जीनोम वाईड एसोसिएशन चने में उपज गुणों का मानचित्रण

[*सीकर एरिएटिनम* एल.]

GENOME WIDE ASSOCIATION MAPPING OF YIELD TRAITS IN CHICKPEA [*CICER ARIETINUM* L.]

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GENOME WIDE ASSOCIATION MAPPING OF YIELD TRAITS IN CHICKPEA [*CICER ARIETINUM* L.]

By

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C E R T I F I C A T E

This is to certify that the thesis entitled, **"Genome Wide Association Mapping of yield traits in chickpea [*Cicer arietinum* L.]"** submitted to the Faculty of the Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY IN GENETICS** is a record of *bona-fide* research work carried out by **Ms. Philanim W.S, Roll No. 10458** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma.

It is further certified that all the assistance and help availed during course of investigation as well as all sources of information have been duly acknowledged by her.

Date: 11.12.2017
Place: New Delhi

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Dedicated

To

My Parents

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ABBREVIATIONS

ADW	Autoclaved Distilled Water
AFLP	Amplified Fragment Length Polymorphism
AM	Association Mapping
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis Of Variance
ARF	Auxin Responsive Factor
bHLH	basic Helix-Loop-Helix
bp	base pair
bZIP	basic leucine Zipper
CD	Critical Difference
CDS	Coding DNA Sequences
CMLM	Compressed Mixed Linear Model
CTAB	Cetyl Trimethyl Ammonium Bromide
CV	Coefficient of Variation
df	degrees of freedom
DNA	Deoxyribo Nucleic Acid
dNTP	deoxy Nucleotide Triphosphate
DRR	Downstream Regulatory Region
DTF	Days To Flowering
DTM	Days To Maturity
DUF	Domain of Unknown Function
E	Environment
EDTA	Ethylene Diammine Tetra Acitic acid
FDR	False Discovery Rate
EMMAX	Efficient Mixed Model Association eXpedited
FST	Fixation index for population SubsTructure
G	Genotype
GA	Genetic Advance
GAI	Gibberellic Acid Insensitive
GAPIT	Genome Association and Prediction Integrated Tool
GBS	Genotyping By Sequencing
GCV	Genotypic Coefficient of Variation
GEI	Genotype Environment Interaction

GEn	Genotype Environment noise
GEs	Genotype Environment signal
GLM	General Linear Model
GWAS	Genome Wide Association Study
h^2	heritability
ICARDA	International Centre for Agricultural Research in the Dry Areas
ICRISAT	International Crop Research for Semi Arid Tropics
IPCA	Interaction Principal Component Analysis
ISSR	Inter Simple Sequence Repeat
K	Kindship
L	Ladder
LD	Linkage Disequilibrium
LOB	Lateral Organ Boundaries
µl	Micro litre
Myb	Myeloblastosis
ng	nano gram
NGS	Next Generation Sequencing
PC	Principal Components
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PH	Plant Height
POP	Population
P/Pl	Pods per Plant
Q	Population Structure
Q-Q	Quantile-Quantile
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RBD	Randomized Block Design
RE	Restriction Enzyme
RFLP	Restriction Fragment Length Polymorphism
SANT	Switching-defective protein 3 (Swi3)-Adaptor 2 (Ada2)-Nuclear receptor corepressor Transcription factor IIIB

SD	Standard Deviation
SNF	Sucrose Non Fermenting
SNP	Single Nucleotide Polymorphism
SPAGeDi	Spatial Pattern Analysis of Genetic Diversity
SPBP	Squamosal Promoter Binding Protein
S/Pl	Seeds per plant
SSLPs	Simple Sequence Length Polymorphisms
SSRs	Simple Sequence Repeats
STMSs	Sequence Tagged Microsatellite Sitess
STR	Short Tandem Repeat
100SW	Hundred Seed Weight
SY/Pl	Seed Yield Per Plant
TASSEL	Trait Analysis by aSSociation, Evolution and Linkage
TE	Tris EDTA Buffer
TF	Transcription Factor
US	Unanchored Scaffolds
WANA	West Asia and North Africa
ZF	Zinc Finger

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INTRODUCTION

CHAPTER 1 INTRODUCTION

Chickpea is one of the world's most important pulse crop, ranking third in world food legume production. The global area under chickpea production is about 13.98Mha, with total production of 13.7 Mt and productivity of approximately 967.6 kg/ha (FAOSTAT, 2014).

India is the world's biggest producer, with an annual production of around 9.8 Mt, covering an area of 9.9 Mha (FAOSTAT, 2014). Chickpea offers significant benefits for human health. The seed is high in protein (20-30%) and dietary fibre, contains approximately 40% carbohydrates and only 3-6% oil (Gil *et al*. 1996). Furthermore, chickpea is a good source of essential minerals such as calcium, magnesium, potassium, phosphorus, iron zinc and manganese, and has been recognized as one of the nutritionally best composed dry legumes for human consumption (Ibrikci *et al*. 2003). Chickpea is a diploid species i.e. 2n = 2x = 16. Its genome size is approximately 738 Mb (Varshney *et al*. 2013). It is highly self pollinated with an out crossing rate of less than 1%. Chickpea serves as an important source of protein in human diet and thus plays an important role in food economy of the country. These are low in fats and most of this is polyunsaturated. One hundred grams of mature boiled chickpeas contain 164 calories, 2.6 g of fat, and 7.6 g of dietary fiber and 8.9 g of proteins. Chickpeas also provide dietary phosphorous (49-53 mg/100g). According to ICRISAT, chickpeas contain on an average 23% proteins, 64% total carbohydrates (47% starch, 6% soluble sugars), 5% fats, 6% crude fibers, 3% ash. There is also high reported mineral content i.e. phosphorous (340mg/100g), Ca, Mg (140mg/100g), Fe (7mg/100g) and zinc (3mg/100g).

Being a rich and economic source of proteins, vitamins and minerals, chickpea seeds are an important commodity, especially in the diet of poor and vegetarian population of developing countries (Verma *et al*. 2015). India is the largest producer of chickpea in the world sharing about 72% of area and production. Despite major efforts, the gap between the potential yield (3-5 t ha^{-1}) and average yield (world average 0.9 t ha^{-1}) still remains large. Inspite of India being the largest chickpea producing country, a deficit exists in domestic production and demand, which is met through imports. On an average, India imports 186,000 tonnes ($US 74 million) p. a.

of chickpea (1998-2007). There is a need to increase yield of chickpea in order to meet the ever increasing demand. Yield being a complex trait, its *per se* selection is very difficult for the breeders. Indirect selection of component traits like number of pod per plant, number of seeds per pod, seed number and seed weight is generally practiced. However, two of the most important component traits that exert maximum effect on yield are seed weight and seed number per plant. Path analysis confirmed that the number of seeds per plant and 100-seed weight had the highest positive direct influence on grain yield per plant. Both traits also displayed a positive indirect effect considerably to biological yield per plant and harvest index. Thus, combined selection for seed number and weight would be fruitful to improve yield potential of chickpea (Monpara and Gaikwad 2014).

Conventional breeding methods can be a way to increase the yield of the crop but the component traits being quantitative in nature, genetic gains through direct selection for these traits were never successful. Also, the conventional breeding methods takes more time, labour and are less effective. Molecular strategies therefore can be seen as an effective method in order to overcome this bottleneck. Over the years, selection for traits using genome based methods like biparental mapping has been utilized. Linkage mapping has been a key tool for identifying the genetic basis of quantitative traits in plants. However, the linkage mapping also comes with its own disadvantage. For linkage studies, suitable crosses, sometimes limited by low polymorphism or small population size, are required. In addition, only two alleles per locus and few recombination events are considered to estimate the genetic distances between marker loci and to identify the causative genomic regions for quantitative trait loci (QTL), thereby limiting the mapping resolution (Cerda *et al*. 2012). To circumvent this the concept of association mapping has been introduced.

Association Mapping (AM) refers to the significant association of a marker locus with a phenotype trait (Gupta *et al*. 2005). Association mapping is based on the concept of linkage disequilibrium simply known as LD. Thus, AM is actually an application of LD. In other words, two markers in LD represent a non-random association between alleles, but do not necessarily correlate/associate with a particular phenotype, whereas association implies a statistical significance and refers to the covariance of a marker and a phenotype of interest (Cerda *et al*. 2012). The concept of LD was first described by Jennings in 1917, and its quantification (*D*) was developed

by Lewontin in 1964 (Abdurakhmonov and Abdukarimov 2008). The simplified explanation of the commonly used LD measure, *D* or *D′* (standardized version of *D*), is the difference between the observed gametic frequencies of haplotypes and the expected gametic frequencies of haplotype under linkage equilibrium. $D = P_{AB} - P_A P_B$, Where P_{AB} is the frequency of gametes carrying allele A and B at two loci; P_A and P_B are the product of the frequencies of the allele A and B, respectively. In the absence of other forces, recombination through random mating breaks down the LD with $D_t = D_0 (1 - r)$ t, where D_t is the remaining LD between two loci after *t* generations of random mating from the original D_0 (Zhu *et al*. 2008). Several statistics have been introduced for LD, and these measurements usually differ in how they are affected by marginal allele frequencies and sample sizes. The two most utilized statistics for LD are *D′* (Lewontin 1964) and r^2, the square of the correlation coefficient between two loci (Hill and Robertson 1968). They reflect different aspects of LD and perform differently under various conditions. *D′* only reflects the recombinational history and is therefore a more accurate statistic for estimating recombination differences, whereas, r^2 summarizes both recombinational and mutational history (Flint-Garcia *et al*.2003). For two biallelic loci, *D′* and r^2 have the following formula: $D' = |D| / D_{max}$ where; $D_{max} = \min (P_A P_b, P_a P_B)$ if $D > 0$; $D_{max} = \min (P_A P_B, P_a P_b)$ if $D < 0$ and $r^2 = D^2 / P_A P_a P_B P_b$.*D* is limited because its range is determined by allele frequencies. *D′* was developed to partially normalize *D* with respect to the maximum value possible for the allele frequencies and give it a range between 0 and 1 (Zhu *et al*. 2008). The r^2 statistic has an expectation of $1/(1+4Nc)$, where *N* is the effective population size and *c* is the recombination rate, and it also varies between 0 and 1 (Hill and Robertson 1968). Choosing the appropriate LD statistics depends on the objective of the study. Most studies on LD in animal populations used *D′* to measure population-wide LD of microsatellite data (Du *et al*. 2007). However, *D′* is inflated by small sample size and low allele frequencies; therefore, intermediate values of *D′* are unsafe for comparative analyses of different studies and should be verified with r^2 before being used for quantification of the extent of LD (Oraguzie *et al*. 2007). Although r^2 is still considered to be allele frequency dependent, the bias due to allele frequency is considerably smaller than in *D′* (Ardlie *et al*. 2002). Currently, most LD mapping studies in plants use r^2 for LD quantification because it also provides information about the correlation between markers and QTL of interest (Flint-Garcia *et al*. 2003 and Gupta *et al*. 2005).

Typically, r^2 values of 0.1 or 0.2 are often considered the minimum thresholds for significant association between pairs of loci and to describe the maximum genetic or physical distance at which LD is significant (Zhu *et al*. 2008).

Linkage disequilibrium is affected by many factors including inbreeding, population size, genetic isolation between lineages, population structure, recombination rate, linkage, artificial selection, population admixture, genetic drift, population bottleneck and natural selection. Some factors may increase the LD and others may reduce the LD.

Important statistical methods utilized for association analysis would be linear regression, analysis of variance (ANOVA), 't' test or chi-square test. There is a problem of population structure that gives conspicuous results which is always associated with association mapping. Therefore, many statistical approaches have been developed in order to combat this problem. General linear and mixed model approaches have been designed recently (Yu *et al*. 2006) and these methods have been further incorporated in TASSEL (Bradbury *et al*. 2007). For the purpose, random or gene based markers are first used in order to estimate Q and a relative kinship matrix (K), which are then fit into a mixed model bodywork for testing the marker-trait association.

The potential of localizing a QTL with high resolution is the primary advantage of AM as compared to linkage mapping. It also can identify more and superior alleles and provides detailed marker data in a large number of lines which could be of immediate application in breeding (Yu and Buckler 2006). Furthermore, AM uses breeding populations including diverse and important materials in which the most relevant genes should be segregating. Complex interactions (epistasis) between alleles at several loci and genes of small effects can be identified, pinpointing the superior individuals in a breeding population (Tian *et al*. 2011). Sample size and structure are not required to be as large as for linkage studies making it more convenient of handling. More number of alleles can be considered, if broader population is included for investigation and allele mining can be attempted by exploitation of genetic diversity in a reference population (Flint-Garcia *et al*. 2003 and Hall *et al.* 2010). AM not only identifies and maps the QTL but also identifies causal polymorphisms within a gene that are responsible for the difference between two phenotypes (Palaisa *et al*. 2003).

Considering the above facts, the present study has been undertaken with following objectives:

i. To find out the extent of genetic variation of yield traits in the association mapping population.

ii. Association mapping of yield traits in the association panel.

iii. Validation of candidate genes for seed weight in contrasting genotypes.

REVIEW OF LITERATURE

CHAPTER 2 REVIEW OF LITERATURE

With constant bulge in the world population, it is a consistent endeavor to increase the overall world food production to make food a sufficiency. Since the inception of Green revolution in the 1960's, there has been a marked increase in the food grain production. But there is still an impending gap in the production. Pulses are an important grain legume that provides high quality protein complementing cereal proteins. Beginning with the domestication of lentils (*Lens esculenta* now L. *culinaris*) in Iran 9,500 to 8,000 years ago to being used as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and utilization by the Roman Empire as a vital food source and further contribution to the soil improvement, legumes have manifested great agricultural significance for thousands of years (Graham and Vance 2003). In India, pulses can be produced with a minimum use of resources and hence, it becomes less costly even than animal protein. In comparison to other vegetables, pulses are rich in protein which are less expensive and can be cultivated as an inter-crop and also as mixed crop. Pulses are mostly cultivated under rainfed conditions and do not require intensive irrigation facility and this is the reason why pulses are grown in areas left after satisfying the demand for cereals/cash crops. Even in such conditions, pulses give better returns. Apart from this, pulses possess several other qualities such as they are rich in protein, improve soil fertility and physical structure, fit in mixed/inter-cropping system, crop rotations and dry farming and provide green pods for vegetable and nutritious fodder for cattle as well (Prospects of Pulses 2016).

Chickpea is one of the most important grain legumes. Domesticated C. *arietinum* have been deduced to have derived from its wild progenitor C. *reticulatum*, with the perceived centre of origin to be Fertile Crescent region (Ladizinsky and Adler 1976). Cultivated chickpea (C. *arietinum*) can be easily distinguished on the basis of seed size and colour as kabuli and desi. The kabuli types have larger, smoother seed and creamy in colour generally grown in the Mediterranean region including Southern Europe, Western Asia and Northern Africa and the desi types are smaller, angular, and brown seeded, grown mainly in Ethiopia and Indian subcontinent (Pundir *et al*. 1985). Covering a global area of 13.98 Mha with the overall production of 13.7 Mt, comes second only after dry beans in world food

legume production (FAOSTAT,2014). India is the world's biggest producer, with an annual production of around 9.8 Mt, representing 67% of total world chickpea production covering an area of 9.9 Mha (FAOSTAT,2014). Chickpea offers richest and cheapest benefits for human health. The seed is high in protein (20-30%) and dietary fibre , contains approximately 40% carbohydrates and only 3-6% oil (Gil *et al*. 1996). Furthermore, chickpea is a good source of essential minerals such as calcium, magnesium, potassium, phosphorus, iron, zinc and manganese, and has been recognised as one of the nutritionally best composed dry legumes for human consumption (Ibrikci *et al*. 2003). Besides, nutritive value of pulses in human diet, food legumes tend to fix atmospheric nitrogen to N- compounds to the tune of 72 to 350 kg per hectare per year and provide soil cover that helps to sustain soil health (Prospects of pulse 2016).

Though valuable in its nutritional point of view, there has not been significant increase in area and production from 1950's till 2010. However, from the last five years (2011-2015) significant growth in area and production have been recorded. India is the still by and large vegetarian in dietary habit and heavily depends upon vegetative source to meet out its daily protein requirement. India is bound to be a global leader in terms of production and consumption of pulses. Since, India is leading importer of pulses, production of pulse/ legume crops has been stagnant over the years. Consequent upon this there is widening gap between demand and supply. About 20 % of the total pulses demands are met by imports only (Singh *et al*. 2015). The existing production in India is insufficient to meet increasing demand and on average India imports 5,38329 tonnes p. a. of chickpea (FAOSTAT 2014). The Recommended Dietary Allowances (RDA) for adult male and female is 60 g and 55 g per day. The per capita availability of pulses is @ 42 g per day (Pulse status 2016). Thus, there is a huge gap on demand and supply front and on the required dosage of the legume required per person. Also the present productivity (0.9 tons/ha) for chickpea in India slacks much behind the potential productivity (3-5 tons/ha) that can be achieved.

PEM (Protein Energy Malnutrition) is a term that is constantly associated with world malnutrition. According to World Health Organization, protein energy malnutrition (PEM) refers to "an imbalance between the supply of protein and energy and the body's demand for them to ensure optimal growth and function" (Bhutia

2014). The incidence of protein energy malnutrition in children have been the subject of extensive research for several decades, and studies shows that protein energy malnutrition affects the growth and development of children especially (0-5years) (Chukwu *et al*. 2017). PEM is measured in terms of underweight (low weight for age), stunting (low height for age) and wasting (low weight for height) (Bhutia 2014). For all the indicators (wasting, stunting and underweight) the most favourable situation--low or moderate prevalences occurs in Latin America; in Asia most countries have high or very high prevalences and in Africa a combination of both these circumstances is found. Globally 161 million under five year olds were estimated to be stunted and about half of all the stunted children lived in Asia and over one third in Africa in 2013. Globally 51 million under five years olds were wasted and 17 millions were severely wasted. Approximately two third of all wasted children lived in Asia and almost one third in Africa, with similar proportion for severely wasted children in 2013 (Wardlaw *et al*. 2014). It was assumed that 7.8% of all childhood deaths result from malnutrition, of which two thirds will be attributable to low birthweight, one third directly to malnutrition (Bailey *et al*. 1996). Protein calorie malnutrition is observed in infants and young children in developing countries and includes a range of pathological conditions arising due to lack of protein and calories in the diet (Haider and Haider 1984). Malnutrition affects about 170 million people especially preschool children and nursing mothers of developing countries in Asia and Africa (Iqbal *et al*. 2006). Common protein deficiency diseases includes kwashiorkor (protein malnutrition predominant), marasmus (insufficient calorie intake) and marasmickwashiokar (both signs present for protein deficiency and calorie insufficiency and sometimes referred to as most severe form of malnutrition). Children with protein energy malnutrition may also indicate deficiencies of vitamins, essential fatty acids and trace elements, all of which may contribute to the illness. Considering all the lacks the country is currently facing, from the problem of malnutrition to insufficiency in the present chickpea production in meeting the countries demand, a necessity for a strategic and an effective effort arises. To bridge the gap nutritional wise, health wise and production wise, it would be a prudent effort to improve and increase the overall production of chickpea. Keeping that in mind, the study has been undertaken having three objectives under the purview of the experiment. A brief account of literature has been reviewed under the following heads.

2.1 Studying Variability, heritability, correlation, path analysis and AMMI analysis.

2.1.1 Variability, heritability and genetic advance

Lack of adequate variability has been viewed as one of the main difficulty in enhancing the productivity of chickpea. Study of extent and magnitude of genetic variability and its parameters in crop is required to frame suitable breeding method for the improvement of the crop. It also justifies if a trait can be subjected to experimentation to produce improved cultivars with desirable characteristics. With the preceding idea about genotype and phenotype that came from Johannsen in 1909 it was vaguely known that a trait is controlled by two entities *viz.* genotype and phenotype (environment being a part of the total phenotypic expression). Prominent efforts put forth by researchers like Nilsson-Ehle (1908) and East (1916) who gave the concept of multiple factor hypothesis proved the inheritance of quantitative characters which led to the fact that variability is due to the interaction between genotype and environment. Further understanding of traits came when Fisher (1930) first presented the method to separate the effects due to the genotype and environment. The extent of genotypic variability was presented by him with appropriate statistical method and the value was expressed as genotypic coefficient of variation. Thus it was made clear that understanding variability in a crop was paramount as traits were influenced by both genotype and environment. Variability is measured by a number of parameters *viz*. genetic coefficient of variation, phenotypic coefficient of variation, heritability estimates and genetic advance, path coefficient analysis.

Dumbre *et al*. (1984) worked out the genetic variability for nine quantitative characters in sixteen cultivars of chickpea. Pods per plant (10.4 – 95.8) exhibited the highest range of variability followed by days to maturity (87.0 -125.0). Lowest was expressed by grain per pod (1.01 – 1.4). Seed yield per plant showed highest genotypic coefficient of variation showing large amount of variability in the material for this character. High heritability with higher genetic advance was observed for the characters, seed yield per plant, 100 seed weight and seeds per pod.

Genetic variability in one hundred forty cultivars of chickpea was studied by Islam *et al*. (1984). Pods per plant and yield per plant showed wide variability where as narrow variability was shown by days to flowering and days to maturity.

Genetic variability in desi x kabuli chickpea crosses was studied by Agrawal (1985). From the study significant differences for days to flowering and maturity and 100 seed weight from P1, P2, F2s and F3s of (1) Jg-315 (Desi) x L-550 (Kabuli) and (2) TLM (Desi) x L-550 were observed. Genotypic effect played major part of the variation in these traits. In (1) days to flowering showed highest heritability of 98.8 per cent followed by days to maturity with 97.8 per cent and 88.5 per cent for 100 seed weight and in (2) these values were 99.8, 98.2 and 83.7 per cents, respectively. These together with high genetic advance recorded for these traits implied predominance of additive genetic variance and improvement of these traits by simple selection was recommended.

Agrawal (1986) conducted study of genetic variability in populations of chickpea crosses. Evaluation was done for traits like days to flowering, days to maturity, plant height, pods per plant primary branches per plant, secondary branches per plant, seeds per plant, seeds per pod, 100 seeds weight and seed yield per plant. High variability at both phenotypic and genotypic levels for some crosses was observed for seeds per plant and pods per plant. High GCV is an indication of the extent of fixable variation present in the population. For days to flowering and days to maturity, GCV and PCV estimates were similar for all crosses; and 100 seed weight, seeds per plant and plant height for some crosses, indicating the major part of variation shared by genetic component. Greater influence of environment on a trait is indicated by wide differences between GCV and PCV. Days to flowering and days to maturity for all the crosses expressed high genetic advance as percentage of mean coupled with high heritability estimates, implying additive gene action controlling the major portion of genotypic variation.

Study on genetic variability by considering stability parameters was done by Maloo and Sharma (1987) for seven characters in twenty one advance strains of chickpea. Seed yield, number of pods per plant and number of primary branches per plant expressed high genetic advance as per cent of mean coupled with high heritability.

Jivani and Yadavendra (1988) reported high genotypic coefficient of variation and phenotypic coefficient of variation for number of pods per plant and 100 seed weight. Forty two genetically diverse genotypes of chickpea were assessed for variability. Plant height, pods per plant, days to flowering and maturity, 100 seed weight and harvest index expressed high heritability estimates. The greatest genetic gains were expected for pods per plant, days to flowering and 100 seed weight.

Mishra *et al*. (1988) observed high heritability for all the characters in a study on genetic variability in one hundred and seventeen genotypes of chickpea. Number of secondary branches per plant, seed yield per plant, number of pods per plant, biological yield per plant and harvest index exhibited high heritability coupled with high genetic advance.

Variability in twelve characters of forty five varieties of chickpea in four environments was studied by Govil and Kumar (1989). They found high genetic advance coupled with high heritability estimates for days to flowering. Seed wrinkling showed highest genotypic coefficient of variation and lowest was reported for number of days to maturity.

Sadhu and Mandal (1989) observed considerable range of variability for seed number plant height, seed yield pod number and seed weight in fortyeight diverse chickpea lines. They further observed that seed weight expressed high heritability estimates coupled high expected genetic gain.

Genetic variability in twenty four genetically diverse lines of chickpea was studied by Samal and Jagdev (1989). Seed mass and yield showed high phenotypic coefficient of variation and genotypic coefficient of variation and moderately high genetic variability for plant height and days to flowering. They also reported high heritability coupled with high expected genetic advance for seed mass and yield.

Singh and Rao (1991) studied genetic variability in chickpea genotypes. Five F_2 population along with six parents were evaluated for ten yield components. Substantial genetic variability for all the characters studied were reported. The heritability estimates are also high for all the characters except pod bearing length and primary branches per plant and plant spread.

One hundred and four genotypes of chickpea evaluated genetic variability were Vijaykumar *et al*. (1991). They observed significant difference for all the eleven

characters. Seed yield per plant, total pods per plant, harvest index and plant spread exhibited highest genotypic coefficient of variation. Lowest value of both genotypic and phenotypic coefficient of variation was observed for days to maturity. High genotypic coefficient of variation along with high heritability were observed for the characters such as 100 seed weight, harvest index and to some extent, effective pods per plant. Harvest index, seed weight and effective pods per plant had high heritability with high expected genetic advance. Low heritability and expected genetic advance values were exhibited by seed yield per plant.

Rao *et al*. (1994) studied forty four varieties of chickpea. Results showed highest genetic coefficient of variation for 100 seed weight followed by secondary branches per plant, pods per plant and seed yield per plant. Also 100 seed weight and plant height exhibited high heritability coupled with high genetic advance. However they observed low heritability with high genetic advance for seed yield, pods per plant and secondary branches per plant.

Thirty random F3 progenies derived from F4 bulks of four crosses was used by Wanjari *et al*. (1996) to study the components of variability. They reported high phenotypic variation and genotypic variation for seed yield per plant and pod weight. High heritability estimates was exhibited by 100 seed weight in all the cases whereas seed yield expressed moderate to low heritability. The estimated genetic advance was very poor in all the cases.

Genetic variability was studied by Sood and Kumari (2000) in thirty two genotypes. Analysis of variance implied highly significant difference among genotypes for plant height, seeds per plant and 100 seed weight. For all characters phenotypic coefficient of variation were higher than the corresponding genotypic ones. Yield per plot and days to flowering recorded a wide range of phenotypic variability for while narrowest was recorded for seeds per pod. Maximum genetic variation was expressed by days to flowering followed by seed per plant, yield per plot and 100 seed weight. Number of seeds per plant, days to flowering and 100 seed weight exhibited high estimates of heritability coupled with genetic advance. Selection for such traits would be thus highly effective with high genetic gain. Days to maturity showed lowest genetic coefficient of variation.

Sable *et al*. (2000) studied variability in thirty genotypes. Genotypic coefficient of variation ranged from 6.37 per cent for protein content to 33.98 per cent for seed yield per plant. High estimated of genotypic coefficient of variation were exhibited by characters *viz*., seed per plant, 100 seed weight and biological yield per plant indicating high degree of variation due to genetic factors. High estimates of genetic advance accompanied with estimates of heritability were expressed for characters *viz*., seed yield per plant, 100 seed weight and biological yield per plant exhibited.

Forty genetically diverse genotypes of chickpea were evaluated for genotypic and phenotypic variability heritability and genetic advance by Arora and Jeena (2001) in 18 quantitative characters. 100 seed weight expressed highest genetic variability followed by seeds per plant. High genetic advance with highest heritability was exhibited by 100 seed weight.

Gumber *et al*. (2002) studied variability in thirty genotypes of chickpea. Pods per plant (78.0%) exhibited highest heritability followed by secondary branches (71.5%) and 100 seed weight (69.4%). The estimate of phenotypic coefficient of variation was highest for seed yield (38.64%) and seeds per pod (13.33%)

Arshad *et al*. (2003) studied 24 advance lines of chickpea for variability, heritability, genetic advance, correlation coefficients and path coefficients for yield and its components. Days to flowering, days to maturity and 100 seed weight showed high heritability with low genetic advance of indicated the influence of dominant and epistatic genes for these traits. High heritability coupled with high genetic advance of secondary branches and biological yield showed that additive gene effects plays predominant role in determining these characters.

Ozveren *et al*. (2006) investigated fifteen Kabuli chickpea genotypes for 2 years to determine the variability, heritability and correlations between yield and yield components. Direct and indirect effects of yield components on seed yield per plant were investigated. 100 seed weight showed highest genotypic variance followed by seed number per plant. Heritabilities for seed number, 1000 seed weight and number of full pods were greater than those for the other traits. Broad-sense heritabilities ranged from 5.47 per cent (days to flowering) to 51.66 per cent (seed number per plant).

Fifty genotypes of chickpea were considered by Meena *et al*. (2006) to investigate the extent of genetic variability and association of traits related to drought tolerance. For all the characters influence of environmental forces on the expression of traits was suggested by the higher magnitude of phenotypic coefficient of variation than the corresponding genotypic coefficient of variation. 100 seed weight exhibited highest phenotypic coefficients of variation and genotypic coefficient of variation followed by seed yield

Forty five genetically diverse chickpea genotypes were taken to investigate genetic variability by Singh (2006) in Pantnagar. Genotypic and phenotypic variabilities were assessed in twelve quantitative traits *viz*., Days to 50 per-cent flowering, days to maturity, reproductive period, plant height primary branches per plant, secondary branches per plant, seeds per pod, pods per plant, 100 seed mass, biological yield, seed yield per plant and harvest index. A higher coefficient of phenotypic variability than the genotypic variability indicated the influence of environmental component. Biological yield, 100-seed weight, pods per plant and seed yield per plant exhibited moderate to high heritability coupled with high genetic advance. Thus, selection based on these traits would lead to improved yield in chickpea.

Genetic variability study in chickpea genotypes was done by Ali *et al*. (2008). Assessment was done in twenty elite chickpea lines for traits like number of plant height, days to flowering, number of days to maturity, number of primary branches per plant, number of secondary branches per plant, and seed yield per plant (g). Varietal differences among the genotypes were significant ($P<0.01$). Phenotypic and genotypic variances were higher for plant height (33.29 & 32.45) and seed yield per plant (13.47 & 13.11). Highest broad sense heritability was exhibited by plant height (97.4) and seed yield per plant (97.3). Seed yield per plant (27.42) showed highest genetic advance followed by plant height (14.51). Predominance of additive effects for these characters was understood from high heritability for both the traits coupled with high genetic advance.

Three hundred sixty chickpea land races and lines were studied by Farshadfar and Farshadfar (2008) to determine the genetic variability. The traits considered were plant height, days taken for 50 per cent flowering, growth type, number of leaflet per

leaf, leaflet size, flower color, pod size, flowering period, days to maturity, pod per plant, seed color, seed shape, 100 seed weight and seed numbers per pod.

Fifty-three genotypes of chickpea were evaluated for variability, heritability and genetic advance under different environments in Himachal Pradesh by Thakur and Sirohi (2008). Analysis of variance indicated considerable genetic variability among the genotypes of all the traits. A high phenotypic and genotypic coefficient of variations were observed for 100-seed weight, seed yield per plant, pods per plant, biological yield per plant and high heritability coupled with high genetic advance were recorded for pods per plant, seed yield per plant, biological yield per plant, 100 seed weight, and plant height which indicated the predominance of additive gene effects in the expression of yield traits. seeds per pod, days to 50% flowering and days to 75% maturity exhibited high heritability with low to moderate genetic advance showed the importance of dominance and epistatic effects of these inherited traits.

A set of 30 chickpea genotypes was assessed by Borate *et al*. (2010) for range, mean, phenotypic and genotypic variances, PCV and GCV and genetic advance for 13 agronomic characters. Range of variability was appreciable for, secondary branches, plant height, days to first flowering, dry matter and grain yield. Number of pods exhibited highest values of genotypic and phenotypic variance while lowest for seed PCV showed higher values than GCV for all characters.

Twenty chickpea genotypes for various yield parameters under field conditions were studied by Malik *et al*. (2010). Significant differences between genotypes for six out of nine traits studied were revealed by Analysis of variance of yield and its components. Pods per plant recorded maximum variation followed by secondary branches per plant, biological yield, grain yield and harvest index.

Forty genotypes of chickpea (*Cicer arietinum* L.) were studied by Saki *et al*. (2010) for assessment of genetic variability. Field experiment was conducted at the experimental field of BARI, Joydebpur, during the year 2004-2005. Number of pods per plant, days to flower, number of branches per plant, plant height, 100-seed weight and seed yield per plant exhibited significant genetic variation. The highest genotypic variability was observed for number of seeds per pod and seed yield, followed by branches per plant and number of pods per plant, whereas days to maturity showed the lowest genotypic co-efficient of variability. Phenotypic variances were higher than

the genotypic variances in all the traits. Pods per plant, 100-seed weight, dry weight per plant and seed yield per plant showed high heritability coupled with high genetic advance, that indicated that usefulness of this traits to carry out effective selection. Considerable heritability was observed for number of seeds per pod, days to flowering and pod length.

Genetic variability, heritability and interrelationships for seed yield and its components (days taken to 50 per cent flowering, plant height, number of pods per plant, days taken to 90 per cent maturity, 100 seed weight) were estimated by Akhtar *et al*. (2011) in twenty advance genotypes of chickpea collected from various sources along with one check variety (Pb-2000). Analysis of Variance showed highly significant differences for all the traits among the genotypes tested. Highest seed yield of 2396 kg per ha was recorded for genotype BRC-61 whereas low yield of only 2068 kg per ha was recorded for the check variety Bunjab-2000. Genotype BRC-61 had highest weight of 100 seeds and was the earliest in maturity. Broad sense heritability ranged from 89.61 (seed yield) to 99.99 per cent for 100 seed weight. Hundred seed weight and number of pods per plant showed greatest heritabilities as compared to other traits. Phenotypic coefficient of variation (PCV) for plant height, days taken to flowering, days taken to maturity, and seed yield were higher than genotypic coefficient of variation (GCV) which means that the expression of these traits is influenced by environmental factors. Therefore, 100 seed weight and number of pods per plant as selection criterion would be effective to improve the grain yield in chickpea.

Genetic variability study in chickpea genotypes was carried out by Babbar *et al*. (2012). Forty four promising lines of chickpea were grown under late sown season in RBD with three replication. Damaged pod percentage, total number of seeds per plant and total number of pods per plant exhibited maximum genotypic coefficient of variation. High heritability coupled with medium genetic advance as percentage of mean, was shown by plant height, days to 50% flowering, days to maturity, seed yield per plant and 100 seed weight whereas, number of seeds per plant, damage pod percentage and number of pods per plant showing medium heritability and high genetic advance as percentage of mean.

Study of genetic diversity in four hundred and ninety-five accessions of chickpea collected from different agro-ecological zones of India, was carried out by

Sewak *et al*. (2012). Wide range of variability was shown by both qualitative and quantitative traits. Moderate to high heritability and genetic advance was observed for days to maturity, 100-seed weight and number of primary branches. Selection based on these characters will be useful for the improvement in yield. A considerable genetic variation for seed yield per plant was indicated by the diversity index.

Twenty two genotypes of chickpea were studied for genetic divergence and path coefficient analysis among in the mid-altitude of Meghalaya by Pandey *et al*. (2013). Significant amount of genetic variability was observed for days to flowering, plant height, number of branches per plant, 100 seed weight and number of pods per plant. Moderate to high genotypic variance was recorded for number of branches per plant, plant height, 100 seed weight, days to flowering and number of pods per plant along with moderate to high heritability and genetic advance.

Genetic variability for qualitative and quantitative traits of economic importance in one hundred and seventy nine genotypes of chickpea was carried out by Ramanappa *et al*. (2013). Highly significant differences were recorded for plant height, number of primary branches, days to fifty percent flowering, number of pods per plant, 100 seed weight, days to maturity, harvest index and grain yield per plant. A good amount of variation between genotypes was also observed for traits such as early plant vigour, testa texture seed color, seed shape and growth habit.

Studies in thirty genotypes of chickpea on genetic variability, heritability and genetic advance was done by Yadav and Yadav (2013). Characters studied were days to primary branches per plant, secondary branches per plant, 50% flowering, days to maturity, plant height, seeds per pod, 100-seed weight, pods per plant, seed yield per plant. High and significant variation was recorded for all the characters except seeds per pod. Seed yield per plant followed by pods per plant and seeds per pod exhibited maximum coefficient of variation at genotypic and phenotypic levels while minimum coefficient of variation was observed for days to maturity. High heritability coupled with high genetic advance was exhibited by characters as pods per plant, secondary branches per plant, plant height 100-seed weight and seed yield per plant. The study indicated the importance of this characters for effective selection for improvement of seed yield in chickpea.

A study on sixty genotypes of chickpea was done by Mallu *et al*. (2014) for evaluating genetic variation and heritability for plant height, days to 50 percent flowering, days to 75 percent maturity, number of pods per plant, 100 seed weight and seed yield per ha. Highest yield per hectare was showed by ICC 9636 while ICC 9002 gave the lowest. High broad sense heritability was recorded for most of the traits studied except days to 75 percent maturity and pods per plant during long rains. Agronomic traits with high broad sense heritability can be used as an effective selection criterion for crop improvement.

Study of genetic variability, correlation and path analysis for seed yield and its components was carried out in 100 advance breeding lines of chickpea under heat stress environment of Jabalpur by Kuldeep *et al*. (2014). Analysis of variance (ANOVA) revealed significant differences among the genotypes for all the trait which implied the presence of sufficient variability among the genotypes of various traits. High GCV and PCV were recorded for number of secondary branches, 100 -seed weight, number of pods per plant, seed yield per plant, total number of pods per plant and harvest index. Seed yield per plant, followed by 100 seed weight, harvest index, number of effective pods per plant and total number of pods per plant revealed high heritability and a high genetic advance.

Thirty six chickpea accessions to study the magnitude of genetic variability, heritability and genetic advance for yield and yield contributing traits were done by Peerzada *et al*. (2014). An evaluation of desi chickpea for yield and yield contributing traits was carried out. Highest mean was obtained for plant height by genotype NBeG13 and highest mean was obtained for number of pods per plant and biological yield per plant by the genotype RKG135. Genotype RVSSG1 revealed highest mean performance for seed yield per hectare while the genotype H 04-75 exhibited highest mean for days to maturity. Genotype BG3004 exhibited the highest mean score for 100 seed weight, and number of branches per plant, the highest mean score for protein content was revealed by BGD1053. Seed yield per hectare showed a high heritability coupled with high genetic advance. Number of pods per plant had high genotypic and phenotypic coefficient of variance. Number of pods per plant exhibited the highest genetic gain. Subsequent proportion of total variability was observed in these traits were due to effect of genetic causes. Hence, these may be used as an effective selection parameter in crop improvement programmes.

Twelve chickpea accessions were grown in restricted soil moisture condition. A study entitled "Evaluation of chickpea genotypes for variability in seed protein content and yield components under restricted soil moisture condition" was conducted by Singh *et al*. (2014). Highest percentage of seed protein was exhibited by Pusa1103 followed by BG 1101, Pusa 362 and BGD 112. Pusa 362 and Pusa 1103 can be used for improvement of protein content in the seed under restricted soil moisture condition. Genotypic, phenotypic coefficient of variation, heritability, genetic advance, correlation coefficients and path coefficients analysis were conducted for yield and its contributing traits along with protein and cold tolerance index. Significant differences among the genotypes were observed by ANOVA. Closer values obtained from phenotypic coefficient variation and genotypic coefficient variation indicated a lower environmental effect on the expression of various traits. High heritability with high genetic advance as per cent of mean was recorded for biomass per plant and pods per plant. This indicated the importance of these characters as selection criteria for crop improvement. A significant positive association was exhibited by biological yield and harvest index with grain yield.

Biological yield per plant, days to 50% flowering, plant height pods per plant, primary branches per plant, days to maturity and seed yield per plant, showed excellent potential in normal sowing condition, whereas harvest index, per cent crude protein and 100-seed weight under late sowing. Seed yield per plant, 100-seed weight and pods per plant showed high heritability with high genetic advance in normal sowing condition. Study of genetic variability and yield stability was done among eight cultivars of chickpea by Mohammad *et al*. (2015) in River Nile State, Sudan. Moderate differences among phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) with high heritability for traits, seed per plant, biomass, and harvest index, implied the genetic influence and thus having the possibility of genetic improvement in the trait of interest.

Twelve genotypes of kabuli chickpea were studied for phenotypic and genotypic variances, heritability in broad sense (h^2), genetic advance and genetic gain for yield and yield contributing traits for 11 characters by Peerzada *et al*. (2015). The experiment was conducted at IIPR, Kanpur, India, in a randomized block design with three replications. High value of genetic advance was recorded for, biological yield/plant and harvest index and number of pods per plant. Highest genetic gain was

recorded for number of pods per plant. Days to maturity, 100-seed weight and number of pods per plant were characterized by high broad sense heritability. seed yield (q/ha), 100-seed weight, number of pods/plant, protein content and seed yield/plant showed high genotypic and phenotypic coefficients of variability. Hence, these characters indicate the presence of a considerable proportion of total variability due to genetic causes and may be used as effective selection parameter during breeding programme for crop improvement.

Forty five genotypes of chickpea were used to study the genetic variability, heritability (h^2) and genetic advance (GA) for yield traits by Yadav *et al*. (2015). Number of primary branches per plant, number of secondary branches per plant, and number of pods per plant exhibited high genotypic and phenotypic coefficients of variability. Hundred seed weight and seed yield per plant had high genotypic and phenotypic coefficients of variability. A low genotypic and phenotypic coefficient of variability was recorded for days to 50% flowering days to maturity, seeds per pod and number of leaflets per leaf. A coupling action of high heritability with high genetic advance was also recorded for width of leaflet seed per plant and primary branches per plant, which indicated the presence of substantial amount of total variability due to genetic causes specially the additive gene effects important for determining these traits. High heritability with moderate genetic advance were exhibited by number of primary branches, number of secondary branches, pod length, plant height and pods per plant showed which indicated the influence of environment for these traits. Low heritability percentage with low and moderate genetic advance for days to maturity indicated greater influence of environment.

Bharadwaj *et al*. (2016) observed high heritability coupled with high genetic advance as per cent of mean for days to fifty per cent flowering and 100 seed weight, while high heritability with moderate genetic advance for days to maturity. Plant height and grain yield per plant showed low to moderate heritability coupled with low to moderate genetic advance as per cent mean.

Dehal *et al*. (2016) studied twenty-five chickpea accessions under two different environments at Palampur location. The study was done to estimate correlation and path coefficient to determine effective selection criteria. ANOVA revealed a high and significant differences showed by the genotypes in both environments for all the characters. Biological yield per plant, days to 50%

flowering, plant height pods per plant, primary branches per plant, days to maturity and seed yield per plant, displayed excellent potential in normal sowing condition, whereas harvest index, per cent crude protein and 100-seed weight for late sowing. Seed yield per plant, 100-seed weight and pods per plant showed high heritability with high genetic advance in normal sowing condition.

One hundred and forty four diverse genotypes of chickpea were evaluated for 11 quantitative characters by Kumar *et al*. (2016). The experiment was laid out at Student's Instructional Farm of Narendra Deva University of Agriculture and Technology, Narendra Nagar, Kumarganj, Faizabad (U.P.) during *rabi* season of 2011-12. Number of pods per plant followed by seed yield per plant and 100 seeds weight showed high PCV and GCV implying the importance of these traits as main yield contributing characters. High heritability estimates were observed for plant height, days to 50% flowering, number of seeds per pod, pods per plant, 100 seeds weight and seed yield per plant. seed yield per plant, number of pods per plant, 100 seeds weight and number of seeds per pod showed high expected genetic advance as per cent of mean. High genetic advance as per cent of mean coupled with high heritability for 100 seeds weight and number of pods per plant would be helpful for indirect selection for improvement in seed yield.

A variability study in chickpea genotypes was conducted by Kumar *et al*. (2016). It revealed maximum days to maturity (151) in BG 1107 and Pusa 1063 and minimum days (136) to maturity in three genotypes (Pusa 209, BG 2002 and Pusa 1090). Good range of variation in biological yield among genotypes that ranged from 19.43 g to 36.83 g was also observed. BG 1105 exhibited maximum number of pods (68) and the range varied from 45.53 to 68. Number of branches ranged from 9.37 to 15.33. The variation for 100-seed weight ranged from 17.05 g to 32.31 g. The highest seed yield plant-1 was recorded for genotype BG 2002 (12.68 g), Pusa 362 (12.73g) and AT-2-1184 showed minimum seed yield (7.83 g). Number of pods per plants, number of branches (88.12), harvest index, protein content (78.65), plant height and biological yield showed high heritability estimates. High heritability coupled with high genetic advance was observed for 100-seed weight, pods per plant, number of branches per plant, biological yield and harvest index. protein content and plant height showed high heritability with low genetic advance.

A study to estimate heritability for seed yield and its attributing traits in seventeen diverse genotypes of desi chickpea varieties was done by Salgotra (2016). High heritability with high genetic advance were observed for number of pods per plant, 100 seed weight and pod length where as plant height and seed yield per plant exhibited high heritability with moderate genetic advance implying the predominance of additive-gene action in these traits.

Heritability and genetic advance for yield contributing traits in population of chickpea was studied by Singhal *et al*. (2016). Heritability is a relative degree at which a trait or a character is transmitted to offspring. Days to fifty percent flowering and 100 seed weight showed high heritability with high genetic advance where as high heritability with moderate genetic advance was observed for days to maturity. They reported low to moderate heritability coupled with low to moderate genetic advance for plant height and seed yield per plant. Traits with high heritability and high genetic advance implies the predominance of additive gene action with good response to selection.

Study of genetic variability for grain yield and its attributing characters was conducted in thirty eight chickpea varieties by Tiwari *et al*. (2016). Both genotypic and phenotypic variances were highly significant for all the traits with little higher phenotypic coefficient of variation. The low phenotypic and genotypic coefficients of variations differences revealed low environmental influences on the expression of these characters. Seeds per pod, seed yield per plant, total number of pods per plant, days to maturity and harvest index showed high heritability with high genetic advance.

Sowjanya *et al*. 2017 studied genetic variability, heritability and genetic advance in chickpea. High grain yield per plant was exhibited by the genotype C-210 (10.03g) followed by C-206 (8.34g), C-226 (7.51g) and C-207 (7.13g) based on the mean performance. High significant difference among 13 chickpea genotypes for all characters was revealed by ANOVA indicating the presence of high variability. Seed yield per plant (22.16, 29.87), biological yield (19.39, 24.82) and harvest index (17.64, 25.69) exhibited high genotypic coefficient of variation (GCV) and phenotypic coefficient variation (PCV). High estimates of heritability coupled with high genetic advance was observed for number of pods per plant, harvest index and biological yield. Genetic advance as percent of mean was highest for biological yield

(31.2) followed by harvest index (24.97), number of pods per plant (24.23), number of branches per plant (22.31), seed yield per plant (21.39) and seed index (21.39). High heritability with high genetic advance as % mean was exhibited by number of pods per plant (71.2, 24.23) and biological yield (61, 31.20).

An investigation on 18 chickpea genotypes including one local check to estimate the genetic variability and character association during *rabi* 2016-17 under randomized block design having three replications was done by Srivastava *et al*. (2017). Data was recorded for plant height, number of primary branches per plant, days to 50% flowering, days to maturity, biological yield per plant, number of pods per plant, harvest index, seed-index and seed yield per plant. Analysis of variance indicated significant differences among the genotypes for all the characters studied. High estimates of genetic advance expressed as percent of mean was recorded for seed-index and number of pods per plant. Number of pods per plant followed by days to maturity and seed-index revealed high estimates of heritability (bs) as well as genetic advance. Seed yield per plant exhibited significant and positive correlation both at genotypic and phenotypic level with biological yield per plant, harvest-index and only at genotypic level with number of pods per plant in the correlation study. Hence, these characters may serve as effective selection criterion for yield improvement in chickpea.

4.1.2 Correlation analysis

Renukadevi and Subbalakshmi (2006) studied correlation and path coefficient for eleven traits including seed yield in fifty genotypes of chickpea. Harvest index, plant height, number of pods per plant, biological yield per plant, number of primary branches, 100 seed weight was positively and significantly correlated withseed yield.

Talebi *et al*. (2007) studied thirty six genotypes during 2005-2006 seasons for their yield performance. From the study, significant and positive relationships were observed between the number of secondary branches and plant height, between 100 seed weight and plant height, between day to maturity and number of primary and secondary branches, between day to heading and day to maturity between seed yield and number of pod per plant and number of seeds per pod, between seed yield and biomass and harvest index were determined significantly. Significant but negative

relationships were revealed between seeds per pod and number of secondary branches and between number of pod per plant and 100 seed weight.

Vaghela *et al*. (2009) studied character association and path analysis for seed yield in chickpea. The study inferred the higher magnitudes of genotypic correlation coefficients as compared to their corresponding phenotypic correlation coefficients for most of the traits. Positive and significant correlation was recorded between seed yield per plant and number of seeds per pod, biological yield, harvest index, number of primary branches per plant, number of pods per plant and 100-seed weight at both genotypic and phenotypic levels.

Gaikwad and Monpara (2012) studied correlation among yield and its component traits in chickpea and observed that seeds per plant, biological yield per plant, and 100 seed weight were positively and significantly correlated with seed yield per plant in all the populations. A highly significant positive association was found among seeds per plant, pods per plant and biological yield per plant. However, negative association within index were implied pairs of important traits like pods per plant and harvest index, biological yield and harvest index. Therefore, combining these traits into one genotype are likely to have problems. There was a high contribution of direct effects on seed per plant followed by 100-seed weight. Seeds per plant showed high indirect contribution through pods per plant, plant height, primary branches per plant and biological yield per plant, therefore, while deciding selection criteria of genotypes in chickpea breeding, these parameters should be given more consideration.

Correlation among various quantitative traits in chickpea was studied by Naveed *et al.* (2012). A positive and significant correlation at genotypic level was obtained for number of secondary branches per plant, biomass per plant, number of seeds per pod, 100 seed weight and pod number per plant, but the same was obtained positive and highly significant at phenotypic level

Avinash *et al*. (2013) studied correlation in twenty two genotypes of chickpea. Days to flowering, number of branches per plant, plant height, number of pods per plant and 100 seed weight showed positive and significant correlation with grain yield per plant. Days to flowering, number of pods per plant, and 100 seed weight were the

main traits that contributed to genetic diversity in chickpea as analysed by percentage contribution of individual characters.

Study of correlation and path coefficient analysis with a set of 105 chickpea accession was done by Jivani *et al*. (2013). Significant correlation between seed yield per plant and number of pods per plant, biological yield per plant and harvest index were observed in both genotype and phenotype levels. Hundred seed weight, number of pods per plant, and plant height exhibited significant and positive association with biological yield per plant.

Kuldeep *et al*. (2014) carried out correlation study for seed yield and its components in advance breeding lines of chickpea. The results showed positive significant correlation between seed yield per plant and plant height, number of pods per plant, number of primary branches, number of secondary branches, hundred seed weight and number of effective pods per plant indicated the importance of these traits contributing to yield.

Forty eight genotypes of chickpea were used by Desai *et al*. (2015) to study character association for yield and its component traits. The analysis between various traits revealed higher magnitude of genotypic correlation as compared to their corresponding phenotypic correlations. This revealed the inheritance of relationship among the characters. Number of effective branches per plant, number of pods per plant and harvest index at genotypic as well as phenotypic levels exhibited a positive and significant association with seed yield per plant.

Mukesh *et al*. (2016) conducted correlation study in chickpea genotypes. The results indicated positive and significant phenotypic correlation of seed yield per plant with number of branches, number of pods per plant, biological yield per plant, 100-seed weight and harvest index.

Salgotra *et al*. (2016) studied seventeen diverse genotype of desi chickpea variety and reported positive and significant correlation was observed for seed yield per plant with plant height and number of pods per plant, number of seeds per pod, 100-seed weight. Seed yield per plant showed a negative correlation with days to 50% flowering and number of secondary branches. Plant height followed by days to maturity, number of primary branches per plant, number of seeds per pod and 100-seed weight exhibited highest direct and positive effect on seed yield.

Srivastava *et al*. (2016a) conducted correlation study for quantitative traits in a mapping population developed for seed traits in chickpea (*Cicer arietinum* L.). The result obtained indicated a significant positive and high correlation in the recombinant inbred lines for 100-seed weight and number of seeds per pod with yield.

Srivastava *et al*. (2016b) studied variability in the RIL population of the cross SBD 377 and BGD 112 and indicated that the developed RILs could be used for mapping seed yield traits. Seed yield per plant being a dependent trait cannot be selected *per se* and selection therefore needs to be exercised through 100 seed weight and total number of seeds. Lines having greater seed number than the best parent with more seed weight could be obtained when diverse crosses are made. This indicated that the negative correlation between seed weight and seed number can be broken without causing any yield penalty up to a level where the source does not become limiting. However, this requires extensive wide crossing and careful evaluation of the segregants.

Tiwari *et al*. (2016) conducted correlation study in thirty eight chickpea genotypes. Genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients in most of the traits. Hundred seed weight, harvest index, number of effective pods per plant, total number of pods per plant, seeds per pod, number of primary branches and plant height were the most important characters that indicated positive association with seed yield per plant.

4.1.3 Path coefficient analysis

Mardi *et al*. (2003) studied four hundred eighteen landraces of desi chickpea at Karj, Iran in 1995-96. The examination of traits showed that, 100 seed weight, seed number per plant and seeds per pod varied highly. The results of analysis indicated that seeds plus pod weight and number of seed per plant had greatest direct effect on seed yield.

Arshad *et al*. (2003) studied path coefficients for yield and its components in 24 advance lines of chickpea. High direct effects were contributed by biological yield and harvest index although the later had negative association with grain yield. Two parameters (biological yield and harvest index) should be given more consideration while deciding the selection criteria of genotypes for rainfed conditions as high

indirect contribution via biological yield was noticed by most of the yield components.

Path coefficient analysis was studied by Talebi *et al*. (2007) in thirty six genotypes during for their yield performance. Harvest index showed the greatest direct effect on seed yield (p.c. = 0.901**). Further, its indirect effect on seed yield was more positive through number of pod per plant, number of seeds per pod and biomass plant heights but negative and low through 100 seed weight, days to heading and maturity and number of primary branches. Thus the two traits biomass (biological yield) and harvest index should be considered while selection for high seed yields in kabuli chickpea.

Path analysis were studied by Singh (2007) in 45 diverse genotypes of chickpea (*Cicer arietinum* L.). Biological yield per plant and pods per plant had highly significant correlation and high direct effect on seed yield. Indirect contributory component to seed yield were 100 seed-mass, pods per plant, harvest index and secondary branches per plant.

Path analysis for seed yield in chickpea was studied by Vaghela *et al*. (2009). Path coefficient analysis showed highest positive direct effect of biological yield per plant followed by harvest index towards seed yield. The indirect effects were higher and positive for most of the traits through biological yield per plant. Biological yield per plant and harvest index were found to be the most important trait for selection to improve seed yield in chickpea.

Gohil and Patel (2010) evaluated twenty-two accessions of chickpea under rainfed conserved soil moisture condition for character association and path analysis. Hundred seed weight and harvest index showed a positive and significant association with seed yield. The maximum direct positive effect to seed yield was recorded from biological yield per plant where as days to maturity revealed maximum negative effect to seed yield. Therefore, harvest index, 100-seed weight, earliness and pods per plant could be considered as the most important traits that can be used in advancement of yield in chickpea under rainfed condition.

Path coefficient analysis under Mediterranean conditions in chickpea was studied by Yucel and Anlarsal (2010) in twenty two chickpea genotypes. The traits studied were seed weight, plant height (cm), branch number per plant, seed number

per plant, total pod number per plant, full pod number per plant, first pod height and empty pod number per plants. A positive and significant association was observed among seed yield and harvest index and seed number. Harvest index exhibited the greatest direct effect on seed yield as revealed by path coefficient analysis. Both correlation and path analysis showed harvest index as a major direct contributor to seed yield.

Zali *et al*. (2011) studied seventeen chickpea genotypes number of seeds per plant and 100-seed weight explained 96% of total yield variation. It can be concluded that seed yield in chickpea can be improved by selecting an ideotype having greater number of secondary and primary branches per plant, as well as higher number of pods per plant, number of seeds per plant and 100-seed weight.

Naveed *et al.* (2012) studied path coefficient for various quantitative traits in chickpea. The results indicated that higher direct effects were found for 100 seed weight and biomass per plant on grain yield per plant. Number of days taken to flowering and maturity had higher direct effects. 100-seed weight, number of grains per plant, and grain yield per plant was found to be useful as selection criteria for crop improvement breeding programme.

Study of path coefficient analysis in 105 chickpea accession was conducted by Jivani *et al*. (2013). Maximum positive direct effect was recorded for harvest index, followed by number of pods per plant, biological yield per plant and 100-seed weight towards seed yield, through path coefficient analysis. Thus they were considered to be most important characters for selection to get higher yield in chickpea.

Dehal *et al*. (2016) conducted path coefficient analysis in twenty-five chickpea accessions and recorded high direct effect towards seed yield per plant through biological yield and harvest index. Primary branches per plant and number of pods per plant showed negligible direct effect but had high indirect via biological yield and harvest index. Hence, the study indicates selecting plants with high biological yield, pods per plant, primary branches per plant and high test weight for greater seed yield would help in realising rewarding selection in chickpea.

Srivastava *et al*. 2016c studied path coefficient in RIL population derived chickpea crosses and reported that direct selection for the grain yield could be

illusory. However, indirect selection through yield contributing traits with high heritability might be more effective than the direct selection for yield. Maximum direct positive contribution to seed yield was from 100 seed weight and maximum direct negative was from days to flowering. 100-seed weight, total number of seeds and pods per plant were most important characters that can be used to improve yield in chickpea.

Path coefficient analysis in thirty eight genotypes by Tiwari *et al*. (2016) revealed that among the different yield contributing characters, days to 50% flowering, 100- seed weight, seeds per pod, total number of pods per plant, number of secondary branches per plant, harvest index, plant height, days to maturity influenced seed yield per plant directly. Thus selection for yield through these traits would be effective.

4.1.4 Cluster analysis

Jeena *et al*. (2002) evaluated forty genotypes of chickpea for 18 quantitative traits by using Mahalanobis' D^2 analysis, which resulted in the formation of 12 diverse clusters. Most of the clusters showed greater diversity among them. Maximum intra-cluster distance was recorded for cluster IV, while highest inter-cluster distance was observed between cluster VIII and XII.

Jeena *et al.* (2005) evaluated eighty chickpea genotypes based on D^2 values eighty genotypes were grouped into eleven clusters. The highest numbers of genotypes were included in cluster I followed by cluster II. No definite relationship was observed between genetic diversity and geographical distribution. Based on inter-cluster distances, crossing between BGM-419 and KPG33 are expected to produce a broad spectrum of variability for yield and its components.

Dwevedi and Gabriyal (2009) studied twenty five genotypes of chickpea using Mahalanobis's D^2 Statistics. The twenty five chickpea genotypes were grouped into six clusters. The cluster I showed largest cluster with eight genotypes. Highest inter cluster distance was observed between cluster III and cluster VI, followed by cluster I and VI. Three characters *viz*. harvest Index, 100 seed weight and number of pods per plant contributed maximum in manifestation of genetic diversity.

Forty four promising lines of chickpea were grown in RBD with three replications under late sown condition and subjected to D^2 cluster analysis by Babbar

et al. (2012). Depending upon the genetic constitution of the genotypes, forty four genotypes were grouped into nine clusters. The maximum intra cluster distance was found in cluster IV followed by cluster I, cluster VI and cluster VIII. Inter cluster values varied from 2.75 to 9.02. Days to maturity, total pods per plant, biological yield, 100 seed weight and seed yield per plant considered as selection criteria, while selecting superior genotypes under late condition. High yielding advanced breeding lines *viz*., Phule G 00108, JG14, JSC56, JG 9602974, BG3005, AKG70, PG03110, were found suitable under late sown condition.

Prakash and Shekhawat (2012) studied thirty genotypes of chickpea. Genotypes grouped then into nine clusters based on D^2 values. Cluster III and IX were more divergent. Genotype GNG 2000 formed mono genotypic cluster with earliest flowering and maturity. Number of pods per plant contributed most towards genetic divergence followed by 100- seed weight and days to 50 % flowering.

Genetic diversity in 27 chickpea genotypes was studied by Syed *et al*. (2012) using Mahalanobis D^2 analysis. The genotypes under study were grouped into five clusters. Cluster II had the highest number of genotypes (11) and Cluster I contained the lowest. Cluster I produced the highest mean value for number of pods per plant. The inter cluster distances were much higher than the intra cluster distances indicating presence of large amount of variability between the genotypes for the traits. Cluster V exhibited the highest intra cluster distance while the lowest distance was observed in cluster I. Lowest inter cluster distance was recorded between cluster III and V and largest inter cluster distance between cluster I and II. Considering all the characters, it was suggested that the genotypes BD6603, BD6548 and BD6549 could be used as parents for future breeding programs to develop high yielding varieties of chickpea.

Jivani *et al*. (2013) studied genetic diversity in 105 diverse genotypes of chickpea (*Cicer arietinum* L.) applying Mahalanobis' D^2 analysis which resulted in 13 diverse clusters. Cluster I had the maximum (46) genotypes followed by cluster II (18), cluster III (16) and cluster IV (12). Four clusters namely V, IX, X and XI were important with respect to construct plant ideotype including seed yield per plant which could be utilized for further crop improvement programme. While cluster VII to XIII were solitary clusters. Maximum genetic distance (D) was found between cluster V and XI followed by between cluster V and X. Three traits *viz*,, 100-seed

weight, seed yield per plant and number of pods per plant contributed more than 63% of towards total genetic divergence.

Jayalakshmi *et al*. (2014) studied 113 chickpea genotypes which were grouped into eight clusters. Cluster I was the largest with sixty nine genotypes followed by cluster III with twenty one genotypes. Maximum inter cluster distance was observed between VII and cluster VIII followed by clusters VI and VII whereas, days to 50 % flowering followed by 100 seed weight, plant height and number of pods contributed maximum towards genetic diversity.

Malik *et al*. (2014) studied genetic diversity in 113 desi chickpea genotypes through cluster analysis. High variances were observed for days to flowering, maturity, plant height, pods per plant, biological yield and harvest index. cluster analysis revealed the genotypes grouped in four clusters. Genotypes with early flowering and early maturity were gathered in cluster I while cluster II showed dominant contribution for harvest index, grain yield per plant and number of pods per plant. The grouping of genotypes would be of practical value to chickpea breeders in identifying the genotype with desired trait for utilization in breeding program for genetic improvement of chickpea.

Parhe *et al*. (2014) studied fifty one genotypes of chickpea for genetic divergence using Mahalanobis's D^2 Statistics. The fifty one chickpea genotypes were grouped into five clusters. The maximum inter-cluster distance was observed between cluster I and V, followed by cluster II and V. Three characters *viz*. 100 seed weight, number of pods per plant and days to 50% flowering contributed maximum in manifestation of genetic diversity.

Temesgen *et al*. (2015) conducted cluster analysis in forty-nine kabuli chickpea genotypes planting them in 7 x 7 simple lattice design at Jari in 2006/07. Cluster analysis grouped 49 kabuli chickpea genotypes into eight clusters. Except between cluster I and IV distances between these clusters are significantly different for all the cluster combinations. This indicated that there was an opportunity to bring about improvement through hybridization of genotypes from different clusters and subsequent selection from the segregating generations. Thus, crosses involving Cluster V with cluster VII and Cluster III with Cluster VII, were suggested to exhibit high heterosis and could result in segregants with higher seed yield.

Kumar *et al*. (2016) conducted studies to estimate genetic diversity for quantitative traits in chickpea (*Cicer arietinum* L.) in the field of the department of Genetics and Plant Breeding, Janta Vedic College, Baraut, Baghput (U.P.) during the crop season 2003 to 2004 under rainfed condition. Cluster I, VII, III and VI, respectively possessed 12, 10, 6 and 5 genotypes. Means of various traits for each character showed that genotypes with maximum 100-seed weight, number of branches, biological yield, pods per plant and seed yield per plant were placed together in cluster II. Genotypes with maximum harvest index were placed in cluster VI and genotypes with maximum days to maturity were placed in cluster VII.

4.1.5 AMMI analysis

Selection and identifying chickpea (*Cicer arietinum* L.) cultivars with wide adaptability across diverse farming environments is required before recommending them to achieve a high rate of cultivar adoption. Sabaghpour *et al*. (2012) carried out multi environment trials over 3 years at 5 locations taking 17 genotypes of autumn chickpea in Iran. Additive main effect and multiplicative interaction (AMMI) were used to understand the GE interaction pattern. 68.36% of the total sum of squares was attributable to environmental effects, only 15.9% to genotypic effects and 13.55% to GE interaction effects as showed by Analysis of variance (ANOVA) of grain yield. AMMI1 Biplot between first principal component and mean grain yields for genotypes and environments implied that high yielding genotypes were not stable cultivars regarding final yield. Four chickpea mega environments in Iran were identified by AMMI2 mega-environment analysis. The first mega environment contained locations, Ghachsaran and Lorestan, where genotype Arman was the winner; the second mega environment contained locations at Gorgan, where genotype FLIP 98-126C was superior. The tertiary mega environment contained locations in Ilam, where genotype FLIP 98-82C was superior and the location of Kermanshah made up the other mega environment, with FLIP 98-201C as superior.

The genotype × environment interaction has a direct influence on genotypes stability and adaptability in different environmental conditions. Plant breeders breed for genotypes that show general adaptability or for genotypes that have specific adaptability for specific environments. Twenty chickpea genotypes grown in a randomized complete block design with three replications under two rainfed and irrigated conditions for 4 consecutive growing seasons (2008-2011) were studied by

Rashidi *et al*. (2013) for understanding the stability of the genotypes. AMMI analysis showed the predominance and significant ($p<0.01$) influence of genotypes (G), environments (E) and genotype × environment interaction (GEI) on chickpea grain yield indicating the presence of genetic variation and possible selection of stable entries. The 81.62% of the total sum of squares was justified by environmental fluctuations exhibiting maximum variation for the trait and that the environments were diverse, with large differences among environmental means causing most of the variation in grain yield. GEI was further partitioned by principal component analysis. The first three multiplicative axis terms (PCA1, PCA2 and PCA3) explained 48.37, 25.54and 16.17% (90.08%) of GEI sum of squares, respectively. According to AMMI1 biplot G2, G8, G11, G14 and G19 in adaptive group 1 revealed specific adaptability for rainfed environments: E3, E5 and E7 with grain yield less than mean and positive interaction. Genotypes G4, G5, G6, G12, G16 and G17 in adaptive group 2 showed specific adaptation for irrigated environments E4, E6 and E8 with high grain yield more than mean yield and positive interaction. The accessions G3, G7, G13, G18 and G18 in adaptive group 3 on the IPCA= 0 exhibited stability and general adaptability with grain yield close to mean yield and negligible interaction. The entries G1, G9 and G10 in adaptive group 4 were identified with specific adaptability for irrigated environment E2 with positive interaction and G20 (adaptive group 5) was screened with general adaptability for stress and non-stress environments (close to IPCA = 0) with high grain yield more than mean yield and negligible interaction.

Different methods have been developed and employed to estimate of genotype by environment interaction (GEI) to understand stability in crop plants. Fourteen kabuli type chickpea genotypes were assessed for seed yield in four stations over three successive years (2010-2013) at west highlands of Iran by Kanouni *et al*. (2015). Randomized complete block design was used in all test environments with four replicates. significant differences between genotypes, locations, and interaction between these two sources were revealed by combined analysis of variance for seed yield. V4 and V2 had the highest (1163.58 kg ha^{-1}) and the lowest seed yield (759.07 kg ha^{-1}), respectively as exhibited by the mean seed yield of genotypes averaged over environments. Significant GE interaction indicated that chickpea genotypes showed various responses to different environments and the stability analysis could be performed. Several stability parameters were employed to investigate GEI and to

identify the best performing stable genotypes. V5, V8 and V3 were identified as the most stable genotypes according to Wricke's ecovalance, stability variance, Plaisted method, and genotypic stability. Based on CV, regression coefficient and MS(GE), V1 and V5 were found to be stable and adapted to diverse environments, and the other genotypes distributed among stability statistics. Twelve test environments were divided into two mega environments based on the AMMI biplot. These mega environments included very cold districts like Maragheh and similar areas, and relatively softened regions of Kurdistan and similar environments. V6 and V4 exhibited better adaptability for these two mega environments. Hence, two genotypes, V4 (FLIP 00-39C) and V6 (FLIP 99-26C) could be recommended as new cultivars to chickpea farmers for autumn sowing in west areas of Iran.

Chickpea is the major pulse crop cultivated in Ethiopia. However, its production is constrained due to genotype instability due to environmental variability. A study was conducted to understand the magnitude of environmental effect on yield of chickpea genotypes and to investigate the stability and adaptability of genotypes under different agro-ecologies by Tilahun *et al*. (2015). Randomized complete block design (RCBD) with four replications was used to evaluate seventeen genotypes in five locations. Various stability indices were used to assess stability and genotype by environment performances. Highly significant ($P \leq 0.01$) differences for genotypes, environments and their interaction were revealed by combined analysis of variance (ANOVA) for yield and yield components. The significant interaction showed genotypes respond differently across environments. DZ-2012-CK-0001 (2933 kg/ha), Arerti (3219 kg/ha), Arerti (3560 kg/ha) DZ- 2012-CK-0013 (2675 kg/ha) and Arerti (2019 kg/ha), respectively were top performing genotypes at Akaki, ChefeDonsa, Debre Zeit, Dembia and Haramaya. 74.45% of the variance were explained by the first two PCs. Based on ASV value, DZ-2012-CK-0002 were most stable genotypes. Arerti and DZ-10-4 were most widely adapted genotypes based on the AMMI biplot. Dembia and Haramaya were most discriminative environments for genotypes. Favorable environment for genotype were Debre Zeit and ChefeDonsa. Genotypes DZ-2012-CK-0004, DZ-2012-CK-0010, DZ-2012-CK-0013, DZ-2012-CK-0007 and DZ- 10-4 are recommendable to Akaki, ChefeDonsa, Debre Zeit, Dembia and Haramya, respectively.

Five elite chickpea genotypes along with three check varieties were studied for stability by Balapure *et al*. (2016). The study was conducted in eight environments during *rabi* 2011-12 season at Pulses Improvement Project, Mahatma Phule Agricultural University, Rahuri to check their stability and randomized block design with three replications was used as the layout of the experiment. The AMMI analysis of variance suggested broad range of diversity among genotypes for seed yield as the mean sum of square for genotypes was significant. G x E mean sum of square was significant for seed yield which indicates that the performance of genotypes was differential over the environments. The proportion of sum of square for G x E for seed yield kg/plot was 26.04 %. The environmental variances are highly significant for all the characters. Stable performance over all environment (non-interacting) for seed yield kg/plot was exhibited by three genotypes *viz*., Phule G-07102, Phule G-09103 and Digvijay. The environments E3 (sowing date 1/11/ 2011), E4 (sowing date 16/11/2011) and E5 (sowing date 1/12/2011) had good conditions for most of the genotypes. The PCA score for these three environments were nearly zero indicating all genotypes produced fairly stable seed yield.

A set of 40 pearl millet genotypes along with one check, Dhanshakti (G30), were evaluated at three different agro climatic zones during the year 2014 for grain iron (Fe) and zinc (Zn) contents using Atomic Absorption Spectrometry by Anuradha *et al*. (2017). The genotypic effect contributed 58.3% and 52.8% of the total variation for grain Fe and Zn content, respectively. Interaction component (G X E) contribution was to the total variation was also relatively high (39.7% and 32.5% for grain Fe and Zn). Stable genotypes were identified using both AMMI and GGE biplot analysis. Genotypes; PPMI 708 (G40), PPMI 1102 (G25) and PPMI 683 (G39) for grain Fe content, whereas PPMI 708 (G40), PPMI 1116 (G24) and PPMI 683 (G39) for grain Zn content were found to be desirable. The Pearson correlation coefficient for grain Fe and Zn content showed that both traits are highly associated ($r = 0.8$, $p < 0.01$) and these traits did not associate significantly with grain yield. Hence, there is possibility for simultaneous improvement of both grain Fe and Zn content without compromising for grain yield.

Study of stability with AMMI analysis was done in twelve chickpea genotypes by Bharadwaj *et al*. (2017). High significance of main effect due to years, locations

and first order interactions (year x location) was indicated by standard multi-factor analysis of variance. The main effect for genotype, first order interaction (varieties x locations), (variety x year) and second order interaction (varieties x locations x year) were highly significant. The highly significant interactions indicated selection of stable variety and reported that varieties need to be tested in severe years and locations. The two stability components, IPCA 1 and IPCA 2 axes also found highly significant ($P<0.01$). Partitioning of the variance component (%) showed that genotypic effects contributed 14.21% to the total variation. 39.50% were due to environmental influence and 46.29% due to GEI. Chickpea varieties JG-226 (0.67), JG-130 (1.03) and Vaibhav (1.33) were the three most stable varieties with higher than grand mean yield, as shown by AMMI stable value (ASV). All these chickpea varieties were early maturing. Portrait shows the JG-14 (G4) exhibited specific adaptability for environments: E1, E2, E3, E7, E8 and E9 with grain yield less than mean. Positive interaction between varieties and environments of first adaptive group were implied by the same sign on the IPCA axis. Varieties JG-63 (G6), JG-130 (G8), JG-226 (G9), Vaibhav (G10), JAKI-9218 (G11) and Vishal (G12) (adaptive group 2) revealed specific adaptation for environments E10, E11 and E12 with high grain yield, more than mean yield and also showed positive interaction.

A study to determine the yield stability and adaptability of desi type chickpea genotypes (*Cicer arietinum* L.) by understanding the interaction between chickpea genotypes with the environment (GxE) was conducted by Funga *et al*., 2017. Seventeen chickpea genotypes were evaluated for two cropping years (2012-2013 and 2013-2014) at four locations i.e., eight environments (locations x years combination). Chickpea grain yield was significantly ($p<0.01$) affected by genotypes, the environments and GXE interaction, indicating that the varieties and the test environments are diverse. The first two principal components IPCA1 and IPCA2 explained 32.7% and 20.4% respectively explaining cumulatively 53.1% of the total variation. This indicates that the interaction of 17 chickpea genotypes with eight environments was predicted by the first two principal components. AMMI1 biplot analysis showed five adaptive categories of genotypes based on the similarities in their performance across environments. The AMMI2 biplot generated by using genotypes and environmental scores for the first two IPCAs revealed positioning of the five genotype groups into four sectors of the biplot. Two genotypes in GC5 (G5

and G11) were found suitable with high yield across environments, low IPCA1 score, low AMMI stability value (ASV) and yield stability index (YSI). G5 was released as a new variety, 'Dimtu' and registered in official varieties catalogue of Euthopia, 2016.

2.2 Association mapping of yield traits in the association panel and to validate candidate genes for seed weight in contrasting genotype

Marker trait association can be studied by both family based linkage analysis and association mapping. In family based linkage analysis, the precision of gene mapping depends on the size of mapping population, genetic variation present in the population, and number of molecular markers used. Despite linkage mapping being used for gene mapping in crop plants, it gives low resolution results, is costlier and deciphers fewer alleles in a relatively longer time scale (FlintGarcia *et al*. 2003, Gupta *et al*. 2005, Stich *et al*. 2006 and Rossa *et al*. 2007). Low resolution in family based linkage mapping is the result of fewer recombination events that happens since experimental crossing in the near past (Jannink and Walsh 2002). Linkage disequilibrium-based association mapping is an alternative approach to overcome the limitations of family based linkage approach (Rossa *et al*. 2007).

There is a switch in the preference for gene mapping from conventional FBL based mapping to LD based association mapping (Goldstein and Weale 2001), which is the most powerful approach to utilize natural variation in the form of ex situ conserved crop genetic resources. Association mapping is an approach that utilizes the principle of LD to bird eye natural population for discerning marker-trait associations (Flint-Garcia *et al*. 2003). LD refers to historically increased nonequilibrium (reduced level of recombinations) of specific alleles at various loci. From determining complex trait variation down to the sequence level by taking advantage of historical and evolutionary recombination events at the population level has been possible by the inception of association mapping, also known as linkage disequlibrium (LD) mapping (Nordborg and Tavare 2002). The concept of LD has been vastly incorporated in human study to map and finally clone genes conferring complex genetic traits by deliberating the degree of LD level statistically (Risch and Merikangas 1996, Weiss and Clark 2002, Chapman *et al*. 2003 and Taniguchi *et al*. 2006). This idea was extended to plants in 2001 and a markedly enhanced mapping resolution over F1-derived mapping populations was accounted (Thornsberry *et al*. 2001).Association mapping offers so much more over conventional FBL mapping.

The accessibility of large genetic variation in the form of germplasm gives greater allele coverage and economizes time and input compared to tedious and expensive biparental mapping populations, and most significantly provides higher resolution due to the exploitation of relatively higher number of recombination events throughout the history of germplasm development. Furthermore, AM also offers the possibility of using historically measured phenotypic data (Kraakman *et al*. 2004 and Kraakman *et al*. 2006). The general approach of association mapping (AM) includes six steps as outlined (i) a collection of diverse genotypes as association panel selected that may include landraces, elite cultivars, wild relatives and exotic accessions, (ii) a comprehensive and accurate phenotypic data's collected for the traits such as, yield, stress tolerance or quality related traits of the selected genotypes over the years or locations (iii) the panel is then scanned with suitable molecular markers (AFLP, SSRs, SNPs) most commonly used is SSRs (iv) population structure and kinships are determined to avoid false positives. Further population structure arising from recent migration and population admixture can be controlled by Genomic Control (Devlin *et al*. 2004). Other methods used *viz*, Structured Association (Pritchard *et al*. 2000) where population structure is studied and Unified Mixed Model (Yu *et al*. 2006) that utilizes the population structure and relative kinship studies followed by (v) quantification of LD level using different statistics like D, D' or r^2. Finally, (vi) genotypic and phenotypic data are correlated by handling effective statistical software enabling tagging of molecular marker positioned in close proximity of gene(s) underlying a specific trait. Consequently, the tagged gene can be mobilized between different genotypes and/or cloned and annotated for a precise biological function (Al-Maskri *et al*. 2012).

Mapping power is the probability of detecting the true marker-trait associations in a set of association panel using association mapping approach and is dependent on (i) the kind of gene action of the trait of interest (ii) size and composition of population (iii) the evolution and extent of LD in the genomic region harboring the loci for trait(s) being mapped and mapping population (iv) accuracy and efficiency of phenotyping, genotyping and method of data analysis and field design. A more efficient data collection and larger population size can increase the power of AM. Special statistical tools aids in determining the false recovery rates (Benjamini and Hochberg 1995) or false positives (Type 1 error) such as permutation (Churchill

and Doerge 1994). The presence of population structure in the panel can give obscure results and therefore to overcome the slack; Pritchard *et al*. (2000) developed a technique known as structured association (SA). Structured association (SA) uses Bayesian approach (Marttinen and Corander 2010) principal to search for sub-populations using Q matrix to derail any false positives. The program STRUCTURE (Pritchard and Wen 2004) is used to estimate Population structure (Q-matrix) and kinship coefficient (K-matrix) in the subpopulations. Recently, another approach called a mixed linear model (MLM) established by Yu *et al*. (2006) to bloc structure information (Q-matrix) and kinship information (K-matrix) in AM analysis. Further the Q+K MLM model performance was more efficient in highly structured population of Arabidopsis in comparison to any other model that used Q- or K-matrix alone (Zhao *et al*. 2007). Some mixed model approaches are also used in combination to QTL and LD, wherein, QTLs or already known genes are used as a priori information in association mapping (Thumma *et al*. 2005). This is the efficient approach in AM that reduces the number of markers and populations size and increasing the accuracy and power of marker-trait associations (Ball 2005). Association mapping after its successful application in human genetics has found its way in plant genetics to help decipher complex quantitative traits. LD-based powerful association mapping tools has demonstrated a remarkable flourish in the field of crop genomics since its inception. With the current methodological developments to minimize spurious associations and false positives in structured populations, applications and study of AM has been extended from model plant Arabidopsis to field crops such as rice, wheat, maize, barley, sugarcane and forage grasses. The increasing number of AM studies in crop species indicates the potential of this approach in all plant species in near future. Furthermore, advancements to develop more cost-effective sequencing technologies for efficient genome sequencing of crop plants will certainly accelerate progress in genome-wide association studies and also help in achieving the discovery of rare and common alleles (Estivill and Armengol 2007) and epigenomic information about the trait of interest. This will enhance the power of LD-based association mapping for deciphering true associations to make possible its effective utilization in crop breeding programs.

A method called genome wide GBS (Genotyping by Sequencing) is most efficiently and preferably used for the purpose. This is a method to delineate single

nucleotide polymorphisms (SNP) so as to perform genotyping studies, such as genome-wide association studies (GWAS) in the arena of genetic sequencing, using restriction enzymes to reduce genome complexity and genotype multiple DNA samples. It is relatively inexpensive and has been used in plant breeding. GBS was first developed by Elshire *et al*. (2011). In brief the protocol includes extraction of high molecular weight DNAs and digestion with specific restriction enzyme (RE) defined previously by frequent cutting in the major repetitive fraction of the genome. *ApeKI* is the most commonly used RE. Sticky ends of the DNA fragments are then ligated using barcoded adapter. PCR amplification is then performed to increase the fragment pool. GBS libraries are further sequenced and next-generation sequencing technology is performed producing about 100 bp single-end reads. The generated raw and initial sequence data are filtered, sorted and aligned to a reference genome. SNPs are then deciphered from the aligned tags and scored to obtain genotypic information. Once a large-coverage, species-wide SNP production has been identified, it is possible to quickly and efficiently call known SNPs in newly sequenced samples. GBS method have been widely used in chickpea to unveil candidate genes for plant height, seed protein content, seed iron and zinc content, drought tolerance in chickpea have been delineated in the past (Kujur *et al*. 2016, Upadhyaya *et al*. 2016a, Upadhyaya *et al*. 2016b and Thudi *et al*. 2014).

GBS has now become an attractive method to decipher candidate genes for different traits not only in chickpea but in many other crops as well. Though a recent development, GBS has laid the foundation of better possibility in understanding the trait to its genotypic core. Genotyping-by-sequencing is a novel application of NGS protocols for discovering and genotyping SNPs for crop improvement. The low cost and affordability of GBS makes it an attractive approach to be utilized in different mapping study and saturate breeding populations with a high density of SNP markers. As the quantity and quality of sequence information generated per run keeps projecting and improving, which allows better multiplexing and lower costs per samples, GBS has become a cost-competitive alternative to other whole genome genotyping platforms. Thus, GBS would facilitate the transfer and incorporation of the validated candidate genes for desirable traits to different genetic background through appropriate breeding methods.

The screening and genotyping of informative markers in individual genotypes/whole association panels for trait association mapping requires massive costs in terms of time, labour and resources due to low genetic polymorphism in chickpea. Kujur *et al*. (2014) combined pooled DNA analysis (with 616 genic microsatellite markers) and individual genotype (large structured association panel) genotyping as an alternative time-saving and cost-effective pool-based trait association mapping approach. Identification of candidate genes for complex quantitative traits in chickpea through association mapping would require high throughput genotyping and large-scale validation of numerous informative genic microsatellite markers. Through this approach seven seed weight-associated transcription factor gene-derived microsatellite markers (with minor allele frequency [15 %) were identified in desi and kabuli chickpea. In the contrasting desi and kabuli genotypes strong marker allele effects of the five transcription factors with increasing seed weight were apparent. Nine such markers linked with three major quantitative trait loci that explained 23.5–34.7 % of the total phenotypic variance of on chromosomes 1 (CaqSW1.1: 73.5–74.5 cM and CaqSW1.2: 79.3–81.3 cM) and 2 (CaqSW2.1: 65.7–67.5 cM) controlling 100-seed weight in chickpea were deciphered by bi-parental linkage mapping by incorporating 241 of the informative gene-based microsatellite markers. Further four transcription factor genes (DUF3594, bZIP, DUF1635 and SBP) controlling seed weight in desi and kabuli chickpea were revealed by the combinatorial approach of trait association mapping, bi-parental linkage mapping, differential expression profiling, and high-resolution microsatellite-single nucleotide polymorphism marker-based haplotyping/linkage disequilibrium mapping. The new cost effective approach can be used in the rapid identification of candidate genes by large-scale trait association mapping and for traits improvement that is of agricultural importance in crop species including chickpea through the development of functional markers.

Thudi *et al*. 2014 studied the genetic basis of tolerance to drought and heat stresses in 300 accessions, including 211 mini-core collection accessions of chickpea through a comprehensive association mapping approach. Phenotypic data were collected on the panel for drought tolerance related root traits, heat tolerance, yield and yield component traits in India (Patancheru, Kanpur, Bangalore) and three locations in Africa (Nairobi, Egerton in Kenya and Debre Zeit in Ethiopia) for 1–7

seasons. Three sub-populations were identified using admixture model in STRUCTURE. The pairwise linkage disequilibrium (LD) estimated using the squared-allele frequency correlations (r^2; when $r^2<0.20$) was found to decay rapidly with the genetic distance of 5 cM. Both the genome-wide and candidate gene-sequencing based association mapping approaches were conducted to establish marker-trait associations (MTAs) using 1,872 markers (1,072 DArTs, 651 single nucleotide polymorphisms [SNPs], 113 gene-based SNPs and 36 simple sequence repeats [SSRs]) with phenotyping data employing mixed linear model (MLM) analysis with optimum compression with P3D method and kinship matrix. From the analysis, 312 significant MTAs were identified where a maximum number of MTAs (70) was identified for 100-seed weight. A total of 18 SNPs from 5 genes (*ERECTA*, 11 SNPs; *ASR*, 4 SNPs; *DREB*, 1 SNP; *CAP2* promoter, 1 SNP and *AMDH*, 1SNP) were significantly associated with different traits. This study provide significant marker trait associations for drought and heat tolerance in chickpea that can be employed after proper validation, in molecular breeding to develop superior varieties with enhanced drought and heat tolerance.

Two inter-specific mapping populations (Pusa 1103 × ILWC 46 and Pusa 256 × ILWC 46) to scan the major genomic region(s) underlying QTL(s) governing pod number trait in chickpea was done by Das *et al*. (2015). The study used a whole-genome, NGS resequencing-based mQTL-seq (multiple QTL-seq) strategy. >8 million high-quality homozygous SNPs with respect to the reference kabuli chickpea were revealed for low and high pod number-containing parental accessions and homozygous individuals (constituting bulks) from each of these two mapping populations through the whole-genome resequencing. From the identified 2,264 non-synonymous and 23,550 regulatory SNPs, the functional significance of the physically mapped SNPs was evident with 8–10% of these SNPs-carrying genes associated to transcription factors and disease resistance-related proteins. Two major genomic regions harbouring robust pod number QTLs (Caqa PN4.1: 867.8 kb and Caqa PN4.2: 1.8 Mb) were narrowed down into the high-resolution short QTL intervals (Caqb PN4.1: 637.5 kb and Caqb PN4.2: 1.28 Mb) on chickpea chromosome 4 by the utilization of these mined SNPs in Δ (SNP index)-led QTL-seq analysis and their correlation between two mapping populations based on mQTL-seq. The regulatory (C/T) and coding (C/A) SNPs-containing one pentatricopeptide repeat (PPR) gene

corresponding to pod number at a major QTL region in chickpea was delineated by the combine analysis of mQTL-seq-derived one novel robust QTL with QTL region-specific association analysis. High pod number-containing parental accessions and homozygous individuals of two mapping populations revealed pronounced anther, mature pollen and pod-specific expression of the target gene especially during pollen and pod development. Thus, vital usefulness for expediting genomics-assisted breeding and genetic enhancement of crop plants, including chickpea can be realized by the proposed mQTLseq-driven integrated strategy that shows promise in rapid genome-wide scanning of potential candidate gene(s) underlying trait-associated high-resolution robust QTL(s).

Chickpea (*Cicer arietinum* L.) comes as the second most important cool season food legume cultivated in arid and semiarid regions of the world. A set of 187 genotypes representing both desi and kabuli types comprising of both international and exotic collections, and with protein content ranging from 13.25% to 26.77% was used by Jadhav *et al*. (2015) to understand the extent of variation for protein content in chickpea germplasm, and to find markers associated with the trait. For the analysis twenty-three SSR markers representing all eight linkage groups (LG) amplifying 153 loci were used. Population structure analysis identified three subpopulations. General linear and mixed linear models were used to take care of population structure in the analysis and to avoid any spurious results by incorporating corresponding Q values of principal components in the analysis. Nine significant associations representing four QTLs in the entire population was shown by marker-trait association (MTA) analysis identified. Further ten significant MTAs representing five QTLs were identified by subpopulation analyses, four of which were common with that of the entire population. Two most significant QTLs linked with markers TR26.205 and CaM1068.195 were present on LG3 and LG5. Gene ontology search identified in the region of significant MTAs, 29 candidate genes on LG3. The findings will be helpful in identification of closely linked markers for protein content in chickpea concentrating on LG3 and LG5 for and their further utilization in nutritional quality improvement through molecular breeding programme.

High quality SNPs (26785 and 16573) differentiating two parental genotypes of a RIL mapping population were discovered by Kujur *et al*. (2015a) by GBS based assay using reference desi and kabuli genome. An ultra-high density (0.20–0.37 cM)

intra-specific chickpea genetic linkage maps were constructed by intergrating 3625 and 2177 SNPs out of the total into eight desi and kabuli chromosomes. Identification of 33 major genomic regions harbouring 35 robust QTLs (PVE:17.9–39.7%) associated with three agronomic traits was possible through one of the constructed high-resolution genetic map and were mapped within, 1 cM mean marker intervals on desi chromosomes. Rather than a traditional QTL map-based cloning method, the extended LD (linkage disequilibrium) decay (,15 cM) in chromosomes of genetic maps have made it feasible to use a rapid integrated approach (comparative QTL mapping, expression profiling, QTL-region specific haplotype/LD-based trait association analysis, and gene haplotype-based association mapping) to narrow-down one major seed weight (SW) robust QTL region. The study revealed favourable natural allelic variants and superior haplotype-containing one seed-specific candidate embryo defective gene regulating SW in chickpea. This vital and thorough information on ultra-high-resolution genetic maps, QTLs/genes and alleles/haplotypes-related genomic information generated and integrated strategy for rapid QTL/gene identification gives the possibility for their genetic enhancement of crop plants including chickpea by expediting genomics-assisted breeding applications.

Ninety two diverse chickpea accessions were sequenced by Kujur *et al*. (2015b) and identified 44844 high-quality SNPs by pertaining to a seed and pod trait-specific association panel using reference genome- and de novo-based GBS (genotyping-by-sequencing) assays. An association panel of 211 chickpea genotypes including the 92 sequenced accessions were formed and GWAS (genome-wide association study) was performed to delineate 22 major genomic loci showing significant association (explaining 23–47% phenotypic variation) with 100-seed weight, seed number/plant and pod number/plant.Through QTL mapping eighteen trait-regulatory major genomic loci underlying 13 robust QTLs were validated and mapped on an intra-specific genetic linkage map. Favorable natural allelic variants and one superior haplotype in the upstream regulatory region of a CesA-type cellulose synthase (Ca_Kabuli_CesA3) gene regulating high pod and seed number/ plant (explaining 47% phenotypic variation was revealed by a combinatorial approach of GWAS, QTL mapping and gene haplotype-specific LD mapping and transcript profiling in chickpea. Higher cellulose accumulation for normal pollen and pollen tube growth was the result of the up-regulation of the superior gene haplotype

correlated with increased transcript expression of Ca_Kabuli_CesA3 gene in the pollen and pod of high pod/seed number accession. Therefore, a combinatorial genome-wide SNP genotyping-based approach has the potential to understand complex quantitative agronomic traits and decipher trait-regulatory genomic loci (candidate genes) for genetic enhancement in chickpea.

Verma *et al*. (2015) studied a segregating population of 177 RILs derived from an intra-specific cross between C. *arietinum* SBD377 (Desi bold seeded, 100 seed weight- 48 g, seed no./ plant- 31) and C. *arietinum* BGD112 (Desi small seeded, 100 seed weight-15.4 g, seed no./plant- 153) grown in the fields at NIPGR, India to map candidate genes for seed traits in chickpea. Genotyping-by-Sequencing (GBS) was used for large-scale SNP discovery and simultaneous genotyping of recombinant inbred lines (RILs) of an intra-specific mapping population of chickpea. Out of the total 119,672 raw SNPs discovered, 3,977 high quality SNPs of which 39.5% were present in genic regions were obtained after stringent filtering. One of the most saturated intra-specific genetic linkage maps of chickpea was constructed using the SNP genotyping data having 3,363 mapped positions including 3,228 SNPs on 8 linkage groups spanning 1006.98cM at an average inter marker distance of 0.33cM. The map was utilized to identify 20 quantitative trait loci (QTLs) associated with seed traits which accounted for phenotypic variations ranging from 9.97% to 29.71%.684 putative candidate genes were revealed through analysis of the genomic sequence corresponding to five robust QTLs whose expression profiling indicated that 101 genes exhibited seed specific expression.

Genotyping by sequencing (GBS) method was used by Upadhyaya *et al*. (2016a) to delineate candidate for seed protein in 336 diverse accessions of chickpea. The study revealed eight major genomic regions harbouring robust QTLs on six chromosomes of an intra-specific high-density genetic linkage map (1.56 cM map-density) governing seed-Fe and Zn concentrations (39.4% combined phenotypic variation explained/ PVE). A structured population of 92 sequenced desi and kabuli accessions was taken to genotype 24620 SNPs discovered from genome-wide GBS (genotyping-by-sequencing) and 13 known cloned Fe and Zn contents-related chickpea gene-orthologs. 16 genomic loci/genes associated (29% combined PVE) with seed-Fe and Zn concentrations were identified by large-scale genotyping of 16591 SNPs and phenotyping-based GWAS (genome-wide association study).

Further QTL mapping validated SNPs in the genes linked tightly with eight QTLs associated for 11 trait-associated. Differential-regulation with seed-specific expression, including of 16 trait-associated genes particularly in accessions/mapping individuals with contrasting level of seed-Fe and Zn contents was evident. Novel functional non-synonymous and regulatory SNP allelic-variants from 16 known/candidate genes, including three strong trait-associated genes (encoding late embryogenesis abundant and yellow stripe-like 1 protein, and vacuolar protein sorting-associated protein) and eight major QTLs regulating seed-Fe and Zn concentrations in chickpea were deciphered by the rapid integrated genomic strategy. These informations and results are vital to be incorporated and deployed in marker-assisted genetic enrichment to develop nutritionally-rich iron/zinc-biofortified chickpea cultivars.

Delineating potential genes/alleles for complex seed-protein content (SPC) is essential for quality trait improvement of chickpea through marker-assisted breeding. A structured population of 336 sequenced desi and kabuli accessions [with 150–200 kb LD (linkage disequilibrium) decay] was utilized in an integrated genomics-assisted breeding strategy encompassing trait association analysis, selective genotyping in traditional biparental mapping population and differential expression profiling was utilized for the first-time by Upadhyaya *et al*. (2016b). The study was done to understand the complex genetic architecture of quantitative SPC trait in chickpea. High-throughput genotyping information of 16376 genome-based SNPs (single nucleotide polymorphism) discovered from the panel was subjected to GWAS (genome-wide association study). SPC trait associated seven most effective genomic loci (genes) [10-20% with 41% combined PVE (phenotypic variation explained)] in chickpea was deciphered. A comparable level of association potential of the identified seven genomic loci with SPC trait was observed regardless of the diverse desi and kabuli genetic backgrounds. The integrated approach discovered in six potential candidate genes regulating SPC trait in chickpea, a diverse naturally occurring novel functional SNP allelic variants. Of these, a zinc finger transcription factor gene associated non-synonymous SNP allele exhibiting strong association with SPC trait was found to be the most promising in chickpea. To accelerate marker assisted genetic improvement by developing nutritionally rich chickpea cultivars with enhanced SPC the informative functionally relevant molecular tags scaled-down is recommendable.

A high throughput multiple QTL-seq strategy was employed in two intra (ICC 4958 × *C. arietinum* kabuli accession ICC 8261)-specific and inter (*Cicer arietinum* desi accession ICC 4958 × *C. reticulatum* wild accession ICC 17160) - RIL mapping populations. The study was done to identify the major QTL genomic regions governing flowering time in chickpea by Srivastava *et al*. (2017). Identification of functionally relevant potential genomic loci using a user-friendly, economical, effective and simpler genomics-assisted breeding strategy is vital for rapid genetic dissection of complex flowering time quantitative trait in chickpea. The bulks were constituted by pooling the homozygous individuals of extreme flowering time phenotypic trait from each of two aforesaid RIL populations. Whole genome resequencing delineated 1635117 and 592486 SNPs exhibiting differentiation between early and late flowering mapping parents and bulks. On chickpea chromosome 4, two longer (907.1 kb and 1.99 Mb) major flowering time QTL genomic regions into the high-resolution shorter (757.7 kb and 1.39 Mb) QTL intervals were narrowed down by the multiple QTL-seq analysis using these mined SNPs in two RIL mapping populations. This essentially identified GI (GIGANTEA) genes regulating flowering time in chickpea and regulatory as well as coding (non-synonymous/synonymous) novel SNP allelic variants from two efl1 (early flowering 1). Interestingly significant impact of evolutionary bottlenecks on these loci during chickpea domestication were deduced from the strong natural allelic diversity reduction (88–91%) of two known flowering genes especially mapped at major QTL intervals as compared to that of background genomic regions (where no flowering time QTLs were mapped; 61.8%) in cultivated vis-à-vis wild Cicer gene pools. Higher association potential of coding non-synonymous and regulatory SNP alleles mined from efl1 (36–49%) and GI (33–42%) flowering genes for early and late flowering time differentiation among chickpea accessions was evident. Delineation from multiple intra-/inter-specific mapping populations of chickpea inferred the robustness, importance and validity of two functional allelic variants-containing genes localized at major flowering time QTLs was apparent. Natural allelic diversity-based domestication pattern of flowering time and genomics-aided crop improvement to develop early flowering cultivars of chickpea can be expedite from the functionally relevant molecular tags deciphered.

MATERIALS AND METHODS

CHAPTER 3 MATERIALS AND METHODS

3.1. Plant material

The Association Mapping population consisted of 380 chickpea genotypes which included germplasm lines, landraces obtained from WANA (West Asia and North Africa) region through ICARDA, training population developed by ICRISAT and released varieties, breeding lines from different institutes (ANNEXURE I). Association mapping was done on this panel of 380 genotypes through Genome Based Sequencing (GBS). A subset of 96 genotypes based on three year phenotypic studies was formed. This was used to validate the candidate gene for seed weight and seed number with earlier reported and selected gene based markers for the traits.

3.2. To find out the extent of genetic variation of yield traits in the association mapping population

Phenotypic data on three hundred and eighty lines grown in Randomised Block Design (RBD) at 2 locations viz., genetics fields of IARI (28.0800^{o} N, 77.1200 E) and IARI Regional Research Centre, Dharwad (15.4602^{o} N, 75.0102 E) were recorded for 3 consecutive years (2014-15, 2015-16, 2016-17) during *rabi* and off seasons at different growth stages of the crop *viz.*, at vegetative stage, reproductive stage, maturity stage and at harvest stage. Data was recorded on seven yield contributing traits viz., (i) Days To Flowering (DTF) (ii) Days To Maturity (DTM) (iii) Plant Height (PH) (iv) Number of Pods per Plant (PP) (v) Number of Seeds per plant (SN) (vi) 100 Seed Weight (SW) (vii) Seed Yield per plant (GY). The data was recorded on 5 random plants for all the characters except for grain yield which was taken on row basis.

Table 3.2: Set of observations recorded for seed yield and its related component in 380 association panel

S.No.	Agronomic Traits	Mode of observation
1.	**Days To 50% Flowering**	Number of days from planting to the completion of 50%flowering in the population
2.	**Days To Maturity**	The number of days from sowing to the physiological maturity was observed as days to maturity.
3.	**Plant Height**	Measured in centimeters (cm) at maturity from ground level to canopy.
4.	**No. of Pods per Plant**	The total number of pods per plant.
5.	**Number of Seeds per Plant**	The total number of seeds per plant
7.	**100 Seed Weight**	Weight of randomly selected (50-100) seeds.
8.	**Seed Yield**	Weight of total seeds produced from one plant.

3.2.1. Statistical analysis

The phenotypic data in respect of different traits studied were subjected to the following analysis.

3.2.1.1. Analysis of variance

The data for different traits were statistically analysed on the basis of model described by Cochran and Cox (1950) for randomised block design. The significance was tested by referring to t-table (Fisher and Yates 1963) for randomised block design.

$$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{g}_i + \mathbf{r}_i + \mathbf{e}_{ij}$$

where, Yij=Phenotypic observation on i^{th} genotype in j^{th} replication.

$\boldsymbol{\mu}$ = general mean

$\mathbf{g}_i$ = effect of i^{th} genotype

r_j= effect of j^{th} replication.

$\mathbf{e}_{ij}$= random error associated with i^{th} genotype in j^{th} replication.

Table 3.3: Analysis of variance (ANOVA) table for Randomised Block Design (RBD)

Source of Variation	D.F	Sum of squares	Mean sum of squares.	Expected M.S.S	F value
Replication	(r-1)	R.S.S	R.M.S.S (M_r)	$\sigma^2e + g\sigma^2r$	R.M.S/E.M.S (M_r / M_e)
Treatments	(t-1) OR (g-1)	Tr. S.S	Tr. M.S.S(M_g)	$\sigma^2e + r\sigma^2g$	Tr. M.S/E.M.S (M_g / M_e)
Error	(r-1)(g-1)	E.S.S	E.M.S.S (M_e)	σ^2e	
Total	(rg-1)				

where, r = number of replications,

g = number of genotypes or treatments.

d.f = degrees of freedom

S.S = Sum of squares.

M.S.S = Mean sum of squares.

M_r, M_g and M_e = Mean sum of squares due to replication genotype and error, respectively.

Error variance (σ^2e)

Genotype variance (σ^2g) = Tr. M.S.S - E.M.S.S/ r , 'OR' ($M_g - M_e / r$)

where Tr. M.S.S = Treatment mean sum of squares.

E.M.S.S = Error mean sum of squares.

r = number of replications.

Phenotypic variance (σ^2p) = $\sigma^2g + \sigma^2e$

where, σ^2g= Genotypic variance

σ^2e = Environmental variance

M.S. due to genotypes were tested against the error variance using 'F' test at P = 0.05 or P = 0.01

3.2.1.2. Mean

Mean value of each trait was obtained by dividing the sum of sample values by corresponding number of observations.

$$\text{Mean} = \sqrt{\Sigma X_{ij}}/\mathbf{N}$$

Where

x_{ij} = any observation in i^{th} genotype and j^{th} replication,

N = Total number of observations.

3.2.1.3. Range

Lowest and highest values for traits were recorded.

3.2.1.4. Standard Error

Standard error of difference of two means was calculated with the help of error mean square from the analysis of variance table.

$$\text{Standard error (m}\pm) = \sqrt{2EMS/r}$$

Where,

EMS = error mean sum of square,

r = number of replications

3.2.1.5. Critical difference (CD)

Critical differences for all the traits were calculated to compare the treatment means. Critical differences were calculated with the help of standard error for the differences of two means and tabulated value of 't' at 5 per cent level of significance and at error degree of freedom.

Critical difference (CD) = $\sqrt{2EMS}$ / r x t at 5 % probability at error degree of freedom.

3.2.1.6. Standard Deviation

Standard deviation (SD, also represented by σ) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points is close to the expected value (mean) of the set, whereas a high standard deviation indicates that the data points are spread out over a

wider range of values. The SD of a random variable, statistical population, the data set is the square root of its variance.

$$\sigma = \sqrt{\Sigma(x-\mu)^2/N}$$

3.2.1.7. Estimation of phenotypic (PCV) and genotypic (GCV) coefficients of variation

The coefficients of variation were calculated by using the formula suggested by Burton and Devane (1953).

- Genotypic co-efficient of variation (GCV)

  GCV (%) $= (\sqrt{\sigma^2 g} / \text{Mean}) \times 100$

- Phenotypic co-efficient of variation (P.C.V)

  PCV (%) $= (\sqrt{\sigma^2 p} / \text{Mean}) \times 100$

  where, $\sigma^2 g$ = Genotypic variance

  $\sigma^2 p$ = Phenotypic variance

  μ = Mean of the trait.

3.2.1.8. Heritability (h^2)

Heritability in a broad sense was estimated according to the formula given by Allard (1960) and expressed as a percentage.

$$\mathbf{(h^2)} = \frac{\sigma g2}{\sigma p2} \times 100$$

Where, $\sigma^2 g$ = Genotypic variance and

$\sigma^2 p$ = Phenotypic variance

Heritability (broad sense) estimates were categorized into high, moderate and low by Robinson *et al*., (1963).

Heritability (%)	Classification
5-10	Low
10-30	Medium
30-60	High
>60	Very high

3.2.1.9. Genetic advance

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It measures genetic gain under selection.

$$GS = K \times h^2 \times \delta_p$$

Where, K = standardised selection differential

H_2= heritability of the character under selection

δ_p = phenotypic standard deviation

The estimates of GS have the same unit as those of mean. The genetic advance from mixture of purelines or clones should be calculated using h^2 (bs) and from segregating populations using narrow sense heritability h^2 (ns) estimates.

3.2.1.10. Correlation

Correlation coefficient is an important and frequently preferred used statistical tool for assessing associations among traits (Kumar *et al*. 2012). Different opinions have been put forth of obtaining such a correlation, but currently the correlation coefficient is usually estimated using the between-group and within-group mean squares in an analysis of variance (Steel and Torrie 1980). Correlation is a method of statistical evaluation used to understand the strength of a relationship between two, numerically measured, continuous variables (say, height and weight). If a correlation is found between two variables, it means that a systematic change in one variable would bring a systematic change in the other variable. The variables alter together over a certain period of time. If there is correlation found, depending upon the numerical values measured, this can be either 'Positive' or 'Negative'.

- A positive correlation occurs if one variable increases simultaneously with the other, i.e. the high numerical values of one variable relate to the high numerical values of the other.
- A negative correlation occurs if one variable decreases when the other increases, i.e. the high numerical values of one variable relate to the low numerical values of the other.

The most used measure of dependence between two quantities is the Pearson correlation coefficient commonly called as the "correlation coefficient". It is deduced by dividing the covariance of the two variables by the product of their standard deviation. In simple words, Pearson's product-moment coefficient is the measurement of the linear dependence between two variables X and Y giving a value between +1 and -1. +1 indicates the strongest positive correlation possible, and -1 indicates the strongest negative correlation possible. Therefore, the closer the coefficient to either of these numbers the stronger the correlation of the data it represents. On this scale 0 indicates no correlation, hence values closer to zero highlights weaker or poorer correlation than those closer to +1/-1.

The population correlation coefficient $\rho_{X,Y}$ between two random variables X and Y with expected values μ_X and μ_Y and standard deviations σ_X and σ_Y is defined as

$$\rho_{XY} = \mathbf{corr}\,(X,Y) = \frac{\mathbf{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E[(X-\mu_X)(Y-\mu_Y)]}{\sigma_X \sigma_Y}$$

Where, E is the expected value operator, cov means covariance, and corr is widely used as an alternative notation for the correlation coefficient.

3.2.1.11. Path coefficient analysis

The direct and indirect contribution of various traits to yield were calculated through path coefficient analysis as suggested by Wright (1921) and elaborated by Dewey and Lu (1959).

The following set of simultaneous equations were formed and solved for estimating direct and indirect effects.

$$r_1Y = P_1Y + r_{12} P_2Y + r_{13} P_3Y + \ldots\ldots\ldots + r_{1i} P_i Y$$

$$r_2Y = r_{21}P_1Y + P_2Y + r_{23} P_3Y + \ldots\ldots\ldots + r_{2i} P_i Y$$

$$r_kY = r_{k1}P_1Y + r_{k2}P_2Y + r_{k3}P_3Y + \ldots\ldots\ldots + P_kY$$

Where,

r_1Y to r_kY = coefficients of correlation between casual factors 1 to i and dependent character Y.

P_1Y to P_kY = direct effects of characters 1 to i on character Y.

r_{12} to r_k-1,1 = Coefficient of correlation among casual factors.

The above equations were written in a matrix form as under.

$$\begin{pmatrix} r_1Y \\ r_2Y \\ . \\ . \\ . \\ r_kY \end{pmatrix} = \begin{pmatrix} 1 & r_{12} & r_{13}.....r_{1i} \\ r_{21} & 1 & r_{23}.....r_{2i} \\ . & & \\ . & & \\ . & & \\ r_{k1} & r_{k2} & r_{k3}.....1 \end{pmatrix} \begin{pmatrix} P_1Y \\ P_2Y \\ . \\ . \\ . \\ P_kY \end{pmatrix}$$

Then $B = [C]^{-1} . A$

Where

$$[C]^{-1} = \begin{pmatrix} C_{11} & C_{12} & C_{13}.....C_{1i} \\ C_{21} & C_{22} & C_{23}.....C_{2i} \\ . & & \\ . & & \\ . & & \\ C_{i1} & C_{i2} & C_{i3}.....C_{ii} \end{pmatrix}$$

Then direct effects were calculated as follows:

$$P_1Y = \sum_{i=1}^{K} C_{1i} r_i y$$

$$P_2Y = \sum_{i=1}^{K} C_{2i} r_i y$$

$$P_kY = \sum_{i=1}^{K} C_{ki} r_k y$$

Later, the path coefficients were rated based on the scales given below (Lenka and Misra, 1973).

>1.00	Very high
0.3 - 0.99	High

0.2 - 0.29	Moderate
0.1 - 0.19	Low
0.0 - 0.09	Negligible

3.2.1.12. Cluster analysis

Mahalanobis (1928) D^2 statistic was used to study the genetic divergence between different populations. The D^2 analysis was carried out using the data recorded on germplasms. Mahalanobis generalized distance (D^2) between any two populations is obtained from the formula:

$$D^2 = \sum \lambda_{ij} \sigma_i \sigma_j$$

Where,

D^2 = Square of generalized distance

λ_{ij} = Reciprocal of the common dispersal index

$\sigma_i = \mu_{i1} - \mu_{i2}$

$\sigma_j = \mu_{j1} - \mu_{j2}$

μ = General mean

Since the formula for computation requires inversion of higher order determinants, transformation of the original correlated unstandardised character mean (Xs) to standard uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the corresponding uncorrelated (Ys) values of any two uncorrelated genotypes (Rao, 1952).

3.2.1.12.1. Clustering D^2 values:

All the n (n-1)/2D^2 values were clustered using Toucher's method (Rao, 1952).

3.2.1.12.2. Intra and inter-cluster distance:

The intra and inter-cluster distances were calculated by following the formula described by Singh and Choudhary (1977).

$$\text{Square of intra cluster distance} = \sum D_i^2 / N$$

Where,

$\sum D_i^2$ = Sum of distances between all possible combinations of the entries included in the cluster

N = Number of all possible combinations

$$\text{Square of inter-cluster distance} = \sum D_{ij}^2 / n_i n_j$$

Where,

$\sum D_{ij}^2$ = Sum of distances between all possible combinations ($n_i n_j$) of the entries included in the cluster

n_i= Number of entries in the cluster i

n_j = Number of entries in the cluster j

3.2.1.12.3. Determination of population constellations:

Population clusters were determined using Tocher's method described by Rao (1952). A cluster or constellation may be explained as a group of populations or genotypes such that any two populations belonging to the same cluster showed, on the average, a smaller D^2 value than those belonging to different clusters. Rao (1952) suggested that two closely related populations of low D^2 value be pooled together and then a third population of similar D^2 value be added to this group such that it did not increase the average D^2 value appreciably. This process is continued. Any population, which substantially increases the average D^2 value, should not be included in that group. After formation of first cluster, the process is repeated to form second, third, etc., clusters using remaining populations until all populations are included in one or the other cluster. After cluster formation average intra and inter-cluster distances were calculated. The square root of corresponding average D^2 values represents the distance within and between groups.

3.2.1.13. AMMI analysis

Additive Main Effects and Multiplicative Interaction (AMMI) was given by Gauch (1988) and is efficiently and vastly utilized for multilocation trials. GE signal and GE noise were calculated from the ANOVA as per Gauch (2013) to understand whether AMMI analysis is worthwhile to the data set. Then, GE noise (GE_N) was calculated by multiplying the error mean sum of square by the degrees of freedom (df) for GEI and then this value is subtracted from sum of squares of GEI to get GE_S. After finding the suitability for conducting AMMI, then further proceeded with AMMI model equation as follows:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n \Upsilon_{gn} \delta_{en} + \rho_{ge} + \kappa_{r(e)} + \varepsilon_{ger}$$

where,

Y_{ger} is yield of g genotype in e environment and r replication,

μ is the grand mean,

α_g is the effect of genotype,

β_e is the effect of environmental,

λ_n is the singular value for (IPC) n and correspondingly λ^2_n is its eigen value,

Υ_{gn} is the eigen vector value for genotype g and component n,

δ_{en} is the eigen vector value for environment e and component n,

with both eigen vectors scaled as unit vectors and ρ_{ge} is the residual,

$\kappa_{r(e)}$ is the r replication effect within e environment and

ε_{ger} is errror.

The statistical significance is tested for *'F test'* with degrees of freedom (df) for principal components (PCs) assigned according to method of Gollob (1968). AMMI1 biplot using main effect means *vs* first Interaction Principal Component Analysis (IPCA) score as described by Zobel *et al*. (1988) and AMMI2 biplot using first two IPCA scores were used to identify stable and specifically adapted genotypes.

3.2.2. Association mapping of yield traits in the association panel

3.2.2.1. Genotyping by Sequencing (GBS) association study

GBS was first developed by Elshire *et al*. (2011). For the study a genome wide GBS (genotyping-by-sequencing) was employed. High molecular weight DNAs of 92 lines were extracted and digestion with specific restriction enzyme (RE) was carried out known previously by frequent cutting in the major repetitive fraction of the genome. Most commonly used RE is *ApeKI*. Barcoded adapters are ligated to the sticky ends of the DNA fragments. PCR amplification is then carried out. GBS libraries obtained are further sequenced through next-generation sequencing technology to produce about 100 bp single-end reads. The generated raw and initial sequence data are filtered, sorted and aligned to a reference genome. From the GBS data, information on structural/functional annotation of SNPs and genotyping data mapped on chromosomes and scaffolds of chickpea genome especially derived from TF genes were acquired. The TF genes with SNPs selected from GBS data were further resequenced using the genomic DNA of 326 diverse desi and kabuli chickpea germplasm accessions (association panel) employing the multiplexed amplicon resequencing method (TruSeq Custom Ampliconv1.5) of Illumina MiSeqnext-generation sequencer (Illumina, USA). The custom oligo probes targeting the CDS (coding DNA sequences)/exons, introns, 2 kb-URRs (upstream regulatory regions)

and 2 kb-DRRs (downstream regulatory regions) of TF genes were designed and synthesized using Illumina Design Studio. Pooling into a custom amplicon tubeall the probes producing amplicons with an average size of 500 bp per reaction. Sample-specific indices were added to each library by PCR using common primers to constitute template libraries. The uniquely tagged pooled amplicon libraries were normalized and generated clusters were sequenced by Illumina MiSeq platform. The visualization/mapping of sequenced TF gene amplicons and discovery of high-quality sequence variants among accessions were performed as per Saxena *et al*. (2014) and Malik *et al*. (2016). The pseudomolecules of kabuli chickpea genome (Varshney *et al*. 2013) were used as a reference to map the high-quality gene amplicon sequence reads of each chickpea accession. Accordingly, the TF gene-derived high quality SNPs were detected among chickpea accessions as per Saxena *et al*. (2014) and Kujur *et al*. (2015a).

For candidate gene association study, genotypic SNPs data were correlated with the phenotypic data and PCA (principal component analysis), population structure (Q) and kinship (K) matrix for seed weight (SW) and seed number (SN) of 326 desi and kabuli accessions from the association panel. GAPIT (Lipka *et al*. 2012) and SPAGeDi 1.2 (Hardy and Vekemans 2002), respectively were used to measure the PCA and K matrix among accessions. To perform association analysis, the CMLM (compressed mixed linear model) (P+K, K and Q+K) along with P3D [population parameters previously determined (Zhang *et al*. 2010; Kang *et al*. 2010)] interfaces of GAPIT were employed following Thudi *et al*. (2014); Kujur *et al*. (2015b) and Kumar *et al.* (2015). Quantile-quantile (Q-Q) plot-based false discovery rate (FDR cut-off ≤ 0.05) corrections (Benjamini and Hochberg 1995) for multiple comparisons between observed/expected -log10(P) values and adjusted Pvalue threshold of significance were performed as per Kujur *et al*. (2015b) were employed for determining precision and robustness TF gene-derived SNP marker-trait association. The TF gene-derived SNP loci exhibiting significant association with SW and SN traits at a lowest FDR adjusted P values (threshold $P < 1 \times 10 -6$) and highest R^2 were identified in chickpea. The R2 representing the magnitude of SNP marker-trait association is estimated based on model with the SNPs and adjusted P values following FDR-controlling method.

3.2.3. Validation of candidate genes for seed weight in contrasting genotype

3.2.3.1. Genomic DNA Extraction

Fresh young leaves about 100 mg were collected from the 96 genotype from the field at sixty days after germination in *rabi* 20014-15 and cryo-preserved for the extraction of DNA. High throughput, reliable and inexpensive protocol was developed using a minor modification in CTAB extraction method of Doyle and Doyle (1987). Leaf DNA of 96 genotypes were isolated using a new protocol which was high throughput, relatively rapid, reliable and inexpensive protocol for the genomic DNA isolation from chickpea developed from chickpea molecular breeding laboratory Kumar *et al.* (2013).

3.2.3.2. CTAB extraction buffer

The extraction buffer was composed of 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.75 M NaCl, 2% (w/v) CTAB and 0.5% β-mercaptoethanol. The CTAB buffer was incubated at 65^{o}C before use for 10 min.

3.2.3.3. Extraction procedure:

The DNA isolation protocol consist of the following steps

(i) A hundred milligrams of leaf tissue was mixed with 1.0 ml of pre-warmed (10 min at 65^{o}C) extraction buffer in a 2.0 ml of eppendorf tubes, two stainless steel balls were added to the tube and the tubes were placed in 96 well rack of the indigenously designed Genogrinder (Innovative Biosciences).

(ii) Samples were efficiently grinded in Genogrinder 4-5 times pertaining to the manufacturer's instructions at 500 strokes per 4.5 minutes for 5 times at an interval of 2 minutes. The homogenized leaf sample obtained was incubated in water bath at 65^{o}C for 30 minutes and the contents were mixed three to four times by inverting the tubes gently.

(iii) Steel balls were removed with blunt end forceps and 600 µl of Chloroform: isoamylalcohol (24:1, v/v) was added to each tube and mixed by gentle inversion for about 5 min.

(iv) The mixture was centrifuged at 12,000 rpm for 10 minutes at 25^{o}C. Upper clear aqueous layer (~1000 µl) was conveyed to a fresh 2.0 ml eppendorf tubes to which 10 µl of RNase (10 mg/ml) was added and mixed well. The tubes were kept in water bath at 37^{o}C for 30 minutes.

(v) 700 µl of isopropanol was added and by continuous inversion gently mixed to precipitate the DNA. Samples were kept in the -20^{o}C for 30 minutes and centrifuged at 12,000 rpm for 10 minutes at 4^{o}C.

(vi) The DNA pellet was separated out by carefully discarding the supernatant. Then air dried for 30 min at room temperature and dissolve in 500 µl of sterile distilled water.

(vii) 50 µl of 3 M Sodium acetate (pH 5.2) and 600 µl of 70% ethanol was added to the DNA pellet and mixed well by gentle inversion and incubated at 20 for 10 minutes.

(viii) The precipitate was spun down at 12,000 rpm for 10 minutes at 4^{o}C and the supernatant was discarded and the pellet was washed with 70% ethanol.

(ix) The DNA pellet was air-dried inside the laminar airflow. The dried pellet was dissolved in 200 µl of TE buffer and stored at −200C till further use.

The modified protocol is also very convenient for extracting high-molecular-weight DNA from leguminous plant materials rich in polyphenols, tannins, and polysaccharides without using expensive liquid nitrogen and toxic phenols. The final DNA obtained by the present protocol is of good quality, without any coloured pigment and contaminants.

3.2.3.4. DNA quantification

DNA quantification was carried out in an agarose gel electrophoresis by loading DNA samples on a 0.8% agarose gel. The DNA was normalized to a concentration 5 ng/µl concentration and was compared with the standard λ DNA molecular weight markers (2.5 ng/µl, 5 ng/µl, 10 ng/µl) were visualized on 0.8% agarose gel.

Table 3.4: Composition of CTAB extraction buffer

Component	**Stock concentration**	**Working solution**	**Final volume (100 ml)**
Tris (pH 8.0)	1M	100mM	10ml
EDTA (pH 8.0)	0.5M	20mM	4ml
NaCl	5M	1.4 M	35ml

CTAB	10%	2%	20ml
ß-mercaptoethanol			0.2ml
ADW			30.8ml

Table 3.5: Composition of TE buffer

Content	Final volume (100ml)
1M Tris (Ph 8.0)	1ml
0.5M EDTA (pH 8.0)	200µl
ADW	98.8ml

3.2.3.5. Preparation of working DNA

A part of the stock DNA was diluted with appropriate amount of ADW (autoclaved distilled water) to yield a working concentration of 10ng/µl and further stored at -20^0c for future use.

3.2.3.6. Polymerase Chain Reaction (PCR)

3.2.3.6.1. Primer selection

21 gene based markers for seed weight previously reported were incorporated in the study. The information on 21 gene based primers with forward and reverse sequences are provided in Table 4.12.

3.2.3.6.2. PCR amplification

For the gene based marker set G storm thermal cycler,Englandwas used to carry out amplifications in PCR tubes containing a 10µl volumes of 20-25 ng/µl plant genomic DNA, 10 x Taq buffer of Bangalore Genei, India, 10 mM dNTP mix, 1.0 µl primer and 0.3 µl of 1U μl^{-1}Taq (Bangalore Genei, India). PCR analysis was taken up by having preparation of 150 seconds at 90ºC followed by 18 cycles of denaturation at 94ºC for 20 seconds, annealing for 50 seconds at 50ºC (touchdown of 0.5ºC for every repeat cycle) and 1 minute elongation at 72ºC for 50 seconds. Further 20 cycles of denaturation at 94ºC for 20 seconds, annealing for 50 seconds at 55ºC and 50 seconds elongation at 72ºC were given and finally extension at 72ºC for 7 minutes were performed.

Table 3.6: Components of a PCR reaction

Components	Working solution concentration	Each reaction requirement
Genomic DNA	20-25ng/µl	1µl
Taq buffer	10x	1.6µl
ADW	-	4.1µl
dNTP	10mM	1µl
Forward primer	10µM	1µl
Reverse primer	10µM	1µl
Taq polymerase	1U	0.3µl
Total		10µl

3.2.3.7. Gel electrophoresis

For the purpose of checking the size difference and polymorphism in the amplified PCR product, a 3% agarose gel was prepared in 1 x TBE buffer with 20µl (4µl/100ml) of ethidium bromide for each 500 ml of volume prepared. The gel was then allowed to cool and set. A 3 µl of 1 x loading dye was added to each of the PCR well. The amplified PCR product was then loaded onto the gel placed inside the electrophoresis unit. The electrophoresis was runned for 3 hours at 120 V till the bromophenol blue dye travelled more than 2/3rd the length of the gel. The gel was then visualized under UV transilluminator and photographed using a Gel Documentation system.

3.2.3.8. SSR data analysis

To score the SSR alleles, the sizes of the bands were scored manually with respect to their positions relative to the 100 bp ladder sequentially from the smallest to the largest sized bands. Scoring for each of the SSR marker was done for all the genotypes as per the requirement of the softwares used, viz. in binomial figure, 0 and 1, in base pairs (allele size) and in AB format. In the base pair format, the size of the bands correspondence to the size of the allele and the missing band is designated as '_' when scored. Diffused bands or bands showing ambiguity or missing bands were taken as missing data and designated as '9' under the binomial format. Further, '1' signifies the presence of bands and '0' signifies the absence of bands.

3.2.3.9. Contrasting genotype for validation

96 lines formed the association mapping panel for the present study. The genotypes were selected based on their 100 seed weight ranging from lowest of 10 to the highest of 50. 21 genic markers for seed weight was used to validate in the present population.

3.2.3.10. Population structure and estimation of K value

The software STRUCTURE, that utilizes a Bayesian algorithm to identify any number of historical subpopulation within the association mapping panel was used (Prichard *et al*. 2000). For the estimation of Q matrix which is the core output of the software STRUCTURE, molecular data of 80 polymorphic SSR markers were used. It assigns fractional membership of each of K historical subpopulations to each member of the panel. The Q matrix can be used as a covariate in the mapping study as it provides information on the subpopulation within the panel (Myles *et al*. 2009). For each run in the software STRUCTURE 2.3, K varied from 2 to 10 with 3 iteration for each K value. In each run, a burning period of 1,50,000 iterations was followed by Markov Chain Monte Carlo (MCMC) repetitions of 2,00,000 iterations. To choose the best K value, it used the pointers that the values of In P(D) stop varying much from its succeeding as compare to its preceding as given in the STRUCTURE manual. Alongside this estimation, the source of genotype from where they have been collected was also considered while concluding the K value.

3.2.3.11. Measure of Linkage Disequilibrium (LD)

The resolution of association studies in a test sample depends on the structure of LD across the genome. LD, or the correlation between alleles at different sites, is generally dependent on the history of recombination between polymorphisms (Remington *et al*.2001). The degree of LD present in the population influences the possibility to identify markers closely linked to the casual locus (Caldwell *et al*. 2006). Several statistics have been proposed for LD and these measurements largely differ in how they are affected by marginal frequencies and small sample sizes (Chao *et al*. 2010). TASSEL 3 was used to quantify LD between each pair of polymorphic loci, more specifically using D' (Farnir *et al*. 2000) which is the standardised disequilibrium coefficient or r^2 (Hill and Robertson 1968) which is the correlation coefficient between the alleles of two loci. Also using TASSEL, the significance of LD coefficient was estimated using 1000 permutations (Bradbury *et al*. 2007). To

display the linkage disequilibrium in the whole SSR set, a disequilibrium plot with P and r^2 was generated using TASSEL. All pair comparisons were made to estimate average r^2 and percent of observations P<0.01 significance levels (chi square).

3.3.3.12. Marker- Trait association analysis

Seed weight in grams of 96 chickpea genotypes along with the 21 polymorphic gene based SSR data across the genotypes was used for mapping QTLs. Association of traits with markers was studied in TASSEL v3 (http://www.maizegenetics.net) with general linear model (GLM) having no control over kinship (K) but using population structure (Q) as a covariate (Yu *et al*. 2006).

GLM equations is given as (Tadesse *et al*. 2015).

$$y = Xa + Qb + e \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{ GLM}$$

y is phenotype vector,

a is markers vector with fixed effects,

b is a vector with fixed effects

e is a residuals vector,

X denotes the accessions/genotypes at the marker,

Q is the Q-matrix, result of STRUCTURE software

The significant association between the SSR marker locus and seed weight were deduced on the basis of P and r^2 values. The markers were considered significantly associated with seed weight, at highly significant probability value (P= <0.01).

RESULTS

CHAPTER 4 RESULTS

The experimental results are presented in this chapter. An association panel of 380 genotypes were studied for seven different phenotypic traits *viz*., Days to flowering (DTF), days to maturity (DTM), plant height (PH), pods per plant (P/Pl), seeds per plant (S/Pl), 100 seed weight (100 SW), seed yield per plant (SY/Pl). The results are described in the following sections and sub headings.

4.1 Objective 1: To find out the extent of genetic variation of yield traits in the association mapping population

4.1.1. Analysis of variance

The analysis of variance (Table 4.1) revealed significant differences among the genotypes for all the seven traits. The significant differences have been indicated with asterisk. Double asterisk indicates highly significant differences at 1% level of significance among the 380 genotypes. The mean performance of the 380 genotypes for all the seven traits are given in ANNEXURE-I. Mean performance of genotypes showing lowest and highest range are presented in Table 4.2. Graphical representation of mean performance of 380 genotypes for all the seven traits is presented in Figure 4.1.

4.1.2. Estimation of Genetic variability, Heritability and Genetic advance.

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability and genetic advance were calculated for all the seven traits and shown in Table 4.2. Graphical representations for GCV, PCV, heritability and genetic advance are presented in Figs 4.2, 4.3, 4.4 and 4.5.

4.1.2.1 Days to 50% flowering

Days to flowering ranged from (44 to 144), 44 days as lower value exhibited by ICCV92311 while higher value was 144 days exhibited by ILC157 with mean of 78 days. The analysis revealed high genotypic coefficient of variation (22.55) and phenotypic coefficient of variation (23.25). High heritability (94.03) coupled with high genetic advance (35.32) was observed for this trait.

4.1.2.2 Days to maturity

Days to maturity ranged from lowest value of 123 days exhibited by ICCV08310 to highest value of 157 days showed by ILC5357. The mean value for the trait was 136 days. Moderate phenotypic and genotypic coefficient of variation (PCV and GCV) of 14.99 and 15.49 respectively were observed. High magnitude of heritability (93.66%) with moderate level of genetic advance (16.18) was revealed.

4.1.2.3 Plant height

Plant height showed good variability ranging from the minimum value of 33.44 cm (IG5869) and maximum value of 82 cm (ICCV14307) with mean of 54.12 cm. Higher phenotypic coefficient of variation (PCV) value of 41.95 as compared to its genotypic coefficient of variation (GCV) value of 34.44 explains higher influence of environment on the expression of this trait. High broad sense heritability (82.11) with low genetic advance (5.47) was observed.

4.1.2.4 Number of Pods per plant

Number of Pods per plant revealed good variability with mean of 26 and higher value of 58 exhibited by ICCV97404 and lower value of 9 exhibited by IG5901. Genotypic and phenotypic coefficient of variation was 32.86 and 36.07. This trait showed high heritability (83%) coupled with good genetic advance (16.56).

4.1.2.5 Number of Seeds per plant

Number of Seeds per plant ranged from minimum of 6 (ILC8804) to maximum of 57 (ICCV00303) and mean value of 24. Phenotypic coefficient of variation (GCV) was 31.51 and genotypic coefficient of variation (GCV) was 28.18. Moderate heritability (79.98%) with moderate amount of genetic advance (12.91) was recorded for this trait.

4.1.2.6 Hundred seed weight

Hundred seed weight recorded minimum value for ILC8797 (11.80) and maximum value for IG5986 (49.14) with mean of 31.86 indicating good variability for these trait. Very low difference between the values of genotypic coefficient of variation (23.44) and phenotypic coefficient of variation (23.47) indicates very less environmental influence on the trait. This trait exhibited very high heritability of 99% and moderate genetic advance of 15.85.

4.1.2.7 Seed yield per plant

Seed yield per plant ranged from minimum value of 1.48g exhibited by ILC8804 to maximum value of 16.25g exhibited by IG6003. The mean value was 7.77g. For the trait, higher phenotypic coefficient of variation (9.07) as compared to genotypic coefficient of variation (7.16) was recorded. Moderate heritability (62.24%) with low genetic advance (5.47) was revealed for this trait.

4.1.3. CORRELATIONS

Correlation analyses for yield per plant with various yield components was performed. Important yield contributing traits *viz*., days to flowering, days to maturity, plant height, number of pods per plant, number of seeds per plant and 100 seed weight were correlated with plant yield. The results are presented in Table 4.3. The correlation of seed yield with its yield components is shown in the form of scatter plot in Figures.

4.1.3.1 Days to Flowering

Days to flowering showed highly significant and negative association with seed yield per plant (-0.520). Significant and positive correlation with days to maturity (0.594) and plant height (0.269) was also recorded. This trait had negative but significant association with number of pods per plant (-0.264), number of seeds per plant (-0.284) and 100 seed weight (-0.450).

4.1.3.2 Days to maturity

Days to maturity had positive correlation with plant height (0.299). Negative and significant association was observed for this trait with pods per plant (-0.206), seeds per plant (-0.223), 100 seed weight (-0.097). The trait exhibited highly significant and negative association with seed yield per plant (-0.281).

4.1.3.3 Plant height

Plant height showed negative and non-significant correlation with pods per plant (-0.022), seeds per plant (-0.039), 100 seed weight (0.045), seed yield per plant (-0.035).

4.1.3.4 Pods per plant

Pods per plant showed positive and highly significant association with seed yield per plant (0.816). Phenotypic correlation coefficient for these traits was

negatively and significantly correlated with 100 seed weight (-0.144). It had positive and significant association with seeds per plant (0.971).

4.1.3.5 Seeds per plant

Seeds per plant had highly significant and strong positive correlation with seed yield per plant (0.825). It was negatively but significantly associated with 100 seed weight (-0.51).

4.1.3.6 100 seed weight

100 seed weight showed positive and highly significant correlation with seed yield per plant (0.381).

4.1.4 Path coefficient analysis

Path coefficient analysis was carried out for seed yield per plant and six yield contributing traits in 380 genotypes. The result of the analysis and estimates of path coefficients are showed in Table 4.4. Only those traits with significant variability and were correlated with yield significantly were considered to understand the direct and indirect effects of the traits on yield. Results on each trait are presented under the following sub headings.

4.1.4.1 Days to flowering

Days to flowering was negatively and significantly correlated with seed yield per plant (r=-0.520). It exhibited negative and negligible amount of direct effect on yield (-0.037). This trait exhibited negligible and negative indirect effects through days to maturity (-0.006), plant height (-0.002) and pods per plant (-0.030). Days to flowering also had negative and moderate amount of indirect effects on seed yield through seeds per plant (-0.211) and 100 seed weight (-0.234).

4.1.4.2 Days to maturity

This trait was negatively but significantly correlated with seed yield (r=-0.281). Days to maturity had low indirect effect through plant height (-0.005), pods per plant (-0.030) and seeds per plant (-0.143). Low direct effect (-0.022) was induced by this trait.

4.1.4.3 Plant height

Plant height exhibited low direct effect (-0.013). Negative indirect effect was induced by pods per plant (-0.042). Plant height had low and positive effect on seed yield through seeds per plant (0.024) and 100 seed weight (0.021). This trait was negatively correlated with seed yield (r= -0.035).

4.1.4.4 Pods per plant

Pods per plant revealed highly significant, strong positive correlation with seed yield per plant (r=0.816). The trait showed low and positive direct effect (0.124) and strongly effected yield through seeds per plant (0.731). Low and negative indirect effect through 100 seed weight was observed (-0.067).

4.1.4.5 Seeds per plant

Highest direct effect on yield was exhibited by seeds per plant (0.769). Strong, highly significant and positive correlation between seeds per plant and yield was observed (r=0.825). Indirect effect through seed weight was negligible and negative (-0.073).

4.1.4.6 Hundred seed weight

This trait had significant positive correlation with yield (r=0.381). Hundred seed weight showed high direct effect (0.489).

4.1.5. Cluster analysis

Cluster analysis was performed in 380 panels to help group the genotypes on the basis of seven morphological traits. Cluster analysis grouped 380 genotypes into 24 clusters (Fig 4.6) and the cluster means for the seven traits are given in Table 4.7. The genotypes in each cluster are represented in ANNEXURE II. Each of the clusters are further explained under the following subheadings. The intra and inter cluster distance for the association panel are presented in Table 4.5 and Table 4.6.

4.1.5.1 Cluster 1

Cluster 1 consist of genotype ILC8797 which is a landrace and is characterized by late days to maturity (144), late days to flowering (113), shortest plant height (34.22), less pods per plant (16.00), less seed number per plant (14.11), lowest hundred seed weight (11.80) and lowest seed yield per plant (1.94). Cluster 1 was nearest to cluster 18 (27.8) and was farthest from cluster cluster 22 (90.6).

4.1.5.2 Cluster 2

Cluster 2 is the largest of all the groups and consists of one hundred ninety-two genotypes of landraces, genomic population and varieties. The group is characterized by early days to flowering 58); early days to maturity (127), medium plant height (51.80), moderate number of pods per plant (28.68), moderate number of seeds per plant (27.08), medium seed yield per plant (9.10) and bold seeded type with high hundred seed weight (34.01). Cluster 2 was nearest to cluster 12 (23.8) but farthest from cluster 10 (69.5).

4.1.5.3 Cluster 3

Cluster 3 includes ten genotypes; nine of which are landraces (IG5999, IG5872, IG5898, IG6006, IG5871, IG5884, IG5997, IG5899, IG5900) and KEB-F2-13, a variety. This group is identified by medium plant height (50.18), moderate pod number per plant (28.03), moderate seed number per plant (26.53); late days to flowering (101); moderate seed yield per plant (9.49); early days to maturity (130) and high seed weigh (35.66). Cluster 3 was nearest to cluster 7 (18.5) but was farthest to cluster 24 (58.0).

4.1.5.4 Cluster 4

Cluster 4 is marked by late days to flowering (108), medium plant height (56.50), late days to maturity (145), moderate number of pods per plant (20.41), moderate seeds per plant (26.53), moderate hundred seed weight (27.07) and low seed yield per plant (4.51). The group has ninety-five genotypes composed totally of landraces. Cluster 4 was farthest from cluster 22 (82.6) but nearest to cluster 16 (14.6).

4.1.5.5 Cluster 5

Cluster 5 consist of ten genotypes; IG5839, IG5890, IG6000, IG5893, IG5867, IG5868, IG5844 (a), IG5849, IG5856, IG5889 which are landraces. This group is characterized by high pods per plant (42.34), high seed number per plant (39.9), moderate hundred seed weight (28.77), high seed yield per plant (11.52) and late days to maturity (145) and medium plant height (58.21) and late days to flowering (108). Cluster 5 was closest to cluster 17 (16.2) while farthest from cluster 22 (88.4).

4.1.5.6 Cluster 6

Cluster 6 is consist of three genotypes namely ILC10070, IG5869, IG5870, all landraces and is specified by early days to maturity (126), short plant height (36.25), moderate pods per plant (29.81), moderate seeds per plant (24.44) and moderate hundred seed weight (27.22), medium seed yield per plant (6.43) and late days to flowering (109). Cluster 6 was farthest to cluster 22 (100.1) but nearest to cluster 4 (16).

4.1.5.7 Cluster 7

Cluster 7 is characterized by late days to maturity (140) and high hundred seed weight (34.49), late days to flowering (96), moderate number of pods per plant (23.75) and medium plant height (56.27). The group marked low seed yield per plant (5.01) and less seed number per plant (14.41) and compose of four genotypes; IG5892, IG5896, ILC5911, ICCV96323. Cluster 7 was closest to cluster 16 (19.8) and farthest from cluster 22 (80.3).

4.1.5.8 Cluster 8

Cluster 8 includes two landrace genotypes (IG5904, IG6003) and is marked by late days to maturity (147) and tall plant height (73.44), high seed yield per plant (13.79), late days to flowering (108), high pod number per plant (35.27), high hundred seed weight (40.42) and high seed number (33.55). Cluster 8 was closest to cluster 5 (21.6) but farthest placed from cluster 22 (86.6).

4.1.5.9 Cluster 9

Cluster 9 is specified by low seeds per plant (17.51), low seed yield per plant (5.44), late days to flowering (111), moderate pods per plant (26.00), high hundred seed weight (31.57), longest days to maturity (148) and tall plant height (79.18). This group includes three landrace genotypes, IG5991, IG5851 (a), IG6001. Cluster 9 showed maximum divergence with cluster 22 (86.9) but was closest to cluster 8 (23.0).

4.1.5.10 Cluster 10

Cluster 10 has four landrace genotypes, ILC157, IG5877, IG5984 and IG5902. The group is characterized by genotypes having longest days to flowering

(119), low pods per plant (15.13), less number of seeds per plant (12.55), low seed yield per plant (5.26), late days to maturity (146), tall plant type (66.88) and high hundred seed weight (45.22). Cluster 10 exhibited closest relationship with cluster 9 (23.2) while was farthest to cluster 22 (90.1).

4.1.5.11 Cluster 11

Cluster 11 shows shortest plant type (53.03), low seed yield per plant (5.84), low pods per plant (16.46), late days to maturity (147), less hundred seed weight (5.84), low seeds per plant (13.26) and earliest days to flowering (53). This group encompasses five landraces, ILC6062, IG5985, IG5987, IG5986 and IG5982. Cluster was placed closest to cluster 12 (24.3) and farthest from cluster 23 (72.7).

4.1.5.12 Cluster 12

Cluster 12 consist of twenty seven genotypes, IG5878, IG5880, IG5879, IG5980, IG5909, IG5906, IG5905, ICSN1, ICSN2, ICSN3, ICSN4, ICSN5, ICSN6, ICSN7, ICSN8, ICSN9, ICSN10, ICSN11, ICSN12, ICSN13, ICSN14, ICSN15, ICSN16, ICSN17, ICSN18, ICSN19, ICSN20 and is marked by early days to flowering (63), medium plant height (53.77), medium pods per plant (28.93), moderate number of seeds per plant (26.90), medium seed yield per plant (9.86), longest days to maturity (150) and high hundred seed weight (37.09). Cluster 12 was placed farthest to cluster 1 (62.7) but exhibited closest relationship with cluster 2 (23.8).

4.1.5.13 Cluster 13

Cluster 13 is specified by late days to flowering (104), low plant height (45.22), lowest pods per plant (11.55), lowest seeds per plant (9.22), low seed yield (4.15), late days to maturity (145) and highest hundred seed weight (48.74). The group has one landrace genotypeILC6025. Cluster 13 was farthest from cluster 22 (82) but closest to cluster 7 (24.3).

4.1.5.14 Cluster 14

Cluster 14 have three genotypes (ILC211, ILC11889, ILCO (Syria) and marked by low plant height (42.33), low hundred seed weight (13.46) and low seed

yield (5.54), late days to flowering (99) and moderate pods per plant (35.11), late days to maturity (141) and high seeds per plant (42.33). Cluster 14 was closest to cluster 23 (21.2) and farthest from cluster 22 (88.7).

4.1.5.15 Cluster 15

Cluster 15 consist of four genotypes, IG5855, ICCV97402, ICCV97404, ICCV00303 and these genotypes are characterized by highest pods per plant (55.00), medium plant height (57.55), highest number of seeds per plant (54.05), early days to flowering (72) and early days to maturity (131) and medium hundred seed weight (22.62) and high seed yield (12.71). Cluster 15 exhibited maximum divergence from cluster 22 (96.5) and closest to cluster 23 (29.4).

4.1.5.16 Cluster 16

Cluster 16 have three landrace genotypes (IG5861, IG5848, IG5857) and specified by late days to flowering (109), medium hundred seed weight (24.61), moderate pods per plant (33.59), low seeds per plant (19.59), medium plant height (53.92), low seed yield (4.80) and late days to maturity (140). Cluster 16 was closest to cluster 4 (14.6) and farthest from cluster 22 (88.2).

4.1.5.17 Cluster 17

Cluster 17 is specified by late days to maturity (147), late days to flowering (116), medium plant height (63.71), high pods per plant (33.62), high seeds per plant (38.37), moderate hundred seed weight (20.34) and medium seed yield (7.88). This group encompasses five landrace genotypes, IG5894, ILC6891, ILC10771, ILC3280 and IG5858. Cluster 17 was placed farthest from cluster 22 (91) and placed closest to cluster 5 (16.2).

4.1.5.18 Cluster 18

Cluster 18 consists of single landrace genotype ILC182. The group is marked by late days to maturity (142), low pods per plant (15.77), low seeds per plant (14.22), low hundred seed weight (13.10), low seed yield per plant (2.14), late days to flowering (111) and medium plant height (61.88). Cluster 18 was placed closest to cluster 4 (16.7) and showed maximum divergence from cluster 22 (90).

4.1.5.19 Cluster 19

Cluster 19 is characterized by late days to maturity (141), low pods per plant (18.44), low seed yield (23.66), low hundred seed weight (16.92), late days to flowering (114), medium plant height (63.66) and low seeds per plant (23.66) and have a single landrace genotype ILC10768. Cluster 19 was farthest from cluster 22 (91.5) and closest to cluster 4 (15.3).

4.1.5.20 Cluster 20

Cluster 20 have a single landrace genotype, IG5851 (b) in the group. The group is specified by early days to flowering (56), medium seed yield per plant (6.87), medium pods per plant (28.11), medium seeds per plant (27.11), moderate hundred seed weight (25.73), longest days to maturity (151) and tall plant height (78.88). Cluster 20 was closest to cluster 12 (28.6) and farthest from cluster 1 (76.3).

4.1.5.21 Cluster 21

Cluster 21 consist of three landrace genotypes ILC9793, IG5865, ILC5588 and is marked by low seed yield per plant (2.33), low seeds per plant (10.00), earliest days to maturity (125) and low pods per plant (12.40). It is further characterized by late days to flowering (109), moderate hundred seed weight (25.42) and medium plant height 60.59). Cluster 21 showed closest relationship with cluster 18 (21.8) but farthest placed from cluster 22 (101.0).

4.1.5.22 Cluster 22

Cluster 22 is specified by early days to flowering (60), moderate pods per plant (20.77), low seeds per plant (17.66), medium seed yield (6.26) and medium plant height (49.55), also marked by high hundred seed weight (36.10) and late days to maturity (140). The group has a single genotypeICCV08303. Cluster 22 was closest to cluster 12 (62.1) and farthest from cluster 23 (101.6).

4.1.5.23 Cluster 23

Cluster 23 is marked by single genotype Phule G96006, a variety and shows late days to flowering (95), early days to maturity (127), medium plant height (51.44), low hundred seed weight (12.71), low seed yield per plant (5.71), high seeds per plant (44.88) and high pods per plant (46.88). Cluster 23 was farthest from cluster 22 (101.6) and closest to cluster 5 (29.7).

4.1.5.24 Cluster 24

Cluster 24 is specified by early days to flowering (55), early days to maturity (129). Further with tallest plant height (82.00), highest seed yield per plant (14.40), high seeds per plant (38.55), high hundred seed weight (37.32) and high pods per plant (40.11) and consist of single genotype ICCV14307. Cluster 24 was placed closest to cluster 20 (30.6) and farthest from cluster 22 (92.9)

4.1.5. AMMI analysis

Pooled ANOVA for seed weight and seed number across the years was performed where different years were taken as random effects and genotypes were considered as fixed effects. The result shows Genotypes (G), Environmental (E) and interaction (GEI) effects being highly significant ($P<0.01$) for seed weight and seed number thus indicating the prominence of all the three types of effects which is merely not random or due to chance (Table 4.8). Maximum variation was accounted by genotypic effect due to seed weight and seed number, contributing 93.08% for both respectively followed by G X E effects with 4.1 % for the two traits. The minimum variation was accounted by the environmental influence with 0.37 % for both the traits respectively.

Genotype Environment signal (GEs) was calculated (Gauch 2013) to deduce the appropriateness of the data to AMMI analysis. GEs was calculated by substracting GEn (GE noise) from GEI. For calculating GE_N, error mean sum of square and degrees of freedom (df) for GE is required. Thus the first step included calculation of GE_N by multiplying the error mean sum of square with the degrees of freedom for GE (2.6 x 758 = 1970.8 for both seed weight and seed number). Further, GEs was computed (10132-1970.8 = 8162.2 for both seed weight and seed number). The reference here was that when SS due to GE_N is almost equal to SS due to GEI obtained in ANOVA, then GEI is said to be buried in the noise and thus considered signal poor. However, in this study, SS due to GE_N were far lesser than GEI sum of squares. Thus, the interaction was almost signal rich and not buried in the noise. This pronounced the usefulness of AMMI analysis in the study.

Ascertaining high yielding and stable genotypes

To understand main effects and interactions for seed weight and seed number, AMMI biplot was constructed. AMMI1 biplot is a plot between the mean and the

IPCA1 of GEI (Fig 4.8a & 4.8b). The elucidation from the biplot is that if main effects have IPCA score nearing to zero, it indicates negligible interaction between the genotype and the environment and when a genotype and an environment have the same sign on the IPCA axis, it shows positive interaction; negative interaction if different. In the figure, G369 was identified as the most stable genotype for seed weight with IPC1 score nearing zero (-0.009) and having a good mean seed weight of 37.33 g/100 seeds. G59 has the highest mean seed weight (49.1 g) with good stability (IPC1 score of 0.28). G60 and G61 have high mean seed weight (48.7 and 48.7 respectively) and with IPC1 score nearing zero (0.09 and 0.25). The most unstable genotype identified was G182 (IPC1 score of 0.78) and mean seed weight of 24.8 g. For seed number per plant, genotype with the highest mean seed number was G182 (57.67) was less stable (IPCA1 score of -0.78). The most stable genotype was G57 with IPCA1 score close to zero (0.03) and mean seed number of 38.44. Further the most unstable genotype was identified to be G301 with mean seed number of 27.11. GenotypesG80 and G140 showed good stability (score of -0.28 and -0.37) with high seed number (50.44 and 53.56 respectively).

AMMI2 biplot for seed weight revealed 61.01% of the interaction accounted by IPC1 and 38.99% accounted by IPC2. Further, for seed number first component, IPC1 explained 78.02% of the genotype and environment interaction and IPC2 described 21.98%. For both the traits the first two interaction components explained 100 % of the G X E variation leaving no residue or noise (Table 4.9). From the biplot, G378, G34, G22, G85, G289, G312, G32 are scattered close to the origin indicating minimal interactions with the environment for seed weight (Fig 4.9a & Fig 4.9b). Genotypes that are scattered far away from the origin viz., G275, G201, G2 shows prominent G X E interaction thus are less stable. For seed number, higher sensitivity to environment was shown by genotypes G70 and G267.

4.2 Objective 2: Association mapping of yield traits in the association panel.

4.2.1 Discovery, Genotyping and Annotation of TF Gene-Derived SNPs

NGS-based amplicon resequencing strategy targeting TF genes (scanned previously by GBS assay) with high sequencing-depth coverage was employed in the present study for large-scale discovery and genotyping of SNPs in 326 desi and kabuli germplasm accessions belonging to an association panel of chickpea. Primarily, this strategy successfully validated all 1029 GBS derived SNPs mined from 736 chickpea TF genes. Further, the kabuli reference genome-based GBS and targeted gene amplicon resequencing-led high-throughput SNP genotyping in 326 desi and kabuli accessions (association panel) altogether discovered 1611 SNPs from 736 TF genes (representing 30 TF gene family) with an average density of 2.2 SNPs per TF (ANNEXURE III, Fig 4.12). Maximum of 551 and 484 SNPs were discovered from the 257 and 221 TF-encoding genes especially belonging to Zinc finger and DUF (domain of unknown function) TF gene families, respectively (Fig. 4.11). Of these, 1497 and 114 SNPs derived from 683 and 53 TF genes were physically mapped across eight chromosomes and unanchored scaffolds of kabuli genome, respectively (Fig 4.11). All eight chickpea chromosomes contained maximum frequency of SNPs from the TF genes belonging to Zinc finger and ARF (auxin responsive factor) TF gene families (Fig 4.11). The comprehensive structural annotation of 1611 SNPs in 736 TF genes exhibited presence of highest and lowest proportion of SNPs in the exons/CDS (58.3%, 939 SNPs) and DRRs (0.4%,7) of 422 and 3TFs, respectively (Fig 4.13). The coding SNPs contained 529 (56.3%) synonymous and 410 (43.7%) non-synonymous (missense and nonsense SNPs) SNPs from 204 and 218 TF genes, respectively (Fig. 4.13). The abundance of non-synonymous SNPs derived from the TF genes representing Zinc finger and ARF TF gene families was evident.

4.2.2 Association Mapping to identify natural SNP allelic variants of TF genes regulating yield traits

For association mapping, the genotyping information of 1611 informative SNPs (differentiating the 326 desi and kabuli chickpea germplasm accessions) discovered from 736 TF genes using genome-wide GBS and targeted gene amplicon resequencing assays were utilized. The use of 1611TF gene-based SNPs primarily in neighbour-joining phylogenetic tree and population genetic structure construction as well as principal component analysis (PCA) classified 326 desi and kabuli chickpea germplasm accessions (association panel) into two distinct population groups- POP I (173 desi and 29 kabuli accessions) and POP II (33 desi and 91 kabuli accessions)

(Fig.4.14). A wider degree of significant population divergence (mean FST 0.47at P<0.001) was observed between POP I and POP II, while FST-led population differentiation was maximum in POP I (0.39). All the accessions with their 81% inferred ancestry were derived from one of the model-based population and rest 19% contained admixed ancestry. Maximum and minimum admixed ancestry was observed in POP II and POP I, respectively. A significant deviation was observed from population assignment of 326 germplasm accessions (representing 58 diverse geographical regions of the world) based on desi and kabuli cultivar specific classification which was more pronounced in cultivated kabuli accessions (29%) belonging to POP I . This is possibly due to a greater effect of geographical origin and adaptive environment rather than cultivar-types of accessions on their assignment to a specific population group. The multiple domestication events (evolutionary bottlenecks) followed by a complex breeding history coupled with strong adaptive selection pressure might have influenced their population group assignment resulting in numerous admixtures among accessions especially within POP II. The comprehensive analysis of multi-location/year field phenotyping data revealed a normal frequency distribution along with a broader phenotypic variation for six major seed yield component traits (DF, PH, BN, PN, SN and SW) in a constituted association panel comprising of 326 desi and kabuli chickpea germplasm accessions (Table 4.8). Maximum CV was observed for SN (57.2–68.2%) followed by PN (52–54%) and minimum for PH (10.2–13.9%) (Table 4.10). Heritability (H^2) was estimated to be ≥ 80% for all six yield traits with highest in case of SW (85–86%). A higher significant positive correlation between PN and SN ($r = 0.96$ with $P < 0.0001$) followed by SW and PH ($r = 0.61$ with $P < 0.0001$), whereas a lower negative correlation between SW and SN ($r = -0.65$ with $P < 0.0001$) followed by SN and PH ($r = -0.56$ with $P < 0.0001$) among 326 chickpea accessions based on Pearson's coefficient (r) was evident. This indicates that the 326 desi and kabuli germplasm accessions representing 58 diverse geographical regions of the world selected by us are rich in natural phenotypic diversity for all six major pod and seed yield component traits (DF, PH, BN, PN, SN and SW). The CMLM and P3D/EMMAX-based candidate gene based association analysis at a FDR cut-off ≤ 0.05 detected 27 TF gene-derived SNPs that were significantly associated with six major pod and seed yield component traits (DF, PH, BN, PN, SN and SW) at a $P \leq 10-6$ (Fig.4.15, Table 4.11). All these 27 trait-associated SNPs were physically mapped on seven

chromosomes (except chromosome 2) and unanchored scaffolds of kabuli genome (Table 2). A maximum of 10 trait-associated TF gene-derived SNP loci were mapped on chromosome 3. Twenty-three and four of 27 trait-associated genic SNP loci were represented from diverse coding (20 synonymous and three non-synonymous SNPs) and noncoding (four intronic) sequence components of 16 genes, respectively (Table 4.11). The phenotypic variation for six major pod and seed yield component traits (DF, PH, BN, PN, SN and SW) explained by 27 TF gene-derived maximum effect SNP loci varied from 10 to 23% R^2 (P 1.7×10^{-6} to 0.3×10^{-7}) among 326 desi and kabuli chickpea germplasm accessions belonging to an association panel (Table 2). The combined phenotypic variation for six major pod and seed yield traits explained by all significant 27 TF gene-based SNP loci was 32%. Notably, six TF gene-derived SNPs identified to be associated commonly with PN and SN traits in a constituted association panel of chickpea. One SNP derived from a TF gene encoding SNF2 (sucrose non-fermenting 2) was associated with both BN and PH traits, whereas another SNP mined from a TF gene encoding B3-domain protein was associated both SW and PH traits in chickpea.

4.2.3 Identification of TF gene based natural allelic variants for flowering time

Four SNPs derived from the diverse coding (two non-synonymous SNPs) and intronic (two SNPs) sequence components of four TF genes exhibited significant association (13–23% R^2 with P 2.0×10^{-6} to 0.3×10^{-7}) with DF trait (Fig. 4.15**,** Table 4.11). Four DF-associated TF gene-based SNPs were physically mapped on two kabuli chickpea chromosomes (3 and 4) with a maximum of 3 SNPs on the chromosome 3. The proportion of DF phenotypic variation explained by four SNP loci derived from four TF genes [encoding bZIP (basic leucine zipper), SBP (squamosal promoter binding protein), bHLH (basic helix-loop-helix) and Myb (myeloblastosis) TFs] in an association panel (380 desi and kabuli accessions) varied from 13 to 23% R^2 (Fig. 4.15, Table 4.11). All four significant SNP loci in combination explained 29% DF phenotypic variation. One intronic SNP (T/A) in a bZIP TF gene and another non-synonymous SNP (A/T) causing amino acid substitutions from cysteine (TGT) to serine (AGT) in a SBP TF gene (20–23% R^2 with P 0.3– 1.1×10^{-7}) exhibited strong association with DF trait in chickpea (Fig. 4.15**,** Table 4.11).

4.2.4 Identification of TF gene based natural allelic variants for plant height

Four non-synonymous SNPs derived from the diverse coding sequence components of four TF genes revealing significant association (10–20% R^2 with P 2.3×10^{-6} to 1.2×10^{-7}) with PH trait were detected (Fig. 4.15**,** Table 4.11). The PH-associated four TF gene-based SNPs were physically mapped on two kabuli chickpea chromosomes (6 and 7) with a maximum of 2 SNPs on the chromosome 6. The proportion of PH phenotypic variation explained by four SNP loci derived from four TF genes [encoding SNF2, WD40 (Trp-Asp 40), B3 and Tify-domain proteins] in an association panel varied from 10 to 20% R2 (Fig. 3b, Table 2). All significant four SNP loci in combination explained 25% PH phenotypic variation. Strong association of one nonsynonymous SNP (A/C) causing amino acid substitutions from histidine (CAC) to proline (CCC) in a WD40-domain protein-coding TF gene (20% R^2 with P 1.2×10^{-7}) with PH trait was observed in chickpea (Fig. 4.15, Table 4.11).

4.2.5 Identification of TF gene based natural allelic variants for branch number

Six SNPs derived from the diverse coding (five nonsynonymous SNPs) and intronic (one SNP) sequence components of six TF genes exhibited significant association (12–20% R^2 with P 2.4×10^{-6} to 0.7×10^{-7}) with BN trait (Fig. 4.15**,** Table 4.11**).** Six BN-associated TF gene-based SNPs were physically mapped on five kabuli chickpea chromosomes (1, 3, 4, 5 and 7) with a maximum of 2 SNPs on the chromosome 5. The proportion of DF phenotypic variation explained by six SNP loci derived from six TF genes [encoding GRAS [Gibberellic acid insensitive (GAI)Repressor of GAI (RGA)-SCARECROW (SCR)], RING type Zinc finger, DUF827 (domain of unknown function 827), SNF2, C2H2-Zinc finger and SANT [switching-defective protein 3 (Swi3)-adaptor 2 (Ada2)-nuclear receptor corepressor (N-CoR), transcription factor (TF)IIIB]-domain proteins] in an association panel varied from 12 to 20% R^2 (Fig. 4.15 , Table 4.11). All significant six SNP loci in combination explained 26% BN phenotypic variation. Two nonsynonymous SNPs- (C/A) and (C/T) causing amino acid substitutions from alanine (GCA) to serine (TCA) and leucine (CTT) to phenylalanine (TTT) in GRAS and SNF2 TF genes (20% R^2 with P $0.7–1.0 \times 10^{-7}$), respectively, revealed strong association with BN trait in chickpea (Fig. 4.15**,** Table 4.11).

4.2.6 Identification of TF gene based natural allelic variants for pod and seed number

Six SNPs derived from the diverse coding (four nonsynonymous and one synonymous SNPs) and intronic (one SNP) sequence components of six TF genes revealed significant association (10–21% R^2 with P 2.2×10^{-6} to 0.3×10^{-7}) with both PN and SN traits (Fig. 4.15, Table 4.11). Six PN/SN associated TF gene-based SNPs were physically mapped on three kabuli chickpea chromosomes (3, 5 and 7) with a maximum of 4 SNPs on the chromosome 3. The proportion of PN and SN phenotypic variation explained by six SNP loci derived from six TF genes [encoding DUF3437, LOB (lateral organ boundaries)-domain protein, C2H2-Zinc finger, WD40, ZF (zinc finger)-HD (homeodomain) homeobox protein and homeobox TFs]in an association panel varied from 10 to 21% **(**Fig.4.15**,** Table 4.11). All significant six SNP loci in combination explained 25% PN and SN phenotypic variation. One nonsynonymous SNP (A/G) causing amino acid substitutions from asparagine (AAC) to aspartic acid (GAC) in a LOB domain protein-coding TF gene (21% R^2 with P 0.3×10^{-7}) exhibited strong association with PN and SN traits in chickpea (Fig. 4.15**,** Table 4.11**)**.

4.2.7 Identification of TF gene based natural allelic variants for seed weight

Seven SNPs derived from the diverse coding (five nonsynonymous and two synonymous SNPs) sequence components of seven TF genes revealed significant association (10– 20% R^2 with P 1.7×10^{-6} to 0.8×10^{-7}) with SW trait (Fig.4.15**,** Table 4.11). Six SW-associated TF gene-based SNPs were physically mapped on four kabuli chickpea chromosomes (1, 3, 6 and 8) with a maximum of 2 SNPs each on the chromosomes 1 and 3. The proportion of SW phenotypic variation explained by seven SNP loci derived from seven TF genes [encoding bZIP, SBP, Jumonji, RING-type Zinc finger, Med12 (mediator complex), B3 and WRKY-domain protein] in an association panel variedfrom10to 20% (Fig.4.15**,** Table 4.11). All significant seven SNP loci in combination explained 30% SW phenotypic variation. One non-synonymous SNP (G/T) causing amino acid substitutions from threonine (ACG) to lysine (AAG) in a bZIP TF gene (20% R^2 with P 0.8×10^{-7}) exhibited strong association with SW trait in chickpea (Fig. 4.15, Table 4.11).

4.3 Objective 3: To validate candidate genes for seed weight in contrasting genotypes.

The squared value of pearson correlation, R^2 is considered more accurate compared to D^1 for LD measurement since the former accounts mutations and The LD measured in R^2 ranged from 0 to 1 with a mean of 0.016 and D' ranged from 0 to 1 with a mean of 0.274. Genic SSR markers for seed weight previously reported by researchers (Table 4.12) were utilized in the study to know the soundness of the reported markers for seed weight in the present association panel. All the markers showed amplification out of which 21 markers amplified polymorphic (Table 4.12). Association of SSR markers with seed weight was studied using General Linear Model (GLM). GLM considers population structure while MLM takes care of both population structure and kinship. Three years phenotypic data for seed weight with three replications for 96 selected genotypes and the genotypic data of 21 genic markers were used to analyze the trait-marker association in TASSEL. The markers were considered to be significantly associated with seed weight at probability level $p<0.01$.

Association with seed weight

Analysis with GLM (Table 4.13) revealed eight genic markers that had highly significant association with seed weight. Qsw 2.1 is significantly highly associated ($p = 3.63 \times 10^{-11}$) with seed weight having R^2 of 44.8 %, followed by QTL'HGWW2 ($p= 2.51 \times 10^{-9}$) with $R^2$of 38.4% and Qsw2 ($p= 5.19 \times 10^{-9}$) with R^2 of 33.6 %. Genic markers Caqsw1.2' and HGWH also exhibited highly significant association with the trait with p value of 2.94×10^{-6} and 4.04×10^{-7} and R^2 of 25.3 % and 33.2 %. Significant association was also revealed by HGWH4, QTL2 and Caqsw1.2 with marker correlation coefficient value of 17.4%, 18.9% and 19.1 %. The electrophoretic profile of PCR amplified products of the 5 validated genic SSR markers are presented in Fig 4.16, 4.17, 4.18, 4.19 and 4.20.

DISCUSSION

CHAPTER 5 DISCUSSION

Chickpea (*C. arietinum* L.) is an ancient crop first grown in India about 4000 B.C. In India chickpea accounts for 45% of the pulse production. It is an important source of dietary protein and also plays a major role in biological nitrogen fixation thereby contributing to crop rotation and sustaining soil productivity. There are two distinct types of cultivated chickpea, *Desi* and *Kabuli*. Desi types have pink flowers, anthocyanin pigmentation on stems and brown colored and thick seed coat. The kabuli types have white flowers, lack anthocyanin pigmentation on stem, white or cream colored seeds, thin seed coat and smooth seed surface. Comparing the chemical composition of desi and kabuli types of chickpea indicates that they differ primarily in content of protein, fiber, poly-phenols, and carbohydrates. The energy value of Desi variety grains is 327 kcal/100g while for Kabuli variety it is 365kcal/100g (Maheri-Sis *et al*. 2008). Chickpea flour has a higher content of protein, fat, ash and fiber (Khan *et al*. 1995 and Hulse 1991). Despite its importance, the crop has not received the attention it deserves. Efforts at conventional breeding have been going on, however, its productivity has remained historically low and unstable and the crop continues to suffer from diseases such as *Fusarium* wilt and *Ascochyta* blight and abiotic stresses such as drought and cold. Breeders have been working tirelessly towards increasing the yields, however, they have not succeeded in substantially increasing the productivity of chickpea. Yield being a complex trait is very difficult for *per se* selection by the breeders. Indirect selection through selecting for component traits like seed weight, number of seeds per plant are the most important yield parameters among all others viz., days to 50% flowering, days to maturity, number of primary branches per plant, number of secondary branches per plant, number of pods per plant and number of seeds per pod. However, being quantitative traits complete gain for them is very difficult. In the absence of major breakthroughs coming in from the use of conventional methods, the challenge now is to use the recently developing molecular marker techniques to improve the efficiency of chickpea breeding. Use of molecular markers has shown great potential in accelerating plant breeding especially in disease resistance, insect resistance and quality traits. It is also equally important that for map generation, we identify and develop our own novel set of markers such as the STMS markers, SNPs etc. Such efforts at characterization of markers and mapping would prove useful in

phylogenetic analysis, inheritance and evolutionary studies, tagging of important traits, identification of quantitative traits and marker assisted introgression of economically important traits, all of which would go a long way in bringing about an improvement in chickpea.

This investigation was primarily attempted with the following three objectives:

1. To find out the extent of genetic variation of yield traits in the association mapping population.
2. Association mapping of yield traits in the association panel.
3. To validate candidate genes for seed weight in contrasting genotype.

5.1 To find out the extent of genetic variation of yield traits in the association mapping population.

5.1.1 Variability

Knowledge of nature and magnitude of genotypic and phenotypic variability present in any crop species plays an important role in formulating a successful breeding programme. In case the variability is very much limited or exhausted due to continued selection, it is warranted to plan for a recombination breeding programme for future genetic amelioration. The findings of this investigation would help in the selection of genotypes based on there, per se performance or in further carrying out selections among the wide and diverse genotypes giving high amount of heterosis. The idea of variability in segregating generations gives a scope for the breeder to carry out selection and generation advancement (Bharadwaj *et al.* 2004). The presence of genetic variability is of utmost importance for any breeding programme and due to this reason the plant breeders have emphasized the evaluation of germplasm for the improvement of crop yield as well as for utilization in further breeding programmes. Evaluation of plant genetic resources is a pre-requisite for which the future breeding work is based (Reddy *et al*. 2012).

5.1.1.1 Analysis of variance

The mean sum of squares were highly significant for all the characters studied indicating the presence of considerable amount of variation in the association panel (Sewak *et al*. 2012, Jivani *et al*. 2013, Peerzada *et al*. 2015, Kumar *et al*. 2016, Tiwari *et al*. 2016 and Srivastava *et al*. 2017). Such a large amount of variability would help

in the structure analysis of the association mapping panel and discerning the characters direct and indirect effect.

5.1.1.2 Variation for yield and yield components

In general all the parameters studied recorded higher values of phenotypic coefficient of variation than their corresponding genotypic coefficients indicating environmental influence on the expression of these traits. A wider range for all the characters studied was observed indicating presence of sufficient amount of variability present among the genotypes. Thus it provides the basis for selection of desirable genotypes from the diverse population for enhancement of chickpea production. These results were in consonance with the findings of Vaghela *et al*. (2009), Malik *et al*. (2010), Babber *et al*. (2012) and Ramanappa *et al*. (2013).

The difference between phenotypic coefficient of variation and genotypic coefficient of variation was low for days to 50% flowering, days to maturity and 100 seed weight. This suggests that the expression of these traits was least affected by the environmental factors and their phenotype is the true representative of its genotype. Further, the selection on the basis of *per se* performance will be effective.

5.1.1.3 Heritability and genetic advance

Heritability estimate in broad sense is the ratio of genotypic variance to the phenotypic variance and is expressed in percentage. The relative degree with which a character is transmitted to offspring is indicated by heritability. It is to be considered that the magnitude of predicted unit of measurement influences genetic advance, which is actually based on heritability estimates. A higher heritability estimate for a character renders it positive to selection as it indicates lower degree of influence of environment in its expression and such traits can be improved by simple selection methods.

In the present study, heritability estimates have been calculated in the broad sense, which includes variance due to all types of gene expressions. The information obtained on the magnitude of heritability estimates for the association mapping panel is also helpful in determining the extent of possible gains through selection. The classification of heritability estimates suggested by various workers was arbitrary. Hence an attempt has been made in present investigation to fix the limits and draw valid conclusion. Heritability estimates have been classified as very high (>90%),

high (80-90%), moderately high (70-80%), moderate (50-70%) and low (<50%) as per Bharadwaj (2004).

Those characters having high heritability and high genetic advance generally indicate that there is a predominance of additive gene action in these traits (Panse 1957). A response to selection could be anticipated in improving these traits and simple selection procedures can bring about improvements this trait. The traits with high heritability and low genetic advance and those with low heritability and high genetic advance indicate the presence of both additive and non additive gene effects with preponderance of the latter. This indicates the environmental influence on this traits in considerable amounts.

The traits exhibiting a low heritability and low genetic advance such as seed yield per plant, the preponderance of non additive gene action in the control of these traits was inferred. Yield is a complex character and an outcome of the interaction of all the other characters. Such characters are highly influenced by environment. Therefore, direct selection for this trait will not be useful. Multilocation, multi environment studies over the years greatly reduce the G X E interactions and E interactions (Mather and Jinks 1977). Comparisons of heritability estimates and prediction of genetic advance by using already obtained in other investigations may not be valid for the current population under study as these are strictly valid for that particular population from which these estimates are inferred. Hence, considerable variation in reports of workers may be generally found for the same character. Though, these may be helpful in hypothetical testing but it is important that heritable estimates be drawn afresh for the population in consideration before making any selections. Comparisons have been made here under with those obtained by previous workers in chickpea to get an overall picture of range of heritability estimates observed in chickpea for yield and yield contributing traits.

Very high and moderately high heritability coupled with high genetic advance was observed for days to 50% flowering, days to maturity, number of seeds per plant, number of pods per plant and hundred seed weight. Jivani *et al*. (2013), Kumar *et al*. (2016), Singhal *et al*. (2016), Tiwari *et al*. (2016) and Srivastava *et al*. (2017) also reported similar observation. Medium heritability with low genetic advance for plant height was reported by Kumar *et al*. (2016) and Srivastava *et al*. (2017). Low

heritability with low genetic advance was revealed for seed yield per plant. This was in consistence with the findings by Kumar *et al*. (2016).

5.1.1.4 Character association analysis

Correlation studies are carried out to understand the association between different traits. Knowledge on correlation among various traits is helpful in selection of any particular trait which may affect related traits in bringing desirable or undesirable changes. As such direct selection of yield may not be very rewarding as it is a complex quantitative trait with low heritability. Also such studies assess the relation of a trait on genotype and genotype x environment interaction. Understanding the association of different yield component traits with themselves and with yield is important to obtain maximum gain through selection. It is therefore a necessity to build a sound genetic basis of correlation to propel an effective breeding programme.

Estimation of genetic correlations along with phenotypic associations not only provides the information about the extent of inherent association but also indicates how much of the phenotypically expressed correlation is influenced by the environment. Though plant breeders are least interested in correlation, nevertheless it provides information about the relationship of characters irrespective of genotypic differences in the plant material. We have discussed below only significant correlations.

Number of pods per plant, number of seeds per plant and hundred seed weight were significantly and positively correlated with seed yield per plant but the association between seeds per plant and pods per plant with hundred seed weight was negative. This indicates that selection for more number of seeds per plant would directly affect the hundred seed weight as the limited photosynthates are available at the source and have to be distributed over increased seed number thereby reducing hundred seed weight. The negative linkage between seed number and hundred seed weight can be broken by maintaining a large population size so that transgressive segregants that have higher seeds per plant and hundred seed weight can be selected. The other approach is to select for other component traits that have indirect positive effect on seed weight to increase the overall seed yield. Since the association between these three traits with seed yield is positive and high, seeds per plant, pods per plant and hundred seed weight should be considered as important component traits to select

genotypes with high seed yield in chickpea. Such association between seeds per plant, pods per plant and hundred seed weight with seed yield was also reported by Renukadevi and Subbalakshmi (2006), Vaghela *et al*. (2009), Gohil and Patel (2010), Yucel and Anlarsal (2010), Pandey *et al*. (2013), Desai *et al*. (2015), Kumar *et al*. (2016) and Salgotra (2016).

Days to flowering was positively and significantly correlated to days to maturity and plant height and negatively correlated with pods per plant, seeds per plant, hundred seed weight and seed yield which was in accordance with result obtained by Salgotra (2016). Number of pods per plant was significantly and positively correlated with number of seeds per plant indicating that increase in the number of pods in the plant would automatically increase the number of seeds per plant. Days to maturity was significantly but negative with seed yield per plant, number of pods per plant and number of seeds per plant. The result indicates that though increase in days to maturity gives a proper time for seed setting in chickpea and usually with longer the days to maturity higher the seed set is expected but due to the terminal heat and drought experienced in the northern parts of India, the seed sets were poor. Similar association was also reported by Ramanappa *et al*. (2013).

5.1.3 Path coefficient analysis

Path coefficient analysis is a useful statistical tool uniquely designed to understand the inter-relationships of different components. Further, also it enables to study the character measures of direct and indirect effects on the seed yield. Through this method, yield-contributing trait components can be ranked and useful traits producing a given correlation coefficient can be revealed. These will in turn dissect the true association existing between a component trait and seed yield and a change in any one yield component will to disturb the whole network of cause and effect. Thus, each component has two paths of action *viz*., (1) The direct influence of that trait on seed yield (2) Indirect effects of that trait through components which are not revealed from the correlation coefficient studies. The path analysis was first suggested by Wright (1921) and provides an effective and useful measure of direct and indirect causes of association. It also depicts the relative importance of each factor involved in contributing to the final product that is, seed yield. In order to obtain such relations, the cause and effect of relationship between yield per se, six yield components were studied in chickpea through path coefficient analysis.

Path analysis has been inferred by Li (1956) and many other workers that it provides a complete visualization of causal factors involved in determining the final outcome of the end point, in the present study, seed yield per plant. However, in view of enormous complexity of factors involved in the expression of yield, it is never possible to have such a complete and inclusive path (Rao and Morton 1980). One way of overcoming this difficulty is by restricting the attributes to the immediate components of yield and treating this residual path as being uncorrelated with all the other components of yield. Though this may not be justifiable in many cases but a comprehensive picture of yield contributing traits can be obtained which of primary relevance in this investigation. However, the paths may differ as to the attributes depending upon the need and the objective of investigation planned.

Path coefficient analysis revealed seeds per plant, hundred seed weight and pods per plant had significant direct effect on seed yield. These characters also showed positive and significant association with seed yield per plant. Obviously direct selection for these characters would lead to increase yields. These findings were in similarity with those of Udin *et al*. (1990), Yucel *et al*. (2006) and Ali *et al*. (2009) for direct effects of number of seeds per plant and Ali *et al*. (2009) for direct effects of number of pods per plant and hundred seed weight on seed yield.

Number of pods per plant had high indirect effects on seed yield per plant through number of seeds per plant. This result was in conformity with the findings by Yucel *et al*. 2006. Since pods per plant also significant positive correlation with seed yield and therefore improvement through direct section or indirect selection through seeds per plant would be possible for this trait. Therefore in a strategic improvement of seed yield per plant in chickpea it would be advantageous to consider component traits like hundred seed weight, number pods per plant and number of seeds per plant for indirect selection for seed yield and make this parameters an integral part of effective selection criteria leading to yield enhancement in chickpea.

5.1.4 Cluster Analysis

The multivariate analysis giving the D^2 value with the dissimilarity level at 30% dissimilarity level grouped the 380 genotypes into twenty four clusters. The results showed the inter-cluster distances greater than intra-cluster distances, elucidating that considerable amount of genetic diversity existed among the

genotypes. Average intra-cluster distance revealed that cluster 3, which contained ten genotypes had maximum intra-cluster distance. The largest cluster group, cluster 2 had minimum intra cluster distance revealing that these genotypes were closely related in their evolutionary process and were passed through similar evolutionary factors.

Inter-cluster distance is the main criterion for selection of genotypes for hybridization programme. Genotypes belonging to the clusters with maximum inter-cluster distance are genetically more divergent and hybridization between genotypes of divergent clusters is likely to produce wide spectrum of genetic variability with desirable segregates. The maximum inter-cluster distance of 101.6 was recorded between cluster 22 and cluster 23 followed by cluster 6 and 22 (100.1 and cluster 21 and 22 (100) suggesting that the diverse genotypes from these diverse groups if used in breeding programme would produce a wide range of genetic variability in the population. The lowest inter-cluster distance of 14 was recorded between cluster 4 and 16 which indicates that the genotypes of these cluster are genetically less diverse and were almost with same genetic makeup.

For improving a particular trait of a variety which is otherwise suitable, donor parent can be selected from clusters with suitable characteristics. This information can be readily obtained from the estimates of cluster mean. Cluster means for the twelve traits of all the nine clusters were worked out. Cluster 15 had highest mean values of number of pods per plant and seeds per plant indicating that cluster 15 may be selected for higher seed and pod number genotypes. Cluster 1 had lowest mean value for plant height and therefore this cluster may be selected for developing short statured chickpea variety. Cluster 13 had highest mean value for hundred seed weight and so can be selected for bold seed size genotypes. The genotypes of cluster 21 may be selected for early maturing genotypes and cluster 11 for early flowering genotypes. Cluster 24 had highest mean value for seed yield per plant and the genotypes of this cluster will be useful for the development of high yielding genotypes in chickpea. To improve any particular trait donor may be selected from these clusters for hybridization program to evolve high yielding strains.

Table 5.1 The following genotypes of marked mean performance from the selected clusters may serve as parents for hybridization programmes

Clusters	Characters	Genotypes
1	Short stature	ILC8797
11	Days to flowering	ILC6062, IG5985, IG5987
13	Hundred seed weight	ILC6025
15	Pods per plant and seeds per plant	IG5855, ICCV97402, ICCV97404, ICCV00303
21	Days to maturity	ILC9793, IG5865, ILC5588
24	Seed yield	ICCV14307

5.1.5 AMMI analysis

To understand main effects and interactions for seed weight and seed number, AMMI biplot was constructed**.** AMMI1 biplot is a plot between the mean and the IPCA1 of GEI. The elucidation from the biplot is that if main effects have IPCA score nearing to zero, it indicates negligible interaction between the genotype and the environment and when a genotype and an environment have the same sign on the IPCA axis, it shows positive interaction; negative interaction if different. AMMI2 is a plot of IPCA1 vs IPCA2 and elucidates the magnitude of interaction of each of the genotype with the environment. The AMMI Analysis of variance showed that the genotype, environment and interaction effects are significant ($p < 0.01$) indicating difference in the genotypes behavior in the environments. It justifies understanding the behavior of the genotypes to rationalize the magnitude and extent of interaction with the environments (Gauch 1992). Estimation of phenotypic stability in this study was thrived by the significance of GE interaction (Farshadfar and Sutka 2006 and Osiru *et al*. 2009). Selection for yield stability across environments defined as location year combinations would help cope with genotype-year or genotype-location year interaction effects (Annicchiarico 1997). Many earlier reports on AMMI analysis have been made by McLaren *et al*. (1998), Ise *et al*. (2001), Kumar *et al*. (2001), Mahalingam *et al*. (2006), Das *et al*. (2009), Mukherjee *et al*. (2013), Akhter *et al*. (2014) in rice; Nachit *et al*. (1992), Tarakanovas and Ruzgas (2006), Mohammadi *et al*.(2007), Rad *et al*. (2013), in wheat, Shinde *et al*.(2002) and Pawar *et al*. (2012), Anuradha *et al*. (2017) in pearl millet, Sobaghpour *et al. (*2012), Balapure *et al*. (2016), Kanouni *et al*. (2015) in chickpea. These entire workers observed significant

G x E interaction for grain yield and stressed upon the usefulness of AMMI analysis for identifying and selection of promising stable genotypes for specific locations or environmental conditions. ANOVA revealed maximum variation explained by the genotypic effect similar to the studies shown by Akter *et al*. (2014) in rice and Anuradha *et al*. (2016) in pearl millet. Contradictory observation was made by Saboghpour *et al*. (2012), Rashidi *et al*. (2013), Balapure *et al*. (2016) and Kanouni *et al*. (2015) where largest contribution to total variation was by environmental effects and the genotype had little effect. The usefulness of the AMMI model is clear as they use overall fitting, impose no restrictions on the multiplicative terms and result in least square fit (Freeman 1990). Gauch and Zobel (1996) emphasized the informativeness of AMMI1 with IPCA1 and AMMI2 with IPCA1 and IPCA2 biplots and the graphical representation of axes, either as IPCA1 or IPCA2 against main effects or IPCA1 against IPCA2. The first two IPCs, IPCA1 and IPCA2 could explain 100% of the interaction effect for seed weight and seed number per plant leaving no residue. This observation supported the findings of Gauch and Zobel (1996), which recommended that the most accurate model for AMMI can be predicted using the first two IPCAs.G369 and G57 were identified as the most stable genotypes for seed weight and seed number per plant deduced from their IPC scores from the biplot. A genotype is best suited to a given environment when it presents high positive interactions with the specific environment making it invariably more suited to exploit the ecological and management conditions of the environment. The study of G X E interaction discerns the stability of a genotype to different environments and the above study clearly showed the convenience of AMMI model in deciphering the most stable and most unstable genotypes for different environments. It will further aide in developing environment wise adaptable genotypes depending on the extent of the genotype interaction with the environment. Less interactive genotypes for general adaptation and greater interacting genotypes for specific environments can be identified from this study.

5.2 Association mapping of yield traits in the association panel

Molecular marker research in chickpea is fairly recent. Isozyme analysis revealed low levels of polymorphism in chickpea (Tayyar and Waines 1996 and Labdi *et al*. 1996). Various DNA based molecular markers were also used to study polymorphisms such as RFLPs (Udupa *et al*. 1993), RAPDs (Ahmad 1999, Sant *et al*.

1999 and Iruela *et al.* 2002), ISSRs (Iruela *et al*. 2002) and AFLP but did not reveal sufficient levels of polymorphism. However, considerable genetic variation has been demonstrated using short microsatellite oligonucleotides for fingerprinting (Sharma *et al*. 1995). Moreover, the most promising DNA based marker system in chickpea has been the microsatellite based STMS technique. These markers were first developed in chickpea and used for mapping by Winter *et al*. 1999 and later more markers were added by Winter *et al*. 2000. At the national level also the group of Dr. Rajeev Varshney of ICRISAT and Dr Sabhyta Bhatia of NIPGR has done commendable work with molecular markers in chickpea. They have not only contributed in map generation but also in mapping disease resistance traits. Molecular analysis of the pathogens of chickpea was done using SSR markers (Barve *et al*. 2001). Genetic variability using DNA based molecular markers was also studied in chickpea (Sant *et al*. 1999, Udupa *et al*.1999 and Sant *et al*. 2000). Molecular maps were developed by most of the chickpea workers using biparental mapping population. Biparental mapping has the advantages of requiring fewer markers to detect QTL and gives high statistical power in detecting a QTL. However, the low accuracy of QTL mapping studies and inadequate validation of QTLs come in the way of practical utility of this QTL information for crop improvement. In contrast association mapping has the of potential of high resolution in localizing a QTL conferring a trait of interest which is the primary advantage of AM as compared to linkage mapping. AM has the potential to decipher superior alleles and provide detailed marker data in a large number of genotypes which could be of immediate application in breeding (Yu & Buckler 2006). Furthermore, AM uses breeding populations including diverse germplasm in which the most relevant genes should be segregating. Complex interactions (epistasis) between alleles at several loci and genes of small effects can be identified, pinpointing the superior individuals in a breeding population (Tian *et al*. 2011). Finally, AM has the potential not only to identify and map QTL but also to identify causal polymorphisms within a gene that are responsible for the difference between two phenotypes (Palaisa *et al*. 2003).

A diverse array of TF-encoding genes is known to regulate multiple growth, development and yield-related traits in crop plants including chickpea (Udvardi *et al*. 2007, Century *et al*. 2008, Libault *et al*. 2009, Kujur *et al*. 2013,2014, Saxena *et al*. 2014a, b, Yu *et al*. 2010). Therefore, it would be interesting to determine the

association potential of these TF genes in governing diverse major pod and seed yield component traits in chickpea through a Genome Wide Association Study (GWAS). This can be primarily achieved by large-scale genotyping of novel synonymous and non-synonymous coding and non-coding intronic and regulatory SNP allelic variants discovered from the TF genes in a diverse set of phenotypically well-characterized natural desi and kabuli germplasm accessions (association panel) of chickpea. In the current investigation, considering the pros and cons of GBS assay, a high sequencing-depth coverage targeted gene amplicon resequencing strategy coupled with GBS assay was utilized to discover and genotype TF gene-based high quality SNPs uniformly across 326 desi and kabuli germplasm accessions (association panel) of chickpea. Primarily, the genotyping and sequencing of 92 accessions selected from 326 desi and kabuli chickpea accessions using GBS assay discovered 1029 high-quality SNPs in 736 TF genes annotated on kabuli reference genome. A NGS-led GBS assay is proficient enough in fast large-scale discovery and high-throughput genotyping of SNPs simultaneously at a genome-wide scale for genomics-assisted breeding applications including association mapping to scan potential genes/ QTLs regulating vital agronomic traits in chickpea (Deokar *et al*. 2014, Bajaj *et al*. 2015a, b,c 2016a, b, Jaganathan *et al*. 2015, Kujur *et al*. 2015a, b, c, 2016a and Upadhyaya *et al*. 2015). However, reduced potential of GBS assay to generate non erroneous and high-quality homozygous SNP genotyping information uniformly across accessions genotyped with high genome/gene coverage is quite apparent. This could restrain the use of GBS assay in high-resolution association mapping study in a large chickpea genome with narrow genetic base. Henceforth, revalidation of GBS-derived SNP genotyping information as well as discovery and large-scale genotyping of novel SNPs covering the whole genome/gene regions at a high-resolution scale using numerous germplasm accessions are essential prior to deployment of these markers in genomics-assisted breeding applications and genetic enhancement of chickpea. The novel TF gene-based natural SNP allelic variants discovered from a diverse set of desi and kabuli chickpea germplasm accessions can be employed for multiple genomics-assisted breeding applications in chickpea. Especially, this involves marker-trait association and quick identification of functionally relevant molecular tags (markers, TFs and alleles) as well as regulatory signatures governing diverse traits of agronomic importance for marker-aided genetic enhancement of chickpea. Notably, six TF gene-derived SNPs identified to be associated commonly with PN and SN traits in a

constituted association panel of chickpea. One SNP derived from a TF gene encoding SNF2 (sucrose non-fermenting 2) was associated with both BN and PH traits, whereas another SNP mined from a TF gene encoding B3-domain protein was associated both SW and PH traits in chickpea. This could be due to high phenotypic correlation as observed in our study between PN vs. SN and SW vs. PH traits in a constituted association panel. Therefore, complex genetic architecture of these PH, PN, SN and SW traits was apparent, which were dissected efficiently in this study through high-resolution association mapping involving functionally relevant informative natural SNP allelic variants discovered from the TF genes of chickpea. The non-synonymous coding SNPs in the TF genes associated with six major pod and seed yield component traits (DF, PH, BN, PN, SN and SW) delineated in our studying high-resolution candidate gene-based association mapping, have functional significance for quantitative dissection of these complex yield traits in chickpea. This information can be useful for establishing rapid marker-trait linkages and efficient identification of potential TFs and natural allelic variants governing diverse pod and seed yield component traits in chickpea. Among six major yield trait-associated 27 TF gene-derived SNPs, merely two, four and three TFs-based SNP allelic variants governing SW, BN and DF traits, respectively delineated by us, have also been documented in our earlier studies for similar pod and yield component traits through integrating association analysis with QTL mapping, expression profiling and gene-based molecular haplotyping in chickpea (Kujur *et al*. 2013, 2014, 2015a, b, c, 2016a, Bajaj *et al*. 2015a, b, c, 2016a, b and Upadhyaya *et al*. 2015). The validation of these molecular tags in two of our independent studies infers the functional relevance and robustness of the identified TF gene-based natural SNP allelic variants in governing major pod and seed yield traits in chickpea. The six major pod and seed yield trait-associated 27 TF genes with SNPs delineated by association mapping in chickpea are reported to be involved in transcriptional regulation of growth, development and yield traits in multiple crop plants (Manning *et al*. 2006, Agarwal *et al.* 2007, Udvardi *et al*. 2007, Nijhawan *et al*. 2008, Libault *et al*. 2009, Heang and Sassa 2012, Martínez-Andújar *et al*. 2012, Hudson and Hudson 2015, Wang *et al*. 2015, Wang and Wang 2015 and Zhang *et al*., 2015). Therefore, functionally relevant novel as well as earlier documented TF gene-based molecular signatures (TFs and natural SNP alleles) regulating six major yield traits delineated in our study, once comprehensively

validated and characterized, will essentially be employed for marker-assisted genetic enhancement to develop high pod and seed yielding cultivars of chickpea.

5.3 Validation of candidate genes for seed weight in contrasting genotype

Microsatellites, or simple sequence repeats (SSRs), simple sequence length polymorphisms (SSLPs), short tandem repeats (STRs), sequence target microsatellites (STMs), are a class of repetitive sequences which are widely-distributed in all eukaryotic genomes and some prokaryotic genomes. They are reported to occur in both euchromatic and heterochromatic regions depending upon species. They consist of arrays of tandemly repeated short nucleotide motifs of 1-5 bases known as mono-, di-, tri-, tetra- or pentanucleotide repeats respectively. It has been shown that such arrays of short DNA elements repeated in tandem tend to be imprecisely replicated during DNA synthesis, and generate new alleles with different numbers of repeating units. Variable number of repeats between individuals is a result of slippage of the DNA polymerase during DNA replication (Tautz *et al*. 1986). This length variation is the basis of polymorphism even between closely related individuals. The microsatellite sequences are flanked by unique sequences which can be used for PCR amplification of the microsatellite regions (Sequence Tagged Microsatellite Sites-STMS). Hence using a pair of flanking, locus-specific, oligonucleotides as primers one can detect DNA length polymorphisms (Litt and Luty 1989 and Weber and May 1989). Further, these microsatellite markers that are specific to a single genetic locus, are codominant and, most importantly, they are multiallelic and detect a much higher level of DNA polymorphism than any other marker system.

The power of association mapping is the probability of detecting the true associations within the mapping population. 21 previously reported gene based SSR markers for seed weight were tested for their validation through their association in a subset of 96 genotypes selected based on their variability to seed weight from the association panel. The results obtained from the analysis revealed a highly significant ($p<0.001$) association between five genic SSR markers with seed weight. These five genic markers viz., TAA 137, CaIISSR25, CaSSR53, GA11 and CEST47 were previously reported by Hossain *et al*. (2010), Gupta *et al*. (2015) to be associated with the trait in biparental populations. The validation of such previously reported genic microsatellite markers by screening them in the association panel for rapidly establishing marker-trait association and identifying markers linked to QTLs for an

important quantitative trait of chickpea will expedite their utility in other populations. Such validated QTLs now can be routinely used in marker based breeding applications for improvement of yield in chickpea.

SUMMARY & CONCLUSIONS

CHAPTER 6 SUMMARY AND CONCLUSION

The present investigation was carried out to find out the extent of genetic variation of yield traits in the association mapping population, association mapping of yield traits in the association panel and to validate candidate genes for seed weight in contrasting genotype. The plant material for the study consist of 380 association mapping panel consisting of landraces, training population and released varieties of both desi and kabuli types from West Asia and North Africa region, ICRISAT and IARI. All the genotypes were evaluated in RCBD (Randomised Complete Block Design) in the field and the data were collected for three years with three replications. The experiment was carried out during the month of *rabi* for three consecutive years, 2014-15, 2015-16 and 2016-17 in Genetics field, IARI.

The salient features of the experimental findings are as follows:

1. The analysis of variance for the 380 genotypes indicated the presence of considerable amount of genetic variability for yield and its component traits for all the three years.
2. The phenotypic coefficient of variance was higher than the corresponding genotypic coefficient variation for most of the traits.
3. The phenotypic coefficient of variance was recorded high for plant height, number of seeds per plant and number of pods per plant.
4. Very high heritability estimates with high genetic advance was observed for most of the traits including days to 50% flowering (94%, 35.32), days to maturity (93.6%, 16.18) and hundred seed weight (99%, 15.85).
5. High to moderate heritability coupled with high genetic advance was recorded for number of pods per plant and number of seeds per plant.
6. High degree of association was observed for seeds per plant, pods per plant and hundred seed weight with seed yield per plant. High correlation was recorded for plant height and hundred seed weight and between pods per plant and seeds per plant.
7. High negative correlation was between days to 50% flowering with hundred seed weight and seed yield per plant.
8. Path coefficient analysis revealed that pods per plant, seeds per plant and hundred seed weight had significant positive direct effect on yield.

Number of pods per plant exerted significant positive indirect effect on seed yield through number of seeds per plant.

9. AMMI1 biplot showed maximum variation was accounted by genotypic effect due to seed weight and seed number, contributing 93.08% for both respectively followed by G X E effects with 4.1 % for the two traits.
10. AMMI analysis identified ICCV14307 and IG5982 as the most stable genotypes for hundred seed weight and genotypes ICCV00309 and IG5893 for seed number.
11. Cluster analysis identified genotypes ILC8797 for short stature plant, ILC6062, IG5985 andIG5987 for early days to 50% flowering, ILC6025 for high hundred seed weight, IG5855, ICCV97402, ICCV97404 and ICCV00303 for high seeds per plant and pods per plant, ILC9793, IG5865 and ILC5588 for early days to maturity and ICCV14307 for high seed yield.
12. Maximum of the trait associated SNPs were mapped through GWAS in the exons or CDS (coding non synonymous region) of the genome (58.3%) and minimum in downstream regulatory regions (DRRs) with only 0.4%. The coding SNPs contained 529 (56.3%) synonymous and 410 (43.7%) non-synonymous (missense and nonsense SNPs) SNPs from204 and 218 TF genes, respectively.
13. Genome wide association mapping for six major seed yield traits identified 27 TF gene-derived SNPs exhibiting significant association with days to 50% flowering, plant height, branch number, pod number, seed number and seed weight. This traits individually and in combination explained 10-23% and 32% phenotypic variation.
14. Maximum TF gene derived SNPs for all the six traits were mapped in chromosome 3 with 10 SNPs and minimum of 2 SNPs each were mapped in chromosome 4 and unanchored scaffold genomic region of chickpea.
15. Five genic microsatellite markers TAA137, CaIISSR25, CaSSR53, GA11 and CEST47 were found to be significantly associated with seed weight in chickpea through association study in 96 subset panel using 21 genic markers.

Conclusion

It can be concluded from the study that there exists considerable variation within the association mapping panel. Presence of additive gene effect for most of the traits infers a positive response to selection. Important traits like pods per plant, seeds per plant and hundred seed weight were significantly associated with seed yield per plant and therefore selection for seed yield can be carried out keeping in mind the selection through these traits would be rewarding. Interestingly from the present investigation through GWAS, novel non-synonymous coding SNP allelic variants in five potential candidate TF genes encoding SBP (squamosal promoter binding protein), SNF2 (sucrosenon-fermenting2), GRAS [Gibberellic acid insensitive (GAI)-Repressor of GAI (RGA)-SCARECROW (SCR)], bZIP (basic leucine zipper) and LOB (lateral organ boundaries)-domain proteins associated strongly with days to 50% flowering, plant height, branch number, pod number, seed number and seed weight traits respectively were found most promising in chickpea. The functionally relevant molecular signatures (TF and natural SNPs alleles) delineated have the potential to accelerate marker-assisted genetic enhancement by developing high pod and seed yielding cultivars of chickpea.

Suggestions for future line of work:

1. Heritability estimates and realized heritability needs to be worked out afresh in the new population or panel in order to form a correct idea of the character inheritance.
2. Stable genotypes identified through AMMI analysis can further be analyzed in other stability models like GGE to establish true stability.
3. Genic SSR markers amplified in this study can be further studied with higher resolving systems like PAGE or SNPs identification in that region to identify functional polymorphism within the gene.
4. Comprehensive validation and characterization of functionally relevant novel TF gene-based molecular signatures (TFs and natural SNP alleles) for its essential utilization in marker-assisted genetic enhancement to develop high seed yielding cultivars of chickpea.

ABSTRACT

Genome Wide Association Mapping of yield traits in Chickpea [*Cicer arietinum* L.]

ABSTRACT

Identification of potential transcription factor (TF) gene-derived natural SNP allelic variants regulating pod and seed yield component traits by large-scale mining and genotyping of SNPs in natural germplasm accessions coupled with high resolution association mapping is vital for understanding the complex genetic architecture of quantitative yield traits in chickpea. In this perspective 380 diverse chickpea genotypes formed the association panel and were phenotyped for three consecutive years (2014, 2015 and 2016). Presence of significant difference among the genotypes and sufficient amount of variability in the association panel was indicated by variability study for days to 50% flowering (DTF), days to maturity (DTM), plant height (PH), pods per plant (P/Pl), seeds per plant (S/Pl), hundred seed weight (100SW) and seed yield per plant (SY/Pl). Very high to moderate heritability coupled with high genetic advance was observed for days to 50% flowering, days to maturity, hundred seed weight, number of pods per plant and number of seeds per plant. High positive association and significant positive direct was observed from seeds per plant, pods per plant and hundred seed weight on seed yield per plant indicating direct selections of this traits for yield would be rewarding. Stability analysis by AMMI method revealed ICCV14307 and IG5982 as the most stable genotypes for hundred seed weight and genotypes ICCV00309 and IG5893 for seed number. A genome-wide GBS (genotyping-by-sequencing) and targeted gene amplicon resequencing-based simultaneous SNP discovery and genotyping assays, which discovered 1611 novel SNPs from 736 TF genes physically mapped on eight chromosomes and unanchored scaffolds of kabuli chickpea genome. A high-resolution genetic association analysis was performed by correlating the genotyping information of 1611 TF gene-based SNPs with multi-location/years field phenotyping data of six major pod and seed yield traits evaluated in a constituted association panel (326 desi and kabuli germplasm accessions) of chickpea. This essentially identified 27 TF gene-derived SNPs exhibiting significant association with six major yield traits, namely days to 50% flowering (DF), plant height (PH), branch number (BN), pod number (PN), seed number (SN) and seed weight (SW) in chickpea. These trait-associated SNPs

individually and in combination explained 10–23% and 32% phenotypic variation respectively for the studied yield component traits. Interestingly, novel non-synonymous coding SNP allelic variants in five potential candidate TF genes encoding SBP (squamosal promoter binding protein), SNF2(sucrosenon-fermenting2),GRAS [Gibberellic acid insensitive (GAI)-Repressor of GAI (RGA)-SCARECROW (SCR)], bZIP (basic leucine zipper) and LOB (lateral organ boundaries)-domain proteins associated strongly with days to 50% flowering (DTF), plant height (PH), branch number (BN), pod number (PN), seed number (SN) and hundred seed weight (100SW) traits respectively were found most promising in chickpea. Validation study with 21 SSR genic markers in 96 subset panel through association study deciphered five genic microsatellite markers TAA137, CaIISSR25, CaSSR53, GA11 and CEST47 significantly associated with seed weight in chickpea.

जीनोम वाईड एसोसिएशन चने में उपज गुणों का मानचित्रण [*सीकर एरिएटिनम* एल.]

अमूर्त

संभावित प्रतिलेखन कारक (टीएफ) की पहचान जीन-व्युत्पन्न प्राकृतिक एसएनपी एलिलिक वेरिएंट पॉड और बीज उपज घटक को विनियमित करते हैं जो बड़े पैमाने पर खनन और एसएनपी के जीनोटाइपिंग को प्राकृतिक जर्मप्लाज्म अभिगमों में उच्च संकल्प एसोसिएशन मैपिंग के साथ जटिल जटिल आनुवंशिक वास्तुकला को समझने के लिए महत्वपूर्ण है। चना में मात्रात्मक उपज गुण। इस दृश्य में 380 विविध चना जीनोटाइप ने एसोसिएशन पैनल का गठन किया और उन्हें लगातार तीन वर्षों (2014, 2015 और 2016) के लिए फेनोटाइप किया गया। एसोसिएशन पैनल में जीनोटाइप और पर्याप्त मात्रा में परिवर्तनशीलता के बीच महत्वपूर्ण अंतर की उपस्थिति को दिन में 50% फूल (डीटीएफ), परिपक्वता (डीटीएम), पौधे की ऊंचाई (पीएच), फली प्रति पौधे (पी / पीएल), बीज प्रति संयंत्र (एस / पीएल), सौ बीज वजन (100 एसडब्ल्यू) और बीज उपज प्रति संयंत्र (एसआई / पीएल)। उच्च आनुवांशिक अग्रिम के साथ मिलकर बहुत ऊंची उदारता से देखा गया दिन 50% फूलों, परिपक्वता के लिए दिन, सौ बीजों का वजन, पौधों की संख्या और पौधों की संख्या प्रति बीज के लिए मनाया गया। उच्च सकारात्मक संघ और महत्वपूर्ण सकारात्मक प्रत्यक्ष बीज प्रति संयंत्र, फली प्रति पौधे और प्रति बीज उपज पर सौ बीज के वजन से देखा गया था जिससे उपज के लिए इस गुण के प्रत्यक्ष चयन को पुरस्कृत किया जाएगा। एएमएमआई पद्धति द्वारा स्थिरता विश्लेषण ने आईसीसी 141477 और आईजी 5982 को बीज के नंबर के लिए सौ बीजों और जीनोटाइप आईसीसी वी 0030 9 9 और आईजी 5893 के लिए सबसे स्थिर जीनोटाइप के रूप में बताया। एक जीनोम-व्यापी जीबीएस (जीनोटाइपिंग-बाय-सिकेंजिंग) और लक्षित जीन एम्प्लिकॉन पुनः अनुक्रमण -आधारित एक साथ एसएनपी डिस्कवरी और जीनोटाइपिंग एल्स, जो 736 टीएफ जीनों से 1611 उपन्यास एसएनपी की खोज की गईं, जो शारीरिक रूप से आठ गुणसूत्रों पर मैप किए गए और काबुली चनेपी जीनोम के असंबद्ध स्कैफोल्ड एक उच्च-रिज़ॉल्यूशन आनुवंशिक एसोसिएशन विश्लेषण 1611 टीएफ जीन आधारित एसएनपी की जीनोटाइपिंग जानकारी के साथ-साथ गठित एसोसिएशन पैनल (326 देसी और काबली जर्मप्लाज्म) के मूल्यांकन के छह प्रमुख पॉड्स और बीज उपज गुणों के बहु-स्थान / वर्ष क्षेत्र के फेनोटाइपिंग डेटा के साथ संबंध

द्वारा किया गया था। चने का) इसने हाल ही में 27 टीएफ जीन-व्युत्पन्न एसएनपी की पहचान की जो छह प्रमुख उपज गुणों के साथ महत्वपूर्ण सहयोग का प्रदर्शन करती है, अर्थात् 50% फूल (डीएफ), पौधे की ऊंचाई (पीएच), शाखा संख्या (बीएन), पोड संख्या (पीएन), बीज संख्या (एसएन)) और चने में बीज वजन (एसडब्ल्यू)। ये गुण-संबद्ध एसएनपी अलग-अलग और संयोजन में अध्ययनित उपज घटक गुणों के लिए क्रमशः 10-23% और 32% फ़िनोटीपिक भिन्नता को समझाते हैं। दिलचस्प बात यह है कि पांच संभावित उम्मीदवार टीएफ जीन एसबीपी (स्कैमोजल प्रमोटर बाइंडिंग प्रोटीन), एसएनएफ 2 (एसक्रिफ़ोन), जीआरएएस [जीबीबीरेलिक एसिड असेंसिटिव (जीएआई) - जीएआई (आरजीए) के संरक्षक, आरजीए -SCARECROW एससीआर)], बीजीआईपी (मूल लियूसीन ज़िप) और लोब (पार्श्व अंग की सीमाएं) -डोमेन प्रोटीन दिन के 50% फूल (डीटीएफ), पौधे की ऊंचाई (पीएच), शाखा संख्या (बी एन), पोड संख्या (पीएन) बीज संख्या (एसएन) और सौ बीजों का वजन (100 एसडब्ल्यू) लक्षण क्रमशः चने में सबसे अधिक आशाजनक पाए गए। 21 एसएसआर जीनिक मार्करों के साथ सत्यापन अध्ययन एसोसिएशन के अध्ययन के माध्यम से 96 सबसेट पैनल में पांच जननिक माइक्रोसाईटलाइट मार्करों टीएए 137, सीएआईएसएसआर 25, सीएएसएसआर 53, जीए11 और सीईएसटी 47 में उल्लेखनीय रूप से चने में बीज के वजन के साथ जुड़ा हुआ है।

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ANNEXURE