea for magnitude of genetic diversity using Mahalanobis's  $D^2$  analysis considering eight yield contributing characters. They were grouped into 14 clusters. They recommended crossing between genotypes of divergent clusters namely ICC 11321 and L 550 (cluster VI) with ICC 11316 (Cluster XI) for improving productivity.

Mishra *et al.*, (1988) studied the genetic variability as estimated by  $D^2$  and metro glyph analysis using 12 yield components in 177 genotypes, which were grouped into 13 clusters

Salimath *et al.*, (1985) subjected eighty genotypes comprising of k*abuli* and *desi* types from India and nine other countries to divergence analysis by using Mahalanobis's  $D^2$  statistic, a clear demarcation between k*abuli* and *desi* cultivars based on yield and nine yield components.

Adhikari and Pandey, (1983) conducted a study involving 36 varieties from ten chickpea growing states of India and concluded that k*abuli* and d*esi* types tended to occupy separate clusters. The study which considered seed yield and 16 yield related traits formed 9 clusters, with all the kabuli types.

Katiyar, (1978) grouped thirty cultivars into 7 clusters on the basis of flowering time, leaf weight, number of pods per plant and seed weight per plant. Maximum diversity was contributed by pod number per plant.

Upadhyaya *et al.*, (2006) assembled a global composite collection of 3,000 accessions from entire collection of chickpea germplasm preserved in ICRISAT and ICARDA which included trait donor parent lines, landraces, elite germplasm lines, cultivars and wild *Cicer* species representing a wide spectrum of genetic diversity.

Upadhyaya *et al.*, (2002) developed a core subset of 1956 accessions (10% of the entire collection) from the entire collection at ICRISAT, which contained 1465 desi, 433 kabuli and 58 intermediate types of accessions. The evaluation of the core subset revealed that kabuli accessions in general had broad plant width, matured late, and had low pod number; high seed weight and low yield.

Upadhyaya and Ortiz, (2001) postulated the "mini core" concept (10% of the core collection or 1% of entire collection) representing entire species diversity and mini core accessions have been selected and used as a gateway for germplasm utilization.

# 2.3 Drought related traits

Drought is economically the most important abiotic constraint to crop production in the world (Araus *et al.*, 2002; Boyer, 1982). Chickpea frequently suffers from drought stress towards the end of the growing season in rain-fed conditions. Ninety percent of the world's chickpea is produced in areas relying upon conserved, receding soil moisture. Therefore, crop productivity is largely dependent on efficient utilization of available soil moisture (Kumar and Van Rheenen, 2000). In both Mediterranean and sub-tropical climates, seed filling in chickpea is subjected to terminal drought, which limits seed yield (Turner *et al.*, 2001).

In chickpea, the focus of drought resistance research is on the ability to sustain greater biomass production and crop yield under seasonally increasing water deficit, rather than the physiological aptitude for plant survival under extreme drought shock (Serraj and Sinclair, 2002). This has led to the focus on escape and avoidance strategies such as early maturity (Kumar and Abbo, 2001) and large root systems (Saxena *et al.*, 1995; Singh *et al.*, 1995; Kashiwagi *et al.*, 2006).

#### 2.3.1 Root system in chickpea

Roots have a major role in dehydration avoidance as deep root system is able to obtain moisture from the deeper soil layers even when the upper soil layer becomes dry. Sponchiado *et al.*, (1980) and Pandey *et al.*, (1984) hypothesized that the ability of a plant to change its root distribution in the soil and it is an important mechanism for drought avoidance. Benjamin and Nielsen (2006) reported that greater root surface area to weight ratio in chickpea as compared to field pea and soybean which indicates either a finer root system or roots with lower specific density. Sponchiado *et al.* (1980) reported the ability of common bean to change root distribution to avoid drought stress that varied by cultivar. A large root system leads to a fall in harvest index because there is much less assimilate available for grain growth. Hence a more efficient root system is to be preferred.

Studies in various crops have shown the importance of a deep root system for extracting moisture under terminal drought stress (Ludlow and Muchow, 1990; Saxena and Johansen, 1990; Turner et al., 2001). Field studies in legumes (Saxena and Johansen, 1990; Turner et al., 2001) showed that both dense root systems extracting more of the water in upper soil layers and longer root systems extracting soil moisture from deeper soil layers are important for maintaining yield under terminal drought stress. A higher ratio of deep root weight to shoot weight was also found to maintain higher plant water potentials and have a positive effect on yield under stress (Mambani and Lal, 1983). Ludlow and Muchow (1990) recommended traits that are suited for intermittent stress conditions in modern agriculture and also three top priorities in order to match plant phenology to water supply, osmotic adjustment, and rooting depth. Roots at the deeper soil layer contributed more to root length or surface area than to root weight (Follett et al., 1974). Deep root systems in sorghum demonstrated increased yield under drought conditions (Jordan et al., 1983; Sinclair, 1994). A high ratio of root weight to shoot weight also maintained higher plant water potential and had a positive effect on yield under drought stress conditions (Mambani and Lal, 1983).

Farshadfar *et al.* (2001) observed highly significant differences among 21 chickpea lines for stress tolerance index (STI), stress susceptibility index (SSI), tolerance index

(TOI) and mean productivity (MP) and correlated between these indices, out of these MP and STI are the most suitable criteria for screening under rainfed environments.

Deshmukh and Kushwa (2002) studied simple traits like relative water content (RWC) and membrane injury index (MII) for screening 20 genotypes for drought tolerance and found that RWC and MII of a genotype measured during early phase provide an indication of its relative MII during reproductive stage and these genotypes can be used to screen large number of populations for drought tolerance.

Krishnamurthy *et al.*, (2003) identified ICC 4958 as a drought avoidant variety with most prolific root system and Kashiwagi *et al.*, (2005) identified ICC 8261 with high root to total plant ratio and deepest root system as most promising by evaluating chickpea mini-core collection (211 accessions) for drought avoidance root traits.

Deshmukh *et al.* (2004) suggested that the genotypes with high DTE, Least DSI and minimum reduction in yield due to stress indicated drought tolerance under field condition.

Kashiwagi *et al.* (2006) found substantial variation in root length density among 12 diverse kabuli and desi chickpea genotypes at different soil moisture levels and reported that the proportion of the roots at the lower depth was also important in water absorption from deeper soil layers.

Kashiwagi *et al.*, (2007) reported that fifteen out of fifty kabuli accessions had more than 50g of 100-seed weight, and Root Length Density (RLD) as large as that of ICC 4958 ( $0.252 \text{ cm cm}^{-3}$ ).

Toker *et al.*, (2007) reported that all 68 accessions were significantly superior to annual wild and cultivated chickpeas including the best drought tolerant chickpea cultivar, ICC 4958.

Kashiwagi *et al.*, (2008) evaluated sixteen diverse chickpea germplasm accessions based on transpiration in chickpea and reported a significant positive correlation between relatively cool canopy area and seed yield under rainfed conditions.

### 2.4 Pod borer resistance related traits

Chickpea is a major pulse crop, rich in protein and is susceptible to a number of insect pests, which attacks on roots, foliage and pods. Gram Pod borer (*Helicoverpa armigera* Hübner) constitutes a worldwide pest of great economic importance on this

crop. It is a highly polyphagus pest, feeding on a wide range of food, oil and fiber crops. This pest is the major constraint in chickpea production causing severe losses upto 100% inspite of several rounds of insecticidal applications (Singh & Yadav, 2006). In chickpea, it feeds on buds, flowers and young pods of the growing crop, the crop often fails to recover and yield is extremely poor. The pest status of this species has increased steadily over the last 50 years due to agro-ecosystem diversification by the introduction of winter host crops such as chickpea (Knights *et al.*, 1980; Passlow, 1986). The noctuid *H. armigera* Hübner and *H. punctigera* Wallengren are among the most damaging pests of field crops (Fitt, 1989; Zalucki *et al.*, 1994). Commercial chickpea crops are important sources of habitat for *Helicoverpa* species (White *et al.*, 1995). Sequeira *et al.*, (2001) reported chickpea attractive to oviposition of *Helicoverpa* moths from 14 days after planting and throughout the growth period. Of all *Helicoverpa* species larvae recorded from the entire samples and crop combinations, 98.3% were found on chickpea.

Direct pollution due to agricultural activities is mainly related to increased use of chemical inputs such as fertilizers and pesticides. But the use of pesticides has lead to the development of pesticide resistant strains in insects, accumulation of pesticide residues in the agricultural commodities, and poisoned food, water, air and soil (Lateef, 1985; Forrester et al., 1993). Moderate levels of resistance in C 235 and L 550 were reported among the eight genotypes evaluated in the laboratory for feeding preference by the fifth instar *H. armigera* larvae (Olla and Saini, 1999). Using three parameters, the number of larvae, number of pods and percentage pod damage, Singh and Yadav, (1999a, b), screened 70 desi chickpea genotypes under normal sown and late sown conditions and reported that the genotypes were more tolerant and as good as common cultivars in late sown conditions. Gumber et al., (2000) reported that the pod borer damage was positively correlated to the total number of pods and pod length by screening 62 chickpea germplasm accessions and six approved cultivars. Bhatt and Patel (2001) evaluated 11 cultivars and reported the cultivars with highest larval population showed significantly higher pod damage. Sharma et al., (2005c) standardized a cage technique to screen chickpeas for resistance to H. armigera and reported that leaf feeding by the larvae and larval weights was lower on ICC 506 than on ICCC 37 at the flowering stage, across growth stages and infestation levels. Sanap and Jamadagni (2005) screened twenty-five promising chickpea genotypes under pesticide-free field conditions at Mahatma Phule Krishi Vidyapeeth, Rahuri, and Maharashtra with resistant check, ICC 506EB and reported the genotypes with fairly good resistance/ tolerance against pod borer. Harminder *et al.*, (2005) reported large pod damage among all the entries; insect infestation was very high in 64 susceptible genotypes. While forty five genotypes were moderately resistant by evaluating among 184 genotypes scored to find donor for pod borer and wilt resistance, together. Singh and Yadav (2006) reported that spreading types were more susceptible to *Helicoverpa* damage than erect types and kabuli types compared to desi types, by evaluating 1600 desi and 1400 kabuli for yield losses arising from pod borer infestation under rainfed conditions. Narayanamma *et al.*, (2007) reported that the genotypes showed antixenosis, antibiosis, and tolerance mechanism of resistance to *H. armigera* by evaluating a set of diverse chickpea genotypes and their F<sub>1</sub> hybrids. Patil *et al.*, (2007) screened screening twenty-five promising chickpea under pesticide-free field conditions with resistant check, ICC 506EB. Sarwar *et al.*, (2009) reported the least sensitive and least productive genotypes by checking the response of 10 chickpea lines to gram pod borer *H. armigera* at the farm conditions.

## 2.5 Quality traits

### 2.5.1 Flavonoids

Flavonoids, a diverse group of low molecular weight secondary metabolites found throughout the plant kingdom, play a key role in a variety of developmental programs, biochemical processes, and environmental responses (Bruce *et al.*, 2000) and are widely distributed group of plant phenolics, with more than 9000 compounds described (Martens and Mithofer, 2005). Accumulation of some flavanoid compounds in plant tissues can be observed as pigmentation of different organs (Winkel-Shirley 2002).

Anthocyanins, isoflavoids (isoflavones, pterocarpans), flavones (in aerial parts), flavondiols and tannins have been detected in chickpea seeds (Harborne, 1994; Bravo, 1998). The flavone 3, 7, 4'-trihydroxyflavanone was named 'garbanzol' after its discovery in chickpea (Kuhnau, 1976).

### 2.5.1.1 Anthocyanins

Anthocyanins are water-soluble plant pigments often responsible for the orange to red (sometimes blue, violet or magenta) colour of flowers, fruits and seed of higher plants. Anthocyanins are the glycosides of anthocyanidins (e.g. pelargonidin, malvidin, cyanidin) and play an important role in pollinator attraction and seed

dispersal. Relatively little work has been done on anthocyanins as a dietary component (Kong *et al.*, 2003), on the health-promoting benefits of anthocyanins outlining their antioxidant, anti-inflammatory, anti-oedema, anti-ulcer and anti-tumour activities. Hence, anthocyanins may play a role in the prevention of coronary heart disease, inflammatory diseases and some cancers.

## 2.5.2 Protein

Chickpea is an important source of protein for millions of people in developing countries. In addition to having high protein content, it is used as a protein rich animal feed and the vegetative biomass is used as fodder. The crude protein content of chickpea seed varies from 17-24% which extremes from 12.4-31.5%, and is commonly 2-3 times higher than cereal grains. Chickpea has been specifically used to treat protein malnutrition and kwashiorkor in children (Krishna Murti, 1975). Factors that cause variation in chickpea seed protein content include genotypes growing environment, field conditions and agronomic practices. These also affect the nutritional quality of protein (Singh *et al.*, 1974; Kumar *et al.*, 1983; Singh *et al.*, 1983).

Chickpea seed also contains an appreciable amount of nonprotein nitrogen (NPN) and total seed nitrogen (Singh and Jambunathan 1981). A large variation in NPN would overestimate the true protein content of the sample and would consequently affect the estimated protein intake in diet.

### 2.6 Molecular diversity

Traditionally, genetic variation is inferred by morphological/phenotypic variation or the growth response of the organism. Classical methods of establishing genetic diversity and /or relatedness among groups of plants relied upon phenotypic (observable) traits. However, these had two disadvantages: First, the quantitative traits are greatly influenced by environmental and genotype x environment interaction, and secondly the levels of polymorphism (allelic variation) that could be looked at are limited. These limitations were significantly overcome by deployment of environment–neutral biochemical makers (Isozymes) and protein electrophoresis and molecular markers that focus directly on the variation controlled by genes or on the genetic material (DNA itself). The higher resolution of molecular markers makes them a valuable tool for finger printing, protection of breeders rights, facilitating appropriate choice of parents for breeding programmes, analyzing quantitative traits, detection of Quantitative Trait Loci (QTL), gene mapping, marker assisted selection, gene transfer, understanding evolutionary pathways and for the assessments of genetic diversity.

The range of molecular markers that can be used on most plant germplasm is quite extensive (Mohan *et al.*, 1997; Gupta and Varshney, 2000). Techniques vary from identifying the polymorphism in the actual DNA sequence to the use of DNA hybridization methods used to identify RFLPs (Restriction Fragment Length Polymorphisms) or the use of PCR based (Polymerase Chain Reaction) technology to find polymorphism using RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) or combination techniques like AFLP (Amplified Fragment Length polymorphism). The different methods differ in their cost, ease of application, type of data generated (whether it provides dominant or co-dominant markers) the degree of polymorphism they reveal, the way they resolve genetic difference, and their utilization for taxonomic studies (Karp *et al.*, 1997).

The applications of different techniques for genetic diversity analysis have been well reviewed (Malyshev and Karte, 1997; Newbury and Ford-Lloyd, 1997: Westman and Kresovich, 1997; Karp et al., 1998). Some applications of diversity analysis using molecular marker tools includes, identifying areas of higher genetic diversity (Hamrich and Godt, 1990), determining collection priorities and sampling strategies (Schoen and Brown, 1991), guiding the designation of in-situ or on-farm conservation strategies (Bonierbale et al., 1997), monitoring genetic erosion (Robert et al., 1991) or vulnerability (Adams and Demeke, 1993), to guide the management of ex-situ collection, maximizing the genetic diversity in core collection, comparing agronomically useful regions of the genomes of different crops (Paterson et al., 1995), monitoring the movement of genetic resources, assisting in taxonomic evolution, enhancing understanding of relationships between crop gene pools (Gepts, 1995), achieving accurate identification of germplasm at the species/ subspecies levels (Wang and Tanksley, 1989; Virk et al., 1995; Martin et al., 1997; Zhu, 1998), and identifying duplicates with in collections particularly in gene banks (Virk et al., 1995).

There are various types of DNA markers available to evaluate DNA polymorphism in sample genomes. Selection of a correct marker system depends upon the type of study to be undertaken and whether that marker system would fulfill at least a few of the mentioned characteristics such as easy availability, highly polymorphic nature, Mendelian inheritance, frequent occurrence in genome, selective neutral behavior, easy and fast assay, high reproducibility, free of epistasis and pleiotropy etc, (Weising *et al.*, 1995). The invention of PCR, which is a very versatile and extremely sensitive technique, uses a thermostable DNA polymerase (Saiki *et al.*, 1988) has changed the total scenario of molecular biology and has also brought about a multitude of new possibilities in molecular marker research.

#### 2.6.1 Microsatellite markers:

SSR markers are considered the markers of choice for plant genetics and breeding applications (Gupta and Varshney, 2000). In case of chickpea, only few hundred SSR markers were available (Table 3). It is also important to note that majority of these markers were developed from targeted SSRs for assaying variation in particular repeat motifs. Hence in order to increase the molecular marker repertoire and to develop genome wide SSR markers, ICRISAT in collaboration with University of Frankfurt, Germany, developed 311 SSR markers from SSR-enriched libraries (Nayak *et al.*, 2010) and 1344 SSR markers from BAC-end sequence mining approaches in collaboration with University of California, Davis, USA (Table 3). As EST sequences from various tissues and developmental stages of chickpea have also been reported (Boominathan *et al.*, 2004; Romo *et al.*, 2004; Buhariwalla *et al.*, 2005; Coram and Pang, 2005; Varshney *et al.*, 2009b, Choudhary *et al.*, 2009b, Choudhary *et al.*, 2009b, Choudhary *et al.*, 2005, Varshney *et al.*, 2009b, Choudhary *et al.*, 2005, SSR markers representing the entire chickpea genome are available.

## 2.6.2 Diversity Array Technology (DArT) markers

DArT are one of the new generation markers. DArT provides high quality markers that can be used for diversity analyses and to construct medium-density genetic linkage maps. The high number of DArT markers generated in a single assay not only provides a precise estimate of genetic relationships among genotypes, but also their even distribution over the genome offers real advantages for a range of molecular breeding and genomics application. DArT was first developed in rice (Jaccoud *et al.,* 2001). Subsequently, it was developed for different crops and used in linkage map construction and diversity analysis. The important plant species for which DArT has been developed include rice (Xie *et al.,* 2006), barley (Wenzel *et al.,* 2004, 2006),

*Arabidopsis* (Witenberg *et al.*, 2005), eucalyptus (Lezar *et al.*, 2004), wheat (Semagn *et al.*, 2006; Akbari *et al.*, 2006), cassava (Xia *et al.*, 2005), sorghum (Mace *et al.*, 2008), in collaboration with DArT Pty Ltd, Australia extended DArT arrays with 15,360 features for chickpea have been developed at ICRISAT (Varshney *et al.*, 2010a).

Marker type	Number of	References
	markers	
Genomic SSR	28	Hüettel et al., 1999
	174	Winter et al., 1999
	10	Sethy et al., 2003
	233	Lichtenzveig et al., 2005
	13	Choudhary et al., 2006
	85	Sethy et al., 2006a, b
	63	Qadir <i>et al.</i> , 2007
	311	Nayak <i>et al.</i> , 2010
	1344	ICRISAT and UC-Davis, USA
EST-derived SSR	60	Choudhary et al., 2009
	77	Varshney et al., 2009b
	106	Buhariwalla et al., 2005
CAPS	12	Rajesh et al., 2008
	5	Varshney et al., 2007
DArT	15,360	DArT Pty Ltd, Australia and ICRISAT
SNP	Ca. 9,000 identified and	ICRISAT, UC-Davis, USA and NCGR,
	768 on Golden Gate assay	USA

Table 3: Genomic resources available for chickpea

\*UC-Davis - University of California, Davis, USA

NCGR - National Center for Genome Resources, New Mexico, USA

ICRISAT - International Crop Research Institute for Semi-Arid Tropics, Hyderabad, India

# 2.6.3 Transcript sequences and SNP markers

Molecular marker technologies, however, are currently undergoing a transition from largely serial technologies based on separating DNA fragments according to their size (SSR, AFLP), to highly parallel, hybridization-based technologies that can simultaneously assay hundreds to tens of thousands of variations especially in genes. This transition has already taken place in several major crop species like rice (Nasu *et al.*, 2009), maize (Yan *et al.*, 2009), soybean (Wu *et al.*, 2010), and common bean (Hyten *et al.*, 2010). In case of chickpea, only few hundred ESTs and some reports on identification of SNPs were available until recently. Recent years have witnessed significant progress in development of comprehensive resource of transcripts by using Sanger sequencing as well as 'next generation sequencing' (NGS) technologies (Varshney *et al.*, 2009c) that are being deployed for understanding genome dynamics as well as development of SNP markers.

Sanger sequencing of a number of cDNA libraries constructed from drought- and salinity-challenged tissues has provided about 20,000 ESTs (expressed sequence tags) in chickpea (Varshney *et al.*, 2009b). Combined analysis of Sanger ESTs together with 454/FLX transcript reads provided 103,215 tentative unique sequences (TUSs) in chickpea. Selected set of SNPs are being used to develop large-scale SNP genotyping platform in chickpea that will augment recently developed GoldenGate assay platforms for 768 SNPs by University of California-Davis, USA, National Centre for Genome Resources (NCGR), USA and ICRISAT.

### 2.6.4 Assessment of Allelic Diversity in Germplasm Collections

Crop breeders are reluctant to select parental lines from thousands of available germplasm lines without knowing their performance especially for quantitative traits which are highly environment sensitive. Selecting a few lines from these vast pools of germplasm is like searching for a needle in a hay stack. Obviously it is more appropriate and attractive to have a small sample of a few hundred germplasm lines, based on critical evaluation, representing the entire diversity of the species. Genomic tools such as molecular markers developed may be useful to select such a representative set of diversity that can be useful in breeding programme (Glaszmann *et al.*, 2010).

# 2.6.5 Genetic diversity studies in Chickpea

Almost all kinds of molecular markers have been used for analysis of genetic diversity in chickpea germplasm. Majority of these studies however employed RAPD and AFLP markers. Although a limited number of genotypes were used for diversity analyses in majority of these studies, the main outcome of these studies was availability of a low level of genetic diversity in cultivated germplasm as compared to wild species. Some of these studies have been mentioned in Table: 4 below.

Some diversity studies have also provided a general consensus about the members of the first crossability group which contains *C. arietinum* along with *C. reticulatum* (Ahmad, 1999; Iruela *et al.*, 2002; Rajesh *et al.*, 2002; Sudupak *et al.*, 2002, 2004; Javedi and Yamaguchi, 2004; Nguyen *et al.*, 2004), suggested to be the annual progenitor of chickpea (Ladizinsky and Adler, 1976) and *C. echinospermum*, suggested to have played a significant role in the evolution of cultivated chickpea (Tayyar and Waines, 1996). The second crossability group contained *C. bijugum*, *C.* 

*judaicum* and *C. pinnatifidum* (Ahmad, 1999; Sudupak *et al.*, 2002, 2004; Sudupak, 2004; Nguyen *et al.*, 2004). The last three species, *C. yamashitae*, *C. chorassanicum* and *C. cuneatum*, were either not included in many studies or were differentially positioned with respect to the cultivated germplasm.

Marker	Material	Outcome	Reference
RAPD			-
75 RAPD	9 annual <i>Cicer</i> species (1 cultivated, 8 wild)	A total of 115 reproducibly scorable RAPD markers were generated, all except 1 polymorphic were utilized to deduce genetic relationships among the annual <i>Cicer</i> species. In addition to, species-diagnostic amplification four distinct clusters were observed.	Ahmad, 1999
7 RAPD primers	43 wild and cultivated accession representing ten species of <i>Cicer</i>	The dendrogram contained two main clusters, one of which comprised accessions of the four perennial species together with the accessions of the three annual species and the other cluster included the remaining three annual species	Sudupak <i>et al.,</i> 2002
42 RAPD primers	19 wild <i>Cicer</i> accessions representing seven annual <i>Cicer</i> spp. ( <i>C. echinospermum, C.</i> <i>reticulatum,</i> <i>C. pinnatifidum, C.</i> <i>judacium,</i> <i>C. cuneatum, C.</i> <i>yamashitae,</i> <i>C. arietinum</i> )	Diversity analysis provided three groups. The Group I included the cultivated species <i>C. arietinum</i> , <i>C.</i> <i>reticulatum</i> and <i>C. echinospermum</i> . Within this group, <i>C. reticulatum</i> accessions were clustered closest to the <i>C. arietinum</i> , <i>C. yamashitae</i> . The Group II was separated from the other clusters. Group III (the annual tertiary group) included <i>C. judaicum</i> , <i>C.</i> <i>pinnatifidum</i> and <i>C. cuneatum</i> .	Talebi <i>et al.,</i> 2009
16 RAPD	30 genotypes	No significant differences were observed between the mean percentage of the presence of RAPD markers between commercial cultivars and landraces.	Ahmad <i>et al.</i> , 2010
ISSR			
15 ISSR markers	6 annual and 7 perennial wild species ( <i>C.</i> <i>acanthophyllum</i> , <i>C.</i> <i>pungens</i> , <i>C. nuristanicum</i> , <i>C.</i> <i>anatolicum</i> , <i>C. microphyllum</i> , <i>C.</i> <i>oxyodon</i> )	The clustering pattern was in agreement with the data based on crossability, seed storage protein, isozyme, allozyme and RAPD marker analysis. 39% molecular variance was observed among annual and perennial groups. The results also suggested the monophyletic origin of	Rajesh <i>et al.</i> , 2003

wild annual chickpea.

#### Table 4: Some genetic diversity studies in chickpea

Marker	Material	Outcome	Reference
10 ISSR primers	12 chickpea genotypes (released cultivars and breeding lines)	In addition to the diversity analysis, one unique band was produced by the GGAGA primer in the BCP-15 genotype. This band may be linked to temperature tolerance phenotype.	Bhagyawant and Srivastava, 2008
AFLP			
AFLP( <i>Eco</i> RI and <i>Mse</i> I) 306 positions	47 accessions representing four perennial and six annual species	AFLP-based grouping of species revealed two clusters, Cluster I, includes three perennial species and <i>C. anatolicum</i> , while Cluster II consists of two subclusters, one including one perennial, along with three annuals from the second crossability group and the other one comprising three annuals from the first crossability group	Sudupak <i>et al.</i> , 2004
214 AFLP marker loci	95 accessions that represented 17 species of <i>Cicer</i>	Three main species groups were identified; Group I included the cultivated species <i>C. arietinum, C.</i> <i>reticulatum</i> and <i>C. echinospermum.</i> Group II consists of <i>C. bijugum, C.</i> <i>judaicum</i> and <i>C. pinnatifidum.</i> While Group III contained all nine perennial species assessed and two annual species	Nguyen <i>et al.</i> , 2004
455AFLP	146 wild annual <i>Cicer</i> accessions (including two accessions of perennial <i>C. anatolicum</i> and six cultivars of chickpea)	Maximum genetic diversity of <i>C.</i> <i>reticulatum, C. echinospermum, C.</i> <i>bijugum</i> and <i>C. pinnatifidum</i> was found in southeastern Turkey, while Palestine was identified as the centre of maximum genetic variation for <i>C.</i> <i>judaicum.</i>	Shan <i>et al.,</i> 2005
8 AFLP primer pairs	28 chickpea accessions from diverse origin	Greatest genetic diversity was found among accessions from Afghanistan, Iran and Lebanon.	Talebi <i>et al.</i> , 2008b
SSR			
12 SSRs	78 genotypes (72 landraces, 4 cultivars, 2 wild species- <i>C. reticulatum</i> and <i>C.</i> <i>echinospermum</i> )	All the 76 accessions of cultivated chickpea could be readily distinguished with these markers. A significant positive correlation between the average number of repeats (size of the locus) and the amount of variation was observed.	Udupa <i>et al.,</i> 1999
90 SSRs	40 accessions (39 annual, 1 perennial)	The degree of conservation of the primer sites varied between species depending on their known phylogenetic relationship to chickpea, ranging from 92.2% in <i>C. reticulatum</i> , chickpea's closest relative and potential ancestor, down to 50% for <i>C. cuneatum</i>	Choumane <i>et</i> <i>al.</i> , 2000

Marker	Material	Outcome	Reference
11 SSRs 74 STMS	29 accessions 10 accessions (9	Efficient marker transferability (97%) of the <i>C. reticulatum</i> STMS markers across other species of the genus was observed as compared to microsatellite markers from the cultivated species. Phylogenetic analysis clearly distinguished all the accessions The high levels of intra-specific	Sethy <i>et al.</i> , 2006a Sethy <i>et al.</i> ,
74 5 1 145	cultivated, 1 wild <i>C. reticulatum</i> )	genetic polymorphism in chickpea were clearly evident from dendrogram analysis. Sequence analysis of these amplicons suggested random point mutations followed by the subsequent expansion by replication slippage.	2006b
48 SSRs	3000 accessions of composite collections	This was the most comprehensive genetic diversity studies in chickpea. In total, 1683 alleles were detected in 2915 accessions, of which, 935 were considered rare, 720 common and 28 most frequent. A number of group- specific alleles were detected: 104 in Kabuli, 297 in desi, and 69 in wild <i>Cicer</i> ; This is an ideal set of germplasm for allele mining, association genetics, mapping and cloning gene(s), and in applied breeding for the development of environments.	Upadhyaya <i>et</i> <i>al.,</i> 2008
10 EST-SSRs	58 accessions	Crossability-group-specific sequence variations were observed among <i>Cicer</i> species that were phylogenetically informative. The neighbor joining dendrogram clearly separated the chickpea cultivars from the wild <i>Cicer</i> and validated the proximity of <i>C. judaicum</i>	Choudhary <i>et</i> <i>al.</i> , 2009
10 SSRs	47 chickpea ( <i>C. arietinum</i> ) accessions including 21 induced mutation lines, 17 hybrid lines, 5 local cultigens, and 4 non-nodulating lines	UPGMA and ME (minimum evolution) trees classified the accessions into 6 groups and all but 6 accessions could be clearly separated. Grouping was mostly the same in the two phylogenetic trees, but the branching order differed greatly. Recent introgression among the parental lines is suggested for this reason.	Khan <i>et al.</i> , 2010
Miscellaneous	•	·	-
12 RAPD, 8 ISSR	75 accessions belonging to 17 species of <i>Cicer</i>	The dendrogram showed the variability between species was related to both growth habit and geographical origin	Iruela <i>et al.</i> , 2002

Marker	Material	Outcome	Reference
17 random genomic and five heterologous probes in 65 probe-enzyme combinations	Five <i>desi</i> and five <i>kabuli</i> type chickpea cultivars	No polymorphism in chickpea varieties was detected with four RAPD markers studied. However, some degree of polymorphism between <i>C. arietinum</i> and its wild relative <i>C. reticulatum</i> was detected.	Udupa <i>et al.</i> , 2003
Microsatellite derived-RFLP	30 accessions	Greatest genetic diversity was observed in Pakistan, Iraq, Afghanistan, south-east Russia, Turkey and Lebanon. Lower genetic diversity was found in Iran, India, Syria, Jordan and Palestine	Serret <i>et al.</i> , 2006
60 RAPD and 10 ISSR primers	19 chickpea cultivars and five accessions of its wild progenitor <i>C. reticulatum</i> Ladizinsky	The ISSR analysis clearly indicated that only six polymorphic markers are reliable for estimation of genetic diversity, while nearly 30 RAPD primers are required for the same.	Rao <i>et al.</i> , 2007
33 RAPD and 9 morphological traits	36 genotypes	Correlation between the genetic distances was obtained with RAPD and morphological traits, indicating that there is a strong multi-locus association between molecular and morphological traits in these cultivars.	Talebi <i>et al.,</i> 2008a
15 AFLP and 18 STMS primer pairs	21 cultivars of <i>C</i> . <i>arietinum</i>	The genetic similarity between cultivars varied from 0.30 to 0.85 for AFLP and 0.22 to 0.83 for STMS markers. Association of varietal type and flower colour was observed as cultivars E 100Ymu and Nabin (both Desi type and pink flower) clustered together in the dendrogram.	Singh et al., 2008

# 2.7 Population structure and Association mapping

Chickpea is a cool season grain legume with high nutritive value. It belongs to the family Fabaceae and is a self-pollinated diploid (2n=2x=16) with a relatively small genome of 750 Mbp (Arumuganathan and Earle, 1991). One of the major goals of plant breeders is to develop genotypes with high yield potential and the ability to maintain the yield across environments. With the development of molecular markers, breeders have a complimentary tool to traditional selection and markers linked to variation in a trait of interest which could be used to assist the breeding programs. Availability of DNA marker based maps for the genomes of many crops facilitated mapping of QTLs of interest and marker-assisted selection (Winter and Kahl, 1995). QTL mapping analysis has provided an effective approach for locating and subsequently manipulating the QTLs associated with different quantitative traits in plants (Rachid *et al.*, 2004). However, a DNA marker map of sufficient density for use in QTL mapping of important traits is still lacking in chickpea but however,

Nayak *et al.*, (2010) developed a first SSR based high density intra specific genetic map (ICC 4958 x ICC 1882) with 255 marker loci.

Linkage analysis and association mapping are the two most commonly used tools for dissecting complex traits (Zhu et al., 2008). Linkage analysis in plants typically localizes QTLs in 10 to 20 cM intervals because of the limited number of recombination events that occur during the construction of mapping populations and evaluating a large number of lines (Doerge, 2002; Holland, 2007). Alternatively, association mapping has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level (Nordborg and Tavare, 2002; Risch and Merikangas, 1996). Choice of population for association mapping and appropriate marker density are crucial decisions for accuracy of association mapping. Different methods and software tools have been developed to correct the results for population structure usually by dividing the germplasm collections into subgroups or adjusting the probability of the null hypothesis (Rafalski, 2010). Presence of population structure within an association mapping population can be an obstacle to the application of association mapping as it often generates spurious genotype-phenotype associations (Yu and Buckler, 2006; Zhu et al., 2008). To account for population structure in association analysis, two major statistical methods, genome control (Devlin and Roeder, 1999; Zheng et al., 2005) and structure association (SA) (Pritchard et al., 2000a, b) were applied in early studies, both of which used random markers spaced throughout the genome, but incorporated them into statistical analysis in different approaches (Yang et al., 2010). Yu and Buckler, 2006 developed a general linear model (GLM) and a mixed linear model (MLM) approaches to perform association analysis. The MLM approach, accounting for both population structure (Q) and relative kinship (K), can be performed with the TASSEL software package (Bradbury et al. 2007), which is most common method of association analysis in plants and has been successfully applied in rice (Agrama et al., 2007; Wen et al., 2009; Borba et al., 2010), wheat (Breseghello and Sorrells, 2006; Neumann et al., 2011), sorghum (Murrary et al., 2009), Arabidopsis (Zhao et al., 2007) and potato (Malosetti et al., 2007). However, until now, the reports of QTLs for chickpea are limited except the QTLs governing grain yield and other agronomic traits would increase our understanding of the genetic control of the characters and to use them effectively in breeding programs. Some of the agronomic and yield influencing traits like doubleflower (Yadav et al., 1978; Rao et al., 1980; Pawar and Patil, 1983; Singh and van Rheenen, 1994; Kumar et al., 2000), flowering time (Or et al., 1999), chilling tolerance during flowering (Clarke and Siddique, 2003), flowers per axis (Srinivasan et al., 2006), double-podding and other morphological characters (Rubio et al., 1999, 2004; Cho et al., 2002; Rajesh et al., 2002; Lichtenzveig et al., 2006) and nutritional traits like  $\beta$ -carotene and lutein content (Abbo *et al.*, 2005) have been extensively studied in chickpea. A QTL flanked by marker TAA170 and TR55 on LG4A identified for root length (Chandra et al., 2003). Or et al. (1999) suggested a major photoperiod response gene (Ppd) affecting time to flowering. Cho et al. (2002) identified a single QTL for days to 50% flowering on LG3 with a LOD score of 3.03. Lichtenzveig et al. (2006) identified two QTLs on LG1 and LG2 linked to time to first flower. Cho et al. (2002) also identified a QTL for seed weight on LG4 accounting for 52% of the total phenotypic variation. Nayak et al. (2010) reported a total of 8 QTLs for root traits with phenotypic variation 4-54%. These reports generated information on QTLs for important traits which can be used for stress breeding in chickpea. Until now, association mapping using the existing natural variation present in the germplasm for the detection of QTL was not been reported in chickpea and QTL reported by the earlier studies and linkage mapping based on mapping population using the RFLP probes were used to identify QTL. Hence, there is a need for the identification and development of more SSR markers and QTLs in chickpea for various agronomic traits which contribute to yield and its improvement.

Material and Methods

## **3. MATERIALS AND METHODS**

A large number of chickpea germplasm accessions (more than 98,000) are conserved in several genebanks in the world (Gowda et al., 2011). ICRISAT maintains the largest collection of 20,267 accessions of 60 countries. Geographic distribution of chickpea germplasm at ICRISAT are given in Table 5. The germplasm at ICRISAT includes 18,392 land races, 98 advanced cultivars, 1293 breeding lines and 288 accessions of wild species. Inspite of vast germplasm accessions available in different genebanks, there has been very limited use of these accessions in crop improvement programs (Upadhyaya et al., 2006). To enhance use of germplasm in crop improvement a core collection of 1956 accessions (Upadhyaya et al., 2001) was developed representing the variability of the entire collection. However, size of core collection was also not convenient for multilocational replicated evaluation. To achieve this Upadhyaya and Ortiz, (2001) proposed the 'minicore' concept and developed chickpea minicore consisting 211 accessions (1% of entire, 10% of core collection), representing entire species diversity and used as a gateway for germplasm utilization. Upadhyaya et al (2006), developed a global composite collection of 3,000 accessions representing a wide spectrum of genetic diversity captured from entire collection of chickpea germplasm preserved in ICRISAT and ICARDA, beside other important genetic stocks and cultivars. Furthermore, based on the 48 SSR markers allelic diversity data, on global composite collection of chickpea, a 'reference set' of most diverse 300 accessions was selected (Upadhyaya et al., 2008) to facilitate identification of diverse germplasm with beneficial traits for enhancing the genetic potential of chickpea globally and broaden the genetic base of cultivars.

#### **3.1. PHENOTYPIC DIVERSITY**

#### **3.1.1 Genetic materials**

Chickpea reference set (Upadhyaya *et al.*, 2008) of 300 accessions consisting of 194 desi accessions, 88 kabuli accessions, 11 pea or intermediate type and 7 Wild accessions was used for this research (Figure 2). Geographically, the reference set includes accessions from South and East Asia (105 accessions), West Asia (93), Mediterranean region (56), Africa (21), North America (6), the Russian Federation (6), South America (4), Europe (3), and accessions with no information on biological status (6). The country of origin, passport and characterization data of reference set are given in the Table 6. Graphical representation of geographic distribution of

chickpea reference set accessions is represented in the Figure 1 and listed in Table 7.

#### 3.1.2 Evaluation of chickpea reference set for agronomic traits

The reference set was evaluated for agronomic traits in four post-rainy or winter rainfed environments Viz., 2006/2007 (E1), 2007/2008 (E2) and 2008/2009 (E3) at ICRISAT (altitude: 545m above the mean sea level, latitude: 17°27'N, longitude: 78°28' E), Patancheru, Andhra Pradesh (Plate 1); 2008/2009 (E4) at UAS (University of Agricultural Sciences), Dharwad (Plate 2) and one late sown (E5), spring irrigated environment during 2008/09 at ICRISAT; along with 5 control cultivars (Annigeri, G 130, ICCV 10, KAK 2, and L 550) as common for all environments. Agro-climatic details of all five seasons are given in Table 8. Annigeri (ICC 4918) is an early maturing desi cultivar, cultivated in large areas of peninsular India (Ali and Kumar, 2003). ICCV 10 (Bharti) is an early-maturing, semi-erect desi cultivar, resistant to Fusarium wilt and dry root rot (Ali and Kumar, 2003). G 130 is a medium tall, erect and late-maturing desi cultivar suitable for irrigated and adequate rainfall areas of Punjab region of India (Singh, 1987). L 550 is a semi-errect, medium tall, smallseeded bushy kabuli cultivar released for all chickpea growing regions in India. It is tolerant to root knot nematode but susceptible to wilt and blight (Dua et al., 2001). KAK 2 is a semi-errect type, bushy, medium tall, large seeded kabuli cultivar, resistant to Fusarium wilt (Zope et al., 2002).

The experiment was carried out on vertisol (Kasireddypally series- Isohyperthermic Type Pellustert) in a solarized field (Swaify *et al.*, 1985) at ICRISAT farm in high input management of 100 kg ha<sup>-1</sup> diammonia phosphate as basal dose and full protection against weeds, insect pests and diseases. Experiment was planted in an alpha Design in all four normal sown winter (E1, E2, E3 and E4) environments (date of sowing 3<sup>rd</sup> week of October) with two replications and in an augmented design in late sown spring environment (date of sowing 3<sup>rd</sup> week of January). Planting was done in each plot on ridges with a row length of 3m and spacing of 60 cm between rows and 10 cm between plants, at a uniform depth. A post-sowing irrigation was given to support germination in all environments. In normal sown environment, two irrigations of 5 cms each were given at 49 days after sowing (DAS) (pre-flowering), and at 78 DAS (pod filling stage). In late sown spring irrigated environment, four irrigations at 23 DAS (vegetative stage), 40 DAS (flowering stage), 55 and 67 DAS (pod development) were given as per the crop requirement. Five pesticide sprays, two

during the vegetative stage, one at flowering stage and two at the pod-filling stage were given to protect the crop from the pod borer, *Helicoverpa armigera* (Hübner).

#### 3.1.2.1 Observations recorded

Observations were recorded on seven qualitative (Table 9) and 17 quantitative (Table 10) traits following the IBPGR, ICRISAT and ICARDA (1993) descriptors for chickpea. The data on all qualitative traits (growth habit, plant pigmentation, flower color, seed color, seed shape, seed dots and seed texture) were recorded on plot basis. Out of 17 quantitative traits, observations on days to 50 percent flowering, flowering duration, days to grain filling, days to maturity, 100-seed weight, plot yield and per day productivity (kg ha<sup>-1</sup>day<sup>-1</sup>) were recorded on plot basis. The data on remaining 10 quantitative traits *viz.*, plant height, plant width, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, tertiary branches, seeds per pod, pods per plant, yield per plant, were recorded on five randomly selected representative plants in a plot. Average values of these five plants was added to plant yield.

#### 3.1.3 Evaluation of Chickpea reference set for drought tolerance related traits

# **3.1.3.1** Soil Plant Analysis Development (SPAD) Chlorophyll Meter Readings (SCMR) in Chickpea reference set

The chickpea reference set along with five controls cultivars (Annigeri, G 130, ICCV 10, KAK 2, and L 550) was evaluated for SCMR, a trait related to drought tolerance, in a precision vertisol field (fine montmorillonitic isohyperthermic typic pallustert) at ICRISAT, during the 2008/09 post rainy (E3) and 2008/09 spring (E5) seasons. The experiment was carried out in an Alpha Design in 2008/09 post rainy in two replications and in the 2008/09 spring season in an augmented design with repeated control cultivars.

The SCMR measurement were taken at 62 DAS by using SPAD-502 meter (Minolta Konica Co.Ltd., Japan) on the third leaf from top on main branch of the five representative plants, as the third leaf was considered as representative of the plant canopy for SCMR measurement (Kashiwagi *et al.*, 2006). The adaxial side of the leaves was placed towards the emitting window of the chlorophyll meter and major veins of the leaf are avoided.

Specific Leaf area: After SPAD measurement, leaves were detached from the plants

and collected immediately and kept in cool (~ $0^{\circ}$ C) condition, then the number of leaflets of five leaves from five representative plants were counted. The leaflets were seperated from the rachis and then spread on the screen to avoid overlapping. The leaf area of all the leaflets was measured by an automatic 'LI-COR area meter'. Subsequently, these leaflets were oven dried at 70°C for 48 hrs to estimate leaf dry weight with the help of a precision balance (in grams).

## 3.1.3.2 Drought tolerance related root traits-Cylinder culture System

Two hundred ninety three test entries (other than wild species ) along with 6 (ICC 4958, Annigeri, G 130, ICCV 10, KAK 2, and L 550) control cultivars were planted in cylinder culture system under a rain out shelter during the 2007/08 (E2) and 2008/09 (E3) seasons at ICRISAT. ICC 4958, (a desi, drought-resistant, short duration, high yielding (under terminal drought) with 30% more root weight than the standard cultivar Annigeri (Saxena, 1987, Krishnamurthy *et al.*, 2003) was used as a control for root traits related with drought tolerance.

#### 3.1.3.2.1 Cylinder culture System

The chickpea accessions were evaluated in 18 cm diameter, 120 cm tall PVC cylinders (Kashiwagi et al., 2005) under rain out shelter in an alpha design with 3 replications and each plot consists of 38 blocks in both the trials. Each block consists of eight PVC cylinders (rows). Plot size ranged from 1.0m width (4 rows) and 2.0m length (flat seeded bed). Plants were 15 cm apart within rows and 20cm between rows. The cylinders where placed in 1.2m deep cement pits with a spacing of  $0.05 \text{ m}^{-2}$ cylinder<sup>-1</sup> to avoid incidence of direct solar radiation on the cylinders. The cylinders (except the top 15 cm) were filled with an equi-mixture (w/w) of vertisol and sand, mixed with di-ammonium phosphate. The soil water content of the mixture was equilibrated to 70% field capacity to create the conditions similar to those in the field at sowing time, where the soil and sand was used to decrease the soil bulk density and facilitate root growth and extraction. The top of the cylinder was filled with the same dried soil-sand mixture. Four seeds of each genotype were sown in the cylinder. The cylinders were irrigated with 150ml of water three times on alternate days (equivalent water for the top 15 cm soil to reach 100% field capacity) until seedlings uniformly emerged, and then no more irrigations was applied to the cylinders. Immediately after sowing, all cylinders were supplied with a rhizobial inoculum (Mesorhizobium ciceri, strain IC 59) as a water suspension. The plants were thinned to 3 plants per cylinder at 7 days after sowing (DAS). Plants were harvested at 35 DAS in both the seasons.

#### 3.1.3.2.2 Observations recorded

At 35 DAS, the shoots were harvested, and the cylinders were placed horizontally and the sand-soil mixture was removed gently with the help of running water. When approximately three-quarters of the filled soil-sand mixture were washed away, the cylinder was erected gently on a sieve so that the entire root system could be easily slipped down. After washing the root and the soil particles, the roots are stretched to measure their length as an estimate of root depth (RDp). The root system was then sliced into portions of 30 cm (0-30cm, 30-60cm, 60-90cm, 90-120cm), to measure the root length (RL) at each of the 30 cm depth of the root system, using an image analysis system (WinRhizo, Regent Instruments INC., Canada). Root length density (RLD) in each 30cm layer was obtained by dividing root length by volume of a 30cm section of the cylinder. The root dry weight (RDW) and shoot dry weight (SDW) were recorded after drying the roots and shoots in a hot air oven at 80°C for 72 hours. Total plant dry weight (TDW) is sum of root and shoot dry weights. Root to total plant dry weight ratio (RDW/TDW %) was calculated as an indicator for biomass allocation to roots on dry weight basis. In addition, the indicator for the effectiveness of roots in shoot production was calculated by shoot to root length density ratio since root length density is the relevant trait associated with water and nutrition uptake than root dry weight (Krishnamurthy et al., 1996; Kashiwagi et al., 2005).

## 3.1.4 Evaluation of reference set for pod borer resistance

Three hundred diverse reference set accessions along with 7 control cultivars (Annigeri, G 130, KAK 2, ICC 506EB-resistant, ICC 3137-susceptible, ICCV 10-moderately resistant, and L 550-susceptible) were planted in Randomized Complete Block Design (RCBD) during the 2007/08 (E2), 2008/09 (E3) post rainy seasons at ICRISAT.

## 3.1.4.1 Insect Culture

Larvae of *Helicoverpa armigera* used in bioassays were obtained from a laboratory culture maintained at ICRISAT. Larvae were reared on chickpea based artificial diet (Armes *et al.*, 1992) at 27°C. Field collected larvae of *H. armigera* were reared in the laboratory on the natural host for one generation before being introgressed or

transferred into the laboratory culture to avoid contamination with the nuclear polyhedrosis virus, bacteria, or fungi. The *H. armigera* neonates were reared in groups of 200-250 in 200 ml plastic cups (having 2 to 3 mm layer of artificial diet on the bottom and sides) for five days.

#### 3.1.4.2 Detached leaf assay

The unsprayed plants grown in field were bioassayed during vegetative stage under controlled conditions in the laboratory (27  $\pm$ 2°C, 65 to 75% RH and a 12 hour photoperiod) by using detached leaf assay (Sharma *et al.*, 2005). Plastic cups of 4.5 x 11.5 cm diameter were used for detached leaf assay. The 10 ml of agar-agar (3%) was boiled and poured into plastic cups, kept in a slanting manner. The solidified agar-agar served as a substratum for holding a chickpea terminal branch with 3 to 4 fully expanded leaves and a terminal bud in a slanting manner. Care was taken to see that the chickpea branches did not touch the inner walls of the cup. Ten neonate *H. armigera* larvae were released on the chickpea leaves in each cup, and then covered with a lid immediately. This system kept the chickpea terminals in turgid condition for one week. The experiment was terminated when more than 80 percent of the leaf area was consumed in the susceptible control or generally 5 to 6 days after releasing the larvae on the leaves.

#### 3.1.4.3 Observations recorded

Detached leaf bioassay was conducted with unsprayed plants at vegetative stage. The data was recorded on leaf damage score, larval survival and mean larval weight. Leaf feeding by *H. armigera* larvae was evaluated visually by 1 to 9 scale (1 = <10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41to 50%, 6 = 51 to 60% 7 = 61to 70%, 8 = 71 to 80 and 9 = >80% leaf area damaged). The number of larvae survived after the feeding period was recorded, and the larvae were then placed in 25ml plastic cups individually and the weights were recorded, 4 hours after weaving them from the food. The data were expressed as percentage of larval survival and mean weight of the larvae in each treatment (genotype).

# 3.1.5 Evaluation of chickpea reference set for Quality traits3.1.5.1 Estimation of Anthocyanins

The seed samples of 300 accessions of reference set along with 5 control cultivars were evaluated in 2006/2007 (E1), to estimate anthocyanins at ICRISAT, Patancheru.

The method of estimation of both methanol extract anthocyanins and acidified methanol extract anthocyanins is given below.

## **Principle:**

The anthocyanins are determined by ionizing the middle ring of flavonoids by acid, yielding a pink color. The intensity of pink color is directly proportional to the concentration of flavon-4-ols.

Chickpea seed samples were treated with methanol and the phenolic compounds are then adsorbed in polyvinyl pyrrolidone (PVP) layers. The PVP is subsequently cleaned and treated with acid to ionize the flavanoid ring, if any. The results in all cases are expressed as A  $_{550}$  g<sup>-1</sup> on moisture free basis.

## **Reagents:**

- 1. Methanol
- 2. Methanol-HCL 1%: Mix 1 ml conc. HCL in methanol and make up the solution to 100 ml with methanol.
- 3. Butanol
- 4. Hydrochloric acid
- 5. Acetic acid
- 0.1 N acetic acid: Dilute 5.71 ml glacial acetic acid to water and make up the solution to 1 L
- 7. Water-saturated butanol: Take 300 ml butanol in a 500 ml separating funnel and add 150 ml water. Shake vigorously and let it stand for overnight. Remove the top layer and mix in a bottle with HCl in ratio 70:30.
- Mix water saturated butanol, methanol and N/10 acetic acid in ratio 70:15:15.
   Use this reagent for sample blank.

## Procedure

#### Flavon-4-ols: Anthocyanidines

- 1. 200 mg of defatted sample is weighed into screw cap test tube.
- 2. 5 ml methanol is added to the sample.
- 3. The tubes are placed on a Staurt tube rotator (TR-2) and mixed for about 1 h.
- 4. After centrifugation the supernatant is collected in a vial, steps 2 to 4 are repeated using the residue and all the extracts in the above vial are pooled. This is referred to as methanol extract.

- 5. To the residue 5 ml methanol-HCL (Reagent 2) is added and steps 3 and 4 are repeated.
- 6. The residue is re-extracted with additional 5 ml of methanol-HCL again and then pooled, which is further used for the estimation. This is referred to as acidic methanol extract.
- 7. 0.5 ml of sample extract is taken (both methanol and acidic methanol extracts can be analyzed separately) and 7 ml of water-saturated butanol is added.
- 8. Using the mixture of water saturated butanol, methanol and N/10 acetic acid in ratio 70:15:15, a blank is prepared and then the tubes are placed on a test tube rotator for about 1 h.
- 9. The absorbance of the sample and blank at 550 nm is recorded by using Spectrophotometer.

## 3.1.5.1.1 Calculation:

Results are reported as methanol extract anthocyanins and acidified methanol extract anthocyanins,  $A_{550}g^{-1}$  by reading the absorbance of samples at 550 nm using Spectrophotometer.

## 3.1.5.2 Estimation of Protein

The seed samples of 300 accessions of reference set along with 5 control cultivars, evaluated in the post rainy 2006/2007 (E1), 2007/2008 (E2), 2008/2009 (E3) post rainy and 2008/09 (E5) spring seasons were analyzed for protein. Protein content was estimated by the micro Kjeldahl digestion and distillation method for determining nitrogen (N) content, which is multiplied by 6.25 for obtaining the protein percent.

## **Reagents:**

Tri acid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1v/v)

## Procedure

The seed samples were finely ground (< 60 mesh for seed samples) using cyclone mill then oven dried at 60°C for 48 h before analysis.

- 1. Ground and dried seed samples of 0.5 g were transferred to 125 ml conical flasks.
- Twelve ml of tri-acid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1(v/v)) were added to the flasks.
- 3. The flour samples were digested in a room temperature for 3 h followed by digestion for 2 to 3 hours on a hot plate, until the digest was clear or colorless.

- 4. The flasks were allowed to cool and contents were diluted to an appropriate volume.
- 5. The digests were used for estimation of N using Atomic Absorption Spectrophotometry (AAS).

#### 3.1.5.2.1 Calculation:

Protein percent was calculated by multiplying 6.25 to the estimated N .

## **3.1.6 STATISTICAL ANALYSIS**

Data for each environment was analysed separately considering genotypes as random using residual (or restricted) maximum likelihood (REML; Patterson and Thompson, 1971) in GenStat 12 (available at <u>http://www.vsni.co.uk</u>; verified 29 Sept, 2010). Pooled analysis for all environments was performed using REML Meta analysis (DetSimonian and Liard, 1986; Hardy and Thompson, 1996; Whitehead, 2002). Genotype were considered random and season as fixed. Variance components due to genotypes ( $\sigma^2 g$ ), genotype × environment ( $\sigma^2 ge$ ), error component ( $\sigma^2 e$ ), and their standard errors were estimated. Significance of differences among seasons was tested using Wald (1943) statistics. Best linear unbiased predictors (BLUPs) (Schonfeld and Werner, 1986) were determined for all quantitative traits.

The correlation coefficients among all traits were estimated for each environment separately as well as on the basis of combined BLUP values obtained from pooled analysis.

For each character, PCV and GCV were computed from variance components based on the methods given by Burton (1952).

$$PCV = \frac{\sqrt{Phenotypic \text{ var } iance}}{Grandmean} \times 100$$

$$GCV = \frac{\sqrt{Genotypic \text{ var } iance}}{Grandmean} \times 100$$

The broad-sense heritability  $(h_b^2)$  was estimated for each environment separately and for over all the environments. Heritability in the broad sense  $(h^2b)$  was calculated according to Lush (1940).

$$(h_b^2) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

where

 $\sigma^2 p$ =phenotypic variance  $\sigma^2 g$ =genotypic variance.

Stability analysis based on Eberhart and Russell's (1966) model was performed to identify stable genotypes. A phenotypic distance matrix was created by calculating the differences between each pair of entries for each characteristic. The diversity index was calculated by averaging all the differences in the phenotypic values for each trait divided by respective range (Johns *et al.*, 1997). The diversity index (H') of Shannon and Weaver (1949) was calculated and used as a measure of phenotypic diversity of each trait. The index was estimated for each character over all entries in three types. Principal component analysis (PCA) was performed for dimensional reduction and to know the importance of different traits in explaining multivariate polymorphism. Cluster analysis was done following the minimum variance method of Ward (1963) to group together similar genotypes based on principal component (PC) scores. Mean and variances of clusters were tested for significance following the Newman-Keuls procedure (Newman, 1939; Keuls, 1952) and Levene (1960) test, respectively.

## **3.2 MOLECULAR DIVERSITY**

The chickpea reference set was planted in the 3rd week of October 2007 in glass house at ICRISAT and DNA was extracted from a single representative plant in each accession. A set of 100 SSR markers located across eight chromosomes of chickpea were selected based on the chickpea linkage map reported by Winter *et al.*, (2000).

## 3.2.1 Genomic DNA isolation

DNA was extracted from the single seedling of each 300 accessions along with five checks by using a high-throughput mini- DNA extraction method (Mace *et al.*, 2003) as described below:

#### **Reagents required**

- 3% CTAB (Cetyl Trimethyl Ammonium Bromide) buffer having 10mM Tris,
   1.4M NaCl, 20mM EDTA and 3% CTAB. The pH was adjusted to 8.0 using HCl. Just before use, mercaptoethanol (0.17%) was added.
- 2. Chloroform-isoamyl alcohol mixture (24:1) stored in the dark at room temperature
- 3. Ice-cold isopropanol

- RNase-A (10 mg/ml) dissolved in solution containing 10mM Tris (pH 7.5) and 15mM NaCl stored at -20°C; working stocks were stored at 4°C.
- 5. Phenol-chloroform-iso-amyl alcohol mixture (25:24:1)
- 6. 3 M sodium acetate (pH 5.2)
- 7. Ethanol (absolute and 70)
- 8.  $T_1E_{0.1}$  buffer (10mM Tris and 1mM EDTA)
- 9.  $T_{10}E_1$  buffer (0.5M Tris and 0.05M EDTA)

## High-throughput mini- DNA extraction

## (i) Sample preparation

- 1. Steel balls (4-mm in diameter and 3 numbers per extraction tube) (Spex CertiPrep, USA), pre-chilled at  $-20^{\circ}$ C for about 30 minutes, were put into the  $12 \times 8$ -well extraction tubes with strip caps (Marsh Biomarket, USA), which were kept on ice.
- 2. The CTAB buffer was pre-heated in 65°C water bath before start of DNA extraction.
- Leaf samples (Final weight of 20-30mg) were cut into pieces (1mm in length). These cut leaves were transferred to the extraction tubes, which were fitted into a 96-tube box.

## (ii) Grinding and extraction

- 4. A volume of 450μl of pre-heated CTAB buffer was added to each extraction tube containing a leaf sample.
- Leaf tissues were disrupted to release DNA into the buffer solution using a Sigma GenoGrinder<sup>™</sup> (Spex CertiPrep, USA) at 500 strokes/minute for 5 minutes.
- 6. Grinding of leaf tissues was repeated until the color of the buffer solution became pale green and the leaf tissue were sufficiently macerated.
- After grinding, the tube box was fixed in a locking device and incubated at 65°C in a water bath for 20 minutes with occasional shaking.

## (iii) Solvent extraction

8. A volume of  $450\mu$ l of chloroform-isoamyl alcohol mixture (24:1) was added to each tube and the samples were centrifuged at 6200 rpm for 10 minutes (Sigma centrifuge model 4K15C with Qiagen rotor model NR09100: 2 × 1120 g SW).  After centrifugation the aqueous layer (approximately 300 μl) was transferred to a fresh strip tube (Marsh Biomarket).

## (iv) Initial DNA precipitation

- To the tube containing aqueous layer, 0.7 volumes (approximately 210µl) of cold isopropanol (kept at -20°C) was added. The solutions were carefully mixed and the tubes were kept at -20°C for 10 minutes.
- 11. The samples were centrifuged at 6200rpm for 15 minutes.
- 12. The supernatant was decanted under a fume-hood and pellets were allowed to air dry (minimum 20 minutes).

## (v) RNase-A treatment

- 13. In order to remove co-isolated RNA, 200 $\mu$ l of low salt TE buffer (T<sub>1</sub>E<sub>0.1</sub>) and 3 $\mu$ l of RNase-A (stock 10mg/ $\mu$ l) were added to each tube containing dry pellet and mixed properly.
- 14. The solution was incubated at 37°C for 30 minutes.

## (vi) Solvent extraction

- After incubation, 200µl of phenol-chloroform-isoamyl alcohol mixture (25:24:1) was added to each tube, carefully mixed and centrifuged at 5000 rpm for 10 minutes.
- 16. The aqueous layer was transferred to fresh tubes and chloroformisoamylalcohol (24:1) mixture was added to each tube, carefully mixed and centrifuged at 5000rpm for 10 minutes. The aqueous layer was transferred to fresh tubes.

## (vii) DNA precipitation

- 17. To the tubes containing aqueous layer, 15µl (approximately 1/10<sup>th</sup> volume) of 3M sodium acetate (pH 5.2) and 300µl (2 volume) of absolute ethanol (kept at -20°C) were added and the tubes were subsequently placed in a freezer (-20°C) for 5 minutes.
- Following incubation, the box containing tubes was centrifuged at 6200 rpm for 15 minutes.

## (viii) Ethanol wash

19. After centrifugation, supernatant was carefully decanted from each tube having ensured that the pellets remained inside the tubes and 200µl of 70 per

cent ethanol was added to the tubes followed by centrifugation at 5000 rpm for 5 minutes.

#### (ix) Final re-suspension

- 20. Pellets were obtained by carefully decanting the supernatant from each tube and then allowed to air dry for one hour.
- 21. Completely dried pellets were re-suspended in  $100\mu$ l of  $T_{10}E_1$  buffer and incubated overnight at room temperature to allow the pellets to dissolve completely.
- 22. Dissolved DNA samples were stored in 4°C.

## 3.2.2 DNA quantification and quality check

The quality and quantity of DNA were checked by agarose gel electrophoresis as described below

#### **Reagents required were:**

- 1. Agarose
- 2. 1X TBE buffer

For 10X TBE buffer, 109g of Tris and 55g of boric acid were dissolved one by one in 800 ml distilled water; then 40ml of 0.5M EDTA (pH 8.0) was added. The volume was made up to 1 liter with distilled water and sterilized by autoclaving. This was stored at 4°C. To prepare working solution (1X), the stock solution was diluted 10 times

3. Ethidium bromide (10 mg/ml)

A quantity of 100 mg ethidium bromide was dissolved in 10 ml of distilled water. The vessel containing this solution was wrapped in aluminium foil and stored at 4°C

4. Orange loading dye

0.5 M EDTA (pH 8.0)	10ml
5 M NaCl	1ml
Glycerol	50ml
Distilled water	39ml

Orange dye powder (Orange G, Gurr Certistain<sup>®</sup>) was added till the color became sufficiently dark

#### Procedure

A quantity of 0.8g of agarose was added to 100ml of 1X TBE buffer and the slurry was heated using microwave oven until the agarose was completely dissolved. After cooling the solution to about 60°C, 5µl of ethidium bromide solution was added and the resulting mixture was poured into the gel-casting tray for solidification. Before the gel solidified, an acrylic comb of desired well number was placed on the agarose solution to form wells for loading samples. Each well was loaded with 5µl of sample aliquot having 3µl distilled water, 1µl Orange dye and 1µl of DNA sample. The DNA samples of known concentration (lambda DNA of 50ng/µl, 100ng/µl and 200ng/µl) were also loaded on to the gel to estimate the DNA concentration of the experimental samples. The gel was run at 70V for 20 minutes. After completing the electrophoresis run, DNA on the gel was visualized under UV light and photographed. If the DNA was observed as a clear and intact band, the quality was considered good, whereas a smear of DNA indicated poor quality. The band intensity was compared with lambda DNA to know the approximate quantity of DNA.

#### 3.2.3 Optimization of SSR primers

One hundred and twenty SSR markers (Winter *et al*, 1999, Huettel *et al*, 1999) were initially optimised on two diverse accessions, Annigeri (ICC 4918), an early maturing desi (Ali and Kumar, 2003), and ICCV 2 (Sweta), early maturing, small seeded kabuli cultivar (Kumar *et al.*, 1985) by using modified Taguchi method (Cobb and Clarkson, 1994). One hundred SSR primer pairs which produced polymorphic alleles among two diverse accessions were chosen to genotype the entire reference set.

#### 3.2.4 SSR genotyping

91 SSR markers out of one hundred polymorphic markers were selected for genotyping 305 (300 reference set accessions along with 5 check cultivars) accessions, which were mapped on 8 chromosomes of chickpea (Winter *et al*, 2000) based on high polymorphism and amplification rate.

#### **3.2.5 Amplification of SSR markers**

PCR reactions were conducted in 96-well and 384-well micro-titer plates in a GeneAmp PCR system 9700 (Applied Biosystems, USA) thermocycler. Each PCR reaction was performed in 5  $\mu$ l volume in 384-well PCR plates.

Component Stock Concentration Volume

DNA	5 ng/µl		1.0 µl
Primers	10 pm/ µl		0.5 µl
MgCl <sub>2</sub>	25 mM		1.0 µl
dNTPs	2 mM		0.25 µl
Buffer	10X		0.5 µl
Enzyme	0.3 U/µl		0.2 µl
(AmpliTaq Gold <sup>®</sup> , A	pplied Biosystems, US	A)	
Water			1.55 µl
		Total	5.0 µl

PCR reactions were carried out in GeneAmp<sup>®</sup>, PCR System 9700 thermal cycler (Applied Biosystems, USA) with a touchdown (65-60) program using the following cyclic conditions:

Step 1:	Denaturation at 94°C for 15 min
Step 2:	Denaturation at 94°C for 15 sec
Step 3:	Annealing at 60 °C for 20 sec
	(1 °C decrease in temperature per cycle)
Step 4:	Extension at 72 °C for 30 sec
Step 5:	Go to Step 2 for 10 times
Step 6:	Denaturation at 94°C for 10 sec
Step 7:	Annealing at 54 °C for 20 sec
Step 8:	Extension at 72 °C for 30 sec
Step 9:	Go to Step 6 for 40 times
Step 10:	Extension at 72 °C for 20 min.
Step 11:	Store at 4 °C

The amplified products were tested on 1.2% agarose gel(Plate 1).

## 3.2.6 Capillary electrophoresis

## i. Sample preparation

After confirming the PCR amplification on 1.2 per cent agarose gel, the PCR products were size-separated by capillary electrophoresis using an ABI Prism 3730 DNA analyzer (Applied Biosystems Inc.). A set of 30 PCR multiplex sets were constructed based on the estimated allele size and the type of forward primer label of the markers. Each set consisted of four SSR markers with different labels and allele size. For post

PCR multiplexing, 1µl PCR product of each of 6-FAM, VIC, NED and PET-labeled products were pooled (according to above mentioned criteria) and mixed with 7 µl of Hi-Di formamide (Applied Biosystems, USA), 0.2 µl of the LIZ-500 size standard (Applied Biosystems, USA) and 2.8 µl of distilled water. The pooled PCR amplicons were denatured 5 minutes at 95°C and cooled immediately on ice.

## ii. SSR fragment analysis

Raw data produced from ABI 3730*xl* Genetic Analyser was analysed using GeneScan 3.1 software (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan  $500^{TM}$  LIZ<sup>®</sup>. GeneScan<sup>®</sup> analysis software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped onto the curve and the sample data is converted from migration times to fragment size. Genotyper 3.7 (Applied Biosystems) was used for allele calling. The peaks were displayed with base pair and height (amplitude) values in a chromatogram and the allelic data were exported in to Excel spread sheet for further analysis (Plate 2).

#### 3.2.7 Molecular data analysis

The fragment sizes for all markers were used for analysis using PowerMarker version 3.25 (Liu and Muse, 2005) (<u>http://www.powermarker.net</u>), including the polymorphic information content (PIC), allelic richness as determined by total number of the detected alleles and number of alleles per locus, gene diversity and occurrence of unique, rare, common, and most frequent alleles, and heterozygosity (%).

#### **Polymorphic Information Content (PIC)**

The polymorphic information content (PIC) was estimated as below (Botstein *et al.* 1980).

$$\widehat{PIC}_{l} = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}^{2} - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}^{2} \tilde{p}_{lv}^{2}$$

#### Gene diversity

Gene diversity often referred to as expected heterozygosity, is defined as the probability that two randomly chosen alleles from the population are different. An unbiased estimator of gene diversity at the  $l^{\text{th}}$  locus is

$$\hat{D}_{l} = (1 - \sum_{u=1}^{k} \tilde{p}_{lu}^{2}) / (1 - \frac{1+f}{n}),$$

#### Heterozygosity

Heterozygosity is the proportion of heterozygous individuals in the population. At a single locus it is estimated as

$$\hat{H}_l = 1 - \sum_{u=1}^k \tilde{P}_{huu}$$

#### Allele and genotype frequencies

The sample allele frequencies are calculated as  $\tilde{p}_u = n_u / (2n)$ , with the variance estimated as

$$\operatorname{var}(\tilde{p}_u) \triangleq \frac{1}{2n} (\tilde{p}_u + \tilde{P}_{uu} - 2\tilde{p}_u^2)$$

Where

where  $\triangleq$  means "estimated by".

The sample genotype frequencies  $\tilde{P}_{uv}$  are calculated as  $n_{uv}/n$ . Both the  $\tilde{p}_u$ s and  $\tilde{P}_{uv}$ s are unbiased maximum likelihood estimates (MLEs) of the population frequencies. Confidence intervals for allele and genotype frequencies are formed by resampling individuals from the data set.

## Unique, rare and common alleles

Unique alleles are those that are present in one accession or in one group of accessions but absent in other accessions or group of accessions. Rare alleles are those whose frequency is  $\leq 1$  per cent in the investigated materials. Common alleles are those occurring between 1-20 per cent in the investigated materials while those occurring >20 per cent was classified as most frequent alleles.

#### Dissimilarity matrix and construction of dendrogram

Genetic dissimilarities among chickpea accessions present in reference set were calculated and dendrogram was constructed using un-weighted pair group method with arithmetic mean (UPGMA) as implemented in DARwin 5.0.156 programme (http://darwin.cirad.fr/darwin).

#### **Principle Coordinate analysis**

The Principal Co-ordinate analysis (PCoA) was carried out with dissimilarity matrix using DARwin5 version 5.0.156 programme (<u>http://darwin.cirad.fr/darwin</u>).

#### 3.2.8 Population structure analysis

In order to infer the population structure of the reference set of chickpea without considering the pre-existing classification or geographical information, the analysis were performed using the software package STRUCTURE 2.3.2. The program STRUCTURE implements a model based clustering method for inferring population structure using genotype data consisting of unlinked markers to identify K clusters to which the program then assigns each individual genotype. The method was introduced by Pritchard et al. (2000a) and extended by Falush et al. (2003, 2007). To determine most appropriate K value, burn-in Markov Chain Monte Carlo (MCMC) replication was set to 10,000 and data were collected over 100,000 MCMC replications in each run. Five independent runs were performed setting the number of population (K) from 2 to 20 using a model allowing for no admixture and correlated allele frequencies. The basis of this kind of clustering method is the allocation of individual genotypes to K clusters in such a way that Hardy-Weinberg equilibrium and linkage equilibrium are valid within clusters, whereas these kinds of equilibrium are absent between clusters. The K value was determined by LnP(D) in STRUCTURE output and an ad *hoc* statistic  $\Delta K$  based on the rate of change in LnP(D) between successive K (Evanno et al., 2005). Once K value had been determined, burn-in period of 1, 00,000 and 2, 00,000 replications were used. The clustering matrices (Q) of closely related clusters/ subdivisions using Bayesian approach, is obtained which is used in association mapping.

### 3.2.9 Association mapping

Association of SSR marker genotypes with trait of interest was tested using the general linear model (GLM) based on chosen Q-matrix derived from STRUCTURE suggested by Yu et al., (2006) was implemented using Q-matrix and the kinship-Matrix, which was also calculated considering all mapped markers. The kinship-Matrix is generated by using the software program TASSEL 2.1(http://www.maizegenetics.net/), by converting the distance matrix calculated from TASSEL's Cladogram function to a similarity matrix. This method simultaneously takes into account multiple levels of both gross level population structure (Q) and finer scale relative kinship (K). The statistical model can be described in Henderson's notations (Henderson, 1975) as follows:

$$\mathbf{y} = \mathbf{X}\mathbf{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

Where  $\mathbf{y}$  is the vector of observations;  $\mathbf{\beta}$  is an unknown vector containing fixed effects including genetic marker and population structure ( $\mathbf{Q}$ );  $\mathbf{u}$  is an unknown vector of random additive genetic effects from multiple background QTL for individuals or lines;  $\mathbf{X}$  and  $\mathbf{Z}$  are the know design matrices; and  $\mathbf{e}$  is the unobserved vector of random residuals. Each of the marker allele is fit as a separate class were heterozygotes fits as additional marker class. The resulting marker effect is not decomposed into additive and dominance effects but simply tested for overall significance. The  $\mathbf{u}$  and  $\mathbf{e}$  vectors are assumed to be normally distributed with null mean and variance of

$$\operatorname{Var}\begin{pmatrix}\mathbf{u}\\\mathbf{e}\end{pmatrix} = \begin{pmatrix}\mathbf{G} & \mathbf{0}\\\mathbf{0} & \mathbf{R}\end{pmatrix}$$

where  $\mathbf{G} = \sigma_a^2 \mathbf{K}$  with  $\sigma_a^2$  as the unknown additive genetic variance and K as the kinship matrix.

The population structure matrix (Q) was constructed by running Structure at K=13. The Q-Matrix and kinship-matrix were also calculated using TASSEL considering all mapped markers. The EMMA method (Kang *et al.*, 2008) was chosen and the MLM parameters were left at the default setting from TASSEL. The EM method uses an expectation-maximization algorithm to derive a restricted maximum likelihood (REML) estimate of the variance components. Each trait was represented by its mean of the two replications and five environments were separately analyzed and compared with results obtained from pooled mean of five environments. The SSR markers associated with trait of interest were identified based on P value of marker, which determines whether a QTL is associated with the marker. The R<sup>2</sup> %(marker) indicating the fraction of the total variance explained by the marker. Only significant (P≤0.001) SSR markers alone were selected.

## 3.2.10 Analysis of Molecular Variance

Analysis of Molecular Variace (AMOVA) is to partition molecular variance among the sub populations (STRUCTURE) and clusters (DARwin 5.0.156), using the software **GENALEX** 6.41 (Peakall and Smouse, 2006) (http://www.anu.edu.au/BoZo/GenAIEx/). SSR marker data of the entire population including five checks were subdivided into thirteen subpopulations obtained from software STRUCTURE at K=13 and four clusters identified by UPGMA using DARwin 5.0.156.

S.No	Country	Total	Desi	Kabuli	Pea	Wild	Not Recorded
1	Afghanistan	734	217	366	120	23	8
2	Albania	2	-	1	-	-	1
3	Algeria	40	7	32	-	-	1
4	Armenia	3	-	3	-	-	-
5	Australia	5	3	2	-	-	-
6	Azerbaijain	9	-	9	-	-	-
7	Bangladesh	170	170	-	-	-	-
8	Brazil	1	1	-	-	-	-
9	Bulgaria	19	2	14	3	-	-
10	Chile	179	2	174	1	-	2
11	China	29	3	26	-	-	-
12	Colombia	1	-	1	-	-	-
13	Cyprus	60	24	35	1	-	-
14	Czechoslovakia (Former)	9	-	7	2	-	-
15	Ecuador	1	-	1		-	_
16	Egypt	60	10	28	21	-	1
17	Ethiopia	960	870	43	35	8	4
18	France	16	2	12	1	-	1
19	Georgia	2	-	2	-	-	_
20	Germany	14	11	1	-	-	2
21	Greece	31	12	14	2	-	3
22	Hungary	10	4	5	-	-	1
23	ICARDA	20	-	18	2	-	-
24	India	7972	7276	344	282	23	47
25	Iran	5294	3585	1576	89	-	44
26	Iraq	46	6	36	-	1	3
27	Israel	79	21	36	5	15	2
28	Italy	66	12	50	3	-	1
29	Jordan	72	8	58	-	3	3
30	Kenya	1	-	1	-	-	-
31	Kyrgyzstan	4	-	4	-	-	-
32	Lebanon	41	1	24	-	15	1
33	Libyan Arab Jamahiriya	2	-	2	-	_	-
34	Malawi	81	78	3	-	-	-
35	Mexico	457	316	131	8	-	2
36	Moldova	4	-	2	2	_	-
37	Morocco	304	99	186	14	1	4
38	Myanmar	132	123	9	-	-	-
39	Nepal	84	79	2	2	-	1

 Table 5: Geographic distribution of Chickpea germplasm with different seed types from different countries

S.No	Country	Total	Desi	Kabuli	Pea	Wild	Not Recorded
40	Nigeria	3	3	-	-	-	-
41	Pakistan	721	462	200	13	37	9
42	Palestine	1	-	1	-	-	_
43	Peru	4	-	3	-	-	1
44	Portugal	99	2	96	1	-	-
45	Romania	11	5	4	-	-	2
46	Russia & CISs	153	56	68	27	1	1
47	Spain	197	21	154	2	1	19
48	Sri Lanka	4	4	-	-	-	-
49	Sudan	17	3	9	3	-	2
50	Syria	446	57	335	4	44	6
51	Tajikistan	13	6	5	-	-	2
52	Tanzania	97	95	2	-	-	-
53	Tunisia	72	-	69	-	-	3
54	Turkey	972	171	620	18	113	50
55	Uganda	1	1	-	-	-	-
56	Ukraine	14	4	8	2	-	-
57	Unknown	262	146	71	8	23	14
58	USA	126	36	82	1	-	7
59	Uzbekistan	15	1	8	5	-	1
60	Yugoslavia (Former)	25	5	17	-	-	3
	Total	20267	14020	5010	677	308	252

S.n		Land			
0	Reference	type	Source country	Region	species
1	ICC10018	Desi	India	South & East Asia	Cicer arietinum
2	ICC10341	Pea	Turkey	Mediterranean	Cicer arietinum
3	ICC10393	Desi	India	South & East Asia	Cicer arietinum
4	ICC10399	Desi	India	South & East Asia	Cicer arietinum
5	ICC10466	Kabuli	India	South & East Asia	Cicer arietinum
6	ICC1052	Desi	Pakistan	South & East Asia	Cicer arietinum
7	ICC10673	Desi	Turkey	Mediterranean	Cicer arietinum
8	ICC10685	Desi	Turkey	Mediterranean	Cicer arietinum
9	ICC10755	Kabuli	Turkey	Mediterranean	Cicer arietinum
10	ICC1083	Desi	Iran	West Asia	Cicer arietinum
11	ICC10885	Kabuli	Ethiopia	Africa	Cicer arietinum
12	ICC10939	Desi	India	South & East Asia	Cicer arietinum
13	ICC10945	Desi	India	South & East Asia	Cicer arietinum
14	ICC1098	Desi	Iran	West Asia	Cicer arietinum
15	ICC11121	Desi	India	South & East Asia	Cicer arietinum
16	ICC11198	Desi	India	South & East Asia	Cicer arietinum
17	ICC11279	Desi	Pakistan	South & East Asia	Cicer arietinum
18	ICC11284	Desi	Russian Federation	Russian Federation	Cicer arietinum
19	ICC11303	Kabuli	Chile	South America	Cicer arietinum
20	ICC11378	Desi	India	South & East Asia	Cicer arietinum
21	ICC11498	Desi	India	South & East Asia	Cicer arietinum
22	ICC11584	Desi	India	South & East Asia	Cicer arietinum
23	ICC1161	Desi	Pakistan	South & East Asia	Cicer arietinum
24	ICC11627	Desi	India	South & East Asia	Cicer arietinum
25	ICC1164	Desi	Nigeria	Africa	Cicer arietinum
26	ICC11664	Desi	India	South & East Asia	Cicer arietinum
27	ICC11764	Kabuli	Chile	South America	Cicer arietinum
28	ICC1180	Desi	India	South & East Asia	Cicer arietinum
29	ICC11819	Kabuli	Chile	South America	Cicer arietinum
30	ICC11879	Kabuli	Turkey	Mediterranean	Cicer arietinum
31	ICC11903	Desi	Germany	Europe	Cicer arietinum
32	ICC1194	Desi	India	South & East Asia	Cicer arietinum
33	ICC11944	Desi	Nepal	South & East Asia	Cicer arietinum
34	ICC12028	Desi	Mexico	North America	Cicer arietinum
35	ICC12037	Kabuli	Mexico	North America	Cicer arietinum
36	ICC1205	Desi	India	South & East Asia	Cicer arietinum
37	ICC12155	Desi	Bangladesh	South & East Asia	Cicer arietinum
38	ICC12299	Desi	Nepal	South & East Asia	Cicer arietinum
39	ICC1230	Desi	India	South & East Asia	Cicer arietinum
40	ICC12307	Desi	Myanmar	South & East Asia	Cicer arietinum
41	ICC12321	Desi	Unknown	Unknown	Cicer arietinum
42	ICC12324	Kabuli	Unknown	Unknown	Cicer arietinum
43	ICC12328	Kabuli	Cyprus	Mediterranean	Cicer arietinum
44	ICC12379	Desi	Iran	West Asia	Cicer arietinum
45	ICC12492	Kabuli	ICRISAT	South & East Asia	Cicer arietinum
46	ICC12537	Desi	Ethiopia	Africa	Cicer arietinum
47	ICC12654	Desi	Ethiopia	Africa	Cicer arietinum
48	ICC12726	Desi	Ethiopia	Africa	Cicer arietinum

Table 6: List of 300 accessions present in Chickpea reference set and five control cultivars, with seed type, origin, and region

S.n		Land			
0	Reference	type	Source country	Region	species
49	ICC12824	Desi	Ethiopia	Africa	Cicer arietinum
50	ICC12851	Desi	Ethiopia	Africa	Cicer arietinum
51	ICC12866	Desi	Ethiopia	Africa	Cicer arietinum
52	ICC12916	Desi	India	South & East Asia	Cicer arietinum
53	ICC12928	Desi	India	South & East Asia	Cicer arietinum
54	ICC12947	Desi	India	South & East Asia	Cicer arietinum
55	ICC13077	Kabuli	India	South & East Asia	Cicer arietinum
56	ICC13124	Desi	India	South & East Asia	Cicer arietinum
57	ICC13187	Kabuli	Iran	West Asia	Cicer arietinum
58	ICC13219	Desi	Iran	West Asia	Cicer arietinum
59	ICC13283	Kabuli	Iran	West Asia	Cicer arietinum
60	ICC13357	Kabuli	Iran	West Asia	Cicer arietinum
61	ICC13441	Kabuli	Iran	West Asia	Cicer arietinum
62	ICC13461	Kabuli	Iran	West Asia	Cicer arietinum
63	ICC13523	Kabuli	Iran	West Asia	Cicer arietinum
64	ICC13524	Desi	Iran	West Asia	Cicer arietinum
65	ICC1356	Desi	India	South & East Asia	Cicer arietinum
66	ICC13599	Desi	Iran	West Asia	Cicer arietinum
67	ICC13628	Kabuli	Unknown	Unknown	Cicer arietinum
68	ICC13719	Kabuli	Iran	West Asia	Cicer arietinum
69	ICC13764	Kabuli	Iran	West Asia	Cicer arietinum
70	ICC13816	Kabuli	<b>Russian Federation</b>	<b>Russian Federation</b>	Cicer arietinum
71	ICC13863	Desi	Ethiopia	Africa	Cicer arietinum
72	ICC13892	Desi	Ethiopia	Africa	Cicer arietinum
73	ICC1392	Desi	India	South & East Asia	Cicer arietinum
74	ICC1397	Desi	India	South & East Asia	Cicer arietinum
75	ICC1398	Desi	India	South & East Asia	Cicer arietinum
76	ICC14051	Desi	Ethiopia	Africa	Cicer arietinum
77	ICC14077	Desi	Ethiopia	Africa	Cicer arietinum
78	ICC14098	Desi	Ethiopia	Africa	Cicer arietinum
79	ICC14199	Kabuli	Mexico	North America	Cicer arietinum
80	ICC1422	Desi	India	South & East Asia	Cicer arietinum
81	ICC1431	Desi	India	South & East Asia	Cicer arietinum
82	ICC14402	Desi	ICRISAT	South & East Asia	Cicer arietinum
83	ICC14446	Kabuli	Italy	Mediterranean	Cicer arietinum
84	ICC14595	Desi	India	South & East Asia	Cicer arietinum
85	ICC14669	Desi	India	South & East Asia	Cicer arietinum
86	ICC14778	Desi	India	South & East Asia	Cicer arietinum
87	ICC14799	Desi	India	South & East Asia	Cicer arietinum
88	ICC14815	Desi	India	South & East Asia	Cicer arietinum
89	ICC14831	Desi	India	South & East Asia	Cicer arietinum
90	ICC1510	Desi	India	South & East Asia	Cicer arietinum
91	ICC15248	Desi	Iran	West Asia	Cicer arietinum
92	ICC15264	Kabuli	Iran	West Asia	Cicer arietinum
93	ICC15294	Desi	Iran	West Asia	Cicer arietinum
94	ICC15333	Kabuli	Iran	West Asia	Cicer arietinum
95	ICC15406	Kabuli	Morocco	Mediterranean	Cicer arietinum
96	ICC15435	Kabuli	Morocco	Mediterranean	Cicer arietinum
97	ICC15510	Desi	Morocco	Mediterranean	Cicer arietinum
98	ICC15518	Kabuli	Morocco	Mediterranean	Cicer arietinum

S.n		Land			
0	Reference	type	Source country	Region	species
99	ICC15567	Desi	India	South & East Asia	Cicer arietinum
100	ICC15606	Desi	India	South & East Asia	Cicer arietinum
101	ICC15610	Desi	India	South & East Asia	Cicer arietinum
102	ICC15612	Desi	Tanzania	Africa	Cicer arietinum
103	ICC15614	Desi	Tanzania	Africa	Cicer arietinum
104	ICC15618	Desi	India	South & East Asia	Cicer arietinum
105	ICC15697	Kabuli	Syrian Arab Republic	Mediterranean	Cicer arietinum
106	ICC15762	Desi	Syrian Arab Republic	Mediterranean	Cicer arietinum
107	ICC15785	Desi	Syrian Arab Republic	Mediterranean	Cicer arietinum
108	ICC15802	Kabuli	Syrian Arab Republic	Mediterranean	Cicer arietinum
109	ICC15868	Desi	India	South & East Asia	Cicer arietinum
110	ICC15888	Pea	India	South & East Asia	Cicer arietinum
111	ICC16207	Desi	Myanmar	South & East Asia	Cicer arietinum
112	ICC16261	Desi	Malawi	Africa	Cicer arietinum
113	ICC16269	Desi	Malawi	Africa	Cicer arietinum
114	ICC16374	Desi	Malawi	Africa	Cicer arietinum
115	ICC16487	Desi	Pakistan	South & East Asia	Cicer arietinum
116	ICC16524	Desi	Pakistan	South & East Asia	Cicer arietinum
117	ICC16654	Kabuli	China	South & East Asia	Cicer arietinum
118	ICC16796	Kabuli	Portugal	Europe	Cicer arietinum
119	ICC16903	Desi	India	South & East Asia	Cicer arietinum
120	ICC16915	Desi	India	South & East Asia	Cicer arietinum
121	ICC1710	Desi	India	South & East Asia	Cicer arietinum
122	ICC1715	Desi	India	South & East Asia	Cicer arietinum
123	ICC1882	Desi	India	South & East Asia	Cicer arietinum
124	ICC1915	Desi	India	South & East Asia	Cicer arietinum
125	ICC1923	Desi	India	South & East Asia	Cicer arietinum
126	ICC2065	Desi	India	South & East Asia	Cicer arietinum
127	ICC2072	Desi	India	South & East Asia	Cicer arietinum
128	ICC2210	Desi	Algeria	Mediterranean	Cicer arietinum
129	ICC2242	Desi	India	South & East Asia	Cicer arietinum
130	ICC2263	Desi	Iran	West Asia	Cicer arietinum
131	ICC2277	Kabuli	Iran	West Asia	Cicer arietinum
132	ICC2482	Kabuli	Iran	West Asia	Cicer arietinum
133	ICC2507	Desi	Iran	West Asia	Cicer arietinum
134	ICC2580	Desi	Iran	West Asia	Cicer arietinum
135	ICC2593	Kabuli	Iran	West Asia	Cicer arietinum
136	ICC2629	Desi	Iran	West Asia	Cicer arietinum
137	ICC2679	Desi	Iran	West Asia	Cicer arietinum
138	ICC2720	Desi	Iran	West Asia	Cicer arietinum
139	ICC2737	Desi	Iran	West Asia	Cicer arietinum
140	ICC283	Desi	India	South & East Asia	Cicer arietinum
141	ICC2884	Desi	Iran	West Asia	Cicer arietinum
142	ICC2919	Desi	Iran	West Asia	Cicer arietinum
143	ICC2969	Desi	Iran	West Asia	Cicer arietinum
144	ICC2990	Desi	Iran	West Asia	Cicer arietinum
145	ICC3218	Desi	Iran	West Asia	Cicer arietinum

S.n		Land			
0	Reference	type	Source country	Region	species
146	ICC3230	Desi	Iran	West Asia	Cicer arietinum
147	ICC3239	Desi	Iran	West Asia	Cicer arietinum
148	ICC3325	Desi	Cyprus	Mediterranean	Cicer arietinum
149	ICC3362	Desi	Iran	West Asia	Cicer arietinum
150	ICC3391	Desi	Iran	West Asia	Cicer arietinum
151	ICC3410	Kabuli	Iran	West Asia	Cicer arietinum
152	ICC3421	Kabuli	Israel	Mediterranean	Cicer arietinum
153	ICC3512	Desi	Iran	West Asia	Cicer arietinum
154	ICC3582	Desi	Iran	West Asia	Cicer arietinum
155	ICC3631	Desi	Iran	West Asia	Cicer arietinum
156	ICC3761	Desi	Iran	West Asia	Cicer arietinum
157	ICC3776	Desi	Iran	West Asia	Cicer arietinum
158	ICC3892	Desi	Iran	West Asia	Cicer arietinum
159	ICC3946	Desi	Iran	West Asia	Cicer arietinum
160	ICC4093	Desi	Iran	West Asia	Cicer arietinum
161	ICC4182	Desi	Iran	West Asia	Cicer arietinum
162	ICC4363	Desi	Iran	West Asia	Cicer arietinum
163	ICC440	Desi	India	South & East Asia	Cicer arietinum
164	ICC4418	Desi	Iran	West Asia	Cicer arietinum
165	ICC4463	Desi	Iran	West Asia	Cicer arietinum
166	ICC4495	Desi	Turkey	Mediterranean	Cicer arietinum
167	ICC4533	Desi	India	South & East Asia	Cicer arietinum
168	ICC456	Desi	India	South & East Asia	Cicer arietinum
169	ICC4567	Desi	India	South & East Asia	Cicer arietinum
170	ICC4593	Desi	India	South & East Asia	Cicer arietinum
171	ICC4639	Desi	India	South & East Asia	Cicer arietinum
172	ICC4657	Desi	India	South & East Asia	Cicer arietinum
173	ICC4814	Desi	Iran	West Asia	Cicer arietinum
174	ICC4841	Kabuli	Morocco	Mediterranean	Cicer arietinum
175	ICC4853	Kabuli	Unknown	Unknown	Cicer arietinum
176	ICC4872	Pea	India	South & East Asia	Cicer arietinum
177	ICC4918	Desi	India	South & East Asia	Cicer arietinum
178	ICC4991	Desi	India	South & East Asia	Cicer arietinum
179	ICC506	Desi	India	South & East Asia	Cicer arietinum
180	ICC5135	Desi	India	South & East Asia	Cicer arietinum
181	ICC5221	Desi	India	South & East Asia	Cicer arietinum
182	ICC5337	Kabuli	India	South & East Asia	Cicer arietinum
183	ICC5383	Desi	India	South & East Asia	Cicer arietinum
184	ICC5434	Desi	India	South & East Asia	Cicer arietinum
185	ICC5504	Desi	Mexico	North America	Cicer arietinum
186	ICC5613	Desi	India	South & East Asia	Cicer arietinum
187	ICC5639	Desi	India	South & East Asia	Cicer arietinum
188	ICC5845	Desi	India	South & East Asia	Cicer arietinum
189	ICC5878	Desi	India	South & East Asia	Cicer arietinum
190	ICC5879	Pea	India	South & East Asia	Cicer arietinum
191	ICC6263	Kabuli	Russian Federation	Russian Federation	Cicer arietinum
192	ICC6279	Desi	India	South & East Asia	Cicer arietinum
193	ICC6293	Desi	Italy	Mediterranean	Cicer arietinum
194	ICC6294	Desi	Iran	West Asia	Cicer arietinum
195	ICC6306	Desi	Russian Federation	Russian Federation	Cicer arietinum

S.n		Land			
0	Reference	type	Source country	Region	species
196	ICC637	Desi	India	South & East Asia	Cicer arietinum
197	ICC6537	Desi	Iran	West Asia	Cicer arietinum
198	ICC6571	Desi	Iran	West Asia	Cicer arietinum
199	ICC6579	Desi	Iran	West Asia	Cicer arietinum
200	ICC67	Desi	India	South & East Asia	Cicer arietinum
201	ICC6802	Desi	Iran	West Asia	Cicer arietinum
202	ICC6811	Desi	Iran	West Asia	Cicer arietinum
203	ICC6816	Desi	Iran	West Asia	Cicer arietinum
204	ICC6874	Desi	Iran	West Asia	Cicer arietinum
205	ICC6875	Desi	Iran	West Asia	Cicer arietinum
206	ICC6877	Desi	Iran	West Asia	Cicer arietinum
207	ICC7052	Desi	Iran	West Asia	Cicer arietinum
208	ICC708	Desi	India	South & East Asia	Cicer arietinum
209	ICC7150	Desi	Turkey	Mediterranean	Cicer arietinum
210	ICC7184	Desi	Turkey	Mediterranean	Cicer arietinum
211	ICC7255	Kabuli	India	South & East Asia	Cicer arietinum
212	ICC7272	Kabuli	Algeria	Mediterranean	Cicer arietinum
213	ICC7305	Desi	Afghanistan	West Asia	Cicer arietinum
214	ICC7308	Kabuli	Peru	South America	Cicer arietinum
215	ICC7315	Kabuli	Iran	West Asia	Cicer arietinum
216	ICC7323	Pea	Russian Federation	Russian Federation	Cicer arietinum
217	ICC7326	Desi	Unknown	Unknown	Cicer arietinum
218	ICC7413	Pea	India	South & East Asia	Cicer arietinum
219	ICC7441	Desi	India	South & East Asia	Cicer arietinum
220	ICC7554	Desi	Iran	West Asia	Cicer arietinum
221	ICC7571	Kabuli	Israel	Mediterranean	Cicer arietinum
222	ICC762	Desi	India	South & East Asia	Cicer arietinum
223	ICC7668	Kabuli	Russian Federation	Russian Federation	Cicer arietinum
224	ICC7819	Desi	Iran	West Asia	Cicer arietinum
225	ICC7867	Desi	Iran	West Asia	Cicer arietinum
226	ICC791	Desi	India	South & East Asia	Cicer arietinum
227	ICC8058	Kabuli	Iran	West Asia	Cicer arietinum
			United States of		
228	ICC8151	Kabuli	America	North America	Cicer arietinum
229	ICC8195	Desi	Pakistan	South & East Asia	Cicer arietinum
230	ICC8200	Desi	Iran	West Asia	Cicer arietinum
231	ICC8261	Kabuli	Turkey	Mediterranean	Cicer arietinum
232	ICC8318	Desi	India	South & East Asia	Cicer arietinum
233	ICC8350	Pea	India	South & East Asia	Cicer arietinum
234	ICC8384	Desi	India	South & East Asia	Cicer arietinum
235	ICC8515	Desi	Greece	Mediterranean	Cicer arietinum
236	ICC8521	Desi	Italy	Mediterranean	Cicer arietinum
237	ICC8522	Desi	Italy	Mediterranean	Cicer arietinum
238	ICC8607	Desi	Ethiopia	Africa	Cicer arietinum
239	ICC8621	Desi	Ethiopia	Africa	Cicer arietinum
240	ICC867	Desi	India	South & East Asia	Cicer arietinum
241	ICC8718	Desi	Afghanistan	West Asia	Cicer arietinum
242	ICC8740	Kabuli	Afghanistan	West Asia	Cicer arietinum
243	ICC8752	Kabuli	Afghanistan	West Asia	Cicer arietinum
244	ICC8855	Kabuli	Afghanistan	West Asia	Cicer arietinum
245	ICC8950	Desi	India	South & East Asia	Cicer arietinum

S.n		Land			
0	Reference	type	Source country	Region	species
246	ICC9002	Desi	Iran	West Asia	Cicer arietinum
247	ICC9137	Kabuli	Iran	West Asia	Cicer arietinum
248	ICC9402	Kabuli	Iran	West Asia	Cicer arietinum
249	ICC9418	Kabuli	Iran	West Asia	Cicer arietinum
250	ICC9434	Kabuli	Iran	West Asia	Cicer arietinum
251	ICC95	Desi	India	South & East Asia	Cicer arietinum
252	ICC9586	Desi	India	South & East Asia	Cicer arietinum
253	ICC9590	Desi	Egypt	Mediterranean	Cicer arietinum
254	ICC9636	Desi	Afghanistan	West Asia	Cicer arietinum
255	ICC9643	Desi	Afghanistan	West Asia	Cicer arietinum
256	ICC9702	Desi	Afghanistan	West Asia	Cicer arietinum
257	ICC9712	Desi	Afghanistan	West Asia	Cicer arietinum
258	ICC9755	Desi	Afghanistan	West Asia	Cicer arietinum
259	ICC9848	Pea	Afghanistan	West Asia	Cicer arietinum
260	ICC9862	Pea	Afghanistan	West Asia	Cicer arietinum
261	ICC9872	Kabuli	Afghanistan	West Asia	Cicer arietinum
262	ICC9895	Pea	Afghanistan	West Asia	Cicer arietinum
263	ICC9942	Desi	India	South & East Asia	Cicer arietinum
264	ICCV95311	kabuli	ICRISAT	South & East Asia	Cicer arietinum
			Syrian Arab		
265	IG10309	Kabuli	Republic	Mediterranean	Cicer arietinum
			Syrian Arab		
266	IG10419	Kabuli	Republic	Mediterranean	Cicer arietinum
2.67	1010500	¥7 1 1	Syrian Arab		
267	IG10500	Kabuli	Republic	Mediterranean	Cicer arietinum
268	IG10569	Kabuli	Syrian Arab Republic	Mediterranean	Cicer arietinum
200	1010307	Kabun	Syrian Arab	Wediterraitean	
269	IG10701	Kabuli	Republic	Mediterranean	Cicer arietinum
			Syrian Arab		
270	IG11045	Kabuli	Republic	Mediterranean	Cicer arietinum
271	IG5909	Kabuli	Iraq	West Asia	Cicer arietinum
272	IG5949	kabuli	Unknown	Unknown	Cicer arietinum
273	IG6044	kabuli	Sudan	Africa	Cicer arietinum
274	IG6047	kabuli	Afghanistan	West Asia	Cicer arietinum
275	IG6055	kabuli	Iran	West Asia	Cicer arietinum
276	IG6067	kabuli	Turkey	Mediterranean	Cicer arietinum
277	IG6154	kabuli	Iran	West Asia	Cicer arietinum
278	IG6343	Kabuli	Turkey	Mediterranean	Cicer arietinum
279	IG6905	Kabuli	Morocco	Mediterranean	Cicer arietinum
280	IG69438	Kabuli	Cyprus	Mediterranean	Cicer arietinum
281	IG69761	Kabuli	Uzbekistan	West Asia	Cicer arietinum
					Cicer
282	IG69974	Wild	Turkey	Mediterranean	echinospermum
283	IG70826	Kabuli	Greece	Mediterranean	Cicer arietinum
			United States of		
284	IG7087	kabuli	America	North America	Cicer arietinum
285	IG71005	kabuli	France	Mediterranean	Cicer arietinum
201	IC71055	Vabul!	Syrian Arab	Maditamanasa	Ciaan anistico
286	IG71055	Kabuli	Republic	Mediterranean Mediterranean	Cicer arietinum
287	IG7148	Kabuli	Algeria	Mediterranean	Cicer arietinum
288	IG72070	Kabuli	Turkey	Mediterranean	Cicer arietinum

S.n		Land			
0	Reference	type	Source country	Region	species
289	IG72109	Kabuli	Turkey	Mediterranean	Cicer arietinum
290	IG7296	kabuli	Afghanistan	West Asia	Cicer arietinum
291	IG72970	Wild	Turkey	Mediterranean	Cicer reticulatum
292	IG73064	Wild	Turkey	Mediterranean	Cicer echinospermum
293	IG73074	Wild	Turkey	Mediterranean	Cicer echinospermum
294	IG73082	Wild	Turkey	Mediterranean	Cicer reticulatum
295	IG73083	Wild	Turkey	Mediterranean	Cicer reticulatum
296	IG73086	Wild	Turkey	Mediterranean	Cicer reticulatum
297	IG73305	Kabuli	France	Mediterranean	Cicer arietinum
298	IG73458	Kabuli	Syrian Arab Republic	Mediterranean	Cicer arietinum
299	IG74036	Pea	Moldova, Republic of	Europe	Cicer arietinum
300	IG74052	Kabuli	Italy	Mediterranean	Cicer arietinum
Cont	rol cultivars				
301	Annigeri	Desi	India	South & East Asia	Cicer arietinum
302	G130	Desi	India	South & East Asia	Cicer arietinum
303	ICCV10	Desi	India	South & East Asia	Cicer arietinum
304	KAK2	Kabuli	India	South & East Asia	Cicer arietinum
305	L550	Kabuli	India	South & East Asia	Cicer arietinum

S.No	Country	Total	Desi	kabuli	Pea	Wild
1	Afghanistan	16	7	6	3	-
2	Algeria	3	1	2	-	-
3	Bangladesh	1	1	-	-	-
4	Chile	3	-	3	-	-
5	China	1	-	1	-	-
6	Cyprus	3	1	2	-	-
7	Egypt	1	1	-	-	-
8	Ethiopia	14	13	1	-	-
9	France	2	-	2	-	-
10	Germany	1	1	-	-	-
11	Greece	2	1	1	-	-
12	India	93	82	6	5	-
13	Iran	75	53	22	-	-
14	Iraq	1	-	1	-	-
15	Israel	2	-	2	-	-
16	Italy	5	3	2	-	-
17	Malawi	3	3	-	-	-
18	Mexico	4	2	2	-	-
19	Moldova, Republic of	1	-	-	1	-
20	Morocco	6	1	5	-	-
21	Myanmar	2	2	-	-	-
22	Nepal	2	2	-	-	-
23	Nigeria	1	1	-	-	-
24	Pakistan	6	6	-	-	-
25	Peru	1	-	1	-	-
26	Portugal	1	-	1	-	-
27	Russian Federation	6	2	3	1	-
28	Sudan	1	-	1	-	-
29	Syrian Arab Republic	12	2	10	-	-
30	Tanzania	2	2	-	-	-
31	Turkey	20	5	7	1	7
32	United States of America	2	-	2	-	-
33	Unknown	6	2	4	-	-
34	Uzbekistan	1	-	1	-	-
35	Total	300	194	88	11	7

Table 7: Country of origin and seed type of Chickpea reference set accessions

Table 8: Meteorological details of environments in which chickpea reference set was evaluated<br/>during 2006-2007 ,2007-08 postrainy, 2008-09 postrainy and winter seasons at<br/>ICRISAT, Patancheru, India

Planting Season	No. of Entries	No. of Irrigations	Rain fall (mm)	Ev	apora (mm)		ten	Max npera (°C)		Min t	emper (°C)	ature		. of br shine ł	0
			Total	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Normal sown	300 + 5 checks	3	26.6	1.4	6.7	4.18	24	34	29.9	10.2	21.2	14.2	3.7	10.6	8.65
Late sown	300 + 5 checks	5	33.6	3.4	13.7	8.16	29	42	36.1	11.6	27.3	19.5	5.3	10.9	9.13

Note: Design: Alpha Design, No. of replication=2, spacing (cm) =60x10, plot size (m<sup>2</sup>) =3 and fertilizer applied is 16 N; 46 P<sub>2</sub>O<sub>5</sub> (kg ha-1)

S.No	Plant trait	Criteria	Classes
1	Growth Habit	Angle of primary branches, at mid-	Erect (0-15° from vertical)
		pod filling stage	Semi-erect (16-25°)
			Semi-spreading (26-60°)
			Spreading (61-80°)
			Prostrate (flat on ground)
2	Plant pigmentation	Anthocyanin content in the plant	No-anthocyanin
			Low-anthocyanin
			High-anthocyanin
3	Flower color	Color of standard petals of fully	Blue
		opened flowers	Light blue
			Light pink
			Pink
			Dark pink
			White-pink
			White and striped
4	Seed color	Observed on mature seeds	Black
			Brown
			Light brown
			Dark brown
			Reddish brown
			Greyish brown
			Salmon brown
			Grey
			Brown beige
			Beige
			Yellow
			Light yellow
			Yellow brown
			Orange yellow
			Orange
			Yellow beige
			Ivory white
			Green
			Light green
			Variegated
			Black-brown mosaic
5	Seed dots	Minute black dots on seed coat	Absence
5		Windle black dots on seed coat	Presence
6	Seed shape	Angular, ram's head,	Desi cultivars
0	Seed shape	Owl's head	Kabuli cultivars
		Pea shaped	Intermediate types
7	Tasta taxtura	1	
/	Testa texture	Seed texture	Rough
			Smooth
			Tuberculated

# Table 9: List of qualitative characters studied

Table 10:	List of	quantitative	characters	studied
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S.No	Quantitative trait	Description
1	Days to 50% flowering (Days)	Number of days from sowing to the day on which 50% plants started flowering.
2	Flower duration (Days)	Number of days from date of 50% flowering to the day when 50% of the plants stopped flowering.
3	Days to maturity (Days)	Number of days from date of sowing to the day when 90% of pods matured.
4	Days to grain filling (Days)	Number of days from date of 50% flowering to the day when 90% of pods were matured.
5	Plant height (cm)	Height of the plant from ground level to the top of the plant (cm)
6	Plant width (cm)	It is the average spread of five representative plants of each accession, recorded in centimeter.
7	Basal primary branches (number)	Number of branches produced on the main stem, starting from the base to the middle of the plant.
8	Apical primary branches (number)	The number of branches produced on upper half to of the main stem
9	Basal secondary branches (number)	The number of branches produced from the nodes or buds of basal primary branches
10	Apical secondary branches (number)	The number of branches produced from the nodes or buds of apical primary branches
11	Tertiary branches (number)	The number of branches produced from the buds of secondary branches
12	Pods per plant (number)	Average number of fully formed pods per plant from five representative plants.
13	Seed per pod (number)	Average of all pods on five representative plants
14	Yield per plant (g)	Average grain yield of five representative plants in gms.
15	100 seed weight (g)	Weight of 100 randomly selected well developed seeds after sun drying.
16	Plot yield (kg ha-1)	Total seed weight of all the plants in the plot is expressed as seed yield in kg per hectare.
17	Per day Productivity (kg ha-1)	Yield per day computed by dividing total plot yield with no of days to maturity

Chromo Marker some location **Forward Primer** Name **Repeat Motifs Reverse Primer** ATTTTACTTTACTACTTTTTT AATAAATGGA CaSTMS2 6 (TAT)25 CCTTTC GTGTAAATTTCATGTA (AT)6(GT)42A T(GT)5CT(GT) AATATATGAATTGGTTCAGA AAACAAATAATAGA CaSTMS4 3 10 CATC AAATTATGCTCC TACAAACTTTTAAGTTCATA AACTTCTCGA CaSTMS5 3 (GA)19 ATTAGTAAATTAAGTTG AGTTTGA TCTATCTTCCATTATTTCTTG TAATTTACATTCTGA CaSTMS6 9 (TC)14 TTAAGT CTACTTAATCCA CaSTMS7 5 (GA)12 GAGGATTCGGATTCAGAT AAAATCTTGGA AGTGA TTGA G CTTCTATATACATAGTCCTA CaSTMS9 NR (TC)6A(TC)13 ACCTCATAAAGCTGTTAAAG CCTACAC ATAACAAAAAGATATCTCAT AACAATATACAATAAATAACCA CaSTMS10 3 (AG)32 CGACTA AGT GTATTTGTTACTGCATATAC TATTTACTAGGTAAATCCTATTT CaSTMS12 11 (AT)10 TTAATTA ATTG TATGTTAAAAGAGAAAGAA TTTTATTAGTTGTCGA CaSTMS13 (GAA)9 GAAGTGAT AATGTATATCA 1 CaSTMS20 5 (CAA)7CTTNTCGTCATCATCGTTTTG CACCCTACTTTTTTCCACCAC (CT)9ATCT(CT CTACAGTCTTTTGTTCTTCTA ATATTTTTTAAGA CaSTMS21 TT)2(CT)4 1 GCTT GGCTTTTGGTAG GATGAAGATAAAAGCATAA TTTCTTCTTCTATGA CaSTMS23 3 (GT)12 TTAAGG TACACACACT TACACTACTGCTATTGATAT CaSTMS25 (CT)19 GTGGT GA CAATGCCTTTTTCCTT 15 AAACGA CAGA GA GTGGCGA T GA 6 NR (GA)23 ATTTTTCTCCGGTGTTGCAC TCAAAGA TAATATAAAAGGA (CT)16(CA)11 TGA A GA 13 3 GGGCTCATTTACAGGTTACA GA 20 2 (CT)23 TATGCACCACACCTCGTACC TGA CGGA ATTCGTGA TGTGT ATGAGTATCAAGCCAACCTG GA 22 NR GTCCCAACAATTTCTTACATGC (CT)10 A TCATACTCAACAAATTCATTTCC GA 26 13 (CT)28 GATGCTCAAGACATCTGCCA C CCGTTTATAAAGGA TGTAZGA GA 34 6 (CT)11 CCTTTGCATGTATGTGGCAT GA C GA 137 NR (GA)9AA(GA)5 GGGGGAAGATATGTTGGGTT GA TCCAACGGGA ACAAAGA C GCATTGCGAACAAGTGTTAG TTCCTTGA AGA TGA TGA GA **GAA 39** 13 (GAA)10 AT AATACA GAA 40 1 (CTT)9 TTGACGCAGAGAACTCTCAA ATTGGTGTGA TGGGTGGA TT TGATCGGAGAGAGAGGAGG GAA 43 NR (CTT)10 CGTTGA TCCACTGCGA TAGT CATGATGCAACATCTCACCA **GAA 58** NR TGA TTATGCTGTTTTGGGGGG (GAA)8 (TAA)16TGA(T AAATGGAAGAAGAATAAAA TTCCATTCTTTATTATCCATATCA TA2 4 AA)19 ACGAAAC CTACA ATCATTTCAATTTCCTCAAC TCGTTAACACGTAATTTCAAGTA 5 TA5 (TTA)29 TATGAAT AAGA T  $(TAA)_{44}$ AAAATTTGCACCCACAAAAT TA8 1 ATG CTGA AAATTATGGCAGGGA AAC **TA14** (TAA)22ATGA TGACTTGCTATTTAGGGAAC TGGCTAAAGA CAATTAAAGTT (TAA)4T(A)3TА GAT(AAT)5AT T(A)3TGATAA TAAAT(GAT)4 6 (TAA)5 ATTTTCTTTATCCGCTGCAA TTAAATACTGCCTTCGA TCCGT **TA20** (TAA)30T(A)5 TAAT(A)5(TA AT A)7TGA(TAA) 1 20

 Table 11: Details of chickpea SSR markers used to genotype chickpea reference set, chromosome location, repeat motif, forward and reverse primer sequences

Marker	Chromo some			D. D.	
Name			Reverse Primer		
TA21	7	(TAA)51	GTACCTCGAAGATGTAGCCG ATA	TTTTCCATTTAGA GTAGGA TCTTCTTG	
TA21 TA22	6	(ATT) <sub>40</sub>	TCTCCAACCCTTTAGATTGA	TCGTGTTTACTGA ATGTGGA	
11122	0	(111)40	AGTTTAATTGGCTGGTTCTA	AGGA TGA	
TA25	8	(TAA)45	AGATAAC	TCTTTAATAAATCAGA ATGA	
TA27	2	(TAA)21	GATAAAATCATTATTGGGTG TCCTTT	TTCAAATAATCTTTCATCAGTCA AATG	
		(TAA)37CAA(T	TAATTGATCATACTCTCACT	TGGGA ATGA ATATATTTTTGA	
TA28	7	AA)30	ATCTGCC GGAGAAAATGGTAGTTTAA	AGTAAA AAAAATATGA AGA	
TA53	2	(TTA)57	AGAGTACTAA	CTAACTTTGCATTTA	
TA64		(TAA) <sub>39</sub>	ATATATCGTAACTCATTAAT	AAATTGTTGTCATCAAATGGA	
1710-	3	(1111)39	CATCCGC	АААТА	
TA71	5	(AAT) <sub>32</sub>	CGATTTAACACAAAACACA A	CCTATCCATTGTCATCTCGT	
			GAAAGATTTAAAAGATTTTC	TTAGA AGCATATTGTTGGGA	
TA72	4	(ATT)36	CACGTTA	TAAGA GT	
TA78	7	(TTA) <sub>30</sub>	CGGTAAATAAGTTTCCCTCC	CATCGTGA ATATTGA AGGGT	
TA80		(TTA) <sub>23</sub>	CGAATTTTTACATCCGTAAT	AATCAATCCATTTTGCATTC	
	6		G		
		(AT)3(TTA)30(	TGTTTTGGAGAAGAGTGATT		
TA96	2	AT)3	С	TGTGCATGCAAATTCTTACT	
TA103		(ATT) <sub>31</sub>	TGAAATATCTAATGTTGCAA	TATGGA TCACATCAAAGA	
	2		TTAGGAC	AATAAAAT	
TA106	6	(TAA) <sub>26</sub>	CGGATGGACTCAACTTTATC	TGTCTGCATGTTGA TCTGTT	
		(TTA)15ACTA(			
TA108	3	TTA)3ATACT A(TTA)31	AAACCATTATCGAGTTGGAT ATAAAGA	TTTCTAAGTGTTCTTTTCTTAGA GTGTGA	
			ACACTATAGGTATAGGCATT	TTCTTTATAAATATCAGA CCGGA	
TA110	2	(TTA)22	TAGGCAA	AAGA	
TA113	1	(TAA) <sub>26</sub>	TCTGCAAAAACTATTACGTT AATACCA	TTGTGTGTAATGGA TTGA GTATCTCTT	
TA117	7	(ATT) <sub>52</sub>	GAAAATCCCAAATTTTTCTT CTTCT	AACCTTATTTAAGA ATATGA GA AACACA	
TA120		(TTA) <sub>5</sub> CTA(TT	TTTAGAGACTATTTAGGATT	GTTCCATTTTTCTTTCTTTCTTTA	
	6	A) <sub>23</sub>	GTCGT	Т	
			TTGAAATTGAACTGTAACAG	TAGA TAGGTGA TCACAAGA	
TA125	3	(TAA)33	AACATAAA	AGA GA ATG	
TA130	4	(TAA)19	TCTTTCTTTGCTTCCAATGT	GTAAATCCCACGA GA AATCAA	
TA132		(TAA) <sub>28</sub>	CGAATAACTGAGAAAAAGA	TTCTAAAACTTCCTTCTACCATT	
	4		AATTAG	AG	
TA135	3	(TAA)17	TGGTTGGAAATTGATGTTTT	GTGGTGTGA GCATAATTCAA	
<b>TA140</b>	-	(TAA)5TT(A)3(	TTTTGGCATGTTGTAGTAAT	TGA AATGA AAAAGA AAAGGA	
TA140	7	TAA)18		AAAAGTA	
TA142	3	(TTA)15	TGTTAACATTCCCTAATATC AATAACTT	TTCCACAATGTTGTATGTTTTGT AAG	
			TATTTTAATCCGGTGAATAT	GTGGA	
TA144	8	(TAA)27	TACCTTT	GTCACTATCAACAATCATACAT	
TA159		$(TAA)_{11}(CAA)_3$	GCTTCTATATATTCAAACTG	AGTGGTTTTTGTATATCAGA	
	8	$_{1}(TAA)_{22}$	AGCA	TTTGT	
TA176		(TAA)40(GAA)	ATTTGGCTTAAACCCTCTTC	TTTATGCTTCCTCTTCTTCG	
	6	9			
TA180	7	(TAA) <sub>30</sub>	CATCGTGAATATTGAAGGGT	CGGTAAATAAGTTTCCCTCC	
			TCTTTTTAAATTTCATTATGA	CCTCGGGA GA	
TA196	15	(TAA)19	AAATACAAATTATA	GGTAAATGTAATTTC	
T 1 200		(TTA) <sub>37</sub>	TTTCTCCTCTACTATTATGAT	TTGA GA GGGTTAGA	
TA200	2	1	CACCAG	ACTCATTATGTTT	
		(75.4.4.)			
TA200 TA203	1	(TAA) <sub>43</sub>	ATAAAGGTTTGATCCCCATT	TGTGCATTCAGA TACATGCT	
		(TAA) <sub>43</sub> (TTA)43	ATAAAGGTTTGATCCCCATT ATCAAAGAAAGAAACACTT GTTCA	TGTGCATTCAGA TACATGCT TGGTTGGA TACAAAAGA CTGGA	

Marker Name	Chromo some location	Repeat Motifs	Forward Primer	Reverse Primer
			GG	
TAA59	7	(AAT)38	GCAGGAAAGACTCCAGCAA C	TGGA TTAATCGTTTTGCTCATC
TAA169	NR	(TAA)28	CTCAACTTTTCATCTCTTCCA CTACTC	CTATATTACTTCCAATTTTACCCT TCG
TAA194	3	(TTA)22	AACGGTTATCTATAATTAAT TGTGCAAG	AATCTTGTCAACCGCATTAATAA TTT
TAASH	5	(TAA)40	GGTAGACGCAAAAGAGTGG G	GCCACATTGA CCAGGA ATG
TR 1	6	(TAA)40 (TAA)31	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGA AAGT
TR 2	3	(TTA) <sub>36</sub>	sGGCTTAGAGTTCAAAGAGA GAA	sAACCAAGA TTGGA AGTTGTG
TR 7	6	(TTA)25	GCATTATTCACCATTTGGAT	TGTGA TAATTTTCTAAGTGTTTT
			TCAGTATCACGTGTAATTCG	
TR 19	2	(TAA)27	Т	CATGA ACATCAAGTTCTCCA
TR 20	4	(TAA) <sub>18</sub>	ACCTGCTTGTTTAGCACAAT	CCGCATAGCAATTTATCTTC
TR 24	3	(TTA) <sub>29</sub>	AACAACTTCCTCTTATTTTCC A	CAGTAAAAATCAGCCCAAAC
TR 26	3	(ATA) <sub>15</sub>	TCATCGCAGATGATGTAGAA	TTGA ACCTCAAGTTCTCTGG
TR 29	5	(TAA) <sub>8</sub> TAGTA ATAG(TAA) <sub>32</sub>	GCCCACTGAAAAATAAAAA G	ATTTGA ACCTCAAGTTCTCG
TR31	3	(TAA)20T(A)5( TAA)9	CTTAATCGCACATTTACTCT AAAATCA	ATCCATTAAAACACGGTTACCTA TAAT
TR 40	6	(TAA)44	AAGTGAAATATGTCATCCTT ATTACTAACT	AGGA AACTGTGTTTCGTCTTTTATT
TR 43	1	(TAA)24	AGGACGAAACTATTCAAGG TAAGTAGA	AATTGA GA TGGTATTAAATGGA TAACG
TR 56	3	(TAA)21	TTGATTCTCTCACGTGTAAT TC	ATTTTGA TTACCGTTGTGGT
TR 59	5	(TA)3(TAA)17 T(TAA)4	AAAAGGAACCTCAAGTGAC A	GA AAATGA GGGA GTGA GA TG
TS 5	3	(TTA)35	GTTGAATAGTACTTTCCCAC TTGAGTC	TGA GA CTAAAAATCATATATTCCCCC
TS 24	6	(TAA)3TAC(T AA)48	GTAGAAAGAAAACTGACAT GGTTGAG	GCCTAACCCAATAATACCTTCTT TT
TS 35	5	(TAA)9T(A)3(T AA)13	GGTCAACATGCATAAGTAAT AGCAATA	ACTTTCGCGA TTCAGCTAAAATA
TS 43	5	(ATT)33	AAGTTTGGTCATAACACACA TTCAATA	TAAATTCACAAACTCAATTTATT GGC
TS 45	8	(TAA)8(A)3(TA A)18	TGACACAAAATTGTCTCTTG T	TGTTCTTAACGTAACTAACCTAA
TS 46	7	(TAA)46(CAA) 2(TAA)3	GTTGATATTTTTGTGTGTGC GTAG	TAATTACTTGCAAAAATAAATGG A CAC
TS 53	5	(TTA)65	GATCNTTCCAAAAGTTCATT TNTATAAT	TTAAAGA ACTGA TACATTCCGA TTATTT
TS 54	4	(TAA)3TAG(T AA)32(CAA)6	TACAAGTTAAAAAATGAATA AATATTAATA	GA AATTTAGA GA GTCAAGCTTTAC
TS 62	7	(TAA) <sub>33</sub>	ATTATTTTGCTTATTGGGTTC TT	TGCAAGTATAATTTTGTTTACCC
TS 72	11	(ATT)39	CAAACAATCACTAAAAGTAT TTGCTCT	AAAAATTGA TGGA CAAGTGTTATTATG
TS 83	13	COMPOUND OF(TTA),(TAA	AAAAATCAGAGCCAACCAA AAA	AAGTAGGA GGCTAAATTATGGA AAAGT
10 00	10	/	*	



## 4. RESULTS

The Chickpea reference set developed at ICRISAT was evaluated under field conditions and also molecularly profiled using polymorphic SSR markers. The results of the investigation are reported under following topics.

- 5. Phenotypic diversity in chickpea reference set for qualitative, quantitative, grain quality traits, resistance to pod borer and for traits related to drought tolerance
- 6. Genetic diversity and population structure using SSR markers
- 7. Identification of allelic variation associated with beneficial traits using association mapping in the reference set of chickpea
- 8. Identification of most diverse accessions with beneficial traits for use in mapping and improvement of chickpea

# PHENOTYPIC DIVERSITY BASED ON QUALITATIVE AND QUANTITATIVE TRAITS

# **4.1 QUALITATIVE TRAITS**

# **4.1.1 Frequency distribution of qualitative traits**

The frequency distributions of different phenotypic classes of the 7 qualitative traits revealed a large variation for each trait. The results of each trait are presented below.

## 4.1.1.1 Growth habit

Based on the angle of primary branches to main stem at the mid pod-filling stage accessions were grouped into five types viz, erect (0-15° from vertical), semi-erect (16-25°), semi-spreading (26-60°), spreading (61-80°), and prostrate (>80° flat on ground). A larger number of accessions were semi-erect type (62.3%), followed by semi-spreading (33.4%). Spreading and erect types were in equal frequency (2.0% each) (Table: 12). The prostrate type of growth habit was rarely (0.3%) observed. Most of the desi accessions, were semi-erect (60.8%), semi spreading (38.1%), erect (0.5%) and spreading (0.5%), whereas in kabuli type most of the accessions were semi-erect (72.7%), semi spreading (19.3%), erect (4.5%) and spreading (3.4%). In pea type, semi-erect and semi spreading were in equal frequency (45.5% each) and 9.0% were erect type. Prostrate and spreading types were not observed in pea type. Only semi-spreading (57.1%) and spreading (42.9%) types were observed in wild accessions. Semi-erect was the most predominant growth habit among the accessions

included in the reference set.

Region wise, West Asia region had more number of semi-erect (71 accessions, 76.3%), followed by South and East Asia (47 accessions, 44.8%) and Mediterranean region (35 accessions, 62.5%). Semi-spreading type was more frequent in South and East Asia (57 accessions, 54.3%) and West Asia (21accessions, 22.6%). (Table 12 and Figure 4a).

# 4.1.1.2 Plant pigmentation

Out of the three types of plant colours, *viz.*, no-anthocyanin, low-anthocyanin and high-anthocyanin (IBPGR, ICRISAT & ICARDA 1993), low-anthocyanin was dominant in the entire reference set and desi types, no-anthocyanin was prominent in kabuli types. In chickpea reference set on the whole, 53.3% accessions had low-anthocyanin, 44.7% with no-anthocyanin and only 2% showed high-anthocyanin. Among desi low-anthocyanin was observed in 78.9%, no-anthocyanin in 18.5% and high-anthocyanin in 2.6% accessions. No-anthocyanin is the characteristic feature of kabuli type of chickpea, while no-anthocyanin (81.8%), high and low-anthocyanin (9.1% each) was observed among pea type. Wild accessions showed low and no-anthocyanin type of plant pigmentation. (Table 12, Figure 4 b and Plate 3, 4, 5).

The frequency of low-anthocyanin pigmentation was predominant in accessions from South and East Asia region (90 accessions, 85.7%) and no-anthocyanin pigmentation was predominant in accessions from West Asia (54 accessions, 58.1%) and Mediterranean (40 accessions, 71.4%) regions.

# 4.1.1.3 Flower colour

Pink (57.0%), white (31.7%), light pink (10.0%), very light pink (1.0%), white with pink stripes (0.3%) were different flower colours observed in the reference set. Desi types were classified into pink (83.5%), light pink (12.9%), very light pink (1.5%), white (1.5%) and white with pink stripes (0.5%). In pea type, white (45.4%) colour was predominant followed by light pink (36.4%) and pink (18.2%) and in kabuli types only white (98.9%) and light pink (1.1%) coloured flowers were observed. Only pink colour flowers were observed in wild accessions.

Region wise, South and East Asia were dominated with more number of accessions with pink colour flower (93 accessions, 88.6%) followed by West Asia (39

accessions, 41.9%) whereas Mediterranean region was dominated by accessions with white (34 accessions, 60.7%) flower colour. (Table 12, Figure 4 c and Plate 6).

#### 4.1.1.4 Seed colour

Seventeen seed colours were observed in the reference set. Yellow brown (36.0%) was most predominant followed by beige (30.0%), black (7.7%), brown beige (7.3%), dark brown (4.7%), light brown (3.3%), light yellow (3.0%), yellow (1.7%) and greyish brown and yellow beige (1.0% each). Brown and green (0.7% each), reddish brown, salmon brown, light green, orange, light orange (0.3% each) were also observed. (Table 12, Figure 4 d and Plate 9).

Most desi types had yellow brown colour (55.2%), followed by black (11.9%), brown beige (10.8%), dark brown (5.7%), light brown (5.2%), light yellow (4.1%), yellow (1.7%), yellow beige (1.5%), light orange and green (1% each), and beige and light green (0.5% each), whereas kabuli accessions were characterised by beige coloured (98.9%) seed coat; however a single kabuli accession possessed Salmon brown (1.1%) seed coat in the entire reference set. Pea type is represented with all rare coloured seed coats such as beige and salmon brown (18.2% each), brown, brown beige, light orange, light yellow, orange, reddish brown, and yellow brown (9.1% each). Wild accessions were both greyish brown and dark brown (42.9% each) and brown (14.3%).

Beige seed colour which is characteristic feature of kabuli type dominated in accessions from Mediterranean (34 accessions, 60.7%) region followed by West Asia (30 accessions, 32.3%). Brown beige was the prominent seed colour in accessions from West Asia (17 accessions, 18.3%). Yellow brown (71 accessions, 67.6%) which is characteristic feature of desi type dominated in South and East Asia accessions followed by West Asia (19 accessions, 20.4%) and Africa (12 accessions, 57.1%). All the wild accessions were from Mediterranean region.

Desi type was not represented in accessions from Europe and South America. Pea types were from the Mediterranean, Europe, Russian Federation and South and East Asia regions. Rare seed colors such as, Salmon brown were from West Asia while orange, reddish brown, and light green were from South and East Asia. Green seed colour was represented in accessions from both West and South and East Asia regions.

#### 4.1.1.5 Seed shape

Angular or ram's head seed shape (67.0%), which is the characteristic of desi type, dominated reference set followed by owl's head shape (29.3%) and Intermediate or pea shaped (3.7%). Angular seed shape dominated in the South and East Asian collections (93 accessions, 88.6%), followed by West Asia (60 accessions, 64.5%), whereas Mediterranean region represented maximum number of Owl's head shaped seeds (33 accessions, 58.9%) followed by West Asia (30 accessions, 32.3%). Three West Asian accessions were of pea type. All the wild accessions had angular seed shape. (Table 12, Figure 4 e and Plate 9).

#### 4.1.1.6 Seed dots

Minute black dots were present on the seed testa of most desi (71.6%) accessions while some (28.4%) accessions had no dots on seeds (Table 12 and Figure 4 f) and in kabuli types dots were totally absent, whereas in pea type (90.9%) seeds were with black dots and (1.1%) were without dots. In wild accessions, 57.1% were with dots and the remaining, 42.9% accessions were without minute black dots. Overall in the entire reference set, accessions with minute black dots (52%) were slightly more than the accessions without (48%) black dots.

#### 4.1.1.7 Seed surface

Rough (66.0%), smooth (30.0%) and tuberculated (4.0%) are the three types of seed testa classes recorded in the reference set. Among desi type, most accessions (97.4%) were of rough type and only few (2.6%) were tuberculated while in kabuli type (95.5%) had smooth and (4.5%) had rough seed surface. In pea types, about 55% were smooth and 45% were with rough seed surface. All wild accessions were tuberculated. (Table 12 and Figure 4 g). Among the qualitative traits, highest polymorphism was observed for seed colour followed by seed surface.

#### **4.2 QUANTITATIVE TRAITS**

The data on 17 quantitative traits of individual environment and the pooled were analyzed for the entire set of chickpea reference set separately to estimate variance components due to genotype and genotype x environments interactions, to compare mean and variance, estimate phenotypic diversity, Shannon-Weaver diversity-index and perform principle component analysis. The results of various analyses are presented below.

# Traits variability in different environments

For the purpose of summarization of results and discussion, the traits studied were grouped into three broad categories based on the life cycle of the chickpea plant (Gowda *et al.*, 2011).

**Vegetative traits:** plant height, plant width, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches and tertiary branches;

**Reproductive traits:** days to 50 percent flowering, flowering duration, days to maturity;

**Yield and yield component traits:** pods per plant, seeds per pod, 100-seed weight, grain yield and per day productivity.

# **4.2.1 VARIANCE COMPONENTS**

REML analysis for individual environments indicated that genotypic variances were significant for all traits, except pod per plant (PPP) in E1, basal secondary branches (BSB) and tertiary branches (TB) in E2 and seed per pod (SDPD) and yield per plant (YPP) in E5, indicating the presence of high variability among accessions for all of the traits (Table 13). In pooled analysis, variance due to genotype and genotypes x environment (G x E) interactions were significant for all the traits except TB and PPP, indicating differential response of the genotypes to different environments. Wald's statistics was highly significant for all the traits indicating that all the environments were different and appropriate to differentiate accessions.

# 4.2.2 MEAN AND RANGE

Mean and range are simple and important measures of variability (Singh, 1983). Variability among the accessions for different traits was assessed by comparing the values of means and range for each trait, between environments. Mean and range were calculated for each character in individual environment separately as well as the pooled over five environments. Mean values of each environment were tested using the Newman-Keuls procedure to compare the mean values all five environments. The estimates of mean and range are presented below.

# 4.2.2.1 Vegetative traits

#### 4.2.2.1.1 Plant height (PLHT, cm)

Plant height is the important trait related to seed yield and fodder yield. Wide variation for plant height was observed among the accessions in reference set. The mean plant height was higher in E3 (44.9  $\pm$  1.11 cm), E2 (44.5  $\pm$  2.17 cm), E1 (44.4 $\pm$ 2.39 cm), and E4 (43.5 $\pm$  1.00cm) than E5 (37.7 $\pm$  1.64) (Table 14). However a wide range for plant height was observed among the accessions in all environments; in E1 (21.3-86.4 cm), in E2 (18.3-92.5 cm), E3 (17.7-97.5 cm), E4 (17.6-88.6 cm), E5 (16.8-83.4 cm) and overall pooled (26.3-92.4 cm) (Table 14). The accessions were grouped based on plant height as dwarf or short (<45 cm), medium (45-60 cm) and tall (>60 cm) (http://agricoop.nic.in/SeedTestguide/Chickpea.htm). Using this criterion and based on the mean height over five environments eight accessions were considered as tall, sixty accessions were medium tall while 232 accessions were dwarf. The accessions ICC 19011, ICC 19034, ICC 19164, ICC18724, ICC 8740, ICC 20260, ICC 19100 and ICC 8752 were tall; accessions ICC 20265, ICC 19122, ICC 19147, ICC 18983, ICC 8521 and ICC 8200 were of medium height, whereas accessions ICC 12321, ICC 12379, ICC 13469, ICC 7554, and ICC 12851 were short in all the environments. ICC 5434 (17cm) is the only accession with very short stature in chickpea reference set. The wild accessions were medium in height and attained a height of (29-33 cm) in almost all the environments.

#### 4.2.2.1.2 Plant width (PLWD, cm)

Plant width, the average spread of plant and is an important descriptor for chickpea. The mean plant width was similar (65-66cm) in E1, E2, E3 and E4 environments, but more than E5 ( $50.4 \pm 1.32$ ) (Table 14). A wide range for plant width was observed among the accessions in different environments. It was 34.8-76.6 cm in E4, 11.91-59.3 cm in E5 and 45.2-69.4 cm when pooled while the range was similar for E1, E2 and E3 (50.1-73.7) cm (Table 14). The accessions ICC 8515, ICC7308, ICC8521, ICC13357, and ICC16796 had significantly high plant width (68-70cm) in all the environments.

#### 4.2.2.1.3 Basal primary branches (BPB, number)

The mean number of basal primary branches was high in E2 (3.1±0.2), than E1 and E4 (2.9±0.05) than in E3 (2.8±0.62) and E5 (2.6±0.20) (Table 14) with an overall mean of 2.9 ± 0.10. The range differed in all environments (2.2 -3.7 in E1, 2.2- 4.5 in

E2, 1.2 -4.4 in E3, 1.2-5.0 in E4 and 0.5-3.7 in E5) (Table 14). Nine accessions (ICC 12492, ICC 11284, ICC 12299, ICC 4657, ICC 10018, ICC 11198, ICC 7255, ICC 10466, and ICC 11284) produced consistently high number of BPB (3-4) than the control cultivars Annigeri and G 130 (<3 branches) in four (E1, E2, E3, E4) environments and overall the environments, whereas in E5 the accessions ICC 10018, ICC 1180, ICC 7255, ICC 3239, and ICC 11378 had high (3.4-3.7) BPB than the control cultivar G 130 (< 3.1 branches).

# 4.2.2.1.4 Apical primary branches (APB, number)

The mean number of apical primary branches was higher in E3 ( $2.9 \pm 0.95$ ), compared to other environments (2.4-2.6) (Table 14), with an overall the mean of  $2.6 \pm 0.11$ . The range was wider in E3 (1.1-7.1), than in other four environments (0.1-5.4) (Table 14). Only one accession ICC 9942 had consistently high (5) APB than the highest control cultivar L550 (< 4 branches) in all the environments and overall across five environments

# 4.2.2.1.5 Basal secondary branches (BSB, number)

The mean number of basal secondary branches was similar in all environments (2.9-3.4), with a mean of  $3.2 \pm 0.12$  (Table 14). However, the range was wider in E4 (1.1-8.4) followed by E5 (0.3-6.3), E1 (1.1-6.5), E3 (0.3-5.7), E2 (1.2-6.0) and overall environments (1.3-5.7) (Tabl.e 14). Three accessions (ICC 10755, ICC 7308, and ICC 2067) had high (6) BSB in E1, E2 and ICC 11198 in E4 environments, than the control cultivar ICCV 10 (< 5 branches).

# 4.1.2.2.1.6 Apical secondary branches (ASB, number)

The mean number of apical secondary branches was between 4.1-4.4 in all environments (Table 14), with an overall mean of  $4.4 \pm 0.21$ . The range was wide in E3 (3.1 -14.7), followed by E4 (3.3-13.0), E2 (1.2-11.3), E1 (2.7-10.6) and E5 (0.47-9.7) (Table 14). Two accessions each in E3 (ICC 16524, ICC 867) (14.7-11.3), and in E4 (ICC 867, ICC 4991) (11) and one accession ICC 16524 (11) in E1 and overall environments, had high ASB than the control cultivar L550 (< 8 branches).

# 4.2.2.1.7 Tertiary branches (TB, number)

The mean number of tertiary branches was higher in E2 ( $1.8 \pm 0.95$ ) than in other environments (1.3-1.5) (Table 14), with an overall mean of  $1.5 \pm 0.21$ . The range was

wide in E2 (1.6-6.9), followed by E1 (1.0-4.2), E4 (0.3-5.4), E3 (0.0-3.2), and E5 (0.3-4.2) (Table 14). Two accessions, ICC 5135, ICC 7308, in E1, E2 and overall environments, one accession, ICC 13719 in E4 and E5 produced high (4) number of TB than the control cultivar Annigeri (< 3 branches).

# 4.2.2.2 Reproductive traits

#### 4.2.2.2.1 Days to 50 percent flowering (DF, days)

Days to 50 percent flowering is an important trait for adaptation. Early flowering is a desirable trait in chickpea, particularly in short crop season environment such as in central and southern India. The mean (59.4) days to 50 percent flowering was similar in E1, E2 and E3 environments. However, overall in the five environments, crop took  $57.5 \pm 0.72$  days for 50% flowering (Table 14). The widest range for days to 50 percent flowering was observed in E4 (34.2-94.7), followed by E5 (35.1-86.5), E2 (37.8-91.6), E1 (40.0-85.3) and E3 (39.2-78.9days) (Table 14).

Some accessions were early flowering than the earliest flowering control cultivar in each environment, ICC 8318 (40 days) in E1 (earliest control KAK2,  $42\pm 1.72$  days), ICC8318 and ICC14595 (38 to 39days) in E2 (KAK2,  $43\pm 1.56$  days), ICC 8318, ICC 14595 and ICC 16374 (39 to 42 days) in E3 (KAK2,  $44\pm 1.71$  days), ICC 14595, ICC 8318, ICC 15618, ICC 16374 and ICC 10393 (35 to 39 days) in E5 (KAK2,  $41\pm 2.15$  days) were identified as promising early flowering accessions whereas in E4 none of the accessions flowered earlier than the control cultivars. ICC 8318 and ICC 14595 were early flowering in all environments. These accessions could provide useful genes for earliness in crop improvement programme for early maturity.

# 4.2.2.2.2 Flowering duration (FD, days)

The mean flowering duration was similar (27.5 days) in all environments and in the pooled analysis (Table 14). The widest range for flowering duration was observed in E4 (18.1-36.9) and E2 (18.3-34.1 days) followed by E5 (20.6-34.2 days), E1 (21.1-35.1 days) and E3 (19.7-32.6 days) (Table 14).

The accessions with shortest flowering duration were ICC 8195, ICC 8521, ICC 12028, ICC 2629, ICC 3421, ICC 5331, ICC 11121, ICC 11198 and ICC 6875 (21 to 23 days) in E1 (shortest control L550,  $26 \pm 1.21$  days), ICC 11121, ICC11198 and ICC11819 (18 days) in E2 (L550,  $25 \pm 1.55$  days); ICC 8195, ICC 11121, ICC11198 and ICC11819 (20 to 21 days) in E3 (shortest control Annigeri,  $21 \pm 0.1$  days); ICC

11121, ICC11198, ICC 8195, ICC6875, ICC 18699 (18 to 20 days) in E4 (shortest control Annigeri recorded 21  $\pm$  0.098 days), ICC11819, ICC 11121, ICC11198 and ICC 8195, (21 days) in E5 (Annigeri 22  $\pm$  0.07 days). Accessions ICC 11121, ICC11198, ICC 8195, ICC 11819 (19-21 days) showed shortest flowering duration in all the five environments.

The accessions with largest flowering duration were, ICC 20174, ICC 20193, ICC 7308, ICC 8752, ICC 9643, ICC 18983 and ICC 20183 (34 to 31 days) in E1 (longest control cultivar KAK2,  $35\pm 1.21$  days), ICC 20183, ICC 20190, ICC 20192, ICC 18983, ICC 20174 and ICC 10393 (32 to 30 days) in E2 (longest control cultivar KAK 2,  $34 \pm 1.55$  days); ICC20193, ICC 1923, ICC 20183, ICC 16374 and ICC 20190 (32 to 31 days) in E3 (longest cultivar control G 130,  $31 \pm 0.1$  days); ICC 10935, ICC 20174, ICC 20193, ICC 1923, ICC 20183 and ICC 20190 (37 to 32 days) in E4 (longest control cultivar Annigeri recorded  $31 \pm 0.098$  days), ICC 20174, ICC 20193, ICC 20183, ICC 20174, ICC 20193, ICC 20193, ICC 20174, ICC 20190 and ICC 1923 (33-31 days) showed longest flowering duration in all the five environments.

# 4.2.2.3 Days to grain filling (DGF, days)

Days to grain filling influences crop duration and is an important trait for adaptation. The mean days to grain filling were nearly same (53.9-55.5 days) in all environments and overall the environments. (Table 14). However, a wide range for days to grain filling was observed all the environments, E5 (30.4-68.9 days), E4 (33.5-71.6 days), E2 (39.0-76.6 days) followed by E3 (40.3-69.8 days) and E1 (43.3- 68.5 days) (Table 14).

The control cultivar, L550 showed the shortest DGF in three environments (50 days in E1, E2 and 48 days in E3) while in E4 Annigeri ( $41\pm 0.83$  days) and in E5, G130 ( $50\pm2.67$  days) showed shortest DGF. A few accessions such as ICC 12299, ICC 19164, ICC11121, ICC2679, ICC11584, ICC 11819, ICC 19147, ICC 2720, ICC 11944, and ICC 5837 had shorter DGF (38-46 days) than L550 in E1, E2, E3. In E4, ICC 10685, ICC 20174, ICC 8521, ICC 13524, ICC 15435 had shorter DGF (34-40 days) than control Annigeri ( $41\pm 0.83$  days), while in E5, ICC 14402, ICC 10685, ICC 506, ICC 1205, ICC 4991, ICC 18847, and ICC 13524 had shorter DGF (30-40 days), compared to the control G130 ( $50\pm2.67$  days).

#### 4.2.2.4 Days to maturity (DM, days)

Overall, the genotypes exhibited the same pattern for days to maturity as that for days to 50% flowering. The genotypes flowered and matured early under late sowing conditions than under the irrigated conditions. The mean days to maturity was  $113.2 \pm$ 1.66 days with a range of 103.6 -126.3 days in E1,  $115.2 \pm 1.59$  days with range of 102.1 -138.2 days in E2, 114.6  $\pm$  1.42 days with a range of 102.4-134.8 days in E3,  $109.2 \pm 0.83$  with a range of 75.6 -129.6 in E4 whereas in E5 mean days to maturity was  $109.5 \pm 1.66$  days which ranged from 72.5-129.5 and however, the combined analysis revealed a mean of  $112.5 \pm 0.59$  with a range of 99.2-130.6 days (Table 14). The promising early maturing accessions compared to the earliest maturing control cultivar (KAK2, 104 days), were ICC 11121(103 days) in E1. ICC11121, ICC 13219, ICC 16903, ICC 8318, ICC 15606, ICC 15697, ICC 1398, ICC 14595, ICC14669 (102-106 days) in E2 (earliest control KAK2, 106 days), ICC11121, ICC 13219, ICC 16903, ICC 8318, ICC 15606, ICC 15697, ICC 10685, ICC 11944, ICC1557 (102-106 days) in E3 (earliest control Annigeri, 108 days), ICC 14402, ICC 10685, ICC 506, ICC 1205, ICC 4991, ICC 12028 (73-93 days) in E5 (earliest control Annigeri, 102 days), ICC 11121, ICC10685, ICC1205, ICC13219, ICC 16903, ICC 11198, ICC 15618, ICC 15606, ICC 15567, ICC 506, ICC 8318, ICC 14402 were the early maturing accessions, overall in all environments.

# 4.2.2.3 Yield and yield component traits

# 4.2.2.3.1 Pods per plant (PPP, number)

The mean number of pods per plant was  $57.4 \pm 9.19$  with range of 30.8-96.5 in E1,  $62.7 \pm 7.01$  (range: 46.2-86.9) in E2,  $58.47 \pm 3.97$  (range: 36.5 -115.5) in E3,  $45.2 \pm 3.03$  (range: 27.3-68.6) in E4, and  $32.2 \pm 2.60$  (range: 19.6-48.6) in E5. However, the combined analysis revealed a mean of  $52.7 \pm 2.1$  with range of 27.2-89.3 (Table 14 and Plate 7, 8).

The normal sown environments (E1, E2, and E3) were conducive for more pods than late sown spring environments. ICC 10018 (96), ICC 10399 (89), ICC 1882 (85), ICC 1510 (82) in E1; ICC 2629 (87), ICC5221 (82), ICC18839 (80), ICC10379 (79), ICC4093 (79) in E2; (ICC2629 (115), ICC6571 (93), ICC4567 (90), ICC5383 (90), ICC10399 (89) in E3; ICC2629 (69), ICC5221 (67), ICC10018 and ICC10399 (66) each, ICC 4991 (64) in E4 and E5, ICC1510 (49), ICC2629 (49), ICC 506 (46), ICC

5221 (46), ICC 1093 9(45) were the top five accessions in each environment with more number of pods per plant. (ICC2629 (89), ICC5221 (78), ICC10399 (77), ICC10018 (76), ICC4593 (69) accessions produced more number of pods per plant, in all environments compared to the control Annigeri (61).

#### 4.2.2.3.2 Seeds per pod (SDPD, number)

The mean number of seeds per pod was higher in E5  $(1.3 \pm 0.12)$ , E2  $(1.3 \pm 0.09)$ , E1  $(1.3 \pm 0.07)$ , and E3  $(1.2\pm0.11)$ , than E4  $(1.1\pm0.02)$  (Table 14), with an overall range of (1.0-2.0). On an average, accessions ICC 4093, ICC 12866, ICC 2864, ICC 3631, ICC 4533) in E1, (ICC 12866, ICC 4657, ICC 6802, ICC 2884, ICC 3631 in E2 and ICC 11378, ICC 11198, ICC 762, ICC 13219, ICC 2507 had 2.0 seeds per pod in all environments. Only one accession (ICC 16207) had two seeds per pod in E5 and all the five control cultivars had only 1 seed per pod in the all environments.

# 4.2.2.3.3 Yield per plant (YPP, g)

The mean yield per plant was more in the E2 ( $15.5\pm2.23g$ ), E1 ( $11.1\pm1.40g$ ), E3 ( $11.3\pm1.64g$ ), than E5 ( $8.4\pm1.44g$ ) and E4 ( $8.0\pm0.38g$ ). ICC 13077 (27g) produced higher yield than control Annigeri (22 g) in E1, while in E2 ICC 13077 (30g), ICC 20267 (21g), ICC 8350 (20g), ICC 1180 (19g), ICC 18679 (19g) produced more yield per plant than Annigeri (18g). ICC 13077 (30g), ICC 18828 (25g) in E3 were high yielding accessions than Annigeri (22g) while in E4, ICC 13077 (29g) produced higher yield than the high yielding control cultivar ICCV 10 (25g) (Table 14).

None of the chickpea reference set accessions showed significantly more yield per plant in E5 than control cultivar L 550 (17g). ICC 13077 (30 g) produced overall higher yield than the high yielding control cultivar Annigeri (22 g) in pooled analysis.

#### 4.2.2.3.4 100-seed weight (SDWT, g)

The trait 100-seed weight was more stable across environments E1, E2, E3 and E4 (normal sown) and reduced significantly in E5 (late sown) as indicated by the environment means: E1 ( $23.6\pm1.32$ ), E2 ( $22.6\pm0.71$ ), E3 ( $22.4\pm0.74$ ), and E4 ( $21.7\pm0.41$ ) (normal sown) and E5 ( $19.3\pm1.16$ ) (late sown)). However, a wide range was observed among accessions for this trait in all the environments (13.4-51.5g in E1, 12.7-55g in E2, 14.7-53.0g in E3, 13.6-51.9g in E4 and 11.0-39.6g in E5 (Table 14). ICC 20266, ICC 19165, ICC 11303, ICC 15518 and ICC 11764 (37-49 g) are the top

five large seeded accessions across E1, E2, E3, and E4 environments. ICC 11303, ICC 15518, ICC 19165, ICC 8151, ICC 11764 (32-40g) in E5 had significantly higher 100-seed weight than large-seeded control cultivar KAK 2 (31g) (Table 14).

# 4.2.2.3.5 Plot yield (PY, kg ha<sup>-1</sup>)

The overall mean grain yield was about 1675 kg ha<sup>-1</sup>, (mean of all five environments). The environment wise mean yields were 1934.4±134.81 kg ha<sup>-1</sup> in E1, 2088.6±206.71 kg ha<sup>-1</sup> in E2, 1808.1±115.20 kg ha<sup>-1</sup> in E3, 1433.1±122.54 kg ha<sup>-1</sup> in E4 and 821±105.64 kg ha<sup>-1</sup> E5 (Table 14). However, a wide range was observed among the accessions for this trait in all the five environments, 365.7 - 3161.4 kg ha<sup>-1</sup> in E1, 566.9 - 3275.4 kg ha<sup>-1</sup> in E2, 657.2 - 4269.9 kg ha<sup>-1</sup> in E3, 296.4-1678.3 kg ha<sup>-1</sup> in E4 and 283.5 t- 1892 kg ha<sup>-1</sup> in E5. Five accessions, ICC 11498, ICC 15510, ICC 8318, ICC 4567, and ICC 10393 that produced > 2300 kg ha<sup>-1</sup> in all the environments, were considered as high yielding accessions. ICC 14446, ICC 12321 and ICC 11279 yielded <1000 kg ha<sup>-1</sup> in all the environments and were considered as poor yielding.

# 4.2.2.3.6 per day productivity (PROD, kg ha<sup>-1</sup> day<sup>-1</sup>)

The overall mean per day productivity was about 14.9 kg ha<sup>-1</sup> day<sup>-1</sup> (mean of all five environments). However while the mean per day productivity among environments varied from: 17.2 kg ha<sup>-1</sup> day<sup>-1</sup> in E1, 18.3 kg ha<sup>-1</sup> day<sup>-1</sup> in E2, 15.9 kg ha<sup>-1</sup> day<sup>-1</sup> in E3, 13.2 in E4 and 7.6 kg ha<sup>-1</sup> day<sup>-1</sup> E5 (Table 14). A wide range was observed among accessions for this trait in all the five environments : 3.3 - 29.8 kg ha<sup>-1</sup> day<sup>-1</sup> in E1, 4.6 - 27.9 kg ha<sup>-1</sup> day<sup>-1</sup> in E2, 5.7 - 36 kg ha<sup>-1</sup> day<sup>-1</sup> in E3, 2.54 - 16.5 kg ha<sup>-1</sup> day<sup>-1</sup> in E4 and 2.5 - 18.3 kg ha<sup>-1</sup> day<sup>-1</sup> in E5 (Table 14). Accessions ICC 11498, ICC 15510, ICC 8318, ICC 4567, and ICC 10393 produced > 20 kg ha<sup>-1</sup> day<sup>-1</sup> in all environments, and they were considered as highly productive accessions. Three accessions, ICC 14446, ICC 12321 and ICC 11279 yielded <8 kg ha<sup>-1</sup> day<sup>-1</sup> in all the environments and were considered to be least productive.

# 4.2.3 Mean performances of the accessions according to their geographical regions

According to Newman- Keuls test, region wise means were not significantly different for most of the traits except for days to 50% flowering and days to maturity (Africa), plant height (Europe), tertiary branches (South America), 100-seed weight (South America), pods per plant and yield per plant (Africa, South America and South and East Asia), and plot yield (Africa and South East Asia) in E1, E2, E3, E4, E5 and when pooled (Table 15). The accessions from Africa flowered earlier (50-54 days), and matured earlier (110-112 days), whereas accessions from Europe flowered late (64-69 days) with short grain filling duration (49-53 days) across environments. The regional mean value for traits such as flowering duration, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, seed per pod were similar across environments. The European accessions had higher mean plant height (46-53 cm) across environments. Higher mean 100-seed weight across environments was in the accessions from South America (32-37 gm). The South East Asian accessions had higher mean yield (2352, 2076, 1933 and 1759 kg ha<sup>-1</sup>) in E1, E2, E3, E4 and overall environments respectively.

The variance of the accessions from different regions were homogeneous for all traits except for days to 50% flowering, flowering duration, days to grain filling, days to maturity and seeds per pod in E1, pods per plant and yield per plant in E2, plant height in E3 and plot yield and per productivity in combined analysis (P = 0.001) according to Levene's test (Table 15).

#### **4.2.4 VARIABILITY STUDIES**

The estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability are furnished in Table 16 and Figure 3. In general, for all the traits, PCV the was slightly higher (0.1-4%) than the GCV. The values were grouped into high (> 20%), medium (10 - 20%) and low (< 10%) based on Sivasubramanian and Madhavamenon, (1973). In pooled analysis PCV was high for tertiary branches (32.42 %), yield per plant (25.24 %), 100-seed weight (24.59 %), productivity (22.19 %), and plot yield (20.60 %). Medium PCV was observed for apical primary branches (18.66%), apical secondary branches (18.31%), basal secondary branches (16.86%), pods per plant (16.67%), plant height (16.43 %), seeds per pod (14.52%), basal primary branches (13.24%) and days to flowering (10.90%). Low PCV was observed for days to grain filling (8.46%), flowering duration (5.70%), plant width (4.77%) and days to maturity (3.94%).

The GCV% was highest for tertiary branches (28.55 %), 100-seed weight (24.25 %), yield per plant (22.87 %) and productivity (21.25%). Medium PCV was observed for plot yield (19.6 %), apical secondary branches (16.91%), plant height (16.53 %), pods per plant (16.49%), apical primary branches (15.89%), basal secondary branches

(14.63%), seeds per pod (11.46%), basal primary branches (11.31%) and days to flowering (10.79%). Low PCV was observed for by days to grain filling (8.02%), Flowering duration (5.14%), plant width (4.51%) and days to maturity (3.72%).

In the present study, all the traits exhibited narrow difference between PCV and GCV indicating the low effect of environment and greater role of genetic factors on the expression of the traits.

The estimates of broad sense heritability  $(h^2b)$  in the chickpea reference set were high (> 70 %) for all traits except PPP and YPP in E2 and seeds per pod on E3 and E5 (Table: 16). For pooled data the  $h^2b$  was more than 85% for PLHT, PLWD, DF, DGF, DM, ASB, PPP, SDWT, YKGH and PROD. The pooled estimates were (62% – 85%) for BPB, APB, BSB, TB, SDPD and YPP. High heritability was observed for more traits in individual as well as overall five environments indicating the reliability of the estimates for variation between entries and selection of material for these traits.

#### **4.2.5 CORRELATION COFFICIENTS**

The correlation coefficients help to understand the degree, nature and extent of association that existed between the different traits in different environments. Phenotypic correlation coefficients were calculated for the chickpea reference set to understand the nature of associations between 17 quantitative traits in all the five environments separately and overall in the five environments. In total, 61 correlations were significant in E1 (Table 17), 55 in E2 (Table 18), 57 in E3 (Table 19), 48 in E4 (Table 20), 50 in E5 (Table 21), and 50 in overall five environments (Table 22).

# 4.2.5.1 Days to 50 percent flowering

Days to 50 percent flowering was significantly and positively correlated with days to maturity (0.597 in E1, 0.694 in E2, 0.620 in E3, 0.599 in E4, 0.525 in E5 and 0.671 in overall), plant width (0.316 in E1, 0.304 in E2, 0.263 in E3, 0.218 in E4, and 0.256 in overall), plant height (0.233 in E1, 0.304 in E2, 0.181 in E3, 0.185 in E4, 0.219 in E5 and 0.240 in overall) and basal primary branches (0.128 in E1, 0.131 in E3, 0.152 in E5 and 0.161 in overall), whereas DF was significantly negatively correlated with flowering duration (-0.345 in E1,-0.121 in E2, -0.211 in E3, -0.159 in E5 and -0.227 in overall), days to grain filling (-0.630 in E1, -0.657 in E2, -0.711 in E3, -0.614 in E4, -0.487 in E5 and -0.716 in overall), apical primary branches (-0.164 in E1, -0.144 in E2, -0.151 in E4, -0.187 in E5 and -0.190 in overall), seeds per pod (- 0.138 in E2), pods per plant (-0.214 in E1, -0.276 in E2, -0.229 in E3, -0.246 in E4 and -0.300 in

overall), yield per plant (-0.158 in E1, -0.173 in E2,-0.220 in E3, -0.135 in E4 and -0.188 in overall), plot yield (-0.360 in E1, -0.345 in E2, -0.360 in E3, -0.326 in E4, -0.293 in E5 and -0.462 in overall) and per day productivity (-0.423 in E1and E2, -0.433 in E3, -0.428 in E4, -0.364 in E5 and -0.537 in overall) (Tables 17 to 22).

# 4.2.5.2 Flowering duration

Flowering duration was significantly and positively correlated with days to grain filling (0.422 in E1, 0.325 in E2, 0.311 in E3, 0.210 in E4, 0.192 in E5 and 0.379 in overall), days to maturity (0.194 in E2, 0.208 in E4), 100-seed weight (0.139 in E1), apical primary branches (0.169 in E4), basal secondary branches (0.137 in E1, 0.136 in E4), tertiary branches (0.127 in E3, 0.180 in E4) and yield per plant (0.284 in E4), whereas significantly negatively correlated with per day productivity (-0.226 in E2 and-0.126 in overall), plot yield (-0.209 in E2, -0.121 in overall), basal primary branches (-0.119 in E4), plant height (-0.145 in E4), apical secondary branches (-0.131 in E2), and seeds per pod (-0.132 in E3) (Tables 17 to 22).

#### 4.2.5.3 Plant height

Plant height was significantly and positively correlated with plant width (0.357 in E1,0.216 in E2, 0.311 in E3, 0.271 in E4, 0.234 in E5 and 0.297 in overall), days to maturity (0.231 in E1, 0.267 in E2, 0.207 in E3, 0.194 in E4, 0.192 in E5 and 0.273 when pooled) and 100-seed weight (0.435 in E1, 0.239 in E2, 0.269 in E3, 0.267 in E5, 0.306 when pooled), whereas significantly negatively correlated with seeds per pod (-0.210 in E1, -0.230 in E2, -0.162 in E3, -0.238 in E5 and -0.201 when pooled), pods per plant (-0.131 in E1, -0.321 in E2, -0.148 in E3 and -0.212 when pooled) plot yield ( -0.216 in E2 and -0.149 when pooled) per day productivity (-0.152 in E1, -0.246 in E2 and -0.189 when pooled), basal secondary branches (-0.124 in E4), yield per plant (-0.158 in E1) and days to grain filling ( -0.125 in E2) (Tables 17 to 22).

#### 4.2.5.4 Plant width

Plant width was significantly and positively correlated with days to maturity (0.336 in E1, 0.248 in E2, 0.244 in E3, 0.291 in E4 and 0.282 in overall) and 100-seed weight (0.200 in E1, 0.227 in E2, 0.236 in E3, 0.219 in E4, 0.145 in E5 and 0.250 in overall), whereas significantly negatively correlated with pods per plant (-0.155 in E1 and - 0.143 in overall), yield per plant (-0.173 in E1) and tertiary branches (-0.155 in E3) (Tables 17 to 22).

## 4.2.5.5 Days to grain filling

Days to grain filling was significantly and positively correlated with plot yield (0.238 in E1, 0.134 in E2, 0.213 in E3, 0.213 in E4 and 0.267 in overall), per day productivity (0.203 in E1, 0.190 in E3, 0.130 in E4 and 0.241 in overall), yield per plant (0.197 in E3 and 0.125 in overall) and days to maturity (0.162 in E1, 0.264 in E4, 0.483 in E5), pods per plant (0.124 in E4), whereas it was significantly and negatively correlated with basal primary branches (-0.136 in E1, -0.125 in E3 and - 0.128 in overall) and tertiary branches (-0.141 in E4). In E2 and E5, days to grain filling was not significantly and negatively correlated with any of the character (Tables 17 to 22).

# 4.2.5.6 Days to maturity

Days to maturity was significantly and positively correlated with 100-seed weight in all the five environments separately and over all in the five environments (0.200 in E1, 0.170 in E2, 0.188 in E3, 0.148 in E4, 0.169 in E5 and 0.216 in overall), basal secondary branches in E5 (0.142), whereas significantly negatively correlated with apical primary branches (-0.199 in E1, -0.138 in E2 and -0.183 when pooled), seeds per pod (-0.134 in E1, -0.148 in E2, -0.123 in E3 and -0.199 when pooled), pods per plant (-0.307 in E1, -0.319 in E2, -0.279 in E3, -0.178 in E4 and -0.335 when pooled), plot yield (-0.358 in E1, -0.407 in E2, -0.305 in E3, -0.191 in E4, -0.194 in E5 and -0.429 when pooled), per day productivity (-0.476 in E1, -0.524 in E2,-0.429 in E3, -0.400 in E4, -0.383 in E5 and -0.560 when pooled), yield per plant (-0.201 in E1, -0.148 in E2, -0.135 in E4) and apical secondary branches (-0.128 in E2) (Tables 17 to 22).

#### 4.2.5.7 Basal primary branches

Basal primary branches were significantly and positively correlated with basal secondary branches (0.219 in E3, 0.343 in E4, and 0.133 in overall), apical secondary branches (0.119 in E1, 0.204 in E4) and tertiary branches (0.155 in E4) whereas significantly negatively correlated with 100-seed weight (-0.130 in E1, -0.144 in E3, -0.122 in E4), yield per plant (-0.137 in E3) and per day productivity (-0.144 in overall). Basal primary branches were not significantly correlated either positively or negatively with any of the character in E2 and E5 (Tables 17 to 22).

## 4.2.5.8 Apical primary branches

Apical primary branches was significantly positively correlated with basal secondary

branches (0.143 in E5), apical secondary branches (0.151 in E1, 0.335 in E3, 0.359 in E5 and 0.283 in overall), seeds per pod (0.129 in E1, 0.147 in E2 and 0.182 in overall), pods per plant (0.169 in E1, 0.139 in E3, 0.187 in E4, 0.222 in E5 and 0.249 in overall), yield per plant (0.161 in E1, 0.210 in E3, 0.271 in E5 and 0.180 in overall), plot yield (0.139 in E1, 0.191 in E3, 0.614 in E4, 0.206 in E5 and 0.232 in overall), per day productivity (0.154 in E1, 0.197 in E3, 0.160 in E4, 0.205 in E5 and 0.240 in overall) and basal primary branches (0.165 in overall) whereas significantly negatively correlated with 100-seed weight (-0.140 in E5). Apical primary branches were not significantly negatively correlated with any of the character in E1, E2, E3, E4 and in overall (Tables 17 to 22).

#### 4.2.5.9 Basal secondary branches

Basal secondary branches was significantly positively correlated with tertiary branches (0.316 in E1, 0.276 in E2, 0.217 in E3, 0.174 in E4, 0.338 in E5 and 0.215 in overall), apical secondary branches (0.281 in E1, 0.161 in E2, 0.274 in E4, 0.355 in E5 and 0.228 in overall), yield per plant (0.176 in E2, 0.179 in E3, 0.200 in E4 and 0.221 in overall) and pods per plant (0.135 in E1, 0.129 in E4 and 0.121 in overall), plot yield (0.153 in E5) and per day productivity (0.172 in E5), whereas significantly negatively correlated with 100-seed weight (-0.140 in E5). Basal secondary branches were not significantly negatively correlated with any of the character in E1, E2, E3, E4 and in overall five environments (Tables 17 to 22).

#### 4.2.5.10 Apical secondary branches

Apical secondary branches was positively correlated with tertiary branches (0.154 in E1, 0.158 in E2, 0.151 in E3, 0.260 in E4, 0.206 in E5 and 0.250 in overall), pods per plant (0.190 in E1, 0.121 in E2, 0.125 in E3, 0.236 in E4, 0.176 in E5 and 0.217 in overall), yield per plant (0.182 in E1, 0.136 in E3, 0.134 in E4, 0.232 in E5 and 0.166 in overall), plot yield (0.177 in E2, 0.127 in E3 and 0.180 in overall) and per day productivity (0.179 in E2, 0.134 in E3 and 0.178 in overall) and negatively correlated with 100-seed weight (-0.131 in E1, -0.161 in E5 and -0.125 in overall. Apical secondary branches were not significantly negatively correlated with any of the character in E2, E3, E4 and in overall five environments (Tables 17 to 22).

#### 4.2.5.11 Tertiary Branches

Tertiary branches were not significantly correlated either positively or negatively with any of the character in E1, E2, and E4 and negatively in E3 and in overall. Number of tertiary branches was positively correlated with yield per plant (0.131 in E3, 0.314 in E5 and 0.258 in overall) whereas it was significantly and negatively correlated with seeds per pod (-0.130 in E5) (Tables 17 to 22).

# 4.2.5.12 Seeds per pod

Seeds per pod was significantly and positively correlated with pod per plant (0.157 in E1, 0.317 in E2, 0.206 in E3 and 0.279 in overall), plot yield (0.210 in E3 and 0.122 in overall), pods per plant (0.139 in E5) and per day productivity (0.215 in E2) and negatively correlated with 100-seed weight (-0.459 in E1 and E2, -0.376 in E3, -0.196 in E4, -0.316 in E5 and -0.508 in overall), yield per plant (-0.216 in E5) (Tables 17 to 22).

# 4.2.5.13 Pods per plant

Pods per plant was significantly positively correlated with three traits viz., yield per plant (0.335 in E1, 0.383 in E2, 0.322 in E3, 0.124 in E4, 0.210 in E5 and 0.277 in overall), plot yield (0.331 in E1, 0.488 in E2, 0.324 in E3, 0.203 in E4 and 0.448 in overall) and per day productivity (0.354 in E1, 0.500 in E2, 0.344 in E3, 0.227 in E4 and 0.466 in overall) and negatively correlated only with 100-seed weight in all environments and when pooled (-0.312 in E1, -0.486 in E2, -0.301 in E3, -0.334 in E4, -0.300 in E5 and -0.448 in overall) (Tables 17 to 22).

# 4.2.5.14 Yield per plant

Yield per plant was significantly positively correlated with two traits in all environments and in overall five environments viz., plot yield (0.159 in E1, 0.269 in E2, 0.169 in E3 and 0.164 in overall), per day productivity (0.181 in E1, 0.269 in E2, 0.177 in E3 and 0.181 in overall) and 100- seed weight (0.136 in E3) and negatively correlated with only 100-seed weight (-0.161 in E1) and Yield per plant were not significantly correlated either positively or negatively with any of the character in E4 and E5, and none of the traits were significantly negatively correlated with yield per plant in E2, E3 and in overall five environments (Tables 17 to 22).

# 4.2.5.15 100-seed weight

100-seed weight was negatively correlated with only one trait, per day productivity (-0.135 in E2) and was not significantly correlated either positively or negatively in E1, E3, E4, E5 and in combined analysis and but was positively in E2 (Tables 17 to 22).

# 4.2.5.16 Plot yield

Plot yield was correlated positively only with per day productivity in all environments and when pooled (0.990 in E1, E2, E3, 0.974 in E4, 0.978 in E5 and 0.987 when pooled) (Tables 17 to 22).

#### 4.2.5.17 Pairs of characters showing meaningful correlation

The numbers of significant correlations were large (316 out of 816 correlations) in the present study and some of them may not be biologically meaningful. Skinner *et al*, (1999) suggested that only those correlations, which are greater than 0.707 or less than -0.707 are biologically meaningful, so that 50 % of the variation in one trait is predicted by the other trait (Snedecor and Cochran, 1980). However with 298 degrees of freedom, the character pairs showing correlation greater than 0.700 or lesser than -0.700 were found biologically meaningful and 2 pairs of characters showed meaningful correlations. The correlations for 1 pair of the characters were positive in all environments and in overall in all environments; plot yield and per day productivity in E1, E2, E3 (0.990), E4 (0.974), E5 (0.978) and in overall. Correlations for 1 pair of the characters were negative in E3 and in overall ; viz., days to 50 percent flowering and days to grain filling in E3 (-0.711), and in overall (-0.716); showed significantly higher and biologically meaningful correlation (Table 23).

However the pairs of traits, viz., days to 50 percent flowering and days to maturity in E1 (0.597), E2 (0.694), E3 (0.620), E4 (0.599), E5 (0.525) and in overall (0.671); pods per plant and per day productivity in E2 (0.500) showed high correlation, and correlations for 1 pair of the characters were negative, days to 50 percent flowering and days to grain filling (-0.614) in E4 (r = 0.50 or more) (Table 23).

## 4.2.6 DIVERSITY ANALYSIS

#### 4.2.6.1 Shannon Weaver Diversity Indices

The Shannon-Weaver diversity (H') indices were calculated to compare values among 17 quantitative traits in each environment separately and also over all the environments. The index is used as a measure of allelic richness and evenness; a low H` indicates an extremely unbalanced frequency class for an individual trait and lack of genetic diversity.

Out of twenty four morphological and agronomic traits studied, dots on seed coat showed lowest H $^{(0.300)}$  in all environments followed by seed shape (0.325), seed surface (0.332), plant color (0.335), growth habit (0.362) and flower color (0.424), however, seed color showed high H $^{(0.807)}$ . Among the quantitative traits, tertiary

branches showed lowest H<sup> $\circ$ </sup> in E1 (0.244), and E2 (0.0797), flowering duration in E3 (0.429) and in E4 (0.312) and seeds per pod in E5 (0.219) environments followed by apical primary branches in E1 (0.468), flowering duration in E2 (0.456) and in E4 (0.305), apical secondary branches in E3 (0.440), yield per plant in E5 (0.413) environments. The traits such as, days to 50 percent flowering in E1(0.631), grain yield in E2 (0.634), days to maturity in E3 (0.631), per day productivity in E4 (0.621) and apical primary branches in E5 (0.623) environments showed highest H<sup> $\circ$ </sup> followed by days to grain filling (0.620), flowering duration (0.602), yield per plant (0.600), apical secondary branches (0.578) in E1, grain yield (0.634), basal primary branches (0.628), per day productivity (0.626), basal secondary branches (0.582) in E3, pod per plant (0.617) in E4 and apical primary branches (0.623), seeds per pod (0.619), days to 50 percent flowering (0.612), 100-seed weight (0.584) and plant height (0.559) in E5.

The combined analysis revealed low H<sup>°</sup> for tertiary branches (0.244) and high H<sup>°</sup> for pods per plant (0.624). Among the environments, E1 (0.577 ± 0.018) revealed high H<sup>°</sup> for the quantitative traits followed by E3 (0.552 ± 0.015), E2 (0.551 ± 0.032), E5 (0.543 ± 0.022) and E4 (0.543 ± 0.019) (Table 24).

# 4.2.6.1.2 Phenotypic diversity of chickpea reference set according to their biological and geographical origin

### 4.2.6.1.2.1 Qualitative traits

A high H was observed for the pea (0.932), followed by desi (0.688) and wild (0.436) accessions for seed color and kabuli accessions for growth habit (0.351) in all environments (Table 25).

Region wise, the accessions from West Asia (0.790), Africa (0.620), South East Asia (0.594) and Mediterranean (0.590) showed high H<sup> $\circ$ </sup> for seed color, and European accessions for growth habit (0.477), North American accessions for flower and seed color (0.378) and accessions from Russian Federation for seed color (0.540), whereas accessions from Africa had high H<sup> $\circ$ </sup> for growth habit and seed dots (0.137); South East Asia for plant color (0.194), Mediterranean for seed shape (0.252), West Asia for seed shape (0.267) for seed shape had low H<sup> $\circ$ </sup> for all traits except growth habit (Table 25).

#### 4.2.6.1.2.2 Quantitative traits

The cultivated type accessions had more diversity than wild type accessions. Among cultivated, desi accessions showed higher diversity (0.574  $\pm$  0.02 in E1, 0.560  $\pm$ 0.03 in E2, 0.552  $\pm$ 0.02 in E3, 0.524  $\pm$  0.03 in E4, 0.529  $\pm$  0.02 in E5 and 0.565  $\pm$  0.02 in overall). Accessions of wild type ( $0.362 \pm 0.02$  in E1,  $0.347 \pm 0.03$  in E2,  $0.393 \pm$ 0.02 in E3,  $349 \pm 0.03$  in E4,  $349 \pm 0.02$  in E5 and  $0.385 \pm 0.02$  in overall) showed low H<sup> $\circ$ </sup> (Table: 26). The traits such days to 50 percent flowering (0.636 in E1), flowering duration (0.527 in E1), plant height (0.602 in E5), plant width (0.620 in E3), days to grain filling (0.629 when combined), days to maturity (0.630 in E2), basal primary branches (0.608 when combined), apical primary branches (0.602 in)E1), basal secondary branches (0.597 in E2), tertiary branches (0.582 in E3), seeds per pod (0.581 in E1), pods per plant (0.632 in E4), yield per plant (0.605 when)combined), 100-seed weight (0.624 in E4), plot yield (0.619 in E2) and per day productivity (0.6255 in E1) in desi accessions, flowering duration (0.562 in E3), basal primary branches (0.628 in E1) and plot yield (0.628 when combined) in kabuli accessions and apical secondary branches (0.562 in E1) in the pea accessions had more H`.

Region wise, the accessions from West Asia  $(0.624 \pm 0.02 \text{ in E1}, 0.639 \pm 0.03 \text{ in E4}, 0.634 \pm 0.02 \text{ in E5}$  and  $0.638 \pm 0.02$  in pooled analysis), and South East Asia  $(0.639 \pm 0.03 \text{ in E2} \text{ and } 0.653 \pm 0.02 \text{ in E3})$  recorded high H<sup>+</sup>, whereas Russian Federation in E1, South East Asia in E2, West Asia in E5, North America accessions in E3, E4, and in overall showed low H<sup>+</sup> (0.196 \pm 0.02 \text{ in all environments and overall) (Table: 27). The traits such as, days to 50% flowering (0.638 when combined), flowering duration (0.624 in E1), plant width (0.630 in E2), apical secondary branches (0.637 in E2), basal primary branches (0.610 in E2), 100-seed weight (0.606 in E5) and yield per plant (0.626 when combined) in the accessions of West Asia, plant height (0.563 in E4), pods per plant (0.635 in E2) in the accessions of Africa, days to maturity (0.639 in E2), tertiary branches (0.582 in E5), seeds per pod (0.610 in E1), plot yield (0.633 in E2) and per day productivity (0.639 in E2) in the accessions of South East Asia , days to grain filling and apical primary branches (0.620 and 0.630 when combined) in the accessions of Mediterranean region had high H<sup>+</sup> in different environments (Table 27).

#### **4.2.6.2** Principal components analysis

Principal component analysis on the mean values of the entire set provides a reduced dimension to the model that could indicate measured differences among the accessions.

#### 4.2.6.2.1 PCA based on environments

The results revealed that in all the five environments and also in the pooled analysis, a large proportion of the total variation was explained by the first seven Principal Components (PCs) in discriminating the entire set of chickpea reference set. The first seven PCs explained 70.41% variation in E1, 69.65% in E2, 69.78% in E3, 66.60% in E4 and 69.79% in E5 (Tables 28, 29, 30, 31, 32). In pooled analysis 71.80% variation was accounted by first seven PCs (Table 33).

The PC1 separated the accessions based on per day productivity, plot yield, days to 50% flowering and days to maturity in all five environments and when pooled, along with pods per plant in E2. PC2 separated the accessions based on days to grain filling and flowering duration in E1, E2, E4 and pooled whereas in E5 based on yield per plant, apical and basal secondary branches and tertiary branches. The PC3 separated the accessions based on flowering duration and plot yield in E1, 100-seed weight and seeds per pod in E2, days to grain filling and flowering duration in E3, apical secondary branches and tertiary branches in E4, days to grain filling and 100-seed weight in E5 and tertiary branches and apical secondary branches when pooled. PC4 separated the accessions based on apical and basal secondary branches in E1, basal secondary branches and tertiary branches in E2, apical primary branches and apical secondary in E3, flowering duration and days to grain filling in E4 and in combined analysis, and plant height, days to grain filling and plant width in E5. The PC5 separated the accessions based on days to maturity in E1, yield per plant in E2, basal secondary branches in E3, 100-seed weight in E4, basal primary branches in E5 and seeds per pod when pooled. Similarly PC6 separated the accessions based on apical primary branches in E1, E2, seeds per pod in E3, E5 and basal primary branches in E4 and when pooled. PC7 separated the accessions based on basal primary branches in E1, E2, E3 and E4, plant width in E5 and apical primary branches when pooled. Scatter plot of first two principal components (PCs) of Chickpea reference set accessions using pooled BLUPs of five environments for yield contributing traits is represented in Figure 5a (Days to 50% flowering (DF) vs. plot yield (YKGH), Figure 5b Days to maturity (DM) vs. Plot yield (YKGH), Figure 5c 100 seed weight vs. Plot yield (YKGH)

#### 4.2.6.3 Phenotypic diversity index

Phenotypic diversity index (Johns et al., 1997) was created by calculating differences between each pair of accessions for each of the 7 qualitative and 17 quantitative traits by averaging all the differences in the phenotypic values for each traits divided by their respective range. Phenotypic diversity differed in different environments. The mean phenotypic diversity index was 0.184 in all environments indicating high variability in the reference set accessions (Table 34). In E1 minimum phenotypic diversity index of 0.002 was observed between ICC 3362 (West Asia) and ICC 1230 (South and East Asia) revealing that these accessions were almost similar. The maximum diversity index was 0.444 between ICCV92311 (South and East Asia) and ICC 11198 (South and East Asia). The cross between these two accessions may result in useful variation. Minimum phenotypic diversity index of 0.002 was observed between ICC 13764 (West Asia) and ICC 12037 (North America) in E2 and ICC 13187 (West Asia) and ICC 12324 (Unknown biological status) in E3 and maximum diversity index was 0.425 between ICC 20266 (Unknown biological status) and ICC 4991 (South and East Asia) in E2 and between Annigeri (South and East Asia) and ICC 16796 (Europe) in E3. In E4 the mean phenotypic diversity was recorded as 0.188, the minimum diversity (0.001) was observed between ICC 9002 (West Asia) and ICC 2065 (South and East Asia) and the maximum diversity (0.430) was observed between Annigeri (South and East Asia) and ICC 16796 (Europe). In E5 the mean phenotypic diversity was recorded as 0.182, the minimum diversity (0.001) was observed between ICC 2065 (South and East Asia) and ICC 12947 (South and East Asia) and the maximum diversity (0.445) was observed between Annigeri (South and East Asia) and ICC 18983 (Mediterranean region). When pooled the mean phenotypic diversity index was 0.184. The maximum diversity (0.425) was observed between ICC 13764 (West Asia) and ICC 12037 (North America). The minimum diversity was 0.001 observed in ICCV92311 (South and East Asia) and ICC 11198 (South and East Asia).

#### 4.2.6.4 Clustering

The hierarchical cluster analysis (Ward, 1963) based on Euclidean distance was conducted using the scores of first three PCs on the pooled data capturing 85%

variation based on geographical origin of reference set accessions.

Grouping of reference set accessions resulted into a dendrogram with four clusters. Accessions from Africa and South East Asia were grouped in to Cluster I, South America origin in Cluster II. Europe and Russian Federation in Cluster III and whereas Mediterranean, unknown, North America and West Africa were grouped together in Cluster IV (Figure 6). Dendrogram of chickpea reference set based on 7 qualitative traits and 17 quantitative traits are represented in Figure 7 and Figure 8 respectively.

# EVALUATION OF CHICKPEA REFERENCE SET ACCESSIONS FOR DROUGHT RESISTANT TRAITS

# 4.3 IDENTIFICATION OF ACCESSIONS WITH HIGH SPAD CHLOROPHYLL METER READINGS (SCMR)

The chickpea reference set along with five check cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) were used to estimate the variation of SPAD Chlorophyll Meter Readings (SCMR) in 2008/2009 post rainy season, normal sown (E3) and 2008/09 spring season, late sown, (E5) at high temperatures at ICRISAT, Patancheru, Andhra Pradesh.

# 4.3.1 Soil Plant Analysis Development (SPAD) Chlorophyll Meter Readings (SCMR)

The mean SCMR reading was 58.21, 62.00, and 60.06 in normal (E3), late sown (E5) environments and for pooled data respectively. The accessions ICC 506 (61.86), ICC 637 (61.61), ICC 11121 (61.13), ICC 7305 (61.02) and ICC 12928 (60.99) had high SCMR when compared with the control cultivar G130 (57.23  $\pm$  1.19) in normal sown conditions. ICC 19095 (71.57), ICC 1510 (67.16), ICC 6874 (66.99), ICC 15567 (66.89) and ICC 2277 (66.68) were better when compared with the control ICCV 10 (58.86  $\pm$  0.60) under late sown environment, whereas in pooled analysis, ICC 19095 (62.78), ICC 6874 (62.45), ICC 506 (62.41), ICC 15618 (62.24) and ICC 12321 (62.10) recorded high SCMR than the control cultivar KAK2 (58.03  $\pm$  1.01) (Table 35).

# 4.4 IDENTIFICATION OF ACCESSIONS RESISTANT TO DROUGHT TOLERANCE

The 293 cultivated diverse accessions of reference set (excluding wild accessions from 300 accessions of chickpea reference set) along with 6 control cultivars (ICC 4958, Annigeri, ICCV 10, G 130, L 550, KAK 2,) were evaluated for drought related root traits during two consecutive post rainy seasons (2007-08 (E2), 2008-09 (E3)) at ICRISAT, Patancheru (Tables 36-37 and Plate 13-14).

# **4.4.1 VARIANCE COMPONENTS**

The REML analysis of data for individual environment revealed significant genotypic variance for all traits in two (E2, E3) environments and in pooled analysis (Tables 36-37).

# 4.4.2 RANGE AND MEAN PERFORMANCE

Mean and range are simple and important measures of variability (Singh, 1983). Variability among the accessions for different traits was assessed by comparing the values of means and range for each trait between environments. Mean and range were calculated for each character in individual environment separately as well as pooled mean of two environments were tested using the Newman-Keuls procedure to compare the mean values within environments. The estimates of mean and range are presented below.

# 4.4.2.1 Shoot dry weight (g)

At 35 DAS the genotypes, ICC 15518 (3.18gm), ICC 18679 (2.94gm), ICC 15406 (2.86gm), ICC 20263 (2.83gm) and ICC 9137 (2.79gm) recorded high shoot dry weight when compared to deep rooted and drought resistant control cultivar ICC 4958 (2.23  $\pm$  0.30) in E2, whereas in E3 the genotypes ICC 15406 (2.68 gm), ICC 15518 (2.56 gm), ICC 14446 (2.49 gm), ICC 11303 (2.48 gm) and ICC 18912 (2.45 gm) recorded high shoot dry weight as compared to control ICC 4958 (2.27  $\pm$  0.24) in E3. In pooled analysis ICC 15518 (2.87 gm), ICC 15406 (2.77gm), ICC 18679 (2.57gm), ICC 20263 (2.56gm) and ICC 11903 (2.50gm) recorded high shoot dry weight as compared to control ICC 15518, ICC 15406 recorded high shoot dry weight as compared to ICC 4958 both in E2 and E3 and also in pooled analysis (Table: 36-37).

Shoot dry weight was significantly positively correlated with the traits RDW, RDp,

TDW, RL, RLD, RSA, RV, S/RLD and significantly negatively correlated with R/T% in both E2 and E3 environments and also when pooled (Tables 38-40).

# 4.4.2.2 Root dry weight (g)

ICC 10885 (0.96gm), ICC 12379 (0.92gm), ICC 20267 (0.90gm), ICC 12492 (0.88gm) and ICC 9862 (0.87gm) recorded high root dry weight than the control cultivar ICC 4958 (0.80  $\pm$  0.11) in E2, whereas in E3 the genotypes ICC 12492 (1.01 gm), ICC 10885 (0.99 gm), ICC 11819 (0.95 gm), ICC 11903 (0.93 gm) and ICC 13187 (0.93 gm) out yielded ICC 4958 (0.76  $\pm$  0.10). In pooled analysis ICC 10885 (0.97 gm), ICC 12492 (0.95 gm), ICC 13187 (0.87gm), ICC 18858 (0.85gm) and ICC 20267 (0.84 gm) recorded high root dry weight than ICC 4958 (0.72  $\pm$  0.086). The genotypes ICC 10885 and ICC 12492 recorded high root dry weight as compared to deep rooted and drought resistant control cultivar ICC 4958 in E2, E3 and also in pooled analysis (Table: 36-37).

Root dry weight was significantly positively correlated with all the traits RDW, RDp, R/T%, TDW, RL, RLD, RSA, RV, S/RLD in both E2 and E3 environments and also when pooled (Tables 38-40).

# 4.4.2.3 Total plant dry weight (g)

The genotypes ICC 15518 (3.99gm), ICC 20267 (3.67gm), ICC 9137 (3.63gm), ICC 15406 (3.62gm) and ICC 18679 (3.61gm) recorded high total plant dry weight as compared to deep rooted and drought resistant control cultivar ICC 4958 (3.03  $\pm$  0.356) in E2, whereas in E3 the genotypes ICC 15406 (3.51 gm), ICC 10885 (3.39 gm), ICC 15518 (3.33 gm), ICC 18912 (3.27 gm) and ICC 11903 (3.26 gm) recorded high total plant dry weight as compared to control cultivar ICC 4958 (0.76  $\pm$  0.10). In pooled analysis ICC 15518 (3.66 gm), ICC 15406 (3.56 gm), ICC 10885 (3.31gm), ICC 18679 and ICC 20263 (3.30gm) recorded high total plant dry weight as compared to ICC 4958 (2.88  $\pm$  0.249) (Tables 36-37).

Total dry weight was significantly positively correlated with all the traits RDW, RDp, TDW, RL, RLD, RSA, RV, S/RLD in both E2 and E3 environments and also when pooled, and significantly negatively correlated with R/T% in E2 and pooled (Table: 38-40).

# 4.4.2.4 Root Depth (cm)

In the E2, genotypes ICC 8740 (136.7cm), ICC 12028 (130cm), ICC 11378, ICC 11498, ICC 15510 and ICC 5845 (128.3cm) recorded high root depth as compared to

control cultivar ICC 4958 (110  $\pm$  10.69), whereas in E3 the genotypes ICC 7819 (133.3 cm), ICC 15610 ICC 2679 and ICC 637 (130cm) and ICC 2242 (128.7cm) recorded high root depth as compared to control cultivar ICC 4958 (0.76  $\pm$  0.10). In pooled analysis ICC 8740 (131.7cm), ICC 11498 (123.5 cm), ICC 18983 (122.6 cm), ICC 15518 and ICC 7819 (122.5 cm) recorded high root depth as compared to control cultivar ICC 4958 (114.16  $\pm$  7.64) (Tables 36-37).

Root depth was significantly positively correlated with the traits (RDW, RDp, R/T%, TDW, RL, RSA, and RV) except S/RLD in E2 and RLD in E3. In combined analysis, root depth was significantly positively correlated with all traits (RDW, RDp, R/T%, TDW, RL, RLD, RSA, RV, S/RLD) and significantly negatively correlated with R/T% in E3 (Tables 38-40).

# 4.4.2.5 Root to total plant dry weight ratio (%)

Root to total plant dry weight ratio(R/T %) is an indicator for biomass allocation to roots on dry weight basis. The genotypes ICC 12492 (34.42%), ICC 9942 (32.47%), ICC 2629 (32.30%), ICC 9434 (31.00%) and ICC 8195 (30.64%) recorded high root to total plant dry weight ratio as compared to ICC 4958 ( $26.48\pm 3.51$ ) in E2, whereas in E3 the genotypes ICC 12492 (43.17%, ICC 15610 (35.27%), ICC 11198 (35.23%), ICC 8384 (34.98%) and ICC 12928 (34.44%) recorded high root to total plant dry weight ratio as compared to deep rooted and drought resistant control cultivar ICC 4958 ( $24.96\pm 3.19$ ). In pooled analysis ICC 12492 (38.95%), ICC 12928 (32.65%), ICC 11198 (32.56%), ICC 2629 (31.55%) and ICC 18858 (31.22%) recorded root to total plant dry weight ratio as compared to ICC 4958 ( $24.28\pm 2.64$ ) (Tables 36-37). Root to total plant dry weight ratio(R/T %) was significantly positively correlated with SDW, TDW, S/RLD in E2 and SDW, S/RLD in E3 (Tables 38-40).

# 4.4.2.6 Root Length (cm)

The genotypes ICC 18828 (6949 cm), ICC 10885 (6848 cm), ICC 15518 (6668 cm), ICC 15785 (6533 cm) and ICC 15510 (6496 cm) recorded high root length as compared to ICC 4958 (5865 $\pm$  1002.4) in E2, whereas in E3 the genotypes ICC 18679 (6804 cm), ICC 10885 (6769 cm), ICC 7819 (6760cm), ICC 3410 (6701 cm) and ICC 20263 (6656 cm) recorded high root length as compared to deep rooted and drought resistant control cultivar ICC 4958 (5433 $\pm$  956.10). In combined analysis ICC 10885

(6818.25 cm), ICC 20267 (6496.14 cm), ICC 3410 (3458.22 cm), ICC 18828 (6377.00cm) and ICC 15518 (6267.60cm) genotypes recorded high root length as compared to ICC 4958 ( $5549\pm751.2$ ) (Tables 36-37).

Root length was significantly positively correlated with all traits in E3, E2 except R/T%, S/RLD and R/T% when pooled (Tables 38-40).

## 4.4.2.7 Root Length Density (cmcm-3)

Root length density is associated with water and nutrition uptake. At 35 DAS in E2 the genotypes ICC 8261 (0.397), ICC 5331 (0.268), ICC 6306 (0.262), ICC 20267 (0.258) and ICC 18912 (0.254) recorded high root length density as compared to control ICC 4958 (0.253 $\pm$  0.029), whereas in E3 the genotypes ICC 8261 (0.422), ICC 15333 (0.285), ICC 20259 (0.281), ICC 15435 (0.278) and ICC 15406 (0.274) recorded high root length density as compared to control ICC 4958 (0.254 $\pm$  0.036). In combined analysis ICC 8261 (0.410), ICC 5337 (0.267), ICC 6306 and ICC 18912 (0.263), ICC 20267 (0.255) genotypes recorded high root length density as compared to control ICC 4958 (0.253 $\pm$  0.0265) (Table: 36-37).

Root length density was significantly negatively correlated with S/RLD in E2, E3 and when pooled and positively correlated with all traits in E2, except RDp in E3 and R/T% when pooled (Tables 38-40).

#### 4.4.2.8 Shoot to Root Length Density ratio (%)

The effectiveness of roots in shoot production was calculated by shoot to root length density ratio. The genotypes ICC 7315 (18.13), ICC 13124 (16.46), ICC 15435 (15.59), ICC 1180 (15.35) and ICC 19011 (15.25) recorded high shoot to root length density as compared to control ICC 4958 ( $8.82\pm 2.234$ ) in E2, whereas in E3 the genotypes ICC 4814 (15.49), ICC 16374 (14.89), ICC 3631 (12.56), ICC 10685 (12.27) and ICC 8718 (11.96) recorded high shoot to root length density as compared to control ICC 4958 ( $8.94\pm 1.98$ ). In pooled analysis ICC 3631 (14.66), ICC 4814 (14.18), ICC 7315 (14.09), ICC 13124 (14.04) and ICC 15697 (13.79) genotypes recorded high root length density as compared to control ICC 4958 ( $8.57\pm 1.52$ ) (Tables 36-37).

Shoot to root length density ratio was significantly positively correlated with SDW, RDW and TDW in E2, SDW, RDW, RDp, TDW and RL in E3 and when pooled. Shoot to root length density ratio is significantly negatively correlated with R/T% and RLD in E2, E3 and when pooled (Tables 38-40).

# EVALUATION OF CHICKPEA REFERENCE SET FOR POD BORERE RESISTANCE TRAITS

## 4.5 IDENTIFICATION OF ACCESSIONS RESISTANT TO POD BORER

Three hundred diverse reference set accessions along with 7 control cultivars (Annigeri, G 130, KAK 2, ICC 506EB-resistant, ICC 3137-susceptible, ICCV 10-moderately resistant, L 550-susceptible) were planted in Randomized Complete Block Design (RCBD) during two consecutive post rainy seasons (2007-08 (E2), 2008-09 (E3)) at ICRISAT, Patancheru (Plate 12).

#### 4.5.1 Leaf Damage score

At vegetative stage in post rainy environments (E2 and E3), the interaction effects were significant for leaf damage in two seasons and in pooled analysis (Tables 41-42). The genotypes ICC 16903 (1.62), ICC 14595 and ICC 20174 (1.92), ICC 15518 (2.08) and ICC 8261 (2.09) showed low leaf damage rating when compared to the resistant control cultivar ICC 506 (2.56) in E2, whereas in E3 the genotypes ICC 20174 (1.28), ICC 14595 (1.29), ICC 16903 (1.55), ICC 15518 (1.91) and ICC 15612 (2.18) had low leaf damage rating as compared to the resistant control cultivar ICC 506 (2.48) in E3 whereas in pooled analysis ICC 20174 (1.39), ICC 16903 (1.42), ICC 14595 (1.45), ICC 15518 (1.94) and ICC 8522 (2.28) recorded low leaf damage rating as compared to the resistant control cultivar ICC 506 (2.61) (Tables 41-42).

# 4.5.2 Larval survival (%)

The interaction effects were significant for larval survival in two seasons and in pooled analysis. Larval survival (%) was lowest in genotypes ICC 3892 (35.99%), ICC 9862 (42.21%), ICC 20192 (43.58%), ICC 7305 (45.02%), ICC 18828 (47.37%) and ICC 7148 (49.8%) when compared to the resistant control cultivar ICC 506 (54.74) in E2. The genotypes ICC 12537 (39.6%), ICC 9590 (39.83%), ICC 7819 (43.01%), ICC 2482 (46.05%) and ICC 14595 (48.71) recorded low larval survival rating as compared to the resistant control cultivar ICC 506 (53.47) in E3 whereas in pooled analysis ICC 7819 (48.83%), ICC 12537 (49.83%), ICC 16903 (50.30%), ICC 15435 (51.65%) and ICC 13764 (52.90%) recorded low larval survival (%) when compared to the resistant control ICC 506 (56.76%) (Tables 41-42).

# 4.5.3 Larval weights

Significant interactions effects were observed for larval weights in two seasons.

Larval weights was lowest in genotypes ICC 1161 (13.2mg), ICC 7305 (13.5mg), ICC 6293 (15.5mg), ICC 8058 (16.3mg), ICC 16915 (16.5) when compared to the resistant control cultivar ICC 506 with (20.2mg) larval weight in E2. The genotypes ICC 20174 (21.2mg), ICC 16903 (23.4 mg), ICC 6877 (24.9mg) recorded low larval weights as compared to the resistant control cultivar ICC 506 (26.2 mg) in E3, when pooled ICC 20174 (21.1mg), ICC 16903 (25.3mg), ICC 6293 (29.2mg) were with lower larval weights when compared to the resistant control ICC 506 recorded (31.0 mg).

The genotypes ICC 20174, ICC 16903, ICC 14595 recorded lowest leaf damage, larval survival and lower larval weights in two environments as well as in pooled analysis (Tables 41-42).

# EVALUATION OF CHICKPEA REFERENCE SET FOR GRAIN QUALITY TRAITS

#### 4.6 IDENTIFICATION OF ACCESSIONS WITH HIGH PROTEIN

The chickpea reference set along with five check cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) were used to estimate protein content by Atomic Spectra Photometric Meter (ASPM) in four seasons 2006/2007 (E1), 2007/2008 (E2), 2008/2009 (E3) post rainy normal sown conditions, 2008/2009 (E5) winter seasons, late sown conditions at ICRISAT, Patancheru, Andhra Pradesh. The mean protein content was 21.07% in E2, 20.47% in E1, 19.45% in E3 and 21.79% in E5. The accessions with high protein content were ICC 12654 (25.82%), ICC 11903 (25.56%), ICC 9418 (25.30%), ICC 19226 (25.23%), and ICC 16654 (25.1), compared to 23.03% of the best control cultivar L 550 in E1. ICC 2737 (25.45%), ICC 12155 (25.14%), ICC 19165 (24.96%), ICC 1161 (24.41%) and ICC 3421 (24.37%), compared to 22.76% of control cultivar L550 in E2. ICC 2737 and ICC 3421 (23.92% each), ICC 3218 (22.97%), ICC 20261 (22.93%) and ICC 19165 (22.77%), compared to 19.15% of the control cultivar KAK2 in E3. The accessions ICC 1161 (26.83%), ICC 9418 (26.64%), ICC 13719 (26.22%), ICC 3218 (25.8%) and ICC 6294 (25.28%), compared to 23.37% of the control cultivar L550 in E5. ICC 3421 (24.72%), ICC 3218 (24.67%), ICC 1161 (24.28%), ICC 19165 (24.08%) and ICC 20261 (23.93%), compared to 22% of the control cultivar ICCV 10 in pooled analysis. Protein content was found to be highest in (E5) late sown conditions compared to (E1, E2, E3) normal sown conditions. Most

of the kabuli from Mediterranean and desi from West Asia and South and East Asia had high protein content compared to other regions.

# 4.7 IDENTIFICATION OF ACCESSIONS WITH HIGH ANTHOCYANIN CONTENT

The chickpea reference set along with five check cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) were used to estimate anthocyanin content by using High Performance Liquid Chromatography (HPLC) at ICRISAT, Patancheru, Andhra Pradesh. The mean anthocyanin content was 1.55 for anthocyanins extracted with acidified methanol and 0.38 for anthocyanins extracted with methanol. The accessions ICC 10939 ( $3.89 \text{ A550g}^{-1}$ ), ICC 4533 ( $3.20 \text{ A550g}^{-1}$ ), ICC 5639 ( $3.08 \text{ A550g}^{-1}$ ), ICC 7272 ( $2.60 \text{ A550g}^{-1}$ ) and ICC 8058 ( $1.84 \text{ A550g}^{-1}$ ) recorded higher anthocyanin content extracted with methanol than the control cultivar L550 recorded highest anthocyanin content ( $1.26 \pm 0.14$ ). The accessions ICC 3892 ( $5.25 \text{ A550g}^{-1}$ ), ICC 11498 ( $4.48 \text{ A550g}^{-1}$ ), ICC 7052 ( $4.08 \text{ A550g}^{-1}$ ), ICC 13524 ( $3.90 \text{ A550g}^{-1}$ ) and ICC 16796 ( $3.64 \text{ A550g}^{-1}$ ) recorded higher anthocyanin content extracted with acidified methanol than the control cultivar L550 with acidified methanol than the control cultivar L550 ( $3.90 \text{ A550g}^{-1}$ ) and ICC 16796 ( $3.64 \text{ A550g}^{-1}$ ) recorded higher anthocyanin content extracted with acidified methanol than the control cultivar L550 ( $3.64 \text{ A550g}^{-1}$ ) recorded higher anthocyanin content extracted with acidified methanol than the control cultivar L550 ( $3.64 \text{ A550g}^{-1}$ ) recorded higher anthocyanin content extracted with acidified methanol than the control cultivar G 130 ( $2.68 \pm 0.41$ ).

The accessions with low acidified methanol anthocyanin content were, ICC 1446, ICC 16374, ICC 2884, ICC 6875 and ICC 7554 (0.25 A550g<sup>-1</sup>). Most of the desi accessions from South and East Asia showed high anthocyanin content extracted with methanol and acidified methanol. West Asian accessions showed high anthocyanin content in desi accessions when compared to other regions.

# 4.8 IDENTIFICATION OF TRAIT SPECIFIC GERMPLASM

By evaluating the chickpea reference set over five environments 2006/07, 2007/08, 2008/09 post-rainy, 2008/09 winter at ICRISAT and 2008/09 post-rainy at UAS, Dharwad, identified a few accessions performed repeatedly better than the best control cultivar for the particular trait(s) in all environments. The number of accessions identified specific for traits, were 2 accessions for early flowering, 11 accessions for early maturing, 17 for more seeds per pod, 35 for more pods per plant, one with more yield per plant, 19 with high 100-seed weight, 119 for high plot yield, 89 for per day productivity, 20 heat tolerant, 13 with high root depth, 42 with high shoot dry weight, 40 with high root dry weight, 11 with high root to total plant dry weight ratio (R-T%), 33 accessions with high root length, 6 accessions for root length

density, twenty five with minimum damage rate to pod borer, 17 with lowest larval survival%, 3 accessions with minimum unit larval weights, 38 with high protein and 40 accessions with high anthocyanin content (Table 43). Extensive evaluation of these accessions in different locations may be useful to reconfirm their genetic worth and use in crop improvement.

#### 4.9 MOLECULAR DIVERSITY IN CHICKPEA REFERENCE SET

In the present study, genotypic diversity and population structure of chickpea reference set was dissected by using 91 polymorphic SSR markers allelic data. The experiment was carried out in different steps and the results are briefly described under the following sub titles,

- 1. Protocol optimization and marker selection
- 2. Genotyping and quality index of markers
- 3. Molecular diversity and population structure of chickpea reference set

4. Identification of allelic variation associated with beneficial traits using association mapping in the reference set of chickpea

#### 4.9.1 Protocol optimization and marker selection

A total of 120 SSR markers mapped on 12 chickpea linkage groups (Winter *et al.*, 2000) were used for screening and PCR protocol optimization. So to get the basic idea of allele range, markers productivity and efficiency by genotyping in the chickpea reference set, these markers were optimized initially by Modified Taguchi method (Cobb and Clarkson, 1994). The optimization of PCR protocol was carried out with two most diverse chickpea accessions (Annigeri and ICCV2) identified from ICRISAT genebank (Plate 10).

Among the 120 markers, 100 markers produced strong and easily scorable polymorphic bands in two genotypes. The PCR products for these markers were analyzed through ABI 3130xl Gene Analyzer which produced first hand on information about the range of the alleles present in the two genotypes. Alleles close in size could be distinguished using different fluorescent dye labels. Equimolar primer concentrations in multiplex PCRs showed uneven amplification in some markers. Similar levels of amplification of each marker was obtained by decreasing the quantity of primer for the strongly amplified fragments, increasing the amount of primers for the poorly amplified fragments and adjusting the concentration of the remaining PCR reagents accordingly. To increase the efficiency of the genotyping,

markers with different labels and allelic range were grouped as a set of multiplex and 33 post PCR multiplex were made. From these 100 markers, based on high polymorphism and amplification rate, 91 SSR markers were selected and 26 multiplex were made to increase the efficiency of genotyping of entire reference set. Raw allelic data was binned through AlleloBin (Indury and Cardon, 1997) to get perfect allele calls based on the repeat length of the marker (Plate 11).

#### 4.9.2 Molecular diversity of Chickpea reference set

#### 4.9.2.1 Allelic richness and diversity in reference set

The ninety one SSR markers detected a total of 2411 alleles in 300 reference set accessions. The number of alleles per locus ranged from 3 (CaSTMS20) to 61 (TS5), with an average of 26.45 alleles per locus (Table 44). The polymorphic information content (PIC) values ranged from 0.021 (CaSTMS20) to 0.969 (TA176), with an average of 0.809. Most of the markers had high PIC (< 4), whereas markers TAA57 (0.166), CaSTMS13 (0.291), TA108 (0.361) and CaSTMS23 (0.392) showed low polymorphism. Gene diversity is defined as the probability that two randomly chosen alleles from the population are different. It varied from 0.021 (CaSTMS20) to 0.969 (TA176) with an average of 0.825 in the reference set. Distribution of number of alleles per locus among 91 SSR markers used for genotyping chickpea reference set id represented in Figure 8.

Significant and positive relationships was observed between allele size range and the amount of variation at SSR loci (as measured by alleles per locus and gene diversity) which indicate that SSR loci with large allele range (resulting from large number of SSR units) show greater variation, and agree with the idea that replication slippage plays an important role in the generation of new alleles at SSR loci.

#### 4.9.2.2 Heterozygosity in germplasm accessions

Chickpea is a self pollinated crop. Moreover, in this study, a single plant from each accession was harvested and parts of the seeds obtained from such plants were sown in field to raise seedlings for DNA extraction. Extreme care was taken to avoid inadvertent seed mixtures. In spite of this, a wide range of heterozygosity (%) was detected in the investigated materials, from 0.00 % to 2.87 %, with an average of 0.15 %. Most of the SSR loci detected no heterozygosity, while the markers TS45, TA64, TA28 detected >1% while TS62, TA53, TA72, detected >2%, TA113, TA71, TA117 detected <2% heterozygosity. A large collection of landraces was involved in this

study and it is possible that these accessions still possess some residual heterozygosity at least at some SSR loci reported. A landrace is defined as an autochthonous (primitive) variety with a high capacity to tolerate biotic and abiotic stresses, resulting in high yield stability and an intermediate yield level under a low input agricultural system (Zeven, 1998) (Table 45).

#### 4.9.2.3 Biological and geographical diversity in the chickpea reference set

Biologically, the 300 (2411 alleles) accessions were grouped into cultivated (1978 alleles) and wild types (433 alleles) and among cultivated accessions, desi (2009 alleles), kabuli (1572 alleles) and pea (544 alleles) types. Geographically, West Asia (1578 alleles) showed maximum alleles followed by South and East Asia (1489 alleles), Mediterranean (1401 alleles), Africa (755 alleles), Russian Federation (333 alleles), North America (286 alleles), South America (239 alleles), Europe (179 alleles) and accessions with unknown biological status (316 alleles). Though cultivated accessions showed similar mean gene diversity, the desi accessions as a group were genetically more diverse (high range of gene diversity, 0.000 - 0.97) than other cultivated such as kabuli and pea types (Tables 46) Interestingly, accessions from West Asia (0.00 – 0.96), South and East Asia (0.00 – 0.96), Mediterranean (0.11 – 0.96) and Africa (0.00 – 0.92) were genetically more diverse (high range in mean gene diversity) than other regions.

This study detected many rare, common, and frequent alleles within each group. A total of 2299 alleles were detected in cultivated types and 433 alleles in wild types of chickpea reference set, of which 1980 were unique in cultivated, 114 in wild accessions and 319 alleles were common among wild and cultivated. In the cultivated group, desi accessions contained the largest number of unique alleles (864) followed by kabuli (836) and pea type (52).

The PIC values ranged from 0.00 to 0.97 in desi, 0.00 to 0.95 in kabuli and 0.00 to 0.89 with an average of 0.73 in pea type, 0.80 in desi and 0.79 in kabuli. Gene diversity averaged 0.82, ranging from 0.00 to 0.97 in desi, whereas in kabuli accessions, it varied from 0.00 to 0.96 with an average of 0.81. In pea type, the gene diversity ranged from 0.00 to 0.89 with an average 0.73. Desi types exhibited maximum mean gene diversity and PIC than kabuli and pea types.

The mean PIC was higher in the accessions from West Asia and Mediterranean regions (0.800) followed by South and East Asia (0.770) and Africa (0.734), whereas

low PIC was observed in the accessions from Europe (0.329). The other regions, with mean PIC value were Russian Federation (0.582), unknown origin (0.542), North America (0.502) and South America (0.464) (Table 46).

## 4.9.2.5 Rare alleles in the reference set

Alleles were considered as rare alleles, when the frequency is less than 1% in the population. These rare alleles may possess genes responsible for specific traits like pest and disease resistance and tolerance to drought. In the reference set 2424 rare alleles were observed from 91 SSR markers. It ranged from 2.0 to 90.0. The markers TS5 (90 alleles), TR1 (82 alleles), TR43 (76 alleles), TR7 (74 alleles) showed high number of rare alleles, whereas markers GAA43, TAA57 (each 2 rare alleles) showed low number of rare alleles. The rare allele loci, number of rare alleles, observed frequency of each rare allele of reference set were presented in the Table 46

#### 4.9.3 Unweighted neighbor-joining tree

Neighbour-joining tree based on simple matching dissimilarity matrix between 297 accessions of the chickpea reference set was broadly clustered accessions into four clusters namely CI to CIV respectively. CI contained 89 accessions of which 64 were desi type, which is dominant in this cluster whereas 24 were kabuli, one accession was pea type. CII consisted of 30 accessions, desi type dominated with 20 accessions along with 9 kabuli and one pea type accession. CIII represented by 87 accessions dominated with 76 desi type of accessions followed by 9 kabuli and two pea type accession. CIV consisted of 91 accessions dominated by 46 kabuli accessions along with 34 desi, 7 pea and 4 wild accessions. The results from the neighbor-joining phylogenetic tree corresponded well with the classification based on three biological statuses of chickpea. CI, CII, CIII dominated with desi type of accessions whereas CIV dominated with kabuli accessions. (Table 47, Figure 9a, 9b).

#### 4.9.3.1 Allelic richness and genetic diversity

The ninety one SSR markers detected a total of 1601 alleles in CI, 1006 in CII, 1547 in CIII and 1715 in CIV. The number of alleles ranged from 1-40 in CI, 1-19 in CII, 1-43 in CIII and 2-37 in CIV with an average of 17.6, 11.1, 17.0 and 18.8 in CI to CIV respectively. The polymorphic information content was 0.961 in CI, 0.929 in CII, 0.960 in CIII and 0.957 in CIV. Gene diversity was 0.962 in CI, 0.933 in CII, 0.962 in CIII and 0.959 in CIV. Heterozygosity was maximum in CII (0.071) compared to CI (0.023), CIII (0.047) and CIV (0.049). The allelic composition revealed the

predominance of common allele (10559 in CI, 3432 in CII, 10145 in CIII and 10937 in CIV) as compared to most frequent alleles (3789, 1456, 3915 and 3628 in CI to CIV respectively). Rare alleles were not seen only in CII whereas in CI (2), CIII (1), CIV (7) alleles were seen (Table 47).

#### 4.9.3.2 Geographical diversity

Majority of the accessions in the reference set were from Asia and Africa. Clustering did not follow the country of origin clearly. But in some clusters accessions from some particular origin were predominant (Table 48)

Cluster I had accessions predominantly from South Asia accessions (43 accessions) followed by West Asia (14 accessions), Africa (13 accessions) and Mediterranean region (7 accessions). The limited number of accessions from North America (3 accessions), South America (3 accessions), Russian Federation (2 accessions), Europe (1 accession), and unknown origin (3 accessions) were spreaded through out the clusters.

Cluster II was dominated by accessions from South Asian (12 accessions) followed by Mediterranean region (7 accessions), Africa (5 accessions), West Asia (14 accessions) and Europe (1 accession).

Cluster III had predominantly accessions from West Asia (43 accessions), followed by South Asian accessions (34 accessions), Mediterranean region (6 accessions), Russian Federation (2 accessions), North America and accessions with unknown origin (1 accession each). Accessions from Africa, South America, and Europe were not represented in cluster III.

Cluster IV had predominantly accessions from West Asia and Mediterranean region (32 accessions each), followed by South Asian accessions (16 accessions), Africa (3 accessions), Russian Federation, North America and accessions with unknown origin (2 accessions each), South America, and Europe (I accession each) were represented in cluster III.

#### 4.9.3.3 Factorial analysis

The factorial analysis based on biological status, has been given in Figure 4. It illustrates the high divergence among genotypes of the reference set based on biological status. The desi type accessions clustered together (quadrant I and II) and wild were in another cluster (quadrant III and IV), kabuli accessions were clustered in quadrant III and IV. Accessions with Pea seed type were distributed in overall the

four quadrants.

#### **4.9.4 Population Structure analysis**

STRUCTURE analysis can help to identify the presence of population structure and also distinct genetic population, assigning the individuals to populations and identify migrants and admixed individuals. Analysis of population structure using 91 SSR markers provided evidence for the presence of significant population structure in the chickpea reference set. The k value was determined by LnP(D) in STRUCTURE output and an *ad hoc* statistic  $\Delta k$  based on the rate of change in LnP(D) between successive k. The final subpopulation were determined based on rate of change in LnP(D) between successive k, stability of grouping pattern across five run and germplasm information about the material under study (Figure10 and Table 49). Based on this information, k=13 chosen as the optimal grouping and burn-in period of 1,00,000 and 2,00,000 replications was selected to assign the posterior membership coefficient (Q) to each accessions. A graphical bar plot was than generated with the posterior membership coefficient were presented in Figure 6. Biological race and geographic origin information was used to assist with the clustering. The clustering matrices (Q) of closely related clusters/ subdivisions using Bayesian approach, was obtained from STRUCTURE and used in association mapping

	<b>19</b> Average logarithm of the pro ea reference set	obability of	data likelihoods ( <i>LnP</i> ( <i>D</i> )) of
K	Average <i>Ln P(D)</i>	K	Average $Ln P(D)$
10	-103288	15	-104828
11	-101982	16	-101299
12	-100232	17	-97693.2
13	-99532.7	18	-96499.3
14	-101130	19	99513.1

Table 50 Overall proportion of membership of the sample in each of the 13subpopulations													
Inferred subpopulations													
SP1	SP2	SP3	SP4	SP5	SP6	SP7							
						0.042							
0.145	0.082	0.074	0.061	0.070	0.094								
SP8	SP9	SP10	SP11	SP12	SP13								
0.154	0.046	0.064	0.042	0.041	0.083								

In the present study, population structure was dissected for 300 accessions by using 91SSR markers allelic data by using the software program STRUCTURE. The

reference set was grouped in to thirteen subpopulations (Figures 12a, 12b). Thus, the thirteen subpopulations as inferred from the STRUCTURE analysis denoted as SP1 (Red), SP2 (Green), SP3 (Dark Blue), SP4 (Yellow), SP5 (Pink), SP6 (Sea blue), SP7 (Brown), SP8 (Maroonish brown), SP9 (Light brown), SP10 (Dark sea blue), SP11 (blue), SP12 (Light green), SP13 (Grey) respectively and SP refers to subpopulation. Overall proportion of membership of the sample in each of the four subpopulations is 0.145, 0.082, 0.074, 0.061, 0.070, 0.094, 0.042, 0.154, 0.046, 0.064, 0.042, 0.041 and 0.083 respectively (Table 50).

The subpopulation 1 contained with 48 accessions, of which kabuli dominated with 28 accessions followed by desi with 17 accessions, pea with 2 accessions and one accession was wild. Geographically, Mediterranean region - 15 accessions, West Asia- 7, Africa -5, South East Asia, Russian Federation and unknown origin - 3 each, North America and Europe by 1 each were represented in this subpopulation.

Subpopulation 2 contained 25 accessions, of which desi dominated with 24 accessions and kabuli by 1 accession. Geographically, South East Asian region - 15 accessions, West Asia - 6, Mediterranean region - 3 and Africa only one accession were represented in this subpopulation.

Subpopulation 3 represented with 24 accessions, of which desi dominated with 17 accessions followed by kabuli with 6 accessions and pea with one accession. Geographically, South East Asia – 12 accessions, West Asia-6, Mediterranean region -3, Africa, South America and unknown accessions – 1 each were represented in this subpopulation.

Subpopulation 4 contained only 14 desi accessions. Geographically, South East Asia – 8 accessions, West Asia-4, Mediterranean region and Africa- 1 each were represented in this subpopulation.

Subpopulation 5 contained only 15 desi accessions. Geographically - South East Asia – 12 accessions and West Asia-3 were represented in this subpopulation.

Subpopulation 6 contained with 28 accessions of which kabuli dominated with 23 accessions followed by desi with 5accessions. Geographically - Mediterranean region -9 accessions, West Asia-6, Africa, South East Asia and North America- 3 each, South America -2, Europe and unknown origin -1 each were represented in this subpopulation.

Subpopulation 7 contained 13 accessions, of which desi dominated with 9 accessions followed by 4 Wild accessions. Geographically - Mediterranean region -7

accessions, West Asia-6 accessions. Maximum numbers of wild accessions are represented in subpopulation 7.

Subpopulation 8 contained highest number of 56 accessions, of which desi dominated with 33 accessions followed by kabuli with 17 accessions, pea with 5 accessions and one accession was wild. Geographically- South and East Asia-23 accessions, Mediterranean region- 13, West Asia -9, Africa-9, South America and Russian Federation – 1 each were represented in this subpopulation.

Subpopulation 9 contained 12 accessions, of which desi dominated with 10 accessions followed by kabuli and pea with one accession each. Geographically- West Asia - 11 accessions and North America - 1 accession were represented in this subpopulation.

Subpopulation 10 with 15 accessions, of which kabuli dominated with 7 accessions followed by desi with 5 accessions, pea 2 with accessions and one accession was wild. Geographically–Mediterranean region – 3 accessions, West Asia-7, South East Asia-2, Russian Federation, North America and unknown origin – 1 each were represented in this subpopulation.

Subpopulation 11 contained only 9 desi accessions. Geographically- South and East Asia-8 accessions and West Asia -1 accession were represented in this subpopulation. Subpopulation 12 contained only 12 desi accessions. Geographically- West Asia -10 accession, South and East Asia and Mediterranean region- 1 accession each were represented in this subpopulation.

Subpopulation 13 contained 29 accessions, of which desi dominated with 24 accessions followed by kabuli with 5 accessions. Geographically - South and East Asia-18 accessions, West Asia -7, Africa, Europe, Mediterranean region and Russian Federation-1 accession each were represented in this subpopulation.

#### 4.9.4.1 Genetic diversity of subpopulations

The reference set was grouped in to thirteen subpopulations with 91SSR markers allelic data by using the software program STRUCTURE. The 91 SSR markers detected a total of 1199 alleles in SP1, 720 in SP2, 778 in SP3, 483 in SP4, 527 in SP5, 803 in SP6, 749 in SP7, 1301 in SP8, 544 in SP9, 574 in SP10, 348 in SP11, 428 in SP12 and 759 in Sp13. Highest number of alleles was detected by SP8 with a mean of 11.4, which ranged from (0 to 26). Lowest number of alleles was detected by SP11 with a mean of 3.1, which ranged from (0-7). PIC values ranged from 0.00 to 0.946 in SP1, 0-0.930 in SP2, 0-0.922 in SP3 and 0-0.891 in SP4, 0-0.877 in SP5, 0-0.945

in SP6, 0-0.902 in , 0-0.947 in SP8, 0-0.863 in SP9, 0-0.890 in SP10, 0-0.819 in SP11, 0-0.863 in SP12 and 0-0.947 in SP13, with an average 0.727, 0.649, 0.667, 0.535, 0.538, 0.653, 0.737, 0.715, 0.612, 0.693, 0.527, 0.517 and 0.690 in SP1 to SP13 respectively. Maximum mean PIC value was detected in SP8 and minimum in SP11 when compared with other sub-populations. Maximum mean gene diversity value was detected in SP7 (0.765) and minimum in SP4 (0.560) when compared with other sub-populations. The average number of alleles per locus and PIC were higher in SP8 compared to other sub-populations. Rare alleles are detected only in SP1 (32) and SP8 (2). Accessions from SP8 consist of 2 rare, 7087 common and 3881 most frequent alleles when compared with other sub-populations (Table 51). Graphical representation of allelic pattern across the population is represented in Figure 6.

#### 4.9.4.2 Genetic relationship among the population

Pairwise comparison on the basis of the values of  $F_{st}$  could be interpreted as standardized population distance between two populations. The pairwise  $F_{st}$  value in this study ranged from 0.102 between SP7 and SP7 to 0.362 between SP11 and SP5 with an average pairwise  $F_{st}$  of 0.206. The pairwise  $F_{st}$  was highest between SP11 and SP5 (0.362) followed by between SP11 and SP9 (0.349) (Table 51). The genetic distance data agreed with the  $F_{st}$  estimate with the mean genetic distance was 0.702. SP2 showed the lowest genetic distance with SP1 (0.172) and SP8 showed the lowest genetic distance with SP5 (0.267) whereas SP11 showed the greatest genetic distance with SP5 (1.391) followed by SP5 with SP4 (1.291) (Table 52-53 and Figure 12)

## 4.9.5 Analysis of molecular genetic variance between and within the subpopulations

The distribution of molecular genetic variation among and within the thirteen subpopulations of accessions was estimated by analysis of molecular variance, AMOVA (Table 54). AMOVA revealed that 20 per cent of the total variance was among the subpopulations, while 80 per cent was among individuals within the subpopulations. The same trend was observed when the AMOVA estimated based on three types of chickpea in reference set.

•	Table 54 Analysis of molecular variance (AMOVA) based on 13 subpopulations(SP1 to SP13) identified by software STRUCTURE												
Source Df SS MS Est. Var. %													
Among Pops	12	8995.045	749.587	28.322	20%								
Within Pops         287         33259.412         115.886         115.886         80%													

1					
	Total	299	42254.457	144.208	100%
	- • • • • •				

#### 4.9.6 Principal coordinates analysis (PCoA)

In this study, principal coordinate analysis and Unweighted neighbor-joining phylogenetic analysis was conducted to further assess the population subdivisions identified using STRUCTURE. The first three PCs explained 81.71 per cent of variation of which PC1 explained 36.48 per variation and PC2 explained 33.38 per cent of the SSR variation among the 300 accessions of chickpea reference set including five control cultivars. Plotting the first two PCs and colour coding genotypes based separated the chickpea reference set accessions into four clusters which was identified by STRUCTURE analysis (Table 55).

#### 4.10 ASSOCIATION ANALYSIS

#### 4.10.1 Association of markers in reference set with phenotypic traits

A general linear model (GLM) was implemented by using TASSEL 2.1 as suggested by Yu *et al.* (2006) to conduct the association analysis and to identify the SSR markers associated with the qualitative, quantitative and grain quality traits, resistance to pod borer and for traits related to drought tolerance in chickpea reference set based on population structure (Q matrix) and relatedness relationship. Each trait was represented by its mean of the two replications. Association analysis was carried for over five environments and over all the five environments. MTAs detected in pooled data were considered as reference, and were compared with the MTAs detected from individual environments. The results of association analysis using simple linear regression markers, and their association with traits, linkage group and position, Fvalue and probability and percentage of phenotypic variance explained by each MTA ( $\mathbb{R}^2$ ,%) and details of MTAs detected by pooling the five environments is presented below:

#### 4.10.1.1 Association of markers with Qualitative traits

Number of significant marker trait associations (MTAs) were 27 (  $P \le 0.001$ ) for qualitative traits involving 21 markers (Table 56), out of which 17 SSR markers were associated with one trait and 4 SSR markers were associated with more than one trait. Of which major MTAs (>20% phenotypic variation) detected were five (two for growth habit and three for seed surface). Maximum numbers of MTAs (5) were detected on chromosome number 6. Seed surface showed detected maximum number

of MTAs (12) whereas minimum number of MTAs was detected for dots on seed coat (1). No significant MTAs were detected for growth habit and seed color. Both Maximum and minimum phenotypic variation was observed for seed surface (22.71) and (6.4), respectively.

Five MTAs were detected for seed shape and were distributed on chromosome 1(TR20), 3(TR24), 5(TS35) and 6(TA22). One unmapped (CaSTMS9) MTA were detected for seed shape. Five MTAs were detected for flower color that were distributed on chromosome 4(TA2), 6(TA22) and 7(TA180, TA21, TS62). Four MTAs were detected for plant color and were distributed on chromosome 1(TA113), 4(TA2, TR20), and 8(TA159). Only one MTA was detected for dots on seed coat on chromosome 6(TA106).Twelve MTAs were detected for seed surface on chromosome 1(CaSTMS13, TA113), 2(TA96, TA27), 3(TA135), 4(TR20), 5(CaSTMS20, CaSTMS7), 6(TR40, TA22), and 13(GAA39). One unmapped (GAA58) MTAs was also detected for seed surface.

Overall the SSR markers, TA22 (Seed shape, flower color and seed surface) and TR20 (Seed shape, seed surface and plant color) detected three MTAs each, whereas TA113 (Plant color and seed surface) and TA2 (flower color and plant color) detected two MTAs each.

Of all the 27 significant MTAs ( $P \le 0.001$ ) detected in pooled analysis for 7 the qualitative traits, five were major MTAs (>20% phenotypic variation), of these two MTAs were detected for Growth habit (TS 35-23.6% and TA159-21.2%) and three MTAs were Seed surface (TR 40-22.7%, TR43-21.6%, TA176-20.2%).

#### 4.10.1.2 Association of markers with Quantitative traits

64 significant (P $\leq$ 0.001) MTAs were detected involving 49 SSR markers in E1, with maximum phenotypic diversity of 43.4% for anthocyanin content. 86 significant MTAs were detected involving 46 SSR markers in E2 and maximum phenotypic diversity of 42% for tertiary branches whereas in E3, 76 significant MTAs with 50 SSR markers and maximum phenotypic diversity of 42.9% for leaf area, in E4 74 significant MTAs with 52 SSR markers and maximum phenotypic diversity of 45.4% for apical secondary branches and in E5 56 significant MTAs with 44 SSR markers and maximum phenotypic diversity of 34.8% for plant width. Marker trait associations (MTAs) (P<=0.05, P<=0.01 & P<=0.001) detected for different Quantitative traits in the chickpea reference set in five environments and in pooled analysis are represented in Table 57. Number of significant MTAs detected in individual environments from E1 to E5 is represented in Tables 58-62.

In pooled analysis, number of significant MTAs were 76 (P $\leq$ 0.001) for quantitative traits (Table 63), of which major MTAs (>20% phenotypic variation) detected were 39. Flowering duration detected highest maximum number of MTAs (14) and maximum number of major MTAs (7), whereas apical primary branches and seeds per pod (1) detected minimum number of MTAs. Maximum phenotypic variation was observed for tertiary branches (37.4%) and minimum was observed for per day productivity (4.13%).

#### Traits variability in different environments

For the purpose of summarization of results and discussion, the traits studied were grouped into three broad categories based on the life cycle of the chickpea plant (Gowda *et al.*, 2011).

**Vegetative traits:** plant height, plant width, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches and tertiary branches;

**Reproductive traits:** days to 50 percent flowering, flowering duration, days to maturity;

**Yield and yield component traits**: pods per plant, seeds per pod, 100-seed weight, grain yield and per day productivity.

4.10.1.2.1 Vegetative traits

#### Number of MTAs in pooled analysis has been presented:

#### **Plant height**

Eight MTAs were detected for plant height and were distributed on chromosomes 1(TR43), 4(TA132), 5(TS43), 6(GA9), 7(TS46, TA28), 8(TA25) and 13 (GAA39).

#### **Plant width**

Five MTAs were detected for plant width that were distributed on chromosomes 1(CaSTMS21), 7(TA180, TA78) and 15 (CaSTMS25). One unmapped (GA22) MTA s was detected for plant width.

#### Apical primary branches

Single MTA on chromosome 6(TS24) was detected for apical primary branches.

#### **Basal secondary branches**

Two MTAs were detected for basal secondary branches on chromosome 1 (CaSTMS 13) and 6(TS24).

#### **Apical secondary branches**

Four MTAs were detected for apical secondary branches on chromosome 1 (GAA40), 2(TA53) and 6 (TS24, CaSTMS2).

#### **Tertiary branches**

Nine MTAs were detected for tertiary branches on chromosomes 1 (TR43, CaSTMS21), 3(TS5, TAA194), 6(TR1, CaSTMS2), 7(TS46, TA78) and 11(CaSTMS12). Phenotypic variation was observed to be highest (34.28%) for this trait than any other quantitative traits using the marker TA78.

#### 4.10.1.2.2 Reproductive traits

#### Days to 50 percent flowering

Eight MTAs were detected for days to 50% flowering on chromosomes 2 (TAA58, TA27), 3(TA64, TA125), 4 (TS54, TA130) and 5(CaSTMS7, TR29)

#### **Flowering duration**

Fourteen MTAs were detected for flowering duration on chromosomes 2 (TA110, TA27), 4 (TS54, TA72, TA132), 5(CaSTMS20, TA5, TS35, CaSTMS7), 6(TR40), 8(TA159), 13(TS83) and 15(CaSTMS25). One unmapped (GAA43) MTA was also detected for flowering duration using GLM.Flowering duration detected highest number of MTAs than all other quantitative traits in chickpea reference set when pooled.

#### Days to maturity

Two MTAs were detected for days to maturity on chromosomes 4 (TA130) and 5(CaSTMS7).

#### 4.10.1.2.3 Yield and yield component traits

#### Pods per plant

Four MTAs were detected for pods per plant on chromosomes 3(CaSTMS5), 4(TAA57), 6(TA106), and 7(TAA58).

#### Seeds per pod

One MTA on chromosome 2 (TA27) was detected for seeds per pod.

#### Yield per plant

Five MTAs were detected for yield per plant that were distributed on chromosomes 2 (TA96), 3(TA142) and 7(TS46, TA117). One unmapped (CaSTMS9) MTA was detected using GLM for yield per plant.

#### 100-seed weight

Five MTAs were detected for 100-seed weight on chromosomes 1 (CaSTMS21), 3(TR56) and 6(TS24, TA22, TA106).

#### **Plot yield**

Four MTAs were detected for plot yield on chromosomes 3(TA108) and 5(CaSTMS7, CaSTMS20, TS35).

#### Per day productivity

Four MTAs were detected for plot yield on chromosomes 3(TA108) and 5(CaSTMS7, CaSTMS20, TS35).

Of all the 76 significant MTAs ( $P \le 0.001$ ) detected in pooled analysis for 17 quantitative traits, 39 were major MTAs (>20% phenotypic variation), of these two major MTAs were detected for Days to 50 percent flowering and apical secondary branches, seven for flowering duration and tertiary branches, six each for plant height, three for plant width, one each for apical primary branches, basal secondary branches and plot yield, three for 100-seed weight and four for yield per plant. Maximum phenotypic variation was observed for tertiary branches (37.4%). TS24 detected maximum of 4 MTAs among the 17 quantitative traits.

## 4.10.1.3 Association of markers with SPAD Chlorophyll Meter Readings (SCMR)

In pooled analysis, only one significant MTAs were detected ( $P \le 0.001$ ) for SCMR (Table 63), distributed on chromosome 7 (TAA 59) and one more for SLA and is distributed on chromosome 13 (TS83) and phenotypic variation was observed to be 16.95 and 18.32 % respectively for both traits using GLM

#### 4.10.1.4 Association of markers with quality traits

#### 4.10.1.4.1 Association of markers with protein related traits

In pooled analysis, only one MTA was detected for protein content ( $P \le 0.001$ ) on chromosome 13(GA26) and phenotypic variation was observed to be 11.04% using GLM (Table 63).

#### 4.10.1.5 Association of markers with pod borer resistance traits

In pooled analysis, two significant MTAs were detected ( $P \le 0.001$ ) with only one trait (Damage rating %) related to Helicoverpa resistance at  $P \le 0.001$ . No MTAs were detected for Leaf damage score and larval survival percentage. Two MTAs were distributed on chromosomes, 3(CaSTMS23) and 4(TA132), and phenotypic variation was 7.09% and 19.63 % respectively for these two markers.

#### 4.10.1.6 Association of markers with drought related traits

In pooled analysis, numbers of significant MTAs detected were 21 (P $\leq$ 0.001) (Table 63), for drought tolerance related root traits and maximum numbers of MTAs (7) were detected for shoot dry weight and total dry weight. Minimum numbers of MTAs were detected for root surface area and root volume (1 each). Maximum phenotypic variation was expected by MTAs for root length density (30%) with TAA59 on chromosome 7 and minimum was for total plant dry weight ratio (7.9%) with CaSTMS 9.

#### Number of MTAs in pooled analysis has been presented:

#### **Root Traits Association**

#### Shoot dry weight

Seven MTAs were detected for shoot dry weight on chromosomes 1 (TA113), 3(CaSTMS5), 5(TA20, TaaSH) and 6(TA22). One unmapped (CaSTMS9) MTAs was detected for the trait shoot dry weight using GLM.

#### Root dry weight

Three MTAs were detected for root dry weight on chromosomes 1 (TA20), 3(CaSTMS5) and 6(TA22).

#### Total plant dry weight

Seven MTAs were detected for total plant dry weight on chromosomes 1 (TA113, 3(CaSTMS5), 5(TA20, TaaSH), 6(TA22) and 13(GA26) One unmapped (CaSTMS9) MTA was detected for the trait total plant dry weight.

#### **Root Length Density**

Two MTAs each were detected for root length density on chromosomes 4(TA130) and 7(TAA59). Maximum phenotypic variation was observed for root length density (30%) with the marker TAA59.

#### **Root surface area and Root volume**

Only MTA was detected for both root surface area and root volume on chromosomes 3(CaSTMS5) and chromosome 6(TA22) respectively.

Of all the 21 significant MTAs ( $P \le 0.001$ ) detected in pooled analysis for the10 drought tolerance related root traits 8 were major MTAs (>20% phenotypic variation), of these one each was detected for shoot dry weight and root volume and two major MTAs each were detected for root dry weight, total dry weight and root length density. Maximum phenotypic variation was observed for root length density (30%) with the marker TAA59. TA25 and TA22 detected maximum of 3 major MTAs each among the 8 major significant root traits.

# 4.10.1.7 Association of markers with more than one trait in reference set with quantitative, quality (anthocyanin and protein traits), pod borer resistant and drought related root traits:

In pooled analysis, a total of 27 markers were found to be associated with more than one trait among quantitative, quality, pod borer resistant and drought related root traits and maximum of the these were detected on chromosome number 1 and 5 (5 each) (Table 64). 5 traits each were found to be associated with the 3 markers CaSTMS 5 (pods per plant, shoot dry weight, root dry weight, total dry weight and root surface area), CaSTMS 7 (productivity per day, days to 50 percent flowering, flowering duration, days to maturity and plot yield) and TA22 (100-seed weight, shoot dry weight, root dry weight, root dry weight, not dry weight, shoot dry weight, root dry weight, not dry weight, shoot dry weight, root dry weight, not dry we

Two markers, TA20 (Leaf area, shoot dry weight, root dry weight and total dry weight) and TS24 (apical primary branches, basal secondary branches, apical secondary branches and 100-seed weight) were found to be associated with 4 traits each

Eleven markers, CaSTMS21 (Tertiary branches, 100-seed weight and plant width), TA27 and TS54 (days to 50 percent flowering, flowering duration and seed per pod), TA108 (plot yield, flowering duration and per day productivity), TA132, TS35 and CaSTMS20 (flowering duration, plant height and damage rate%), TA130 (days to 50% flowering, days to maturity and root length density), TS46 (plant height, tertiary branches and yield per plant), GA26 (protein %, shoot dry weight and total dry

weight) and CaSTMS 9 (yield per plant, shoot dry weight and total dry weight) were found to be associated with 3 traits each.

Eleven markers, TA113, TaaSH (shoot dry weight and total dry weight), TA8 (Leaf Dry weight and leaf area), TR43 (plant height and tertiary branches), TA106 (pods per plant and 100-seed weight), CaSTMS2 (apical secondary and tertiary branches), TAA59 (Root length density and SPAD), TAA58 (pods per plant and days to 50 percent flowering), TA78 (plant width and tertiary branches), TS83 (flowering duration and Specific leaf area) and CaSTMS25 (plant width and flowering duration) were found to be associated with 2 traits each.

Hence, these most significant MTAs were believed to be associated with colocalized/pleiotropic QTLs. The co-localization of specific genes/QTLs/markers could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response and pod borer resistance traits. The presence of several colocalized/pleiotropic QTLs verified the complex quantitative nature of drought tolerance, pod borer resistance in chickpea and allowed the identification of some important genomic regions for traits related to high yield, good protein percent, drought tolerance and resistance to pod borer. The markers associated with more than one trait may be efficiently utilized in improvement of more than one trait simultaneously through marker assisted selection (MAS).

### Tables

			Тур	es	-				G	eographical re	gions		_	
Trait	Entire	Desi	Kabuli	Pea	Wild	Afric a	Euro pe	Mediter ranean	North America	Russian Federation	South & East Asia	South America	Unkn own	West Asia
Growth Habit														
Erect	6 (2.0%)	1(0.5%)	4(4.5%)	1(9.0%)	-	-	1	3	-	1	-	-	-	1
Prostrate	1 (0.3%)	1(0.5%)	-	-	-	-	-	-	-	-	1	-	-	-
Semi-erect	187 (62.3%)	118(60.8%)	64(72.7%)	5(45.5%)	-	13	2	35	5	5	47	4	5	71
Semi-Spreading	100 (33.4%)	74(38.1)	17(19.3%)	5(45.5%)	4(57.1%)	8	-	12	1	-	57	-	1	21
Spreading	6 (2.0%)	-	3(3.4%)	-	3(42.9%)	-	-	6	-	-	-	-	-	-
Plant pigmentation	n													
High- anthocyanin	6(2.0%)	5(2.6%)	_	1(9.1%)	_	3	-	-	_	-	2	_	-	1
Low-anthocyanin	160 (53.3%)	153(78.9%)	-	1(9.1%)	6(85.7%)	13	-	16	1	1	90	-	1	38
No-anthocyanin	134 (44.7%)	36(18.5%)	88(100.0%)	9(81.8%)	1(14.3%)	5	3	40	5	5	13	4	5	54
Flower color														
Light pink	30 (10.0%)	25(12.9%)	1(1.1%)	4(36.4%)	-	-	1	1	1	-	1	-	1	25
Pink	171 (57.0%)	162(83.5%)	-	2(18.2%)	7(100.0%)	17	-	19	1	1	93	-	1	39
Very light pink	3 (1.0%)	3(1.5%)	-	-	-	1	-	1	-	-	1	-	-	-
White	95 (31.7%)	3(1.5%)	87(98.9%)	5(45.5%)		3	2	34	4	5	10	4	4	29
White with pink strips	1(0.3%)	1(0.5%)	-	-	-	-	-	1	-	-	-	-	-	-
Seed color														
Beige	90 (30.0%)	1(0.5%)	87(98.9%)	2(18.2%)	-	2	2	34	4	3	7	4	4	30
Black	23 (7.7%)	23(11.9%)	-	-	-	2	-	2	-	1	4	-	-	14
Brown	2 (0.7%)	-	-	1(9.1%)	1(14.3%)	-	-	1	-	-	1	-	-	-
brown beige	22 (7.3%)	21(10.8%)	-	1(9.1%)	-	-	1	3	1	-	-	-	-	17
Dark brown	14 (4.7%)	11(5.7%)	-	-	3(42.9%)	-	-	6	-	-	6	-	-	2
Green	2 (0.7%)	2(1.0%)	-	-	-	-	-	-	-	-	1	-	-	1
Greyish brown	3 (1.0%)	-	-	-	3(42.9%)	-	-	3	-	-	-	-	-	-
Light brown	10 (3.3%)	10(5.2%)	-	-	-	1	-	-	-	-	7	-	-	2
Light green	1 (0.3%)	1(0.5%)	-	-	-	-	-	-	-	-	1	-	-	-

**Table 12:** Frequency distribution of accessions for various qualitative traits in different seed types and geographical regions in the Chickpea reference set

			Тур	es					G	eographical re	gions			
Trait	Entire	Desi	Kabuli	Pea	Wild	Afric a	Euro pe	Mediter ranean	North America	Russian Federation	South & East Asia	South America	Unkn own	West Asia
Light orange	3 (1.0%)	2(1.0%)	-	1(9.1%)	-	1	-	-	-	-	1	-	-	1
Light yellow	9 (3%)	8(4.1%)	-	1(9.1%)	-	1	-	-	1	1	2	-	1	3
Orange	1 (0.3%)	-	-	1(9.1%)	-	-	-	-	-	-	1	-	-	-
Reddish brown	1 (0.3%)	-	-	1(9.1%)	-	-	-	-	-	-	1	-	-	-
Salmon brown	3 (1.0%)	-	1(1.1%)	2(18.2%)	-	-	-	-	-	-	-	-	-	3
Yellow	5 (1.7%)	5(2.6%)	-	-	-	2	-	-	-	1	2	-	-	-
Yellow beige	3 (1.0%)	3(1.5%)	-	-	-	-	-	1	-	-	-	-	1	1
Yellow brown	108 (36%)	107(55.2%)	-	1(9.1%)	-	12		6	-	-	71	-	-	19
Seed shape														
Angular	201 (67.0%)	194(100.0%)	-	-	7(100.0%)	19	1	22	2	2	93	-	2	60
Owl's Shape	88 (29.3%)	-	88(100.0%)	-	-	2	1	33	4	3	7	4	4	30
pea	11 (3.7%)	-	-	11(100.0%)	-	-	1	1	-	1	5		-	3
Seed dots														
Absent	156 (52.0%)	55(28.4%)	88(100.0%)	10(90.9%)	3(42.9%)	6	2	41	5	6	21	4	4	67
Present	144 (48.0%)	139(71.6%)	-	1(9.1%)	4(57.1%)	15	1	15	1	-	84	-	2	26
Seed Surface														
Rough	198 (66.0%)	189(97.4%)	4(4.5%)	5(45.5%)	-	19	1	16	2	2	94	1	2	61
Smooth	90 (30.0%)	-	84(95.5%)	6(54.5%)	-	2	2	33	4	4	7	3	4	31
Tuberculated	12 (4.0%)	5(2.6%)	-	-	7(100.0%)	-	-	7	-	-	4	-	-	1

Numbers in parenthesis indicate percentage of accessions in each group

	E1		E2		E3		E4	1	E5		Pool	ed	Poole	ed
Trait	$\sigma^2 g$	SE	$\sigma^2 g$	SE	$\sigma^2 g$	SE	$\sigma^2 g x e$	SE						
DF	30.89**	3.12	42.42**	3.66	38.24**	3.37	44.55**	3.64	40.78**	3.95	38.04**	3.17	0.81**	0.26
FD	5.80**	1.08	1.79**	0.17	0.65*	0.28	3.11**	0.27	1.81**	0.31	2.04**	0.20	0.98**	0.10
PLHT	46.48**	5.15	44.86**	4.09	64.33**	5.32	50.39**	4.12	46.94**	4.10	51.12**	4.26	1.71**	0.31
PLWD	7.366**	0.80	10.26**	0.99	8.169**	0.70	14.11**	1.25	19.07**	1.77	7.29**	0.69	3.22**	0.24
DGF	16.92**	2.91	20.60**	1.83	21.83**	2.06	30.02**	2.50	35.73**	4.25	18.34**	1.68	5.13**	0.48
DM	14.69**	2.40	22.34**	2.06	20.75**	1.77	29.29**	2.42	42.57**	3.77	17.06**	1.59	6.87**	0.48
BPB	0.09**	0.04	0.23**	0.02	0.31**	0.03	0.36**	0.03	0.25**	0.03	0.11**	0.01	0.12**	0.01
APB	0.28**	0.04	0.43**	0.04	0.64**	0.05	0.42**	0.03	0.38**	0.04	0.17**	0.02	0.27**	0.01
BSB	0.49**	0.05	0.54	0.00	0.47**	0.04	0.63**	0.05	0.51**	0.05	0.21**	0.02	0.28**	0.02
ASB	1.74**	0.18	1.06**	0.09	1.17**	0.10	1.06**	0.10	0.72**	0.06	0.54**	0.05	0.31**	0.02
TB	0.43**	0.05	1.00	1.13	0.28**	0.02	0.48**	0.04	0.21**	0.03	0.32**	0.12	0.08	0.26
SDPD	0.01*	0.00	0.06**	0.01	0.03**	0.00	0.10**	0.01	0.02	0.01	0.02**	0.00	0.01**	0.00
PPP	45.70	39.30	97.21**	17.90	113.17**	10.64	57.89**	5.66	38.25**	4.28	82.57**	8.08	3.43	4.27
YPP	9.93**	0.94	6.78**	2.57	13.43**	1.38	12.22**	1.00	1.89	1.97	6.57**	0.82	2.16**	0.83
SDWT	36.71**	3.23	37.14**	3.06	31.55**	2.60	29.34**	2.39	19.61**	1.75	28.01**	2.36	3.59**	0.21
YKGH	164440**	17239	227535**	22821	210055**	17998	37818**	12353	73552**	7738	95440**	10009	68916**	5452
PROD	14.44**	1.50	20.28**	1.98	17.57**	1.50	4.5**	1.13	6.74**	0.71	8.98**	0.91	5.481**	0.43

**Table 13:** Variance due to genotypes ( $\sigma^2 g$ ) and genotype x environment interaction ( $\sigma^2 g e$ ), and residual, ( $\sigma^2 e$ ) in different environments for the quantitative traits in the chickpea reference set

*#* - trait significant in all environments and pooled

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

			Mean (±	<b>S.E</b> )					Ran	ige		
Trait	<b>E1</b>	E2	E3	E4	E5	Pooled	E1	E2	E3	E4	Е5	Pooled
DF	59.2±1.7	59.6±1.6	59.2±1.7	54.4±0.6	54.9±2.2	57.5±0.7	40.0-85.3	37.8-91.6	39.2-78.9	34.2-94.7	35.1-86.5	36.5-89.3
FD	27.2±1.2	27.6±0.5	27.4±0.8	27.5±0.4	27.8±0.8	27.6±0.5	21.1-35.1	18.3-34.1	19.7-32.6	18.1-36.9	20.6-34.2	19.3-32.9
PLHT	44.4±2.4	44.5±1.7	44.9±1.1	43.5±1.0	37.7±1.6	43.6±0.8	21.3-86.4	18.3-92.5	17.7-97.5	17.6-88.6	16.8-83.4	26.3-92.4
PLWD	65.6±0.9	65.4±1.2	65.7±0.7	64.9±1.1	50.4±1.3	63.4±0.6	52.8-72.1	50.1-73.4	53.3-73.7	34.8-76.6	11.9-59.3	45.2-69.4
DGF	53.9±2.0	55.6±0.7	55.4±1.5	54.7±0.8	54.6±2.7	55.0±1.0	43.4-68.3	37.9-77.9	39.6-70.5	33.5-71.6	30.4-68.9	41.2-70.4
DM	113.2±1.8	115.2±1.3	114.6±1.2	109.2±0.8	109.5±1.7	112.5±0.6	103.6-126.3	102.1-138.2	102.4-134.8	75.6-129.6	72.5-129.5	99.2-130.6
BPB	2.9±0.2	3.1±0.2	2.8±0.1	2.9±0.1	2.6±0.2	2.9±0.1	2.2-3.7	2.2-4.5	1.2-4.4	1.2-5.0	0.5-3.7	2.1-3.9
APB	2.4±0.2	2.5±0.1	2.9±0.1	2.6±0.1	2.5±0.3	2.6±0.1	0.7-4.3	0.1-4.9	1.1-7.1	0.4-5.4	0.4-4.7	1.4-4.9
BSB	3.2±0.3	3.4±0.2	3.0±0.2	3.2±0.1	2.9±0.2	3.2±0.1	1.1-6.5	1.2-6.0	0.3-5.7	1.1-8.7	0.3-6.3	1.3-5.7
ASB	4.2±0.4	4.4±0.2	4.4±0.3	4.4±0.4	4.1±0.2	4.4±0.2	2.7-10.6	1.2-11.3	3.1-14.7	3.3-13.0	0.4-9.7	2.9-10.1
TB	1.5±0.3	1.8±1.0	1.4±0.1	1.5±0.2	1.3±0.2	1.5±0.2	1.0-4.2	1.6-6.9	0.0-3.2	0.3-5.4	0.3-4.2	1.1-12.3
SDPD	1.26±0.07	1.27±0.09	1.23±0.11	1.14±0.02	1.29±0.12	1.20±0.07	1.1-1.6	1.0-2.0	1.1-1.7	1.0-2.0	1-1.5	1.0-1.6
PPP	57.4±9.2	62.7±7.0	58.5±4.0	45.2±3.0	32.2±2.6	52.7±2.1	30.8-96.5	46.2-86.9	36.5-115.5	27.3-68.6	19.6-48.6	27.2-89.3
YPP	11.1±1.4	15.5±2.2	11.3±1.6	8.0±0.4	8.4±1.4	11.2±0.1	6.1-26.8	13.4-25.1	5.5-30.2	1.2-29	6.9-16.7	5.9-29.9
SDWT	23.6±1.3	22.6±0.7	22.4±0.7	21.7±0.4	19.3±1.2	22.0±0.4	13.4-51.5	12.7-55	14.7-53.0	13.6-51.9	11.0-39.6	13.5-49.4
YKGH	1934.1±134.8	2088.6±206.7	1808.1±115.2	1433.1±12	821.7±105.6	1675.0±57.0	365.7-3161.1	566.9-3215.4	657.2-4269.9	296.4-1678.3	283.5-1892.1	771-3176
PROD	17.2±1.3	18.3±1.8	15.9±1.0	13.2±1.4	7.6±1.0	14.9±0.5	3.3-29.8	4.6-27.9	5.6-36.0	11.1-16.5	2.5-16.5	6.8-27.2

**Table: 14:** Mean ( $\pm$  Standard error) and range values for quantitative traits in different environments and pooled over environments in the chickpea reference set

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

Trait	DF (days)	FD (days)	PLHT (cm)	PLWD (cm)	DGF (days)	DM (days)	BPB (no)	APB (no)	BSB (no)	ASB (no)	TB (no)	SDPD (no)	PPP (no)	YPP (g)	SDWT (g)	YKGH (kg ha- 1)	PROD (kg ha-1 day-1)
E1 (2006-07 post rainy)																	
Africa(21)	55.41	26.72	42.87	65.62	55.99	111.79	2.95	2.58	3.05	4.31	1.49	1.31	59.69	12.05	20.82	2125.06	19.11
Europe(3)	67.41	25.80	52.19	68.00	48.96	115.13	2.82	2.30	2.94	4.00	1.26	1.22	49.64	8.80	30.03	1425.88	12.44
Mediterranean(56)	60.02	27.56	45.65	66.00	54.03	114.61	2.94	2.32	3.24	3.99	1.43	1.22	52.51	9.81	27.38	1688.30	14.82
North America(6)	61.26	26.55	45.76	65.17	52.59	113.96	2.84	2.39	2.83	4.48	1.22	1.21	47.54	10.95	28.91	1972.40	17.28
Russian Federation(6)	61.69	26.79	46.59	67.03	54.61	116.17	3.01	2.30	2.76	4.02	1.54	1.23	46.84	11.60	24.77	1764.71	15.20
South and East Asia(105)	57.84	27.02	42.41	64.88	53.93	111.91	2.93	2.40	3.32	4.34	1.53	1.27	62.83	12.07	21.41	2076.35	18.64
South America(4)	62.44	28.34	46.66	67.02	52.29	114.22	2.85	2.52	3.77	4.55	1.67	1.19	47.06	11.65	34.67	1734.95	15.19
Unknown(6)	60.78	27.41	46.92	66.08	52.97	113.57	3.05	2.53	3.08	4.17	1.22	1.25	52.21	9.85	27.08	1556.13	13.69
West Asia(93)	60.50	27.22	45.60	66.00	53.69	113.98	2.93	2.34	3.04	4.13	1.36	1.27	54.96	10.62	23.04	1870.45	16.45
Variance	30.89	5.801	48.495	7.367	15.792	14.884	0.097	0.289	0.49	1.744	0.435	0.013	541	8.003	36.58	204982	17.64
E2 (2007-08 post rainy)																	
Africa(21)	54.88	27.51	43.43	65.28	57.58	112.46	3.10	2.67	3.22	4.39	1.78	1.44	66.59	15.20	19.71	2240.83	19.99
Europe(3)	68.77	27.36	51.86	69.11	50.97	119.74	2.87	2.48	3.37	3.75	1.74	1.13	53.53	17.86	32.59	1601.50	13.33
Mediterranean(56)	60.97	28.06	45.63	65.91	56.05	117.02	3.13	2.37	3.49	4.01	1.78	1.16	57.67	15.10	25.09	1701.78	14.64
North America(6)	62.02	27.29	45.74	65.82	55.73	117.75	2.92	2.29	2.87	4.50	1.75	1.14	55.84	14.99	29.69	2001.69	17.04
Russian Federation(6)	62.65	27.22	46.74	67.96	55.47	118.12	3.09	2.54	3.05	4.27	1.78	1.18	58.34	15.45	25.01	2002.26	17.08
South and East Asia(105)	57.82	27.44	42.18	64.52	55.82	113.63	3.03	2.46	3.55	4.62	1.80	1.30	66.07	15.68	20.92	2349.73	20.81
South America(4)	62.69	24.88	46.50	67.92	52.77	115.46	2.89	2.63	4.10	4.45	3.07	1.09	51.66	14.93	35.63	1943.48	16.87
Unknown(6)	61.47	27.66	46.58	66.34	56.28	117.75	3.14	2.79	2.96	4.53	1.75	1.22	59.77	15.14	26.52	1552.67	13.17
West Asia(93)	60.92	27.59	45.86	65.75	55.05	115.98	3.02	2.48	3.26	4.34	1.77	1.30	62.07	15.26	22.19	2030.09	17.57
Variance	42.429	1.795	48.649	10.26	21.644	23.152	0.228	0.432	0.537	1.068	1.001	0.062	97.5	6.749	37.03	266254	23.32
E3 (2008-09 post rainy)																	
Africa(21)	54.75	27.68	43.28	65.44	57.76	112.51	2.68	2.99	2.93	4.56	1.50	1.27	60.60	12.19	19.99	2023.31	18.07

Table: 15: Means and variance for quantitative traits in different geographical regions of chickpea reference evaluated in different environments and overall in pooled analysis

Trait	DF (days)	FD (days)	PLHT (cm)	PLWD (cm)	DGF (days)	DM (days)	BPB (no)	APB (no)	BSB (no)	ASB (no)	TB (no)	SDPD (no)	PPP (no)	YPP (g)	SDWT (g)	YKGH (kg ha- 1)	PROD (kg ha-1 day-1)
Europe(3)	61.32	26.17	52.60	70.43	59.53	120.85	2.42	2.26	2.79	4.38	1.01	1.18	45.72	11.06	28.87	1430.77	11.91
Mediterranean(56)	60.44	27.82	46.49	65.92	56.26	116.70	2.73	2.83	3.01	4.04	1.43	1.17	50.72	11.10	24.67	1634.23	14.14
North America(6)	62.02	27.21	46.27	65.73	54.23	116.25	2.70	2.82	2.73	4.68	1.34	1.17	49.86	10.13	28.54	1927.01	16.58
Russian Federation(6)	62.02	27.34	47.31	67.85	55.74	117.76	2.59	3.02	2.70	3.99	1.51	1.22	51.11	12.56	23.34	1478.25	12.62
South and East Asia(105)	57.57	27.25	42.37	64.98	55.41	112.99	2.78	3.02	2.99	4.66	1.41	1.24	63.54	12.32	20.93	1935.33	17.20
South America(4)	62.02	25.42	47.48	67.17	53.44	115.46	2.18	2.95	2.44	4.18	1.66	1.14	48.73	11.98	37.17	1527.56	13.19
Unknown(6)	60.78	27.54	46.99	66.77	56.58	117.36	2.73	2.64	2.74	3.94	1.29	1.22	57.83	10.00	27.00	1683.09	14.34
West Asia(93)	60.68	27.33	46.48	66.19	54.89	115.58	2.85	2.76	2.97	4.25	1.33	1.23	57.38	10.35	21.71	1730.94	15.02
Variance	38.243	2.648	66.521	8.17	23.116	20.838	0.318	0.639	0.474	1.167	0.28	0.03	117	13.27	31.55	224707	18.73
E4(2008-09 UAS post rainy)																	
Africa(21)	49.94	27.61	42.02	64.61	57.67	107.66	2.88	2.66	3.03	4.29	1.31	1.29	45.33	7.66	19.17	1447.49	13.46
Europe(3)	66.07	26.08	51.51	68.16	51.27	117.18	2.62	2.65	2.82	4.14	2.29	1.33	37.80	7.19	28.09	1435.54	12.95
Mediterranean(56)	56.02	27.97	44.43	65.77	53.74	109.82	2.98	2.46	3.29	4.25	1.54	1.08	42.19	7.36	23.89	1299.77	11.97
North America(6)	56.40	27.50	44.70	65.03	53.37	109.74	3.21	2.62	2.70	4.46	1.38	1.19	36.07	7.68	28.11	1431.66	13.16
Russian Federation(6)	57.48	27.66	46.22	66.96	56.20	113.66	2.90	2.67	2.84	3.93	1.43	1.27	40.69	10.20	23.99	1412.79	12.74
South and East Asia(105)	52.53	27.29	41.58	63.85	54.91	107.46	2.89	2.67	3.33	4.57	1.55	1.15	48.56	8.87	20.15	1441.54	13.38
South America(4)	58.59	26.68	44.48	66.90	54.91	113.41	3.42	1.95	3.60	5.22	1.74	1.00	37.08	11.61	35.72	1442.10	13.17
Unknown(6)	55.57	27.58	45.31	66.20	56.23	111.78	3.33	2.42	3.20	4.45	1.41	1.33	46.07	9.10	26.00	1420.43	12.92
West Asia(93)	55.80	27.52	44.85	65.37	54.45	110.25	2.94	2.55	3.09	4.32	1.41	1.10	44.42	6.97	21.05	1426.55	13.06
Variance	0.606	0.421	1.00	1.112	0.831	0.746	0.053	0.074	0.078	0.444	0.15	0.02	3.04	0.388	0.416	122.547	1.43
E5 (2008-09 spring)																	
Africa(21)	50.09	27.86	36.23	50.48	57.22	107.52	2.57	2.68	2.84	4.23	1.26	1.30	32.27	8.26	17.46	877.85	8.18
Europe(3)	63.84	26.91	45.80	52.98	52.58	116.52	2.38	2.44	2.84	4.32	1.05	1.23	25.56	8.20	23.61	938.97	8.19
Mediterranean(56)	56.59	28.10	39.29	51.24	54.13	110.75	2.59	2.34	2.95	3.88	1.31	1.26	29.52	8.30	20.20	750.62	6.87
North America(6)	56.47	27.82	38.52	52.37	52.92	109.19	2.68	2.51	2.81	4.22	1.08	1.30	28.22	8.26	24.48	923.15	8.48
Russian Federation(6)	57.78	27.74	40.06	51.04	55.85	114.02	2.61	2.39	2.58	3.95	1.50	1.30	30.57	8.77	21.43	698.84	6.21
South and East Asia(105)	52.96	27.76	35.90	49.94	54.61	107.46	2.59	2.67	2.90	4.26	1.29	1.30	34.93	8.67	18.34	858.74	8.07
South America(4)	57.92	25.74	39.74	53.63	53.26	111.14	2.27	2.17	2.36	3.53	1.33	1.25	27.26	9.00	31.81	676.48	6.06

Trait	DF (days)	FD (days)	PLHT (cm)	PLWD (cm)	DGF (days)	DM (days)	BPB (no)	APB (no)	BSB (no)	ASB (no)	TB (no)	SDPD (no)	PPP (no)	YPP (g)	SDWT (g)	YKGH (kg ha- 1)	PROD (kg ha-1 day-1)
Unknown(6)	55.75	27.88	39.53	50.91	56.09	112.15	2.79	2.54	2.84	4.00	1.10	1.24	29.02	8.53	19.52	829.80	7.40
West Asia(93)	56.44	27.75	38.65	50.15	54.33	110.80	2.63	2.39	2.82	3.99	1.26	1.29	31.62	8.12	19.23	811.47	7.36
Variance	2.159	0.772	1.645	1.325	2.672	1.669	0.206	0.248	0.224	0.215	0.211	0.118	2.61	1.443	1.165	105.64	1.004
Pooled																	
Africa(21)	52.72	27.58	41.47	62.25	57.41	110.27	2.85	2.73	3.01	4.35	1.45	1.33	53.42	10.76	19.37	1780.57	16.13
Europe(3)	65.84	26.43	51.20	66.02	52.58	118.20	2.61	2.42	2.95	4.10	1.42	1.19	38.74	11.90	28.88	1329.00	11.23
Mediterranean(56)	58.91	28.01	44.71	62.99	54.54	113.80	2.89	2.46	3.21	4.02	1.47	1.17	44.94	10.17	24.31	1410.02	12.42
North America(6)	59.84	27.32	44.35	62.80	53.77	113.50	2.86	2.52	2.77	4.47	1.31	1.20	40.56	9.90	28.14	1640.83	14.37
Russian Federation(6)	60.45	27.43	45.76	64.35	55.73	116.13	2.86	2.58	2.77	4.01	1.51	1.23	43.63	11.84	23.77	1448.83	12.47
South and East Asia(105)	55.67	27.41	40.73	61.57	54.96	110.63	2.85	2.65	3.22	4.50	1.54	1.26	55.73	11.57	20.32	1759.72	15.89
South America(4)	60.90	26.03	45.02	64.63	53.23	114.03	2.71	2.44	3.26	4.43	4.35	1.12	38.33	11.74	35.39	1470.75	12.83
Unknown(6)	58.97	27.69	45.16	63.34	55.74	114.63	3.04	2.59	2.95	4.22	1.30	1.23	48.17	10.29	25.38	1361.67	11.83
West Asia(93)	58.94	27.53	44.44	62.73	54.47	113.37	2.88	2.50	3.03	4.21	1.40	1.24	49.46	9.87	21.43	1550.90	13.65
Variance	0.7236	0.521	0.7672	0.6449	1.103	0.7684	0.092	0.104	0.097	0.1798	0.177	0.072	2.01	1.0	0.443	67.4	0.612

Numbers in parenthesis indicates number of accessions in each region Variance homogeneity was tested by Levene's test.

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

		Ι	Heritability	7			GCV%	PCV%
Trait	<b>E1</b>	E2	E3	E4	E5	Pooled	Poo	oled
DF	94.8	94.2	92.3	99.1	93.9	98.0	10.79	10.90
FD	84.7	87.6	78.2	94.3	80.1	81.1	5.13	5.70
PLHT	93.7	93.8	98.1	99.0	97.0	98.4	16.31	16.43
PLWD	93.5	85.3	94.3	91.2	95.0	89.4	4.51	4.76
DGF	85.4	97.3	90.8	97.7	88.4	90.5	8.02	8.42
DM	87.2	92.6	93.5	98.1	96.5	89.8	3.70	3.90
BPB	69.0	80.6	98.7	99.2	90.8	72.9	11.31	13.24
APB	88.7	95.9	98.5	98.7	90.9	72.5	15.89	18.66
BSB	93.0	91.0	90.8	99.0	94.7	75.2	14.63	16.86
ASB	94.8	95.2	93.5	81.4	96.6	85.2	16.91	18.31
TB	92.0	96.5	98.4	95.3	88.1	77.6	28.55	32.42
SDPD	78.3	86.7	63.2	99.5	53.5	62.2	11.46	14.52
PPP	91.2	49.5	86.5	84.2	90.4	95.8	16.32	16.67
YPP	85.5	56.9	79.7	98.7	70.4	83.8	23.11	25.24
SDWT	97.4	98.6	98.6	99.4	96.2	97.6	24.30	24.59
YKGH	95.2	83.9	94.0	86.4	91.9	89.8	17.79	18.76
PROD	95.3	85.5	94.4	84.7	92.0	91.3	19.52	20.42

**Table: 16** Heritability, genotypic (GCV) and phenotypic coefficient of variance (PCV) in the chickpea reference set evaluated in different environments and overall in pooled analysis

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

E1	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.345**															
PLHT	0.233**	-0.017														
PLWD	0.316**	-0.090	0.357**													
DGF	-0.630**	0.422**	-0.060	-0.031												
DM	0.597**	0.025	0.231**	0.336**	0.162**											
BPB	0.128*	-0.020	-0.079	-0.057	-0.136*	0.056										
APB	-0.164**	0.088	-0.010	-0.067	0.043	-0.199**	-0.024									
BSB	-0.104	0.137*	-0.062	-0.008	0.052	-0.024	0.075	-0.006								
ASB	0.013	0.024	0.048	0.017	-0.078	-0.110	0.119*	0.151**	0.281**							
ТВ	0.021	0.087	-0.054	-0.022	0.006	0.089	0.008	-0.009	0.316**	0.154**						
SDPD	-0.104	-0.077	-0.210**	-0.065	0.011	-0.134*	-0.006	0.129*	-0.006	0.033	-0.054					
PPP	-0.214**	-0.006	-0.131*	-0.155**	0.062	-0.307**	0.055	0.169**	0.135*	0.190**	0.009	0.157**				
YPP	-0.158**	0.050	-0.158**	-0.173**	0.050	-0.201**	-0.053	0.161**	0.090	0.182**	0.085	0.053	0.335**			
SDWT	0.076	0.139*	0.435**	0.200**	0.117	0.200**	-0.130*	-0.071	-0.058	-0.131*	-0.089	-0.459**	-0.312**	-0.161**		
YKGH	-0.360**	-0.069	-0.116	0.050	0.238**	-0.358**	-0.075	0.139*	0.068	0.082	-0.002	0.090	0.331**	0.159**	-0.047	
PROD	-0.423**	-0.069	-0.152**	-0.007	0.203**	-0.476**	-0.079	0.154**	0.068	0.087	-0.015	0.101	0.354**	0.181**	-0.077	0.990**

**Table: 17:** Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set evaluated during 2006-2007 postrainy season (E1), at ICRISAT, Patancheru, India.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

E2	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.121*															
PLHT	0.304**	-0.047														
PLWD	0.260**	-0.036	0.216**													
DGF	-0.657**	0.325**	-0.125*	-0.070												
DM	0.694**	0.194**	0.267**	0.248**	0.062											
BPB	0.107	-0.081	-0.058	-0.035	-0.071	0.076										
APB	-0.144*	0.069	0.092	-0.016	0.067	-0.138*	-0.015									
BSB	-0.019	0.029	-0.065	-0.024	0.019	-0.010	0.038	-0.040								
ASB	-0.058	-0.131*	-0.052	-0.082	-0.040	-0.128*	0.020	0.107	0.161**							
ТВ	-0.051	-0.057	0.027	0.048	0.045	-0.020	0.013	0.102	0.276**	0.158**						
SDPD	-0.138*	-0.072	-0.230**	-0.083	0.049	-0.148*	-0.004	0.147*	-0.018	0.052	-0.027					
PPP	-0.276**	0.017	-0.321**	-0.100	0.075	-0.319**	0.025	0.062	0.083	0.121*	-0.011	0.317**				
YPP	-0.173**	0.098	-0.074	0.066	0.099	-0.148*	-0.035	-0.022	0.176**	0.048	0.077	-0.040	0.383**			
SDWT	0.115	-0.010	0.239**	0.227**	0.044	0.170**	-0.024	-0.046	-0.055	-0.110	0.010	-0.423**	-0.486**	0.082		
YKGH	-0.345**	-0.209**	-0.216**	0.003	0.134*	-0.407**	-0.039	0.056	0.071	0.177**	0.008	0.210**	0.488**	0.269**	-0.117	
PROD	-0.423**	-0.226**	-0.246**	-0.041	0.116	-0.524**	-0.048	0.068	0.070	0.179**	0.009	0.215**	0.500**	0.269**	-0.135*	0.990**

**Table: 18**: Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set evaluated during 2007-2008 postrainy season (E2), at ICRISAT, Patancheru, India.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

E3	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.211**															
PLHT	0.181**	-0.078														
PLWD	0.263**	-0.062	0.390**													
DGF	-0.711**	0.311**	-0.004	-0.091												
DM	0.620**	0.078	0.207**	0.244**	0.097											
BPB	0.131*	-0.012	-0.089	-0.065	-0.125*	0.060										
APB	-0.047	0.048	0.067	-0.028	-0.022	-0.103	0.059									
BSB	-0.016	0.097	-0.087	-0.051	0.010	0.012	0.219**	-0.079								
ASB	-0.084	0.051	0.069	-0.042	0.013	-0.118*	0.065	0.335**	-0.053							
TB	-0.056	0.137*	-0.013	-0.158**	0.064	0.012	0.079	0.061	0.224**	0.143*						
SDPD	-0.063	-0.132*	-0.162**	-0.068	-0.033	-0.123*	0.095	0.070	0.048	0.076	-0.055					
PPP	-0.229**	-0.020	-0.148*	-0.104	0.052	-0.279**	0.046	0.139*	0.051	0.125*	-0.026	0.206**				
YPP	-0.220**	0.115	0.002	0.027	0.197**	-0.094	-0.137*	0.210**	0.179**	0.136*	0.129*	0.021	0.322**			
SDWT	0.083	-0.010	0.269**	0.236**	0.082	0.188**	-0.144*	-0.013	-0.101	-0.084	-0.018	-0.376**	-0.301**	0.136*		
YKGH	-0.360**	-0.021	-0.004	0.102	0.213**	-0.305**	-0.084	0.191**	0.001	0.127*	-0.042	0.019	0.324**	0.169**	0.069	
PROD	-0.433**	-0.028	-0.039	0.065	0.190**	-0.429**	-0.090	0.197**	-0.004	0.134*	-0.044	0.033	0.344**	0.177**	0.038	0.990**

**Table: 19:** Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set evaluated during 2008-2009 postrainy season (E3), at ICRISAT, Patancheru, India.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling,

DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches,

TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity.

E4	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.013															
PLHT	0.185**	-0.145*														
PLWD	0.218**	0.084	0.271**													
DGF	-0.614**	0.210**	-0.027	0.026												
DM	0.599**	0.208**	0.194**	0.291**	0.264**											
BPB	0.06	-0.119*	-0.07	-0.026	-0.021	0.05										
APB	-0.151**	0.052	0.008	-0.085	0.141*	-0.044	0.038									
BSB	-0.099	0.039	-0.124*	-0.074	0.015	-0.105	0.051	0.06								
ASB	-0.036	-0.041	0.047	0.039	0.004	-0.042	0.026	0.103	0.274**							
ТВ	0.069	-0.112	0.028	-0.06	-0.141*	-0.054	0.059	-0.03	0.174**	0.260**						
SDPD	0.01	-0.091	-0.03	-0.018	-0.116	-0.106	0.082	0.072	-0.03	-0.021	0.004					
PPP	-0.246**	-0.033	-0.031	-0.085	0.124*	**-0.178	-0.055	0.187**	0.129*	0.236**	0.057	0.049				
YPP	-0.135*	-0.075	-0.078	-0.006	0.028	*-0.135	0.04	0.097	0.200**	0.134*	0.113	0.101	0.124*			
SDWT	0.033	-0.044	0.267**	0.219**	0.109	**0.148	-0.022	-0.06	-0.03	-0.048	0.022	-0.196**	-0.334**	0.084		
YKGH	-0.326**	-0.028	-0.062	0.035	0.213**	**-0.191	-0.078	0.164**	0.153**	0.087	-0.02	0.027	0.203**	0.046	0.053	
PROD	-0.428**	-0.071	-0.102	-0.036	0.130*	**-0.400	-0.078	0.160**	0.172**	0.087	0.001	0.05	0.227**	0.081	0.009	0.974**

**Table: 20:** Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set evaluated during 2008-2009 postrainy season (E4), at UAS, Dharwad India.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

E5	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.159**															
PLHT	0.219**	-0.034														
PLWD	0.077	-0.008	0.234**													
DGF	-0.487**	0.192**	-0.022	-0.054												
DM	0.525**	0.022	0.191**	0.041	0.483**											
BPB	0.152**	-0.058	-0.09	-0.076	-0.037	0.114										
APB	-0.187**	0.169**	-0.097	-0.068	0.1	-0.109	0.046									
BSB	0.046	0.136**	-0.081	-0.035	0.113	0.142**	0.343**	0.143*								
ASB	0.054	0.007	0.051	-0.02	-0.092	-0.043	0.204**	0.359**	0.355**							
TB	0.051	0.18**	0.016	0.031	0.011	0.045	0.155**	0.102	0.338**	0.206**						
SDPD	-0.058	-0.097	-0.238**	-0.059	-0.019	-0.074	0.021	0.108	0.076	0.03	-0.130*					
PPP	-0.072	0.063	-0.048	-0.091	-0.002	-0.082	-0.051	0.222**	-0	0.176**	0.034	0.139*				
YPP	-0.03	0.284**	0.128*	0.029	0.072	0.001	-0.012	0.271**	0.117	0.232**	0.314**	-0.216**	0.210**			
SDWT	0.056	-0.081	0.217	0.145*	0.116	0.169**	-0.122**	-0.140*	-0.140*	-0.161**	-0.09	-0.316**	-0.300**	0.032		
YKGH	-0.293**	-0.007	-0.051**	-0.034	0.102	-0.194**	-0.078	0.206**	0.024	0.05	-0.1	0.063	0.08	-0.034	0.038	
PROD	-0.364**	-0.003	-0.085	-0.037	-0.02	-0.383**	-0.1	0.205**	-0.02	0.05	-0.11	0.067	0.08	-0.032	0.008	0.978**

**Table: 21:** Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set evaluated during 2008-2009 spring season (E5), at ICRISAT, Patancheru, India.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

Pooled	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH
FD	-0.159**															
PLHT	0.219**	-0.034														
PLWD	0.077	-0.008	0.234**													
DGF	-0.487**	0.192**	-0.022	-0.054												
DM	0.525**	0.022	0.191**	0.041	0.483**											
BPB	0.152**	-0.058	-0.09	-0.076	-0.037	0.114										
APB	-0.187**	0.169**	-0.097	-0.068	0.1	-0.109	0.046									
BSB	0.046	0.136**	-0.081	-0.035	0.113	0.142*	0.343**	0.143*								
ASB	0.054	0.007	0.051	-0.02	-0.092	-0.043	0.204**	0.359**	0.355**							
ТВ	0.051	0.180**	0.016	0.031	0.011	0.045	0.155**	0.102	0.338**	0.206**						
SDPD	-0.058	-0.097	-0.238**	-0.059	-0.019	-0.074	0.021	0.108	0.076	0.03	-0.130**					
PPP	-0.072	0.063	-0.048	-0.091	-0.002	-0.082	-0.051	0.222**	-0	0.176**	0.034	0.139*				
YPP	-0.03	0.284**	0.128**	0.029	0.072	0.001	-0.012	0.271**	0.117	0.232**	0.314**	-0.216**	0.21**			
SDWT	0.056	-0.081	0.217**	0.145*	0.116	0.169**	-0.122*	-0.140*	-0.140*	-0.161**	-0.09	-0.316**	-0.300**	0.032		
YKGH	-0.293**	-0.007	-0.051	-0.034	0.102	-0.194**	-0.078	0.206**	0.024	0.05	-0.1	0.063	0.08	-0.034	0.038	
PROD	-0.364**	-0.003	-0.085	-0.037	-0.02	-0.383**	-0.1	0.205**	-0.02	0.05	-0.11	0.067	0.08	-0.032	0.008	0.978**

Table: 22: Phenotypic correlation coefficients between 17 quantitative traits in chickpea reference set in pooled analysis.

(\*Significant at P < 0.05, \*\* Significant at P < 0.01)

		Correlation
Pair of traits	Environment	coefficient
Plot yield and per day productivity	(2006-07) E1	0.99
	(2007-08) E2	0.99
	(2008-09) E3	0.99
	(2008-09) E4	0.974
	(2008-09) E5	0.978
	Pooled	0.978
Days to 50% flowering and days to grain filling	(2008-09) E3	-0.711
	pooled	-0.716
Traits showed high correlation (r=0.05 or more)		
days to 50% flowering and days to maturity	(2006-07) E1	0.597
	(2007-08) E2	0.694
	(2008-09) E3	0.62
	(2008-09) E4	0.599
	(2008-09) E5	0.525
	pooled	0.671
pods per plant and per day productivity	(2007-08) E2	0.5
Days to 50% flowering and days to grain filling	(2008-09) E4	-0.614

**Table: 23:** Meaningful correlation (r> 0.500) for quantitative traits in the chickpea reference set evaluated in five environments and in pooled analysis

**Table: 24:** Shannon-weaver diversity (H') for qualitative and quantitative traits in chickpea reference set evaluated during E1 (2006-07), E2 (2007-08), E3 (2008-09) post-rainy season at ICRISAT Centre, E4 (2008-09) post-rainy season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT, Patancheru and pooled analysis

Qualitative traits	H'					
Seed Shape	0.325					
Flower color	0.424					
Plant color	0.335					
Seed color	0.807					
Growth habit	0.362					
Dots on seed						
coat	0.301					
Seed surface	0.332					
Mean±S.E	0.412±0.067					
Quantitative						
traits	E1	E2	E3	E4	E5	Pooled
DF	0.631	0.602	0.607	0.598	0.612	0.598
FD	0.602	0.456	0.429	0.305	0.312	0.515
PLHT	0.545	0.532	0.539	0.546	0.559	0.539
PLWD	0.577	0.598	0.613	0.566	0.477	0.600
DGF	0.620	0.613	0.600	0.614	0.554	0.610
DM	0.626	0.619	0.631	0.595	0.558	0.612
BPB	0.614	0.628	0.564	0.607	0.512	0.600
APB	0.468	0.608	0.514	0.564	0.623	0.596
BSB	0.580	0.617	0.531	0.518	0.553	0.566
ASB	0.578	0.572	0.440	0.419	0.524	0.518
ТВ	0.327	0.080	0.582	0.386	0.547	0.244
SDPD	0.617	0.460	0.467	0.219	0.619	0.543
PPP	0.614	0.612	0.582	0.617	0.599	0.624
YPP	0.600	0.545	0.573	0.543	0.413	0.556
100-SDWT	0.579	0.560	0.535	0.550	0.584	0.558
YKGH	0.613	0.634	0.580	0.620	0.591	0.621
PROD	0.618	0.626	0.597	0.621	0.593	0.614
Mean±S.E	0.577±0.018	0.551±0.032	0.552±0.015	0.523±0.029	0.543±0.019	$0.560 \pm 0.022$

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

Trait/Types/Origin	Growth Habit	Plant pigmentation	Flower color	Seed color	Seed Shape	Seed surface	Seed dots
Seed Types				-			-
Desi	0.314	0.256	0.245	0.688	0.000	0.051	0.257
Kabuli	0.351	0.000	0.027	0.027	0.000	0.079	0.000
Pea	0.406	0.261	0.450	0.932	0.000	0.299	0.132
Wild	0.297	0.178	0.000	0.436	0.000	0.000	0.297
Mean	0.342	0.174	0.180	0.521	0.000	0.107	0.172
Geographical origin							
Africa	0.136	0.258	0.398	0.619	0.288	0.259	0.136
Europe	0.477	0.276	0.000	0.276	0.276	0.276	0.276
Mediterranean	0.326	0.384	0.259	0.589	0.443	0.252	0.403
North America	0.276	0.376	0.195	0.376	0.195	0.195	0.276
Russian Federation	0.439	0.195	0.195	0.539	0.195	0.000	0.276
South & East Asia	0.201	0.193	0.211	0.594	0.319	0.222	0.189
South America	0.000	0.000	0.000	0.000	0.000	0.000	0.244
Unknown	0.276	0.376	0.195	0.376	0.195	0.276	0.276
West Asia	0.329	0.469	0.317	0.789	0.256	0.257	0.300
Mean	0.274	0.281	0.197	0.462	0.241	0.193	0.264

**Table: 25:** Shannon-weaver diversity (H') observed for qualitative traits in different seed types and geographical regions in the chickpea reference set.

**Table: 26:** Shannon-weaver diversity (H') in different seed types observed for quantitative traits in chickpea reference set evaluated during E1 (2006-07), E2 (2007-08), E3 (2008-09) post-rainy season at ICRISAT Centre, E4 (2008-09) post-rainy season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT Patancheru and in overall pooled analysis

Seed type	Season	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH	PROD	Mean
Desi	E1	0.637	0.527	0.548	0.605	0.597	0.614	0.585	0.602	0.521	0.520	0.386	0.581	0.620	0.591	0.593	0.603	0.626	0.574
	E2	0.597	0.352	0.572	0.611	0.624	0.630	0.602	0.601	0.597	0.556	0.232	0.519	0.621	0.587	0.597	0.619	0.606	0.560
	E3	0.597	0.375	0.535	0.620	0.598	0.630	0.569	0.483	0.533	0.428	0.582	0.508	0.567	0.603	0.591	0.570	0.593	0.552
	E4	0.604	0.266	0.564	0.577	0.598	0.603	0.606	0.539	0.533	0.417	0.375	0.247	0.632	0.542	0.600	0.601	0.609	0.524
	E5	0.624	0.380	0.602	0.499	0.545	0.534	0.563	0.521	0.488	0.560	0.339	0.545	0.620	0.395	0.624	0.574	0.587	0.529
	Pooled	0.613	0.450	0.558	0.602	0.629	0.610	0.608	0.542	0.580	0.459	0.354	0.545	0.611	0.605	0.608	0.610	0.616	0.565
kabuli	E1	0.602	0.551	0.533	0.563	0.610	0.594	0.628	0.478	0.514	0.561	0.395	0.470	0.602	0.516	0.581	0.610	0.621	0.554
	E2	0.605	0.280	0.478	0.602	0.565	0.539	0.611	0.541	0.548	0.535	0.053	0.421	0.579	0.512	0.571	0.623	0.606	0.510
	E3	0.614	0.562	0.462	0.595	0.590	0.579	0.603	0.513	0.588	0.464	0.367	0.403	0.618	0.501	0.560	0.582	0.597	0.541
	E4	0.576	0.322	0.543	0.451	0.585	0.597	0.543	0.541	0.539	0.413	0.437	0.145	0.617	0.511	0.557	0.597	0.605	0.505
	E5	0.579	0.367	0.416	0.436	0.465	0.527	0.496	0.467	0.525	0.486	0.265	0.575	0.599	0.423	0.589	0.604	0.601	0.495
	Pooled	0.598	0.509	0.495	0.583	0.604	0.599	0.603	0.574	0.593	0.423	0.079	0.386	0.593	0.492	0.586	0.628	0.618	0.527
pea	E1	0.406	0.449	0.330	0.539	0.539	0.473	0.549	0.450	0.505	0.562	0.450	0.261	0.473	0.449	0.330	0.583	0.562	0.465
	E2	0.487	0.398	0.374	0.583	0.330	0.539	0.487	0.299	0.562	0.539	0.330	0.261	0.549	0.330	0.398	0.539	0.394	0.435
	E3	0.450	0.505	0.398	0.432	0.539	0.549	0.449	0.539	0.487	0.449	0.398	0.374	0.549	0.330	0.398	0.549	0.549	0.467
	E4	0.487	0.449	0.374	0.583	0.508	0.539	0.374	0.450	0.432	0.487	0.132	0.132	0.549	0.505	0.261	0.505	0.505	0.428
	E5	0.505	0.406	0.374	0.385	0.394	0.539	0.330	0.505	0.285	0.398	0.330	0.508	0.539	0.505	0.539	0.505	0.505	0.444
	Pooled	0.562	0.398	0.330	0.394	0.394	0.539	0.508	0.539	0.539	0.539	0.255	0.385	0.562	0.330	0.330	0.583	0.539	0.454
wild	E1	0.346	0.469	0.469	0.436	999	0.415	0.555	999	0.415	0.415	0.469	0.178	0.469	0.260	0.346	0.501	0.415	0.362
	E2	0.346	0.415	0.346	0.346	0.501	0.346	0.178	0.178	0.178	0.555	0.469	0.415	0.436	0.260	0.415	0.346	0.178	0.347
	E3	0.178	0.555	0.469	0.436	0.555	0.436	0.469	999	0.415	0.415	0.469	0.178	0.415	0.469	0.346	0.436	0.436	0.393
	E4	0.178	0.415	0.469	0.346	0.415	0.346	0.469	999	0.415	0.415	0.469	0.178	0.436	0.346	0.346	0.346	0.346	0.349
	E5	0.346	0.415	0.469	0.436	0.469	0.415	0.469	999	0.415	0.415	0.415	999	0.469	0.346	0.415	0.260	0.346	0.359
	Pooled	0.178	0.346	0.501	0.415	0.555	0.346	0.415	0.178	0.415	0.555	0.469	0.346	0.469	0.415	0.346	0.346	0.260	0.385

Region	Season	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH	PROD
	E1	0.494	0.489	0.553	0.556	0.437	0.534	0.567	0.424	0.565	0.527	0.375	0.561	0.549	0.507	0.455	0.553	0.548
	E2	0.616	0.258	0.496	0.530	0.495	0.465	0.537	0.424	0.549	0.629	0.424	0.534	0.635	0.523	0.337	0.583	0.541
Africa	E3	0.600	0.466	0.501	0.600	0.502	0.474	0.473	0.486	0.392	0.455	0.415	0.564	0.530	0.561	0.218	0.542	0.617
Anica	E4	0.604	0.218	0.563	0.530	0.437	0.564	0.501	0.337	0.486	0.543	0.415	0.260	0.564	0.582	0.246	0.567	0.527
	E5	0.526	0.392	0.535	0.525	0.433	0.561	0.301	0.427	0.476	0.507	0.212	0.496	0.517	0.582	0.336	0.561	0.536
	Pooled	0.633	0.495	0.534	0.517	0.437	0.520	0.525	0.486	0.565	0.564	0.337	0.561	0.536	0.560	0.316	0.588	0.561
	E1	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276
	E2	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276
Europe	E3	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	999	0.276	0.276	0.276	0.276	0.276	0.276
Europe	E4	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276
	E5	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276
	Pooled	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276	0.276
	E1	0.577	0.563	0.481	0.593	0.513	0.577	0.565	0.538	0.487	0.579	0.402	0.425	0.589	0.593	0.570	0.569	0.550
	E2	0.522	0.535	0.465	0.597	0.548	0.513	0.589	0.613	0.561	0.576	0.526	0.397	0.585	0.566	0.539	0.569	0.563
Mediterranean	E3	0.556	0.494	0.486	0.599	0.620	0.562	0.534	0.542	0.623	0.611	0.403	0.443	0.620	0.500	0.521	0.522	0.513
Mediterranean	E4	0.496	0.404	0.523	0.605	0.567	0.550	0.560	0.555	0.555	0.540	0.446	0.280	0.583	0.575	0.529	0.578	0.565
	E5	0.546	0.499	0.503	0.591	0.585	0.490	0.524	0.502	0.567	0.509	0.304	0.506	0.584	0.561	0.564	0.545	0.582
	Pooled	0.543	0.535	0.493	0.609	0.618	0.605	0.594	0.630	0.568	0.585	0.404	0.449	0.586	0.580	0.546	0.555	0.565
	E1	0.439	0.196	0.377	0.439	0.477	0.276	0.377	0.276	0.439	0.439	0.377	0.377	0.439	0.276	0.439	0.377	0.439
	E2	0.439	0.377	0.196	0.540	0.439	0.196	0.439	0.196	0.477	0.439	0.377	0.439	0.439	0.301	0.439	0.439	0.439
North America	E3	0.439	0.439	0.196	0.439	0.377	0.439	0.439	0.439	0.439	0.439	0.276	0.439	0.540	0.439	0.439	0.439	0.439
Norui America	E4	0.477	0.477	0.439	0.439	0.196	0.196	0.477	0.377	0.196	0.439	0.196	0.196	0.439	0.477	0.439	0.477	0.439
	E5	0.377	0.439	0.377	0.439	0.196	0.196	0.439	0.377	0.439	0.439	0.196	0.377	0.439	0.477	0.276	0.477	0.439
	Pooled	0.439	0.439	0.377	0.439	0.377	0.196	0.439	0.196	0.439	0.377	0.196	0.439	0.439	0.477	0.439	0.439	0.439
	E1	0.196	0.439	0.439	0.439	0.439	0.377	0.377	0.196	0.439	0.477	0.439	0.276	0.377	0.196	0.196	0.276	0.276
	E2	0.439	0.276	0.439	0.439	0.439	0.439	0.439	0.377	0.439	0.439	0.377	0.276	0.439	0.276	0.439	0.439	0.439
Russian Federation	E3	0.439	0.377	0.439	0.439	0.477	0.439	0.439	0.439	0.439	0.196	0.439	0.377	0.439	0.377	0.439	0.439	0.301
Kussiali Pederatioli	E4	0.439	0.276	0.439	0.439	0.439	0.276	0.477	0.377	0.276	0.477	0.196	0.377	0.439	0.196	0.439	0.377	0.377
	E5	0.377	0.196	0.439	0.377	0.439	0.439	0.301	0.377	0.301	0.439	0.439	0.477	0.439	0.196	0.439	0.439	0.439
	Pooled	0.439	0.477	0.439	0.439	0.477	0.439	0.439	0.196	0.477	0.439	0.377	0.439	0.439	0.196	0.439	0.477	0.477
South & East Asia	E1	0.563	0.591	0.468	0.516	0.597	0.614	0.590	0.528	0.562	0.429	0.382	0.613	0.617	0.557	0.574	0.604	0.597

**Table: 27:** Shannon-weaver diversity (H') based on geographical origin observed for quantitative traits in chickpea reference set evaluated during E1 (2006-07), E2 (2007-08), E3 (2008-09) post-rainy season at ICRISAT Centre, E4 (2008-09) post-rainy season at UAS, Dharwad, E5 (2008-09) spring at ICRISAT, Patancheru and in overall pooled analysis.

Region	Season	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	SDWT	YKGH	PROD
	E2	0.589	0.395	0.535	0.558	0.612	0.638	0.595	0.550	0.634	0.461	0.192	0.533	0.610	0.554	0.551	0.633	0.639
	E3	0.579	0.505	0.484	0.593	0.602	0.616	0.605	0.492	0.653	0.365	0.569	0.508	0.590	0.543	0.539	0.580	0.578
	E4	0.605	0.453	0.556	0.575	0.572	0.580	0.603	0.592	0.502	0.319	0.527	0.261	0.629	0.512	0.515	0.575	0.600
	E5	0.610	0.434	0.541	0.615	0.552	0.561	0.552	0.529	0.451	0.462	0.582	0.597	0.623	0.255	0.586	0.569	0.582
	Pooled	0.586	0.484	0.505	0.540	0.611	0.611	0.575	0.561	0.583	0.459	0.316	0.576	0.606	0.500	0.522	0.586	0.619
	E1	0.452	0.244	0.244	0.452	0.452	0.244	0.452	0.244	0.244	0.244	0.452	0.244	0.452	0.452	0.452	0.452	0.452
	E2	0.452	0.244	0.452	0.452	0.452	0.452	0.301	0.244	0.244	0.244	0.244	0.452	0.244	0.244	0.452	0.244	0.244
South America	E3	0.452	0.452	0.244	0.244	0.452	0.452	0.244	0.452	0.452	0.452	0.301	0.244	0.452	0.452	0.452	0.452	0.244
South America	E4	0.452	0.301	0.452	0.452	0.452	0.452	0.452	0.452	0.452	0.452	0.452	999	0.244	0.244	0.452	0.244	0.244
	E5	0.452	0.452	0.244	0.452	0.452	0.452	0.452	0.244	0.452	0.452	0.244	0.452	0.244	0.244	0.452	0.452	0.452
	Pooled	0.452	0.452	0.244	0.452	0.452	0.452	0.244	0.452	0.452	0.452	0.244	0.244	0.244	0.244	0.452	0.452	0.452
	E1	0.621	0.624	0.477	0.592	0.616	0.611	0.578	0.521	0.520	0.534	0.407	0.488	0.581	0.558	0.539	0.625	0.586
	E2	0.599	0.518	0.494	0.630	0.591	0.603	0.610	0.415	0.597	0.637	0.419	0.483	0.615	0.580	0.480	0.628	0.602
West Asia	E3	0.626	0.506	0.411	0.605	0.600	0.636	0.494	0.474	0.583	0.561	0.381	0.472	0.471	0.602	0.517	0.589	0.593
West Asia	E4	0.615	0.224	0.503	0.476	0.611	0.639	0.527	0.563	0.581	0.615	0.421	0.172	0.572	0.574	0.586	0.593	0.602
	E5	0.635	0.339	0.447	0.370	0.574	0.578	0.544	0.508	0.438	0.607	0.271	0.527	0.578	0.600	0.606	0.565	0.534
	Pooled	0.638	0.480	0.456	0.530	0.613	0.628	0.586	0.592	0.571	0.595	0.467	0.506	0.542	0.626	0.519	0.590	0.594
	E1	0.377	0.196	0.439	0.439	0.377	0.377	0.276	0.377	0.439	0.540	0.377	0.377	0.477	0.439	0.196	0.439	0.439
	E2	0.377	0.377	0.439	0.439	0.377	0.377	0.439	0.439	0.439	0.377	0.439	0.196	0.439	0.377	0.196	0.439	0.439
	E3	0.196	0.439	0.477	0.439	0.377	0.439	0.439	0.301	0.276	0.439	0.439	0.377	0.439	0.439	0.196	0.439	0.439
Unknown	E4	0.377	0.439	0.196	0.477	0.377	0.477	0.276	0.377	0.439	0.377	0.377	0.276	0.439	0.196	0.196	0.276	0.276
	E5	0.439	0.439	0.196	0.439	0.377	0.377	0.196	0.377	0.196	0.377	0.196	0.196	0.439	0.196	0.439	0.439	0.439
	Pooled	0.196	0.196	0.439	0.439	0.377	0.439	0.377	0.377	0.439	0.439	0.439	0.196	0.439	0.377	0.196	0.439	0.439

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity

Percentage of variation explained (%)		Principle components										
	1	2	3	4	5	6	7	8	9	10		
	20.35	11.81	10	9.32	6.83	6.41	5.73	5.24	4.79	3.84		
Latent vectors	3.46	2.01	1.70	1.58	1.16	1.09	0.98	0.89	0.81	0.65		
DF	-0.389	0.294	0.214	0.186	0.037	0.058	-0.076	-0.165	0.116	0.034		
FD	0.068	-0.325	-0.475	0.011	-0.050	0.141	0.200	-0.040	-0.069	0.012		
PLHT	-0.229	-0.244	0.206	0.280	-0.247	0.238	0.095	0.053	-0.197	-0.370		
PLWD	-0.194	-0.164	0.316	0.293	0.310	0.299	0.040	-0.017	-0.068	0.054		
DGF	0.173	-0.469	-0.283	-0.068	0.303	0.183	0.057	-0.201	0.023	0.084		
DM	-0.369	-0.081	-0.117	0.145	0.370	0.220	-0.050	-0.338	0.183	0.079		
BPB	-0.032	0.245	-0.101	0.121	0.091	-0.251	0.772	-0.256	0.252	-0.035		
APB	0.174	0.019	-0.016	0.080	-0.367	0.521	0.157	0.258	0.569	-0.086		
BSB	0.100	0.044	-0.276	0.461	0.169	-0.178	-0.033	0.252	-0.316	-0.250		
ASB	0.109	0.177	-0.090	0.489	-0.178	0.139	0.175	0.134	-0.290	0.592		
TB	0.025	0.077	-0.288	0.398	0.190	-0.168	-0.369	0.207	0.476	-0.183		
SDPD	0.164	0.275	0.010	-0.202	0.362	0.497	0.038	0.217	-0.172	-0.040		
PPP	0.312	0.158	0.043	0.148	-0.078	0.128	0.043	-0.432	-0.200	-0.567		
YPP	0.228	0.115	-0.108	0.149	-0.287	0.131	-0.379	-0.567	0.026	0.228		
SDWT	-0.204	-0.469	0.099	0.121	-0.293	-0.155	0.035	0.002	0.025	0.017		
YKGH	0.382	-0.186	0.387	0.164	0.208	-0.108	0.030	-0.037	0.149	0.092		
PROD	0.412	-0.162	0.377	0.129	0.148	-0.140	0.030	0.007	0.117	0.077		

**Table: 28:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set evaluated during 2006-07 (E1) post-rainy season at ICRISAT Centre, Patancheru, India

Percentage of variation explained (%)		Principle components										
	1	2	3	4	5	6	7	8	9	10		
	21.68	10.34	9.44	8.18	7.49	6.78	5.74	5.54	4.92	4.17		
Latent vectors	3.69	1.76	1.60	1.39	1.27	1.15	0.98	0.94	0.84	0.70		
DF	-0.362	0.430	-0.124	0.060	0.233	0.053	-0.020	0.032	0.117	0.069		
FD	-0.057	-0.507	-0.004	0.182	0.351	0.072	-0.083	-0.132	0.209	-0.015		
PLHT	-0.247	0.114	0.269	-0.034	-0.058	0.368	-0.086	-0.140	0.068	0.503		
PLWD	-0.135	0.191	0.336	-0.062	0.326	0.278	0.259	0.271	-0.142	-0.388		
DGF	0.142	-0.575	0.178	0.052	0.039	0.020	0.347	0.227	0.132	0.077		
DM	-0.366	-0.009	-0.033	0.155	0.342	0.072	0.270	0.239	0.267	0.134		
BPB	-0.029	0.141	-0.154	0.143	0.003	-0.270	0.730	-0.531	-0.050	0.017		
APB	0.081	-0.083	0.024	0.131	-0.232	0.696	0.105	-0.372	-0.040	0.086		
BSB	0.064	0.076	0.149	0.586	0.039	-0.258	-0.092	0.148	-0.084	0.503		
ASB	0.130	0.203	0.033	0.342	-0.293	0.068	-0.033	0.030	0.753	-0.339		
ТВ	0.027	0.058	0.213	0.551	-0.230	0.062	0.061	0.185	-0.435	-0.258		
SDPD	0.217	0.054	-0.389	0.045	0.134	0.347	0.143	0.247	-0.177	0.026		
PPP	0.366	0.091	-0.126	0.100	0.390	0.047	-0.068	-0.140	0.026	-0.067		
YPP	0.138	0.050	0.354	0.140	0.444	-0.029	-0.278	-0.434	-0.065	-0.175		
SDWT	-0.214	-0.056	0.522	-0.195	-0.160	-0.135	0.123	-0.078	0.017	-0.086		
YKGH	0.410	0.212	0.240	-0.167	0.086	0.009	0.183	0.138	0.137	0.217		
PROD	0.436	0.198	0.225	-0.180	0.028	-0.009	0.129	0.097	0.086	0.188		

**Table: 29:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set evaluated during 2007-08 (E2) post rainy, at ICRISAT Centre, Patancheru, India.

Percentage of variation explained (%)	Principle components										
	1	2	3	4	5	6	7	8	9	10	
	19.01	12.09	10.28	8.93	7.13	6.4	5.94	5.1	4.53	4.33	
Latent vectors	3.23	2.06	1.75	1.52	1.21	1.09	1.01	0.87	0.77	0.74	
DF	-0.434	0.003	0.315	0.207	0.110	0.001	-0.067	0.227	-0.239	0.178	
FD	0.075	0.029	-0.461	0.161	-0.059	-0.101	0.281	0.405	-0.407	-0.313	
PLHT	-0.130	0.405	0.114	0.194	-0.055	-0.112	0.098	-0.448	0.067	-0.367	
PLWD	-0.124	0.397	0.235	0.084	0.227	-0.296	0.166	-0.105	-0.134	-0.251	
DGF	0.242	0.166	-0.493	-0.086	-0.044	-0.269	0.305	-0.173	0.201	0.213	
DM	-0.354	0.160	-0.130	0.200	0.115	-0.293	0.244	0.154	-0.126	0.494	
BPB	-0.057	-0.268	0.080	0.269	0.269	0.217	0.497	0.139	0.502	-0.086	
APB	0.162	0.051	0.181	0.430	-0.379	0.027	0.058	0.259	0.204	0.154	
BSB	0.033	-0.172	-0.170	0.288	0.624	0.048	-0.081	-0.123	0.074	-0.185	
ASB	0.149	-0.014	0.116	0.442	-0.428	0.080	0.144	-0.150	-0.065	-0.141	
ТВ	0.042	-0.081	-0.250	0.415	0.067	0.359	-0.169	-0.417	-0.363	0.281	
SDPD	0.101	-0.333	0.219	0.010	-0.009	-0.464	0.140	-0.364	0.048	0.315	
PPP	0.309	-0.160	0.188	0.102	0.123	-0.325	-0.169	0.224	-0.093	-0.123	
YPP	0.221	0.122	-0.122	0.338	0.101	-0.348	-0.531	0.143	0.221	0.016	
SDWT	-0.096	0.493	-0.111	0.019	0.039	0.221	-0.191	0.104	0.384	0.194	
YKGH	0.416	0.261	0.228	-0.041	0.231	0.149	0.185	0.067	-0.182	0.218	
PROD	0.445	0.225	0.232	-0.069	0.202	0.181	0.141	0.048	-0.154	0.138	

**Table: 30:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set evaluated during 2008-09 (E3) post rainy, at ICRISAT Centre, Patancheru, India

				Р	rinciple coi	mponents				
Percentage of variation explained (%)	1	2	3	4	5	6	7	8	9	10
explained (70)	17.6	10.81	9.48	8.48	7.2	6.69	6.34	5.68	5.15	4.66
Latent vectors	2.99	1.84	1.61	1.44	1.22	1.14	1.08	0.97	0.88	0.79
DF	-0.436	-0.148	0.227	-0.051	0.293	-0.198	0.236	0.011	0.185	-0.112
FD	-0.035	0.257	-0.220	0.399	0.092	-0.324	0.247	-0.253	0.103	0.219
PLHT	-0.164	0.196	0.370	-0.149	0.179	0.227	-0.439	0.058	0.052	0.023
PLWD	-0.158	0.312	0.330	-0.029	0.251	0.029	0.099	-0.237	-0.348	-0.165
DGF	0.184	0.472	-0.150	0.367	-0.136	0.261	-0.085	0.119	-0.251	0.192
DM	-0.348	0.296	0.121	0.315	0.216	0.019	0.202	0.130	-0.030	0.065
BPB	-0.040	-0.169	0.055	0.082	-0.102	0.445	0.480	0.543	-0.196	-0.178
APB	0.181	0.041	0.019	0.224	0.271	0.349	0.002	0.078	0.774	0.023
BSB	0.188	-0.142	0.271	0.302	-0.192	-0.234	0.264	-0.070	0.053	-0.219
ASB	0.132	-0.154	0.417	0.347	0.067	-0.110	-0.161	0.104	-0.097	0.005
ТВ	0.027	-0.275	0.385	0.122	-0.184	-0.140	-0.075	0.153	-0.028	0.665
SDPD	0.053	-0.240	-0.045	-0.133	0.375	0.392	0.249	-0.343	-0.196	0.476
PPP	0.278	-0.131	0.029	0.265	0.371	0.015	-0.347	0.035	-0.201	-0.255
YPP	0.146	-0.133	0.227	0.159	-0.248	0.328	0.109	-0.602	0.018	-0.226
SDWT	-0.104	0.359	0.324	-0.183	-0.436	0.139	0.026	-0.078	0.194	0.070
YKGH	0.424	0.258	0.189	-0.247	0.196	-0.157	0.255	0.113	0.011	0.034
PROD	0.472	0.168	0.151	-0.301	0.136	-0.154	0.202	0.073	0.019	0.014

**Table: 31:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set evaluated during 2008-09 (E4) post rainy, at UAS, Dharwad, India

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, ASB

TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity

				F	rinciple c	omponent	8			
Percentage of variation explained (%)	1	2	3	4	5	6	7	8	9	10
explained (70)	15.88	13.76	10.55	8.75	8.14	6.95	5.76	5.06	4.52	4.11
Latent vectors	2.70	2.34	1.79	1.49	1.38	1.18	0.98	0.86	0.77	0.70
DF	-0.394	0.122	-0.229	0.312	0.133	0.181	0.274	0.314	0.282	0.031
FD	0.092	0.198	0.326	-0.133	-0.276	-0.199	-0.045	0.439	0.486	-0.287
PLHT	-0.197	-0.026	0.219	0.453	-0.053	0.253	-0.050	-0.006	-0.199	-0.032
PLWD	-0.111	-0.058	0.115	0.353	-0.067	0.070	-0.750	0.115	-0.080	-0.303
DGF	0.055	0.059	0.526	-0.445	0.190	0.243	-0.132	-0.108	-0.204	0.018
DM	-0.341	0.181	0.284	-0.125	0.319	0.416	0.140	0.212	0.081	0.049
BPB	-0.053	0.287	-0.170	-0.027	0.427	-0.219	0.050	-0.126	-0.124	-0.631
APB	0.303	0.271	0.078	0.068	-0.082	0.190	0.039	-0.360	0.408	-0.117
BSB	0.051	0.418	0.030	-0.001	0.371	-0.190	-0.145	0.105	-0.064	0.103
ASB	0.145	0.394	-0.111	0.275	0.083	0.114	-0.034	-0.378	0.044	0.097
ТВ	-0.019	0.375	0.132	0.132	-0.049	-0.389	-0.036	0.199	-0.320	0.445
SDPD	0.163	0.142	-0.328	-0.146	0.044	0.424	-0.390	0.076	0.175	0.300
PPP	0.195	0.180	-0.080	0.000	-0.328	0.387	0.290	0.162	-0.502	-0.306
YPP	0.068	0.316	0.283	0.219	-0.344	-0.006	0.146	-0.081	0.029	0.069
SDWT	-0.169	-0.232	0.372	0.230	0.141	-0.070	0.126	-0.383	0.125	0.052
YKGH	0.455	-0.176	0.141	0.235	0.340	0.086	0.112	0.261	0.007	0.031
PROD	0.492	-0.205	0.074	0.257	0.249	-0.007	0.089	0.215	0.008	0.012

**Table: 32:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set evaluated during 2008-09 (E5) spring, at ICRISAT Centre, Patancheru, India

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, ASB

TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity

					Principle con	nponents				
Percentage of variation explained (%)	1	2	3	4	5	6	7	8	9	10
	22.58	11.92	10.08	9.53	6.45	5.76	5.48	4.68	4.57	4.10
Latent vectors	3.84	2.03	1.71	1.62	1.10	0.98	0.93	0.80	0.78	0.70
DF	-0.391	-0.246	0.165	0.261	0.046	0.100	0.069	-0.120	-0.246	0.034
FD	0.026	0.238	0.138	-0.525	0.277	0.074	0.023	-0.064	-0.265	-0.006
PLHT	-0.191	0.237	0.163	0.288	0.290	-0.066	-0.294	-0.007	0.299	0.197
PLWD	-0.138	0.224	0.119	0.361	0.328	0.302	0.386	0.144	0.026	-0.222
DGF	0.198	0.416	-0.045	-0.371	0.149	0.248	-0.031	0.199	0.235	0.164
DM	-0.360	0.076	0.193	-0.066	0.215	0.392	0.079	0.039	-0.113	0.208
BPB	-0.063	-0.293	0.088	-0.097	-0.336	0.577	-0.213	-0.037	0.515	0.092
APB	0.207	-0.004	0.293	0.032	0.339	-0.051	-0.497	-0.339	0.055	-0.389
BSB	0.115	-0.064	0.428	-0.131	-0.292	0.315	-0.049	0.067	-0.398	-0.342
ASB	0.148	-0.100	0.439	0.158	0.030	-0.137	-0.341	0.292	-0.131	0.393
ТВ	0.041	0.049	0.482	-0.044	-0.166	-0.317	0.289	0.482	0.268	-0.085
SDPD	0.172	-0.397	-0.016	-0.021	0.408	0.053	0.166	0.107	0.326	-0.348
PPP	0.339	-0.185	0.076	0.057	0.182	0.103	0.201	-0.234	-0.085	0.500
YPP	0.169	0.135	0.378	0.008	-0.160	-0.098	0.413	-0.608	0.220	0.083
SDWT	-0.168	0.497	0.014	0.185	-0.294	0.011	-0.137	-0.161	0.101	-0.154
YKGH	0.396	0.151	-0.093	0.334	-0.042	0.260	0.008	0.111	-0.120	0.000
PROD	0.424	0.126	-0.117	0.312	-0.080	0.173	0.000	0.089	-0.097	-0.037

**Table: 33:** Percentage of variation (%) and vector loading explained by first ten Principle component (PCs) estimated for 17 quantitative traits in chickpea reference set in overall pooled analysis.

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity

**Table 34:** Phenotypic diversity index in chickpea reference set evaluated

 in different environments at ICRISAT, Patancheru and UAS, Dharwad, India.

E1 (2006-07)	Diversity	Germplasm accessions
		ICCV92311:ICC11198
Maximum diversity	0.444	(India) (India)
		ICC3362:ICC1230
Minimum diversity	0.002	(Iran) (India)
Mean diversity	0.186	
E2 (2007-08)		
		ICC 20266: ICC 4991
Maximum diversity	0.425	(Unknown) (India)
		ICC 13764: ICC 12037
Minimum diversity	0.002	(Iran) (Mexico)
Mean diversity	0.187	
E3 (2008-09)		
		ICC 4918: ICC 16796
Maximum diversity	0.425	(India) (Portugal)
		ICC 13187: ICC 12324
Minimum diversity	0.002	(Iran) (Unknown)
Mean diversity	0.188	
E4 (2008-09)		
· · · · ·		ICC 4918: ICC 16796
Maximum diversity	0.43	(India) (Portugal)
		ICC 9002: ICC 2065
Minimum diversity	0.001	(Iran) (India)
Mean diversity	0.188	
E5 (2008-09)		
		ICC4918: ICC 18983
Maximum diversity	0.445	(India) (Greece)
		ICC2065:ICC12947
Minimum diversity	0.001	(India) (India)
Mean diversity	0.182	
Pooled		
		ICC 13764: ICC 12037
Maximum diversity	0.001	(Iran) (Mexico)
		ICCV92311:ICC11198
Minimum diversity	0.425	(India) (India)
Mean diversity	0.184	

E1= 2006-07, E2=2007-08, E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru, E4=2008-09 post rainy seasons at UAS, Dharwad

**Table: 35:** Mean (± Standard error), variance component and heritability in Chickpea Reference set evaluated during (E3) 2008-09 post-rainy, (E5) spring season for SPAD Chlorophyll Meter Readings (SCMR) related traits

	E3	E5	Pooled	E3	E5	Pooled	E3		E5		Pooled		Pooled	
				. 2	. 2	. ?	2	a F	2	a F	2	a F		a F
Trait	Mean $(\pm S.E)$	Mean $(\pm S.E)$	Mean $(\pm S.E)$	$h^2$	$h^2$	hĩ	σg	SE	σg	SE	σ~g	SE	σ2g x e	SE
SPAD	58.21±1.18	62±0.59	60.1±1.01	77.4	98.4	60.6	**3.30	0.51	**12.24	1.03	0	0.473	**3.94	0.72
Leaf Area	23.07±1.35	8.08±2.57	15.6±1.38	99.0	45.3	45.9	**74.94	6.23	9.44	5.15	**16.08	3.306	**35.22	3.20
Dry Weight	0.15±0.01	0.1±0.01	0.12±0.01	97.1	77.7	36.8	**0.001	0.00	**0.001	0.00	**0.00	0.00	**0.001	0.00

SPAD = Soil Plant Analysis Development.

E3=2008-09 post rainy, E5=2008-09 spring seasons at ICRISAT centre, Patancheru.

<b>Table: 36:</b> Expression of drought tolerance related traits in chickpea reference set
evaluated in cylinders during (E2) 2007-08, (E3) 2008-09 post-rainy season at
ICRISAT Patancheru, India

Trait	Trial mean	Range of mea	ans	σ²g	S.E	Heritability
	mean	Minimum	Maximum			
Shoot Dry Wei	ght (g)					
E2	1.89	1.34	2.77	0.097**	0.0119	68.6
E3	1.65	1.1	2.41	0.0815**	0.0092	73.7
<b>Root Dry Weig</b>	ht (g)					
E2	0.6	0.47	0.79	0.007**	0.0011	52.2
E3	0.58	0.39	0.89	0.013**	0.0015	70.2
Root Depth (cn	n)					
E2	107.81	99.37	117.13	27.3**	7.7	32.3
E3	107.57	98.52	116.02	26.6**	7.4	32.8
Root – Total dr	y weight (%	/0)				
E2	24.4	22.09	27.81	2.89**	0.83	32
E3	25.97	20.88	35.05	5.91**	0.95	53.7
Total Dry wt R	latio					
E2	2.5	1.85	3.53	0.146**	0.0176	69.9
E3	2.23	1.45	3.19	0.145**	0.0162	75.3
Root Length (c	m)					
E2	4776.79	4008.26	5523.25	262986**	69483	34.4
E3	4671.71	4024.55	5350.81	213585**	61299	31.8
Root Length D	Density (cm	cm-3)				
E2	0.18	0.14	0.21	0.0003**	0.00006	42.7
E3	0.21	0.18	0.23	0.0003**	0.00009	31.6
<b>Root Surface</b> A	Area (cm2)			•		
E2	748.73	565.63	930.35	9360**	2072	40.4
E3	802.96	629.57	1003.9	11971**	2292	46
Root Volume (	(cm3)					
E2	9.32	6.76	13.75	2.69**	0.54	43.9
E3	11.7	8.55	15.85	3.91**	0.74	46.6

E2=2007-08, E3=2008-09 post rainy seasons at ICRISAT centre, Patancheru.

		Range						
Trait	Mean	Minimum	Maximum	PCV	GCV	$\sigma_{g}^{2}$	$\sigma_{p}^{2}$	$\mathbf{h}^2$
SDW	1.77	1.073	2.869	48.085	45.649	0.653	0.725	90.12
RDW	0.592	0.3353	0.9739	50.146	46.221	0.075	0.088	84.96
RDp	107.69	83.8	131.7	19.686	17.23	344.3	449.45	76.6
R_T_%	25.194	16.98	38.95	29.529	26.294	43.87	55.32	79.29
TDW%	2.362	1.45	3.662	45.577	43.314	1.047	1.158	90.32
RL	4724.2	2846	6818	41.722	36.024	2896000	3884666	74.55
RLD	0.192	0.1345	0.4098	40.157	36.002	0.005	0.006	80.38
RSA	775.84	466	1149.3	47.695	42.308	107734	136915.5	78.69
RV	10.51	5.44	19.41	60.659	54.251	32.51	40.643	79.99

**Table: 37** Expression of drought tolerance related traits in chickpea reference set

 evaluated in cylinders in overall pooled analysis

SDW=Shoot Dry Weight, RDW=Root Dry Weight (RDW), RDp=Root Depth, R\_T\_%=Root to Total Plant Dry Weight ratio, TDW%=Total Plant Dry Weight, RL=Root Length, RLD=Root Length Density, RSA=Root surface area, RV=Root Volume.

**Table: 38:** Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set during, E2 (2007-08) post rainy season at ICRISAT, Patancheru, India.

Trait	SDW	RDW	RDp	R_T_%	TDW%	RL	RLD	RSA	RV
SDW									
RDW	0.658**								
RDp	0.300**	0.482**							
R_T_%	-0.456**	0.343**	0.196**						
TDW%	0.983**	0.785**	0.363**	-0.291**					
RL	0.536**	0.618**	0.739**	0.056	0.591**				
RLD	0.607**	0.606**	0.253**	-0.046	0.647**	0.513**			
RSA	0.588**	0.822**	0.525**	0.220**	0.684**	0.711**	0.711**		
RV	0.476**	0.774**	0.434**	0.292**	0.580**	0.550**	0.598**	0.924**	

Significant level indicated with asterisks as follows: \*P<0.005, \*\*P<0.01.

SDW=Shoot Dry Weight, RDW=Root Dry Weight (RDW), RDp=Root Depth, Root to Total Plant Dry Weight ratio (R/T %), TDW=Total Plant Dry Weight, RL=Root Length, RLD=Root Length Density, RSA=Root surface area, RV=Root Volume, and Shoot to Root Length Density ratio (S/RLD).

**Table: 39:** Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set during E3 (2008-09) post rainy season at ICRISAT, Patancheru, India

Trait	SDW	RDW	RDp	R_T_%	TDW%	RL	RLD	RSA	RV
SDW									
RDW	0.706**								
RDp	0.263**	0.408**							
R_T_%	-0.201**	0.535**	0.238**						
TDW%	0.975**	0.845**	0.326**	0.015					
RL	0.529**	0.608**	0.688**	0.196**	0.589**				
RLD	0.602**	0.576**	0.089	0.087	0.634**	0.387**			
RSA	0.653**	0.811**	0.464**	0.345**	0.746**	0.615**	0.734**		
RV	0.592**	0.798**	0.399**	0.393**	0.695**	0.499**	0.624**	0.943**	

Significant level indicated with asterisks as follows: \**P*<0.005, \*\**P*<0.01.

SDW=Shoot Dry Weight, RDW=Root Dry Weight (RDW), RDp=Root Depth, Root to Total Plant Dry Weight ratio (R/T %), TDW=Total Plant Dry Weight, RL=Root Length, RLD=Root Length Density, RSA=Root surface area, RV=Root Volume, and Shoot to Root Length Density ratio (S/RLD).

**Table: 40:** Phenotypic correlation coefficients between drought tolerance related traits in chickpea reference set in pooled analysis.

Trait	SDW	RDW	RDp	R_T_%	TDW%	RL	RLD	RSA	RV
SDW									
RDW	0.721**								
RDp	0.311**	0.456**							
R_T_%	-0.335**	0.392**	0.206**						
TDW%	0.983**	0.837**	0.367**	-0.160**					
RL	0.623**	0.666**	0.656**	0.075	0.670**				
RLD	0.667**	0.620**	0.230**	-0.038	0.693**	0.564**			
RSA	0.689**	0.853**	0.551**	0.241**	0.773**	0.757**	0.722**		
RV	0.608**	0.834**	0.482**	0.317**	0.703**	0.609**	0.612**	0.943**	

Significant level indicated with asterisks as follows: \*P<0.005, \*\*P<0.01.

SDW=Shoot Dry Weight, RDW=Root Dry Weight (RDW), RDp=Root Depth, Root to Total Plant Dry Weight ratio (R/T %), TDW=Total Plant Dry Weight, RL=Root Length, RLD=Root Length Density, RSA=Root surface area, RV=Root Volume, and Shoot to Root Length Density ratio (S/RLD).

Table: 41: Expression of resistance to <i>H.armigera</i> using detached leaf assay during flowering stage in Chickpea Reference
set evaluated during (E2) 2007-08, (E3) 2008-09 post-rainy season at ICRISAT Patancheru, India.

	E2	E3	Pooled	E2	E3	Pooled
Trait	Mean ( ± S.E)	Mean (± S.E)	Mean (±S.E)	Range	Range	Range
Damage Score	3.99±0.336	3.76±0.349	4.035±0.23	1.62-8.55	1.28-7.77	1.38-7.85
larval survival%	70.53±5.54	71.66±3.84	70.9±3.71	35.99-92.88	33.47-106.58	36.76-91.05
Unit larval wt	3.13±0.54	6.43±0.63	4.789±0.52	1.32-7.38	2.62-12.02	3.10-6.96

**Table: 42**: Expression of resistance to *H.armigera* using detached leaf assay during flowering stage in Chickpea Reference set evaluated during (E2) 2007-08, (E3) 2008-09 post-rainy season at ICRISAT, Patancheru, India.

	E2	E3	Pooled	E2		E3		Pooled		Pooled	
Trait	$h^2$	h <sup>2</sup>	h <sup>2</sup>	$\sigma^2 g$	SE	$\sigma^2 g$	SE	$\sigma^2 g$	SE	$\sigma^2 g x e$	SE
Damage Score	95.542	95.026	93.18	1.22**	0.12	1.3**	0.126	0.94**	0.10	0.36**	0.15
larval survival%	85.845	95.146	91.76	127.12**	20.05	162.69**	15.640	21.67*	9.93	122.25**	14.34
Unit larval wt	85.385	92.438	88.67	1.17**	0.19	2.84**	0.310	0.053	0.13	0.85*	0.39

## Table 43: List of trait specific germplasm in the chickpea reference set

Early flowering accessions (2 accessions)	ICC 8318, ICC 14594 (37-38 days)
Early maturing accessions (11 accessions)	ICC 11121, ICC 10685, ICC1205, ICC 13219, ICC 16903, ICC 11198, ICC 15618, ICC 15606, ICC 15567, ICC 506, ICC 8318, ICC 14402 (99- 104 days)
100- seed weight (19 accesions)	ICC 19165, ICC 20266, ICC 11303, ICC 15518, ICC11764, ICC 16796, ICC 9137, ICC 14199, ICC 12328,ICC 16654 (Top ten accessions with highest seed size-49-35gm
Plot yield (119 accessions)	ICC 11498, ICC 15510, ICC 8318, ICC 4567, ICC 10393, ICC 3362, ICC 15868, ICC 10018, ICC 5383, ICC 3325 16654 (Top ten accessions with highest seed yield-3172-2116 kg ha <sup>-</sup>
Heat Tolerant accessions (20 accessions)	ICC 3362, ICC 3582, ICC 11498, ICC 3776, ICC 8318, ICC 15510, ICC 10393, ICC 8384, ICC 3391, ICC 12328
Protein content (38 accessions)	ICC7668, ICC708, ICC11819, ICC67, ICC2629, ICC13187, ICC8515, ICC7052, ICC7184, ICC2919 (Top ten accessions with highest protein%-30.3-26.6%
Anthocyanin content (40 accessions)	ICC 3892, ICC 11498, ICC 7052, ICC 13524, ICC 16796, ICC 12916, ICC 6263, ICC 3325, ICC 4814, ICC 2720 (Top ten accessions with highest anthocyanin content 5.3-3.4
Shhot dry weight (42 accessions)	ICC 15518, ICC 15406, ICC 18679, ICC 20263, ICC 11903, ICC 14446, ICC 12328, ICC 18699, ICC 15435, ICC 18912 (Top ten accessions with highest Shoot dry weight – 2.9-2.4 gm)
Root dry weight (40 accessions)	( ICC 10885, ICC 12492, ICC 13187, ICC 18858, ICC 20267, ICC 11819, ICC 12379, ICC 15333, ICC 18912, ICC 19011 (Top ten accessions with highest root dry weight – .97-0.82 gm),
Root depth (13 accessions)	ICC 8740, ICC 11498, ICC 18983, ICC 15518, ICC 7819, ICC 10885, ICC 2679, ICC 12028, ICC 16207, ICC 13524 (Top ten accessions with highest Root Depth 131.7-119.8 cm
Root to total plant dry weight ratio (R-T) % (11 accessions)	ICC 12492, ICC 12928, ICC 11198, ICC 2629, ICC 18858, ICC 16207, ICC 15610, ICC 19226, ICC 12037, ICC 9434, ICC 1230- 39.0-30.2%
Root length (33 accessions)	ICC 10885, ICC 20267, ICC 3410, ICC 18828, ICC 15518, ICC 18679, ICC 20263, ICC 8521, ICC 3512, ICC 8318- Top ten accessions with highest root length – 6818.3-6008.4
Root length density (6 accessions)	ICC 8261, ICC 5337, ICC 6306, ICC 18912, ICC 20267, ICC 14446 – 0.41-0.26)
Damage rating (25 accessions)	ICC 20174, ICC 16903, ICC 14595, ICC 15518, ICC 8522, ICC 9590, ICC 9875, ICC 9712, ICC 9895, ICC 4182, ICC 15435- Top ten accessions with minimum damage rating – 1.4 – 2.4
Larval survival % (17 accesions)	ICC 7819, ICC 12537, ICC 6903, ICC 15435, ICC 13764, ICC 18828, ICC 9862, ICC 4533, ICC 14595, ICC 11498 - Top ten accessions with lowest larval survival % - 48.8-54.1%),
Unit larval weight (3 accessions)	ICC 70826, ICC 16903, ICC 6293 (2.1-2.9 gm) compared to the control cultivar ICC 506 – 3.1gm.

**Table 44:** Allelic richness, major allele frequency, gene diversity, heterozygosity,polymorphic information content (PIC), allele range, rare, common and most frequentalleles of 91 SSR loci in the chickpea reference set (300 accessions)

	Allele	Major.Allele.	Gene	Hetero	DIG	Allele	Rare	Common	Frequent
Marker	No	Frquency	Diversity	zygosity	PIC	range	alleles	alleles	alleles
CaSTMS2	26	0.121	0.936	0.00	0.932	210-326	18	562	0
CaSTMS4	19	0.239	0.873	0.00	0.861	209-275	14	380	124
CaSTMS5	13	0.491	0.699	0.00	0.668	202-242	12	214	218
CaSTMS6	10	0.594	0.538	0.00	0.460	200-232	16	18	404
CaSTMS7	8	0.727	0.443	0.00	0.413	150-177	10	142	404
CaSTMS9	11	0.538	0.597	0.00	0.531	100-138	14	40	362
CaSTMS12	7	0.496	0.564	0.00	0.469	140-152	12	24	476
CaSTMS13	8	0.816	0.318	0.00	0.296	100-156	10	88	434
CaSTMS20	3	0.990	0.021	0.00	0.021	144-153	6	0	570
CaSTMS21	12	0.434	0.724	0.00	0.686	162-207	16	198	380
CaSTMS23	6	0.632	0.484	0.00	0.390	101-151	6	8	518
CaSTMS25	17	0.429	0.732	0.00	0.698	101-186	22	170	372
GA9	13	0.580	0.610	0.00	0.574	177-218	16	230	340
GA13	5	0.577	0.508	0.00	0.402	109-127	6	8	558
GA20	27	0.122	0.927	0.00	0.922	143-213	22	554	0
GA22	31	0.261	0.823	0.00	0.801	110-361	48	198	268
GA26	17	0.211	0.855	0.00	0.838	180-236	12	280	210
GA34	41	0.149	0.918	0.00	0.912	116-363	64	446	0
GA137	18	0.335	0.817	0.00	0.799	117-482	12	216	298
GAA39	15	0.600	0.576	0.00	0.529	209-255	22	60	448
GAA40	9	0.445	0.705	0.00	0.660	199-244	12	150	328
GAA43	4	0.659	0.477	0.00	0.401	100-109	2	24	520
GAA58	9	0.482	0.620	0.00	0.551	219-249	6	58	392
TA2	22	0.121	0.929	0.00	0.924	119-189	14	480	0
TA5	26	0.112	0.920	0.00	0.915	158-440	28	544	0
TA8	24	0.143	0.919	0.00	0.914	131-252	12	448	0
TA14	29	0.139	0.920	0.00	0.915	210-348	18	574	0
TA20	43	0.159	0.949	0.00	0.947	119-347	42	500	0
TA21	34	0.103	0.942	0.00	0.939	276-422	40	540	0
TA22	43	0.071	0.965	0.00	0.963	314-312	28	566	0
TA25	42	0.093	0.952	0.00	0.950	190-373	38	524	0
TA27	28	0.310	0.848	0.00	0.836	177-334	32	360	176
TA28	52	0.089	0.960	0.41	0.958	110-433	67	425	0
TA53	32	0.140	0.936	1.65	0.933	169-269	32	454	0
TA59	18	0.169	0.906	0.00	0.899	216-268	12	426	0
TA64	38	0.084	0.951	0.40	0.949	119-462	35	465	0

	Allele	Major.Allele.	Gene	Hetero	DIC	Allele	Rare	Common	Frequent
Marker	No	Frquency	Diversity	zygosity	PIC	range	alleles	alleles	alleles
TA71	34	0.093	0.946	2.71	0.944	121-275	15	575	0
TA72	30	0.168	0.899	1.71	0.891	150-263	34	550	0
TA78	34	0.148	0.924	0.00	0.920	151-259	30	566	0
TA80	29	0.168	0.920	0.00	0.915	125-268	20	576	0
TA96	32	0.149	0.932	0.00	0.929	175-429	24	498	0
TA103	24	0.157	0.902	0.00	0.894	144-202	26	484	0
TA106	41	0.177	0.933	0.00	0.929	131-480	40	502	0
TA108	8	0.788	0.365	0.00	0.349	101-156	12	98	410
TA110	21	0.088	0.941	0.00	0.938	185-244	8	490	0
TA113	18	0.220	0.886	2.41	0.876	112-240	15	439	128
TA117	32	0.116	0.939	2.87	0.936	116-366	32	526	0
TA120	19	0.189	0.876	0.00	0.863	123-126	22	550	0
TA125	29	0.131	0.922	0.00	0.917	168-256	40	404	0
TA130	26	0.272	0.876	0.00	0.867	147-476	32	390	158
TA132	39	0.215	0.908	0.00	0.902	104-484	50	358	112
TA135	34	0.224	0.898	0.00	0.891	107-481	42	422	134
TA140	27	0.208	0.879	0.00	0.868	102-192	38	274	218
TA142	39	0.181	0.918	0.00	0.913	103-263	58	484	0
TA144	22	0.122	0.926	0.00	0.921	198-290	10	530	0
TA159	45	0.077	0.958	0.00	0.956	100-494	56	486	0
TA176	56	0.088	0.969	0.00	0.969	150-356	40	554	0
TA180	27	0.151	0.922	0.00	0.917	153-373	18	526	0
TA196	20	0.192	0.876	0.00	0.864	175-230	22	436	0
TA200	29	0.115	0.933	0.00	0.929	139-343	20	556	0
TA203	39	0.054	0.964	0.00	0.963	108-294	28	560	0
TAA57	4	0.902	0.180	0.00	0.169	128-352	2	48	458
TAA58	39	0.071	0.960	0.00	0.958	206-335	26	454	0
TAA59	28	0.517	0.716	0.00	0.706	145-410	32	228	278
TAA169	20	0.211	0.880	0.00	0.868	152-398	18	378	106
TAA194	21	0.344	0.835	0.00	0.822	116-278	16	324	178
TaaSH	26	0.118	0.932	0.00	0.928	366-463	8	570	0
TR1	52	0.097	0.939	0.00	0.936	107-492	82	516	0
TR2	32	0.081	0.954	0.00	0.952	103-289	18	522	0
TR7	44	0.137	0.917	0.00	0.911	109-465	74	380	0
TR19	34	0.082	0.956	0.00	0.954	106-484	24	466	0
TR20	12	0.221	0.853	0.00	0.836	149-196	8	416	120
TR24	32	0.196	0.919	0.00	0.914	111-242	28	502	0
TR26	11	0.690	0.458	0.00	0.395	129-456	18	10	494

Montron	Allele	Major.Allele.	Gene	Hetero	PIC	Allele	Rare	Common	Frequent
Marker	No	Frquency	Diversity	zygosity	PIC	range	alleles	alleles	alleles
TR29	30	0.152	0.927	0.00	0.923	142-395	28	498	0
TR31	35	0.173	0.906	0.00	0.899	117-426	50	550	0
TR40	27	0.123	0.918	0.00	0.912	190-285	24	546	0
TR43	60	0.083	0.966	0.00	0.965	140-495	76	524	0
TR56	17	0.280	0.832	0.00	0.813	227-381	12	224	242
TR59	15	0.260	0.863	0.00	0.850	102-203	6	376	134
TS5	61	0.178	0.944	0.00	0.943	100-425	90	336	0
TS24	31	0.414	0.787	0.00	0.772	100-363	54	212	188
TS35	46	0.076	0.962	0.00	0.961	200-262	50	452	0
TS43	38	0.306	0.885	0.00	0.881	171-290	28	344	164
TS45	31	0.169	0.925	0.36	0.920	140-393	16	540	0
TS46	29	0.100	0.946	0.00	0.944	157-270	28	392	0
TS53	9	0.310	0.738	0.00	0.691	155-400	8	62	466
TS54	35	0.144	0.936	0.00	0.933	149-422	34	506	0
TS62	30	0.095	0.953	1.24	0.951	175-272	12	472	0
TS72	22	0.152	0.919	0.00	0.913	213-304	14	474	0
TS83	26	0.184	0.884	0.00	0.873	113-383	32	500	0
Mean	26	0.264	0.825	0.15	0.809		26.6	374	129.5
Min	3	0.054	0.021	0.00	0.021		2	0	0
Max	61	0.990	0.969	2.87	0.969		90	576	570

**Table 45:** Allelic richness, major allele frequency, gene diversity, heterozygosity, polymorphic information content (PIC), allele range, rare, common and most frequent alleles of 91 SSR loci of biological races in the chickpea reference set (300 accessions)

			Des	si			Kabı	uli			Pe		
S.No	Marker	Allele no	Gene Diversity	Hetero zygosity	PIC	Allele no	Gene Diversity	Hetero zygosity	PIC	Allele no	Gene Diversity	Hetero zygosity	PIC
1	CaSTMS2	26	0.94	0.00	0.93	17	0.91	0.00	0.90	9	0.88	0.00	0.86
2	CaSTMS4	16	0.83	0.00	0.82	13	0.85	0.00	0.84	8	0.86	0.00	0.84
3	CaSTMS5	8	0.69	0.00	0.66	10	0.64	0.00	0.61	3	0.57	0.00	0.49
4	CaSTMS6	5	0.51	0.00	0.42	5	0.51	0.00	0.46	2	0.48	0.00	0.36
5	CaSTMS7	5	0.44	0.00	0.40	4	0.37	0.00	0.35	3	0.53	0.00	0.47
6	CaSTMS9	7	0.55	0.00	0.46	7	0.58	0.00	0.54	5	0.77	0.00	0.73
7	CaSTMS12	5	0.54	0.00	0.44	5	0.59	0.00	0.51	2	0.48	0.00	0.36
8	CaSTMS13	5	0.33	0.00	0.30	3	0.27	0.00	0.25	1	0.00	0.00	0.00
9	CaSTMS20	1	0.00	0.00	0.00	1	0.00	0.00	0.00	1	0.00	0.00	0.00
10	CaSTMS21	10	0.68	0.00	0.64	7	0.78	0.00	0.74	4	0.55	0.00	0.50
11	CaSTMS23	3	0.47	0.00	0.37	4	0.50	0.00	0.40	2	0.35	0.00	0.29
12	CaSTMS25	11	0.69	0.00	0.65	10	0.75	0.00	0.72	4	0.70	0.00	0.65
13	GA9	10	0.59	0.00	0.55	9	0.63	0.00	0.59	3	0.56	0.00	0.48
14	GA13	3	0.50	0.00	0.38	5	0.53	0.00	0.43	2	0.42	0.00	0.33
15	GA20	23	0.91	0.00	0.90	21	0.93	0.00	0.93	7	0.81	0.00	0.79
16	GA22	24	0.84	0.00	0.82	13	0.73	0.00	0.69	5	0.72	0.00	0.68
17	GA26	13	0.83	0.00	0.81	12	0.85	0.00	0.83	6	0.79	0.00	0.76
18	GA34	35	0.91	0.00	0.91	20	0.90	0.00	0.89	8	0.86	0.00	0.84
19	GA137	17	0.82	0.00	0.80	15	0.81	0.00	0.78	4	0.66	0.00	0.61
20	GAA39	11	0.59	0.00	0.54	6	0.45	0.00	0.41	3	0.57	0.00	0.49
21	GAA40	8	0.71	0.00	0.66	5	0.56	0.00	0.51	3	0.59	0.00	0.53
22	GAA43	3	0.49	0.00	0.42	3	0.41	0.00	0.34	2	0.49	0.00	0.37
23	GAA58	7	0.59	0.00	0.51	4	0.61	0.00	0.55	3	0.43	0.00	0.39
23	TA2	20	0.92	0.00	0.91	15	0.90	0.00	0.89	8	0.86	0.00	0.84
25	TA5	20	0.92	0.00	0.91	18	0.91	0.00	0.90	5	0.69	0.00	0.65
26	TA8	21	0.92	0.00	0.91	20	0.92	0.00	0.90	6	0.78	0.00	0.05
20	TA14	24	0.92	0.00	0.91	19	0.89	0.00	0.91	7	0.84	0.00	0.75
27	TA14 TA20	37	0.92	0.00	0.95	30	0.94	0.00	0.87	9	0.88	0.00	0.82
28	TA20	26	0.93	0.00	0.93	28	0.95	0.00	0.93	8	0.86	0.00	0.84
30	TA21 TA22	40	0.96	0.00	0.96	20	0.92	0.00	0.94	9	0.88	0.00	0.86
31	TA25	35	0.95	0.00	0.94	24	0.92	0.00	0.95	9	0.88	0.00	0.86
31	TA25	23	0.93	0.00	0.94	19	0.95	0.00	0.95	7	0.83	0.00	0.80
33	TA27 TA28	38	0.84	0.00	0.85	33	0.85	0.00	0.84	8	0.81	0.00	0.79
33	TA53	28	0.90	0.01	0.90	22	0.93	0.00	0.93	9	0.86	0.00	0.85
35	TA59	16	0.93	0.00	0.93	14	0.93	0.00	0.92	-	0.80	0.00	0.85
35	TA59 TA64	34	0.89	0.00	0.88	21	0.88	0.00	0.87	6 9	0.81	0.00	0.79
30	TA04 TA71	32	0.93	0.01	0.93	21	0.92	0.00	0.92	9	0.88	0.00	0.86
37	TA71 TA72	32 27	0.94	0.03	0.94	18	0.92	0.02	0.92	9 7	0.88	0.10	0.80
38 39	TA72 TA78	27	0.90	0.02	0.89	24	0.88	0.00	0.87	9	0.82	0.00	0.80
40	TA/8 TA80	26	0.90	0.00	0.89	17	0.94	0.00	0.94	6	0.88	0.00	0.86
	TA80 TA96		0.93		0.92	22	0.89		0.88	7	0.76	0.00	0.73
41		26		0.00				0.00		7			
42	TA103	21	0.90	0.00	0.89	16	0.89	0.00	0.88		0.84	0.00	0.82
43	TA106	34	0.93	0.00	0.92	24	0.91	0.00	0.90	7	0.84	0.00	0.82
44	TA108	8	0.41	0.00	0.39	4	0.17	0.00	0.17	3	0.59	0.00	0.50
45	TA110	20	0.94	0.00	0.94	19	0.93	0.00	0.92	7	0.84	0.00	0.82
46	TA113	15	0.87	0.01	0.86	13	0.87	0.04	0.85	7	0.81	0.00	0.79
47	TA117	26	0.93	0.02	0.93	26	0.94	0.03	0.94	8	0.86	0.09	0.84
48	TA120	18	0.89	0.00	0.88	13	0.83	0.00	0.81	6	0.76	0.00	0.73
49	TA125	22	0.91	0.00	0.91	20	0.92	0.00	0.92	5	0.73	0.00	0.70
50	TA130	21	0.86	0.00	0.85	16	0.87	0.00	0.86	5	0.74	0.00	0.70
51	TA132	28	0.86	0.00	0.85	30	0.95	0.00	0.94	9	0.88	0.00	0.86

			Des	si			Kabı	uli			Pe	Pea		
S.No	Marker	Allele no	Gene Diversity	Hetero zygosity	PIC	Allele no	Gene Diversity	Hetero zygosity	PIC	Allele no	Gene Diversity	Hetero zygosity	PIC	
52	TA135	27	0.89	0.00	0.88	19	0.89	0.00	0.88	6	0.79	0.00	0.76	
53	TA140	23	0.88	0.00	0.87	15	0.86	0.00	0.84	6	0.81	0.00	0.79	
54	TA142	30	0.90	0.00	0.90	26	0.92	0.00	0.91	4	0.73	0.00	0.68	
55	TA144	20	0.93	0.00	0.92	16	0.86	0.00	0.85	8	0.84	0.00	0.82	
56	TA159	36	0.94	0.00	0.94	32	0.94	0.00	0.93	9	0.88	0.00	0.87	
57	TA176	53	0.97	0.00	0.97	29	0.95	0.00	0.94	9	0.86	0.00	0.85	
58	TA180	24	0.90	0.00	0.90	22	0.93	0.00	0.92	7	0.82	0.00	0.80	
59	TA196	17	0.88	0.00	0.86	14	0.84	0.00	0.82	5	0.74	0.00	0.70	
60	TA200	28	0.93	0.00	0.93	19	0.91	0.00	0.90	8	0.84	0.00	0.83	
61	TA203	37	0.96	0.00	0.96	29	0.95	0.00	0.94	8	0.86	0.00	0.84	
62	TAA57	4	0.19	0.00	0.18	2	0.16	0.00	0.14	2	0.17	0.00	0.15	
63	TAA58	27	0.95	0.00	0.95	32	0.95	0.00	0.95	8	0.86	0.00	0.84	
64	TAA59	24	0.72	0.00	0.71	18	0.73	0.00	0.71	2	0.35	0.00	0.29	
65	TAA169	17	0.87	0.00	0.86	10	0.83	0.00	0.82	6	0.80	0.00	0.77	
66	TAA194	17	0.83	0.00	0.81	19	0.83	0.00	0.82	5	0.78	0.00	0.74	
67	TaaSH	24	0.92	0.00	0.92	21	0.93	0.00	0.93	7	0.83	0.00	0.80	
68	TR1	34	0.92	0.00	0.92	31	0.94	0.00	0.93	8	0.86	0.00	0.84	
69	TR1 TR2	32	0.93	0.00	0.95	24	0.94	0.00	0.94	10	0.80	0.00	0.84	
70	TR2 TR7	32	0.93	0.00	0.95	24	0.94	0.00	0.94	7	0.89	0.00	0.88	
70	TR19	32	0.90	0.00	0.89	23	0.92	0.00	0.92	5	0.84	0.00	0.82	
				-		9				5				
72	TR20 TR24	11	0.82	0.00	0.80	9 19	0.82	0.00	0.80	6	0.74	0.00	0.70	
73		27	0.89	0.00	0.89		0.93	0.00	0.92	-	0.79	0.00	0.76	
74	TR26	7	0.45	0.00	0.38	5	0.39	0.00	0.35	3	0.58	0.00	0.49	
75	TR29	27	0.92	0.00	0.91	20	0.90	0.00	0.90	7	0.82	0.00	0.80	
76	TR31	28	0.90	0.00	0.89	17	0.88	0.00	0.87	9	0.88	0.00	0.86	
77	TR40	21	0.90	0.00	0.89	19	0.90	0.00	0.89	7	0.84	0.00	0.82	
78	TR43	51	0.96	0.00	0.96	33	0.92	0.00	0.92	10	0.89	0.00	0.88	
79	TR56	13	0.77	0.00	0.74	13	0.88	0.00	0.87	5	0.74	0.00	0.70	
80	TR59	14	0.85	0.00	0.84	12	0.86	0.00	0.84	6	0.78	0.00	0.75	
81	TS5	48	0.92	0.00	0.92	35	0.96	0.00	0.95	8	0.88	0.00	0.86	
82	TS24	27	0.78	0.00	0.77	12	0.75	0.00	0.72	5	0.79	0.00	0.76	
83	TS35	36	0.96	0.00	0.96	27	0.95	0.00	0.94	8	0.84	0.00	0.82	
84	TS43	32	0.86	0.00	0.86	23	0.92	0.00	0.92	4	0.66	0.00	0.61	
85	TS45	27	0.92	0.01	0.92	23	0.91	0.00	0.91	5	0.71	0.00	0.66	
86	TS46	26	0.94	0.00	0.93	22	0.94	0.00	0.93	8	0.86	0.00	0.85	
87	TS53	8	0.73	0.00	0.68	6	0.75	0.00	0.70	3	0.62	0.00	0.55	
88	TS54	32	0.94	0.00	0.94	22	0.91	0.00	0.90	6	0.79	0.00	0.76	
89	TS62	27	0.95	0.01	0.94	25	0.94	0.01	0.94	4	0.67	0.00	0.61	
90	TS72	20	0.89	0.00	0.89	16	0.92	0.00	0.91	7	0.82	0.00	0.80	
91	TS83	22	0.86	0.00	0.85	13	0.86	0.00	0.85	6	0.79	0.00	0.76	
	Mean	22	0.82	0.00	0.80	17	0.81	0.00	0.79	6	0.73	0.00	0.70	
	Min	1	0	0	0	1	0	0	0	1	0	0	0	
	Max	53	0.97	0.03	0.97	35	0.96	0.04	0.95	10	0.89	0.1	0.88	

_	#	Allele	informatio	n	Gene dive	rsity	PIC valu	ue	Heterozygosity	
Category	Accessions	# Alleles	Range	Avg	Range	Avg	Range	Avg	Range	Avg
Ref-211+89	300	2411	3-61	26	0.021-0.969	0.825	0.021-0.969	0.809	0.00-2.87	0.15
	•			Biolo	gical status					
Desi chickpea	194	2009	1-53	22	0-0.97	0.820	0-0.97	0.80	0-0.03	0.00
Kabuli chickpea	88	1572	1-35	17	0-0.96	0.810	0-0.95	0.79	0-0.04	0.00
Pea-shaped chickpea	11	544	1-10	6	0-0.89	0.73	0-0.89	0.73	0-0.01	0.00
Wild species	7	433	1-8	5	0-0.86	0.73	0-0.84	0.69	0-0.33	0.01
				Geogra	phical regions					
South East Asia	110	1489	1-36	16	0-0.96	0.79	0-0.22	0	0-0.96	0.770
West Asia	93	1578	1-37	17	0-0.96	0.820	0-0.06	0.001	0-0.96	0.800
Mediterranean	56	1401	2-30	15	0.11-0.96	0.817	0.107-0.96	0.800	0-0.10	0.006
Africa	21	755	1-15	8	0-0.92	0.760	0-0.13	0.010	0-0.91	0.730
North America	6	286	0-6	3	0-0.83	0.560	0-0.50	0.005	0-0.81	0.502
Russian Federation	6	333	0-6	4	0-0.83	0.640	0-0.25	0.030	0-0.81	0.590
South America	4	239	1-4	3	0-0.75	0.540	0-0	0.000	0-0.70	0.460
Europe	3	179	0-4	2	0-0.72	0.410	0-0.33	0.000	0-0.067	0.340
Unknown	6	316	1-6	3	0-0.83	0.590	0-0.25	0.000	0-0.81	0.540

**Table 46:** Range and average gene diversity of both biological status and geographical regions in the chickpea reference set

**Table 47:** Details of the accessions present in four clusters identified by unweighted neighbor joining tree based on 91 SSR markers in the chickpea reference set

Cluster I : Total 89 accessions + 2 control cultivars
64 desi accessions 24 kabuli accessions 1 pea type
+ 2 controls
ICC 4918
ICC 4948
Cluster II : Total 30 accessions
20 desi accessions 9 kabuli accessions 1 pea type
Cluster III : Total 87 accessions
76 desi accessions 9 kabuli accessions 2 pea type
<b>Cluster IV</b> : Total 91 accessions + 3 control cultivars
34 desi accessions 46 kabuli accessions 7 pea type
+1  control + 2  controls
ICC 15996 ICCV 92311,ICC 4973

Category	Cluster-I	Cluster-II	Cluster-III	Cluster-IV
Total number of alleles	1601	1006	1547	1715
Allele range	1-40	1-19	1-43	2-37
Average number of alleles	17.6	11.1	17	18.8
PIC	0.961	0.929	0.96	0.957
Gene Diversity	0.962	0.933	0.962	0.959
Heterozygosity	0.023	0.071	0.047	0.049
Rare alleles	2	0	1	7
Common alleles	10559	3432	10145	10937
Frequent alleles	3789	1456	3915	3628
Biological Status				
Desi	64	20	76	34
Kabuli	24	9	9	46
Pea	1	1	2	7
Wild	0	0	0	4
Geographical origin				
Africa	13	5	0	3
Europe	1	1	0	1
Mediterranean	7	8	6	32
North America	3	0	1	2
Russian Federation	2	0	2	2
South East Asia	43	12	34	16
South America	3	0	0	1
Unknown	3	0	1	2
West Asia	14	4	43	32

**Table 48:** Range and average Gene diversity of both biological status and geographical regions in the chickpea reference collection

			Ν	/Iean				R	lange				
Pop ulati ons	Total no of alleles	Samp le Size	Allel eNo	Gene Diversi ty	Heteroz ygosity	PIC	Allele range	Gene Dversity	Heterozy gosity	PIC	Rare alleles	commo n alleles	Freque nt alleles.
1	1199	48	11	0.739	0.0023	0.727	0-25	0-0.948	0-0.667	0-0.946	32	5816	2824
2	720	25	6	0.668	0.0059	0.649	1-18	0-0.934	0-0.080	0-0.930	0	2183	2297
3	778	24	7	0.685	0.0011	0.667	0-16	0-0.926	0-0.041	0-0.922	0	2122	1556
4	483	14	4	0.560	0.0006	0.535	0-10	0-0.900	0-0.071	0-0.891	0	783	1151
5	527	15	5	0.564	0.0006	0.538	0-11	0-0.888	0-0.066	0-0.877	0	960	1926
6	803	28	7	0.670	0.0035	0.653	0-21	0-0.947	0-0.071	0-0.945	0	2311	1717
7	749	13	7	0.765	0.0027	0.737	1-11	0-0.909	0-0.091	0-0.902	0	1393	871
8	1301	56	11	0.731	0.0006	0.715	0-26	0-0.950	0-0.018	0-0.947	2	7087	3881
9	544	12	5	0.650	0.0013	0.612	1-9	0-0.876	0-0.000	0-0.863	0	865	1241
10	574	15	5	0.714	0.0018	0.693	0-12	0-0.898	0-0.071	0-0.890	0	1160	1130
11	348	9	3	0.561	0.0058	0.527	0-7	0-0.840	0-0.000	0-0.819	0	324	1340
12	428	12	4	0.567	0.0067	0.517	1-9	0-0.876	0-0.083	0-0.863	0	552	1462
13	759	29	7	0.707	0.0017	0.690	0-21	0-0.949	0-0.087	0-0.947	0	2177	1961

**Table 51:** Summary statistics of the chickpea reference set accessions based onsubpopulations detected by STRUCTURE analysis using 91 SSR markers

SP	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9	SP10	SP11	SP12	SP13
SP1	0.000												
SP2	0.157	0.000											
SP3	0.229	0.244	0.000										
SP4	0.239	0.255	0.253	0.000									
SP5	0.292	0.317	0.284	0.326	0.000								
SP6	0.237	0.265	0.220	0.253	0.313	0.000							
SP7	0.162	0.185	0.165	0.197	0.161	0.186	0.000						
SP8	0.225	0.248	0.215	0.241	0.116	0.235	0.102	0.000					
SP9	0.198	0.224	0.195	0.211	0.184	0.202	0.102	0.114	0.000				
SP10	0.271	0.298	0.264	0.279	0.334	0.254	0.216	0.259	0.235	0.000			
SP11	0.205	0.214	0.292	0.319	0.362	0.313	0.219	0.286	0.268	0.349	0.000		
SP12	0.200	0.227	0.229	0.255	0.243	0.245	0.124	0.172	0.158	0.269	0.274	0.000	
SP13	0.280	0.292	0.257	0.271	0.337	0.253	0.226	0.268	0.243	0.271	0.346	0.290	0.000

SP- Subpopulations

SP	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9	SP10	SP11	SP12	SP13
SP1	0.000												
SP2	0.172	0.000											
SP3	0.772	0.743	0.000										
SP4	0.679	0.643	0.710	0.000									
SP5	1.282	1.218	1.192	1.291	0.000								
SP6	0.703	0.799	0.529	0.552	1.298	0.000							
SP7	0.809	0.864	0.863	0.961	0.761	0.886	0.000						
SP8	1.069	1.063	1.016	0.995	0.267	1.020	0.552	0.000					
SP9	0.861	0.937	0.831	0.803	0.767	0.760	0.690	0.501	0.000				
SP10	0.721	0.806	0.630	0.518	1.188	0.404	0.916	0.949	0.761	0.000			
SP11	0.373	0.311	0.928	0.863	1.391	0.952	1.001	1.244	1.130	1.003	0.000		
SP12	0.694	0.751	0.920	0.942	1.017	0.933	0.728	0.807	0.786	0.834	0.956	0.000	
SP13	0.721	0.665	0.527	0.439	1.081	0.392	0.940	0.935	0.764	0.297	0.872	0.957	0.000

SP- Subpopulations

Axis	PC1	PC2	PC3	ICC18912	0.191	1.331	0.088
% Variation	36.48	33.38	11.85	ICC5434	-0.236	-0.32	-0.32
Cumulative %	36.48	69.86	81.71	ICC4918	-0.349	-0.34	-0.35
Eigen values	188.6	66.97	40.7	ICC14595	-0.151	0.017	-0.42
ICC8522	1.055	-0.09	0.382	ICC5878	-0.554	-0.7	1.041
ICC13283	1.118	-0.19	-0.19	ICC1083	-0.173	-0.33	-0.39
ICC8058	1.069	-0.28	-0.03	ICC5613	-0.308	-0.35	-0.44
ICC12537	1.139	-0.13	0.079	ICC8318	-0.456	-0.67	1.186
ICC8261	1.197	-0.09	0.207	ICC9702	-0.438	-0.2	1.483
ICC19100	1.127	-0.15	-0.02	ICC9590	-0.15	0.062	-0.29
ICC10755	1.043	-0.05	-0.09	ICC6279	-0.227	-0.22	-0.43
ICC13764	0.967	-0.19	-0.02	ICC16374	1.165	-0.24	0.178
ICC20262	1.079	-0.02	0.064	ICC6802	-0.435	-0.19	-0.39
ICC13441	1.201	-0.12	0.055	ICC6811	-0.325	-0.27	1.351
ICC19011	1.28	-0.17	-0.01	ICC14669	-0.324	-0.23	-0.46
ICC7668	1.088	-0.09	0.1	ICC5845	-0.925	-0.07	-0.47
ICC14199	1.131	-0.05	-0.1	ICC11498	-0.902	-0.23	-0.24
ICC7326	1.106	-0.16	0.068	ICC2720	-0.961	-0.05	-0.5
ICC6294	1.016	-0.1	-0.15	ICC791	-1.119	-0.08	-0.42
ICC8607	0.7	-0.19	0.42	ICC6579	-1.125	-0.18	-0.18
ICC15762	0.949	0.028	0.312	ICC5639	-0.98	0.038	-0.25
ICC7571	1.128	-0.16	-0.12	ICC12928	-0.967	-0.09	-0.43
ICC8515	1.214	-0.17	-0.04	ICC440	-0.836	-0.03	-0.33
ICC7305	1.025	-0.2	-0.15	ICC9586	-1.001	-0.08	-0.02
ICC14098	1.171	-0.17	0.078	ICC6571	-0.966	-0.09	-0.18
ICC18858	1.091	-0.05	-0.09	ICC1715	-1.023	-0.06	-0.15
ICC15510	1.184	-0.13	-0.03	ICC16524	-1.055	-0.07	-0.19
ICC2737	1.043	-0.08	0.114	ICC1161	-0.987	-0.14	-0.33
ICC8855	1.001	-0.03	-0.11	ICC11627	-0.986	-0.06	-0.33
ICC19164	1.075	-0.34	-0.08	ICC4567	-1.042	-0.07	-0.08
ICC20259	1.161	-0.24	-0.09	ICC7255	-0.288	-0.32	-0.19
ICC9402	1.153	-0.19	0.05	ICC18720	-0.315	-0.36	-0.36
ICC12866	1.134	-0.1	0.032	ICC13719	-0.316	-0.16	-0.4
ICC12321	1.042	-0.17	-0.08	ICC12324	-0.405	-0.27	-0.16
ICC15802	0.893	-0.12	-0.14	ICC18828	-0.098	-0.03	-0.31
ICC2679	1.1	-0.22	0.044	ICC12328	-0.333	-0.19	-0.29
ICC9643	1.042	-0.09	0.14	ICC11819	-0.392	-0.5	1.013
ICC20261	1.053	-0.19	-0.1	ICC16654	-0.308	-0.19	1.517
ICC7323	1.044	-0.29	-0.16	ICC4841	-0.102	-0.24	-0.31
ICC4853	1.101	-0.15	-0.01	ICC8151	-0.122	-0.02	-0.5
ICC12492	0.984	-0.27	0.146	ICC7308	-0.341	-0.24	-0.24
ICC3421	1.288	-0.19	-0.11	ICC11879	-0.074	-0.28	-0.11
ICC13077	1.004	0.025	0.184	ICC15248	-0.366	-0.4	0.292
ICC19226	1.072	-0.28	-0.12	ICC12037	-0.344	-0.08	-0.39
ICC3410	0.998	-0.31	-0.01	ICC19034	-0.317	-0.24	-0.24
ICC20265	1.077	-0.12	-0.04	ICC20264	-0.413	-0.41	1.021

**Table 55:** Principal Coordinates Analysis (PCoA) in the chickpea reference set accessions using 91 SSR markers based on estimates of Nei (1973) distance

ICC20267	1.152	-0.12	-0	ICC5337	-0.024	-0.06	-0.19
ICC13187	1.122	-0.09	0.294	ICC15333	-0.217	-0.36	0.234
ICC20190	0.974	-0.25	0.158	ICC16796	-0.313	-0.05	0.487
ICC6263	1.224	-0.25	0.081	ICC18847	-0.252	-0.09	-0.46
ICC4093	1.01	-0.17	-0.07	ICC18699	-0.369	-0.35	1.015
ICC3218	1.056	-0.16	-0.01	ICC20260	0.238	1.28	0.081
ICC4495	1.129	-0.34	0.04	ICC12824	-0.461	-0.11	-0.3
ICC6816	1.3	-0.2	-0.07	ICC7150	-0.287	-0.53	1.273
ICC3230	1.232	-0.07	-0.2	ICC13892	-0.273	-0	-0.32
ICC8950	1.034	-0.21	-0.06	ICC18836	-0.37	-0.61	1.054
ICC1710	1.206	-0.33	0.015	ICC12851	-0.195	-0.4	-0.19
ICC1923	1.021	0.079	0.108	ICC2277	-0.195	-0.05	-0.36
ICC1422	1.371	-0.17	-0.09	ICC20195	-0.661	-0.18	0.269
ICC2242	1.202	-0.15	-0.17	ICC20174	-0.147	0.935	-0.01
ICC11664	1.045	-0.16	-0.38	ICC20192	-0.719	-0.06	0.042
ICC15567	1.051	-0.12	-0.08	ICC10685	-0.674	0.111	0.028
ICC3362	1.068	-0.27	0.01	ICC10673	0.243	1.367	0.213
ICC16903	1.074	-0.37	0.048	ICC20183	-0.78	-0.27	0.297
ICC6537	1.052	-0.4	-0.07	ICC7052	-0.043	1.416	-0.05
ICC708	1.208	-0.19	-0.13	ICC9712	0.03	1.246	0.275
ICC16915	1.111	-0.12	-0.13	ICC3892	-0.648	-0.07	-0.08
ICC3325	1.136	-0.06	-0.12	ICC8718	-0.716	-0.01	-0.05
ICC14778	1.132	-0.12	-0.02	ICC3582	0.908	-0.15	0.085
ICC10393	1.198	-0.08	-0.14	ICC7184	0.959	-0.02	0.121
ICC1194	1.158	-0.18	-0.13	ICC4363	-0.718	0.092	0.028
ICC16487	1.094	-0.33	-0.12	ICC9137	-1.034	-0.02	0.148
ICC1230	1.123	-0.36	0.056	ICC15518	-0.86	0.103	0.267
ICC6874	1.072	0.026	-0.18	ICC8740	-0.915	-0	-0.11
ICC1098	1.049	-0.09	0.035	ICC10341	-0.89	0.16	0.078
ICC19122	1.049	0.036	0.061	ICC15612	-1.134	-0.18	-0.35
ICC16269	1.074	-0.25	-0.09	ICC4872	-0.893	-0.11	-0.08
ICC4639	0.149	1.222	0.231	ICC15435	-0.712	0.194	0.496
ICC12916	0.261	1.451	0.003	ICC11944	-0.955	0.055	-0.15
ICC13863	0.067	1.275	0.085	ICC6875	-0.719	0.088	0.026
ICC2629	0.083	1.435	-0.06	ICC20194	-0.962	-0.01	0.405
ICC11198	0.196	1.349	-0.03	ICC10399	-1.172	-0.03	-0.27
ICC11378	0.053	1.401	0.056	ICC12299	-0.852	0	-0.03
ICC1398	0.137	1.525	-0.05	ICC15606	-1.064	-0.05	-0.34
ICC13523	-0.01	1.236	0.305	ICC7272	-0.48	-0.21	0.383
ICC11121	0.04	1.437	-0.09	ICC13124	-0.8	0.054	0.263
ICC8521	0.188	1.258	0.129	ICC16261	-0.955	-0.02	-0.22
ICC15888	0.217	1.449	0.17	ICC20263	-0.788	-0.09	-0.1
ICC10018	0.134	1.462	0.004	ICC10885	-0.812	-0.06	0.172
ICC4593	-0.22	-0.24	1.19	ICC19165	-0.766	-0.07	-0
ICC5135	-0.01	1.206	-0.11	ICC14077	-0.81	-0.02	-0.09
ICC9755	0.108	1.323	0.444	ICC10945	-0.88	0.046	-0.17
ICC15697	0.107	1.363	0.393	ICC4533	-0.806	-0.15	0.138
ICC13628	0.277	1.149	-0.2	ICC12726	-0.924	-0.25	0.22

0.01	1 10 4	0.07	100(20)	0.711	0.02	0.1
						-0.1
						0.207
						-0.02
					-0.04	0.413
0.178	1.293	0.105	ICC7413	-0.939	-0.07	-0.01
0.203	1.444	0.264	ICC8200	-0.811	0.096	0.018
-0.92	-0.16	-0.14	ICC18983	-0.213	-0.08	-0.43
-0.96	0.02	-0.14	ICC11284	-0.376	-0.48	-0.08
-0.76	-0.06	0.123	ICC3391	-0.22	-0.3	-0.27
-0.96	-0.08	0.102	ICC1392	-0.209	-0.1	-0.34
-0.9	0.084	-0.06	ICC1397	1.138	-0.03	-0.23
-0.9	-0.12	-0.2	ICC12947	1.176	0.029	-0.22
-0.76	0.055	-0.02	ICC1431	1.177	-0.11	0.074
-0.93	0.011	-0.2	ICC1510	1.114	-0.26	-0.15
-0.74	-0.06	-0.1	ICC5383	1.098	-0.11	-0.04
-1.05	-0.07	-0.16	ICC283	1.09	-0.2	-0.13
-0.99	-0.13	0.059	ICC2580	1.195	-0.23	0.017
-0.87	-0.17	-0.02	ICC456	1.022	-0.07	-0.14
-0.98	0.01	-0.17	ICC1356	1.009	-0.2	-0.17
-0.95	-0.11	-0.03	ICC3631	0.973	-0.13	0.048
-0.87	0.117	-0.17	ICC2507	1.128	-0.24	0.073
-1.02	-0.08	-0	ICC3776	1.06	-0.23	0.054
-0.92	-0.05	-0.03	ICC4814	1.122	-0.2	-0.04
-1.01	-0.21	-0.05	ICC4182	-0.309	0.05	0.452
-0.8	-0.09	-0.04	ICC4463	1.003	-0.14	0.004
-1.06	-0.05	-0.12	ICC3761	-0.815	0.104	0.268
-0.93	-0.1	0.215	ICC1052	-0.265	-0.07	-0.47
-0.88	-0.13	-0.12	ICC13524	0.111	1.27	0.199
-0.98	-0.04	0.048	ICC2884	-0.933	-0.06	-0.06
-0.98	-0.03	-0.28	ICC6293	-0.832	0.007	0.206
-0.83	-0.02	-0.12	ICC4418	-0.246	-0.1	-0.3
-0.94	-0.02	-0.22	ICC1180	-0.336	-0.46	-0.3
		-0.13	ICC2065			-0.47
			ICC8384			-0.46
			ICC67			-0.57
-0.94	0.078	0.134	ICC95	-0.358	-0.59	0.925
	-0.06	0.611	ICC13816	-0.312	-0.22	-0.49
-0.85	0.046	-0.1	ICC2263	-0.203	-0.2	-0.43
		0.34	ICC3946			0.907
						-0.48
-0.35	-0.17	-0.12	ICC1164	-0.319	-0.3	-0.25
						-0.25
						-0.29
						-0.51
						-0.4
	0.1					-0.53
-0.45	-0.57	1.07/4		-0 -19	-0.20	
-0.45 -0.21	-0.57 0.035	1.074 -0.11	ICC15868 ICC762	-0.339 -0.416	-0.28 -0.2	-0.13
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td=""><td>0.131.3160.294ICC188390.1021.319-0.13ICC109390.0531.29-0.02ICC58790.1781.2930.105ICC74130.2031.4440.264ICC8200-0.92-0.16-0.14ICC18983-0.960.02-0.14ICC1392-0.96-0.080.102ICC1392-0.97-0.080.102ICC1397-0.98-0.012-0.2ICC12947-0.99-0.12-0.2ICC1431-0.930.011-0.2ICC1510-0.74-0.06-0.1ICC5383-1.05-0.07-0.16ICC283-0.99-0.130.059ICC2580-0.930.01-0.17ICC1356-0.94-0.05-0.03ICC456-0.95-0.11-0.03ICC3631-0.87-0.17-0.02ICC3776-0.92-0.05-0.03ICC4814-1.01-0.21-0.05ICC4182-0.92-0.05-0.12ICC3761-0.93-0.130.215ICC1052-0.88-0.03-0.12ICC4463-1.04-0.02-0.12ICC4463-1.05-0.03ICC4463-1.06-0.02-0.12ICC4463-1.070.025ICC13524-0.98-0.040.245ICC6293-0.84-0.02-0.12ICC4463-0.95-0.13ICC3761<td< td=""><td>0.131.3160.294ICC18839-0.7470.1021.319-0.13ICC10939-0.6860.0531.29-0.02ICC5879-0.8660.1781.2930.105ICC7413-0.9390.2031.4440.264ICC8200-0.811-0.92-0.16-0.14ICC18983-0.213-0.960.02-0.14ICC1394-0.376-0.76-0.060.123ICC3391-0.22-0.96-0.080.102ICC13971.138-0.9-0.12-0.2ICC14311.177-0.930.011-0.2ICC14311.177-0.930.011-0.2ICC15101.114-0.74-0.06-0.1ICC53831.098-1.05-0.07-0.16ICC2831.099-0.99-0.130.059ICC25801.195-0.87-0.17-0.02ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.03ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.17ICC25071.128-1.02-0.08-0.03ICC4182-0.309-0.88-0.03ICC4182-0.305-0.93-0.14ICC135240.111-0.94-0.05ICC13524-0.131-0.95-0.12ICC4418-0.246-0.94-0.24-0.22ICC1180</td><td>0.131.3160.294ICC18839-0.747-0.020.1021.319-0.13ICC10939-0.6860.0350.0531.29-0.02ICC5879-0.866-0.040.1781.2930.105ICC7413-0.939-0.070.2031.4440.264ICC8200-0.8110.0960.92-0.16-0.14ICC18983-0.213-0.080.950.02-0.14ICC1392-0.29-0.31-0.960.021.012ICC1392-0.29-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.930.011-0.2ICC15101.114-0.26-0.74-0.06-0.1ICC53831.098-0.11-1.05-0.07-0.16ICC2831.09-0.23-0.99-0.130.059ICC25801.195-0.23-0.98-0.11-0.02ICC4561.022-0.07-0.980.01-0.17ICC25071.128-0.24-0.29-0.05-0.03ICC4182-0.39-0.13-0.91-0.12ICC37611.061-0.23-0.92-0.05-0.12ICC37611.061-0.24-0.93-0.14ICC1524-0.14</td></td<></td></t<></td></td<>	0.131.3160.2940.1021.319-0.130.0531.2930.1050.1781.2930.1050.2031.4440.2644-0.92-0.16-0.14-0.960.02-0.14-0.960.020.123-0.96-0.080.102-0.970.084-0.06-0.98-0.12-0.2-0.760.055-0.02-0.930.011-0.2-0.74-0.06-0.11-0.930.011-0.21-0.74-0.06-0.11-0.75-0.17-0.02-0.74-0.07-0.161-0.93-0.17-0.02-0.94-0.17-0.02-0.95-0.11-0.03-0.95-0.11-0.03-0.95-0.11-0.03-0.95-0.12-0.04-0.94-0.05-0.12-0.95-0.13-0.02-0.93-0.11-0.12-0.94-0.05-0.12-0.93-0.13-0.12-0.94-0.03-0.12-0.95-0.03-0.12-0.94-0.02-0.22-0.84-0.02-0.12-0.94-0.04-0.12-0.95-0.14-0.12-0.94-0.04-0.13-0.95-0.14-0.12-0.94-0.02-0.23-0.95-0.14-0.12-0.94-0.14-0.12 <t< td=""><td>0.131.3160.294ICC188390.1021.319-0.13ICC109390.0531.29-0.02ICC58790.1781.2930.105ICC74130.2031.4440.264ICC8200-0.92-0.16-0.14ICC18983-0.960.02-0.14ICC1392-0.96-0.080.102ICC1392-0.97-0.080.102ICC1397-0.98-0.012-0.2ICC12947-0.99-0.12-0.2ICC1431-0.930.011-0.2ICC1510-0.74-0.06-0.1ICC5383-1.05-0.07-0.16ICC283-0.99-0.130.059ICC2580-0.930.01-0.17ICC1356-0.94-0.05-0.03ICC456-0.95-0.11-0.03ICC3631-0.87-0.17-0.02ICC3776-0.92-0.05-0.03ICC4814-1.01-0.21-0.05ICC4182-0.92-0.05-0.12ICC3761-0.93-0.130.215ICC1052-0.88-0.03-0.12ICC4463-1.04-0.02-0.12ICC4463-1.05-0.03ICC4463-1.06-0.02-0.12ICC4463-1.070.025ICC13524-0.98-0.040.245ICC6293-0.84-0.02-0.12ICC4463-0.95-0.13ICC3761<td< td=""><td>0.131.3160.294ICC18839-0.7470.1021.319-0.13ICC10939-0.6860.0531.29-0.02ICC5879-0.8660.1781.2930.105ICC7413-0.9390.2031.4440.264ICC8200-0.811-0.92-0.16-0.14ICC18983-0.213-0.960.02-0.14ICC1394-0.376-0.76-0.060.123ICC3391-0.22-0.96-0.080.102ICC13971.138-0.9-0.12-0.2ICC14311.177-0.930.011-0.2ICC14311.177-0.930.011-0.2ICC15101.114-0.74-0.06-0.1ICC53831.098-1.05-0.07-0.16ICC2831.099-0.99-0.130.059ICC25801.195-0.87-0.17-0.02ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.03ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.17ICC25071.128-1.02-0.08-0.03ICC4182-0.309-0.88-0.03ICC4182-0.305-0.93-0.14ICC135240.111-0.94-0.05ICC13524-0.131-0.95-0.12ICC4418-0.246-0.94-0.24-0.22ICC1180</td><td>0.131.3160.294ICC18839-0.747-0.020.1021.319-0.13ICC10939-0.6860.0350.0531.29-0.02ICC5879-0.866-0.040.1781.2930.105ICC7413-0.939-0.070.2031.4440.264ICC8200-0.8110.0960.92-0.16-0.14ICC18983-0.213-0.080.950.02-0.14ICC1392-0.29-0.31-0.960.021.012ICC1392-0.29-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.930.011-0.2ICC15101.114-0.26-0.74-0.06-0.1ICC53831.098-0.11-1.05-0.07-0.16ICC2831.09-0.23-0.99-0.130.059ICC25801.195-0.23-0.98-0.11-0.02ICC4561.022-0.07-0.980.01-0.17ICC25071.128-0.24-0.29-0.05-0.03ICC4182-0.39-0.13-0.91-0.12ICC37611.061-0.23-0.92-0.05-0.12ICC37611.061-0.24-0.93-0.14ICC1524-0.14</td></td<></td></t<>	0.131.3160.294ICC188390.1021.319-0.13ICC109390.0531.29-0.02ICC58790.1781.2930.105ICC74130.2031.4440.264ICC8200-0.92-0.16-0.14ICC18983-0.960.02-0.14ICC1392-0.96-0.080.102ICC1392-0.97-0.080.102ICC1397-0.98-0.012-0.2ICC12947-0.99-0.12-0.2ICC1431-0.930.011-0.2ICC1510-0.74-0.06-0.1ICC5383-1.05-0.07-0.16ICC283-0.99-0.130.059ICC2580-0.930.01-0.17ICC1356-0.94-0.05-0.03ICC456-0.95-0.11-0.03ICC3631-0.87-0.17-0.02ICC3776-0.92-0.05-0.03ICC4814-1.01-0.21-0.05ICC4182-0.92-0.05-0.12ICC3761-0.93-0.130.215ICC1052-0.88-0.03-0.12ICC4463-1.04-0.02-0.12ICC4463-1.05-0.03ICC4463-1.06-0.02-0.12ICC4463-1.070.025ICC13524-0.98-0.040.245ICC6293-0.84-0.02-0.12ICC4463-0.95-0.13ICC3761 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td=""><td>0.131.3160.294ICC18839-0.7470.1021.319-0.13ICC10939-0.6860.0531.29-0.02ICC5879-0.8660.1781.2930.105ICC7413-0.9390.2031.4440.264ICC8200-0.811-0.92-0.16-0.14ICC18983-0.213-0.960.02-0.14ICC1394-0.376-0.76-0.060.123ICC3391-0.22-0.96-0.080.102ICC13971.138-0.9-0.12-0.2ICC14311.177-0.930.011-0.2ICC14311.177-0.930.011-0.2ICC15101.114-0.74-0.06-0.1ICC53831.098-1.05-0.07-0.16ICC2831.099-0.99-0.130.059ICC25801.195-0.87-0.17-0.02ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.03ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.17ICC25071.128-1.02-0.08-0.03ICC4182-0.309-0.88-0.03ICC4182-0.305-0.93-0.14ICC135240.111-0.94-0.05ICC13524-0.131-0.95-0.12ICC4418-0.246-0.94-0.24-0.22ICC1180</td><td>0.131.3160.294ICC18839-0.747-0.020.1021.319-0.13ICC10939-0.6860.0350.0531.29-0.02ICC5879-0.866-0.040.1781.2930.105ICC7413-0.939-0.070.2031.4440.264ICC8200-0.8110.0960.92-0.16-0.14ICC18983-0.213-0.080.950.02-0.14ICC1392-0.29-0.31-0.960.021.012ICC1392-0.29-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.930.011-0.2ICC15101.114-0.26-0.74-0.06-0.1ICC53831.098-0.11-1.05-0.07-0.16ICC2831.09-0.23-0.99-0.130.059ICC25801.195-0.23-0.98-0.11-0.02ICC4561.022-0.07-0.980.01-0.17ICC25071.128-0.24-0.29-0.05-0.03ICC4182-0.39-0.13-0.91-0.12ICC37611.061-0.23-0.92-0.05-0.12ICC37611.061-0.24-0.93-0.14ICC1524-0.14</td></td<>	0.131.3160.294ICC18839-0.7470.1021.319-0.13ICC10939-0.6860.0531.29-0.02ICC5879-0.8660.1781.2930.105ICC7413-0.9390.2031.4440.264ICC8200-0.811-0.92-0.16-0.14ICC18983-0.213-0.960.02-0.14ICC1394-0.376-0.76-0.060.123ICC3391-0.22-0.96-0.080.102ICC13971.138-0.9-0.12-0.2ICC14311.177-0.930.011-0.2ICC14311.177-0.930.011-0.2ICC15101.114-0.74-0.06-0.1ICC53831.098-1.05-0.07-0.16ICC2831.099-0.99-0.130.059ICC25801.195-0.87-0.17-0.02ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.03ICC4561.022-0.980.01-0.17ICC36310.973-0.870.117-0.17ICC25071.128-1.02-0.08-0.03ICC4182-0.309-0.88-0.03ICC4182-0.305-0.93-0.14ICC135240.111-0.94-0.05ICC13524-0.131-0.95-0.12ICC4418-0.246-0.94-0.24-0.22ICC1180	0.131.3160.294ICC18839-0.747-0.020.1021.319-0.13ICC10939-0.6860.0350.0531.29-0.02ICC5879-0.866-0.040.1781.2930.105ICC7413-0.939-0.070.2031.4440.264ICC8200-0.8110.0960.92-0.16-0.14ICC18983-0.213-0.080.950.02-0.14ICC1392-0.29-0.31-0.960.021.012ICC1392-0.29-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.90.084-0.06ICC13971.138-0.03-0.9-0.12-0.2ICC14311.177-0.11-0.930.011-0.2ICC15101.114-0.26-0.74-0.06-0.1ICC53831.098-0.11-1.05-0.07-0.16ICC2831.09-0.23-0.99-0.130.059ICC25801.195-0.23-0.98-0.11-0.02ICC4561.022-0.07-0.980.01-0.17ICC25071.128-0.24-0.29-0.05-0.03ICC4182-0.39-0.13-0.91-0.12ICC37611.061-0.23-0.92-0.05-0.12ICC37611.061-0.24-0.93-0.14ICC1524-0.14

ICC9434	-0.12	-0.25	-0.33	ICC14799	-0.217	-0.15	-0.57
ICC2482	-0.4	-0.29	0.166	ICC15610	-0.105	-0.03	-0.4
ICC19147	-0.28	-0.48	0.553	ICC9002	-0.288	-0.31	-0.59
ICC9862	-0.24	-0.32	-0.26	ICC2210	-0.428	-0.19	-0.4
ICC8752	-0.24	-0.06	-0.24	ICC16207	-0.282	-0.15	1.078
ICC20266	-0.38	-0.29	0.598	ICC12307	-0.389	-0.37	1.393
ICC5504	-0.35	-0.46	0.105	ICC2969	-0.229	-0.42	-0.45
ICC1915	-0.14	-0.25	-0.14	ICC15618	-0.353	-0.29	-0.4
ICC10466	-0.23	-0.32	0.842	ICC14402	-0.261	0.125	-0.57
ICC7315	-0.08	-0.25	-0.34	ICC11903	-0.244	-0.46	1.084

Trait	Locus	Chr_pos	F_Marker	p-perm_Marker	p-adj_Marker	Rsq_Marker
Seed Shape	CaSTMS9	NN	4.2958	9.99E-04	9.99E-04	0.0963
	TR20	1	4.1727	9.99E-04	9.99E-04	0.1015
	TA22	6	2.2348	0.005	9.99E-04	0.1862
	TA180	7	2.161	0.007	0.038	0.1208
	TR24	3	2.4717	0.007	9.99E-04	0.156
	TR40	6	2.0625	0.014	0.1049	0.1163
	TS35	5	1.9204	0.014	9.99E-04	0.1768
	TS45	8	1.8641	0.016	0.2098	0.1249
	GAA40	1	2.8805	0.021	0.1339	0.0562
	TR31	3	1.9896	0.021	0.0689	0.139
	TAA169	NN	1.9899	0.034	0.4895	0.0855
	TR26	3	2.2594	0.04	0.6913	0.0545
	TA8	1	1.8121	0.042	0.7393	0.0935
Flower color	TA21	7	2.2003	0.003	9.99E-04	0.1557
	TS62	7	2.1283	0.003	9.99E-04	0.1478
	CaSTMS9	NN	2.9198	0.004	0.023	0.0711
	GAA58	NN	2.8298	0.004	0.0689	0.0573
	TA2	4	2.3332	0.004	9.99E-04	0.1107
	TA22	6	1.969	0.004	9.99E-04	0.1758
	TR24	3	2.0424	0.004	0.024	0.1392
	TA180	7	2.2434	0.005	9.99E-04	0.1289
	CaSTMS5	3	2.1461	0.014	0.5045	0.0631
	TR20	4	2.0499	0.025	0.8052	0.056
	TA159	8	1.6127	0.027	0.6583	0.1542
	TA103	2	1.6831	0.034	0.9201	0.0909
	TA106	6	1.5965	0.034	0.7892	0.1402
	TR7	6	1.5612	0.034	0.8232	0.1504
	GA20	2	1.668	0.038	0.9091	0.1008
	TR31	3	1.5498	0.044	0.971	0.1176
	TS46	7	1.5593	0.046	0.99	0.1018
	TS43	5	1.4864	0.048	0.996	0.1262
Plant color	TA2	4	2.6652	9.99E-04	9.99E-04	0.1065
	TR20	4	4.1693	9.99E-04	9.99E-04	0.0905
	TA159	8	2.1148	0.004	9.99E-04	0.1627
	TA113	1	2.4382	0.006	9.99E-04	0.103
	TA180	7	2.1228	0.007	0.039	0.1062
	GAA58	NN	3.1655	0.012	0.045	0.0546
	TR24	3	1.8287	0.012	0.2727	0.1097
	TA200	2	1.9488	0.018	0.1748	0.1056
	TA22	6	1.7799	0.023	0.1958	0.1403
	CaSTMS4	3	2.0267	0.025	0.4196	0.0738
	TR43	1	1.6972	0.025	0.2138	0.1793
	TR59	5	2.0244	0.028	0.7473	0.059
	TA14	6	1.692	0.038	0.8322	0.0939
	TA14 TA28	7	1.583	0.04	0.8322	0.0535
Seed color	CaSTMS21	1	2.4354	0.004	0.1179	0.0523
2004 00101	TA180	7	2.4334	0.004	0.0579	0.0323

**Table 56:** Marker trait associations (MTAs) detected for different qualitative traits in the Chickpea reference set

Trait	Locus	Chr_pos	F_Marker	p-perm_Marker	p-adj_Marker	Rsq_Marker
	CaSTMS5	3	2.3383	0.009	0.1888	0.0544
	TA106	6	1.8253	0.014	0.1518	0.1243
	TR20	4	2.3149	0.017	0.3586	0.05
	TA200	2	1.7615	0.023	0.5654	0.0901
	TA130	4	1.6973	0.041	0.8881	0.0788
	GAA58	NN	2.0544	0.043	0.975	0.034
	TA159	8	1.545	0.049	0.9221	0.1191
Growth	TS35	5	1.807	0.006	0.0699	0.2356
habit	TaaSH	5	1.8065	0.011	0.3916	0.1402
	CaSTMS20	5	4.7386	0.012	0.1289	0.0438
	TR40	6	2.0829	0.013	0.049	0.1634
	TA203	1	1.6119	0.032	0.7502	0.1858
	GAA43	NN	2.9434	0.034	0.8352	0.0367
	CaSTMS25	15	2.0065	0.035	0.6364	0.1031
	TA120	6	1.8079	0.035	0.8831	0.1044
	TS43	5	1.6812	0.035	0.6004	0.1876
	TA159	8	1.6563	0.037	0.5774	0.212
	GA26	13	1.8615	0.042	0.8971	0.0964
	TA2	4	1.6954	0.045	0.972	0.1134
	TA8	1	1.6224	0.047	0.989	0.1185
	TA27	2	1.7943	0.047	0.6693	0.1493
	TAA194	3	1.6454	0.047	0.994	0.1057
	TR2	3	1.5516	0.049	0.99	0.1498
Dots on	CaSTMS21	1	2.6347	0.003	0.031	0.0592
seedcoat	TA106	6	2.0049	0.004	9.99E-04	0.1406
	CaSTMS5	3	2.5415	0.005	0.0569	0.0617
	TA130	4	1.9768	0.005	0.1159	0.0944
	TA8	1	2.1707	0.006	0.035	0.0948
	TA180	7	2.1346	0.006	0.034	0.1041
	TAA169	NN	2.0851	0.007	0.1459	0.0775
	TS35	5	1.8285	0.008	0.0799	0.1484
	TA120	6	2.0908	0.012	0.2118	0.0741
	CaSTMS9	NN	2.2263	0.013	0.5025	0.0468
	GAA58	NN	2.4217	0.013	0.4406	0.0417
	TA108	3	2.4599	0.013	0.5105	0.0378
	TR20	4	2.2106	0.014	0.4715	0.0505
	TA159	8	1.7564	0.017	0.2178	0.1384
	TR24	3	1.802	0.02	0.3756	0.1058
	TS53	5	2.2545	0.021	0.7552	0.039
	TA203	1	1.6821	0.023	0.5225	0.12
	TAA59	7	1.6931	0.023	0.7622	0.0888
	TA22	6	1.6067	0.032	0.6983	0.1266
	TA71	5	1.5994	0.037	0.8182	0.118
	CaSTMS4	3	1.6917	0.044	0.989	0.0614
	TA64	3	1.6044	0.046	0.8711	0.1128
Seed surface	CaSTMS13	1	7.567	9.99E-04	9.99E-04	0.1243
	CaSTMS20	5	9.5593	9.99E-04	9.99E-04	0.064
	GAA58	NN	4.8945	9.99E-04	9.99E-04	0.0954
	TR40	6	4.6543	9.99E-04	9.99E-04	0.2271
	CaSTMS7	5	5.5069	0.002	9.99E-04	0.0951
	TA96	2	2.5801	0.002	9.99E-04	0.1705

Trait	Locus	Chr_pos	F_Marker	p-perm_Marker	p-adj_Marker	Rsq_Marker
	TA135	3	2.8	0.002	9.99E-04	0.1906
	TR20	4	3.3294	0.002	9.99E-04	0.0885
	GAA39	13	3.0198	0.004	9.99E-04	0.0996
	TA27	2	2.3971	0.004	9.99E-04	0.1435
	TA22	6	1.974	0.005	9.99E-04	0.18
	TA113	1	2.3731	0.006	9.99E-04	0.1195
	CaSTMS4	4	2.2634	0.011	0.0839	0.0964
	TS54	NN	1.9732	0.012	0.046	0.15
	CaSTMS23	3	3.5979	0.013	0.049	0.0498
	TR43	1	1.7331	0.015	0.1129	0.2159
	TS35	5	1.8284	0.016	0.1149	0.1804
	TS83	13	2.0295	0.017	0.1179	0.1173
	CaSTMS9	NN	2.5288	0.018	0.2328	0.0639
	GA26	13	2.2089	0.018	0.2298	0.0852
	TS46	7	1.8131	0.021	0.4306	0.1181
	TA176	6	1.6824	0.022	0.2507	0.202
	CaSTMS6	9	2.8833	0.024	0.0779	0.0658
	TA144	8	1.9597	0.029	0.4356	0.0976
	CaSTMS25	15	1.9596	0.034	0.6803	0.0766
	TaaSH	5	1.8051	0.035	0.6424	0.1063
	TA14	6	1.7455	0.038	0.6853	0.1144
	TA21	7	1.6895	0.04	0.6953	0.1291
	TA180	7	1.6917	0.044	0.8871	0.1042
	TA130	4	1.839	0.045	0.6194	0.108
	TA64	3	1.5759	0.05	0.9401	0.1352

Traits	Locus	E1		E2		E3		E4		E5		Total	poole	ed
	CaSTMS7	***		***		*		***		***		5	***	
	GAA39	***		*				***		***		4		
	TA27	***		***				***		***		4	***	
	TA64	***		***		*		***		***		5	***	
	TA125	***		***		*		***		***		5	***	
	TA130	***		***		*		***		***		5	***	
	TA135	***		**				***		***		4	***	
	TAA58	***		***				***		***		4	***	
	TR26	***						*		*		3	*	
	TR29	***		***		***		***		***		5	***	
	TS45	***		**				***		***		4	**	
	TS54	***		***				***		***		4	***	
	GA20	*				**						2		
	GA34	*		**				***				3	*	
Darra 45 500/	TA144	*		*				***		*		4	*	
Days to 50% flowering	TaaSH	*		**				*		*		4	*	
nowening	TR20	*		*		**		*		*		5	*	
	TR40	*										1		
	TS43	*						**				2		
	CaSTMS2			*		*				*		3	*	
	CaSTMS20							*				1		
	TA72									*		1	*	
	GA26			***						*		2		
	TA59			**				*				2		
	TAA194			***								1		
	TA78					*						1		
	TA106					***						1		
	TA159					**						1		
	CaSTMS13					**				**		3		
	TA80							*				1		
	TOTAL		19		19		12		20		19	90		17
	CaSTMS20	**		***		*		**		*		5	***	
	TAA194	***										1		
	TS54	***				**		**		*		4	***	
	CaSTMS6			*								1		
	CaSTMS13			*								1		
	GAA58			*								1	*	
	TAA57			*								1		
	TR31			*								1		
Flowering	CaSTMS5			***								1		
Duration	CaSTMS7			***				***				2	***	
	CaSTMS25			***				***		***		3	***	
	TA5			***		***		***		***		4	***	
	TA20			***						***		2		
	TA27			***		***		***		***		4	***	
	TA72			***		***		**				3	***	
	TA110			***		***		***		***		3	***	
	TA132			***						***		2	***	
	TA159			***		***				***		3	***	

**Table 57:** Marker trait associations (MTAs) (P<=0.05, P<=0.01& P<=0.001) detected for different Quantitative traits in the chickpea reference set in five environments and in overall pooled analysis

Traits	Locus	E1	E2	E3	E4	E5	Total	pooled
	TAA59		***			***	2	
	TR1		***		**		2	
	TR43		***				1	
	TS35		***	*			2	***
	TS83		***				1	***
	CaSTMS4			***	***	**	3	*
	GA20			*			1	
	GAA43			**	**	***	3	***
	TA103			**	***		2	
	TR40			***	***	*	3	***
	TA125				***		1	
	TR29				***			
	TR59					*	1	
	TOTAL	3	21	13	15	14	64	16
	GA9	***	***	***	***	***	5	***
	GAA39	***	***	***	***	***	5	***
	TA25	***	***	***	***	***	5	***
	TA28	***	***	***	***	***	5	***
	TS43	***	***	***	***	***	5	***
	TS46	***	***	***	***	***	5	***
	CaSTMS21	*		***	*		3	*
	CaSTMS25	*	*		*	*	4	
Plant height	GAA43	*		*	*		3	
Plant neight	TA5	*		*	***	***	4	
	TA78		*				1	
	TA180		*				1	
	TA132		***	***	*	*	4	***
	TR43		***	***	***	***	4	***
	GA20			*			1	
	CaSTMS13				*		1	
	CaSTMS20				*		1	
	TOTAL	10	11	12	14	10	57	9
	CaSTMS25	***		***	***		3	***
	GAA40	**					1	
	TA180	***		***	***		3	***
	TAA169	**		*			2	
	TR43	**	***				2	
	TS35	**		**	*		3	*
	TS83	**		**			2	
	CaSTMS6	*					1	
	CaSTMS9	*					1	
Plant width	CaSTMS21	*	***		***	***	4	***
	GAA39	*	***	*	*		4	
	GAA58	*		***			2	
	TA25	*		**			2	
	TA78	*	*	***	***		4	***
	TA132		***				1	
	TA28		*				1	
	TA110		*				1	
	TS46		*				1	
	CaSTMS4				***	***	2	*

Traits	Locus	E1		E2	E3		E4	E5	Total	pooled
	GA22						***	***	2	***
	TA142						***	*	2	
	TS53						***	*	2	
	TA130						*		1	
	TR40						*	*	2	
	TA176							*	1	
	TA22									*
	TOTAL		14	8		9	12	7	50	8
	CaSTMS4	**							1	*
	TS54	***		*					2	
	TA120	*							1	
	TA180			**					1	
	TAA59			*				*	2	
	CaSTMS13				*				1	
Days tograin	TR20				*				1	
filling	TS83				*		**		2	*
	TAA169						*		1	
	TAA194						*	*	2	
	TA21						**		1	
	TA64						**		1	*
	TA132						**		1	*
	TOTAL		3	3		3	6	2	17	4
	CaSTMS7	**			***		**		3	***
	TA22	**							1	
	TA27	*			*				2	*
	TA130	*			***		*		3	***
	TA159	*							1	
	TA180	*		***	***				3	
	TAA194			***				*	2	
	TaaSH			***					1	
	TA64			*	***				2	*
	TS24			*					1	
	TAA58				***		*		2	
Days to maturity	TR40				**				1	
maturity	TS45				**				1	*
	CaSTMS20				*				1	
	CaSTMS21				*				1	
	GAA39				*				1	
	TA25				*			***	2	**
	GAA58						*		1	
	TA21						***		1	
	TA71						***		1	
	GAA40							*	1	
	TA103							*	1	
	TOTAL		6	5		12	6	4	33	6
	TS24	***	5	**			***	**	4	***
	GAA40				***				4	
Apical					*			**		
primary	TAA194	-			*			ጥጥ	2	
branches	TaaSH				^ 		*		1	
	TA22						*		1	
	TR29							**	1	

Traits	Locus	E1		E2		E3		E4		E5	Total	pooled
	TOTAL		1		1		3		2	3	10	1
	TA106	*								*	2	
	TA110	*									1	*
Basal	CaSTMS2									**	1	
primary branches	TAA169									**	1	
oralienes	TAA194									*	1	
	TOTAL		2		0		0		0	4	6	1
	GA26	***		***							2	
	TA22	***									1	
	TAA194	***		***		*				***	4	
	TS24	***									1	***
	CaSTMS12	*				**				*	3	
	CaSTMS21	*		*							2	
	TA110	*									1	
	TAA169	*									1	
Basal	TR29	*									1	
secondary branches	CaSTMS7			*							1	
branches	TA27			*							1	
	CaSTMS20			***							1	**
	TAA58			***							1	
	CaSTMS13							***			1	***
	CaSTMS2									*	1	
	GAA40									*	1	
	TA159									*	1	
	TOTAL		9		7		2		1	5	24	3
	GA34	***				***					2	
	TA20	***									1	
	TA103	***		***		***					3	*
	TS24	***		***		***					3	***
	GAA40			***				***			2	***
	TA53			***		***		***			3	***
	TS83			***							1	
	TS5			*				***			2	
Apical secondary	TA25					***					1	*
branches	TA106							***			1	
oraneneo	TA108							***			1	
	TA176							***			1	
	TaaSH							***			1	
	TAA169									**	1	
	TAA194									**	1	
	TR29									***	1	
	CaSTMS2							***				***
	TOTAL		4		6		5		8	3	26	6
			-						÷			-
	CaSTMS2	***		***				***			3	***
	GA22	***									1	
Tertiary	GAA39	***								***	2	
branches	TA140	***									1	
	TR19	***									1	
	CaSTMS12	*		***						***	3	***
	CaSTMS23	*								***	2	

Traits	Locus	E1	E2	E3	E4	E5	Total	pooled
	GA26	*					1	
	GAA43	*				*	2	
	TAA57	*					1	
	TS35	*			***		2	
	TAA58			*	*	***	4	
	TAA59					***	1	
	TA159				***		1	
	TA117					***	1	
	GAA40				***		1	
	TA103				***		1	
	GA20					*	1	
	CaSTMS13					***	1	
	TAA57	*					1	
	TaaSH				***	**	2	
	TA5			*	***		2	
	TA113		*			***	2	
	TA27				*		1	
	TS43		*				1	
	CaSTMS21		***				1	***
	TA78		***				1	***
	TAA194		***				1	***
	TR1		***			***	2	***
	TR43		***				1	***
	TS5		***				1	***
	TS46		***				1	***
	TA25			***		**	2	
	CaSTMS6			*		***	1	
	GA9					**	1	
	CaSTMS9					*	1	
	CaSTMS25					*	1	
	TA130					*	1	
	TA144					*	1	
	TR7					*	1	
	TOTAL	12	11	4	9	20	56	9
	GA34	***					1	*
	TA130	**	***				2	**
	CaSTMS25	*					1	
	GAA58	*					1	
	TA22	*	**				2	
	TA200		***				1	**
	TR56		**				1	
	TA27		*	*	**		3	***
Seeds per	TA27 TA28		*				1	**
pod	TS54		*		***		2	**
	TA96			**			1	
	CaSTMS13			*			1	
	CaSTMS15 CaSTMS4				***		1	**
	CaSTMS4 CaSTMS2					*	1	**
	TS5	+				*	1	
	100					-	1	ala ala
	TA8							**

Traits	Locus	E1	E2		E3		E4	E5	Total	pooled
	TS46									*
	TOTAL	4	5	7		3	3	2	20	11
	CaSTMS9	***	***		***		***	***	5	***
	TA96	***	*		***		***	***	5	***
	TS46	*						*	3	***
	TS54		*						1	
	TR20				**		*		2	
	TA27						***	***	2	
	TA142						***	***	2	***
Yield per	TS62						***	***	2	
plant	TS72						***	***	2	
	CaSTMS13						**	***	2	
	CaSTMS7							*	1	
	TA72							*	1	
	TA130							*	1	
	TA8									*
	TA117									***
	TOTAL		3	3		3	8	11	28	6
	CaSTMS5	*	***			-	*		3	***
	TA22	*	*				*		3	*
	TR20	*	*						2	*
	CaSTMS2		**						1	*
	GA34		**						1	
	TA130		***						1	
	TAA57		**		***				2	***
Pods per	TAA58		**				**		2	***
plant	TR43		**						1	
1			**						1	
	TR59		*						1	
	TA71		*				***	**	1	***
	TA106		*				* * *	**	3	* * *
	TA113		*				*	*	1	
	TR31						*	*	2	di.
	TA27			10						*
	TOTAL		3	13		1	5	2	24	8
	CaSTMS21	***	***		***		***		4	***
	TA22	***	***		***		***	***	5	***
	TA106	***	***		***		***		4	***
	TA113	***							1	
	TR56	***	***		***		***	*	5	***
	TS24	***	***		***		***	*	5	***
	TA159	*			*				2	
100-seed	TAA169	*			ļ				1	
weight	TaaSH	*						**	2	
	TR7	*							1	
	TR20	*							1	
	TR1		***		*				2	
	CaSTMS5		*				*		2	*
	GA26		*		*		*	*	4	*
	TA180		*		*			**	3	
	TA71						*		1	
	TA132							*	1	

Traits	Locus	E1		E2		E3		E4		E5	Total	pooled
	TOTAL		11		9		9		8	7	44	7
	CaSTMS6	***									1	
	CaSTMS7	**									1	***
	CaSTMS20	***									1	***
	TA78	***		*							2	*
	TA135	***									1	**
	TS35	***		***							2	***
	GAA39	*		*							2	*
	TA72	*									1	*
	TA176	*									1	
	TS24	*									1	
	TS83	*									1	
	CaSTMS21			***							1	
Plot yield	TR1			**							1	
	CaSTMS2			*							1	
	GAA43			*							1	
	TA113			*						*	2	
	TAA58			*							1	
	GAA58					***				*	2	*
	TA108					***		***			2	***
	TA159					***					1	
	TA21							*			1	
	TAA59					*					1	*
	TA14										1	*
	TR40											*
	TOTAL		11		9		4		2	2	28	12
		**	11		,				2	2	20	***
	CaSTMS20	***		**								**
	TA78	***		**								*
	TA135	**										ŕ
	TA176			staata								staataata
	TS35	**		**								***
	CaSTMS6	*										
	CaSTMS7	*										***
	TA72	*										*
	TS24	*		*								
per day	CaSTMS2			**								
productivity	CaSTMS21			**								
	TR1			*								
	TA22			*								
	TAA58			*								
	TAA59					*						
	GAA58					**						*
	TA108					***		**				***
	TA159					***						
	TA21							***				
	TR40											*
	TA14											*
	TOTAL		9		8		4		2	0	23	10

Significant level indicated with asterisks as follows: \*P<0.005, \*\*P<0.01, \*\*\*P<0.001

**Table 58:** List of highly significant (P<=0.001) marker trait associations (MTAs) detected in 2005-06 (E1) post rainy season at ICRISAT, Patancheru, India.

		Chromosome	_		_ 2
Trait	Locus	position	<i>P</i>	F_Marker	<b>R<sup>2</sup>%</b>
	CaSTMS7	5	0.000999	5.5705	11.28
	GAA39	13	0.000999	2.8919	11.26
	TA27	2	0.000999	2.4838	17.33
	TA64	3	0.000999	2.5368	22.85
	TA125	3	0.000999	2.1991	16.23
Days to 50% flowering	TA130	4	0.000999	2.7108	17.41
,	TA135	3	0.000999	2.1619	18.43
	TAA58	2	0.000999	2.5195	23.23
	TR26	3	0.000999	3.1193	9.06
	TR29	5	0.000999	2.7847	20.08
	TS45	8	0.000999	2.1166	17.19
	TS54	4	0.000999	2.0665	18.26
Flowering Duration	TAA194	5	0.000999	2.542	15.53
	TS54	4	0.000999	2.0726	20.76
	GA9	6	0.000999	4.5322	14.77
	GAA39	13	0.000999	4.6086	16.91
	TA25	8	0.000999	2.276	23.42
Plant Height	TA28	7	0.000999	2.6793	31.05
	TS43	5	0.000999	3.0621	26.57
	TS46	7	0.000999	3.1852	21.99
D1	CaSTMS25	15	0.000999	2.8059	14.25
Plant width	TA180	7	0.000999	2.4544	19.27
Days to grain filling	TS54	4	0.000999	2.1633	20.91
Apical primary Branches	TS24	6	0.000999	2.3559	21.65
	GA26	13	0.000999	2.8009	14.48
	TA22	6	0.000999	1.9956	25.13
Basal secondary branches	TAA194	5	0.000999	2.9492	18.24
	TS24	6	0.000999	2.1707	20.1
	GA34	6	0.000999	2.219	25.01
Apical secondary	TA20	1	0.000999	2.0784	24.83
branches	TA103	2	0.000999	3.3865	21.86
	TS24		0.000999	6.8883	42.09
	CaSTMS2	6	0.000999	2.2628	17.87
	GA22	NN	0.000999	2.6529	23.62
Tertiary branches	GAA39	13	0.000999	4.4647	19.19
	TA140	7	0.000999	2.9091	22.54
	TR19	2	0.000999	2.797	26.53
Seeds per pod	GA34	6	0.000999	1.9997	19.37
	CaSTMS9	NN	0.000999	6.425	19.37
Yield per plant	TA96	2	0.000999	3.6976	29.36
	1 A90	L	0.000999	3.09/0	29.30

		Chromosome			
Trait	Locus	position	Р	F_Marker	<b>R<sup>2</sup>%</b>
	CaSTMS21	1	0.000999	3.8005	10.26
	TA22	6	0.000999	3.4628	27.32
100-seed weight	TA106	6	0.000999	2.0635	18.06
100-seed weight	TA113	1	0.000999	2.4453	12.67
	TR56	3	0.000999	2.5848	10.1
	TS24	6	0.000999	2.9035	18.76
	CaSTMS6	9	0.000999	3.1643	8.28
	CaSTMS20	5	0.000999	5.6496	4.55
Plot yield	TA78	7	0.000999	2.4441	19.95
	TA135	3	0.000999	2.1628	18.18
	TS35	5	0.000999	1.994	22.25
per day productivity	TA78	7	0.000999	2.4849	19.67
per day productivity	TA135	3	0.000999	2.0659	17.07
	CaSTMS23	3	0.000999	4.8383	9.24
	GA34	6	0.000999	4.6345	42.96
	TA53	2	0.000999	3.7207	32.14
	TA117	7	0.000999	2.0689	25.3
Antho-Methanol	TA120	6	0.000999	3.0983	17.77
	TR19	2	0.000999	2.2202	22.67
	TS5	3	0.000999	2.9183	43.44
	TS24	6	0.000999	2.2105	20.83
	TS62	7	0.000999	2.3317	22.94
Antho-acidifiedmethanol	CaSTMS4	3	0.000999	2.8013	14.95
protein content	TS53	5	0.000999	4.5679	11.37

**Table 59:** List of highly significant (P<=0.001) marker trait associations (MTAs) detected in 2006-07 (E2) post rainy season at ICRISAT, Patancheru, India.

Trait	Locus	Chromosome position	Р	F_Marker	<b>R<sup>2</sup>%</b>
	CaSTMS7	5	0.000999	5.1422	10.39
	GA26	13	0.000999	2.6108	11.41
	TA27	2	0.000999	2.3101	16.15
	TA64	3	0.000999	2.257	20.7
	TA125	3	0.000999	2.4039	17.19
Days to 50% flowering	TA130	4	0.000999	2.6667	16.96
	TAA58	2	0.000999	2.0054	19.38
	TAA194	5	0.000999	2.6096	13.81
	TaaSH	5	0.000999	2.5084	16.16
	TR29	5	0.000999	2.5601	18.59
	TS54	4	0.000999	2.2525	19.26
	CaSTMS5	3	0.000999	2.8692	10.88
	CaSTMS7	5	0.000999	5.2171	11.81
	CaSTMS20	5	0.000999	8.0603	7.12
	CaSTMS25	15	0.000999	6.4995	26.35
	TA5	5	0.000999	4.2982	27.21
	TA20	1	0.000999	3.4779	34.48
	TA27	2	0.000999	3.8349	26.6
Flowering Duration	TA72	4	0.000999	2.8298	24.4
Flowering Duration	TA110	2	0.000999	6.6825	31.33
	TA132	4	0.000999	3.9968	35.02
	TA159	8	0.000999	2.8017	30.54
	TAA59	7	0.000999	3.2373	23.53
	TR1	6	0.000999	2.814	34.82
	TR43	1	0.000999	2.16	32.54
	TS35	5	0.000999	2.2449	27.23
	TS83	13	0.000999	2.3638	17.3
	GA9	6	0.000999	4.9232	16.21
	GAA39	13	0.000999	4.2401	16.22
	TA25	8	0.000999	2.6943	27.04
Plant Height	TA28	7	0.000999	2.3053	28.91
I fant Height	TA132	4	0.000999	2.051	20.88
	TR43	1	0.000999	1.8639	27.86
	TS43	5	0.000999	2.7659	25.41
	TS46	7	0.000999	3.3545	23.44
	CaSTMS21	1	0.000999	3.8324	13.62
Plant width	GAA39	13	0.000999	3.8626	16.69
	TA132	4	0.000999	2.2238	24.63
	TR43	1	0.000999	2.4216	36.61
	TA180	7	0.000999	2.4989	17.04
Days to Maturity	TAA194	3	0.000999	3.2457	16.87
	TaaSH	5	0.000999	2.4763	16.37

Trait	Locus	Chromosome position	Р	F_Marker	<b>R<sup>2</sup>%</b>
	CaSTMS20	5	0.000999	5.7683	5.44
	GA26	13	0.000999	3.6181	17.55
Basal secondary branches	TAA58	2	0.000999	2.3032	25.15
	TAA194	5	0.000999	2.5602	15.91
	GAA40	1	0.000999	3.6095	9.7
	TA53	2	0.000999	2.1213	20.02
Apical secondary branches	TA103	2	0.000999	2.4517	16.94
	TS24	6	0.000999	2.7928	23.42
	TS83	13	0.000999	2.2809	17.16
	CaSTMS2	6	0.000999	3.1193	22.7
	CaSTMS12	11	0.000999	592.7988	39.47
	CaSTMS21	1	0.000999	10.3337	29.75
	TA78	7	0.000999	5.9005	32.32
Tertiary branches	TAA194	3	0.000999	3.4905	20.69
	TR1	6	0.000999	77.6798	30.25
	TR43	1	0.000999	69.2155	30.46
	TS5	3	0.000999	69.5562	30.67
	TS46	7	0.000999	4.0152	29.77
	TA130	4	0.000999	2.5061	15.74
Seeds per pod	TA200	2	0.000999	2.3083	16.23
<b>D</b> 1 1	CaSTMS5	3	0.000999	2.8585	9.72
Pods per plant	TA130	4	0.000999	2.5072	16.27
Yield per plant	CaSTMS9	NN	0.000999	4.1946	13.92
	CaSTMS21	1	0.000999	3.1812	9.16
	TA22	6	0.000999	3.7471	29.87
100 1 1	TA106	6	0.000999	2.5993	22.26
100-seed weight	TR1	6	0.000999	1.864	21.94
	TR56	3	0.000999	3.9934	15.1
	TS24	6	0.000999	3.54	22.54
	CaSTMS21	1	0.000999	2.9326	8.06
Plot yield	TS35	5	0.000999	2.0137	19.71
<i>.</i>	CaSTMS5	3	0.000999	3.5863	11.01
	GA26	13	0.000999	3.3624	12.73
Shoot Dry weight	TaaSH	5	0.000999	2.3699	15.01
	TR40	6	0.000999	2.3496	14.37
Root Dry weight	TA22	6	0.000999	2.015	21.62
	CaSTMS5	3	0.000999	3.4495	10.64
	GA26	13	0.000999	3.3179	12.57
Total dry weight Ratio	TA22	6	0.000999	2.0444	21.07
	TaaSH	5	0.000999	2.4168	15.23
	TA130	4	0.000999	4.126	24.66
Root length Density	TAA59	7	0.000999	3.6877	26.57
Shoot to Root length Density	CaSTMS25	15	0.000999	3.5275	13.94

Trait	Locus	Chromosome position	Р	F Marker	R <sup>2</sup> %
	TA106	6	0.000999	2.1163	21.38
Days to 50% flowering	TR29	5	0.000999	2.6355	19.7
	CaSTMS4	3	0.000999	2.8015	15
	TA5	5	0.000999	2.2456	16.54
	TA27	2	0.000999	2.2748	17.86
Flowering Duration	TA72	4	0.000999	2.185	20.01
	TA110	2	0.000999	3.5928	19.99
	TA159	8	0.000999	2.2627	26.3
	TR40	6	0.000999	2.491	18.6
	CaSTMS21	1	0.000999	3.4221	10.78
	GA9	6	0.000999	4.4869	14.56
	GAA39	13	0.000999	5.2472	18.61
	TA25	8	0.000999	2.0055	21.22
Plant Height	TA28	7	0.000999	2.8668	32.18
	TA132	4	0.000999	2.0461	20.19
	TR43	1	0.000999	1.87	27.05
	TS43	5	0.000999	3.0549	26.37
	TS46	7	0.000999	3.0464	21.16
	CaSTMS25	15	0.000999	3.2702	16.15
	GAA58	NN	0.000999	3.3868	9.35
Plant width	TA78	7	0.000999	2.253	21.99
	TA180	7	0.000999	2.65	20.41
	CaSTMS7	5	0.000999	3.7069	8.1
	TA64	3	0.000999	2.0853	20.35
Days to Maturity	TA130	4	0.000999	2.4205	16.38
	TA180	7	0.000999	2.2047	15.7
	TAA58	2	0.000999	2.1708	21.45
Apical primary Branches	GAA40	1	0.000999	5.2282	13.66
	GA34	6	0.000999	2.9783	29.46
	TA25	8	0.000999	2.1012	23.53
Apical secondary branches	TA53	2	0.000999	2.2345	19.99
	TA103	2	0.000999	3.5924	21.93
	TS24	6	0.000999	6.2566	38.26
Tertiary branches	TA25	8	0.000999	1.966	24.33
Pods per plant	TAA57	4	0.000999	8.8	8.71
Viold non-nlant	CaSTMS9	NN	0.000999	5.4759	16.12
Yield per plant	TA96	2	0.000999	2.7984	23.38
	CaSTMS21	1	0.000999	3.0927	8.89
	TA22	6	0.000999	3.7841	29.9
100-seed weight	TA106	6	0.000999	2.7914	23.28
	TR56	3	0.000999	3.9231	14.81
	TS24	6	0.000999	3.0138	19.99

**Table 60:** List of highly significant (P<=0.001) marker trait associations (MTAs) detected in 2008-09 (E3) post rainy season at ICRISAT, Patancheru, India

		Chromosome			2
Trait	Locus	position	Р	F_Marker	<b>R<sup>2</sup>%</b>
	GAA58	NN	0.000999	3.3682	8.41
Plot yield	TA108	3	0.000999	4.5874	9.94
	TA159	8	0.000999	1.9589	22.4
per day productivity	TA108	3	0.000999	4.0082	8.56
	TA159	8	0.000999	2.0238	22.28
Damage rating	CaSTMS23	3	0.000999	4.7236	8.09
Larval survival (%)	TA125	3	0.000999	2.4485	19.65
	CaSTMS5	3	0.000999	4.3973	12.33
Shoot Dry weight	CaSTMS9	NN	0.000999	3.6979	8.2
Shoot Dry weight	TA20	5	0.000999	2.6213	21.51
	TA113	1	0.000999	2.5173	12.16
Root Dry weight	CaSTMS5	3	0.000999	3.4384	10.58
Root Dry weight	TA20	1	0.000999	2.4314	23.79
	CaSTMS5	3	0.000999	4.7316	13.08
	CaSTMS9	NN	0.000999	3.5982	7.99
Total dry weight Ratio	TA20	5	0.000999	2.5428	23.16
	TA113	1	0.000999	2.6266	12.58
	TaaSH	5	0.000999	2.3259	13.9
Root length Density	TAA59	7	0.000999	3.1209	21.76
Desta	CaSTMS5	3	0.000999	3.3737	10.86
Root surface area	TA20	1	0.000999	1.9751	21.38
Root Volume	TA180	7	0.000999	2.0249	15.2
	TS43	5	0.000999	2.0112	21.11
Shoot to Root length	TS53	5	0.000999	3.221	8.71
Density	TS83	13	0.000999	2.3776	16.41
LeafArea	TA8	1	0.000999	3.0612	19.93
LealAlea	TA20	1	0.000999	2.3606	26.82
Leaf DryWeight	TA8	1	0.000999	2.2668	15.72
	CaSTMS21	1	0.000999	3.1062	11.39
	GA22	NN	0.000999	2.1034	19.37
	TA8	1	0.000999	2.4506	17.44
Specific Leaf Area	TA71	5	0.000999	3.2815	33.09
	TR43	1	0.000999	3.1595	42.89
	TS83	13	0.000999	2.8661	21.19

**Table 61:** List of highly significant (P<=0.001) marker trait associations (MTAs)</th>detected in 2008-09 (E4) post rainy season at UAS, Dharwad, India

Trait	Locus	Chromosome position	P	F_Marker	R <sup>2</sup> %
	CaSTMS7	5	0.000999	6.4118	12.76
	GA34	6	0.000999	2.0349	20.8
	GAA39	13	0.000999	3.2104	12.36
	TA27	2	0.000999	2.4797	17.36
	TA64	3	0.000999	2.4007	22.02
	TA125	3	0.000999	2.4547	17.76
Days to 50% flowering	TA130	4	0.000999	2.9527	18.67
	TA135	3	0.000999	2.3439	19.67
	TA144	8	0.000999	2.4735	13.99
	TAA58	2	0.000999	2.7125	24.56
	TR29	5	0.000999	2.4956	18.53
	TS45	8	0.000999	2.1186	17.25
	TS54	4	0.000999	2.647	22.08
	CaSTMS4	3	0.000999	2.4878	13.44
	CaSTMS7	5	0.000999	3.7057	8.61
	CaSTMS25	15	0.000999	2.7807	13.35
	TA5	5	0.000999	3.1901	21.6
	TA27	2	0.000999	2.7877	20.75
Flowering Duration	TA103	2	0.000999	2.4198	16.21
	TA110	2	0.000999	4.6485	24.05
	TA125	3	0.000999	2.3257	18.57
	TR29	5	0.000999	2.1977	18.29
	TR40	6	0.000999	2.3141	17.36
	GA9	6	0.000999	4.5284	14.9
	GAA39	13	0.000999	5.2405	18.89
	TA5	5	0.000999	2.3497	15.98
	TA25	8	0.000999	2.2892	23.74
Plant Height	TA28	7	0.000999	2.9117	33
	TR43	1	0.000999	1.8998	27.77
	TS43	5	0.000999	3.1667	27.44
	TS46	7	0.000999	3.2535	22.56
	CaSTMS4	3	0.000999	3.7499	20.32
	CaSTMS21	1	0.000999	11.6658	32.62
	CaSTMS25	15	0.000999	2.8474	14.71
D1	GA22	NN	0.000999	4.8097	35.59
Plant width	TA78	7	0.000999	2.2899	22.77
	TA142	3	0.000999	2.1009	24.03
	TA180	7	0.000999	2.8377	22.02
	TS53	2	0.000999	3.937	10.94
Dovo to Mat	TA21	7	0.000999	1.8998	21.54
Days to Maturity	TA71	5	0.000999	2.0764	22.02
Apical primary Branches	TS24	6	0.000999	2.4505	22.12

Trait	Locus	Chromosome position	Р	F_Marker	<b>R<sup>2</sup>%</b>
Basal secondary branches	CaSTMS13	1	0.000999	3.5664	<b>K</b> 70 8.84
Dusur secondary oranenes	CaSTMS15 CaSTMS2	6	0.000999	4.6254	29.87
	GAA40	1	0.000999	9.6394	22.52
	TA53	2	0.000999	3.3392	28.65
Apical secondary	TA106	6	0.000999	1.9906	23.09
branches	TA108	3	0.000999	5.0383	11.96
	TA176	6	0.000999	3.8051	45.41
	TaaSH	5	0.000999	3.5502	24.74
	TS5	3	0.000999	1.9307	32.45
	CaSTMS2	6	0.000999	2.2239	17.93
	GAA40	1	0.000999	4.8423	13.39
	TA5	5	0.000999	3.3003	24.45
Tertiary branches	TA103	2	0.000999	2.292	17.1
	TA159	8	0.000999	2.0606	26.85
	TaaSH	5	0.000999	2.4156	19.17
	TS35	5	0.000999	2.0139	27.44
G 1 1	CaSTMS4	3	0.000999	2.5748	15.01
Seeds per pod	TS54	NN	0.000999	2.1513	22.37
Pods per plant	TA106	6	0.000999	2.0317	20.97
	CaSTMS9	NN	0.000999	6.1243	18.85
	TA27	2	0.000999	2.3271	19.32
Viald new plant	TA96	2	0.000999	2.8181	25.1
Yield per plant	TA142	3	0.000999	2.2633	25.23
	TS62	7	0.000999	2.178	21.2
	TS72	4	0.000999	2.3898	15.91
	CaSTMS21	1	0.000999	2.9984	8.65
	TA22	6	0.000999	3.2459	27.19
100-seed weight	TA106	6	0.000999	2.5688	21.96
	TR56	3	0.000999	3.435	13.29
	TS24	6	0.000999	3.114	20.46
Plot yield	TA108	3	0.000999	4.1362	9.38
per day productivity	TA21	7	0.000999	2.1776	19.38

Tuo 14	Tana	Chromosome	מ	T Marker	<b>R<sup>2</sup>%</b>
Trait	Locus CaSTMS7	position 5	<b>P</b> 0.000999	<b>F_Marker</b> 4.8234	<b>K</b> %
	GAA39	13	0.000999	3.1766	9.9
	TA27	2		2.2515	12.14
	TA27 TA64	3	0.000999		
		3	0.000999	2.1917	20.42
Days to 50%	TA125 TA130	4	0.000999	2.1764 2.8749	16.01 18.13
flowering					
	TA135	3	0.000999	2.1038	17.95
	TAA58	2	0.000999	2.5626	23.39
	TR29	5	0.000999	2.5729	18.81
	TS45	8	0.000999	2.2324	17.83
	TS54	4	0.000999	2.1868	18.98
	CaSTMS25	15	0.000999	2.874	13.56
	GAA43	NN	0.000999	5.5266	6.41
	TA5	5	0.000999	2.8332	19.47
Flowering	TA20	4	0.000999	2.0483	23.47
Duration	TA27	2	0.000999	2.7386	20.21
	TA110	2	0.000999	3.2175	17.92
	TA132	4	0.000999	2.0208	21.34
	TA159	8	0.000999	2.7623	29.49
	TAA59	7	0.000999	2.1772	16.86
	GA9	6	0.000999	4.4992	14.89
	GAA39	13	0.000999	4.811	17.75
	TA5	5	0.000999	2.2039	15.24
Plant Height	TA25	8	0.000999	2.585	25.99
i funt Horgin	TA28	7	0.000999	2.916	33.19
	TR43	1	0.000999	1.9142	28.04
	TS43	5	0.000999	3.2489	28.06
	TS46	7	0.000999	3.0559	21.65
	CaSTMS4	3	0.000999	3.6351	19.82
Plant width	CaSTMS21	1	0.000999	11.1897	31.71
	GA22	NN	0.000999	4.6341	34.75
Days to Maturity	TA25	8	0.000999	1.952	23.16
Basal secondary branches	TAA194	3	0.000999	2.1189	16.54
Apical secondary branches	TR29	5	0.000999	1.9699	19.28

**Table 62:** List of highly significant (P<=0.001) marker trait associations (MTAs) detected in 2008-09 (E5) spring at ICRISAT, Patancheru, India.

		Chromosome			
Trait	Locus	position	P	F_Marker	<b>R<sup>2</sup>%</b>
	CaSTMS6	9	0.000999	3.5545	10.92
	CaSTMS12	11	0.000999	4.747	10.19
	CaSTMS13	1	0.000999	4.7676	11.55
	CaSTMS23	3	0.000999	6.1821	11.2
Tantiany bronchas	GAA39	13	0.000999	3.1316	14.14
Tertiary branches	TA113	1	0.000999	2.369	16.43
	TA117	7	0.000999	2.5047	28.28
	TAA58	7	0.000999	3.0961	31.44
	TAA59	7	0.000999	2.3893	19.72
	TR1	6	0.000999	2.4302	33.58
	CaSTMS9	NN	0.000999	5.5588	17.44
	CaSTMS13	1	0.000999	4.5098	11.02
	TA27	2	0.000999	2.7379	21.97
Yield per plant	TA96	2	0.000999	3.0412	26.56
	TA142	3	0.000999	2.3116	25.64
	TS62	7	0.000999	2.0991	20.61
	TS72	4	0.000999	2.5714	16.92
100-seed weight	TA22	6	0.000999	2.5127	23.25
LeafArea	TA2	4	0.000999	2.3969	14.63
LearArea	TaaSH	5	0.000999	2.2976	16.42
Leaf DryWeight	TA2	4	0.000999	2.4506	15.24
Lear Dry weight	TA130	4	0.000999	2.6076	18.59

		Chromosome			2
Trait	Locus	position	F_marker	Р	<b>R<sup>2</sup>%</b>
	CaSTMS7	5	5.0414	0.001	10.25
	TA27	2	2.2398	0.001	15.83
	TA64	3	2.2874	0.001	21
Days to 50%	TA125	3	2.2706	0.001	16.51
flowering	TA130	4	2.7726	0.001	17.57
	TAA58	2	2.2898	0.001	21.49
	TR29	5	2.567	0.001	18.72
	TS54	4	2.265	0.001	19.43
	CaSTMS7	5	4.244	0.001	9.45
	CaSTMS20	5	8.9955	0.001	7.55
	CaSTMS25	15	3.5169	0.001	15.78
	GAA43	NN	5.436	0.001	6.21
	TA5	5	3.6799	0.001	23.34
	TA27	2	3.3	0.001	22.88
Flowering	TA72	4	2.3486	0.001	20.35
Duration	TA110	2	4.8325	0.001	24.03
	TA132	4	2.32	0.001	23.27
	TA159	8	2.0468	0.001	23.54
	TR40	6	2.6472	0.001	18.77
	TS35	5	2.0449	0.001	24.44
	TS54	4	2.0657	0.001	19.41
	TS83	13	2.2877	0.001	16.16
	GA9	6	4.7028	0.001	15.23
	GAA39	13	4.9861	0.001	18.01
	TA25	8	2.3812	0.001	24.2
	TA28	7	2.8087	0.001	31.99
Plant Height	TA132	4	1.9958	0.001	19.95
	TR43	1	1.9434	0.001	27.94
	TS43	5	3.1599	0.001	27.16
	TS46	7	3.3905	0.001	23.04
	CaSTMS21	1	5.8748	0.001	19.51
	CaSTMS25	15	2.5384	0.001	13.18
Plant width	GA22	NN	2.4388	0.001	21.8
	TA78	7	2.158	0.001	21.5
	TA180	7	2.7401	0.001	21.19
<b>.</b>	CaSTMS7	5	3.6833	0.001	7.93
Days to Maturity	TA130	4	2.3862	0.001	15.93
Apical primary					
Branches	TS24	6	2.9775	0.001	25.6
Basal secondary	CaSTMS13	1	3.4067	0.001	8.4
branches	TS24	6	2.3527	0.001	20.93

**Table 63:** List of highly significant (P<=0.001) marker trait associations detected in overall pooled analysis data</th>

Trait	Locus	Chromosome position	F_marker	P	<b>R<sup>2</sup>%</b>
	CaSTMS2	6	2.4223	0.001	17.55
Apical secondary	GAA40	1	3.4935	0.001	9.17
branches	TA53	2	2.2521	0.001	20.43
	TS24	6	2.9171	0.001	23.56
	CaSTMS2	6	2.4342	0.001	18.53
	CaSTMS12	11	132.9631	0.001	32.81
	CaSTMS21	1	7.1839	0.001	22.65
	TA78	7	4.2099	0.001	34.28
Tertiary branches	TAA194	3	2.643	0.001	16.39
•	TR1	6	18.8468	0.001	36.4
	TR43	1	17.1985	0.001	37.34
	TS5	3	16.4998	0.001	37.35
	TS46	7	2.9863	0.001	23.85
Seeds per pod	TA27	2	2.1944	0.001	15.2
1 1	CaSTMS5	3	2.7992	0.001	8.9
	TA106	6	2.1163	0.001	19.38
Pods per plant	TAA57	4	5.4025	0.001	5.39
	TAA58	7	1.9948	0.001	18.13
	CaSTMS9	NN	10.2197	0.001	27.22
	TA96	2	3.9831	0.001	31.35
Yield per plant	TA117	7	2.27	0.001	25.83
F F	TA142	3	2.3795	0.001	25.62
	TS46	7	2.3887	0.001	19.92
	CaSTMS21	1	3.2055	0.001	8.94
	TA22	6	3.783	0.001	29.11
100-seed weight	TA106	6	2.37	0.001	20.19
6	TR56	3	3.5908	0.001	13.42
	TS24	6	3.1591	0.001	20.14
	CaSTMS7	5	3.8166	0.001	7.33
	CaSTMS20	5	5.9664	0.001	4.41
Plot yield	TA108	3	4.1875	0.001	7.96
	TS35	5	2.0798	0.001	21.11
	CaSTMS7	5	3.7768	0.001	7.08
per day	CaSTMS20	5	5.7128	0.001	4.13
productivity	TA108	3	3.437	0.001	6.5
1 ,	TS35	5	1.928	0.001	19.5
protein content	GA26	13	2.1067	0.001	11.04
1	CaSTMS23	3	4.2451	0.003	7.09
Damage rating	TA132	4	1.9017	0.001	19.63
Leaf DryWeight	TA8	1	2.4949	0.003	15.03
	TA8	1	2.7677	0.001	17.73
LeafArea	TA0	1	1.9768	0.001	22.72
Specific Leaf Area	TS83	13	2.2482	0.001	16.95
SCMR	TAA59	7	2.2482	0.001	18.32

		Chromosome			
Trait	Locus	position	F_marker	P	<b>R<sup>2</sup>%</b>
	CaSTMS5	3	4.613	0.001	12.8
	CaSTMS9	NN	3.8527	0.001	8.49
	GA26	13	3.3841	0.001	12.02
Shoot Dry weight	TA20	5	2.0967	0.001	20.2
	TA22	6	1.9585	0.001	19.21
	TA113	1	2.5203	0.001	12.15
	TaaSH	5	2.5669	0.001	15.04
	CaSTMS5	3	3.1695	0.001	9.71
Root Dry weight	TA20	1	2.1367	0.001	21.39
	TA22	6	2.1294	0.001	21.33
	CaSTMS5	3	4.7487	0.001	13.1
	CaSTMS9	NN	3.5495	0.001	7.88
Total day and alst	GA26	13	3.0547	0.001	11.02
Total dry weight Ratio	TA20	1	2.2564	0.001	21.27
Ratio	TA22	6	2.1079	0.001	20.25
	TA113	1	2.6058	0.001	12.48
	TaaSH	5	2.6486	0.001	15.4
Root length	TA130	4	3.4588	0.001	20.21
Density	TAA59	7	4.7943	0.001	30.0
Root surface area	CaSTMS5	3	3.0982	0.001	10.13
Root Volume	TA22	6	2.0859	0.001	21.52

		Chromosome	
S.No	Marker	position	Traits
1	TA113	1	SDW, TDW
2	CaSTMS21	1	TB, 100sdwt, PLWD
3	TA8	1	LDW,Leaf area
4	TA20	1	Leaf area, SDW,RDW,TDW
5	TR43	1	PLHT, TB
6	TA27	2	DF,FD,SDPD
7	CaSTMS5	3	PPP, SDW, RDW, TDW, RSA
8	TA108	3	PY, FD,Prod
9	TA132	4	FD,PLHT,Damage Rate%
10	TS54	4	DF,FD,SDPD
11	TA130	4	DF,DM,RLD
12	TA20	5	SDW, RDW, TDW, Leaf area
13	TAAsH	5	SDW, TDW
14	CaSTMS7	5	prod,DF,FD,DM,PY
15	CaSTMS20	5	PY,Prod, FD
16	TS35	5	FD,PY,Prod
17	TA106	6	PPP, 100-sdwt
18	TA22	6	100-sdwt, SDW,RDW,TDW,RV
19	TS24	6	APB,BSB,ASB,100-sdwt
20	CaSTMS2	6	ASB,TB
21	TAA59	7	RLD, SPAD
22	TAA58	7	PPP, DF
23	TS46	7	PLHT, TB, YPP
24	TA78	7	PLWD,TB
25	GA26	13	Protein content, SDW,TDW
26	TS83	13	FD,SLA
27	CaSTMS25	15	PLWD,FD
28	CaSTMS9	NN	YPP, SDW,TWD

**Table 64:** List of markers associated with more than one trait evaluated in the chickpea reference set

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity. SPAD = Soil Plant Analysis Development. SDW=Shoot Dry Weight, RDW=Root Dry Weight (RDW), RDp=Root Depth, TDW=Total Plant Dry Weight, RL=Root Length, RLD=Root Length Density, RSA=Root surface area and RV=Root Volume.

Discussion

# **5. DISCUSSION**

A large number of chickpea germplasm accessions (more than 98,000) are conserved in several genebanks in the world (Gowda *et al.*, 2011). ICRISAT maintains the largest collection of 20,267 accessions of 60 countries which include 18392 land races, 98 advanced cultivars, 1293 breeding lines, 288 wild species and 196 accessions with no information on biological status. Inspite of vast germplasm accessions available in different genebanks, there has been very limited use of these accessions in crop improvement programs (Upadhyaya *et al.*, 2006). To enhance use of germplasm in crop improvement, a core collection of 1956 accessions (Upadhyaya *et al.*, 2001) was developed representing the variability of the entire collection. However, size of core collection was also not convenient for multilocational replicated evaluation. To achieve this Upadhyaya and Ortiz, (2001) proposed the 'minicore' concept and developed chickpea minicore consisting 211 accessions (1% of entire, 10% of core collection) representing entire species diversity and used as a gateway for germplasm utilization.

# **Global composite collection of Chickpea**

Upadhyaya *et al.*, (2006) developed a global chickpea composite collection consisting of 3000 accessions. The chickpea composite collection included the 1956 accessions of the ICRISAT core collection (Upadhyaya *et al.*, 2001), 709 cultivated accessions representing unique accessions at ICARDA, 39 advanced breeding lines and released cultivars, 35 distinct morphological variants, 20 wild species (*C. echinospermum* and *C. reticulatum*) accessions and 241 accessions carrying specific traits such as tolerance/resistance to biotic and abiotic stress, important agronomic characters (early maturity, multi-seeded pods, double podded, large-seed size, high seed protein, nodulation and responsiveness to high-input conditions). This global composite collection is composed of 80% landraces, 9% advanced breeding lines, 2% cultivars, 1% wild species and 8% for which precise status is unknown. Geographically, 39% of the composite collection originated from South and South-East Asia, 25% from West Asia, 22% from the Mediterranean and 5% each from Africa and the Americas.

#### **Development of reference set of Chickpea**

A genotype based 300 accessions reference set was developed from composite collection (Upadhyaya *et al.*, 2006) using data on 48 SSR markers, for diverse applications in chickpea genomics and breeding (Upadhyaya *et al.*, 2008).

The objectives of this study was to determine phenotypic diversity using 17 quantitative traits, seven qualitative traits and grain quality traits, resistance to pod borer and for traits related to drought tolerance; genotypic diversity using 91 SSR, to identify allelic variation associated with beneficial traits using association mapping and to identify genetically diverse trait-specific germplasm lines for use in breeding programme to develop cultivars with a broad genetic base.

#### Diversity in chickpea reference set

A wide spectrum of diversity has been captured in the reference set, which consisted of 194 desi, 88 kabuli, 11 pea or intermediate type accessions and 7 wild accessions. Of these 267 were landraces, 13 advanced lines and cultivars, 7 wild *Cicer* accessions, and 13 accessions with unknown biological status. Geographically, the reference set included accessions from South and East Asia (105), West Asia (93), Mediterranean region (56), Africa (21), North America (6), Russian Federation (6), South America (4), Europe (3), and unknown origin (6).

# PHENOTYPIC DIVERSITY BASED ON QUALITATIVE AND QUANTITATIVE TRAITS

# **5.1. QUALITATIVE TRAITS**

#### **5.1.1. Frequency distribution**

Qualitative traits are useful in characterization of accessions, as they show high heritability and stable expression. Out of the seven qualitative traits studied, maximum diversity was observed for seed color indicating importance of this trait in assessing phenotypic diversity. This is not surprising as the classification of chickpea types itself is based on seed color, shape and size. The frequency distributions of different phenotypic classes of the qualitative traits revealed a large variation for each trait. In chickpea reference set, the traits like low anthocyanin plant pigmentation (53.3%), pink flower colour (57.0%), semi-erect growth habit (62.3%), yellow brown seed color (36.0%), angular or ram's head seed shape (67.0%) with minute black dots (52.0%) and rough seed surface

(66.0%) were the most predominant characters.

Most of the qualitative traits are related with type of chickpea, desi or kabuli or intermediate. As desi types dominated entire reference set, the traits that are characteristics of desi type were predominant in the reference set. Among the qualitative traits relatively high polymorphism was observed for seed colour followed by seed surface indicating relatively greater importance of these two traits in phenotypic diversity assessment.

Semi-erect growth habit was most prevalent among accessions across three seed types (Upadhyaya *et al.*, 2001, Chaturvedi *et al.*, 2009), whereas plant pigmentation, flower colour, seed color, seed shape, minute black seed dots and seed surface differed within three seed types. Pink flower color (83.5%) among desi accessions, white flower color (98.9%) in kabuli, both white (45.4%) and light pink (36.4%) in pea type were the most prevalent characters among three seed types. Pink flower color in desi, white flower in kabuli is the characteristics of chickpea seed types, reported by Pundir *et al.*, (1985), Upadhyaya *et al.*, (2001), Chaturvedi *et al.*, (2009).

In the entire reference set low-anthocyanin was dominant over no and high anthocyanin (Rao *et al.*, 1980, Pundir *et al.*, 1985, Upadhyaya *et al.*, 2001). Most of the desi accessions (78.9%) were with low anthocyanin plant pigmentation, whereas kabuli types were with no-anthocyanin, and no-anthocyanin (81.8%) and low-anthocyanin (9.1%) was observed among pea type. Only 2% of the accessions were with high-anthocyanin pigmentation in the entire reference set. Desi accessions (55.2%) predominated with yellow brown and kabuli with beige (98.9%) seed color. Angular or ram's head seed shape (67.0%), which is the characteristic of desi type, dominated reference set followed by owl's head shape (29.3%) and intermediate or pea shaped (3.7%) (Pundir *et al.*, 1985, Upadhyaya *et al.*, 2001, Upadhyaya and Ortiz, 2001).

Minute black dots were present on the seed testa of most desi (71.6%) accessions while (28.4%) accessions had no dots on seeds and totally absent in kabuli type whereas in pea type (90.9%) seeds were with dots and (1.1%) were without dots. Among desi type accessions (97.4%) were of rough type and (2.6%) are tuberculated while in kabuli type (95.5%) had smooth and (4.5%) had rough seed surface. In pea types (54.5%) were smooth and (45.5%) were with rough seed surface (Pundir *et al.*, 1985 and 1988 Upadhyaya *et al.*, 2001).

Region wise, South and East Asia region accessions dominated with lowanthocyanin pigmentation (90 accessions, 85.7%), pink flower colour (93 accessions, 88.6%), yellow brown (71 accessions, 67.6%) seeds along with angular seed shape (93 accessions, 88.6%). Further in West Asian accessions, semi-erect (71 accessions, 76.3%), and no-anthocyanin (54 accessions, 58.1%) features were more common compared to other groups. This suggests the presence of greater variability in Asian material compared to other groups as they are the most preferred types in cultivation indicating the greater role of human selection in this region compared to other regions. Mediterranean region was dominated by accessions with beige seed color, white (34 accessions, 60.7%) flower colour since most of the kabuli and wild accessions originated from this region compared to other regions. The wide variability for these qualitative traits were reported earlier in chickpea with 16,820 accessions at ICRISAT (Upadhyaya et al., 2003), 1956 accessions of core collection (Upadhyaya et al, 2001), 211 accessions of mini core collection (Upadhyaya and Ortiz, 2001) and 88 accessions (Chaturvedi et al., 2009).

#### **5.2. QUANTITATIVE CHARACTERS**

The data on 17 quantitative traits of individual five environments and pooled (meta) were analyzed for the entire reference set to estimate variance components due to genotypes ( $\sigma^2 g$ ) and genotype x environment interactions ( $\sigma^2 g$ e), means and variances, phenotypic diversity and Shannon-Weaver diversity index (H') and PCA. The results of various analyses are discussed below.

#### 5.2.1. Variance components

The statistical procedure REML (Restricted Maximum Likelihood) allows estimating the variance components in a situation of high unbalancing data. Variances of 17 quantitative traits were calculated in individual environments separately and pooled over five environments. The five environments differed significantly as revealed by Wald's statistics; indicating that choice of the environments was appropriate in expressing the variability of reference set. Estimates of variance components due to genotypes were significant for most of the quantitative traits except for days to 50% flowering, flowering duration, days to grain filling, days to maturity and seeds per pod in E1, pods per plant and yield per plant in E2, plant height in E3 and plot yield and productivity in pooled analysis indicating that the reference set had sufficient genetic variation for most of the traits. In the pooled analysis, estimates of variance components due to  $\sigma^2 g$ and  $\sigma^2 g$  were estimated and tested against their respective standard errors and they were significant for all the traits except plot yield indicating the genotypes had variation and their performance differed in different environments. Significant variance in most of the traits in individual and pooled analysis showed that the genotypes in the reference set are diverse and had sufficient scope for selection and utilization in crop improvement programme.

Variance due to genotypes has been reported significant in earlier studies for the qualitative traits such as days to 50 percent flowering (Upadhyaya and Ortiz, 2001, Upadhyaya et al, 2001, 2003, Gowda et al., 2011), flowering duration (Gowda et al., 2011), days to maturity (Upadhyaya et al., 2001, Gowda et al., 2011), days to grain filling (Gowda et al., 2011), plant height and width (Upadhyaya et al. 2003, Gowda et al., 2011), apical primary branches, basal primary branches, apical secondary branches, basal secondary branches (Upadhyaya and Ortiz, 2001, Upadhyaya et al, 2001, 2003, Gowda et al., 2011), tertiary branches (Upadhyaya and Ortiz, 2001, Upadhyaya et al, 2001), 100-seed weight (Upadhyaya et al, 2001, 2003, Chaturvedi et al., 2009, Gowda et al., 2011), seeds per pod (Upadhyaya et al. 2003), pods per plant (Chaturvedi et al., 2009, Gowda et al., 2011), grain yield (Upadhyaya et al, 2001, Gowda et al., 2011), whereas non-significant for seeds per pod (Chaturvedi et al., 2009), yield (Abdel et al., 2005) and significant genotype x environment was observed for all traits except basal primary branches, basal secondary branches and pods per plant (Gowda et al., 2011).

# **5.2.2 Variability Studies**

Genetic variability studies provide basic information regarding the genetic properties of the population based on which breeding methods are formulated for improvement of the crop. These studies are also helpful to know about the nature and extent of variability that can be attributed to different causes, sensitive nature of the crop to environmental influences, heritability of the characters and genetic advance that can be realized in practical breeding. Progress in any crop improvement program depends mainly on the variability existing for the quantitative traits of the base population. Hence, to have a comprehensive idea, it is necessary to have an analytical assessment of yield components and other important agronomic traits.

# **5.2.2.1** Mean performance of the reference set accessions for quantitative traits in different environments

Substantial environmental variation was observed, indicating adequacy of these environments in differentiating the genotypes. The traits, days to 50 percent flowering, flowering duration, days to grain filling, 100- seed weight, plant width and number of branches did not differ significantly between five environments. However, plant height, days to maturity, pods per plant, seeds per pod, yield per plant, per day productivity and grain yield differed significantly between the environments. Mean productivity per day (18.3 kg ha<sup>-1</sup> day<sup>-1</sup>), plot yield (2088.6±206.71 kg ha<sup>-1</sup>), yield per plant (15.5±2.23g), pods per plant (62.7 ± 7.01), days to maturity (115.2±1.59 days) and basal primary branches (3.1±0.2) were maximum in E2 when compared to E1, E3, E4 and E5. Apical primary branches (2.9±0.95) and plant height (44.9±1.11cm) in E3, and 100-seed weight (23.6±1.32g) in E1 showed the maximum mean performance.

The differential response of reference set accessions for different environments was due to the different growing conditions in all five environments. In E1, E2, E3 reference set was grown in irrigated conditions during post rainy seasons 2006/07, 2007/08, 2008/09 at ICRISAT, E4 during post rainy irrigated environment 2008/09 at UAS, Dharwad and E5 during spring irrigated environment 2008/09 at ICRISAT. A review of weather data in different seasons revealed no appreciable difference among environments for sunshine hours, minimum and maximum temperatures, and total pan evaporation during cropping period. The major difference observed during E2 from other environments was the quantity of rainfall received during the cropping season which leads to increased plant height, plant width, number of branches and grain yield while reducing number of days to maturity.

The ten accessions ICCs 8318,14595,16374,9590,15518,15618,4918,6279,4533 and 1083 consistently flowered early (<50 days) in all environments indicating that these accessions could be source of genes for early flowering in breeding of early maturing cultivars. The incorporation of earliness will also ensure in avoiding the more exposure against major biotic and abiotic genotypes

(Chaturvedi *et al.*, 2009). Early flowering accessions were reported in chickpea (Upadhyaya *et al.*, 2007), groundnut (Upadhyaya *et al.*, 2006), pearl millet (Bhattacharjee, 2007) and finger millet (Geetha Rani, 2005).

The ICC 5434 (17 cm) is the only accession with very short stature in reference set, five accessions (ICCs 12321, 12379, 13469, 7554 and 12851) were short (< 45 cm) while eight accessions (ICCs 19011, 19034, 19164, 18724, 8740, 20260, 19100, 8752) were tall (> 60 cm) in all the five environments. Plant height with erect growth habit can play an very important role as it will provide more chances of sunlight to penetrate the lower most part of the plant which will ultimately help in reducing the high humidity in crop canopy during reproductive phase. This will ensure in retaining more number of pods at lower part of the plant also which can be helpful in relation to biomass accumulation (Chaturvedi *et al.*, 2009). Multilocational evaluation of these taller accessions could be used to find their suitability for release as cultivar or use in breeding programme.

The mean number of basal primary branches was high in E2 ( $3.1\pm0.2$ ), apical primary branches in E3 ( $2.9\pm0.95$ ) and tertiary branches in E2 ( $1.8\pm0.95$ ) than in other environments, whereas number of basal secondary branches was similar in all environments with a mean of  $3.2\pm0.12$ , apical secondary branches with an overall mean of  $4.4\pm0.21$ ,. The accessions with extreme number of branches could be used as parents for crossing to improve this particular trait. In general, traits appreciably affected by environmental factors were mostly vegetative, while reproductive components were least affected. Similar reports of differential response of vegetative traits in different seasons were reported in chickpea (Upadhyaya *et al.*, 2001, Gowda *et al.*, 2011).

Means and range of the reference set studied in the present study were similar to the composite collection (Upadhyaya *et al.*, 2006) of chickpea indicating that reference set represented the diversity of composite collection.

The similar range for quantitative traits has been reported earlier in chickpea germplasm characterization with varying number of accessions (25 accessions, Pundir *et al.*, (1991);132 accessions, Khan *et al.*, (1991); 40 accessions, Lokender Kumar and Arora, (1992); 60 accessions, Narendra Kumar, (1997); 108 accessions, Yadav and Sharma, (1999); 33 accessions, Subhash *et al.*, (2001); 1956 accessions, Upadhyaya *et al.*, (2001); 211 accessions, Upadhyaya and Ortiz, (2001); 16820 accessions, Upadhyaya *et al.*, (2003); 81 accessions, Prakash,

(2006); 24 accessions, 3000 accessions, Upadhyaya *et al.*, (2006); 360 accessions, Farshadfar and Farshadfar, (2008), 27 accessions, Bhavani *et al.*, (2009); 88 accessions, Chaturvedi *et al.*, (2009); 25 accessions, Dwivedi and Gaibriyal, (2009); 25 accessions, Malik *et al.*, (2010) and 65 accessions, Gowda *et al.*, (2011).

# **5.2.2.2** Mean performances of the accessions according to their geographical regions

The seven morphological descriptors showed differences among geographical regions in their distribution and range of variation. None of the morphological descriptors was monomorphic and most showed at least two relatively frequent phenotypic classes. Plant colour showed a pattern typical to the regions in which different chickpea types are grown. No-anthocyanin which is characteristic of kabuli chickpeas was less frequent in the Southeast Asia, and Africa, where desi chickpeas having low- or high-anthocyanin accessions are cultivated. Similarly, in Mediterranean region and Europe, the no-anthocyanin accessions are cultivated. The pattern for flower colour, seed colour, seed shape, and seed surface across different regions was similar to plant colour. Thus kabuli characteristics such as white flower, owl's head seed shape, a smooth seed surface, and beige seeds were more frequent in the Mediterranean region and Europe. Accessions with pink flowers, brown or yellow-brown seeds, angular seed shape, and rough seed surface, were abundant in Southeast Asia, and Africa. Erect, prostrate and spreading growth habits had a very low frequency across all the regions except East Asia. Semi-erect and semi spreading growth habits were evenly distributed in South Asia, whereas in the rest of the regions, except Southeast Asia, semi-erect accessions were predominant. Southeast Asia and West Asia showed 100% range for the seven morphological descriptors, and the Mediterranean region showed 100% range variation for all morphological descriptors except for plant colour.

According to Newman- Keuls test, region wise means were not significantly different for most of the traits except for days to 50 percent flowering and days to maturity (Africa), plant height (Europe), tertiary branches (South America), 100-seed weight (South America), pods per plant and yield per plant (Africa, South America and South and East Asia), and plot yield (Africa and South East Asia) in five environments and when pooled. The quantitative traits showed a large range

for different traits in different regions. The accessions from Africa flowered earlier (50-54 days), and matured earlier (110-112 days), whereas accessions from Europe flowered late (64-69 days) with short grain filling duration (49-53 days) across environments. The regional mean value for traits such as flowering duration, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, seed per pod were similar across environments. The European accessions had higher mean plant height (46-53 cm) across environments. Higher mean 100-seed weight across environments was in the accessions from South America (32-37 g) indicating the relative importance of seed size in this regions. The South East Asian accessions had higher mean yield overall in all environments. In Europe, the Mediterranean region, and Americas, large-seeded kabuli cultivars are preferred whereas in Southeast Asia and Africa mostly small-seeded desi cultivars are grown. The similar findings have been reported in chickpea by Upadhyaya *et al.*, (2001, 2003, and 2006), Upadhyaya and Ortiz, (2001).

#### 5.2.2.3 Phenotypic and genotypic coefficient of variation, (PCV and GCV)

The values for phenotypic coefficient of variation (PCV) were found higher than genotypic coefficient of variation (GCV) for all the traits in all environments indicating the influence of environment upon these traits (Subhash *et al.*, 2001) or very low influence of environment in the expression of these traits (Sidramappa *et al.*, 2008). In the present study, the traits tertiary branches, yield per plant, 100-seed weight, productivity, and plot yield showed high estimates of PCV and GCV. Singh *et al.*, 1992, Jahagirdar *et al.*, 1994, Rao *et al.*, 1994, Subhash *et al.*, 2001, Upadhyaya *et al.*, 2001, Upadhyaya and Ortiz, 2001, Saleem *et al.*, 2002, Arshad *et al.*, 2003 and 2004, Khan *et al.*, 2006, Upadhyaya *et al.*, 2007, Chaturvedi *et al.*, 2009, Malik *et al.*, 2010 reported higher estimates of PCV and GCV for most of these traits.

Narrow difference between PCV and GCV was observed in all environments and when pooled indicating greater role of genetic factors on the expression of these traits. Rest of the characters showed moderate to low variability. Moderate PCV and GCV were observed for apical primary branches, apical secondary branches, basal secondary branches, pods per plant, plant height, seeds per pod, basal primary branches and days to flowering. Low PCV and GCV were observed for days to grain filling, flowering duration, plant width and days to maturity. Raju *et al.*, 1978, Agrawal, 1985, Samal and Jagdev, 1989, Singh and Rao, 1991, Chavan *et al.*, 1994, Upadhyaya *et al.*, 2001, Upadhyaya and Ortiz, 2001, Upadhyaya *et al.*, 2007, Ali *et al.*, 2008, Patil *et al.*, 2008 reported moderate to low estimates of PCV and GCV for most of these traits.

#### 5.2.2.4 Heritability and genetic gain

The simple measures of variability like mean, variance and coefficient of variation reveals the extent of variability but not the heritable proportion of the total variation. To have the knowledge of the heritable proportion of variability, it is necessary to estimate the heritability. Heritability is a quantitative measure and also provides information about the correspondence between genotypic variance and phenotypic variance, *i.e.*, the ratio of variance due to hereditary differences ( $\sigma^2 g$ ) to the total phenotypic variance ( $\sigma^2 p$ ) (Singh, 1977), expressed as percent. The knowledge of heritability helps the plant breeder in predicting the behavior of characters in succeeding generations and to difference the effectiveness of selections.

In the present study, the broad-sense heritability was high (>80%) for most of the traits except for pods per plant and yield per plant in E2 and seeds per pod in E3 and E5, plant height, plant width, days to 50 percent flowering, days to grain filling, days to maturity, apical secondary branches, pods per plant, 100-seed weight, grain yield and per day productivity for pooled data indicating the reliability of the selection for these traits in this material. Populations which are genetically more uniform are expected to show lower heritability than the genetically variable population. Also, more variable environmental condition reduces the estimates of heritability, whereas more uniform environmental condition increases the magnitude of heritability (Dabholkar, 1999). Hence, high heritability of the traits under study may be due to highly variable and genetically diverse germplasm and more uniform environmental condition in all five environments.

Since heritability is also influenced by environment, the information on heritability alone may not help in pin-pointing characters for effective selection. Heritability gives the information on the magnitude of inheritance of quantitative traits, while genetic advance will be helpful in formulating suitable selection procedures. Therefore estimates of heritability and genetic advance would give better idea about possible gains of selection (Chavan *et al.*, 1994). The grain yield and its components like days to 50 % flowering, days to maturity, days to grain filling, seeds per pod, pods per plant, yield per plant, 100-seed weight and per day productivity exhibited high genetic advance as per cent of mean coupled with high estimates of broad sense heritability indicating that, the variation is attributable to genetic factors and selection may be effective for improvement of these traits.

The high estimates of heritability coupled with high genetic advance as per cent of mean in chickpea have been reported earlier for days to 50 percent flowering and days to maturity (Chandra, 1968; Joshi, 1972; Samal and Jagdev, 1989; Sharma et al., 1990; Misra, 1991; Singh and Rao, 1991; Panchbhavi et al., 1992; Chavan et al., 1994; Mathur and Mathur, 1996 and Upadhyaya et al., 2007, Gowda et al., 2011), plant height and plant width (Samal and Jagdev, 1989, Sharma et al., 1990, Singh and Rao, 1991, Misra 1991, Chavan et al., 1994 and Mathur and Mathur, 1996) Gowda et al., 2011), number of branches (Sharma et al., 1990 and Jha et al., 1997, Gowda et al., 2011), pods per plant (Joshi, 1972, Mishra et al., 1988; Samal and Jagdev, 1989, Mishra, 1991; Singh and Rao, 1991, Chavan et al., 1994, Mehndi et al., 1994, Mathur and Mathur, 1996, Narayana and Reddy, 2002, Sial et al., 2003 and Gowda et al., 2011), seeds per pod (Iqbal et al., 1994), 100-seed weight (Samal and Jagdev, 1989) Singh and Rao, 1991; Chavan et al, 1994; Jahagirdar et al, 1994; Tripathi, 1998; Kumar et al, 1999; Saleem et al, 2002; Toker, 2004, Gowda et al., 2011), yield per plant (Samal and Jagdev 1989; Jahagirdar et al, 1994; Singh and Rao 1991; Chavan et al, 1994; Gowda et al, 2011) and grain yield (Mehndi et al., 1994, Kumar and Krishna, 1998, Arshad et al., 2003, 2004, Upadhyaya et al., 2007).

#### 5.2.3 Correlations

Phenotypic correlation coefficients were estimated to know the association among traits which could be used as guidelines while making selections to exploit correlated response in the breeding programme. Understanding the interaction of traits among themselves and with the environment is of great use in plant breeding. Correlation studies provide information on the nature and extent of association between quantitative traits and it would be possible to bring out genetic up-gradation by selecting for easily measurable trait. Hence, an attempt was made to study the association prevailing among 17 quantitative traits in chickpea reference set.

Grain yield is a complex character and jointly determined by a number of yield related traits. An insight into the association between grain yield and other traits helps to improve the efficiency of selection. In general, the correlation between yield and other characters as well as among the component characters will vary with the genotype handled by the breeder. In the present investigation, the phenotypic correlations between pairs of characters have been studied to identify the component traits that are closely related to grain yield in chickpea reference set. In the present study, correlations were calculated in each environment separately and also based on the pooled data.

A total of, 61 correlations were significant in E1, 55 in E2, 57 in E3, 48 in E4, 50 in E5, and 50 in overall five environments at P<0.05. Among the 15 independent characters on grain yield, days to grain filling had positive correlation with grain yield in all environments except E5, apical primary branches in all environments except E2, basal secondary branches in E5, apical secondary branches in E2, E3 and pooled, seeds per pod in E3 and pooled, pods per plant in all environments except E5 and yield per plant exhibited significant positive correlation with grain yield in all environments except E4 and E5. However, magnitude of relationship was different in different environments indicating the strong association between the traits without any environmental influence.

It would be inferred that, selection for high yield would be effective through selection for these characters. Besides these characters showed high heritability coupled with high genetic advance as per cent mean, hence selection would be effective. Positive correlation of days to 50 per cent flowering (Vijayalakshmi *et al.*, 2000, Upadhyaya *et al.*, 2001, Saleem *et al.*, 2002), plant height (Tripathi, 1998, Yucel *et al.*, 2006), 100-seed weight (Benjamini, 1981, Singh, 1982, Tomar *et al.*, 1982, Arshad *et al.*, 2002, Saleem, 2002, Narayana and Reddy, 2002, Dobariya, 2003, Sial *et al.*, 2003, Arshad *et al.*, 2004, Hassan *et al.*, 2005), number of branches (Ozdemir, 1996), pods per plant and seeds per pod (Mishra *et al.*, 1988, Sandhu *et al.*, 1988, Sharma and Maloo, 1988, Sandhu and Mandal, 1989, Tagore and Singh, 1990, Uddin *et al.*, 1990, Chavan *et al.*, 1994, Sarvalia and Goyal, 1994, Tripathi, 1998, Bakhsh *et al.*, 2002b, Arshad *et al.*, 2002, Narayana and

Reddy, 2002, Bhaduoria *et al.*, 2003, Dobariya, 2003, Sial *et al.*, 2003, Arshad *et al.*, 2004, Hassan *et al.*, 2005, Yucel *et al.*, 2006, Babar *et al.*, 2008, Malik *et al.*, 2010) and per day productivity (Upadhyaya *et al.*, 2007) with grain yield were reported in chickpea. Days to 50 percent flowering, flowering duration, plant height and days to maturity showed significant negative correlation with grain yield.

From the above results it is seen that most of the traits were associated with grain yield and inter correlated among themselves. It indicates that the selection in any one of these yield attributing traits will lead to increase in other traits, thereby finally boosting the grain yield. Hence, primary selection of these traits may be given importance to obtain genotypes with increased plot yield. In addition, the significant associations between these component traits suggest the possibility of simultaneous improvement of these traits by selection.

In the present study, only those correlations which are greater than 0.500 or smaller than -0.500 were considered as meaningful as at least 25 per cent of the variation in one trait is predicted by the other (Upadhyaya et al., 2010c). The correlations for one pair of the characters were positive in all the five environments and overall, plot yield and per day productivity in E1, E2, E3 (0.990), E4 (0.974), E5 (0.978) and in overall. Correlations for a pair of the characters were negative in E3 and in overall; viz., days to 50 percent flowering and days to grain filling in E3 (-0.711), and in overall (-0.716); showed significantly higher and biologically meaningful correlation. However the pairs of traits, viz., days to 50 percent flowering and days to maturity in E1 (0.597), E2 (0.694), E3 (0.620), E4 (0.599), E5 (0.525) and in overall (0.671); pods per plant and per day productivity in E2 (0.500) showed high correlation, and correlations for one pair of the characters were negative, days to 50 percent flowering and days to grain filling (-0.614) in E4 (r = 0.50 or more). Days to 50 percent flowering was significantly and positively correlated with days to maturity, plant width, plant height and basal primary branches indicates the simultaneous improvement of other traits through the selection in positive direction for days to 50 percent flowering. Upadhyaya et al., (2001) reported the positive correlation of days to 50 percent flowering with flowering duration and days to maturity. Therefore, it can be inferred that selection should be in positive side for days to 50 percent flowering, days to maturity, days to grain filling, number of branches,

seeds per pod, pods per plant and yield per plant and negative side for plant height and width which will in turn automatically increases the grain yield in chickpea and is also, useful in evaluation of large germplasm set which is an easily measurable trait, with high correlation.

#### **5.3 DIVERSITY ANALYSIS**

#### 5.3.1. Shannon Weaver Diversity Indices

The Shannon-Weaver diversity index (H<sup> $\circ$ </sup>) was calculated for different traits in each environment separately and also pooled data over environments. The index is used as a measure of allelic richness and evenness; a low H<sup> $\circ$ </sup> indicates an extremely unbalanced frequency class and lack of genetic diversity.

Out of seven qualitative traits studied, dots on seed coat showed low mean H<sup>`</sup> in all environments indicating relatively unevenness distribution of alleles and low allelic richness for this trait, followed by seed shape, seed surface, plant color, growth habit and flower color. Seed color showed high mean H', indicating relative high diversity for this trait. Among the quantitative traits studied tertiary branches, flowering duration and seeds per pod showed low mean H<sup>\</sup> in all environments followed by apical primary branches, flowering duration, apical secondary branches and yield per plant in all environments. The traits such as, days to 50 percent flowering, grain yield, days to maturity, per day productivity and apical primary branches in all environments showed highest H<sup>^</sup> indicating evenness and richness, followed by days to grain filling, flowering duration, yield per plant, apical secondary branches, grain yield, basal primary branches, per day productivity, basal secondary branches, days to maturity, plant width and tertiary branches, pod per plant and apical primary branches, seeds per pod, days to flowering), 100-seed weight and plant height. Similar results have been reported by Upadhyaya et al., 2001 in core collection (1956 accessions), Upadhyaya and Ortiz, 2001 in mini core collection (211 accessions), Upadhyaya, (2003) in world collection of chickpea germplasm (16,820 accessions) in different regions for seven qualitative traits and 13 quantitative trait, whereas Islam et al., (1984) reported maximum diversity in number of pods and plot yield followed by minimum diversity in days to 50% flowering and days to maturity. The mean and range of H` for all the traits in the present study, is comparable with the H` of composite collection of chickpea (Upadhyaya et al., 2006a) indicating that the reference set represents the entire diversity of composite collection.

# 5.3.2. Phenotypic diversity matrix

Phenotypic diversity index (Johns *et al.*, 1997) was created by estimating differences between each pair of accessions for each of the 7 qualitative and 17 quantitative traits by averaging all the differences in the phenotypic values for each traits divided by their respective range. The entire chickpea reference set evaluated at five different environments, exhibited similar minimum diversity, ranging from 0.001 to 0.002 in all environments.

The maximum diversity index was observed between ICCV92311 (Southeast Asia) and ICC 11198 (Southeast Asia) in E1, between ICC 20266 (Unknown biological status) and ICC 4991 (Southeast Asia) in E2, between ICC 4918 (Southeast Asia) and ICC 16796 (Europe) in E3 and E4, between ICC 4918 (Southeast Asia) and ICC 18983 (Mediterranean) in E5 and between ICC 13764 (West Asia) and ICC 12037 (North America) when pooled. Based on the diversity index, ten most diverse accessions were identified in each environment and pooled data of five environments. Most of the pair of accessions which expressed most diversity in pooled data was also recorded in individual environment as well. Hence, ICCV92311 (Southeast Asia) and ICC 11198 (Southeast Asia), ICC 20266 (Unknown biological status) and ICC 4991 (Southeast Asia), ICC 4918 (Southeast Asia) and ICC 16796 (Europe), ICC 4918 (Southeast Asia) and ICC 18983 (Mediterranean), ICC 13764 (West Asia) and ICC 12037 (North America), ICC 15996 (Southeast Asia) and ICC 19011 (Mediterranean), ICCV 92311 (Southeast Asia) and ICC 16524 (Southeast Asia), ICCV92311 (Southeast Asia) and ICC 11279 (Southeast Asia), ICCV92311 (Southeast Asia) and ICC 5135 (Southeast Asia), ICC4918 (Southeast Asia) and ICC 14446 (Mediterranean) were the ten most diverse pairs of accessions identified based on five environments performance and further exploitation of these widely diverse accessions would help in the development of mapping population to identify QTLs and use in breeding programs to study the segregating generation and selection of superior lines. The results observed in this study are in agreement with earlier reports based on geographical origin (Upadhyaya, 2003) in world collection of chickpea germplasm (16,820 accessions) in different regions.

#### **5.3.3.** Principal component analysis

Principal Component Analysis (PCA) was used to provide a reduced dimension model that would indicate measured differences among groups.

In the present study, in all the five environments and also in the pooled analysis, a large proportion of the total variation was explained by the first seven Principal Components (PCs) and all together explained that, per day productivity, plot yield, days to 50% flowering and days to maturity were most important traits that made contribution in explaining variation in the first seven PCs. It indicated the importance of these traits which contributed more towards divergence in chickpea reference set. These results observed in this study are in agreement with earlier reports based on geographical origin (Upadhyaya, 2003) in world collection of chickpea germplasm (16,820 accessions) in different regions.

#### 5.3.4. Clustering

The hierarchical cluster analysis (Ward, 1963) based on Euclidean distance was conducted using the scores of first three PCs on the pooled data of reference set accessions.

Grouping of reference set accessions resulted into a dendrogram with four clusters. Accessions from Africa and South East Asia were grouped in to Cluster I, South America in Cluster II. Europe and Russian Federation in Cluster III and whereas Mediterranean, unknown, North America and West Africa was grouped together in Cluster IV.

This clustering is not surprising considering the trade of chickpea from the Mediterranean region to the countries in West Asia, and between Europe and Americas, and the preference for light coloured large-seeded cultivars. These links facilitate a flow of particular chickpea types between regions. The accessions from all the member regions of Cluster I were predominantly of desi type with low 100-seed weight whereas most members of Cluster II, III and IV were predominantly of kabuli type with high 100-seed weight The accessions in Cluster I had predominantly low-anthocyanin plants, pink flowers, angular shaped brown or yellow brown seeds with rough seed surface and dots on the seed testa, whereas in Cluster II, III and IV accessions were predominantly non-anthocyanin plants, beige coloured seeds, with smooth seed surface and without dots on seed testa. Both clusters differed significantly for all the 17 agronomic traits. Accessions in

Cluster II, III and IV took more days to 50 percent flowering and maturity had taller plants and more tertiary branches, and higher 100-seed weight than the accessions in Cluster I. Accessions in Cluster I had wider plants, more basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, pods per plant, seeds per pod and higher plot yield than in Cluster II, III and IV. This clustering observed in this study is in agreement with earlier reports based on geographical origin (Upadhyaya, 2003) in world collection of chickpea germplasm (16,820 accessions) in different regions.

# **IDENTIFICATION OF TRAIT SPECIFIC SOURCES**

In any crop, improvement of yield and other traits like quality, biotic and abiotic stresses can be achieved by identifying different gene/trait specific sources. The use of genetic resources in the breeding programs have been mainly as sources of resistance to pests and diseases (Knauft and Gorbet, 1989), or as sources of male sterility, short stature or any such character with simple inheritance. Well known examples are semi-dwarf rice and wheat genotypes which contributed much to the success of green revolution. There have been fewer efforts for identifying germplasm lines for increasing yield potential than for pest resistance and nutritional quality (Halward and Wynne, 1991), because such traits are highly environment interactive and require multi-environment testing to accurately characterize them (Upadhyaya *et al.*, 2010a). Thus identification of promising resources for the environment sensitive quantitative characters is a difficult task.

However, with the use of core (Upadhyaya *et al.*, 2001) and mini core collections of chickpea (Upadhyaya and Ortiz, 2001), sources for high grain yield (Upadhyaya *et al.*, 2007a), tolerance to drought (Kashiwagi *et al.*, 2005) and disease resistance (Pande *et al.*, 2006) have been identified. Evaluation of mini core led to the identification of 39 chickpea accessions for a combination of agronomic traits such as early maturity, seed size and grain yield (Upadhyaya *et al.*, 2007a). Similarly, new sources for tolerance to drought (Upadhyaya, 2005) and low temperature at germination (Upadhyaya *et al.* 2009a), and for early-maturity (Upadhyaya *et al.*, 2006a), were identified in the groundnut core and mini core collections. Upadhyaya *et al.* (2005) identified 15 fastigiata, 20 vulgaris, and 25 hypogaea type groundnut accessions for pod yield and its components upon multi-location evaluation of groundnut core collection for Asia region.

Upadhyaya *et al.* (2010d) evaluated finger millet core collection for grain nutrients and identified accessions rich in Fe, Zn, Ca and protein. Hence, multienvironmental evaluation / characterization of chickpea reference set and identification of trait specific sources for different yield contributing traits will provides new sources for future breeding program in chickpea.

In the present study, chickpea reference set was evaluated in five different environments which showed a wide range of variability for yield and its component traits within and between environments for identification of new trait specific sources. Out of 300 accessions present in chickpea reference set, 2 for early flowering, 17 for more seeds per pod, 35 for more pods per plant, one with more yield per plant, 19 with high 100-seed weight, 119 for high plot yield, 89 for per day productivity, 20 heat tolerant, 13 with high root depth, 42 with high shoot dry weight, 40 with high root dry weight, 11 with high root to total plant dry weight ratio (R/T%), 33 accessions with high root length, 6 accessions for root length density, twenty five with minimum damage rate to pod borer, 17 with lowest larval survival%, 3 accessions with high anthocyanin content, were identified as trait specific for important traits.

The genetically diverse trait-specific accessions identified in the present study can be used in breeding program to develop high yielding adapted cultivars with a broad genetic base. Extensive evaluation of these accessions in different locations may be useful to assess the stability for identifying the stable trait specific accessions.

#### **5.5. MOLECULAR DIVERSITY**

Understanding the distribution of genetic diversity among individuals, populations and gene pools is crucial for the efficient management of germplasm collections and breeding programs. Diversity analysis is routinely carried out using sequencing of selected gene(s) or by molecular markers. Molecular markers are increasingly important tools for genetic and genomic studies, breeding and biodiversity research. In any genome, the number of morphological and biochemical markers are limited when compared to DNA markers which are ubiquitous and numerous. However, several DNA-based molecular markers are available for genetic diversity analysis for most of all the crops. An extensive characterization of plant genetic resources provides an opportunity for structural dissection to mine the allelic variation, and identify diverse accessions for crop improvement (Upadhyaya *et al.*, 2010a). The DNA-based markers are promising and effective tools for measuring genetic diversity in plants germplasm and elucidating their evolutionary relationships (Pervaiz *et al.*, 2009).

Germplasm characterization based on molecular markers has gained importance due to the speed and quality of data generated. A comprehensive study of the molecular genetic variation present in diploid germplasm would be useful for determining whether morphologically based taxonomic classifications reflect patterns of genomic differentiation. It would also provide information on the population structure, allelic richness, and diversity parameters of diploid germplasm to help breeders use genetic resources for cultivar development more effectively (Şakiroglu *et al.*, 2010). Almost all kinds of molecular markers have been used for analysis of genetic diversity in chickpea germplasm. Majority of these studies however employed RAPD and AFLP markers, but now SSRs are preferred.

Amongst the DNA markers, the microsatellites (also known as simple sequence repeats (SSRs)) markers are now the markers of choice in most areas of molecular genetics as they are highly polymorphic even between closely related lines, require low amount of DNA, can be easily automated for high throughput screening, can be exchanged between laboratories and are highly transferable between populations. The SSR markers are co-dominant markers and good for the studies of population genetics and mapping. Microsatellite (SSR) markers were utilized to reveal genetic diversity in apple (Malus spp.) (Hokanson et al., 1998), common beans (Phaseolus vulgaris L.) (Blair et al., 2009) core collections, US peanut mini core collection (Kottapalli et al., 2007) and USDA rice minicore subset (Agrama et al., 2009). Hence in order to increase the molecular marker repertoire and to develop genome wide SSR markers, ICRISAT in collaboration with University of Frankfurt, Germany, developed 311 SSR markers from SSRenriched libraries (Nayak et al., 2010) and 1344 SSR markers from BAC-end sequence mining approaches in collaboration with University of California, Davis, USA. As EST sequences from various tissues and developmental stages of chickpea have also been reported (Boominathan et al., 2004; Romo et al., 2004; Buhariwalla et al., 2005; Coram and Pang, 2005; Varshney et al., 2009b, Choudhary *et al.*, 2009), a few hundred SSR markers have been developed from ESTs (Buhariwalla *et al.*, 2005, Varshney *et al.*, 2009b, Choudhary *et al.*, 2009). As a result of above mentioned efforts, at present >2000 SSR markers representing the entire chickpea genome are available. Genetic diversity in chickpea using microsatellite (SSR) markers are reported by Udupa *et al.*, (1999), Choumane *et al.*, (2000), Sethy *et al.*, (2006a) and (2006b), Upadhyaya *et al.*, (2008), Choudhary *et al.*, (2009), Khan *et al.*, (2010).

#### 5.5.1 Molecular diversity of chickpea reference set

Out of 100 SSR markers in this study, 91 markers mapped on 12 chickpea linkage groups of Winter *et al.*, (2000) produced clear, scorable and polymorphic marker profile.

# 5.5.1.1 Allelic richness and genetic diversity in chickpea reference set

A set of 91 highly informative SSR markers detected a total of 2,411 alleles in 300 reference set accessions. However, the number alleles per locus detected in this study was earlier reported, e.g., 7.6 (Wang et al., 2009) and 4.79 (Shehzad et al., 2009) in sorghum, 8.23 in maize (Yang et al., 2010) and 8.2 (Agrama et al., 2007), 15.8 (Agrama and Eizenga, 2008) and 12.4 (Borba et al., 2010) in rice. Higher average number of alleles per locus was reported in some crops like 16.7 in barley (Malyshera-Otta et al., 2006), 35 in chickpea (Upadhyaya et al., 2008b), whereas (Huettel et al., 1999, Choudhary et al., 2006, Sethy et al., 2006a, b) reported 2 to 6 alleles per marker in chickpea. The difference in SSR allelic richness can be explained by several factors like diversity range of the germplasm, number of accessions used, number of SSR loci and SSR repeat type (Yang et al., 2010). A higher number of lines in the samples leads to a more diverse range of germplasm by sampling, and a larger number of loci (and in particular, the use of dinucleotide repeat SSRs rather than tri- or higher) will leads to a higher number of alleles and higher genetic diversity (Gupta and Varshney, 2000, Yang et al., 2010). In fact, the earlier studies in chickpea also revealed the abundance of TAA/TTA (tri-nucleotide) and TA/GA (di-nucleotide) SSR motifs and the extensive polymorphism was found with markers containing these repeat motifs (Huettel et al., 1999, Udupa et al., 1999, Leichtenzveig et al., 2005). Similar studies in other legumes (Medicago, soybean, Lotus) showed the abundance of trinucleotide (TTC) and di-nucleotide (GA) repeats (Jayashree et al., 2006).

Moreover, the higher number of alleles, gene diversity, and PIC in chickpea reference set is due to more number of tri-nucleotide and di-nucleotide repeat motif markers used in the evaluation.

In the reference set, a total of 2424 rare alleles were observed from 91 SSR markers. It ranged from 2.0 to 90.0. The markers TS5 (90 alleles), TR1 (82 alleles), TR43 (76 alleles), TR7 (74 alleles) showed high number of rare alleles, whereas markers GAA43, TAA57 (each 2 rare alleles) showed low number of rare alleles. Common alleles ranged from 0-576 with a mean of 374. TA80 (576) showed high number of common alleles. Frequent alleles ranged from 0-570 with a mean of 129.5. CaSTMS 20 (570) showed highest number of frequent alleles from 91 SSR markers. 1980 unique alleles were detected among cultivated accessions whereas, 114 in wild accessions and 319 alleles were common among wild and cultivated. In the cultivated group, desi accessions contained the largest number of unique alleles (864) followed by kabuli (836) and pea type (52). However, variable and inconsistent relationship between repeat unit length and SSR polymorphism has been reported in several self pollinated crops (Sorghum, Folkerstma et al., 2005). Information available on the alleles present in different germplasm lines will be very useful for developing the mapping populations for genome analysis as well as applied breeding programmes.

#### 5.5.1.2 Polymorphic information content (PIC).

The relative informativeness of each marker can be evaluated on the basis of its polymorphic information content (PIC) value. The average PIC value in this study was 0.81, this was higher than that reported in sweet sorghum (0.54, Wang *et al.*, 2009) and rice (0.42, Jin *et al.*, 2010), but lower than that reported in chickpea (0.85, Upadhyaya *et al.*, 2008b). Out of 91 markers, 80 markers were highly polymorphic with PIC values more than 0.50. The PIC values ranged from 0.00 to 0.97 in desi, 0.00 to 0.95 in kabuli and 0.00 to 0.89 with an average of 0.73 in pea type, 0.80 in desi and 0.79 in kabuli.

Similar estimates of PIC values were observed in case of earlier microsatellite studies in chickpea (Geleta *et al.*, 2006, Taran *et al.*, 2007). Gupta *et al.* (2003) reported increased PIC with greater number of markers. They obtained PIC of 0.469 with 65 SSRs markers compared to 0.210 with 20 SSRs on 52 wheat genotypes. Most of the self pollinated crops such as sorghum (Folkertsma *et al.*,

2005), barley (Turuspekoy *et al.*, 2001) and wheat (Stepien *et al.*, 2003) produced the optimum PIC range of 0.600 to 0.700. This result indicated that PIC values depend not only on the number of alleles but also the gene diversity (Smith *et al.*, 2000). Normally inbreeding species, the level of polymorphism is expected to be generally lower than in out crossing species (Miller and Tanksley, 1990).

Although, the number of SSR marker in this study was limited, high polymorphism was revealed indicating wide diversity among accessions. The high diversity obtained with SSRs is consistent with their known characteristics, such as more variability, and higher resolution and higher expected heterozygosity than the RFLPs, RAPDs or AFLPs (Pejei *et al.*, 1989; Powell *et al.*, 1996; Taramino and Tingey, 1996). The high levels of polymorphism associated with SSRs are expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz and Renz, 1984; Tautz *et al.*, 1986) rather than by simple mutations, insertions or deletions.

#### 5.5.1.3 Gene diversity

Gene diversity is defined as the probability that two randomly chosen alleles from the population are different. It varied from 0.02 to 0.97, with an average of 0.83. 83 out of 91 SSRs were detected high gene diversity > 0.50 and only 8 SSR markers were <0.50. Gene diversity averaged 0.82, ranging from 0.00 to 0.97 in desi, whereas in kabuli accessions, it varied from 0.00 to 0.96 with an average of 0.81. In pea type, the gene diversity ranged from 0.00 to 0.89 with an average 0.73. Desi types exhibited maximum mean gene diversity and PIC than kabuli and pea types. Random genomic DNA markers (RFLP and RAPD) may assay polymorphism located in the non-coding regions of the genome that are poorly conserved among species, whereas functional markers such as EST/SSR would assay polymorphism that is associated with the coding regions of the genome and detect "true gene diversity" available inside or adjacent to the genes (Maestri *et al.*, 2002, Thiel *et al.*, 2003). High polymorphism, allele number and gene diversity indicated a wide diversity among accessions present in the chickpea reference set.

#### 5.5.1.4 Heterozygosity

Single allele per locus in each genotype was observed in most of the accessions. These observations are as expected as the SSR markers are locus-specific and generally amplify one locus (Gupta and Varshney, 2000). In the present study, a wide range of heterozygosity (%) was detected from 0.00% to 2.87%, with an average of 0.151%. Out of 91 markers, 82 SSR markers detected no heterozygosity indicating that a large collection of landraces was involved in this study and it is possible that these accessions still possess some residual heterozygosity at least at some SSR loci reported (Upadhyaya et al., 2008). A landrace is defined as an autochthonous (primitive) variety with a high capacity to tolerate biotic and abiotic stresses, resulting in high yield stability and an intermediate yield level under a low input agricultural system (Zeven, 1998). The heterozygosity observed at some of the loci could also be due to high mutational rate and mutational bias at SSR loci (Udupa and Baum, 2001). The loci with large number of repeat units (SSR units) tend to show high mutational rate. As a result, any mutations in any one of the alleles may create a heterozygous condition. Many of the loci which displayed heterozygous status have a large number of SSR units. Therefore, SSR markers from other crop/related species exhibited more heterozygosity as compared to SSRs from chickpea.

# 5.5.2 Unweighted neighbor-joining tree

Neighbour-joining tree based on simple matching dissimilarity matrix between 300 accessions of the chickpea reference set along with five checks highlighted broadly four clusters namely CI to CIV, respectively. The CI, CII and CII were dominated by desi accessions, CIV predominated with kabuli accessions. The results from the neighbor-joining phylogenetic tree corresponded well with the classification based on three seed types of chickpea.

# 5.5.3 Pearson Correlations

The correlations coefficients among number of repeat unit, number of alleles per locus, major allele frequency, gene diversity and PIC for 91 SSR markers were estimated. Number of repeat unit were highly significant and positively correlated with number of alleles per locus, gene diversity and PIC, whereas negative and significantly correlated with major allele frequency. Number of alleles per locus was highly significant and positively correlated with gene diversity and PIC, and significantly negatively correlated with major allele frequency. Significant positive correlation between allele per locus and gene diversity was reported in chickpea with 48 SSR markers (Upadhyaya *et al.*, 2008b) and positive correlation

between PIC and number of allele, PIC and repeat unit, number of alleles per locus and repeat unit was reported earlier studies by Jia *et al.* (2009). Highly significant negative correlation of major allele frequency was recorded with gene diversity and PIC. Gene diversity was highly significant and positively correlated with PIC. It could be inferred that the increase in major allele frequency leads to decreases in number of alleles per locus, gene diversity and PIC.

# 5.6 POPULATION STRUCTURE AND ASSOCIATION MAPPING

Chickpea is a cool season grain legume with high nutritive value. It belongs to the family Fabaceae and is a self-pollinated diploid crop (2n=2x=16) with a relatively small genome of 750 Mbp (Arumuganathan and Earle, 1991). One of the major goals of plant breeders is to develop genotypes with high yield potential and the ability to maintain the yield across environments. With the development of molecular markers, breeders have a complimentary tool to traditional selection and markers linked to variation in a trait of interest which could be used to assist the breeding programs. Availability of DNA marker based maps for the genomes of many crops facilitated mapping of QTLs of interest and marker-assisted selection (Winter and Kahl, 1995). QTL mapping analysis has provided an effective approach for locating and subsequently manipulating the QTLs associated with different quantitative traits in plants (Rachid et al., 2004). However, a DNA marker map of sufficient density for use in QTL mapping of important traits is still lacking in chickpea but however, Nayak et al., (2010) developed a first SSR based high density intra specific genetic map (ICC 4958 x ICC 1882) with 255 marker loci.

The phenotypic variation of many complex traits of agriculturally or evolutionary importance is influenced by multiple quantitative trait loci (QTLs), their interaction, the environment and the interaction between QTL and environment. Linkage analysis and association mapping are the two most commonly used tools for dissecting complex traits (Zhu *et al.*, 2008). Linkage analysis in plants typically localizes QTLs in 10 to 20 cM intervals because of the limited number of recombination events that occur during the construction of mapping populations and evaluating a large number of lines (Doerge, 2002; Holland, 2007). Alternatively, association mapping has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary

recombination events at the population level (Nordborg and Tavare, 2002; Risch and Merikangas, 1996). Choice of population for association mapping and appropriate marker density are crucial decisions for accuracy of association mapping. One of the sources of false positives in association mapping is population structure, which is a division of the population into distinct subgroups related by kinship. Different methods and software tools have been developed to correct the results for population structure usually by dividing the germplasm collections into subgroups or adjusting the probability of the null hypothesis (Rafalski, 2010). Presence of population structure within an association mapping population can be an obstacle to the application of association mapping as it often generates spurious genotype-phenotype associations (Yu and Buckler, 2006; Zhu et al., 2008). To account for population structure in association analysis, two major statistical methods, genome control (Devlin and Roeder, 1999; Zheng et al., 2005) and structure association (SA) (Pritchard et al., 2000) were applied in earlier studies, both of which used random markers spaced throughout the genome, but incorporated them into statistical analysis in different approaches (Yang et al., 2010).

Yu *et al.* (2006) developed a general linear model (GLM) and a mixed linear model (MLM) approach to perform association analysis. The MLM approach, accounting for both population structure (Q) and relative kinship (K), can be performed with the TASSEL software package (Bradbury *et al.* 2007), which is most common method of association analysis in plants and has been successfully applied in rice (Agrama *et al.*, 2007; Wen *et al.*, 2009; Borba *et al.*, 2010), wheat (Breseghello and Sorrells, 2006; Neumann *et al.*, 2011), sorghum (Murrary *et al.*, 2009), *Arabidopsis* (Zhao *et al.*, 2007) and potato (Malosetti *et al.*, 2007). However, until now, the reports of QTLs for chickpea are limited except the QTLs governing grain yield and other agronomic traits would increase our understanding of the genetic control of the characters and to use them effectively in breeding programs.

Some of the agronomic and yield influencing traits like double-flower (Yadav *et al.*, 1978; Rao *et al.*, 1980; Pawar and Patil, 1983; Singh and van Rheenen, 1994; Kumar *et al.*, 2000), flowering time (Or *et al.*, 1999), chilling tolerance during flowering (Clarke and Siddique, 2003), flowers per axis (Srinivasan *et al.*, 2006), double-podding and other morphological characters (Rubio *et al.*, 1999, 2004;

Cho *et al.*, 2002; Rajesh *et al.*, 2002; Lichtenzveig *et al.*, 2006) and nutritional traits like  $\beta$ -carotene and lutein content (Abbo *et al.*, 2005) have been extensively studied in chickpea. A QTL flanked by marker TAA170 and TR55 on LG4A identified for root length (Chandra *et al.*, 2003). Or *et al.* (1999) suggested a major photoperiod response gene (*Ppd*) affecting time to flowering. Cho *et al.* (2002) identified a single QTL for days to 50% flowering on LG3 with a LOD score of 3.03. Lichtenzveig *et al.* (2006) identified two QTLs on LG1 and LG2 linked to time to first flower. Cho *et al.* (2002) also identified a QTL for seed weight on LG4 accounting for 52% of the total phenotypic variation. Nayak *et al.*, (2010) reported a total of 8 QTLs for root traits with phenotypic variation 4-54%. These reports generated information on QTLs for important traits which can be used for stress breeding in chickpea.

Until now, association mapping using the existing natural variation present in the germplasm for the detection of QTL was not been reported in chickpea and QTL reported by the earlier studies and linkage mapping based on mapping population using the RFLP probes were used to identify QTL. Hence, there is a need for the identification and development of more SSR markers and QTLs in chickpea for various agronomic traits which contribute to yield and its improvement.

#### 5.6. 1 Population structure in chickpea reference set

#### 5.6.1.1 Allelic richness and genetic diversity of subpopulations

The reference set was grouped in to thirteen subpopulations by using 91SSR markers allelic data by using the software program STRUCTURE. 91 SSR markers detected a total of 1199 alleles in SP1, 720 in SP2, 778 in SP3, 483 in SP4, 527 in SP5, 803 in SP6, 749 in SP7, 1301 in SP8, 544 in SP9, 574 in SP10, 348 in SP11, 428 in SP12 and 759 in SP13. Highest number of alleles was detected by SP8 with a mean of 11.4, which ranged from (0-26). Lowest number of alleles was detected by SP11 with a mean of 3.1, which ranged from (0-7). Maximum mean PIC value was detected in SP8 and minimum in SP11 when compared with other sub-populations. Maximum mean gene diversity value was detected in SP7 (0.765) and minimum in SP4 (0.560) when compared with other sub-populations. Rare alleles are detected only in SP1 (32) and SP8 (2). Accessions from SP8 consist of 2 rare, 7087 common and 3881 most

frequent alleles when compared with other sub-populations.

#### 5.6.1.2 Analysis of molecular genetic variance (AMOVA)

The distribution of molecular genetic variation among and within the thirteen subpopulations was estimated by AMOVA. AMOVA revealed that 20 per cent of the total variance was among the subpopulations, while 80 per cent was among individuals within the subpopulations. The same trend was observed when the AMOVA estimated based on three chickpea types in reference set.

# 5.6.1.2. Principal coordinates analysis (PCoA) and unweighted neighborjoining tree

In order to link the genetic diversity with the phenotypic diversity, efforts were made by analyzing the phenotypic data for seventeen quantitative traits together with genotyping data by using Principle Coordinate Analysis (PCoA) and unweighted neighbor-joining phylogenetic analysis was conducted to further assess the population subdivisions identified using STRUCTURE. The first three PCs explained 81.71 per cent of variation of which PC1 explained 36.48 per variation and PC2 explained 33.38 per cent of the SSR variation among the 300 accessions of chickpea reference set including five checks. Plotting the first two PCs and colour coding genotypes based separated the chickpea reference set accessions into four clusters which was identified by STRUCTURE analysis.

Neighbor-joining tree was constructed based on the simple matching dissimilarity matrix of 91 SSR markers assayed. Color coding was given for the thirteen subpopulations as inferred from the STRUCTURE analysis denoted as SP1 (Red), SP2 (Green), SP3 (Dark Blue), SP4 (Yellow), SP5 (Pink), SP6 (Sea blue), SP7 (Brown), SP8 (Maroonish brown), SP9 (Light brown), SP10 (Dark sea blue), SP11 (blue), SP12 (Light green), SP13 (Grey) respectively, which clearly differentiated subpopulations. Therefore, PCoA and neighbor-joining revealed genetic relationship fairly consistent with the structure based membership assignment for most of the accessions. Varshney (2007a) also reported the similar grouping of early flowering accessions in a USDA collection of chickpea germplasm by SSR marker data. For other traits, phenotypic classes were not associated with regional classification based on SSR markers. Jin *et al.* (2010) also reported the fairly consistent relationship between neighbor-joining tree with STRUCTURE based membership assignment in rice. Şakiroglu *et al.*, 2010

reported the consistent pattern Neighbor-joining tree with the PCoA and population subdivision by STRUCTURE in wild diploid alfalfa (*Medicago sative* L.).

# 5.6.2. Genome- wide Association (GWA) analysis

In total 300 genotypes (chickpea reference set) were used in the marker-trait association analysis. The extent of variability (in terms of CV %) available for different traits indicated suitability of reference set of chickpea for the study of marker-trait associations. The correlation studies revealed the presence of significant positive correlations between most of the qualitative, quantitative and grain quality traits, resistance to pod borer and for traits related to drought tolerance in a structured chickpea reference set under study. This suggests their suitability for the study of marker-trait associations using common set of markers. Association mapping is an innovative linkage disequilibrium based methodology to dissect quantitative traits. Although large number of markers are necessary for detecting association of complex traits using GWA (Genome-wide association), but this method does not require any prior information about genes for the traits of interest. Advantage of GWA over candidate gene sequencing approach, involves the detection of unknown loci associated with the trait. As an alternative to traditional linkage analysis, association mapping offers three advantages- i) increased mapping resolution, ii) reduced research time and iii) greater allele numbers (Yu and Buckler, 2006). Since its introduction to plants (Thornsberry et al., 2001), association mapping has continued to gain acceptance in genetic research. There are limited studies of association mapping in case of plant species. Application of association-mapping approaches in plants is complicated by the population structure present in most germplasm sets to overcome this problem, linear models with fixed effects for sub-populations (Breseghello and Sorrells, 2006) or a logistic regression-ratio test (Pritchard et al., 2000; Thornsberry et al., 2001) can be employed. Owing to the large germplasm sets required for dissecting complex traits, the probability increases that partially related individuals are included. This applies in particular when genotypes selected from plant-breeding populations are used for association mapping (Thornsberry *et al.*, 2001; Kraakman et al., 2004). Association mapping identifies QTLs by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse Germplasm (Zhu *et al.*, 2008). Association analysis was applied using structure (Q)-kinship (K) mixed-model approach (Yu *et al.*, 2006) that promises to correct for linkage disequilibrium (LD) caused by population structure and relatedness relationship.

In the present study, an attempt was made to associate neutral SSR markers to quantitative, qualitative, quality related root traits and pod borer related traits using reference set of chickpea. The likely number of sub-populations was obtained based on the delta K value derived from Evanno's method (Evanno *et al.*, 2005). In the present study, at K=13 there was deep portioning of population into thirteen sub-populations, which might be due to the selection pressure due to domestication and breeding. At K=13, delta K value was found to be maximum and this information was further used in association analysis to avoid false positives.

# 5.6.3 Association of markers in reference set with qualitative, quantitative, quality (anthocyanin and protein traits), pod borer resistant and drought related traits

64 significant (P $\leq$ 0.001) MTAs were detected involving 49 SSR markers in E1, with maximum phenotypic diversity of 43.4% for anthocyanin content. 86 significant MTAs were detected involving 46 SSR markers in E2 and maximum phenotypic diversity of 42% for tertiary branches whereas in E3, 76 significant MTAs with 50 SSR markers and maximum phenotypic diversity of 42.9% for leaf area, in E4 74 significant MTAs with 52 SSR markers and maximum phenotypic diversity of 45.4% for apical secondary branches and in E5 56 significant MTAs with 44 SSR markers and maximum phenotypic diversity of 34.8% for plant width.

In the present study, by pooling the five environments data, number of significant MTAs (P $\leq$ 0.001) were 27 for qualitative traits with 21 markers, 76 (P $\leq$ 0.001) for quantitative trait, two for SCMR, one each for protein content, two for pod borer resistant traits and 21 for drought tolerance related traits and 7 among qualitative, 39 among quantitative, 1 among SCMR and 8 among drought related traits were identified as the major MTAs (>20% phenotypic variation) across all the environments in chickpea reference set

Hence, these most significant MTAs were believed to be associated with colocalized/pleiotropic QTLs. The co-localization of specific genes/QTLs/markers could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response and pod borer resistance traits. The presence of several co-localized/pleiotropic QTLs verified the complex quantitative nature of drought tolerance, pod borer resistance in chickpea and allowed the identification of some important genomic regions for traits related to high yield, good protein percent, drought tolerance and resistance to pod borer. The markers associated with more than one trait may be efficiently utilized in improvement of more than one trait simultaneously through marker assisted selection (MAS). Till date there are no reports of association studies in case of chickpea, however the association studies in other crop species especially in cereals such as maize (Lu et al., 2009), barley (Malysheva-Otto et al., 2006; Cockram et al., 2008), sorghum (Shehzad et al., 2009) and wheat (Neumann et al., 2011) have revealed that the linkage based QTL analyses can be complemented by LD based association studies. Association mapping studies in legumes are limited to soybean and Medicago, where association map consisting of 150 markers was constructed on the basis of differences in allele frequency distributions between the two sub-populations of soybean for seed protein and the genome-wide association studies has been started in Medicago as a part of HapMap (Haplotype Map) project on 384 inbred lines (http://www.medicagohapmap.org/about.php). The phenotypic variation explained using GLM was found to be comparatively higher compared to that computed from MLM in the present study. This was also evident from studies of association mapping in case of wheat (Neumann et al., 2011) where, the GLM and MLM models were compared to give markers-trait associations. The association studies in crop species are taking advantage of development of high-throughput marker technologies like SSRs and advanced statistical tools. Chickpea reference set is genetically diverse and possesses potential variation for economic traits and hence could be extensively evaluated for greater exploitation in breeding programs to improve and to widen the genetic base of chickpea cultivars. Marker trait associations identified in this study using SSR markers and association mapping approach was the first effort in this crop, will provide a preliminary knowledge to the research community for further QTL identification, to identify candidate genes and gene cloning that underlie QTLs in chickpea.

Summary

# 6. SUMMARY

Phenotypic and molecular characterization of germplasm and identification of genetically diverse trait specific sources are important for enhanced utilization of chickpea genetic resources in breeding improved cultivars. Hence, the current study was undertaken to understand the phenotypic and genetic diversity in chickpea reference set, to identify trait specific germplasm and the SSR markers associated with phenotypic variation. The genetic material used in this study was a genotype based reference set of 300 accessions (Upadhyaya *et al.*, 2008) developed from composite collection (Upadhyaya *et al.*, 2006). Reference set and five control cultivars (Annigeri, G 130, ICCV 10, KAK 2, and L 550) were evaluated in five environments [(E1), (E2), (E3), (E5) at ICRISAT, Patancheru, Andhra Pradesh; and (E4) at UAS (University of Agricultural Sciences), Dharwad] in alpha design with two replications. The data were recorded on seven qualitative, 17 quantitative, 10 drought tolerance related, three pod borer resistance and two quality traits. For the molecular characterization of chickpea reference set, 91 SSR markers were used. The results are summarized below.

# **6.1. PHENOTYPIC DIVERSITY**

#### **6.1.1. Qualitative traits**

• In the entire chickpea reference set, low anthocyanin plant pigmentation, pink flower colour, semi-erect growth habit, yellow brown seed color, angular or ram's head seed shape with minute black dots and rough seed surface were the most predominant classes among qualitative traits.

• Most of the qualitative traits are related with type of chickpea, desi or kabuli or intermediate. As desi types dominated entire reference set, the traits that are characteristics of desi type were predominant in the reference set. The proportion of low anthocyanin plant pigmentation, pink flower colour, semi-erect growth habit, yellow brown seed color, angular or ram's head seed shape with minute black dots and rough seed surface were the most prevalent classes across desi types.

• Semi-erect growth habit was most prevalent among accessions across three seed types whereas plant pigmentation, flower colour, seed color, seed shape, minute black seed dots and seed surface differed within three seed types. Pink flower color among desi accessions, white flower color in kabuli, both white and light

pink in pea type were the most prevalent characters among three seed types. Pink flower color in desi, white flower in kabuli is the characteristics of chickpea seed types.

• In the entire reference set low-anthocyanin was dominant over no and high anthocyanin. Most of the desi accessions were with low anthocyanin plant pigmentation, whereas kabuli types were with no-anthocyanin, and no-anthocyanin and low-anthocyanin was observed among pea type. Only 2% of the accessions were with high-anthocyanin pigmentation in the entire reference set. Desi accessions predominated with yellow brown and kabuli with beige seed color. Angular or ram's head seed shape, which is the characteristic of desi type, dominated reference set followed by owl's head shape and intermediate or pea shaped.

• Minute black dots were present on the seed testa of most desi accessions while accessions had no dots or totally absent in kabuli type whereas in pea type seeds were with dots and without dots. Among desi type accessions, seeds were with rough and tuberculated surface while kabuli type were with smooth and rough seed surface and in pea types, smooth and rough seed surface were observed.

• Among the qualitative traits relatively high polymorphism was observed for seed colour followed by seed surface indicating greater importance of these two traits in phenotypic diversity assessment.

•The Shannon-Weaver diversity indices (H') estimates were computed for seven qualitative traits. Seed color showed the maximum H' value in chickpea reference set, followed by seed shape, seed surface, plant color, growth habit and flower color. However, dots on seed coat showed lowest H` in the entire reference set indicating the importance of these qualitative traits in contributing towards diversity in reference set and in the three seed types

# **6.1.2.** Quantitative traits

• REML analysis of data indicated that variance components due to genotype ( $\sigma^2$  g) and genotype × environment ( $\sigma^2$  ge) interaction were significant for all quantitative traits except tertiary branches and pods per plant. This indicated sufficient variability for all the traits in reference set and differential response of the genotypes to different environments.

• The wider range was observed for various traits in different environments and

among different seed types of the chickpea reference set

• High genetic advance as per cent of mean coupled with high estimates of broad sense heritability ( $h^2_{b}$ ) (>60%) were observed in all five environments separately and overall in pooled data indicating that the variation for most traits was heritable and selection would be effective for improvement of these traits.

• Mean days to 50 percent flowering, flowering duration, days to grain filling, 100-seed weight, and plant width did not differ significantly between five environments indicating less influence of the environment on the expression of these traits. However, plant height, days to maturity, pods per plant, seeds per pod, yield per plant, per day productivity ( kg ha<sup>-1</sup> day<sup>-1</sup>), and grain yield (kg ha<sup>-1</sup>) significantly between the environments indicating the greater influence of the environments on the expression of these traits.

• Mean productivity per day, plot yield, yield per plant, pods per plant, days to maturity and basal primary branches were highest in E2 as compared to E1, E3, E4 and E5. Apical primary branches and plant height in E3 and 100-seed weight in E1 showed the highest mean performance.

• Variances were significant for most of the quantitative traits except for days to 50% flowering, flowering duration, days to grain filling, days to maturity and seeds per pod in E1, pods per plant and yield per plant in E2, plant height in E3 and plot yield and productivity when pooled indicating that the reference set had adequate genetic variation for most of the traits.

• Grain yield per plot (Kg ha<sup>-1</sup>) was highly significant and positively correlated with days to grain filling, apical primary branches, basal secondary branches, apical secondary branches, seeds per pod, and pods per plant and yield per plant. It could be inferred that, the selection in positive direction for all the traits (plant height, plant width and number of branches of five plants, single plant yield would reset in correlated response for grain yield per plot (Kg ha<sup>-1</sup>)) for genetic enhancement of grain yield.

• Days to 50 percent flowering, grain yield, days to maturity, per day productivity and apical primary branches in all environments had maximum H` indicating evenness and richness of alleles for these traits, followed by days to grain filling, flowering duration, yield per plant, apical secondary branches, grain yield, basal primary branches, per day productivity, basal secondary branches, days to maturity, plant width and tertiary branches, pod per plant and apical primary branches, seeds per pod, days to flowering, 100-seed weight and plant height. This indicated the importance of these characters in contributing towards divergence.

• Tertiary branches, flowering duration and seeds per pod showed low mean H` in all environments followed by apical primary branches, flowering duration, apical secondary branches and yield per plant in all environments.

• Days to 50 percent flowering, days to maturity, days to grain filling, flowering duration, apical and basal secondary branches, tertiary branches, pods per plant. 100-seed weight, seeds per pod, yield per plant, per day productivity, plot yield occurred in the first three PCs in all five environments separately, indicated their importance for characterization in chickpea germplasm accessions.

• Ten pairs of most diverse accessions were identified based on phenotypic distance for each environment separately and for pooled data of five environments. These diverse accessions could be utilized in development of mapping population and in hybridization program to generate the segregating population for the selection of superior lines.

• The clustering of reference set accessions using scores of first three Principal components (PCs) corresponded well with chickpea regional classification.

# 6.1.3. Drought related traits

**a.** The chickpea reference set along with five check cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) was evaluated to estimate the variation of SPAD Chlorophyll Meter Readings (SCMR) in (E3) and (E5) at ICRISAT, Patancheru, Andhra Pradesh.

There was a significant difference in SCMR among the entries, while 12 accessions showed superior and consistent SCMR in the entire reference set.
b. Cultivated 293 diverse reference set accessions (excluding wild accessions from 300 accessions of chickpea reference set) along with 6 control cultivars (ICC 4958, Annigeri, ICCV 10, G 130, L 550, KAK 2,) were evaluated for drought tolerance related root traits during two consecutive post rainy seasons (E2,E3) at ICRISAT, Patancheru.

• The REML analysis of data for individual environment revealed significant genotypic variance for all traits in two (E2, E3) environments and when pooled. Among reference set, 13 accessions were with deepest root system, 42 were with

superior shoot dry weight, 40 with high root dry weight, 11 with high root to total plant dry weight ratio (R-T%), 33 accessions with high root length, 6 accessions for root length density.

# 6.1.4. Pod borer resistant related traits

The chickpea reference set along with 7 control cultivars (Annigeri, G 130, KAK 2, ICC 506EB-resistant, ICC 3137-susceptible, ICCV 10-moderately resistant, L 550-susceptible) were planted in Randomized Complete Block Design (RCBD) during two consecutive post rainy seasons (2007-08 (E2), 2008-09 (E3)) at ICRISAT, Patancheru.

• At vegetative stage in post rainy environments (E2 and E3), significant  $\sigma^2 g$  in individual environments,  $\sigma^2 g$  and  $\sigma^2 g e$  in pooled analysis was observed for all the three pod borer resistant traits. Accessions twenty five with minimum damage rate to pod borer, 17 with lowest larval survival percentage, 3 accessions with minimum unit larval weights were observed in chickpea reference set.

#### 6.1.5. Quality traits

**a.** The chickpea reference set along with five check cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) were used to estimate protein content by Atomic Spectra Photometric Meter (ASPM) in four seasons 2006/2007 (E1), 2007/2008 (E2), 2008/2009 (E3) post rainy normal sown conditions, 2008/2009 (E5) winter seasons, late sown conditions at ICRISAT, Patancheru, Andhra Pradesh.

The mean protein content was 21.07% in E2, 20.47% in E1, 19.45% in E3 and 21.79% in E5. Thirty eight accessions (30.3-26.6%) with high protein content were identified in the entire reference set from all environments and when pooled.
b. The chickpea reference set along with five control cultivars (Annigeri, ICCV 10, KAK 2, L 550, G130) was used to estimate anthocyanin content by using High Performance Liquid Chromatography (HPLC) at ICRISAT, Patancheru, Andhra Pradesh.

• The mean anthocyanin content was 1.55 A550g<sup>-1</sup> for anthocyanins extracted with acidified methanol and 0.38 A550g<sup>-1</sup> for anthocyanins extracted with methanol. Forty accessions with high anthocyanin content were observed in the entire reference set.

• The trait-specific sources for 19 economically important traits, such as protein, anthocyanin content, pod borer resistant, drought and yield traits mainly (15

accessions for each trait) namely early flowering, seeds per pod, pods per plant, yield per plant, 100-seed weight, plot yield, per day productivity, heat tolerant, high root depth, shoot dry weight, root dry weight, root to total plant dry weight ratio (R-T%), root length, root length density, minimum damage rate to pod borer, lowest larval survival%, unit larval weights, high protein and high anthocyanin content. were identified. Multi-trait specific accessions, which were sources for more than one trait, were identified.

• Finally, 2 accessions for early flowering, 17 for more seeds per pod, 35 for more pods per plant, one with more yield per plant, 19 with high 100-seed weight, 119 for high plot yield, 89 for per day productivity, 20 heat tolerant, 13 with high root depth, 42 with high shoot dry weight, 40 with high root dry weight, 11 with high root to total plant dry weight ratio (R-T%), 33 accessions with high root length, 6 accessions for root length density, 25 with minimum damage rate to pod borer, 17 with lowest larval survival%, 3 accessions with minimum unit larval weights, 38 with high protein and 40 accessions with high anthocyanin content were identified for specific traits.

• Extensive evaluation of these accessions in different locations may be useful to reconfirm their genetic worth and use in crop improvement.

# **6.2. MOLECULAR DIVERSITY**

A total of 100 SSR markers were used initially to genotype chickpea reference set. Of these, 91 SSR markers produced clear, scorable and polymorphic marker profiles and were used for further analysis.

# 6.2.1. Allelic richness and genetic diversity

• The SSR markers used in this study were highly polymorphic and informative, and detected a total of 2411 alleles with an average of 26.45 alleles per locus. A total of 2299 alleles were detected in cultivated types and 433 alleles in wild types of chickpea reference set, of which 1980 were unique in cultivated, 114 in wild accessions and 319 alleles were common among wild and cultivated. In the cultivated group, desi accessions contained the largest number of unique alleles (864) followed by kabuli (836) and pea type (52).

• In reference set 2424 rare alleles were observed ranging from 2.0 to 90.0. The markers TS5 (90 alleles), TR1 (82 alleles), TR43 (76 alleles), TR7 (74 alleles) showed high number of rare alleles, whereas markers GAA43, TAA57 (each 2

rare alleles) showed low number of rare alleles.

• Common alleles ranged from 0-576 with a mean of 374. TA80 (576) showed high number of common alleles. Frequent alleles ranged from 0-570 with a mean of 129.5. CaSTMS 20 (570) showed highest number of frequent alleles.

• The unweighted neighbor-joining tree based on simple matching dissimilarity matrix of 300 accessions of chickpea reference set highlighted broadly four clusters which corresponded well with the classification based on three seed types of chickpea

• Finally, ten pairs of most diverse accessions were identified based on dissimilarity matrix using molecular data indicating the presence of greater genetic diversity in chickpea reference set.

# **6.2.2.** Population structure analyses

• The STRUCTURE analysis provided evidence for the presence of population structure and identified 13 subpopulations (SP1 to SP13). Further, consistency of this population structure was assessed by principal coordinate and un-weighted neighbor joining phylogenetic analysis, which showed consistent relationship with population structure identified by STRUCTURE analysis.

•The general linear model (GLM) was implemented in TASSEL v2.1 and used to find marker traits associations (MTAs) associated with the qualitative, quantitative and grain quality traits, resistance to pod borer and for traits related to drought tolerance in a structured chickpea reference set. The MTAs detected using pooled BLUPs of all environments was considered as final MTAs, since it represents the average performance of the accessions over the all the environments.

# 6.2.3. Association of markers in reference set with phenotypic traits

• Among qualitative traits, a total of 21 SSR markers showed 27 MTAs (P≤0.001)

of which 17 SSR markers were associated with one qualitative trait and 4 SSR markers were associated with more than one trait. Of which major MTAs (>20% phenotypic variation) detected were five (two for growth habit and three for seed surface).

• 64 ( $P \le 0.001$ ) significant MTAs were detected involving 49 SSR markers in E1, with maximum phenotypic diversity of 43.4% for anthocyanin content. Similarly 86 significant MTAs were detected involving 46 SSR markers in E2 and

maximum phenotypic diversity of 42% for tertiary branches whereas in E3, 76 significant MTAs with 50 SSR markers and maximum phenotypic diversity of 42.9% for leaf area, in E4 74 significant MTAs with 52 SSR markers and maximum phenotypic diversity of 45.4% for apical secondary branches and in E5 56 significant MTAs with 44 SSR markers and maximum phenotypic diversity of 34.8% for plant width.

• Using pooled BLUPs of all environments, a total of 76 MTAs ( $P \le 0.001$ ) of which flowering duration showed highest maximum number of MTAs (14) whereas apical primary branches and seeds per pod (1) showed lowest number of MTAs and major MTAs (>20% phenotypic variation) detected were 39. Maximum phenotypic variation was observed for tertiary branches (37.4%) and minimum was observed for per day productivity (4.13%).

• Only one MTA was detected using GLM for protein content ( $P \le 0.001$ ) on chromosome 13(GA26) applying 11.04% phenotypic variation.

• Two significant MTAs were detected (P $\leq$ 0.001) with only one trait (Damage rating %) related to Helicoverpa resistance at P $\leq$ 0.001. No MTAs were detected for Leaf damage score and larval survival percentage. Two MTAs were distributed on chromosomes, 3(CaSTMS23) and 4(TA132), and phenotypic variation was 7.09 and 19.63 % respectively for these two markers.

• Only one significant MTAs were detected ( $P \le 0.001$ ) for SCMR, distributed on chromosome 7 (TAA 59) and one more for SLA and is distributed on chromosome 13 (TS83) and phenotypic variation was observed to be 16.95 and 18.32 % respectively for both traits using GLM

• Numbers of significant MTAs detected were 21 ( $P \le 0.001$ ) for drought related root traits and maximum numbers of MTAs (7) were detected for shoot dry weight and total dry weight. Minimum numbers of MTAs were detected for root surface area and root volume (1 each). Maximum phenotypic variation was expected by MTAs for root length density (30%) with TAA59 on chromosome 7 and minimum was for total plant dry weight ratio (7.9%) with CaSTMS 9.

• Eight major MTAs (>20% phenotypic variation) were detected for drought related root traits, of these one each was detected for shoot dry weight and root volume and two each for root dry weight, total dry weight and root length density. Maximum phenotypic variation expected was for root length density (30%) for the

marker TAA59. TA25 and TA22 detected maximum of 3 major MTAs each among the 8 major significant root traits

• Of the MTAs in pooled data (P $\leq$ 0.001), 27 for qualitative, 76 for quantitative, 2 for pod borer related traits, 1 for protein related traits, 5 for SPAD and 21 for drought related traits were identified as stable and highly significant. Seven for qualitative, 39 for quantitative, 1 for SPAD and 8 for drought related traits were identified as the major MTAs that expected >20% phenotypic variation across all the environments in chickpea reference set

In summary, the chickpea reference set is genetically diverse and possesses potential variation for economic traits and hence could be extensively evaluated for greater exploitation for use in breeding programs. The superior trait specific accessions identified could be utilized in breeding programs to improve traits and to widen the genetic base of chickpea cultivars. Marker trait associations identified in this study using SSR markers and association mapping approach the first effort in this crop, and will provide important information to the research community for further QTL identification, to identify candidate genes and gene cloning that underlie QTLs in chickpea.

Figure: 1 Geographical distribution of 300 chickpea reference set accessions

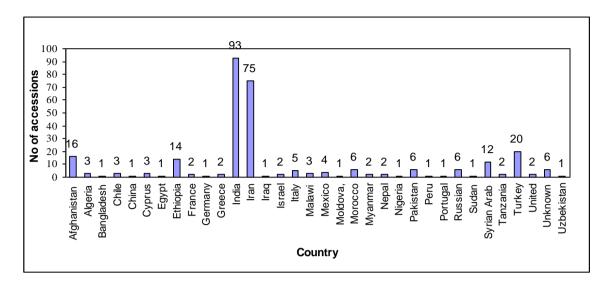


Figure: 2 Number of accessions in different seed types of the chickpea reference set

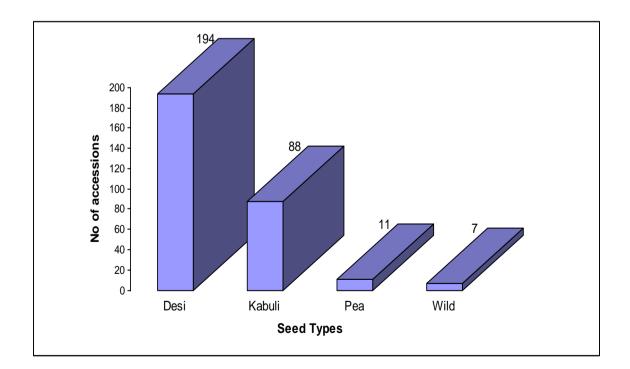


Figure: 3 Heritability, genotypic (GCV) and phenotypic coefficient of variance (PCV) in the chickpea reference set for 17 quantitative traits based on pooled BLUPs of five environments

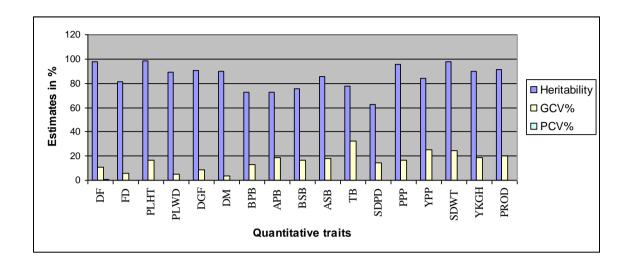


Figure: 4 Frequency distribution of accessions for various qualitative traits in the chickpea reference set

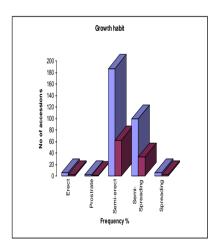


Figure 4a: Growth Habit

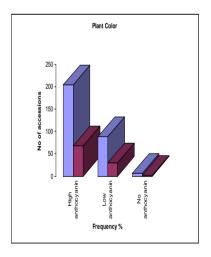
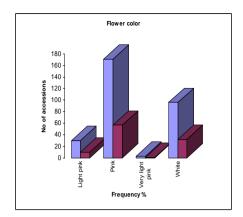


Figure 4b: Plant pigmentation



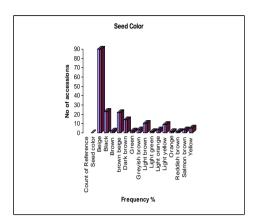
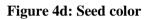


Figure 4c: Flower color



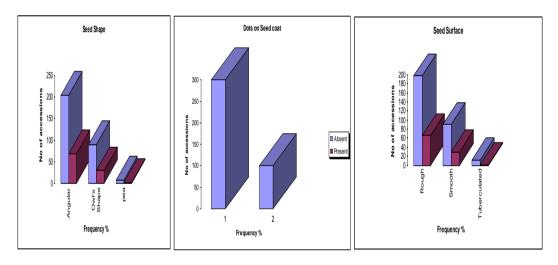


Figure 4e: Seed shape surface

Figure 4f: Seed dots

Figure 4g: Seed

Figure 5: Scatter plot of first two principal components (PCs) of the chickpea reference set accessions using pooled BLUPs of five environments for yield contributing traits

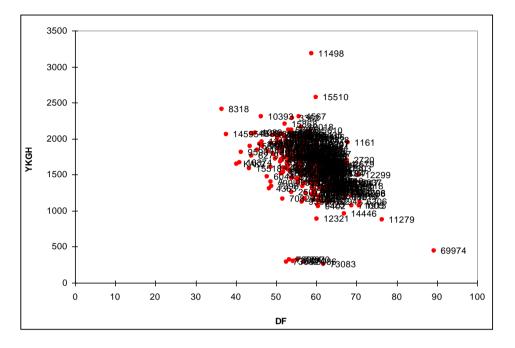


Figure 5a: Days to 50% flowering (DF) vs. plot yield (YKGH)

Figure 5b: Days to maturity (DM) vs. Plot yield (YKGH)

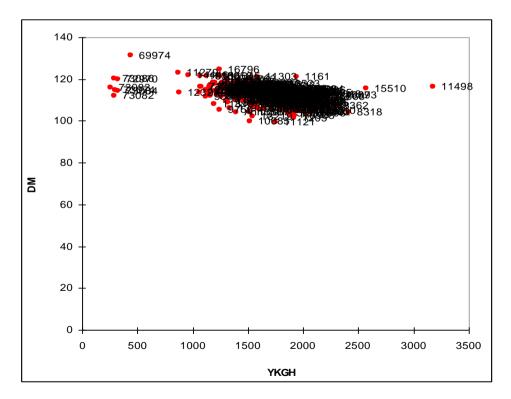
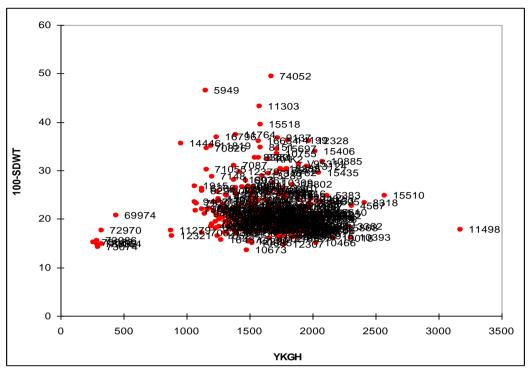
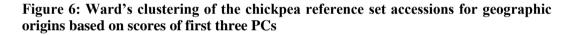


Figure 5c: 100 seed weight vs. Plot yield (YKGH)



100 sdwt = 100 seed weight, YKGH = plot yield



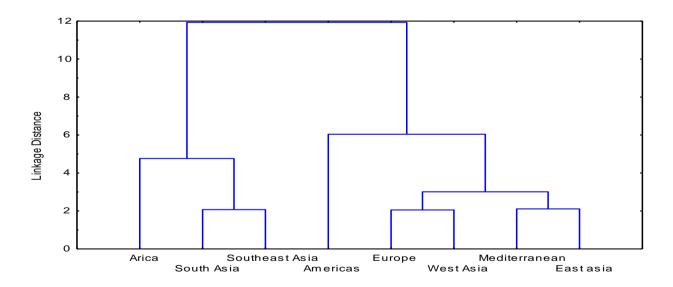
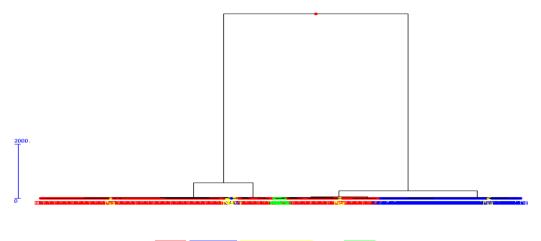


Figure 7: Dendrogram based on 7 qualitative traits of the chickpea reference set accessions based on different seed types (Desi, Kabuli, Pea Shaped and Wild)



Chickpea reference set (Desi, Kabuli, Pea Shaped and Wild)

Figure 8: Distribution of number of alleles per locus among 91 SSR markers used for genotyping the chickpea reference set

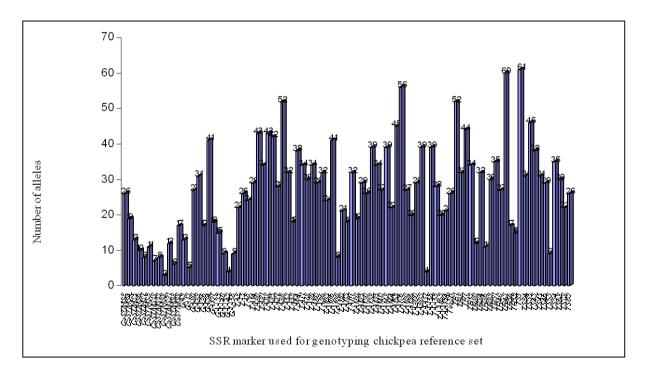
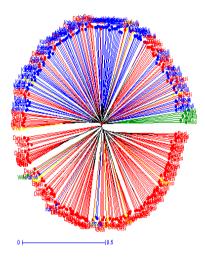
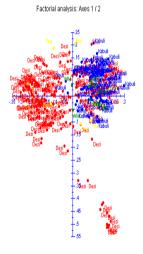


Figure 9a: Unweighted neighbor-joining tree based on the simple matching dissimilarity matrix of 91 SSR markers genotyped across the chickpea reference set



Chickpea reference set (Desi, Kabuli, Pea Shaped and Wild)

Figure 9b: Factorial analysis based on the simple matching dissimilarity matrix of 91 SSR markers genotyped across the chickpea reference set



Chickpea reference set (<mark>Desi, Kabuli, Pea Shaped</mark> and <mark>Wild</mark>)

Figure 10: Rate of change in Ln P(D) between successive K (K averaged over the five run) in the chickpea reference set accessions

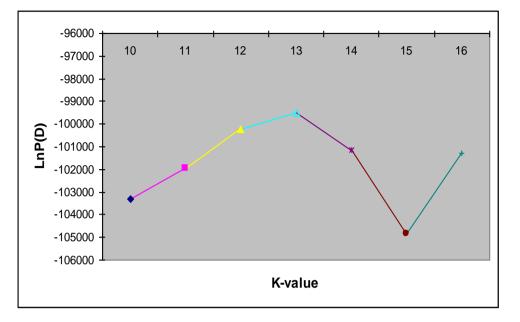


Figure 11a: Population structure of the chickpea reference set based on 91 SSR markers (k=13) revealed by STRUCTURE analysis (Bar plot in single lines)

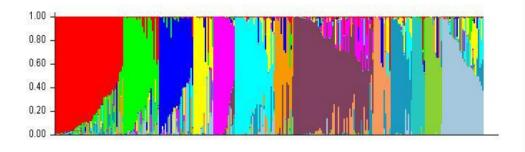


Figure 11b: Population structure of the chickpea reference set based on 91 SSR markers (k=13) revealed by STRUCTURE analysis (Bar plot in multiple lines)

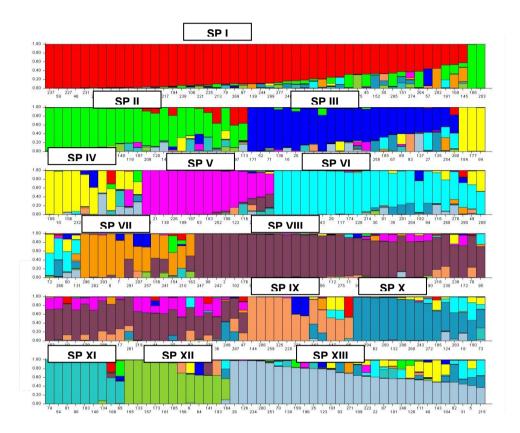
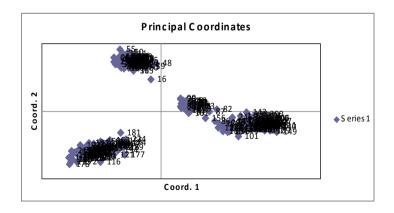


Figure 12: Principal coordinates analysis (PCoA) of the chickpea reference set accessions using 91 SSR markers based on Nei (1973) distance estimates.



1. Field Evaluation of the Chickpea Reference set at ICRISAT, Patancheru, India



2. Field Evaluation of the Chickpea Reference set at UAS, Dharwad, India



3. Diversity in Chickpea Germplasm at ICRISAT, Patancheru, India



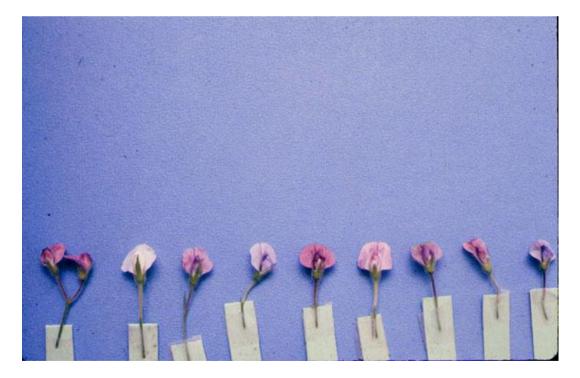
4. Diversity for Foliage Color in Chickpea Reference set



5. Diversity for Leaf and Stem Type and Shape in Chickpea Reference set



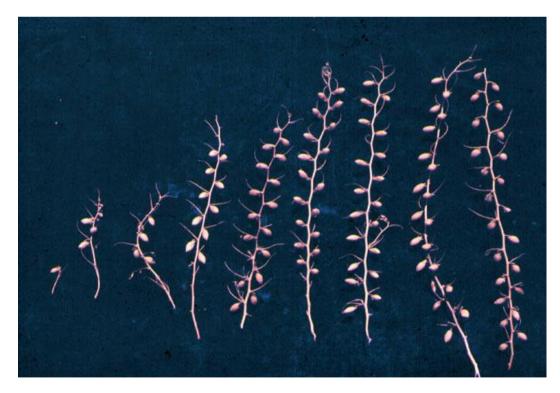
6. Diversity for Flower Shape and Color in Chickpea Reference set



7. Diversity for Pod Shape and Color in Chickpea Reference set



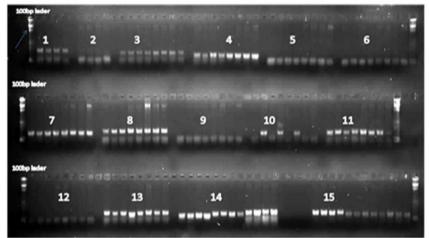
8. Diversity for Pod Number in Chickpea Reference set



9. Diversity for Seed Shape and Color in Chickpea Reference set

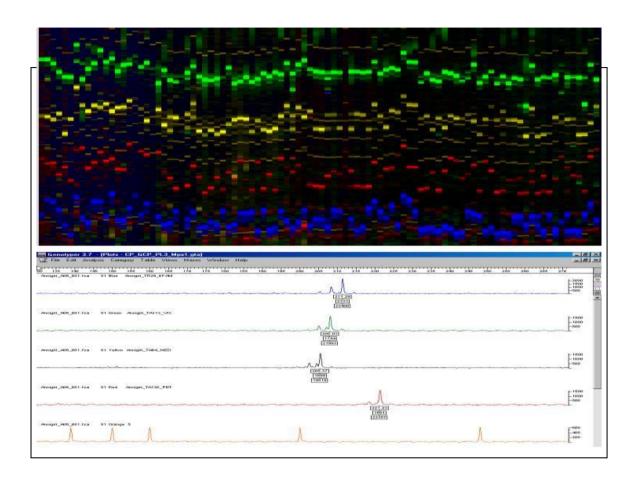


10. PCR products tested for amplification on 1.2 per cent agarose gel in Chickpea Reference set



<sup>(</sup>Numbers on gel represent the list of primesr checked)

11. Allele sizing of the data obtained from ABI 3730xl genetic analyzer using Genotyper software version 4.0 (Applied Biosystems, USA) in Chickpea Reference set



**12.** Pod borer screening of the chickpea reference set accessions- Detached leaf bioassay



**13.** Phenotyping of the chickpea reference set for drought tolerance using PVC cylinder technique



14. Chickpea reference set accessions showing diversity in root lengths



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Appendices

S.No	ICC	SDSH	FLCL	PLCL	SDCL	GH	DOT	SS
1	10018	1	2	2	17	4	2	1
2	10341	3	4	3	1	4	1	2
3	10393	1	2	2	17	3	2	1
4	10399	1	2	2	17	4	2	1
5	10466	2	4	3	1	4	1	2
6	1052	1	2	2	2	3	1	1
7	10673	1	2	2	5	3	2	1
8	10685	1	2	2	5	3	2	1
9	10755	2	4	3	1	3	1	2
10	1083	1	2	2	17	4	2	1
11	10885	2	4	3	1	3	1	2
12	10939	1	2	2	5	4	2	3
13	10945	1	2	2	8	3	2	1
14	1098	1	2	2	17	3	2	1
15	11121	1	2	2	17	3	2	1
16	11198	1	2	2	17	3	2	1
17	11279	1	2	2	8	3	2	1
18	11284	1	4	3	15	3	1	1
19	11303	2	4	3	1	3	1	1
20	11378	1	2	2	17	3	2	1
21	11498	1	2	2	17	3	2	1
22	11584	1	2	2	8	4	2	1
23	1161	1	4	3	15	4	1	1
24	11627	1	2	2	17	3	2	1
25	1164	1	4	3	10	3	1	1
26	11664	1	2	2	17	3	2	1
27	11764	2	4	3	1	3	1	2
28	1180	1	2	2	2	4	1	1
29	11819	2	4	3	1	3	1	2
30	11879	2	4	3	1	3	1	2
31	11903	1	1	3	4	3	2	1
32	1194	1	2	2	17	3	2	1
33	11944	1	2	2	17	3	2	1
34	12028	1	1	3	4	3	1	1
35	12037	2	4	3	1	3	1	2
36	1205	1	2	2	17	3	2	1
37	12155	1	2	2	17	3	2	1
38	12299	1	2	2	17	4	2	1
39	1230	1	2	2	17	4	2	1
40	12307	1	2	2	17	3	2	1
41	12321	1	1	3	16	3	2	1
42	12324	2	4	3	1	3	1	2
43	12328	2	4	3	1	3	1	2
44	12379	1	2	2	17	3	2	1
45	12492	2	4	3	1	3	1	2
46	12537	1	2	2	2	3	1	1

Appendix 1: Scores of seven qualitative traits for 300 accessions in chickpea reference set

47	12654	1	2	2	11	3	2	1
48	12726	1	2	3	17	4	2	1
49	12824	1	2	1	17	3	2	1
50	12851	1	2	2	2	4	1	1
51	12866	1	2	2	17	3	2	1
52	12916	1	2	2	17	3	2	1
53	12928	1	2	2	11	3	2	1
54	12947	1	2	2	17	3	2	1
55	13077	2	4	3	1	3	1	2
56	13124	1	2	2	17	4	2	1
57	13187	2	4	3	1	3	1	2
58	13219	1	2	2	17	3	2	3
59	13283	2	4	3	1	3	1	2
60	13357	2	4	3	1	3	1	2
61	13441	2	4	3	1	3	1	2
62	13461	2	4	3	1	3	1	2
63	13523	2	4	3	1	3	1	2
64	13524	1	2	2	2	3	1	1
65	1356	1	2	2	17	3	2	1
66	13599	1	1	3	4	3	1	1
67	13628	2	4	3	1	4	1	2
68	13719	2	4	3	1	3	1	2
69	13764	2	4	3	1	3	1	2
70	13816	2	4	3	1	3	1	2
71	13863	1	3	3	17	4	1	1
72	13892	1	2	1	17	3	2	1
73	1392	1	2	2	17	3	2	1
74	1397	1	2	2	17	3	2	1
75	1398	1	2	2	17	3	2	1
76	14051	1	2	2	17	3	2	1
77	14077	1	2	1	17	3	2	1
78	14098	1	2	2	17	3	2	1
79	14199	2	4	3	1	3	1	2
80	1422	1	2	2	17	3	2	1
81	1431	1	2	2	17	3	2	1
82	14402	1	2	2	17	4	1	1
83	14446	2	4	3	1	1	1	2
84	14595	1	2	3	17	4	2	1
85	14669	1	2	2	17	4	2	3
86	14778	1	2	2	5	4	2	1
87	14799	1	2	2	8	4	2	1
88	14815	1	2	2	17	4	2	1
89	14831	1	2	2	17	4	2	1
90	1510	1	2	2	17	4	2	1
91	15248	1	1	3	11	3	2	1
92	15240	2	4	3	1	4	1	1
93	15294	1	1	3	4	3	2	1
93	15234	2	4	3	1	3	1	2
94	15555	2	4	3	1	3	1	2
		2	4					2
96	15435			3	1	3	1	
97	15510	1	2	2	17	3	2	1

00	15510	2		2		2		
98	15518	2	4	3	1	3	1	2
99	15567	1	2	2	8	3	2	1
100	15606	1	2	2	17	4	2	1
101	15610	1	2	2	17	3	2	1
102	15612	1	2	2	8	4	2	1
103	15614	1	2	2	17	4	2	1
104	15618	1	2	2	17	4	2	1
105	15697	2	4	3	1	4	1	2
106	15762	1	2	2	4	3	2	1
107	15785	1	5	3	17	4	2	1
108	15802	2	4	3	1	3	1	2
109	15868	1	2	2	17	4	2	1
110	15888	3	4	3	12	4	1	1
111	16207	1	2	2	17	4	2	1
112	16261	1	2	2	15	4	2	1
113	16269	1	2	2	15	4	2	1
114	16374	1	2	2	17	3	2	1
115	16487	1	2	2	11	4	2	1
116	16524	1	2	2	8	3	2	1
117	16654	2	4	3	1	3	1	2
118	16796	2	4	3	1	1	1	2
119	16903	1	2	2	17	4	2	3
120	16915	1	2	2	17	4	2	3
121	1710	1	2	2	17	3	2	1
122	1715	1	2	2	17	4	2	1
123	1882	1	2	2	5	4	2	1
124	1915	1	2	2	2	3	1	1
125	1923	1	2	2	17	3	2	1
126	2065	1	2	2	17	3	2	1
120	2072	1	2	2	17	4	2	1
127	2210	1	2	2	17	3	2	1
120	2242	1	2	2	17	3	2	1
130	2263	1	2	2	17	4	2	1
130	2203	2	4	3	1	4	1	2
131	2482	2	4	3	1	3	1	2
132	2482	1	2	2	1	3	1	1
133	2580	1	2	2	8	3	2	1
134	2580	2	4	3	8	3	1	2
			2	2		4	2	
136	2629	1			8			1
137	2679	1	1	3	11	3	1	1
138	2720	1	2	2	17	3	2	1
139	2737	1	2	2	2	4	1	1
140	283	1	2	2	17	4	2	1
141	2884	1	2	2	2	3	1	1
142	2919	1	1	3	4	3	1	1
143	2969	1	2	2	17	3	1	1
144	2990	1	1	3	4	3	1	1
145	3218	1	1	3	16	3	1	1
146	3230	1	2	2	6	3	2	1
147	3239	1	1	3	4	4	1	1
148	3325	1	2	2	17	4	2	1

					r	[		r
149	3362	1	2	2	17	4	2	1
150	3391	1	1	3	4	3	1	1
151	3410	2	4	3	1	3	1	1
152	3421	2	4	3	1	3	1	2
153	3512	1	2	2	17	3	2	1
154	3582	1	2	2	5	3	2	1
155	3631	1	2	2	2	3	1	1
156	3761	1	2	2	2	3	1	1
157	3776	1	2	1	2	3	1	1
158	3892	1	2	2	2	3	1	1
159	3946	1	2	2	2	4	1	1
160	4093	1	2	2	5	3	2	1
161	4182	1	2	2	2	3	1	1
162	4363	1	2	2	2	3	1	1
163	440	1	2	2	17	4	2	1
164	4418	1	2	2	2	3	1	1
165	4463	1	2	2	2	4	1	1
166	4495	1	2	2	17	3	2	1
167	4533	1	2	2	17	4	2	1
168	456	1	2	2	17	4	2	1
169	4567	1	2	2	17	3	2	1
170	4593	1	2	2	17	3	2	1
171	4639	1	2	2	17	3	2	1
172	4657	1	2	2	17	4	2	1
173	4814	1	2	2	2	4	1	1
174	4841	2	4	3	1	3	1	2
175	4853	2	4	3	1	3	1	2
176	4872	3	2	1	13	4	2	1
177	4918	1	2	2	17	4	2	1
178	4991	1	2	2	15	4	2	1
179	506	1	2	2	17	4	2	1
180	5135	1	2	2	17	3	2	1
180	5221	1	2	2	9	4	1	1
182	5337	2	4	3	1	3	1	2
183	5383	1	2	2	17	3	2	1
184	5434	1	2	2	17	2	2	1
185	5504	1	2	2	11	3	2	1
185	5613	1	2	2	6	3	2	1
180	5639	1	2	2	17	4	2	1
187	5845	1	2	2	17	3	2	1
189	5878	1	3	2	2	4	1	1
190	5879	3	4	3	10	4	1	1
190	6263	2	4	3	10	3	1	2
191	6279	1	2	1	17	4	1	1
192	6293	1	2	2	2	4	1	1
193	6293	1	1	3	4	4	1	1
194	6306	1	2	2	2	3	1	1
		1				4		
196 197	637 6537		2	2	5	3	2	1
	6537	1			17		2	1
198	6571	1	2	2	17	3	2	1
199	6579	1	2	2	17	3	2	1

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200	67	1	2	2	17	3	2	1
201	6802	1	2	2	11	3	2	1
202	6811	1	2	2	17	3	2	1
203	6816	1	2	2	17	3	2	1
204	6874	1	2	2	17	3	2	1
205	6875	1	1	3	4	3	1	1
206	6877	1	1	3	4	3	1	1
207	7052	1	2	2	2	3	1	1
208	708	1	2	2	5	4	2	1
209	7150	1	1	3	4	3	1	1
210	7184	1	2	2	5	3	1	1
211	7255	2	4	3	1	4	1	2
212	7272	2	4	3	1	3	1	2
213	7305	1	1	3	10	3	1	1
214	7308	2	4	3	1	3	1	2
215	7315	2	4	3	1	3	1	2
216	7323	3	4	3	11	1	1	2
217	7326	1	2	2	11	3	2	1
218	7413	3	1	3	3	3	1	1
219	7441	1	2	2	17	4	1	1
220	7554	1	1	3	4	4	1	1
221	7571	2	4	3	1	3	1	2
222	762	1	2	2	17	4	2	1
222	7668	2	4	3	1	3	1	2
223	7819	1	1	3	4	3	1	1
224	7867	1	1	3	4	3	1	1
225	791	1	2	2	17	4	2	1
220	8058	2	4	3	1	3	1	2
228	8151	2	4	3	1	3	1	2
229	8195	1	2	2	5	3	1	1
229	8195	1	2	2	17	3	2	1
			4	3	1	3	1	2
231	8261 8318	2	2	3	17	4	2	1
		-		-			_	-
233	8350	3	2	2	17	4	1 2	1
234	8384				17			1
235	8515	1	2	3	4	3	2	1
236	8521	1	2	3	16	1	2	1
237	8522	1	2	2	2	3	1	1
238	8607	1	2	2	17	4	2	1
239	8621	1	2	2	17	3	2	1
240	867	1	2	2	8	4	2	1
241	8718	1	2	2	17	4	2	1
242	8740	2	4	3	1	4	1	2
243	8752	2	4	3	1	4	1	2
244	8855	2	4	3	1	4	1	2
245	8950	1	2	2	17	4	2	1
246	9002	1	2	2	17	3	2	1
247	9137	2	4	3	1	3	1	2
248	9402	2	4	3	1	3	1	2
249	9418	2	4	3	1	4	1	2
250	9434	2	4	3	1	3	1	2

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251	95	1	2	2	17	4	2	1
252	9586	1	2	2	17	3	2	1
253	9590	1	3	3	17	3	2	1
254	9636	1	1	3	17	3	1	1
255	9643	1	1	3	4	3	1	1
256	9702	1	1	3	4	4	1	1
257	9712	1	1	3	4	3	1	1
258	9755	1	1	3	4	3	2	1
259	9848	3	1	3	4	3	1	2
260	9862	3	1	3	14	3	1	2
261	9872	2	1	3	14	3	1	2
262	9895	3	1	3	14	3	1	2
263	9942	1	2	2	17	4	2	1
264	20267	2	4	3	1	4	1	2
265	18828	2	4	3	1	3	1	2
266	18836	2	4	3	1	3	1	2
267	18839	2	4	3	1	3	1	2
268	18847	2	4	3	1	3	1	2
269	18858	2	4	3	1	3	1	2
270	18884	2	4	3	1	3	1	2
271	18679	2	4	3	1	3	1	2
272	20266	2	4	3	1	3	1	2
272	20262	2	4	3	1	3	1	2
273	20265	2	4	3	1	4	1	2
274	20263	2	4	3	1	3	1	2
275	20263	2	4	3	1	3	1	2
277	20259	2	4	3	1	3	1	2
278	18699	2	4	3	1	4	1	2
279	18720	2	4	3	1	3	1	1
280	18912	2	4	3	1	3	1	2
281	19034	2	4	3	1	1	1	2
282	20174	1	2	2	5	4	1	3
283	18983	2	4	3	1	3	1	2
284	20264	2	4	3	1	4	1	2
285	19226	2	4	3	1	4	1	2
286	19011	2	4	3	1	5	1	2
287	18724	2	4	3	1	4	1	2
288	19095	2	4	3	1	5	1	2
289	19100	2	4	3	1	5	1	2
290	20260	2	4	3	1	4	1	2
291	20183	1	2	2	3	5	2	3
292	20190	1	2	2	5	4	1	3
293	20192	1	2	3	5	5	1	3
294	20193	1	2	2	7	5	2	3
295	20194	1	2	2	7	4	2	3
296	20195	1	2	2	7	4	2	3
297	19122	2	4	3	1	1	1	2
298	19147	2	4	3	1	3	1	2
299	19164	3	4	3	1	3	1	2
300	19165	2	4	3	1	3	1	2
Control cultivars								

301	4918_C	1	2	2	8	4	2	1
302	4948_C	1	2	2	17	3	2	1
303	15996_C	1	2	2	8	3	2	1
304	V92311_C	2	4	3	1	4	1	2
305	4973_C	2	4	3	1	3	1	2

SDSH = Seed Shape, FLCL= Flower colour, PLCL= Plant colour, SDCL= Seed colour, GH = Growth habit, DOT= Dots on seed coat, SS= Seed surface

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
10018	56.31	27.86	38.27	61.87	56.49	112.8	3.854	3.436	4.377	5.188	2.138	1.284	75.69	10.87	16.02	2165	19.09
10341	58.8	28.88	46.07	68.43	53.3	112.1	2.888	2.226	2.794	4.347	1.484	1.117	42.09	8.77	22.12	1646	14.66
10393	46.33	30.25	40.08	59.67	65.87	112.2	2.958	2.837	3.102	4.069	1.505	1.309	67.44	9.3	16.18	2307	20.54
10399	49.42	29.38	38.85	60.65	60.68	110.1	2.433	2.726	3.214	4.083	1.465	1.152	77.08	12.78	16.53	1780	16.09
10466	54.1	26.69	38.99	60.83	55.1	109.2	3.871	2.711	2.627	4.152	1.608	1.392	54.84	9.03	15.03	2028	18.56
1052	59.71	28.25	41.03	61.66	54.09	113.8	3.25	2.683	2.493	4.67	1.595	1.36	36.58	7.49	16.43	1249	10.86
10673	59.45	27.67	41.54	62.51	54.35	113.8	2.467	2.448	3.208	3.945	1.205	1.501	54.76	9.23	13.57	1475	12.88
10685	56.38	28.77	43.31	62.87	43.42	99.8	2.608	2.398	2.118	3.979	1.195	1.247	50.74	8.26	15.08	1519	15.33
10755	57.22	29.08	49.43	64.45	58.28	115.5	2.748	1.796	5.332	5.254	1.524	1.125	47.68	11.69	33.4	1717	14.83
1083	44.77	29.2	39.32	60.54	59.83	104.6	2.903	2.7	3.751	5.071	1.565	1.103	52.3	9.69	19.3	2076	19.81
10885	54.11	29.53	45.45	65.37	59.49	113.6	2.449	2.7	3.183	4.559	2.081	1.081	48.43	11.55	31.77	2083	18.27
10939	57.06	27.76	36.35	58.65	52.04	109.1	2.723	2.912	3.527	4.098	1.309	1.238	65.85	9.05	16.07	1840	16.79
10945	52.65	29.64	37.44	58.3	55.05	107.7	2.547	2.343	2.55	4.38	1.174	1.228	57.13	9.6	18.25	1895	17.53
1098	52.35	27.27	39.74	59.72	57.45	109.8	2.806	2.537	3.015	4.218	1.246	1.416	47.58	8.51	18.38	2025	18.32
11121	56.53	19.38	37.66	58.29	42.67	99.2	2.994	2.442	2.374	3.93	1.205	1.317	55.29	9.65	16.7	1750	17.52
11198	56.53	19.38	38.67	58.58	46.27	102.8	3.732	1.986	4.021	6.105	2.679	1.466	52.89	11.48	17.52	1882	18.21
11279	76.35	25.88	41.03	61.79	46.65	123	2.614	2.288	2.239	4.3	1.37	1.212	45.34	7.62	17.62	874	7.05
11284	60.74	27.84	47.03	67.44	56.56	117.3	3.484	3.438	2.38	4.941	1.37	1.393	53.27	8.42	18.93	1561	13.19
11303	65.65	26.32	52.47	64.56	55.45	121.1	2.475	2.518	3.194	3.947	1.204	1.089	31.03	10.19	43.2	1582	13.07
11378	59.18	26.19	41.54	62.97	55.12	114.3	2.99	2.956	3.229	4.254	1.143	1.621	57.57	9.77	18.3	1823	15.84
11498	58.77	26.95	41.91	61.79	57.63	116.4	2.884	3.452	4.209	5.262	1.432	1.45	57.89	11.04	17.83	3176	27.2
11584	65.54	27.1	40.29	59.45	48.56	114.1	3.113	2.141	3.067	3.69	1.153	1.228	48.75	10.36	19.33	1233	10.67
1161	67.91	27.05	42.16	62.88	52.99	120.9	3.122	2.342	4.13	5.219	1.927	1.106	66.45	9.39	17.82	1942	15.94
11627	61.1	27.67	40.48	61.06	51.4	112.5	3.299	2.099	3.237	4.111	1.164	1.299	54.82	7.84	18.43	1329	11.88
1164	56.34	27.84	38.74	59.3	56.76	113.1	2.658	3.076	3.892	5.246	1.689	1.395	50.85	10.08	17.44	1579	13.86

Appendix 2: Mean performance of 300 accessions in chickpea reference set accessions for 17 quantitative traits based on overall pooled analysis

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
11664	59.07	27.13	40.78	61.27	54.43	113.5	3.071	1.902	3.555	4.08	1.982	1.496	66.35	16.71	17.45	1471	12.9
11764	59.24	27.64	40.56	63.56	55.26	114.5	3.109	2.6	3.319	4.26	1.679	1.086	35.93	10.91	37.3	1391	12.06
1180	60.31	27.13	37.6	61.26	50.69	111	3.834	2.651	3.309	4.289	1.226	1.113	43.79	13.42	17.85	1659	14.79
11819	67.13	21.84	41.09	61.18	40.77	107.9	2.718	2.111	3.149	4.932	2.207	1.09	35.46	10.68	35.04	1195	10.99
11879	58.56	27.47	42.68	61.6	55.64	114.2	3.153	2.221	3.863	4.307	1.277	1.124	43.72	12.44	26.1	1379	11.98
11903	63.51	27.76	40.58	61.26	52.79	116.3	2.284	2.261	3.21	4.255	1.267	1.063	35.99	7.92	28.07	1374	11.76
1194	56.08	27.34	43.38	59.12	51.02	107.1	2.913	2.55	2.721	3.855	1.308	1.179	52.65	10.19	19.71	1672	15.47
11944	59.93	27.1	32.06	57.21	47.27	107.2	3.452	2.308	3.215	4.311	1.205	1.558	54.09	10.28	16.24	1877	17.46
12028	61.16	26.32	42.21	61.98	46.94	108.1	2.563	2.408	2.49	4.08	1.288	1.149	36.84	9.49	22.67	1518	13.9
12037	60.69	27.03	43.62	62.5	54.71	115.4	2.763	2.428	3.223	4.517	1.287	1.317	43.63	9.21	19.53	1783	15.36
1205	55.39	27.57	42.05	64.74	45.81	101.2	2.628	2.248	3.332	4.691	1.328	1.458	52.52	10.97	19.68	1915	18.38
12155	55.32	24.77	38.85	61.4	53.58	108.9	3.316	2.344	3.082	4.071	1.498	1.354	55.93	10.64	15.82	1739	15.87
12299	70.48	27.57	37.57	60.81	47.22	117.7	3.404	2.119	2.944	3.3	1.492	1.267	51.68	7.58	15.27	1496	12.58
1230	56.61	27.3	40.4	61.43	55.69	112.3	2.435	2.444	2.752	3.829	1.196	1.422	57.45	10.59	22.37	1946	17.21
12307	56.11	27.27	37.32	59.99	53.69	109.8	2.495	2.282	3.405	3.992	1.287	1.347	49.98	8.78	14.69	1778	16.17
12321	60.09	27.47	45.36	61.6	53.51	113.6	3.253	2.298	2.92	3.491	1.317	1.259	38.78	7.54	16.47	882	7.76
12324	61.56	27.47	51.79	64.97	53.34	114.9	3.069	2.132	3.219	4.04	1.339	1.099	45.17	9.21	25.3	1645	14.2
12328	60.13	27.42	44.91	62.65	53.57	113.7	3.492	2.312	4.229	3.809	1.628	1.14	43.28	9.34	35.97	1973	17.24
12379	60.32	27.23	45.31	64.72	55.38	115.7	2.641	2.253	2.535	4.878	1.237	1.072	38.21	10.31	29.71	1419	12.14
12492	62.04	26.8	53.38	61.86	54.36	116.4	3.873	3.044	3.283	4.112	1.185	1.191	50.94	10.25	21.46	1306	11.15
12537	53.06	27.37	38.61	59.51	58.04	111.1	2.375	2.109	2.442	4.131	1.185	1.376	45.65	7.75	19.48	1622	14.55
12654	55.44	27.71	43.16	61.5	57.56	113	3	3.305	3.104	4.03	1.288	1.483	51.67	10.39	17.81	1752	15.46
12726	55.74	27.47	42.49	63.94	57.36	113.1	2.798	2.292	3.142	4.488	1.154	1.467	51.03	7.27	17.76	1872	16.45
12824	56.27	27.07	44.07	62.24	54.93	111.2	3.011	2.262	2.327	4.206	2.115	1.32	55.5	11.24	17.76	2016	18.07
12851	55.44	27.03	45.25	61.34	55.76	111.2	3.193	2.222	2.984	4.667	1.391	1.163	62.62	10.14	18.53	1779	15.95
12866	55.81	26.8	41.13	63.06	56.19	112	2.97	2.338	2.57	4.574	1.204	1.443	60.47	10.47	18.53	1655	14.67
12916	62.45	27.23	43.14	63.25	51.25	113.7	2.394	2.348	2.601	4.402	1.348	1.276	50.06	11.37	19.63	1629	14.26
12928	65.74	26.73	47.25	63.69	49.46	115.2	2.828	2.279	3.268	4.173	1.303	1.181	57.49	8.35	20.03	1374	11.81

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
12947	60.23	27.47	44.07	62.46	51.07	111.3	2.573	2.48	3.181	4.338	1.41	1.155	56.43	13.8	22.45	1924	17.17
13077	62.91	27.37	42.02	63.7	54.09	117	2.755	1.642	3.217	3.973	1.558	1.136	53.34	29.97	24.16	1254	10.66
13124	46.53	27.47	40.1	60.49	62.17	108.7	2.459	2.579	2.313	3.79	1.122	1.102	49.55	12.76	30.76	1953	17.85
13187	62.47	27.72	55.74	64.22	56.43	118.9	3.153	2.368	3.119	4.019	1.287	1.072	34.5	8.03	25.88	1383	11.58
13219	53.55	27.46	40.74	59.68	48.75	102.3	2.375	3.204	2.508	3.295	1.185	1.474	57.92	9.47	19.78	1544	15.05
13283	63.61	27.66	51.06	64.9	54.69	118.3	3.134	2.338	2.428	4.309	1.441	1.219	41.21	12.6	28.88	1600	13.44
13357	64.54	27.67	51.34	68.64	53.06	117.6	3.087	2.73	3.114	4.121	1.431	1.081	48.8	12.84	25.65	1522	12.87
13441	63.9	27.1	55.26	61.43	50.5	114.4	2.724	3.124	3.369	3.549	1.393	1.063	54.51	10.05	19.32	1308	11.4
13461	60.94	27.47	45.32	63.4	47.96	108.9	2.795	2.528	3.059	4.204	1.195	1.298	45.59	9.15	17.14	1321	11.69
13523	59.87	27.77	42.65	64.77	57.93	117.8	3.034	2.445	3.135	4.021	1.256	1.063	43.82	11.8	24.51	1788	15.11
13524	60.49	27.55	42.01	63.9	48.01	108.5	3.279	2.308	3.119	4.413	1.496	1.595	46.08	8.61	17.64	1535	13.92
1356	53.74	27.57	40.05	62.84	54.36	108.1	2.964	2.222	3.241	4.311	1.421	1.108	52.68	12.71	19.95	2086	19.19
13599	59.36	27.23	45.59	64.35	54.14	113.5	3.222	2.368	3.086	4.309	1.446	1.063	46.52	10.36	23.72	1254	10.96
13628	60.69	27.47	42.09	64.26	55.51	116.2	2.405	2.438	3.074	4.081	1.452	1.173	50.22	8.64	20.67	1334	11.42
13719	66.34	27.37	42.39	62.9	46.76	113.1	3.236	2.408	3.211	5.112	2.556	1.229	55.73	11.06	24.86	1319	11.52
13764	58.77	27.57	45.13	63.45	57.43	116.2	2.758	2.308	3.06	4.349	1.496	1.337	43.35	9.05	20.58	1547	13.24
13816	56.99	27.47	43.89	63.41	57.51	114.5	2.346	2.408	3.179	4.228	2.043	1.094	36.99	9.8	25.18	1791	15.57
13863	53.4	27.35	41.67	64.13	54.6	108	2.685	2.349	3.248	4.281	1.348	1.573	54.01	12.03	16.83	1839	16.98
13892	53.52	27.27	37.84	62.34	51.58	105.1	2.805	3.385	3.084	5.071	2.176	1.456	48.12	11.7	17.05	1791	16.97
1392	55.08	26.83	42.21	62.4	56.42	111.5	2.614	2.72	3.331	4.162	1.359	1.223	49.26	12.68	23.97	1888	16.72
1397	55.69	27.17	40.6	62	55.41	111.1	3	2.404	2.885	4.284	1.122	1.182	54.03	11.22	20.8	1441	12.93
1398	48.81	27.37	39.21	61.01	57.09	105.9	2.691	2.289	2.87	4.518	1.287	1.151	66.55	14.16	20.75	1335	12.57
14051	48.01	27.57	38.74	62.42	59.69	107.7	2.429	3.139	3.418	4.249	1.227	1.541	60.1	13.63	17.62	1826	16.91
14077	51.51	27.47	38.14	62.85	55.39	106.9	2.878	4.079	3.259	3.988	1.123	1.356	54.32	8.42	17.62	1874	17.41
14098	45.39	27.44	37.56	61.59	59.91	105.3	3.267	2.593	3.066	3.987	1.287	1.223	51.27	9.3	20.8	1839	17.38
14199	59.03	27.35	42.11	63.94	55.37	114.4	2.661	2.533	2.198	4.065	1.832	1.063	27.15	9.93	36.18	1806	15.69
1422	48.72	27.17	38.05	60.65	56.88	105.6	3.182	2.134	2.962	3.273	1.565	1.231	56.11	10.65	21.64	1601	15.12
1431	58.15	27.06	40.16	61.06	53.55	111.7	2.927	2.726	3.082	4.071	1.143	1.141	52.55	12.49	21.59	1577	13.95

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
14402	51.17	27.57	43.39	60.59	53.03	104.2	2.599	2.566	2.654	4.385	1.205	1.389	45.38	10.14	20.54	2069	19.19
14446	67.04	27.13	46.79	62.74	54.86	121.9	2.429	1.461	3.084	3.107	1.143	1.09	31.1	8.87	35.47	959	7.82
14595	37.7	27.37	35.53	62.03	66.7	104.4	2.688	2.19	3.856	4.588	2.155	1.13	47.77	9.81	23.4	2058	19.6
14669	46.3	27.66	37.97	62.88	58.6	104.9	2.797	2.191	3.194	3.973	1.391	1.09	61.21	11.91	21.27	1918	18.27
14778	58.09	27.47	39.87	62.92	52.51	110.6	2.429	2.119	3.06	4.179	1.164	1.289	48.98	9.42	18.17	1635	14.7
14799	58.48	27.34	41.35	63.72	55.02	113.5	2.725	3.075	3.05	4.287	2.434	1.242	54.72	10.44	20.82	1746	15.32
14815	56.39	27.27	42.93	63.24	57.21	113.6	3.222	2.767	3.112	5.015	1.94	1.304	50.44	13.2	19.23	1753	15.36
14831	58.45	27.44	42.16	65.21	53.55	112	2.788	2.625	3.495	4.102	1.102	1.235	59.29	12.73	19.21	1654	14.69
1510	59.54	27.12	51.9	60.64	53.76	113.3	2.48	2.068	3.048	3.88	1.144	1.189	66.62	11.89	21.55	1535	13.47
15248	59.27	27.13	41.42	62.22	52.03	111.3	3.042	2.368	2.937	3.734	1.492	1.102	47.35	9.27	21.54	1513	13.56
15264	55.33	27.39	40.06	61.5	55.67	111	2.805	2.438	3.465	4.686	1.476	1.098	41.66	8.02	25.68	1637	14.7
15294	58.53	27.52	43.79	64.46	58.47	117	2.389	2.574	2.347	5.185	1.102	1.063	44.36	7.61	25.65	1507	12.8
15333	59.47	27.45	55.56	62.53	57.53	117	3.176	2.162	3.819	3.99	1.185	1.078	42.67	10.23	30.4	1749	14.93
15406	59.13	27.15	41.42	63.58	55.57	114.7	2.721	2.19	3.392	3.887	1.595	1.098	39.92	10.8	33.85	2022	17.58
15435	58.53	26.96	47.93	66.44	49.87	108.4	2.528	2.669	2.593	3.776	1.578	1.063	40.67	10.95	29.47	2048	18.98
15510	59.91	27.57	41.37	63.76	55.59	115.5	3.01	2.19	3.243	4.466	2.104	1.111	42.02	8.24	24.72	2569	22.23
15518	43.33	27.84	41.49	63.96	65.07	108.4	2.644	3.053	2.986	3.42	1.163	1.063	41.39	9.95	39.52	1586	14.61
15567	51.71	27.67	37.52	61.76	52.29	104	2.223	2.289	2.891	4.646	1.164	1.126	45.26	7.95	18.55	1519	14.55
15606	54.69	27.07	39.43	61.8	49.11	103.8	2.483	3.076	4.272	5.124	1.123	1.303	53.41	14.26	17.45	2024	19.48
15610	58.44	27.23	41.61	65.22	46.56	105	2.64	2.149	3.066	4.214	1.174	1.414	47.85	11.72	21.39	2114	20.1
15612	51.02	26.93	37.53	64.26	55.18	106.2	2.987	2.434	3.122	3.418	1.123	1.108	54.37	12.15	18.43	1956	18.41
15614	50.45	27.2	34.95	61.69	54.35	104.8	3.159	2.775	3.393	4.3	1.348	1.234	49.18	13.72	17.4	1961	18.71
15618	43.55	27.71	37.49	61.92	60.05	103.6	2.305	3.614	3.223	4.294	1.143	1.487	51.96	10.36	17.29	1899	18.22
15697	55.81	27.3	41.11	64.7	49.39	105.2	2.742	2.563	3.479	3.399	1.184	1.107	43.55	10.06	34.36	1717	16.33
15762	57.02	27.4	40.95	61.43	53.48	110.5	2.378	2.999	2.435	3.589	1.328	1.063	33.05	8.01	29.51	1720	15.48
15785	62.92	27.77	41.02	61.18	48.88	111.8	2.858	2.246	3.128	3.378	1.267	1.077	35.81	8.33	20.35	1260	11.22
15802	58.39	27.81	44.67	63.73	53.81	112.2	3.387	2.149	2.847	4.539	1.473	1.063	40.59	10.15	27.13	1843	16.41
15868	52.26	27.1	40.07	63.92	59.44	111.7	2.384	2.149	3.52	4.473	1.236	1.288	59.74	10.75	18.02	2202	19.69

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
15888	57.62	27.66	38.02	64.14	55.58	113.2	2.718	2.813	3.451	4.588	2.023	1.129	58.51	10.17	20.88	1877	16.53
16207	60.19	27.32	42.87	62.84	54.61	114.8	2.27	2.69	3.371	4.392	1.132	1.547	54.18	11.7	20.32	1917	16.64
16261	59.31	27.47	41.49	64.15	54.49	113.8	3.223	3.129	2.183	5.097	2.238	1.31	60.85	10.46	20.19	1714	14.98
16269	59.27	27.3	40.97	61.28	56.03	115.3	3.136	3.11	3.086	5.036	1.393	1.148	51.01	11.09	20.24	1348	11.66
16374	41.01	28.86	42.37	64.22	70.99	112	2.398	2.441	3.299	3.24	1.349	1.296	43.47	10.09	23.02	1670	14.83
16487	62.8	27.61	40.97	62.35	49.7	112.5	2.849	2.441	4.065	4.348	1.495	1.204	43.57	9.29	15.58	1278	11.31
16524	58.83	27.76	37.83	56.36	50.97	109.8	2.693	1.876	2.377	9.246	2.067	1.258	55.74	11.25	16.04	1662	15.05
16654	59.97	27.46	40.97	61.86	53.03	113	2.788	2.401	3.819	4.393	2.001	1.117	37.85	12.34	35.97	1575	13.91
16796	69.16	27.37	45.24	68.54	55.44	124.6	2.549	2.456	2.626	3.377	1.081	1.081	33.74	11.01	36.8	1241	9.92
16903	45.94	27.71	39.36	60.08	56.56	102.5	2.799	2.626	2.28	4.601	1.122	1.182	61.44	9.06	18.5	1921	18.7
16915	50.31	27.03	38.69	62.68	56.49	106.8	2.614	3.136	3.398	4.34	1.37	1.242	62.27	13.28	16.48	2011	18.7
1710	58.15	26.85	45.6	62.64	54.05	112.2	2.503	2.306	3.48	3.728	1.164	1.389	53.21	9.74	18.2	1861	16.52
1715	61	27.37	38.14	62.08	47.9	108.9	2.927	2.694	3.104	4	1.328	1.229	59.35	11.08	18.82	1738	15.48
1882	50.93	27.34	36.59	60.85	56.77	107.7	2.792	3.079	3.454	5.825	1.163	1.192	62.27	13.64	21.11	2053	18.99
1915	70.7	26.49	47.36	64.63	50.9	121.6	2.201	1.71	2.317	3.707	2.158	1.151	32.68	7.95	26.72	1068	8.82
1923	52.15	31.46	41.12	62.45	59.75	111.9	2.986	2.054	3.121	4.083	1.186	1.094	55.05	9.8	21.17	1589	14.18
2065	58.85	27.51	38.5	62.31	53.75	112.6	2.699	2.623	3.228	3.892	1.205	1.295	55.87	10.01	19.45	1772	15.6
2072	52.76	27.84	37.69	61.8	60.14	112.9	3.037	2.523	2.758	4.08	1.246	1.463	54.45	10.77	19.12	1660	14.61
2210	63.39	27.2	39.19	60.99	49.61	113	2.495	2.352	2.27	4.052	1.102	1.285	49.28	9.91	21.07	1401	12.33
2242	60.32	27.77	41.78	62.23	57.68	118	3.652	2.308	3.383	4.083	1.339	1.134	43.94	9.16	20.16	1451	12.23
2263	58.55	27.4	39.76	62.82	53.85	112.4	2.384	2.712	3.945	4.322	2.002	1.229	63.66	12.37	21.06	1816	16.12
2277	62.98	27.47	44.03	63.38	57.32	120.3	3.134	2.461	2.365	4.296	1.37	1.154	43.42	8.42	24.91	1309	10.83
2482	55.54	28.01	40.24	63.54	58.36	113.9	2.853	2.456	3.794	3.89	1.574	1.193	40.88	9.93	24.1	1993	17.49
2507	53.96	27.57	40.82	63.86	58.44	112.4	2.658	2.255	3.438	4.297	1.349	1.321	46.1	8.05	18.37	1258	11.15
2580	52.42	27.42	39.24	62.78	57.98	110.4	2.849	2.169	4.003	4.068	1.432	1.151	51.24	13.18	23.23	1973	17.76
2593	57.1	27.59	46.03	67.08	57.2	114.3	3.627	2.276	3.179	3.523	1.143	1.32	45.28	9.65	18.63	1383	12.04
2629	60.3	27.69	38.56	62.12	52.9	113.2	2.906	2.481	2.197	4.231	1.204	1.224	89.28	13.05	17.56	1605	14.1
2679	67.79	25.82	42.48	67.1	48.21	116	2.933	2.483	2.851	3.317	1.288	1.199	42.82	8.02	20.72	1649	14.15

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
2720	67.62	26.39	43.62	66.93	45.28	112.9	2.637	2.96	3.415	4.403	1.713	1.309	64.17	9.83	18.61	1702	15
2737	65.25	27.37	39.54	64.07	50.35	115.6	3.004	2.31	3.102	3.579	1.225	1.271	47.67	7.17	18.08	1237	10.66
283	57.58	27.83	39.57	62.73	57.52	115.1	3.174	3.195	3.423	4.092	1.96	1.181	59.24	13.35	22.95	1993	17.28
2884	55.14	27.89	40.96	64.07	56.36	111.5	3.102	2.428	2.294	4.311	1.277	1.458	57.77	10.91	18.35	1444	12.91
2919	59.5	27.83	43.41	66.16	55.8	115.3	2.984	2.981	2.895	4.351	1.215	1.178	50.04	9.71	22.43	1896	16.41
2969	58.49	26.73	40.59	64.14	56.61	115.1	2.889	2.302	3.44	4.113	1.359	1.272	55.83	10.97	20.68	1997	17.26
2990	61.35	27.23	39.88	61.96	57.25	118.6	2.431	2.289	2.587	3.196	1.68	1.063	44.83	8.74	22.31	1301	10.88
3218	69.92	27.52	39.81	59.15	47.28	117.2	2.588	2.637	3.103	4.216	1.698	1.154	46.58	7.49	18.41	1335	11.34
3230	61.84	28.47	36.25	55.57	46.56	108.4	3.097	2.401	3.163	4.326	1.226	1.148	52.32	9.85	17.56	1606	14.81
3239	61.49	28.06	39.24	58.54	53.91	115.4	3.246	2.385	2.959	4.594	2.156	1.115	43.48	8.41	23.05	1300	11.25
3325	53.84	27.45	37.42	62.16	54.76	108.6	2.944	2.64	4.136	5.327	1.112	1.129	65	10.22	20.94	2116	19.44
3362	54.09	27.67	38.66	62.41	53.01	107.1	2.248	2.363	3.555	3.984	1.185	1.346	52.41	13.6	18.41	2290	21.36
3391	53.6	27.57	38.84	59.08	57.2	110.8	3.328	1.99	3.285	3.326	1.206	1.302	40.87	10.26	22.41	1742	15.67
3410	56.52	27.47	40.35	61.91	56.78	113.3	3.142	2.244	3.004	4	1.451	1.264	43.26	9.31	26.18	1769	15.5
3421	62.45	26.53	40.92	61.96	51.25	113.7	2.983	2.342	3.08	3.523	1.637	1.35	43.93	9.99	24.68	1515	13.28
3512	61.77	27.27	36.21	55.48	48.73	110.5	3.114	2.602	2.528	4.164	2.032	1.09	50.78	8.51	22.73	1422	12.82
3582	62	27.47	39.83	65.89	55.5	117.5	2.503	2.852	3.217	3.388	1.638	1.282	50.03	9.92	19.69	1547	13.13
3631	60.94	27.44	42.31	64.38	54.56	115.5	2.93	2.129	3.181	3.96	1.93	1.51	48.33	9.9	20.45	1570	13.58
3761	53.98	27.47	43.33	65.42	55.52	109.5	2.805	2.607	3.257	3.592	1.267	1.426	58.84	10.68	19.22	1464	13.33
3776	54.53	27.82	44.03	66.03	60.07	114.6	2.558	2.84	3.088	5.166	1.143	1.398	47.18	8.28	19.06	1709	14.87
3892	55.54	26.09	38.13	59.07	54.46	110	2.302	2.755	2.926	4.281	1.185	1.333	46.81	5.95	18.76	1385	12.58
3946	61.06	27.57	40.71	66.56	56.44	117.5	2.594	2.915	3.084	4.429	1.257	1.333	51.99	10.98	19.91	1679	14.21
4093	58.68	27.54	43.27	62.02	57.22	115.9	3.105	2.177	3.102	4.265	1.287	1.435	66.21	8.6	19.07	1599	13.74
4182	53.82	26.42	42.51	60.97	56.28	110.1	3.529	2.82	3.05	4.467	1.123	1.436	49.98	9.85	19.9	1452	13.13
4363	48.38	27.93	40.69	59.35	61.42	109.8	2.705	2.516	2.485	2.941	1.236	1.531	48.61	10.31	17.16	1305	11.83
440	60.35	27.13	42.33	62.44	56.35	116.7	3.475	3.777	3.124	5.14	1.533	1.695	52.58	8.93	18.31	1667	14.24
4418	53.84	27.69	43.38	61.96	56.76	110.6	3.024	2.199	2.903	4.268	1.309	1.389	56.64	8.53	18.72	1714	15.44
4463	60.96	27.67	41.65	55.92	56.44	117.4	2.838	2.283	4.357	5.499	1.226	1.343	44.78	7.32	18.43	1194	10.15

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
4495	52.79	27.37	39.31	61.22	57.11	109.9	2.595	3.166	3.131	4.456	1.163	1.144	54.28	14.51	20.55	1750	15.93
4533	44.22	27.57	34.97	55.86	65.18	109.4	2.657	2.737	2.37	3.269	1.308	1.255	52.46	13.46	23.64	2064	18.84
456	58.68	26.91	39.14	62.54	52.92	111.6	2.534	2.325	3.022	4.207	1.164	1.276	65.92	11.78	20.1	1673	14.94
4567	55.69	27.91	43.06	64.25	56.71	112.4	2.375	3.148	3.423	4.524	2.001	1.259	68.19	17.08	22.7	2309	20.54
4593	59.04	27.96	40.16	64.47	54.96	114	2.697	3.373	2.76	3.625	1.37	1.155	68.67	12.12	20.33	1677	14.67
4639	59.95	27.07	39.79	61.77	53.85	113.8	3.344	2.177	3.349	4.336	1.248	1.127	67.41	11.48	20.7	1851	16.17
4657	58.94	28.16	35.06	60.7	49.96	108.9	3.956	2.456	2.982	4.256	1.236	1.403	58.06	8.59	18.94	1639	14.77
4814	56.7	27.03	40.4	59.93	56.5	113.2	3.236	2.758	2.794	4.176	1.514	1.376	43.08	10.3	19.22	1340	11.78
4841	61.49	27.37	40.87	62.71	50.01	111.5	3.566	2.247	3.615	4.207	1.245	1.198	38.57	11.43	26.52	1532	13.59
4853	51.8	27.66	40.99	62.47	61	112.8	3.338	3.632	2.518	4.159	1.329	1.611	60.23	15.82	20.55	1599	14.13
4872	49.19	27.67	36.79	61.56	58.91	108.1	3.118	2.705	3.122	4.841	1.411	1.09	58.13	11.02	24.18	1790	16.49
4918	43.85	28.19	36.88	60.32	61.25	105.1	3.057	2.316	3.617	4.123	1.617	1.157	58.44	13.05	20.29	2074	19.63
4991	57.92	27.49	41.35	63.17	49.48	107.4	2.279	2.813	3.115	7.524	2.499	1.339	68.12	11.03	16.85	1897	17.29
506	50.68	28.18	38.93	63.61	53.22	103.9	2.374	3.341	2.838	4.321	1.536	1.171	62.59	11.4	20.68	1865	17.47
5135	59.79	28.11	37.58	62.62	52.41	112.2	2.182	2.129	2.555	6.157	3.37	1.222	50.53	10.59	20.17	1689	14.98
5221	50.7	27.86	39.15	62.5	60.4	111.1	2.302	2.413	4.099	4.087	1.411	1.161	77.59	11.8	19.74	1935	17.37
5337	69.48	23.93	43.01	63.65	43.32	112.8	2.771	2.591	3.239	4.102	1.248	1.164	51.16	13.91	25.12	1394	12.29
5383	53.16	28.23	37.53	59.41	58.54	111.7	2.879	2.999	3.291	4.166	1.278	1.218	65.34	11.37	24.82	2118	18.96
5434	51.92	27.96	17.31	57.86	57.38	109.3	3.188	2.29	2.976	3.416	1.237	1.232	48.14	10.85	19.8	1535	14.01
5504	58.38	27.57	42.84	63.02	56.42	114.8	2.93	3.385	2.546	5.095	1.206	1.2	47.76	10.75	24.85	1765	15.33
5613	51.33	27.67	39.67	62.36	54.67	106	2.379	2.25	3.254	3.42	1.287	1.287	48.73	9.21	20.69	1802	17.07
5639	51.92	27.96	39.66	61.3	55.98	107.9	2.899	2.611	3.359	4.547	1.185	1.367	60.24	9.4	20.72	1888	17.5
5845	61.52	27.27	39.63	62.03	49.48	111	2.954	3.183	3.088	4.689	1.278	1.41	51.07	8.48	17.82	1634	14.71
5878	53.59	27.57	36.98	60.08	52.51	106.1	2.773	2.98	2.776	4.56	1.288	1.334	59.94	10.15	16.9	1954	18.36
5879	49.77	27.55	37.57	59.67	59.73	109.5	2.904	2.544	3.117	4.627	1.277	1.39	55.07	16.87	19.93	2057	18.77
6263	60.37	26.68	43.55	59.49	53.53	113.9	2.501	2.169	2.762	3.399	1.329	1.135	45.31	9.61	26.36	1392	12.2
6279	43.87	28.33	37.52	57.81	65.33	109.2	2.617	2.19	3.084	4.352	1.122	1.148	51.42	10.69	18.99	1760	16.05
6293	59.6	28.33	41.56	63.73	56.2	115.8	3.084	2.418	3.098	3.936	1.236	1.319	52.9	9.85	18.05	1364	11.7

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
6294	63.46	27.96	44.96	68.22	51.34	114.8	3.193	2.141	3.006	4.062	1.268	1.15	40.25	9.9	25.76	1123	9.74
6306	70.92	26.93	47.72	67.71	50.68	121.6	2.682	2.432	2.747	3.192	1.826	1.356	38.3	21.4	26.28	1126	9.24
637	52.68	27.84	40.6	60.8	57.82	110.5	3.048	2.265	3.066	3.947	1.081	1.122	52.8	9.86	22.29	1741	15.74
6537	54.53	27.77	40.68	61.76	56.57	111.1	2.694	2.416	3.518	4.346	1.972	1.348	56.1	11.28	18.17	1819	16.31
6571	55.09	27.96	41.12	61.92	52.21	107.3	2.682	2.059	3.159	3.941	1.122	1.224	66.08	10.99	18.54	1759	16.35
6579	53.36	27.69	41.1	62.78	54.94	108.3	2.645	2.325	2.197	4.114	1.309	1.536	65.28	12.36	19.13	1794	16.51
67	51.02	27.59	40.55	65.65	56.58	107.6	2.91	3.079	3.341	4.083	2.095	1.355	63.7	13.87	21.29	1675	15.5
6802	60.19	27.99	40.23	64.95	52.81	113	2.867	2.607	2.503	4.319	1.761	1.357	50.02	10.96	19.3	1737	15.25
6811	55.31	27.1	40.88	64.02	51.09	106.4	2.671	2.29	3.024	4.537	1.102	1.592	47.78	7.87	17.88	1578	14.84
6816	49.01	27.5	40.64	63	63.09	112.1	2.651	2.24	3.082	4.67	1.226	1.337	50.33	11.12	17.86	2050	18.18
6874	52.21	28.19	36.88	58.41	56.79	109	3.031	2.379	3.323	4.114	1.122	1.264	66.9	12.65	18.48	2023	18.49
6875	67.16	22.83	41.99	64.14	51.14	118.3	3.196	2.202	3.263	4.152	1.196	1.113	46.91	9.9	21.79	1267	10.64
6877	61.22	26.93	41.05	65.36	51.08	112.3	2.95	2.179	3.172	4.1	1.412	1.063	46.99	11.37	25.24	1748	15.47
7052	61.47	27.79	41.45	62.64	53.33	114.8	2.81	2.448	2.438	3.159	1.185	1.456	42.49	7.35	17.06	1125	9.73
708	60.42	27.69	40.38	63.12	54.98	115.4	2.828	2.359	3.007	3.921	1.185	1.14	61.82	11.62	22.42	1978	17.09
7150	65.26	24.01	44.36	65.95	49.24	114.5	2.997	2.369	3.05	4.006	1.278	1.147	49.86	10.76	23.41	1218	10.58
7184	65.16	27.13	42.35	65.94	50.84	116	3.303	2.331	2.916	4.036	1.226	1.472	54.23	9.59	17.26	1518	13.04
7255	53.5	28.13	44.49	64.92	56.9	110.4	3.873	2.221	3.018	4.746	1.37	1.164	49.37	10.77	30.26	1795	16.24
7272	55.75	28.28	45.7	67.41	56.45	112.2	2.46	2.577	3.119	4.124	2.002	1.063	45.07	11.65	29.96	1776	15.79
7305	60.83	27.54	42.34	62.01	56.07	116.9	3.148	3.088	2.982	3.994	1.195	1.252	53.64	6.85	21.67	1534	13.09
7308	51.56	28.31	45.99	69.22	60.94	112.5	2.52	2.542	3.39	4.576	12.328	1.197	51.08	15.21	26.01	1703	15.11
7315	56.46	27.37	43.9	65.04	57.04	113.5	2.588	2.536	3.186	4.908	1.143	1.169	44.36	11.61	29.38	1651	14.49
7323	60.3	27.47	48.55	63.75	56.2	116.5	2.988	2.568	2.415	4.211	1.309	1.236	41.31	10.29	23.13	1082	9.26
7326	60.2	27.57	43.77	67.56	53.9	114.1	3.137	2.187	3.121	5.228	1.205	1.145	52.74	12.39	22.88	1543	13.49
7413	51.32	27.15	39.92	62.07	59.38	110.7	2.95	3.191	3.345	5.167	1.33	1.063	64.32	9.76	22.12	1850	16.69
7441	53.36	27.66	42.21	61.24	55.64	109	2.189	2.96	3.104	4.39	1.289	1.222	63.04	11.94	20.11	1878	17.2
7554	58.27	27.37	45.26	63.25	56.13	114.4	2.305	2.318	3.164	3.605	1.349	1.165	48.05	12.42	25.11	1732	15.08
7571	58.79	27.87	44.16	64.47	56.71	115.5	3.276	2.24	3.319	4.283	1.349	1.142	39.43	8.54	23.75	1631	14.01

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
762	63.45	28.04	44.11	64.34	56.25	119.7	3.117	2.533	3.08	4.081	2.032	1.292	50.29	11.14	21.56	1512	12.59
7668	53.39	28.18	43.92	64.32	60.01	113.4	3.163	2.468	3.141	4.08	1.207	1.19	46.78	11.54	22.7	1728	15.2
7819	60.72	28.03	43.75	61.6	53.78	114.5	2.278	2.595	3.07	3.535	1.206	1.112	44.37	9.66	23.13	1550	13.51
7867	62.38	26.93	52.35	62.3	51.32	113.7	2.793	2.475	2.163	4.03	1.081	1.085	47.19	8.88	22.36	1396	12.17
791	61.31	27.94	53.84	61.96	56.39	117.7	2.455	2.26	2.884	4.767	1.909	1.154	48.42	8.91	20.52	1663	14.12
8058	70.45	26.21	53.34	61.96	50.95	121.4	2.805	2.284	2.258	4.164	1.277	1.19	51.38	9.17	20.64	1238	10.16
8151	61.31	27.66	49.77	63.17	52.59	113.9	3.196	2.242	2.88	4.123	1.132	1.089	39.34	11.82	34.66	1585	13.88
8195	60.07	21.09	54.07	60.52	50.43	110.5	3.368	1.872	3.248	4.423	1.513	1.335	58.32	10.02	17.71	1436	12.97
8200	60.07	27.86	55.83	60.33	52.43	112.5	2.513	2.564	3.084	4.175	2.443	1.063	49.88	9.02	23.74	1572	13.97
8261	52.95	27.66	53.32	63.82	58.45	111.4	2.903	2.047	3.226	3.952	1.081	1.124	42.12	9.33	32.67	1574	14.09
8318	36.53	28.09	38.62	61.38	67.47	104	2.96	2.598	3.355	3.491	1.328	1.113	54.45	12.87	23.28	2410	23.19
8350	58.63	27.57	51.33	64.06	47.77	106.4	2.924	3.197	3.199	4.423	1.287	1.137	46.5	14.51	32.67	1541	14.41
8384	51.69	27.71	42.26	60.7	63.41	115.1	2.298	2.628	3.287	4.133	1.698	1.255	62.01	13.11	20.23	2062	17.85
8515	67.24	27.88	55.05	69.45	48.36	115.6	2.449	2.924	3.116	4.734	1.081	1.174	45.86	8.38	22.2	1317	11.33
8521	67.24	25.62	56.71	69	51.06	118.3	2.772	1.67	2.437	4.068	1.215	1.195	43.54	9.44	22.18	1182	10.01
8522	51.79	27.34	40.78	60.26	61.91	113.7	2.562	2.437	2.734	4.185	1.194	1.354	51.3	8.7	18.57	1741	15.27
8607	55.29	27.23	53.22	61.92	54.21	109.5	3.035	3.012	2.62	4.03	1.081	1.546	61.74	13.52	19.26	1785	16.29
8621	48.91	27.67	44.57	59.98	58.29	107.2	2.423	2.348	2.84	3.529	1.453	1.155	57.31	11.29	20.18	1936	18.02
867	51.21	27.44	41.13	61.72	60.09	111.3	2.432	1.799	3.187	7.169	1.901	1.099	57.2	12.16	21.9	1707	15.28
8718	60.72	27.54	51.79	64.4	54.38	115.1	3.062	2.099	2.725	4.038	1.492	1.185	54.72	9.71	21.69	1593	13.78
8740	64.91	27.64	64.34	68.21	54.49	119.4	2.329	2.149	2.986	3.615	1.328	1.087	43.62	9.05	21.23	1411	11.76
8752	51.21	28.13	61.02	61.73	65.49	116.7	2.745	2.468	2.815	4.34	1.081	1.259	48.49	10.78	21.83	1513	12.95
8855	53.95	27.23	44.29	62.21	56.95	110.9	3.122	2.941	2.35	4.506	1.369	1.162	48.36	9.31	22.22	1730	15.55
8950	53.95	27.23	41.07	60.62	58.05	112	2.64	3.603	3.327	4.214	1.363	1.416	48.46	10.52	19.47	1940	17.27
9002	55.29	27.86	41.42	59.14	52.91	108.2	2.452	2.508	3.044	4.009	1.492	1.345	56.76	11.09	19.14	1545	14.19
9137	58.38	28.33	44.26	67.92	55.62	114	2.569	2.408	2.373	4.05	1.204	1.072	41.61	11.23	36.61	1725	15.07
9402	60.55	27.96	41.59	62.99	53.05	113.6	3.029	2.468	2.65	3.18	1.122	1.372	48.51	9.67	23.52	1065	9.32
9418	60.55	27.96	42.21	64.22	53.05	113.6	2.914	2.249	3.283	3.346	1.288	1.46	41.25	9.23	21.05	1144	9.99

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
9434	65.39	27.42	43.89	62.98	53.21	118.6	2.827	2.169	2.683	4.282	1.909	1.219	57.42	10.28	21.21	1308	10.95
95	59.39	27.47	37.87	65.22	55.41	114.8	2.588	3.221	3.209	4.421	1.225	1.315	55.71	8.18	19.62	1686	14.65
9586	56.37	27.23	41.32	61.96	55.23	111.6	3.092	2.239	4.051	4.223	1.267	1.095	47.93	10.35	21.99	1122	10
9590	41.28	27.77	43.52	64.15	69.62	110.9	2.768	2.92	3.259	4.237	1.081	1.308	54.49	8.34	18.81	1807	16.29
9636	59.13	28.36	40.36	61.91	53.67	112.8	3.253	2.63	2.924	4.962	1.081	1.379	54.77	12.4	19.45	1312	11.6
9643	59.13	28.77	42.73	64.03	52.97	112.1	3.189	2.348	3.407	4.589	1.287	1.156	50.68	12.79	19.89	1461	13
9702	60.58	27.57	45.78	63.01	54.62	115.2	3.455	2.548	3.325	3.397	1.574	1.165	46.93	10.25	20.57	1691	14.65
9712	59.39	27.47	42.76	61.46	55.31	114.7	3.508	2.787	3.245	5.48	1.554	1.163	46.8	8.79	19.95	1260	10.92
9755	57.46	27.77	42.54	64.21	47.74	105.2	3.168	2.9	2.692	4.071	1.328	1.189	55.84	9.33	20.81	1242	11.66
9848	59.13	27.96	44.19	64.04	55.07	114.2	3.037	2.204	3.409	4.941	1.909	1.085	49.83	9.86	22.88	1486	12.95
9862	55.29	27.72	44.37	63.99	57.71	113	2.433	2.419	3.221	5.324	1.492	1.125	54.51	11.25	19.91	1708	15.03
9872	58.91	27.55	41.96	62.08	52.69	111.6	2.983	2.229	2.991	5.357	1.245	1.157	45.73	10.91	20.83	1510	13.52
9895	55.06	28.36	41.96	62.08	58.74	113.8	3.122	3.164	2.992	4.822	1.328	1.167	58.48	9.27	21.18	2038	17.91
9942	53.13	27.96	37.32	59.52	57.67	110.8	2.546	4.924	4.335	5.121	1.081	1.273	62.17	8.18	18.67	1875	16.9
20267	46.63	28.29	41.22	59.6	62.27	108.9	2.72	2.553	2.98	3.6	1.86	1.047	54.85	15.94	31.28	1891	17.3
18828	53.13	29.62	38.64	62.64	63.37	116.5	2.351	3.182	3.225	4.19	1.081	1.089	46.71	14.39	27.38	1689	14.47
18836	58.91	27.55	41.99	64.39	55.59	114.5	2.711	3.084	3.421	4.275	1.204	1.108	46.61	9.85	18.13	1230	10.75
18839	59.16	28.23	41.28	59.66	53.44	112.6	3.373	2.57	3.111	4.03	1.442	1.063	55.03	12.43	21.88	1654	14.62
18847	58.69	28.63	43.69	61.37	49.71	108.4	3.01	2.424	2.687	4.05	1.379	1.406	41.25	10.92	24.17	1538	14.06
18858	56.27	27.55	52.18	64.39	59.13	115.4	3.408	2.308	4.181	4.941	2.688	1.306	54.24	12.11	23.49	1504	12.99
18884	59.49	27.93	43.69	59.66	62.11	121.6	3.296	2.029	2.938	4.061	1.482	1.072	45.89	11.52	23.83	1251	10.14
18679	57.11	27.96	47.1	68.17	55.99	113.1	2.398	2.924	3.263	3.989	1.453	1.116	47.27	12.27	21.9	1942	17.11
20266	59.49	28.5	46.97	59.18	56.81	116.3	3.008	2.844	2.864	4.34	1.132	1.099	41.95	8.16	46.42	1152	9.86
20262	47.84	28.5	43.23	60.24	67.16	115	2.923	2.139	3	5.159	1.122	1.176	49.57	9.66	19.03	1474	12.77
20265	68.76	27.37	59.91	45.21	46.64	115.4	3.236	3.363	3.307	5.112	1.267	1.329	47.11	7.81	17.45	1384	11.98
20263	59.49	27.66	53.23	60.1	55.51	115	3.413	2.484	3.252	3.926	1.348	1.262	42.87	6.49	20.22	1292	11.19
20261	59.49	27.66	41.54	60.1	52.91	112.4	2.99	3.164	2.862	4.009	1.328	1.106	40.18	8.68	21.49	1217	10.78
20259	63.73	27.59	41.54	60.1	52.17	115.9	2.867	3.184	3.186	5.148	1.081	1.095	40.03	8.65	18.26	1337	11.51

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	TB	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
18699	59.49	24.01	39.86	60.1	53.01	112.5	3.388	2.689	3.531	4.392	2.115	1.106	45.89	9.95	24.99	1741	15.44
18720	57.11	27.96	40.43	62.17	57.99	115.1	3.027	2.884	3.414	4.019	1.909	1.089	50.16	12.41	26.77	1490	12.93
18912	58.91	27.55	35.94	62.06	52.99	111.9	2.648	2.364	2.328	4.216	1.287	1.089	47.69	8.49	26.56	1442	12.86
19034	58.91	27.35	88.99	62.24	55.69	114.6	2.318	2.964	3.264	4.423	1.909	1.072	44.35	10.86	27.89	1468	12.78
20174	89.34	32.35	30.08	66.93	41.892	131.23	3.502	2.064	4.298	3.172	1.164	1.207	45.29	14.50	20.77	438.84	3.34
18983	51.6	30.33	56.68	62.95	65.6	117.2	2.484	2.481	2.753	3.109	1.458	1.093	43.87	14.48	34.52	1159	9.71
20264	58.45	27.96	45.68	62.19	56.05	114.5	3.049	2.149	3.285	4.941	1.102	1.353	48.89	8.24	30.91	1378	11.97
19226	68.76	27.37	45.68	62.19	47.54	116.3	2.617	2.428	3.246	4.154	1.112	1.128	43.97	8.72	21.76	1071	9.14
19011	57.11	28.23	92.45	62.19	57.09	114.2	2.268	2.348	2.805	3.491	1.698	1.063	42.24	5.94	30.14	1162	10.14
18724	68.76	27.37	65.45	62.63	49.64	118.4	3.189	2.708	3.008	4.941	1.247	1.081	41.94	6.74	28.66	1205	10.11
19095	59.49	27.96	54.76	66.62	54.51	114	2.586	2.695	2.41	4.858	1.453	1.111	44.31	9.9	25.6	1244	10.83
19100	55.77	27.3	62.01	63.54	55.23	111	3.017	3.124	3.206	2.929	1.33	1.063	46.76	11.3	21.46	1622	14.54
20260	57.07	29.34	63.48	66.61	54.83	111.9	2.62	2.628	2.592	4.112	1.163	1.089	39.63	8.34	21.86	1165	10.35
20183	55.36	32.81	31.34	58.44	64.486	119.85	3.049	2.179	3.906	3.648	1.427	1.232	37.58	11.79	17.53	323.42	2.69
20190	53.36	31.48	32.28	56.85	60.902	114.26	2.855	2.179	3.47	3.752	2.45	1.214	39.13	11.73	14.83	322.47	2.82
20192	54.33	31.24	30.8	60.32	60.592	114.92	3.212	2.179	4.021	3.395	2.851	1.221	39.55	11.70	14.11	297.86	2.60
20193	52.64	32.9	28.78	59.97	59.308	111.95	3.098	2.179	3.501	3.578	2.171	1.231	35.73	10.01	14.82	289.39	2.63
20194	61.86	26.2	26.34	54.68	54.18	116.04	2.949	2.179	3.584	3.381	2.132	1.223	33.60	9.82	15.19	260.01	2.27
20195	57.05	29.03	31.65	56.72	63.098	120.15	3.003	2.164	3.513	3.524	2.251	1.202	40.69	11.58	15.49	291.48	2.46
19122	60.5	27.71	59.75	67.03	53.7	114.2	2.873	2.234	3.387	4.559	1.248	1.168	45.2	9.31	18.91	1203	10.43
19147	62.11	27.96	59.44	66.2	50.39	112.5	2.865	2.924	2.913	4.013	1.143	1.077	51.18	8.29	16.98	1395	12.35
19164	64.85	24.16	67.78	68.26	49.05	113.9	2.99	2.556	3.006	4.659	1.903	1.418	46.63	16.8	21.77	1365	11.94
19165	58.68	27.69	51.73	66.33	53.72	112.4	2.648	3.241	2.544	4.299	1.225	1.134	44.65	13.36	49.42	1671	14.78
Control o	ultivars																
4918	48.76	27.56	42.13	48.25	55.44	104.2	3.19	4.144	5.472	5.357	3.808	1.288	61.41	21.89	19.88	1395	13.85
4948	59.68	29.92	51.68	59.01	50.72	110.4	3.042	3.398	3.576	5.607	3.397	1.222	58.06	15.89	15.44	1516	13.68
15996	49.81	31.24	45.22	62.91	56.79	106.6	2.537	4.475	5.726	6.791	3.393	1.476	53.72	18.92	16.69	1719	16
V9231	40.25	31.31	49.21	55.97	65.55	105.8	2.327	3.356	1.369	4.987	1.375	1.058	51.36	18.97	32.26	1646	15.58

ICC	DF	FD	PLHT	PLWD	DGF	DM	BPB	APB	BSB	ASB	ТВ	SDPD	PPP	YPP	100-SDWT	YKGH	PROD
1																	
4973	63.16	28.36	54.91	68.31	50.84	114	3.02	4.569	4.468	10.171	3.726	1.375	54.03	18.77	19.09	1401	12.31

DF = days to 50% flowering, FD = flowering duration, PLHT = plant height, PLWD = plant width, DGF=Days to Grain Filling, DM = days to maturity, BPB = basal primary branches, APB = apical primary branches, BSB = basal secondary branches, ASB = apical secondary branches, TB = tertiary branches, SDPD = seed per pod, PPP = pods per plant, YPP = yield per plant, SDWT = 100-seed weight, YKGH = plot yield, PROD = per day productivity