“GENETIC DIVERGENCE STUDIES IN ADVANCE BREEDING LINES OF CHICKPEA (*Cicer arietinum* L.) UNDER RAINFED CONDITIONS”

By

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(J-16-M-440)

Thesis submitted to Faculty of Agriculture
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE IN AGRICULTURE

GENETICS AND PLANT BREEDING

Division of Plant Breeding and Genetics
Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu 180009
2019

M.Sc.

GENETIC DIVERGENCE STUDIES IN ADVANCE BREEDING LINES OF CHICKPEA (*Cicer arietinum* L.) UNDER RAINFED CONDITIONS

SAROJ BALA

2019

CERTIFICATE-I

This is to certify that the thesis entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)** to the Faculty of Post Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is a record of bonafide research, carried out by **Saroj Bala**, Registration No. **J-16-M-440**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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ACKNOWLEDGEMENT

"*Accomplishment of this thesis is the results of benevolence of omnipotent and omniscient Almighty and blessing of my teachers.*

I am overwhelmed with joy to evince my profound sense of reverence and gratitude to my esteemed Major Advisor, Dr. Sanjeev Kumar, Sr. Scientist, Plant Breeding and Genetics, SKUAST-Jammu for his insightful, critical criticis, intellectual guidance, constant encouragement, motivation, indispensable support during course of the investigation. In crisp, he is a real 'mentor'who groomed me as a researcher of today's age. He always gives me positive attitude to face the every situation. It is all because of his open mindedness, objective thoughts in research that has helped me to establish the overall direction of the research. I consider it an honor to work with you Sir.

I express my special gratitude to honorable members of my advisory committee, Dr. S. K. Gupta, Professor & Head, Plant Breeding and Genetics, Dr. Gurdev Chand, Asst. Professor, Division of Plant Physiology, Dr. Magdeshwar Sharma, Sr. Scientist, Entomology (Dean's Nominee) for their generous help and encouragement throughout the course of the study.

I extend my respectful thanks to all the Professor ofPlant Breeding and Genetics, Dr. Bikram Singh, Dr. A. K. Razdan, Dr. Parveen Singh, Dr. Bupesh Kumar, Dr. B. B. Gupta, Dr. Tuhina Dey, Dr. S. K. Rai, Dr. B. S. Jamwal, Dr. M. K. Pandey for their kind attitude, guidance and support.

I feel to express my thanks to supporting staff of my department Mr. Paramjit, Mr. Naresh Choudhary, Mr. Darshan Lal, Mr. Vijay Kumar for their co-operation and help during the work.

I express my intense gratitude to Honorable Vice Chancellor and Director Education for providing all possible facilities for completion of this study.

Sincere and special thanks to my colleagues Chamanpreet Kour and Lekh Raj for their support and cooperation.

I express my special thanks to my seniors and juniors Mrs. Rubby Sandhu, Ms. Heena Attri, Dr. Waniya, Dr. Amardeep Kour, Dr. Ashwani Kumar, Mr. Ashok, Mr.Jafar Ali, Mr. Manmohan Singh, Mrs. Rucku Gupta, Ms. Akashi Sharma, Mr. Sonam Sharma, Mr. Om prakash, Mrs. Surbhi Kohli, Mr. Harpreet Singh for their support and suggestions during the work.

My deepest gratitude goes to my family for their intense love and support throughout my life. I have no suitable words that can fully describe their everlasting love and care for me. I express my heartfelt gratitude to my parents, Sh. Amar Nath and Mrs. Bimla Devi.

I also acknowledge my sincere thanks to the staff members of Central Library, SKUAST-J, Chatha for providing all possible help while collecting the research literature.

Finally, I owe my gratitude to all those whom I could not mention here for their love and affection throughout the course of study.

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ABSTRACT

Title of Thesis	:	**Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions**
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Admission number	:	J-16-M-440
Major Subject	:	Genetics & Plant Breeding
Name and designation of the major advisor	:	Dr. Sanjeev Kumar, Sr. Scientist (Plant Breeding & Genetics)
Degree to be awarded	:	M.Sc. Agriculture (Genetics & Plant Breeding)
Name of the university	:	Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu

ABSTRACT

The cultivated chickpea (*Cicer arietinum* L.) is the first most important pulse crop of India in area, production and productivity (Agrawal *et al.* 2018). Chickpea is one of the major pulse crop grown under rainfed conditions in Jammu and Kashmir during *Rabi* season. In the present investigation, twenty two advanced breeding lines of chickpea along with standard check (SCS-3) were evaluated in Randomized Block Design (RBD) with row length of 5m and spacing 30x10cm^2 under rainfed conditions at the experimental farm of Advanced Center for Rainfed Agriculture (ACRA) Dhiansar during *Rabi* 2017-18 with the objectives to find out genetic variability, heritability, correlation coefficient, path analysis and genetic divergence. The analysis of variance showed highly significant differences for all the characters studied except days to maturity where it was significant at p=0.05. The high estimate of phenotypic and genotypic coefficient of variations were recorded for seed yield / plant. High heritability coupled with high genetic advance as per cent of mean was observed for seed yield / plant, biological yield / plant, test weight, number of pods / plant, number of primary branches / plant, number of secondary branches / plant, number of seeds / pod, relative growth rate, proline content and harvest index which indicated that additive gene effects is important in determining these characters and improvement can be done through selection based on phenotypic values. The most important character i.e. seed yield / plant, was positively and significantly correlated with plant height, number of pods / plant, test weight, biological yield / plant and harvest index. Path analysis revealed that characters *viz*. biological yield / plant, harvest index and plant height exhibited high positive direct effect on seed yield / plant. All the twenty two(22) advance breeding lines and check were grouped into 6 clusters based on D^2 analysis. Cluster I and II were the biggest with 5 and 8 lines followed by cluster II containing 6 lines, respectively.

On the basis of genetic divergence and mean performance, advance breeding lines **SCS-15-D-3, SCS-15-D-11, SCS-15-D-1, SCS-15-D-8 and SCS-15-D-15** have been suggested for further use in chickpea breeding programme.

Keywords: *Cicer arietinum,* variability, correlation coefficients, genetic diversity.

03/10/19

Signature of the Major Advisor **Signature of the Student**

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Introduction

CHAPTER-I

INTRODUCTION

The cultivated chickpea (*Cicer arietinum* L.) is an annual legume of the family *Fabaceae* having 2n=2x=16 chromosomes, with a relatively small genome size of 738.09 Mb (Varshney *et al.,* 2013). It is highly self-pollinated crop with very low level of out crossing due to cleistogamous flowers (Toker *et al.,* 2006). It is also known as gram or Bengal gram, garbanzo or garbanzo bean, and sometimes known as Egyptian pea and chana. Cultivated chickpea has two distinct forms, i.e. *desi* (small seeded, angular shaped with high percentage of fibre) and *kabuli* (large seeded, owl shaped, with a low percentage of fibre).

It is second most important pulse crop world-wide in terms of area under cultivation after dry bean but ranks third in production following dry bean and peas and is currently cultivated more than 50 countries (Reddy *et al.,* 2017). Globally, chickpea is grown in an area of 13.98 Million ha with production of 13.74 Million tonnes with an average productivity of 982 Kg/ha (FAO, 2016). India ranked first in area and production of chickpea in the world with production of 9.08 Million tonnes from an area of 9.54 Million ha with productivity of 951 Kg/ha (Directorate of Economics & Statistics, 2016-17). The area sown under pulses in Jammu and Kashmir is about 26.57 thousand ha, production is about 84.10 thousand quantal with productivity of 3.17 quantal/ha (Jeelani and Choure, 2015)

It is desirable crop due to its good nutritional value as it contains on an average of 4.5% fat, 8% crude fiber, 25-29% protein, 41-50% carbohydrate and 2.7% ash (Wood and Grusak. 2007). Besides its nutritional value, it is an important contributor to soil fertility as it provides nitrogen to soil due to nitrogen fixation ability with help of bacteria (*Rhizobium* sp.) (Gul *et al*., 2011). Despite its high nutritional values and economic importance, the average yield of chickpea production in India is low and unstable. This may be attributed to the evolution of cultivars with narrow genetic base making them vulnerable to constitutional stresses (Bhanu *et al.,* 2017).

About 90% of the world's chickpea is grown under rainfed conditions where the crop grows and matures on a progressively depleting soil moisture profile and experiences terminal drought, a condition in which seed yield of chickpea is low

(Kumar and Abbo, 2001). Drought is one of the most important abiotic stresses, which limits crop production in different parts of the world (Singh *et al.*, 2008). Estimate of yield loss due to drought ranges from 15 to 60 % which depends on geographical region and length of crop season. The early sowing and short duration cultivars are predicted to help chickpea crop to escape terminal drought condition. On the other hand, the combination of long duration cultivars, standard cultivars and early sowing conditions are predicted to increase seed yield of chickpea in areas where high temperature is major crop production constraint (Mohammed *et al.*, 2017).

Evaluation of crop genetic resources is a pre-requisite on which the future breeding work is based. The value of germplasm relies not only on the number of accessions it possesses, but also upon the genetic variability present in those accessions for agronomic and yield components (Reddy *et al.*, 2012). Crop improvement depends largely on the genetic variability for exploitation through various methods of plant breeding (Malik *et al.*, 2010). It is of prime importance to analyze and evaluate the magnitude of genetic variation among quantitative traits and their inter-relationship for breeding and in selecting desirable types with yield as an end product.

In addition to genetic variation, heritability of economically important characters is essential for effective breeding programme and selection of specific traits. Heritability act as predictive tool in expressing the reliability of phenotypic traits and thus high heritability could assist in effective selection of particular characters and devise future breeding programme of chickpea. It is equally important to study other variability parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), and genetic advance over mean (GAM).

In chickpea, association of one or more characters influenced by a large number of genes is elaborated statistically by correlation coefficients. Genotypic correlation coefficient provides a measure of genotypes conjugation between characters. The inter-relationship between important yield components is best estimated by correlation coupled with path coefficient analysis (Agrawal *et al.*, 2018). These techniques are used in the breeding programme to exploit the yield potential for enhancing the productivity of chickpea and to develop high yielding improved varieties. The estimates of correlation coefficient alone may be often misleading due to mutual cancellation of component characters. So, study of correlation coupled with a path analysis is more effective tool in the study of yield contributing characters. Path coefficient analysis is an important technique for partitioning the correlation coefficient into direct and indirect effects of the causal components on the complex components like yield.

The knowledge of genetic diversity helps in the tagging of germplasm, identification of genetic stock and establishment of core collections (Upadhyaya *et al.*, 2007). If the parents selected for hybridization have diverse background, more are the chances of improving the characters under consideration (Chowdhury *et al.*, 2002). Criteria for estimation of the genetic diversity can be different, including morphological traits (Upadhaya *et al.*, 2007) or molecular markers (Rao *et al.*, 2007). Murthy and Arunachalam (1966) stated that multivariate analysis with "Mahalanobis D^2 statistics" is a powerful tool to know the clustering pattern to establish the relationship between genetic and geographic divergence and to determine the role of different quantitative characters towards the maximum divergence. Quantitative traits provide an estimate of genetic diversity and numerical taxonomic techniques including principal component and cluster analyses have been successfully used to classify and measure the pattern of genetic diversity in germplasm (Ghafoor *et al.*, 2001). Principal component and cluster analysis procedures were found to be efficient to assess genetic diversity for agro-morphological traits in chickpea and were reported by many research workers (Parameshwarappa *et al.*, 2011, Gupta *et al.*, 2011, Nihal and Adak, 2012).

In pulses, especially chickpea a very narrow genetic base exists due to some common genotypes are being involved in hybridization programme. There is immediate need to broaden the genetic base of chickpea. Keeping these facts in mind, twenty two diverse lines were developed and evaluated for yield, yield attributing and bio-physiological traits under rain-fed conditions during *Rabi* 2017-18.

The present study entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** was planned with the following objectives:

- To study the genetic variability of different morpho-physiological and quality traits of chickpea.
- To study the correlation between different traits of chickpea and study the cause effect of relationship.
- To assess genetic diversity of chickpea recombinants and identification of diverse parents for future hybridization programme.

Chapter-2

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

The literature pertaining to the present investigation entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** has been reviewed under the following headings:

2.1 Genetic variability

2.2 Correlation and path analysis

2.3 Genetic divergence

2.1 GENETIC VARIABILITY:

Genetic variability refers to the presence of differences among the individuals of plant population. The existing variability is essential for improvement of genetic material. However, it is only genetic variation which is heritable and hence important in any selection programme (Manikanteswara *et al.* 2018). The genetic variability is determined with the help of certain genetic parameters, such as, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance.

Arora (1991) revealed high genotypic coefficient of variation and phenotypic coefficient of variation for pods / plant, test weight and seed yield / plant. High heritability coupled with high expected genetic advance for test weight, seed yield / plant and number of pods / plant. Rao *et al*. (1994) evaluated forty four varieties of chickpea and reported highest genetic coefficient of variation for test weight followed by secondary branches / plant, number of pods / plant and seed yield / plant. Also observed high heritability coupled with high genetic advance for test weight and plant height. Arora and Jeena (2001) used forty genetically diverse genotypes of chickpea and reported highest genotypic variability for test weight followed by seed yield / plant, biological yield / plant, secondary branches / plant and seeds / plant. The test weight also exhibited high genetic advance with high heritability.

Usmani *et al.* (2005) reported that high phenotypic coefficient of variation for seed yield / plant, plant height, harvest index (%) and high genotypic coefficient of variation for number of pods / plant. Durga *et al.* (2007) exhibited that maximum variability was observed for secondary branches / plant, followed by number of pods / plant and seed yield / plant. High heritability coupled with high genetic advance was observed for days to 50% flowering, number of pods / plant and seed yield / plant. Borate *et al*. (2010) were evaluated PCV and GCV and genetic advance for thirteen agronomic traits from 30 chickpea genotypes and reported that range of variability was appreciable for number of secondary branches / plant, plant height and seed yield / plant. Genotypic and phenotypic variance were highest for number of pods / plant, while lowest for seed yield / plant. PCV showed higher values than GCV for all characters.

Akhtar *et al.* (2011) twenty advance genotypes of chickpea collected from various sources along with one check variety (Pb-2000) and reported phenotypic coefficient of variation for days to flowering, days to maturity, plant height and seed yield / plant were high than corresponding genotypic coefficient of variation, which means that the expression of these traits is more influenced by environmental effects. Kanouni *et al*. (2012) revealed that genotypic differences were significant. The phenotypic coefficient of variation (PCV) were found higher than the genotypic coefficient of variation (GCV) and the environmental coefficient of variation (ECV) for all traits. Jadhav *et al.* (2012) found high variability in days to maturity followed by number of pods / plant, days to 50% flowering, harvest index, seed yield / plant, test weight, plant height and number of secondary branches / plant. High heritability along with high genetic advance was also observed for seed yield / plant, secondary branches / plant and test weight.

Parameshwarappa *et al.* (2012) reported high PCV and GCV estimates for the traits under study *viz.,* number of primary branches / plant, secondary branches / plant, number of pods / plant and seed yield / plant. High heritability with moderate genetic advance as %age of mean was recorded for days to 50% flowering in all three environments. High heritability with high genetic advance as %age of mean was recorded for number of pods / plant, test weight and seed yield / plant. Jivani *et al.* (2013) revealed that high heritability coupled with high genotypic coefficient of variation (GCV) and high genetic advance as per cent of mean (GA%) were observed for seed yield / plant, biological yield / plant, harvest index, number of pods / plant and test weight which indicated that response to selection would be very high for these yield components.

Ramanappa *et al*. (2013) one hundred and seventy nine chickpea genotypes were evaluated for genetic variability. revealed genotypes exhibited highly significant differences for days to 50% flowering, plant height (cm), number of primary branches, number of pods/ plant, days to maturity, test weight, harvest index and seed yield/plant (g).The coefficient of variation (PCV) was slightly higher than their corresponding genotypic coefficient variation (GCV) for all the characters indicated least influence of environment in the expression of these traits. Shweta *et al.* (2013) reported high PCV and GCV for seed yield / plant followed by number of pods / plant and number of seeds / pod whereas minimum in days to maturity. High heritability along with high genetic advance as percentage of mean was observed in secondary branches / plant, seed yield / plant, test weight, number of pods / plant and plant height.

Aarif *et al.* (2014) used twenty two genotypes of *kabuli* chickpea to assess variability, heritability, genetic advance between yield and yield components and reported that test weight had the highest magnitude of genotypic coefficient of variation, whereas phenotypic coefficient of variation was found to be high for test weight followed by seed yield / plant and secondary branches / plant. Replicated field experiment was conducted for sixty genotypes of chickpea during long and short rain seasons by Mallu *et al*. (2014) and all the studied traits were highly significant. High broad sense heritability observed for days to 50 % flowering, plant height, test weight, seed yield / plant, number of secondary branches / plant, harvest index, days to maturity and number of pods / plant.

Hagos *et al.* (2015) observed the highest estimates for phenotypic coefficients of variation in number of secondary branches / plant, number of pods / plant and test weight. High GCV found in secondary branches / plant, number of pods / plant in chickpea genotypes. Gupta *et al.* (2016) evaluated six important quantitative traits among twenty five chickpea genotypes to ascertain their potential to grow in new agro-climatic zone of North-Western Himalayas. The chickpea genotypes exhibited sufficient variability for all the traits. High PCV was observed only for seed yield / plant but moderate PCV for plant height, number of branches / plant and number of pods / plant. However, moderate GCV was observed for plant height, number of pods / plant and seed yield / plant. The traits under study exhibited greater influence of environment. Kumar *et al.* (2016) forty chickpea genotypes were evaluated under rainfed conditions to estimate genetic variability among various traits. High heritability was observed for number of branches / plant, protein content, number of pods / plant, harvest index, plant height and biological yield / plant. High heritability coupled with high genetic advance for number of branches / plant, harvest index, number of pods / plant, test weight and biological yield / plant.

Admas and Abeje (2017) under diverse agro-climatic conditions in Ethiopia, eighty chickpea germplasm and four standard checks were used for the quantitative traits to determine the level of chickpea germplasm variation. It showed the existence of genetic variation among chickpea germplasm. There is no wide variation between GCV and PCV for all traits except primary branches / plant which indicates that the variation observed in the genotypes have strong genetic bases.

Kadir *et al.* (2017) reported that GCV values is highest for number of seeds / plant in irrigated conditions while it is lowest for plant height and highest for test weight for rain-fed conditions. Broad sense heritability (h^2) found comparatively high for all measured traits in irrigated conditions. Reddy *et al.* (2017) observed wide variation for all the traits in F_2 population of the chickpea cross BG-212xICCV-07305. PCV and GCV estimates were high for number of pods / plant followed by branches / plant, seed yield / plant, plant height, test weight and days to flowering. The high heritability with high expected genetic advance as %age of mean was observed for all the characters indicating lesser effect of environment on these traits.

Balasaheb *et al.* (2018) investigation was undertaken with thirty five genotypes of chickpea with one check in a RBD design with three replications. High estimates of GCV and PCV observed in economic yield followed by biological yield / plant and number of pods / plant. Highest broad sense heritability recorded highest for economic yield (82%) followed by number of pods / plant (76%) and harvest index (75%). High heritability coupled with high genetic advance as percent of mean is observed in economic yield followed by number of pods / plant and harvest index. Manikanteswara *et al*. (2018) the experimental material comprised of twenty one chickpea genotypes. Results revealed maximum GCV and PCV recorded for seed yield / plant and harvest index. High genetic advance as percent of mean recorded for seed yield / plant. Higher heritability (broad sense) was recorded for characters that is days to maturity (97%) and number of pods / plant (92%).

Paul *et al*. (2018) a set of 296 F_{8-9} recombinant inbred lines (RILs) of the cross ICC 4567 (heat sensitive) x ICC 15614 (heat tolerant) was evaluated under field conditions. Very high GCV and PCV values of total number of filled pods / plot and seed yield under heat stress environment and moderate under non-stress environment indicated large effect of heat stress on the RILs for creating variation among them. However, the GCV and PCV values for test weight in both heat stress and non-stress environment was moderate.

2.2 CORRELATION AND PATH ANALYSIS:

The association of one or more characters influenced by a large number of genes is elaborated statistically by correlation coefficients. Genotypic correlation coefficient provides a measure of genotypes conjugation between characters. The methods of partitioning the correlation into direct and indirect effects by path coefficients analysis was suggested by Wright (1921). Partitioning of the correlation coefficient into its components, one component being the path coefficient that measures the direct effect of a predictor variable upon its response variable; the second component being the indirect effects of a predictor variable on the response variable through another predictor variable is the advantage of path analysis (Dewey and Lu, 1959).

Yadav (1990) reported that seed yield / plant was positively correlated with number of seeds / pod, number of pods / plant, numbers of secondary branches / plant, test weight and plant height, while negative correlation between pods / plant and seed yield / plant. Also indicated that all crosses had highest positive direct effect for number of seeds / plant followed by test weight.

Tripathi and Arora (1991) reported that seed yield / plant was positively associated with biological yield / plant, number of pods / plant, plant height, number of secondary branches / plant, harvest index and number of seeds / pod. Number of secondary branches exhibited positive correlation with number of pods / plant and biological yield / plant but negative relationship with test weight.

Rao *et al*. (1994) and Mathur and Mathur (1996) reported revealed that positive correlation of seed yield / plant with number of primary branches / plant, secondary branches / plant, test weight, harvest index and number of pods / plant while negative correlations with plant height and days to flowering.

Saleem *et al.* (2002) reported that seed yield / plant was positively correlated with days to flowering, total weight of plant, number of pods / plant and test weight both at the genotypic and phenotypic levels. Number of pods / plant had maximum positive direct effect on seed yield. It was concluded that number of pods per plant and test weight could be used as selection criteria to improve the yield. Muhammad *et al*. (2004) studied correlation coefficient for yield and its components in twenty four advance lines of chickpea. Seed yield / plant had positive and significant correlation with plant height, number of pods / plant, test weight and biological yield / plant. Whereas high direct effects were contributed by biological yield / plant and harvest index.

Renukadevi and Subbalakshmi (2006) exhibited that seed yield / plant had positively and significantly correlated with plant height, number of primary branches / plant, number of pods / plant, test weight, biological yield / plant and harvest index. The positive and direct effect on seed yield / plant was exhibited by plant height, number of primary branches / plant, number of pods / plant, biological yield / plant, harvest index and days to maturity.

Zena *et al*. (2008) studied path coefficient analysis in thirty six chickpea genotypes for 12 quantitative traits along with seed yield. And reported that biological yield / plant had shown highest direct effect on the seed yield / plant which was followed by test weight, yield index, maturity period, number of secondary branches / plant and number of seeds / pod. Vaghela *et al.* (2009) reported that seed yield / plant was found to be highly significant and positively correlated with biological yield / plant, number of pods / plant, harvest index, number of seeds / pod, number of primary branches / plant and test weight at both the genotypic and phenotypic levels. The highest direct and positive effect on seed yield was exhibited by biological yield / plant followed by harvest index, while the number of pods / plant, test weight and number of seed / pod exhibited moderate direct positive effects.

Gohil and Patel (2010) twenty two genetically diverse genotype of chickpea were evaluated under rainfed condition to understand the contribution of various characters to seed yield. reported that genotypic correlation coefficient were higher than corresponding phenotypic ones for the most of character combinations. Test weight and harvest index had a significant positive association with seed yield / plant. The harvest index, test weight, pods / plant and earliness were most important characters which can be used to improve yield in chickpea under rainfed conditions.

Zali *et al.* (2011) seventeen chickpea genotypes were used to estimate correlation coefficient and path analysis. Number of seeds / plant and test weight had a positive direct effect on seed yield / plant. Number of seeds / plant, number of secondary branches, test weight, number of pods / plant, number of primary branches and plant height also had positive and highly significant phenotypic correlations with seed yield / plant. Aycicek and Babagil (2013) reported that seed yield / plant positively correlated with plant height, number of pods / plant, seed number / plant and test weight. Positive direct effects of plant height and number of pods / plant with significant correlation with seed yield / plant suggested that these yield components may be a good selection criteria to improve chickpea cultivar.

Padmavathi *et al.* (2013) thirty genotypes of *kabuli* chickpea were used to study the extent of correlation and path analysis for yield and yield contributing characters. Seed yield / plant was significantly and positively correlated with plant height, number of primary branches / plant, number of secondary branches / plant, number of pods / plant, test weight, harvest index and biological yield / plant. Path coefficient analysis indicated that biological yield / plant, number of pods / plant and harvest index had high positive direct effect on seed yield / plant signifying the importance of these traits in improvement of seed yield.

Parhe *et al.* (2014) total fifty one diverse genotypes of chickpea were grown in RBD design with two replications. Seed yield / plant was significantly correlated with traits, *viz.,* number of primary branches / plant, number of secondary branches / plant, plant height, test weight and number of pods / plant. Path coefficient analysis revealed that test weight, number of pods / plant and number of secondary branches / plant had the highest positive direct effects on seed yield / plant. Singh *et al.* (2014) twelve chickpea genotypes were used under restricted soil moisture conditions for the study. Highly significant correlation coefficients observed in biomass / plant and harvest index. Biological yield / plant, harvest index and protein content has positive direct effect and correlation with seed yield / plant, suggesting the possibility of seed yield improvement through direct selection for these traits. Pusa 362 and pusa 1103 can be used in breeding programe for improving protein content in seed under restricted soil moisture conditions.

Edalat *et al.* (2015) Four genotypes, two semi bush types and early mature cultivars (ILC482 and Flip84-42) and two stand types and late mature cultivars (Hashem and Arman) were used under rainfed conditions. It was reported that biological yield / plant had the greatest direct effect on seed yield / plant followed by number of pods / plant. Positive correlation between seed yield / plant and number of pod / plant, number of seed / pod, biological yield and plant height. Shafique *et al.* (2016) estimation of genetic variability and interrelation of various yield component traits by using path analysis and correlation analysis in twenty genotypes of chickpea (*Cicer arietinum* L.) under irrigated conditions. revealed that pods / plant, harvest index, test weight and branches / plant had high positive direct effect on seed yield / plant at both genotypic and phenotypic levels. Correlation coupled with path coefficient analysis for number of pods / plant had a direct relationship with seed yield / plant.

Tadesse *et al.* (2016) twenty genotypes of *desi* chickpea grown under rainfed conditions to determine relationships among yield and some yield components using correlation and path coefficient analysis. Seed yield was significantly and positively correlated with days to flowering, days to maturity, number of pods / plant, number of seeds / pod and plant height. Path analysis revealed that biomass had the greatest direct effect on seed yield/plant (0.0146) and followed by stand count at harvest and plant height. Astereki *et al.* (2017) twenty five chickpea genotypes including advanced lines and commercial varieties were grown under dryland conditions. Significant and positive correlation between seed yield and number of pods / plant and harvest index. Path analysis indicated that days to flowering, days to maturity, number of pods / plant and days to flowering directly and indirectly affected seed yield.

Bhanu *et al.* (2017) studied fifty three germplasm of chickpea to determine relationships among yield. Positive significant relationships were found between seed yield / plant and number of primary branches / plant, number of secondary branches / plant and number of pods / plant. The path coefficient analysis based on seed yield / plant, as a dependent variable, showed that number of pods / plant had the greatest direct effect on seed yield / plant followed by number of secondary branches / plant. Kumar *et al.* (2018) studied genetic association among sixty five treatments for 11 characters in which biological yield / plant, test weight, harvest index and pods / plant showed highly positive significant correlations with seed yield / plant in both timely and late sown environments. Whereas, path analysis showed highly positive direct effects towards expression of seed yield / plant were displayed by biological yield / plant followed by harvest index and test weight. Singh *et al.* (2018) sixteen genotypes were evaluated under dryland condition terminal drought stress. The correlation coefficients indicated that seed yield / plant was positively and significantly correlated to plant height, secondary branches / plant and total number of pods / plant from. Path analysis of seed yield / plant indicated that all the other traits, except days to flowering and number of pods / plant exhibited high positive direct effect.

2.3 GENETIC DIVERSITY:

Genetic diversity on the other hand is a pre-requisite for crop improvement programme (Jakhar *et al.* 2016). Effective hybridization program between genetically diverse parents will lead to considerable amount of heterotic response in F_1 hybrids and broad spectrum of variability in segregating generations. For realizing genetic diversity among the parents with their direct and indirect effects of different traits on seed yield and selection of best diverse parents for chickpea future breeding programme.

Sarvalia and Goyal (1994) 76 genotypes were grouped into 10 clusters on the basis D^2 statistics. Cluster I contained 35 genotypes, cluster II with 20 genotypes, III and IV with 8 and 4 genotypes, respectively, cluster V, VI and VII with 2 genotypes each and remaining 3 clusters consisted of a single genotype each. The maximum inter-cluster distance observed between V and IX and the minimum between I and VII. The genotypes having cluster V were good for most of the yield components. Samal and Jagdev (1996) thirty two cultivars were grouped into six clusters.

Maximum genetic distance was observed between cluster III and VI followed by cluster IV and VI suggesting wide diversity among these groups. Khan (1999) estimated genetic diversity in 36 geologically diverse chickpea genotypes on the basis D^2 statistics. The genotypes were grouped into eight clusters on the basis of yield and yield components. It was concluded that hybridization among the genotypes from cluster I and II may result in high yield progenies.

Kashyap and Rastogi (2003) studied genetic divergence in sixty chickpea germplasm accessions by D^2 statistics method. Germplasm accessions were grouped into 6 clusters and the maximum intra-cluster distance was observed in cluster I comprising 14 genotypes. Cluster III and VI were identified as genetically divergent. Durga *et al.* (2005) used 132 genotypes to assess genetic diversity. Genotypes were grouped into nine clusters. Cluster I was the largest, with 20 genotypes, followed by clusters V and VII with 16 and 15 genotypes, respectively. Maximum intra - cluster distance was observed in cluster VI followed by cluster IV, cluster I and cluster IX. Maximum inter cluster distance was noticed between clusters I and VIII. The crossing of genotypes from the clusters I and VIII may leads to maximum diversity in the segregating populations.

Singh *et al.* (2006) revealed that maximum inter cluster distance observed between cluster I and VI, followed by cluster I and V and I and IV. The genotypes belonging to different cluster should be used as parents for hybridization programme for development of high yielding varieties.

Dwevedi *et al*. (2009) reported that twenty five chickpea genotypes were grouped into six clusters using Mahalanobis's D^2 Statistics. 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, test weight and number of pods / plant contributed maximum. Sreelakshmi *et al.* (2010) reported that 40 genotypes were grouped into 8 clusters. Out of which, cluster I and II had maximum number with seven genotypes each. Among the characters, seed yield contributed maximum towards genetic divergence. Tomar *et al.* (2011) observed maximum intra-cluster distance was observed in cluster VIII, followed by cluster VI, cluster IV and cluster I. Maximum inter-cluster distance was observed between cluster IV and VIII. Crossing between the genotypes from the cluster IV and VIII may lead to maximum diversity in the segregating populations and development of high yielding varieties.

Syed *et al.* (2012) genetic diversity of twenty seven chickpea genotypes was studied through Mahalanobis D^2 and Principal Component analysis. Inter cluster distances in all cases were larger than intra cluster distances suggested wider genetic diversity among the genotypes of different groups. The highest inter cluster distance was observed between clusters I and II followed by cluster I and III, I and IV, I and V which indicated that the genotypes grouped in these clusters were highly divergent from each other. Jain and Indapurkar (2013) investigated the nature and magnitude of genetic divergence using Mahalanobis's D^2 Statistics on thirty genotypes of chickpea and these genotypes were grouped into six clusters. The cluster I shows largest cluster with eight genotypes. Highest inter cluster distance was observed between cluster IV and cluster V, followed by cluster V and VI. The cluster VI was identified for pods / plant and seed yield / plant.

Kuldeep *et al*. (2015) revealed that D^2 analysis established the presence of broad diversity among the inbreds by the formation of 16 clusters from 100 genotypes. Test weight, harvest index, number of pods / plant and seed yield / plant contributed maximum in the manifestation of genetic divergence. Temesgen *et al.* (2015) estimated diversity of forty nine *kabuli* chickpea genotypes and these genotypes were grouped into eight clusters. Distances between these clusters are significantly different for all the cluster combinations except between cluster I and IV. This indicated that there is an opportunity to bring about improvement through hybridization of genotypes from different clusters and subsequent selection from the segregating generations.

Jakhar *et al.* (2016) reported that test weight was the maximum contributor towards divergence followed by number of pods / plant, days to 50% flowering and seed yield / plant, number of secondary branches, number of seeds / pod, protein content and number of primary branches / plant. The days to maturity and plant height were the lowest contributor towards divergence.

Admas and Abeje (2017) studied diversity in eighty chickpea germplasm along with four standard checks. Germplasm grouped into four clusters. Cluster I with 47 accessions, cluster II with 18 accessions, cluster III with 15 accessions and cluster IV with 4 accessions. First two principal components explained 72.9% of the total variation among the genotypes for all quantitative traits. Primary branches / plant, secondary branches / plant, number of pods / plant and days to maturity are the most important contributing traits for the relative magnitudes of eigen vectors for the first principal components. Kushwaha *et al.* (2017) studied eighty four chickpea genotypes to know the nature and magnitude of genetic divergence. All genotypes were classified into eight clusters. Inter-cluster distances were greater than intra-cluster distances and a considerable amount of genetic variability present in the material. Diverse clusters namely, V and VIII consists of diverse parents hold good promise for potential hybrids for desirable characters.

Vijayakumar *et al.* (2017) reported that 169 germplasm lines were grouped into eight different clusters indicated diverse nature of material. Cluster I was the largest with 53 genotypes followed by cluster III (44 genotypes), and cluster II (34 genotypes). The intra-cluster distance was maximum in cluster IV followed by cluster III and V. The widest inter cluster distance was noted between cluster IV and V followed by V and VIII, II and IV indicated wide divergence among the clusters. Balasaheb *et al.* (2018) studied thirty five genotypes with a check were grouped into six clusters. The maximum genetic distance found between the clusters II and V. Greater the divergence between the two clusters, wider is the genetic diversity in the genotypes. On the basis of mean performance of the genotypes, IC-275323 was recorded high yield among 35 genotypes under study.

Gediya *et al.* (2018) indicated wider genetic diversity among fifty eight genotypes of chickpea. The maximum genetic divergence was observed between cluster IX and XVI, followed by cluster IX and XIV, while closest proximity was noticed between cluster III and VIII. The characters viz., seed yield / plant, test weight and number of pods / plant contributed much to the total genetic divergence. On the basis of cluster mean values, cluster XII was superior for seed yield / plant and harvest index, whereas cluster XV was good number of pods / plant and cluster X was good for test weight. Raj *et al.* (2018) revealed that maximum intra cluster distance was exhibited by cluster V followed by cluster I and cluster IV. The maximum inter-cluster distance was observed in cluster II and VII, followed by cluster II and V, cluster III and V suggesting that genetic architecture of the genotypes in one cluster differ entirely from those included in other clusters

2.4 Physiological and biochemical parameters

Plants show a lot of morpho-physiological and metabolic changes in response to drought stress and these changes lead them to adapting to drought stress conditions (Awari *et al.* 2017). Relative water content is one of the important parameter to measure the water status of the tissue. Gradual decrease in RWC with increase in stress and greater reduction afterwards under severe stress has been reported in chickpea crop. Increase in proline content during water stress conditions suggests that proline is one of the common compatible osmolyte under water stress conditions. Relative growth rate (RGR) and crop growth rate (CGR) which are indirect measurement of photosynthetic efficiency of plants also usually decreasing in drought conditions. In present investigation, RWC was moderate and proline content was low under rainfed condition it means crop was free from drought stress.

Rizvi *et al.* (2014) conducted an experiment with five chickpea genotypes under irrigated and rainfed conditions. Relative water content, chlorophyll content and protein content decreased under moisture stress, whereas proline content increased with the increase in moisture stress. The genotypes Pusa-1108, Pusa-362 and Pusa-1103 could be considered as drought tolerant. Ozalkan *et al.* (2010) studied fifteen kabuli chickpea genotypes during four growth stages to determined the relationship between some plant growth parameters and seed yield. Seed yield was significantly and positively correlated with biomass, leaf area index, crop growth rate at linear vegetative stage.

Maniram *et al*. (2017) studied six cultivars of chickpea at growth stage on i.e. 55, 75, 95 DAS. The drought tolerance capacity of tolerant genotypes was associated with retention of high RWC, high total chlorophyll content and higher osmotic potential under stress conditions. Tolerant genotypes recorded less reduction in yield attributes like pods/plant, seeds/plant, test weight, straw yield, harvest index and seed yield under conditions of water stress over the susceptible genotypes. Ulemale *et al.* (2013) conducted an experiment on fourteen chickpea genotypes under moisture stress and non-stress condition to study physiological indices for drought tolerance. Genotypes i.e. Phule G 09103, Phule G 2008-74, Digvijay, Phule G 0302-26 exhibited higher values for drought tolerance efficiency, proline content and lower value for drought susceptibility index.

Chapter-3

Material and Methods

CHAPTER-III

MATERIALS AND METHODS

The present experiment **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** was conducted during *Rabi* 2017-18. The details of the materials used and techniques adopted during the course of investigation are described in this chapter.

3.1 Location and experimental site

The advance breeding lines ($F_{7:8}$) of 22 different cross combinations with a check i.e. SKUAST Check Samba (SCS-3). SCS-3 were raised at Advanced Center for Rainfed Agriculture (ACRA), SKUAST-J, Rakh Dhiansar, Jammu in Randomized Block Design (RBD) with three replications and row length of 5m of each line with spacing of 30x10 cm^2. The experiment was conducted on 10th November 2017 and harvested on 18th April 2018. Experimental field presented in fig.1.

3.2 Experimental material

The experimental material used in the present study comprised of twenty two advance breeding lines along with standard check (SCS-3, having characteristics *viz.*, semi tall, erect plant type with two seeds/pod, tolerant to pod borer and drought, good cooking quality etc). The experimental material was obtained from Pulses Research Sub Station (PRSS) Samba 2017-18. The details are as under:

Table 1: Particulars of chickpea advance breeding lines ($F_{7:8}$) used in the research work

S.No.	Selection	Pedigree	Source
1.	SCS-15-D-1	HCC-5 X GNG 663	IIPR Kanpur
2.	SCS-15-D-2	ICCV 89445 X ICCV 88502	IIPR Kanpur
3.	SCS-15-D-3	SC5 X CSG 8962	IIPR Kanpur
4.	SCS-15-D-4	ICCV 96029 X ICCC37	IIPR Kanpur
5.	SCS-15-D-5	[{(JG74 X WR315) X JG74} -2010-1-3-5-11-15-10-2]	IIPR Kanpur
6.	SCS-15-D-6	RSG-143-1 X JG-315	IIPR Kanpur

7.	SCS-15-D-7	BGD 112 X JSC37	IIPR Kanpur
8.	SCS-15-D-8	SAKI 9516 X C-102	IIPR Kanpur
9.	SCS-15-D-9	Phule G-00109 X GCP 101	IIPR Kanpur
10.	SCS-15-D-10	ICCV97033/FLIP 94-89	IIPR Kanpur
11.	SCS-15-D-11	Avrodhi x NDG30	IIPR Kanpur
12.	SCS-15-D-12	GJG0207 X SAKI9516	IIPR Kanpur
13.	SCS-15-D-13	RSG931 X RSG807	IIPR Kanpur
14.	SCS-15-D-14	Pusa362 x GPF2	IIPR Kanpur
15.	SCS-15-D-15	ICCV04112 X JAKI9218	IIPR Kanpur
16.	SCS-15-D-16	IPC94-132 X RSG905	IIPR Kanpur
17.	SCS-15-D-17	SAKI9516 X FG-716	IIPR Kanpur
18.	SCS-15-D-18	BG1088/FLIP97020-1785-D	IIPR Kanpur
19.	SCS-15-D-19	AIGB-20-01	IIPR Kanpur
20.	SCS-15-D-20	ICCV96029 X JG11	IIPR Kanpur
21.	SCS-15-D-21	JG130 X KAK2 (KABULI CHICKPEA)	IIPR Kanpur
22.	SCS-15-D-22	IPC98-12 X ICC395468	IIPR Kanpur

3.3 Observations:

Ten competitive plants per genotype were taken at random for recording observations on following characters in each replication and averages were worked out for statistical analysis.

3.3.1 Morphological characters:

3.3.1.1 Days to 50% flowering: Number of days from sowing to the date when 50% plants in each plot flowered were recorded and the average number of days for 50% flowering was calculated.

3.3.1.2 Days to maturity: Number of days taken from the day of sowing to the physiological maturity of crop was recorded as days to maturity.

3.3.1.3 Plant height (cm): Height of the plant was recorded at the time of harvesting by measuring the height of a plant from ground level to the top of the main axis.

Figure 1. Experimental field at ACRA, Dhiansar, SKUAST-J

3.3.1.4 Number of primary branches / plant: Branches arising from main stem were considered as basal or primary branches which were counted and recorded at the time of harvest.

3.3.1.5 Number of secondary branches / plant: Fruiting branches arising from primary branches were recorded as secondary branches at the time of harvest.

3.3.1.6 Number of pods / plant: The total numbers of pods were counted from ten randomly selected plants at maturity and average was worked out.

3.3.1.7 Number of seeds / pod: This observation was recorded by taking the seeds of randomly selected ten pods from a plant and the average of ten plants was estimated.

3.3.1.8 Biological yield / plant (g): Test dry weight of above ground portion of the plant was recorded in grams.

3.3.1.9 Seed yield / plant (g): The weight of seeds obtained as a mean of ten observational plants represented seed yield / plant.

3.3.1.10 Harvest index (%): The harvest index was calculated by the following equation:

$$\text{Harvest index (\%)} = \frac{\text{Seed yield}}{\text{Biological yield}} \times 100$$

3.3.1.11 Test weight (g): It was recorded by weighing randomly taken 100 seeds.

3.3.1.12 Protein content (%): It was estimated by the method of Lowry *et al.* 1951.

Reagents:

1. Solution A: 2% sodium carbonate in 0.1N NaOH.

2. Solution B: 0.5% copper sulphate in 1% sodium potassium tartate.

3. Solution C: Mix 50 ml of solution A with 1 ml of solution B just prior to use.

4. FCR: Dilute the commercial reagent (2N) with an equal volume of water.

5. Stock standard protein solution: 50 ml of bovine serum albumin / 50 ml of water.

6. Working standard solution: Dilute 10 ml of the stock solution to 50 ml water to obtain 200 *u*g protein/ ml.

Method:

1. Grind 0.5 g of the sample with suitable solvent.

2. Centrifuge and use the supernatant for protein estimation.

Estimation of protein:

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution into series of test tubes.

2. Pipette out 0.1 ml and 0.2 ml of the sample extract into other test tubes.

3. Make up the volume to 1 ml with water in all the tubes. A tube with 1 ml of water serves as the blank.

4. Add 5 ml of solution C, mix well and incubate at room temperature for 10 minutes.

5. Add 0.5 ml of FCR, mix well immediately and incubate at room temperature in dark for 30 minutes.

6. Read the absorbance at 660 nm against the blank.

3.3.2 Physiological traits:

3.3.2.1 Relative water content: Three leaflets on top, middle and lower part of plant from five plants in each line were taken for measuring relative water content (RWC) at podding stage.

RWC (%) was calculated by the following equation given by Barrs and Weatherley (1962).

$$RWC\ (\%) = [F_W - D_W/\ T_W - D_W] \times 100$$

Where, F_W = Fresh weight

T_W = Turgid weight

D_W = Dry weight

Plate 1a. Fresh Weight of leaf for RWC

Plate 1b. Dry Weight of leaf for RWC

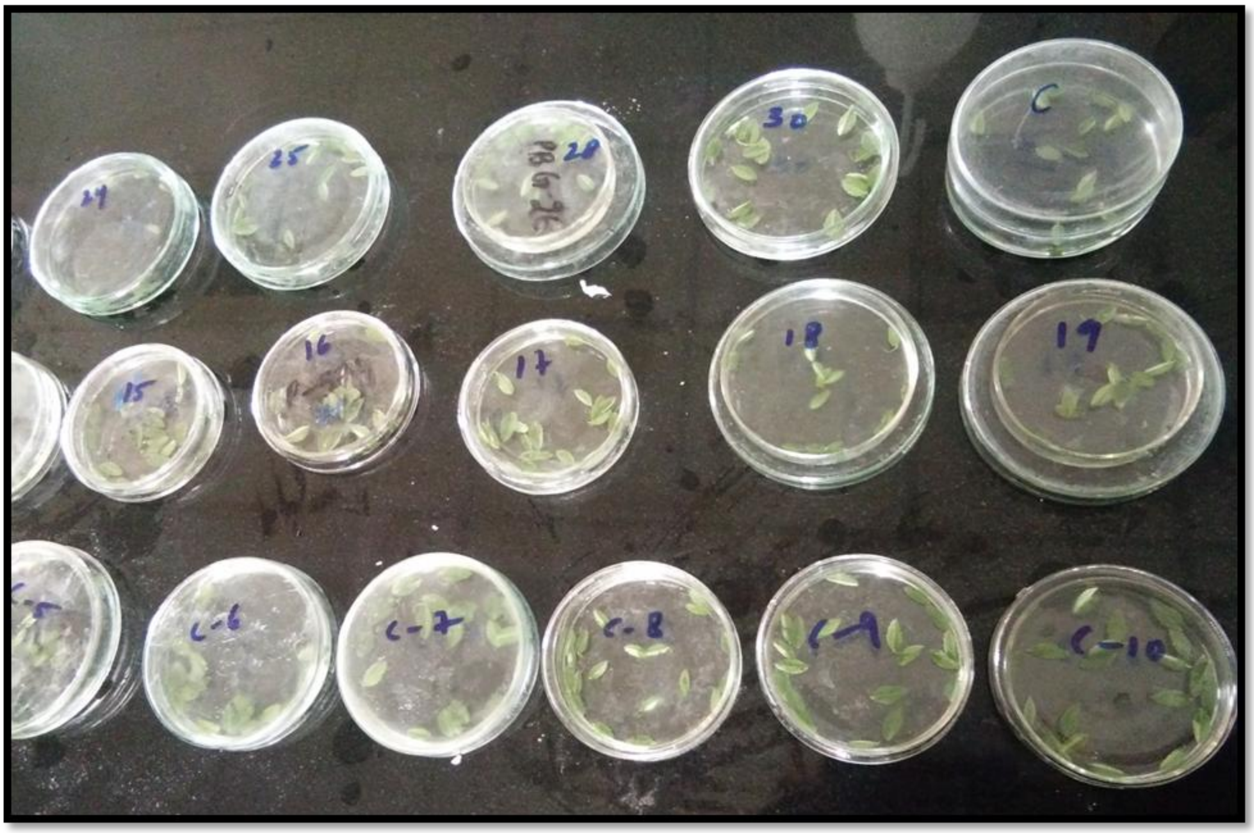


Plate 1c. Turgid Weight of leaf for RWC

Plate 1 : Relative water content of leaves

Protocol of relative water content:

Requirement: Leaves of chickpea, petriplates, distilled water, weighing balance, oven.

Procedure:

1. Took three leaflets of chickpea from top, middle and lower part of the plant

2. Washed the leaves with distilled water.

3. The fresh weight was recorded of the leaves in (g) with the help of weighing balance.

4. For measuring the turgid weight of leaves, place the leaves in petriplate dipped with distilled water for 24 hours.

5. After 24 hours, recorded the turgid weight of the leaves using weighing balance.

6. Then the leaves were dried in the oven at the temperature of $\pm$ 65^0 C for 2 hours, recorded the dry weight of the leaves.

7. Now by applying the given formula i.e., RWC (%) = $F_W - D_W / T_W - D_W$ x 100, relative water content was calculated. Presented in plate 1.

3.3.2.2 Relative growth rate (RGR): It is the rate of increase in the dry weight per unit dry weight already present and is expressed as g.g.day $^{-1}$. Relative growth rate (RGR) was calculated by the equation given by Radford (1967).

$$RGR = \frac{InW_2 - InW_1}{t_2 - t_1}$$

Where,

In = Natural log

W_1 = Dry weight of plant (g) at time t_1

W_2 = Dry weight of plant (g) at time t_2

$t_2 - t_1$ = Time interval in days

3.3.2.3 Crop growth rate (CGR): Represented total dry matter productivity of the community per unit land area over a certain time span. The crop growth rate have been calculated by using following equation given by Watson (1947). CGR expressed as mg m^{-2} day^{-1}.

$$CGR = \frac{W_2 - W_1}{p(t_2 - t_1)}$$

Where,

p = Ground area

W_1 = Dry weight of plant (g) at time t_1

W_2 = Dry weight of plant (g) at time t_2

$t_2 - t_1$ = Time interval in days

3.3.3 Biochemical traits:

3.3.3.1Proline content: Proline content was estimated by using the method of Bates *et al.* 1973.

Reagents:

(i) 3 % aqueous sulphosalicylic acid (w/v)

(ii) Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 2 ml 6.0 M o-phosphoric acid until dissolved)

(iii) Toluene

Procedure:

1. 300mg of fresh leaves were separately homogenized in 5ml of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was taken.

2. 2ml of supernatant was taken in a test tube and 2.0ml reagent acid ninhydrin was added.

3. This mixture was then kept in boiling water bath for 1 hour at 100^0C.

4. Thereafter reaction was terminated by keeping tubes in ice bath.

5. Then 4.0 ml of toluene was added.

6. After vigorous shaking the upper coloured organic phase was taken after attainment of room temperature.

7. Absorbance was recorded at 520nm by using toluene as blank.

The proline content expressed as mg g^{-1} FW.

3.3.3.2 Chlorophyll content: The leaf chlorophyll content was estimated using a SPAD chlorophyll meter (Model 502) at podding stage. SCMR readings were limited to upper most leaves during 9:00 to 11:00 h of day.

Procedure:

1. Three plant randomly selected from each line/ variety

2. SCMR was recorded from the three fully expanded uppermost leaves of each plant.

3. The average of nine SCMR values in each line was considered as the line SCMR value.

3.4 Statistical Analysis

The mean values of five randomly selected observational plants for different traits were used for statistical analysis. The following statistical measures/parameters were worked out for presentation of the data on different quantitative attributes.

3.4.1 Analysis of variance

The analysis of variance was done as suggested by Panse and Sukhatme (1967) in following form.

Table. 2. Analysis of variance (ANOVA).

Source of variations	D. F	MSS	Expected mean squares	“F “value
Replications	(r-1)	MSr	$\sigma^2e + g\sigma^2r$	MSr/ MSe
Treatments	(t-1)	MSt	$\sigma^2e + r\sigma^2g$	MSt/ MSe
Error	(r-1) (t-1)	MSe	σ^2e	
Total	(rt-1)			

Where,

r = number of replication

t = number of treatments

σ^2r = variance due to replication

$\sigma^2 g$ = variance due to treatment/ genotype

$\sigma^2 e$ = variance due to error

The treatment mean square (MSt) was tested against error mean square (MSe) by ‘f’ test for n_1 = (t-1) and n_2 = (r-1)(t-1) degrees of freedom. The characters showing significant differences were subjected to further analysis.

Components of variation:

The phenotypic and genotypic variances were calculated by using the respective mean squares from the variance ANOVA table (Johnson *et al.* 1955) as below.

Environmental variance ($\sigma^2 e$) = MSe

Genotypic variance ($\sigma^2 g$) = $\frac{\text{MSt- MSe}}{r}$

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

Where,

MSt = Treatment/ genotypic mean sum of squares

MSe = Error mean sum of squares

r = Number of replication

3.5 Biometrical analysis

3.5.1 Estimation of phenotypic and genotypic coefficients of variation:

The phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) in per cent were computed by the following formulae given by Burton (1952).

$$\text{PCV\%} = \frac{\sqrt{\sigma^2 p}}{\bar{X}} \times 100$$

Where,

$\sigma^2 p$ = Phenotypic variance

X = Mean of character

$$GCV\% = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100$$

Where,

$\sigma^2 g$ = Genotypic variance

X = Mean of character

3.5.2 Estimation of heritability (h^2):

Heritability in broad sense was estimated by the following formula given by Burton (1952).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

3.5.3 Genetic advance:

Genetic advance was calculated by the formula given by Johnson *et al.* (1955)

(i). $GA = K \times (\sigma^2 g / \sigma^2 p) \times \sigma p$ or $GA = K \times h^2 \times \sigma p$

Where,

K = Selection differential which is 2.06 at 5 percent selection intensity

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

σp = phenotypic standard deviation

h^2 (b.s.) = heritability broad sense

(ii). Genetic advance (G.A) as percentage of mean (GAM)

$$GAM = \frac{G.\,A.}{\bar{X}} \times 100$$

3.5.4 Correlation coefficients:

Phenotypic, genotypic and environmental correlation coefficients between characters were computed utilizing respective components of variance and co-variance, by following formula suggested by Miller *et al.* (1958).

$$r_g = \frac{Cov.XY(g)}{\sqrt{Var.X(g)Var.Y(g)}}$$

Where,

r_g = Genotypic correlation coefficient

Cov.XY = Co-variance of character X and Y,

Var.X = Variance of character x

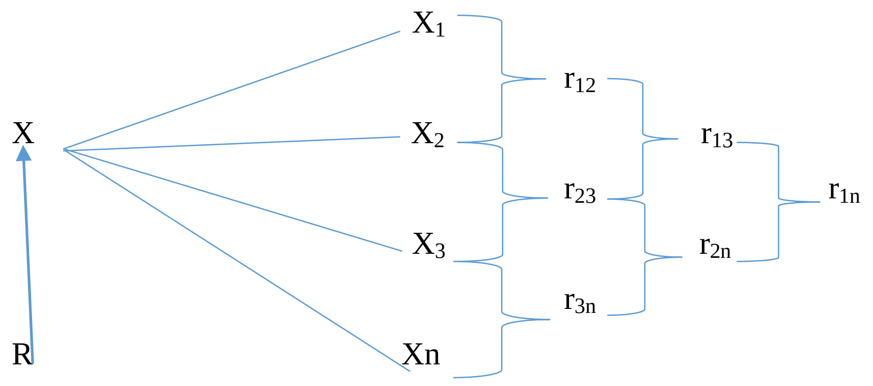
Var.Y = Variance of character y.

To test the significance of phenotypic and environmental correlation coefficients, the estimated values were compared with the tabulated values of 't'at n-2 d. f. at 5% and 1% level of probability.

3.5.5 Path coefficient analysis:

Genotypic and phenotypic correlation coefficient into direct and indirect effects by path analysis as suggested by Dewey and Lu (1959) and developed by Wright (1921).

In the present study, path diagram was prepared by taking yield as the effect *i.e.* function of various components like X_1, X_2, X_3 and these component showed following type of association with each other.



In path diagram the yield is the result of X_1, X_2, X_3 …..Xn and some other undefined factors designated by R. The double arrow lines indicated mutual association as measured by correlation coefficient. The single arrow represents direct influence as measured by path coefficient Pij. Path coefficients were obtained by solving a set of simultaneous equation of the form as per Dewey and Lu (1959).

$$r_{ny} = P_{ny} + r_{n2} P_{2y} + r_{n3} p_{3y} + \ldots\ldots\ldots\ldots\ldots$$

Where,

r_{ny} = represents the correlation between one component and yield

P_{ny} = represents path coefficient between that character and yield

r_{n2} = represents correlation between that character and each of the other components in turn.

Matrix A Matrix B C

$$\begin{bmatrix} r_{1y} \\ r_{2y} \\ r_{ny} \end{bmatrix} = \begin{bmatrix} r_{11} & r_{12} & r_{13} \ldots\ldots r_{1n} \\ r_{21} & r_{22} & r_{23} \ldots\ldots r_{2n} \\ r_{n1} & r_{n2} & r_{n3} \ldots\ldots 1 \end{bmatrix} \begin{bmatrix} P_{1y} \\ P_{2y} \\ P_{ny} \end{bmatrix}$$

Where,

$R_{12} = r_{21}$ and so on

R_{1y} = Correlation between one component character and seed yield

The 'B' matrix was inverted $[B]^{-1}$ and path coefficients (Pij) were obtained as,

$$i.e.\ Pij = (B)^{-1} .A$$

The indirect effects of a particular character through other characters were obtained by multiplication of direct paths and particular correlation between these characters separately.

$$\text{Indirect effects} = r_{ij} \times p_{iy}$$

Where,

i = 1 to 9

j = 1 to 9

$P_{iy} = P_{1y}, P_{2y}, \ldots\ldots\ldots\ldots\ldots\ldots\ldots, P_{ny}$

Path coefficient (p_{ij}), correlation coefficient (r_{ij}) and residual factors (R) were diagrammatically presented. The residual factor *i.e.* variation in yield unaccounted for by these associations was calculated with the following formula:

$$\text{Residual factor (R)} = (1 - R^2)$$

Where,

$R^2 = P_{1y} r_{1y} + P_{2y} r_{2y} + \ldots\ldots\ldots\ldots\ldots + P_{ny} r_{ny}$

$P_{1y}, P_{2y}, \ldots\ldots\ldots\ldots\ldots, P_{ny}$ = Direct path values

R_{1y}, r_{2y}, r_{ny} = Correlation coefficient

3.6 Multivariate analysis:

3.6.1 Estimation of Wilk's (Λ) criterion:

To test the significance of difference between lines, taking all the characters simultaneously, 'V' statistic was calculated which was based on Wilk‟s (Λ) criterion (Wilks, 1932). The sum of squares and sum of products of error and error + variety were utilized for estimation of "Λ".

To calculate the value of "Λ" following relationship was used:

$$\text{"}\Lambda\text{"} = \frac{|E|}{|E+V|}$$

Where,

$|E|$ was the determinant of error sum of squares and sum of products matrix and $|E+V|$ was the determinant of the "error + variety' sum of squares and sum of products matrix.

X^2 was used to test the significance of "Λ" as

$$\chi^2_{pq} = V = -m \log_e \Lambda$$

Where,

$$m = n - \frac{p+q+1}{2} \quad \text{with pq degree of freedom}$$

Where,

n = total number of observations –1,

p = number of characters,

q = k – 1, and

k = number of lines

3.6.2 Genetic diversity:

The generalize distance between two population is defined by Mahalanobis (1936) as

$$D^2 = \lambda\ ij.\ \sigma\ i.\ \sigma\ j$$

Where,

λ ij = reciprocal matrix to the common dispersion matrix

σ i = difference between the mean values of two populations for i^{th} character

σ j = difference between the mean values of two populations for j^{th} character

Estimation of D^2 values from the above formula is very complicated in the present study it was found convenient to work with a set of uncorrelated characters constructed from the original measurements. D^2 with such transformed variables reduced to the evaluation of simple sum of squares. Transformation was done by using pivotal condensation method (Singh and Chaudhary 1977). The coefficients for the transformation were obtained by dividing the first row of reduced matrix by the square root of the corresponding pivotal condensation elements.

3.6.3 Determination of gene constellation:

Tocher's method as described by Rao (1952) was followed for cluster formation. No formal rules can be laid down for finding the clusters because a cluster is not a well defined term the only the criteria appears to be that any two groups belonging to the same cluster should at least on an average show a smaller D^2 than those belonging to the two different clusters. A simple device suggested by K. D. Tocher described by Rao (1952) is to start with the two closely associated groups and find a third group which has the smallest D^2 from the two. Similarly the fourth is chosen to have the smallest D^2 from the first three and so on if at any stage the average D^2 of the group from those already listed appears to be high, then this group does not fit in the former groups and is therefore taken outside the former cluster. The group of first cluster are then omitted and rest are treated similarly it is also useful to calculate the change in average D^2 within a cluster due to inclusion of an additional group if the changes are appreciable, then the newly added group has to be considered as outside the cluster.

3.6.4 Average intra and inter cluster D^2 and D values:

(i) Average intra cluster D^2

$$D^2 = \Sigma Di^2/n$$

Where,

ΣDi^2 is sum of distances between all possible combinations

(n) is the population included in a cluster

(ii) Average inter cluster D^2

$$D^2 = \frac{\Sigma \text{ distances between the population of cluster i and j}}{n_i . n_j}$$

Where,

n_i = number of population in cluster i

n_j = number of population in cluster j

Cluster means: Cluster means were calculated for individual character on the basis of mean performance of the genotypes included in that cluster.

Chapter-4

Results

CHAPTER-IV

RESULTS

The results obtained from the present investigation entitled, **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** are presented under the following headings.

4.1 Analysis of variance

4.2 Mean performance

4.3 Genetic variability parameters

4.4 Correlation studies

4.5 Path analysis

4.6 Genetic diversity (D^2 analysis)

4.1 ANALYSIS OF VARIANCE:

Analysis of variance (Table 3 and 4) showed highly significant differences among the advance breeding lines under study for various traits *viz.*, days to 50% flowering, days to maturity, plant height, number of primary branches / plant, number of secondary branches / plant, number of pods / plants, number of seeds / pod, biological yield / plant, seed yield / plant, harvest index, test weight, protein content, proline content, chlorophyll content, relative water content, relative growth rate and crop growth rate.

4.2 MEAN PERFORMANCE:

The mean value and range of seventeen characters for twenty two advance breeding lines ($F_{7:8}$) of chickpea along with a standard check (SCS-3) have been presented in table 5 and 6, respectively.

4.2.1 Morphological traits:

4.2.1.1 Days to 50% flowering:

The population mean for days to 50% flowering was 100.44 day. The variation in days to 50% flowering ranged from 94.33 to 108 days. Advance breeding line

SCS-15-D-8 flowered in (94.33) days while highest days were taken by SCS-15-D-16 (108). Twelve out of twenty two advance breeding lines were early for days to 50% flowering than average mean of advance breeding lines. Nineteen advance breeding lines exhibited higher mean performance than standard check (SCS-3).

4.2.1.2 Days to maturity:

The range for this character was 148.0 to 158.66 days with average mean of 152.7 days. Advance breeding line SCS-15-D-3 (148.0) matured in least number of days followed by SCS-15-D-20 (149.66), while SCS-15-D-19 was matured late by (158.66) days. Eighteen out of twenty two advance breeding lines were late in maturity than standard check (150.33).

4.2.1.3 Plant height (cm):

The average mean for plant height was 45.96 cm. The maximum plant height was exhibited by advance breeding line SCS-15-D-1 (54.06) while it was minimum in SCS-15-D-18 (39.66) followed by SCS-15-D-21 (39.93). Thirteen lines were significantly superior in height of the plant than average mean, whereas ten out of twenty two advance breeding lines ($F_{7:8}$) found superior in plant height than standard check SCS-3 (46.70).

4.2.1.4 Number of primary branches / plant:

The mean performance for this character was 4.83 and it ranged from 1.03 to 8.66. The advance breeding line SCS-15-D-16 showed the highest (8.66) number of primary branches followed by SCS-15-D-11 (8.00) while it was minimum in SCS-15-D-15 (1.03) followed by SCS-15-D-13 (1.66). Fifteen out of twenty two advance breeding lines exhibited higher values than standard check (3.90) for the trait number of primary branches / plant.

4.2.1.5 Number of secondary branches/ plant:

Eleven advance breeding lines exhibited significantly higher number of secondary branches / plant when compared with average mean value of 15.13. The variation for number of secondary branches / plant ranged between 9.43 to 20.86. The advance breeding line SCS-15-D-21 (20.86) exhibited highest number of secondary branches / plant followed by SCS-15-D-11 (20.30), SCS-15-D-9 (19.83) and SCS-15-

Table 3: Analysis of variance (ANOVA) for morphological traits in advance breeding lines of chickpea

Source of variations	d.f	Mean Sum of Squares (MSS)											
		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches/ Plant	No. of secondary branches/ Plant	No. of pods / plant	No. of seeds / pod	Biological yield / plant (g)	Seed yield/ plant (g)	Harvest index (%)	Test weight (g)	Protein content (%)
Replications	2	0.62	38.97**	213.01**	0.05	0.005	2.13	0.04*	2.17	0.13	38.34	0.53	3.66
Treatments	22	49.26**	18.43**	39.90*	14.77**	34.90**	66.71**	0.18**	121.87**	83.59**	423.11**	59.15**	31.03**
Error	44	7.09	6.62	18.21	0.14	0.37	1.96	0.009	2.45	0.72	32.16	2.55	2.08

*and** indicates significance at 5% and 1% levels, respectively.

Table4: Analysis of variance (ANOVA) for various biochemical and physiological traits in advance breeding lines of chickpea

Source of variations	d.f	Mean Sum of Squares (MSS)				
		Proline content (mg g^{-1} FW)	Chlorophyll content (SPAD)	Relative water content (%)	Relative growth rate (g g^{-1} day^{-1})	Crop growth rate (mg m^{-2} day^{-1})
Replications	2	0.00007	0.82	0.79	0.00002	0.14
Treatments	22	0.058**	148.78**	216.29**	0.0013**	103.87**
Error	44	0.00051	2.53	1.70	0.00002	0.49

*and** indicates significance at 5% and 1% levels, respectively.

D-12 (19.16), while SCS-15-D-14 (9.43) exhibited minimum number of secondary branches / plant. Twenty out of twenty two advance breeding lines revealed highest values than standard check (10.16).

4.2.1.6 Number of pods / plant:

Number of pods / plant ranged from 27.30 to 43.20. The line SCS-15-D-13 exhibited maximum number of pods / plant followed by SCS-15-D-11 (43.00) and SCS-15-D-18 (40.46). Advance breeding line SCS-15-D-20 (27.30) showed minimum value followed by SCS-15-D-1 (27.36). Twelve out of twenty two advance breeding lines showed significantly superior value for number of pods / plant than the mean performance (35.41) whereas, eight lines were found superior than standard check (37.03).

4.2.1.7 Number of seeds / pod:

Number of seeds / pod ranged from 1.2 to 2.0. Mean performance for the character i.e. number of seeds / pod is 1.57. The advance breeding line SCS-15-D-19 (2.0) showed the highest number of seeds / pod followed by SCS-15-D-7 (1.96) and SCS-15-D-22 (1.93) while SCS-15-D-13 showed minimum number of seeds / pod (1.20). Nineteen out of twenty two advance breeding lines of chickpea exhibited higher number of seeds / pod than standard check (1.26).

4.2.1.8 Biological yield / plant (g):

Biological yield / plant ranged from 14.80 to 36.86 g around average mean of 24.43 g. The maximum biological yield / plant was exhibited by SCS-15-D-15 (36.86 g) while minimum showed by SCS-15-D-7 (14.80 g). Six out of twenty two advance breeding lines exhibited maximum mean performance than standard check SCS-3 (28.53 g).

4.2.1.9 Seed yield / plant (g):

Seed yield / plant was ranged from 5.0 g to 23.90 g with a mean of 15.17g. The advance breeding line SCS-15-D-1 (5.0 g) exhibited minimum seed yield / plant followed by SCS-15-D-7 (5.90 g) while the maximum seed yield / plant was exhibited by SCS-15-D-15 (23.90 g) followed by SCS-15-D-9 (21.90 g). Eight out of twenty two advance breeding lines exhibited higher seed yield / plant than standard check (17.60 g).

4.2.1.10 Harvest index (%):

Harvest index ranged from 32.33 to 84.56 % with average mean of 61.25 %. The maximum and minimum harvest index was exhibited by SCS-15-D-22 (84.56%) and SCS-15-D-1 (32.33%), respectively. Fourteen lines recorded high harvest index than the mean performance (61.25%) whereas fourteen out of twenty two advance breeding lines showed superior performance than standard check (61.66 %).

4.2.1.11 Test weight (g):

The maximum test weight was exhibited by SCS-15-D-21 (28.36 g) followed by SCS-15-D-14 (28.10 g) while minimum was exhibited by advance breeding line SCS-15-D-5 (13.0 g). Eleven advance breeding lines were found significantly higher in test weight than the mean performance (20.34 g) while six out of twenty two advance breeding lines were found higher than standard check (23.23 g).

4.2.1.12 Protein content (%):

The range for protein content was varied between 18.16 to 28.66 %. The lowest protein content was recorded by the SCS-15-D-22 (18.16 %) followed by SCS-15-D-13 (18.36%) and SCS-15-D-4 (18.50%) while maximum protein content showed by SCS-15-D-19 (28.66 %) followed by SCS-15-D-12 (28.06%). Eleven lines recorded significantly high protein content than the mean performance (23.22%). Fifteen out of twenty two advance breeding lines exhibited higher values than standard check (21.63 %).

4.2.2 Biochemical and physiological traits:

4.2.2.1 Proline content (mg g^{-1} FW):

The variation for proline content ranged between 0.27 to 0.75 mg g^{-1} FW (Table.6). Minimum proline content was recorded by SCS-15-D-16 (0.27) followed by SCS-15-D-14 (0.28) and SCS-15-D-21 (0.28) while maximum recorded by SCS-15-D-7 (0.75). The mean performance for proline content is 0.47 mg g^{-1} FW. Five out

Table 5: Genetic parameters of morphological traits in advance breeding lines of chickpea

S. No	Characters	Range		Mean+S.E	Phenotypic coefficients of variance (%)	Genotypic coefficients of variance (%)	Heritability in broad sense (%)	Genetic advance	Genetic advance as %age of mean
		Min.	Max.						
1.	Days to 50% flowering	94.33	108.0	100.44+1.53	4.57	3.73	66.46	6.29	6.26
2.	Days to maturity	148.0	158.66	152.7+1.48	2.12	1.29	37.28	2.49	1.63
3.	Plant height(cm)	39.66	54.06	45.96+2.46	10.97	5.85	28.42	2.95	6.42
4.	No. of primary branches/plant	1.03	8.66	4.83+0.21	26.31	25.64	97.14	4.48	50.3
5.	No. of secondary branches/plant	9.43	20.86	15.13+0.35	22.77	22.40	96.82	6.87	45.42
6.	No. of pods /plant	27.30	43.20	35.41+0.80	13.70	13.11	91.65	9.16	25.86
7.	No. of seeds/pod	1.20	2.0	1.57+0.05	16.69	15.49	86.16	0.46	29.62
8.	Biological yield/plant (g)	14.80	36.86	24.43+0.90	26.60	25.81	94.19	12.61	51.61
9.	Seed yield/plant (g)	5.0	23.90	15.17+0.49	35.08	34.63	97.43	10.68	70.42
10	Harvest index (%)	32.33	84.56	61.25+3.27	20.80	18.63	80.21	21.06	34.38
11	Test weight (g)	13.0	28.36	20.34+0.92	22.74	21.34	88.07	8.39	41.26
12	Protein content (%)	18.16	28.66	23.22+0.83	14.74	13.37	82.24	5.80	24.98

of twenty two advance breeding lines exhibited higher mean values than standard check (0.62).

4.2.2.2 Chlorophyll content (SPAD):

The variation for chlorophyll content ranged between 41.43 to 67.53. The minimum chlorophyll content was showed by SCS-15-D-8 (41.43) followed by SCS-3 (42.80) while maximum exhibited by SCS-15-D-16 (67.53). The mean performance for chlorophyll content is 53.80. Twenty one advance breeding lines ($F_{7:8}$) exhibited higher values than standard check (42.80).

4.2.2.3 Relative water content (%):

Relative water content ranged between 35.70 to 65.50 % with mean value of 50.11%. The maximum RWC was recorded by advance breeding line SCS-15-D-5 (65.50%) and minimum recorded by SCS-15-D-14 (35.70%). Eight out of twenty two advance breeding lines were found significantly superior than standard check SCS-3 (52.60%).

4.2.2.4 Relative growth rate (g.g.day^{-1}):

Average mean for RGR was 0.06 and it ranged between 0.03 to 0.10 g.g.day^{-1}. Advance breeding chickpea line SCS-15-D-22 (0.10) exhibited maximum relative growth rate and minimum exhibited by SCS-15-D-16 (0.03) followed by SCS-15-D-21 (0.03) and SCS-15-D-19 (0.03). Two advance breeding lines were found significantly superior than standard check (0.09).

4.2.2.5 Crop growth rate (mg m^{-2} day^{-1}):

Crop growth rate ranged between 15.44 to 39.46 mg m^{-2} day^{-1} with mean value of 23.74. Advance breeding line SCS-15-D-6 (39.46) showed maximum crop growth rate. However, minimum crop growth rate was exhibited by SCS-15-D-15 (15.44). Advance breeding line SCS-15-D-6 (39.46) showed higher crop growth rate than standard check (31.56).

4.3 GENETIC VARIABILITY PARAMETERS

4.3.1 Genotypic and phenotypic coefficients of variation (GCV & PCV):

The estimates of genotypic coefficients of variation have been presented in table 5 and 6, respectively. The highest genotypic coefficient of variation (GCV) was observed for seed yield / plant (34.63 %) followed by relative growth rate (32.67 %), proline content (29.22 %), biological yield / plant (25.81 %), number of primary branches / plant (25.64%), crop growth rate (24.72 %), number of secondary branches / plant (22.40 %), and test weight (21.34%). The characters *viz*., harvest index (18.63 %), relative water content (16.87 %), number of seeds / pod (15.49 %), protein content (13.37%) number of pods / plant (13.11%) and chlorophyll content (12.97 %) exhibited moderate value of GCV. Whereas characters *viz.,* plant height (5.85 %), days to 50% flowering (3.73%) and days to maturity (1.29) exhibited low value of GCV.

Phenotypic coefficient of variation (PCV) exhibited almost similar trend (Table 5 and 6), with maximum PCV exhibited by seed yield / plant (35.08%) followed by relative growth rate (33.45 %), proline content (29.60 %), biological yield / plant (26.60 %), number of primary branches / plant (26.31%), crop growth rate (24.89 %), number of secondary branches / plant (22.77%), test weight (22.74%) and harvest index (20.80 %). The characters *viz*., relative water content (17.07 %), number of seeds / pod (16.69 %), protein content (14.74%) number of pods / plant (13.70%) chlorophyll content (13.31 %) and plant height (10.97 %) exhibited moderate value of PCV whereas characters *viz.,* days to 50% flowering (4.57 %) and days to maturity (2.12 %) exhibited low value of PCV.

The values of PCV are greater than the corresponding GCV for all the traits under studied.

4.3.2 Heritability in broad sense and genetic advance:

The estimates of heritability in broad sense have been depicted in table 5 and 6, respectively. The maximum heritability estimate of 98.59% was exhibited by crop growth rate followed by relative water content (97.68%), seed yield / plant (97.43%), proline content (97.42%), number of primary branches / plant (97.14%), number of secondary branches / plant (96.82%), relative growth rate (95.38%), chlorophyll

Table 6: Genetic parameters of biochemical and physiological traits in advance breeding lines of chickpea

S. No	Characters	Range		Mean±S.E	Phenotypic coefficients of variance (%)	Genotypic coefficients of variance (%)	Heritability in broad sense (%)	Genetic advance	Genetic advance as %age of mean
		Min.	Max.						
1.	Proline content ($mg.g^{-1}$ FW)	0.27	0.75	0.47±0.01	29.60	29.22	97.42	0.28	59.42
2.	Chlorophyll content (SPAD)	41.43	67.53	53.80±0.92	13.31	12.97	95.05	14.02	26.06
3.	Relative Water Content (%)	35.70	65.50	50.11±0.75	17.07	16.87	97.68	17.21	34.36
4.	Relative Growth Rate ($g\ g^{-1}\ day^{-1}$)	0.03	0.10	0.06±0.002	33.45	32.67	95.38	0.04	65.73
5.	Crop Growth Rate ($mg\ m^{-2}\ day^{-1}$)	15.44	39.46	23.74±0.40	24.89	24.72	98.59	12.0	50.56

content (95.05%), biological yield / plant (94.19%), number of pods / plant (91.65%), test weight (88.07%), number of seeds / pod (86.16%), protein content (82.24%), harvest index (80.21%) and days to 50% flowering (66.46%). The moderate heritability recorded for days to maturity (37.28%). The lowest heritability of 28.42% was recorded for plant height.

The maximum genetic advance as percentage of mean was recorded by seed yield / plant (70.42%) followed by relative growth rate (65.73%), proline content (59.42%), biological yield / plant (51.61%), crop growth rate (50.56%), number of primary branches / plant (50.3%), number of secondary branches / plant (45.42%), test weight (41.26%), harvest index (34.38%), relative water content (34.36%), number of seeds / pod (29.62%), chlorophyll content (26.06%), number of pods / plant (25.86%), and protein content (24.98%). The lowest genetic advance as %age of mean was exhibited by days to maturity (1.63%) followed by days to 50% flowering (6.26%) and plant height (6.42%).

High heritability estimates along with high genetic advance as percentage of mean were observed for seed yield / plant, proline content, number of primary branches / plant, crop growth rate, relative water content, number of secondary branches / plant, relative growth rate, chlorophyll content, biological yield / plant, number of pods / plant, test weight, number of seeds / pod and harvest index indicating that additive gene effects are more important in determining these characters and the improvement can be done through mass selection based on phenotypic values. On the other hand, high heritability coupled with low genetic advance was recorded for days to maturity and days to 50% flowering indicating the influence of dominant and epistatic genes (non- additive gene effect) for these traits. Therefore, there appears to be a limited scope of improvement in such traits.

4.4 CORRELATION ANALYSIS:

To know the nature and magnitude of relationship existing between the traits under study, correlation coefficients were carried out at genotypic and phenotypic levels and presented in table 7 and 8, respectively.

Seed yield / plant showed highly significant correlation with biological yield / plant (0.862), number of pods / plant (0.381) whereas it showed negative and significant correlation with plant height (-0.341), number of primary branches / plant (-0.253) and days to 50% flowering (-0.299) at phenotypic level.

4.4.1 Days to 50% flowering:

Days to 50% flowering showed highly significant correlation with days to maturity (0.571) followed by number of seeds / pod (0.326) and number of secondary branches / plant (0.260) while it showed negative but highly significant correlation with biological yield / plant (-0.507) followed by test weight (-0.387), number of pods / plant (-0.359) and seed yield / plant (-0.349) at genotypic level (Table 7). It showed positive and significant correlation with days to maturity (0.280) and number of seeds /pod (0.242) while negative and highly significant correlation with biological yield / plant (-0.357) followed by test weight (-0.259), number of pods / plant (-0.258) and seed yield / plant (-0.299) at phenotypic level.

4.4.2 Days to maturity:

It exhibited positive and significant correlation with yield contributing traits like number of pods / plant (0.382), biological yield / plant (0.366), number of seeds / pods (0.275) while it revealed highly significant and negative correlation with plant height (-0.362), number of secondary branches / plant (-0.265) at genotypic level. At phenotypic level it showed significant correlation with biological yield / plant (0.285).

4.4.3 Plant height (cm):

Plant height showed positive and highly significant correlation with number of pods / plant (0.948), biological yield / plant (0.758), seed yield / plant (0.659), number of secondary branches / plant (0.426). Highly significantly and negatively correlated with harvest index (-0.462) and test weight (-0.301) at genotypic level while at phenotypic level (Table 7) it was highly significant but positively correlated with number of pods / plant (0.412), biological yield / plant (0.354), seed yield / plant (0.341) and number of secondary branches / plant (0.239).

4.4.4 Number of primary branches / plant:

Number of primary branches / plant showed highly significant and positive correlation with number of secondary branches / plant (0.499), number of seeds / pod

Table 7: Genotypic and phenotypic correlation coefficients among morphological traits with seed yield / plant in advance breeding lines of chickpea

Characters		Days to maturity	Plant height (cm)	No. of primary branches/ plant	No. of secondary branches/ plant	No. of pods / plant	No. of seeds / pod	Biological yield / plant (g)	Seed yield/ plant (g)	Harvest index (%)	Test weight (g)	Protein content (%)
Days to 50% flowering	r_g	0.571	0.059	0.018	0.260	-0.359	0.326	-0.507	-0.349	0.041	-0.387	0.150
	r_p	0.280*	0.110	0.003	0.182	-0.258*	0.242*	-0.357**	-0.299*	-0.057	-0.259*	0.192
Days to maturity	r_g		-0.362	-0.120	-0.265	0.382	0.275	0.366	0.181	-0.129	0.036	-0.023
	r_p		-0.104	-0.039	-0.149	0.228	0.111	0.285*	0.127	-0.138	0.052	0.001
Plant height (cm)	r_g			-0.016	0.426	0.948	0.013	0.758	0.695	-0.462	-0.301	-0.274
	r_p			-0.049	0.239*	0.41***	0.008	0.354**	0.341**	-0.218	-0.134	-0.138
No. of primary branches/ plant	r_g				0.499	-0.152	0.411	0.244	-0.254	-0.143	-0.023	0.548
	r_p				0.491**	-0.143	0.373**	0.239*	-0.253*	-0.127	-0.010	0.498***
No. of secondary branches / plant	r_g					0.052	0.223	0.147	0.127	-0.022	-0.127	0.405
	r_p					0.041	0.208	0.142	0.122	-0.024	-0.110	0.361**
No. of pods / plant	r_g						-0.328	0.444	0.394	0.226	-0.020	-0.100
	r_p						-0.256*	0.412***	0.381**	0.208	-0.002	-0.087
No. of seeds / pod	r_g							-0.215	-0.218	-0.117	-0.182	0.140
	r_p							-0.207	-0.199	-0.081	-0.166	0.106
Biological yield / plant (g)	r_g								0.906	-0.405	0.409	-0.055
	r_p								0.862***	-0.265*	0.399**	-0.041
Seed yield/ plant (g)	r_g									0.739	0.319	-0.134
	r_p									0.700***	0.289*	-0.132
Harvest index (%)	r_g										0.044	-0.114
	r_p										0.005	-0.127
Test weight (g)	r_g											-0.134
	r_p											-0.107

*,** and *** indicates significance at 5%, 1% and 0.1% levels, respectively

Table 8: Genotypic and phenotypic correlation coefficients among biochemical and physiological traits with seed yield/plant in advance breeding lines of chickpea.

Characters		Chlorophyll content (SPAD)	Relative water content (%)	Relative growth rate (g g^{-1} day^{-1})	Crop growth rate (mg m^{-2} day^{-1})	Seed yield / plant (g)
Proline content (mg g^{-1} FW)	r_g	-0.200	-0.066	0.234	0.212	-0.124
	r_p	-0.182	-0.068	-0.238*	0.210	-0.119
Chlorophyll content (SPAD)	r_g		0.067	-0.187	-0.198	-0.068
	r_p		0.062	-0.182	-0.190	-0.066
Relative water content (%)	r_g			0.182	0.119	0.027
	r_p			0.184	0.114	0.021
Relative growth rate (g g^{-1} day^{-1})	r_g				0.254	-0.127
	r_p				0.249*	-0.125
Crop growth rate (mg m^{-2} day^{-1})	r_g					0.160
	r_p					0.162

*and** indicates significance at 5% and 1% levels, respectively

(0.411), biological yield / plant (0.244) and negatively correlated with seed yield / plant (-0.254) at genotypic level (Table 7). It exhibited highly significant and positive correlation with number of secondary branches / plant (0.491), number of seeds / pod (0.373) and biological yield / plant (0.239) and showed negative correlation with seed yield / plant (-0.253) at phenotypic level.

4.4.5 Number of pods / plant:

It exhibited significantly high and positive correlation with biological yield / plant (0.444), seed yield / plant (0.394) while it was negatively correlated with number of seeds / pod (-0.328) at genotypic level (Table 7). At phenotypic level, it showed highly significant and positive correlation with biological yield / plant (0.412) and seed yield / plant (0.381) while it was negatively correlated with number of seeds / pod (-0.256).

4.4.6 Biological yield / plant (g):

It showed highly significant and positive correlation with seed yield / plant (0.906) and test weight (0.409) while it exhibited negative and highly significant correlation with harvest index (-0.405) at genotypic level. Biological yield / plant showed highly significant and positive correlation with seed yield / plant (0.862) and test weight (0.399) while it revealed negative and significant correlation with harvest index (-0.265) at phenotypic level

4.4.7 Seed yield / plant (g):

It exhibited highly significant and positive correlation with harvest index (0.739) and test weight (0.319) at genotypic level while revealed highly significant and positive correlation with harvest index (0.700) but it showed positive and significant correlation with test weight (0.289) at phenotypic level.

4.4.8 Proline content (mg g^{-1} FW):

Proline content exhibited negative and significant correlation with relative growth rate (-0.238) at phenotypic level (Table 8) while it revealed positive correlation at genotypic level.

4.4.9 Relative growth rate (g.g.day $^{-1}$):

It showed positive and significant correlation with crop growth rate (0.249) at phenotypic level and exhibited similar results at genotypic level.

4.5 PATH ANALYSIS:

Correlation coefficients, which measure the association between any two characters, may not give a true or comprehensive picture of a rather complex situation. Path coefficient analysis is a means of measuring the direct and indirect effects of one variable through the other variables on the end product. The direct and indirect effects of different independent variables on seed yield / plant (dependent variable) at genotypic and phenotypic levels are estimated and presented in table 9 and 10, respectively.

4.5.1 Days to 50% flowering:

Days to 50% flowering exhibited negative direct effect of -0.019 on seed yield / plant, while it exhibited highest positive indirect effect for days to maturity (0.011), followed by biological yield / plant (0.009), test weight (0.007), number of pods / plant (0.006) and negative indirect effect of -0.006 was exhibited for number of seeds/pod at genotypic level.

4.5.2 Days to maturity:

Direct and positive effect for days to maturity was 0.064 on seed yield / plant while positive indirect effect recorded *via* number of pods /plant (0.023) followed by biological yield / plant (0.018) and harvest index (0.011). The maximum negative indirect effect exhibited *via* days to 50% flowering (-0.001) at genotypic level. At phenotypic level, direct and positive effect for days to maturity was 0.011 while highest positive indirect effect 0.003 was recorded *via* biological yield / plant and number of pods / plant (0.002). The maximum negative indirect effect of -0.003 was showed *via* days to 50% flowering.

4.5.3 Plant height (cm):

Plant height exerted negative direct effect on seed yield / plant (-0.024). It has highest positive indirect effect through number of pods / plant (0.023) followed by

Table 9: Direct and indirect effects of morphological traits on seed yield / plant in advance breeding lines of chickpea

Characters		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches/ plant	No. of secondary branches/ plant	No. of pods / plant	No. of seeds / pod	Biological yield / plant (g)	Harvest index (%)	Test weight (g)	Protein content (%)	Correlation with seed/yield
Days to 50% flowering	**G**	**-0.0192**	0.0110	-0.0011	-0.0004	-0.0050	0.0069	-0.0063	0.0098	-0.0008	0.0075	-0.0029	-0.349
	P	**-0.0289**	0.0081	-0.0032	-0.0001	-0.0053	0.0075	-0.0070	0.0103	0.0017	0.0075	-0.0056	-0.299*
Days to maturity	**G**	-0.0367	**0.0642**	-0.0233	-0.0077	-0.0170	0.0246	0.0177	0.0236	-0.0083	0.0024	-0.0015	0.181
	P	-0.0032	**0.0113**	-0.0012	-0.0004	-0.0017	0.0026	0.0013	0.0032	-0.0016	0.0006	0.0000	0.127
Plant height (cm)	**G**	-0.0015	0.0090	**-0.0249**	0.0004	0.0106	0.0236	0.0003	0.0189	0.0115	0.0075	0.0068	0.695
	P	0.0030	-0.0028	**0.0271**	-0.0013	-0.0068	-0.0113	-0.0002	-0.0096	-0.0059	-0.0036	-0.0038	0.341**
No. of primary branches/ plant	**G**	-0.0001	0.0005	0.0001	**-0.0043**	-0.0022	0.0007	-0.0018	0.0011	0.0006	0.0001	-0.0024	-0.254
	P	-0.0001	0.0015	0.0018	**-0.0371**	-0.0182	0.0053	-0.0139	0.0089	0.0047	0.0004	-0.0185	-0.253*
No. of secondary branches / plant	**G**	0.0288	-0.0293	-0.0471	0.0552	**0.1104**	0.0057	0.0246	0.0162	-0.0025	-0.0141	0.0448	0.127
	P	0.0157	-0.0129	-0.0218	0.0423	**0.0861**	0.0035	0.0179	0.0122	-0.0021	-0.0095	0.0311	0.122
No. of pods / plant	**G**	0.0390	-0.0415	0.1029	0.0166	-0.0056	**-0.1084**	0.0356	-0.0482	-0.0246	0.0023	0.0109	0.394
	P	0.0086	-0.0076	0.0139	0.0048	-0.0014	**-0.0334**	0.0085	-0.0138	-0.0070	0.0001	0.0029	0.381**
No. of seeds / pod	**G**	-0.0253	-0.0213	0.0011	-0.0319	-0.0173	0.0254	**-0.0774**	0.0166	0.0091	0.0141	-0.0109	-0.218
	P	-0.0030	-0.0014	0.0001	-0.0047	-0.0026	0.0032	**-0.0125**	0.0026	0.0010	0.0021	-0.0013	-0.199
Biological yield / plant (g)	**G**	-0.3437	0.2482	-0.5130	-0.1657	0.0995	0.3005	-0.1456	**0.6768**	0.2746	0.2769	-0.0375	0.906
	P	-0.2534	0.2023	-0.2512	-0.1699	0.1008	0.2921	-0.1469	**0.7087**	0.1882	0.2831	-0.0295	0.862***
Harvest index (%)	**G**	0.0194	-0.0609	-0.2173	-0.0672	-0.0107	0.1064	-0.0550	0.1905	**0.4696**	0.0209	-0.0537	0.739
	P	-0.0296	-0.0712	-0.1127	-0.0658	-0.0127	0.1078	-0.0421	0.1370	**0.5159**	0.0027	-0.0658	0.700***
Test weight (g)	**G**	0.0037	-0.0004	0.0029	0.0002	0.0012	0.0002	0.0018	-0.0039	-0.0004	**-0.0096**	0.0013	0.319
	P	-0.0005	0.0001	-0.0003	0.0000	-0.0002	0.0000	-0.0003	0.0008	0.0000	**0.0019**	-0.0002	0.289*
Protein content (%)	**G**	-0.0135	0.0021	0.0247	-0.0493	-0.0365	0.0091	-0.0127	0.0050	0.0103	0.0121	**-0.0900**	-0.134
	P	-0.0081	-0.0001	0.0058	-0.0209	-0.0152	0.0037	-0.0045	0.0017	0.0053	0.0045	**-0.0419**	-0.132

Diagonal figures represent direct effects

Residual effect : Phenotypic= 0.1049 and Genotypic = 0.0743 biological yield / plant (0.018) and harvest index (0.011). Plant height recorded positive direct effect (0.027) while positive indirect effect recorded through days to 50% flowering (0.003). The maximum negative indirect effect showed by number of pods / plant (-0.011) at phenotypic level.

4.5.4 Number of primary branches / plant:

Number of primary branches / plant exhibited negative direct effect (-0.004) on seed yield / plant. The highest positive indirect effect of 0.001 was recorded *via* biological yield / plant while negative indirect effect of -0.002 was revealed *via* protein content at genotypic level. The negative direct effect for number of primary branches / plant was -0.037 while highest positive indirect effect of 0.008 was recorded *via* biological yield / plant and negative indirect effect of -0.018 was seen *via* protein content at phenotypic level.

4.5.5 Number of secondary branches / plant:

At genotypic level, direct positive effect for number of secondary branches / plant was 0.110 on seed yield / plant, whereas positive indirect effect recorded by character number of primary branches / plant (0.055) while negative indirect effect recorded by plant height (-0.047). At phenotypic level, direct positive effect for number of secondary branches / plant was 0.086 while positive indirect effect recorded by character number of primary branches / plant (0.042), protein content (0.031) and negative indirect effect recorded by plant height (-0.021).

4.5.6 Number of pods / plant:

Negative direct effect for number of pods / plant was (-0.108) and positive indirect effect recorded by character plant height (0.102) followed by days to 50% flowering (0.039) and number of seeds / pod (0.035) while negative indirect effect recorded for biological yield / plant (-0.048) at genotypic level. Negative direct effect for number of pods / plant was (-0.033) while positive indirect effect recorded by character plant height (0.013) followed by days to 50% flowering (0.086) and number of seeds / pod (0.085). Maximum negative indirect effect recorded for biological yield / plant (-0.013) at phenotypic level.

4.5.7 Number of seeds / pod:

The direct effect for number of seeds / pod was (-0.077) on seed yield / plant. The maximum positive indirect effect recorded by number of pods / plant (0.025) while maximum negative indirect effect recorded by number of primary branches / plant (-0.031) at genotypic level. Direct effect for number of seeds / pod was (-0.012). The maximum positive indirect effect recorded by number of pods / plant (0.003) while maximum negative indirect effect recorded by number of primary branches / plant (-0.004) at phenotypic level.

4.5.8 Biological yield / plant (g):

At genotypic level, direct positive effect for biological yield / plant was (0.676) on seed yield / plant. The maximum positive indirect effect recorded by number of pods / plant (0.300) followed by test weight (0.276), harvest index (0.274) and days to maturity (0.248), while maximum negative indirect effect showed by plant height (-0.513). At phenotypic level, positive indirect effect exhibited by number of pods / plant (0.292) followed by test weight (0.283), harvest index (0.188) and days to maturity (0.202), while maximum negative indirect effect recorded by days to 50% flowering (-0.253) and plant height (-0.251).

4.5.9 Harvest index (%):

Harvest index exhibited direct positive effect (0.469) on seed yield / plant. The maximum positive indirect effect showed by biological yield / plant (0.190) while maximum negative indirect effect recorded by plant height (-0.217) at genotypic level. The maximum positive indirect effect exhibited for biological yield / plant (0.137), while maximum negative indirect effect showed for plant height (-0.112) at phenotypic level.

4.5.10 Test weight (g):

At genotypic level, direct negative effect for test weight was (-0.009). The maximum positive indirect effect showed by character days to 50% flowering (0.003), while maximum negative indirect effect exhibited by biological yield / plant (-0.003). At phenotypic level, maximum positive indirect effect showed by character

Table 10: Direct and indirect effects of biochemical and physiological traits on seed yield / plant in advance breeding lines of chickpea

Characters		Proline content (mg g^{-1} FW)	Chlorophyll content (SPAD)	Relative water content (%)	Relative growth rate (g g^{-1} day^{-1})	Crop growth rate (mg m^{-2} day^{-1})	Correlation with seed yield/plant
Proline content (mg g^{-1} FW)	**G**	**-0.1456**	0.0292	0.0097	-0.0342	-0.0310	-0.124
	P	**-0.1391**	0.0254	0.0095	-0.0332	-0.0293	-0.119
Chlorophyll Content (SPAD)	**G**	-0.0038	**-0.0894**	-0.0060	0.0168	0.0177	-0.068
	P	0.0034	**-0.0821**	-0.0051	0.0150	0.0157	-0.066
Relative water content (%)	**G**	0.0249	0.0040	**0.0291**	0.0053	0.0035	0.027
	P	-0.0017	0.0015	**0.0225**	0.0042	0.0026	0.021
Relative growth rate (g g^{-1} day^{-1})	**G**	-0.0040	0.007	-0.0015	**-0.1698**	-0.0432	-0.127
	P	-0.009	0.0002	-0.004	**-0.1645**	-0.0410	-0.125
Crop growth rate (mg m^{-2} day^{-1})	**G**	0.0024	-0.0002	0.0014	0.0014	**0.2135**	0.160
	P	0.0025	-0.0001	0.0014	0.0018	**0.2146**	0.162

Diagonal figures represent direct effects
Residual effect : Phenotypic= 0.9602and Genotypic = 0.0743

biological yield / plant (0.0008) while maximum negative indirect effect revealed by days to 50% flowering (-0.0005).

4.5.11 Protein content (%):

Direct negative effect for protein content was (-0.090) on seed yield / plant. The highest positive indirect effect exhibited by plant height (0.024), while maximum negative indirect effect showed by number of primary branches / plant (-0.049) at genotypic level. At phenotypic level, highest positive indirect effect revealed by plant height (0.005) while maximum negative indirect effect exhibited by number of primary branches / plant (-0.020).

Residual effect: Residual effect was recorded 0.089 and 0.104 at genotypic and phenotypic levels, respectively.

4.6 GENETIC DIVERSITY STUDIES (D^2 ANALYSIS) :

4.6.1 Cluster formation:

The multivariate analysis grouped twenty three lines into 6 clusters (Table 11). Among these, cluster II was the largest including eight lines followed by cluster III with six lines, cluster I with five lines, cluster IV with two lines, cluster V and VI had only one line each.

4.6.2 The average intra and inter-cluster distance:

The intra and inter-cluster D values among the six clusters have been presented in table 12. The cluster III (320.37) showed the highest intra-cluster distance, while the lowest intra-cluster distance was exhibited by the cluster V and VI (0.00). The highest inter-cluster distance was observed between cluster IV and VI (904.40) followed by cluster IV and V (718.77), cluster II and IV (566.82), cluster V and VI (519.92), cluster III and V (513.47), and cluster I and IV(503.23). The least inter-cluster distance was recorded between cluster I and V (316.89).

A line diagram depicting Mahalanobis Euclidean Distance of six clusters through inter and intra-clusters distribution in 23 genotypes, dendrogram based on genetic distance between advance breeding lines are presented in Fig. 2 and 3, respectively.

4.6.3 Cluster mean:

The cluster means for all the traits are given in table 13.

4.6.3.1 Days to 50 % flowering:

The cluster mean for character days to 50% flowering varied from 94.33 to 103.53. The advance breeding lines of cluster V showed early flowering as it had taken minimum number of (94.33) days for flowering. The mean of cluster I (103.53) and IV (101.0 days) indicated late flowering.

4.6.3.2 Days to maturity:

The cluster mean for character days to maturity was ranged from 151.27 to 154.33. The late maturing advance breeding lines were found in cluster VI (154.33) whereas early maturing genotypes were found in cluster I (151.27).

4.6.3.3 Plant height (cm):

The cluster mean varied from 43.63 to 50.80. The tallest lines were grouped into cluster IV (50.80) followed by cluster VI (48.63) and cluster I (47.95).

4.6.3.4 Number of primary branches / plant:

Cluster mean for number of primary branches / plant ranged between 1.03 to 7.67. The advance breeding lines had maximum number of primary branches were grouped into cluster V (7.67) and cluster I (6.21) whereas least cluster mean was observed in VI (1.03).

4.6.3.5 Number of secondary branches / plant:

The cluster mean varied from 11.76 to 16.81. Maximum number of secondary branches were grouped in cluster I (16.81) followed by cluster II (16.72) and cluster V (16.13) whereas minimum cluster mean was observed in cluster III (11.76).

4.6.3.6 Number of pods / plant:

The advance breeding lines had maximum number of pods / plant were grouped into cluster II (37.05) and cluster III (36.72). The minimum cluster mean was observed in cluster IV (31.33).

Table 11: Grouping of twenty three advance breeding lines into different clusters on the basis of D^2 analysis

Clusters	No. of advance breeding lines	Advance breeding lines
I	5	SCS-15-D-10, SCS-15-D-16, SCS-15-D-12, SCS-15-D-5, SCS-15-D-3
II	8	SCS-15-D-9, SCS-15-D-21, SCS-15-D-11,SCS-15-D-4, SCS-15-D-18, SCS-15-D-17, SCS-15-D-20, SCS-15-D-19
III	6	SCS-15-D-13, SCS-15-D-14, SCS-15-D-22, SCS-15-D-2, SCS-3, SCS-15-D-6
IV	2	SCS-15-D-1, SCS-15-D-7
V	1	SCS-15-D-8
VI	1	SCS-15-D-15

Table 12: Average intra and inter – cluster distance (D-values)

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**180.26**	374.91	402.11	503.23	316.89	385.97
Cluster II		**286.20**	413.44	566.82	334.21	453.85
Cluster III			**320.37**	496.98	513.47	423.75
Cluster IV				**174.85**	718.77	904.40
Cluster V					**0.00**	519.92
Cluster VI						**0.00**

Tocher Method

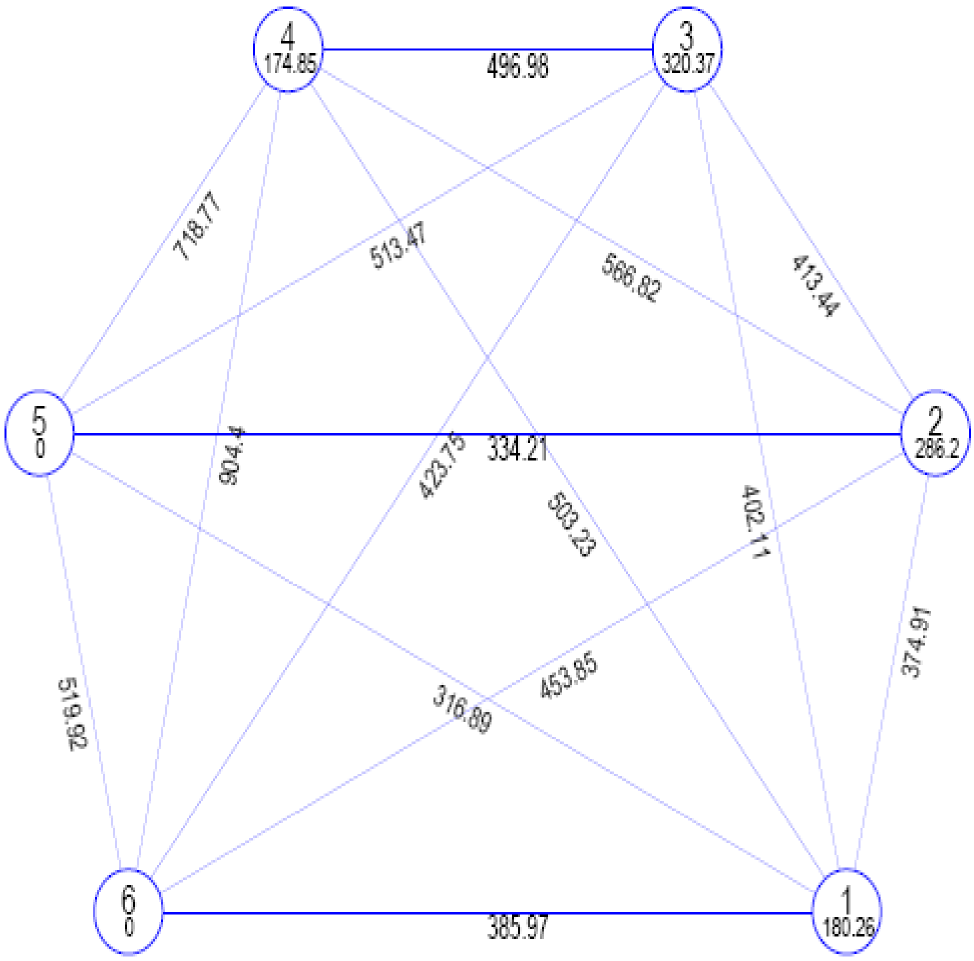


Figure 2. Mahalanobis Euclidean Distance of 6 clusters through inter and intra clusters distribution in 23 genotypes.

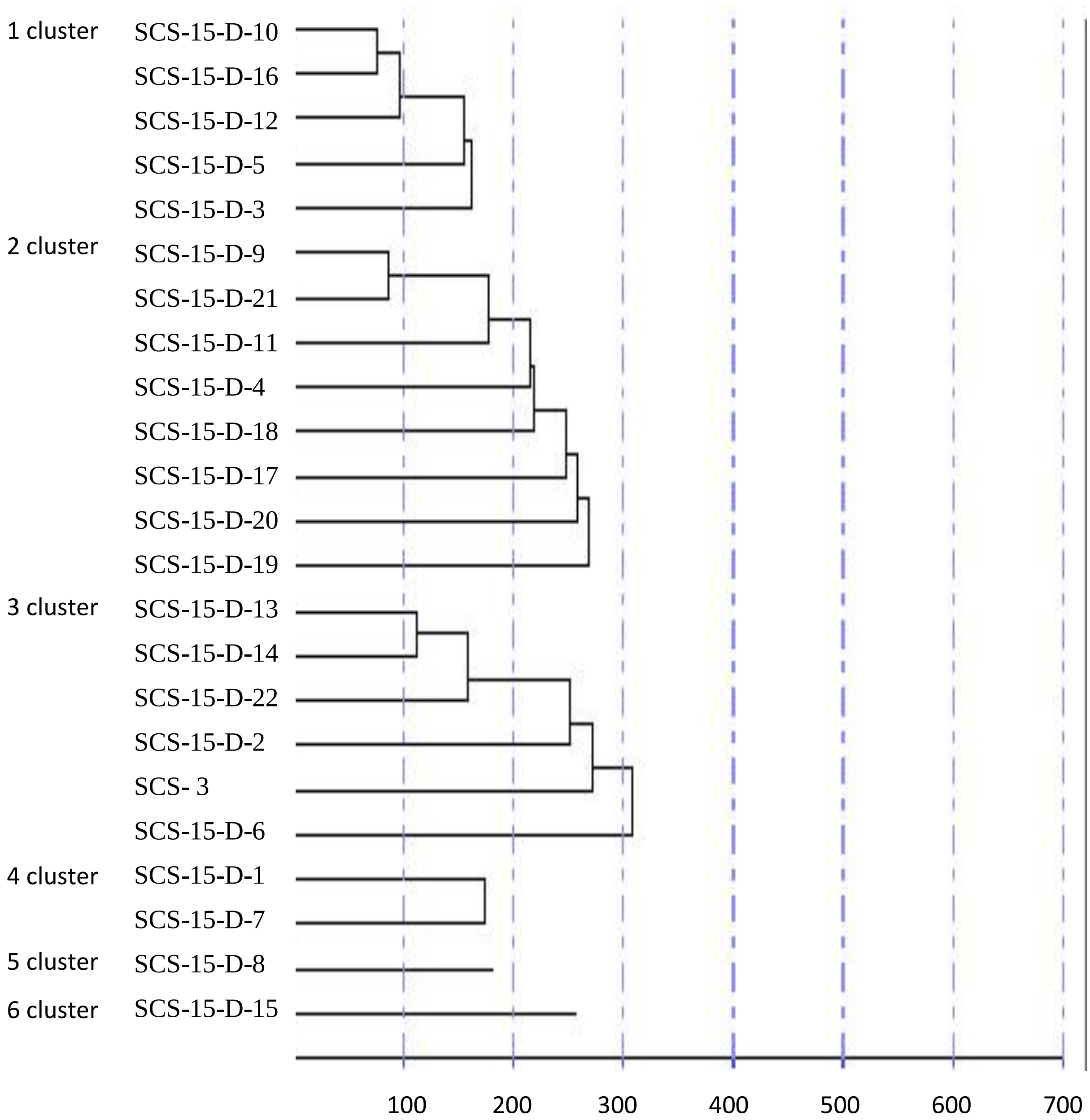


Figure 3. Dendogram of Chickpea advance breeding lines by clustering (Tocher Method)

4.6.3.7 Number of seeds / pod:

The cluster mean for number of seeds / pod varied from 1.03 to 1.83. Cluster V (1.83) showed maximum number of advance breeding lines for number of seeds / pod followed by cluster IV (1.80) whereas cluster VI (1.03) revealed minimum number of seeds / pod.

4.6.3.8 Biological yield / plant (g):

Cluster mean for biological yield / plant varied from 15.15g to 36.87g. Cluster VI (36.87g) showed maximum cluster mean for biological yield / plant, while minimum cluster mean was observed in cluster IV (15.15g) and I (18.53g).

4.6.3.9 Seed yield / plant (g):

Mean for seed yield / plant varied from 5.45g to 23.90g. The maximum cluster mean was observed in cluster VI (23.90g) and minimum cluster mean was observed in cluster IV (5.45g).

4.6.3.10 Harvest index (%):

The cluster mean for harvest index was varied from 36.10 to 69.33. Cluster V (69.33) exhibited maximum cluster mean followed by cluster III (65.28) and cluster VI (64.87). Minimum cluster mean was observed in cluster IV (36.10).

4.6.3.11 Test weight (g):

Test weight of the cluster mean varied from 15.80 to 26.40. The advance breeding lines with maximum test weight grouped into cluster V (26.40) and lines with least test weight grouped into cluster I (15.80).

4.6.3.12 Protein content (%):

The cluster mean for protein content varied from 20.48 to 25.99. The maximum cluster mean was observed in cluster I (25.99) and cluster II (24.22). Minimum cluster mean was observed in cluster IV (20.48) and cluster III (20.65).

4.6.3.13 Proline content (mg g^{-1} FW):

Proline content varied from 0.35 to 0.65. Cluster IV (0.65) showed maximum cluster mean whereas least cluster mean was observed in cluster VI (0.35) followed by cluster V (0.36) and cluster I (0.39).

4.6.3.14 Chlorophyll content (SPAD):

The cluster mean for chlorophyll content varied from 41.43 to 57.52. Cluster I (57.52) exhibited maximum cluster mean, while cluster V (41.43) showed minimum cluster mean.

4.6.3.15 Relative water content (%):

For relative water content cluster mean varied from 43.95 to 60.71. The maximum cluster mean was observed in cluster I (60.71) followed by cluster V (59.40) and cluster VI (58.50). Least cluster mean was observed in cluster IV (43.95).

4.6.3.16 Relative growth rate (g.g.day $^{-1}$):

For relative growth rate, cluster mean varied from 0.05 to 0.08. The maximum cluster mean was observed in cluster III, VI and VI (0.08). Cluster II and V (0.05) exhibited minimum cluster mean.

4.6.3.17 Crop growth rate (mg m^{-2} day^{-1}):

Cluster mean for crop growth rate varied from 15.44 to 30.45. The maximum cluster mean was observed in cluster V (30.45) and minimum cluster mean was observed in cluster VI (15.44).

Table 14: Advance breeding lines of chickpea with desirable traits.

Clusters	**Advance breeding lines**	**Desirable characteristics**	**No. of traits**
I	SCS-15-D-3	Earliness in days to maturity, moderate plant height, moderate number of primary branches / plant, higher number of secondary branches / plant and moderate number of pods /plant	05
II	SCS-15-D-11	Dwarf plant, higher number of primary branches / plant, higher number of secondary branches / plant, higher number of pods /plant and moderate seed yield / plant	05

Table 13:Mean performance of different clusters formorpho-physiological and biochemical traits in advance breeding lines chickpea

Clusters	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches/ plant	No. of secondary branches / plant	No. of pods / plant	No. of seeds/ pod	Biological yield / plant (g)	Seed yield/ Plant (g)	Harvest index (%),	Test weight (g)	Protein content (%)	Proline Content (mg g^{-1} FW)	Chlorophyll Content (SPAD)	Relative Water Content (%)	Relative Growth Rate (g g^{-1} day^{-1})	Crop Growth Rate (mg m^{-2} day^{-1})
I	103.53	151.27	47.95	6.21	16.81	32.85	1.58	18.53	10.85	59.06	15.80	25.99	0.39	57.52	60.71	0.07	23.21
II	99.50	153.29	43.63	5.55	16.72	37.05	1.59	28.11	18.00	64.44	21.47	24.22	0.51	54.22	45.36	0.05	23.35
III	100.67	152.78	45.40	2.69	11.76	36.72	1.49	24.87	16.09	65.28	22.51	20.65	0.49	54.74	46.72	0.08	25.56
IV	101.00	153.17	50.80	5.50	15.27	31.33	1.80	15.15	5.45	36.10	18.55	20.48	0.65	47.32	43.95	0.08	22.04
V	94.33	153.33	45.77	7.67	16.13	34.53	1.83	28.10	19.47	69.33	26.40	23.20	0.36	41.43	59.40	0.05	30.45
VI	96.33	154.33	48.63	1.03	13.23	36.50	1.03	36.87	23.90	64.87	18.67	22.43	0.35	51.63	58.50	0.08	15.44

IV	SCS-15-D-1	Tall plant, moderate number of secondary branches / plant, moderate number of pods / plant	03
V	SCS-15-D-8	Earliness in days to 50% flowering, dwarf plant, higher number of primary branches / plant, higher number of secondary branches / plant, higher number of pods /plant and moderate seed yield / plant	06
VI	SCS-15-D-15	Higher number of pods /plant, high seed yield / plant and high harvest index.	03

Chapter-5

Discussion

CHAPTER-V

DISCUSSION

The discussion of present investigation entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** is confined to genetic variability, heritability and genetic advance, correlation, path analysis and genetic divergence.

The analysis of variance showed significant differences among all 22 advance breeding lines of chickpea along with a standard check (SCS-3) for all the characters under rainfed conditions. Mean sum of squares due to treatments were highly significant for all the traits. Hence, showed the presence of clear significant difference among the advance breeding lines. Similar, findings have been reported in chickpea by Ali *et al.* (2008) and Atta *et al.* (2008)

5.1 GENETIC VARIABILITY:

The estimation of genetic variability is a prerequisite for any breeding programs. The present study revealed significant differences among the advance breeding lines ($F_{7:8}$) of chickpea. The results indicating the existence of large genetic variability for all the traits. Genetic variability with the help of suitable parameters such as genetic coefficient of variation, heritability estimates and genetic advance are absolutely necessary to start an efficient breeding programme. In the present study, it was observed that the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters studied. This indicates the presence of environmental influences in the expression of these characters, but these differences were small (less than 8%) in majority of the characters studied indicating a little influence of the environment in the expression of these characters and selection for improvement of such characters based on phenotype would be rewarding to improve present chickpea advance breeding lines.

In the present investigation, high to moderate estimate of PCV and GCV was recorded for seed yield / plant, relative growth rate, proline content, biological yield / plant, crop growth rate, number of primary branches / plant, number of secondary branches / plant, test weight, harvest index, relative water content, number of seeds / pod, protein content, number of pods / plant and chlorophyll content. Paul *et al.* (2018), Atta *et al.* (2008), Sewak *et al.* (2012) reported high genotypic coefficient of variation and phenotypic coefficient of variation for seed yield / plant. Joshi *et al.* (2018) reported high GCV and PCV for seed yield / plant and biological yield / plant. Atta *et al.* (2008) reported high GCV and PCV for test weight. Kumar *et al.* (2016) reported moderate values of PCV and GCV for harvest index. Ramanappa *et al.* (2013) reported moderate GCV and PCV for number of seeds / pod. Whereas low estimate of GCV and PCV was recorded for characters *viz.,* days to 50% flowering, days to maturity and plant height. Saleem *et al*. (2008) and Khan *et al.* (2011) also reported low PCV and GCV for days to 50% flowering and days to maturity. Hakim *et al*. (2006) and Kumar *et al.* (2016) observed lower PCV and GCV for plant height.

The least phenotypic and genotypic coefficients of variations were observed for days to 50% flowering, days to maturity and plant height and hence, these traits provides practically less chance for selection due to more influence of environment.

5.2 HERITABILITY AND GENETIC ADVANCE:

Heritability is a significant parameter for efficient selection and improvement of advance breeding lines**.** In the present investigation, high heritability estimates were recorded for seed yield / plant, number of primary branches / plant, number of secondary branches / plant, relative growth rate, chlorophyll content, biological yield / plant, number of pods / plant, test weight, number of seeds / pod and harvest index. The results are in agreement with the findings of Dar *et al.* (2012), Singh *et al.* (2012), Yadav *et al.* (2015) for seed yield / plant, number of pods / plant, number of secondary branches / plant, number of primary branches / plant. Johnson *et al.* (1955) suggested that high heritability estimates, combined with high genetic advance as % of mean, is indicative of additive gene action and selection based on these parameters would be more reliable.

In the present investigation, high heritability estimates along with high genetic advance were observed for seed yield / plant, proline content, number of primary branches / plant, crop growth rate, relative water content, number of secondary branches / plant, relative growth rate, chlorophyll content, biological yield

/ plant, number of pods / plant, test weight, number of seeds / pod and harvest index indicating that additive gene effects are more important in determining these characters and the improvement can be done through mass selection based on phenotypic values. Mishra & Yadav (1994) and Atta *et al.* (2008) reported high heritability along with high genetic advance for number of secondary branches / plant, number of pods / plant, test weight and seed yield / plant. Joshi *et al.* (2018) also reported high heritability along with high genetic advance as % of mean for test weight, harvest index, biological yield / plant and seed yield / plant. Chouhan *et al.* (2000) and Arora and Jeena (2001) also observed high heritability estimate along with high genetic advance for biological yield / plant. Padmavathi *et al.* (2013) also reported high heritability along with high genetic advance as % of mean for number of primary branches / plant. On the other hand, high heritability coupled with low genetic advance was recorded for days to maturity and days to 50% flowering. The results are in agreement with the findings of Arshad *et al.* (2004), indicating the influence of dominant and epistatic genes (non- additive gene effect) for these traits. Therefore, there appears to be a limited scope of improvement in such traits.

5.3 CORRELATION COEFFICIENT ANALYSIS:

Seed yield is a polygenic trait governed by many genes with cumulative gene effects. Thus, for improvement in seed yield / plant cannot be achieved by practicing selection in diverse population on *per se* performance basis. For seed yield improvement programme, an another measure is required which is used to find out the degree and direction of relationship between two or more variables, that is, correlation coefficient. It is useful to determine the components traits on which selection can be based for genetic improvement in seed yield / plant. The correlation studies revealed the higher estimates of genotypic correlation coefficients than their corresponding phenotypic correlation coefficients, suggesting a strong inherent relationship between different traits and that the environmental factors had not played much role in transmission of the traits studied.

The most important character (Table 7 & 8) i.e. seed yield / plant, was positively and significantly correlated with plant height, number of pods / plant, test weight and biological yield / plant but it was negatively correlated with days to 50% flowering and number of primary branches / plant revealed importance of these characters in determining seed yield / plant. These results are in close agreement with some earlier reports by Malik *et al.* (1987), Khan *et al.* (1989) and Arshad *et al.* (2004). Agrawal *et al.* (2018) and Vaghela *et al*. (2009) also reported that seed yield / plant was positively and significantly correlated with harvest index. Parhe *et al.* (2014) observed that seed yield / plant was negatively correlated with days to 50% flowering. Singh *et al.* (2017) also reported that days to 50% flowering exhibited significant and positive correlation with days to maturity. Tadesse *et al*. (2016) reported that plant height was positively correlated with number of pods / plant. Number of primary branches / plant was positively and significantly correlated with number of secondary branches / plant. Similar result was obtained by Zali *et al*. (2011).

Biological yield / plant was positively and significantly correlated with plant height, number of primary branches / plant, number of pods / plant and test weight. Similar results were also reported by Padmavathi *et al.* (2013). Biological yield / plant was negatively and significantly correlated with harvest index. The negative associations of character pairs like biological yield / plant vs harvest index reported by Arshad *et al.* (2004).

In drought stress conditions decrease in chlorophyll content is mainly the result of damage to chloroplasts (Smirnoff *et al.* 1955). But in present investigation, average chlorophyll content exhibited by advance breeding lines indicated that plants were not much exposed to stress. Advance breeding lines exhibited very little amount of proline content which indicated plants were stress free. Positive and significant correlation was observed between relative growth rate and crop growth rate. Similar results were also reported by Ozalkan *et al.* (2010).

5.4 PATH ANALYSIS:

Simple correlation coefficient does not give clear information about the interrelationship between the causal and resultant variables. The correlation coefficients has to be partitioned into direct and indirect effects to establish the intensity of effects of independent variables on dependent variable. Path coefficient provides a method for separating direct and indirect effects and measures the relative importance of casual factors, which ultimately effect the trait seed yield / plant.

The positive and high direct effect exhibited by biological yield / plant followed by harvest index on seed yield / plant which supports the findings of Ashraf *et al.* (2002), Noor *et al.* (2003) and Thakur and Sirohi (2009). In the present investigation, biological yield / plant and harvest index had the maximum contribution in determining seed yield / plant in chickpea. It was also observed that high indirect contribution was exhibited *via* biological yield / plant and most of the seed yield components and hence these two traits may be given more emphasis while selecting high seed yielding chickpea advance breeding lines for rainfed conditions.

The negative and direct effect was exhibited by relative growth rate, proline content, protein content, number of seeds / pod, number of pods / plant, number of primary branches / plant. Days to 50% flowering had negative direct effect and indirect effects *via* plant height, number of secondary branches / plant, number of seeds / pod, whereas, all other traits had positive indirect effects. These positive indirect effects *via* days to maturity, number of pod / plant, biological yield / plant and test weight neutralized the negative effects resulting from positive association between days to 50% flowering and seed yield / plant. Singh *et al.* (2014) also observed negative and direct effect of number of pods / plant on seed yield / plant. Singh *et al.* (2017) reported positive direct effect of days to maturity and negative direct effect of days to 50% flowering and number of seeds / pod which is in accordance with the present findings. Therefore, indirect selection through these traits might be helpful in yield improvement. Parhe *et al.* (2014) also observed positive direct effect of number of secondary branches / plant and negative direct effect of number of primary branches / plant on seed yield / plant.

The remaining estimates of indirect effects obtained in path analysis at genotypic level were too low to be considered of any consequence. Similar results were reported by Renukadevi & Subhalakshmi (2006).

5.5 GENETIC DIVERGENCE:

The success of breeding programme through hybridization is highly dependent on genetic divergence of parents involved. The more diverse the parents more are the chances of pronounced heterotic effects and increased spectrum of variability in the segregating generations. The present study was also aimed to analyze the genetic divergence among the twenty two (22) advance breeding lines of chickpea to identify the superior and divergent lines for formulating the hybridization programme. Mahalanobis D^2 statistic is a powerful tool widely used by breeders in quantifying the degree of divergence at genotypic level.

The inter-cluster distances were greater than intra-cluster distances, revealing that considerable amount of genetic diversity was existed among the advance breeding lines of chickpea under study. Grouping of genotypes into cluster I (5), cluster II (8), cluster III (6) and cluster IV (2), indicated that these genotypes within the cluster could be closely related in their evolutionary process and passed through similar evolutionary factors.

Genotypes belonging to the clusters with maximum inter-cluster distance are genetically more divergent and hybridization between genotypes of divergent clusters are likely to produce wide range of variability. The highest inter-cluster distance of 904.40 was recorded between the cluster IV and VI followed by cluster IV and V (718.77), cluster II and IV (566.82), cluster V and VI (519.92), cluster III and V (513.47), and cluster I and IV (503.23). Therefore, it is suggested that if the diverse advance breeding lines from these groups are used in breeding programme, it is expected to throw a wide range of segregants. The minimum inter-cluster distance between clusters indicates that the lines of these clusters are genetically less diverse and were almost with same genetic makeup.

Cluster means for the seventeen traits of six clusters were worked out. It was found that Cluster I had lowest mean value for days to maturity. Cluster II had lowest mean value for plant height. Cluster IV had highest mean value for plant height. Cluster V had lowest mean value for days to 50% flowering and highest mean value for number of primary branches / plant, number of seeds / pod, harvest index and test weight. Cluster VI had highest mean value for seed yield / plant. To improve any particular trait donor for hybridization could be chosen from an appropriate cluster. Based on the study of genetic divergence the following advance breeding lines were identified which can be used in breeding programme (Table 14). Hence, advance breeding line of chickpea SCS-15-D-8 from cluster V have been suggested for further use in chickpea breeding programme.

Chapter-6

Summary and Conclusion

CHAPTER-VI

SUMMARY AND CONCLUSIONS

The present investigation entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** was conducted at Advanced Center for Rainfed Agriculture (ACRA), SKUAST-J, Rakh Dhiansar, Jammu during *Rabi* 2017-18 to assess the nature and extent of genetic variability, association and diversity among 22 advance breeding lines ($F_{7:8}$) of chickpea.

The observations were recorded on days to 50% flowering, days to maturity, plant height, number of primary branches / plant, number of secondary branches / plant, number of pods / plant, number of seeds / pod, biological yield / plant, seed yield / plant, harvest index, test weight, protein content, proline content, chlorophyll content, relative water content, relative growth rate and crop growth rate.

The data on all the characters studied under rainfed conditions of Jammu were subjected to statistical analysis and the following conclusions were drawn:

1. The analysis of variance revealed highly significant differences for all the characters under study except days to maturity where it was significant at p=0.05. This indicated abundant amount of genetic variability for these characters.
2. The high estimate of phenotypic and genotypic coefficients of variation were recorded for seed yield / plant indicated good amount of genetic variation and less differences among PCV and GCV showed a little influence of the environment.

3. High heritability coupled with high genetic advance as per cent of mean was observed for seed yield / plant, biological yield / plant, test weight, number of pods / plant, number of primary branches / plant, number of secondary branches / plant, number of seeds / pod, relative growth rate, proline content and harvest index indicated that additive gene effects are important in determining these characters and improvement can be done through selection based on phenotypic values.

4. The genotypic correlation coefficients were higher than the phenotypic correlation coefficients. This indicated that there is strong inherent association between the various morpho-physiological and biochemical characters studied.

5. The most important character i.e. seed yield / plant, was positively and significantly correlated with plant height, number of pods / plant, test weight, biological yield / plant and harvest index which revealed importance of these characters in determining seed yield.

6. Path analysis revealed that characters *viz*. biological yield / plant, harvest index and plant height showed high positive direct effect on seed yield / plant and these are major yield contributing traits under rainfed conditions.

7. All the twenty three advance breeding lines were grouped into six clusters based on D^2 analysis. Cluster II was biggest with 8 lines followed by cluster III containing 6 lines.

8. The maximum inter-cluster distance was observed between cluster IV and VI followed by cluster IV and V. The lines belonging to these clusters should be used in hybridization programme for chickpea varietal improvement programme.

9. On the basis of genetic divergence and mean performance of the advance breeding lines of chickpea *viz.*, SCS-15-D-3, SCS-15-D-11, SCS-15-D-1, SCS-15-D-8 and SCS-15-D-15 have been suggested for further use in chickpea breeding programme under rainfed conditions.

Implications of research work:

- The genetic variability for different characters should be exploited for chickpea varietal improvement programme under rainfed conditions.

- The characters showed high heritability along with high genetic advance is indicative of additive gene action and selection based on these parameters would be more reliable.

- Advance breeding lines from different clusters, identified for a specific character may be used as parents for breeding programme with an objective to improve the specific character like seed yield / plant under rainfed conditions.

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Appendices

Table : Mean values for morphological traits of chickpea advance breeding lines under rainfed conditions.

S.No.	Advance breeding lines	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches /plant	No. of secondary branches /plant	No. of pods / plant	No. of seeds / pod	Biological yield / plant	Seed yield / plant (g)	Harvest index (%)	Test weight	Protein content (%)
1.	SCS-15-D-1	98.00	154.00	54.06	5.00	12.20	27.36	1.63	15.50	5.00	32.33	18.83	21.63
2.	SCS-15-D-2	101.66	155.66	46.20	2.00	10.70	29.50	1.56	26.26	17.00	64.80	26.10	23.66
3.	SCS-15-D-3	100.00	148.00	47.76	4.03	14.73	31.43	1.26	18.60	11.40	61.33	17.00	24.00
4.	SCS-15-D-4	95.66	157.33	46.90	3.66	15.10	40.26	1.50	30.46	21.06	69.16	15.40	18.50
5.	SCS-15-D-5	102.66	155.00	46.60	6.66	16.63	39.63	1.66	21.80	15.00	69.56	13.00	26.56
6.	SCS-15-D-6	101.00	153.00	43.10	3.00	15.53	37.76	1.70	25.30	15.93	63.06	21.00	23.00
7.	SCS-15-D-7	104.00	152.33	47.53	6.00	18.33	35.30	1.96	14.80	5.90	39.86	18.26	19.33
8.	SCS-15-D-8	94.33	153.33	45.76	7.66	16.13	34.53	1.83	28.10	19.46	69.33	26.40	23.20
9.	SCS-15-D-9	102.33	151.66	44.76	5.66	19.83	32.43	1.40	29.40	21.90	74.56	24.10	24.60
10.	SCS-15-D-10	100.66	152.33	47.63	7.00	15.83	31.53	1.86	16.56	11.00	67.23	15.00	24.33
11.	SCS-15-D-11	97.00	151.00	41.33	8.00	20.30	43.00	1.46	28.30	19.86	70.23	21.40	27.40
12.	SCS-15-D-12	106.33	150.66	50.06	4.66	19.16	29.03	1.56	16.80	8.03	47.46	18.36	28.06
13.	SCS-15-D-13	95.00	154.66	44.83	1.66	11.83	43.20	1.20	25.10	13.00	52.16	20.63	18.36
14.	SCS-15-D-14	103.33	151.66	47.46	2.66	9.43	39.53	1.26	21.53	14.00	65.40	28.10	19.06
15.	SCS-15-D-15	96.33	154.33	48.63	1.03	13.23	36.50	1.30	36.86	23.90	64.86	18.66	22.43
16.	SCS-15-D-16	108.00	150.33	47.66	8.66	17.66	32.60	1.53	18.86	8.80	49.70	15.63	27.00
17.	SCS-15-D-17	99.66	153.66	42.96	4.90	11.20	39.20	1.36	17.53	11.90	67.83	17.53	26.30
18.	SCS-15-D-18	105.66	152.00	39.66	2.33	18.76	40.46	1.76	32.53	19.33	59.50	17.30	25.03
19.	SCS-15-D-19	96.66	158.66	41.03	7.90	14.16	36.70	2.00	30.10	13.66	45.40	24.66	28.66
20.	SCS-15-D-20	101.33	149.66	52.46	6.03	13.50	27.30	1.83	22.10	14.56	65.96	23.00	21.90
21.	SCS-15-D-21	97.66	152.33	39.93	5.90	20.86	37.00	1.36	34.46	21.66	62.86	28.36	21.33
22.	SCS-15-D-22	107.00	151.33	44.10	2.90	12.86	33.26	1.93	22.50	19.03	84.56	16.00	18.16
23.	SCS-3	97.00	150.33	46.70	3.90	10.16	37.03	1.26	28.53	17.60	61.66	23.23	21.63
	Mean	100.44	152.75	45.96	4.83	15.13	35.41	1.57	24.43	15.17	61.25	20.34	23.22
	C.D at 5%	4.38	4.23	7.02	1.62	1.01	2.30	0.16	2.57	2.19	9.33	2.63	2.37
	C.V.	2.65	1.68	9.28	7.83	4.06	3.95	6.20	6.41	5.62	9.25	7.85	6.21

Table : Mean values for biochemical and physiological traits of chickpea advance breeding lines under rainfed conditions

S.No.	Advance breeding lines	Proline content (mg g^{-1} FW)	Chlorophyll con. (SPAD)	Relative water content (%)	Relative growth rate (g g^{-1} day^{-1})	Crop growth rate (mg m^{-2} day^{-1})
1.	SCS-15-D-1	0.55	45.83	38.46	0.072	19.50
2.	SCS-15-D-2	0.57	58.50	56.20	0.050	17.54
3.	SCS-15-D-3	0.45	43.50	62.73	0.085	22.49
4.	SCS-15-D-4	0.56	61.60	50.46	0.060	29.55
5.	SCS-15-D-5	0.44	52.50	65.50	0.085	29.58
6.	SCS-15-D-6	0.46	55.53	44.60	0.092	39.46
7.	SCS-15-D-7	0.75	48.80	49.43	0.095	24.57
8.	SCS-15-D-8	0.35	41.43	59.40	0.046	30.44
9.	SCS-15-D-9	0.31	50.63	41.83	0.064	17.64
10.	SCS-15-D-10	0.37	60.46	60.46	0.057	19.58
11.	SCS-15-D-11	0.64	54.53	42.80	0.041	26.61
12.	SCS-15-D-12	0.42	63.60	55.40	0.078	19.79
13.	SCS-15-D-13	0.44	57.73	40.46	0.075	16.55
14.	SCS-15-D-14	0.28	53.46	35.70	0.063	21.40
15.	SCS-15-D-15	0.34	51.63	58.50	0.080	15.44
16.	SCS-15-D-16	0.27	67.53	59.46	0.038	24.61
17.	SCS-15-D-17	0.50	59.60	44.60	0.041	18.64
18.	SCS-15-D-18	0.69	50.76	51.53	0.056	27.58
19.	SCS-15-D-19	0.39	45.23	39.53	0.038	25.68
20.	SCS-15-D-20	0.65	53.70	46.53	0.047	19.51
21.	SCS-15-D-21	0.28	57.70	45.56	0.034	21.54
22.	SCS-15-D-22	0.51	60.40	50.76	0.107	26.85
23.	SCS-3	0.62	42.80	52.60	0.094	31.56
	Mean	0.47	53.80	50.11	0.065	23.74
	C.D at 5%	0.03	2.62	2.14	0.007	1.15
	C.V.	4.75	2.96	2.60	7.193	2.95

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CERTIFICATE-IV

Certified that all necessary corrections as suggested by external examiner and the advisory committee have been duly incorporated in the thesis entitled **"Genetic divergence studies in advance breeding lines of chickpea (*Cicer arietinum* L.) under rainfed conditions"** submitted by **Saroj Bala**, Registration No. **J-16-M-440.**

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