

INTRODUCTION

CHAPTER- I

Among all the beans grown in India, cluster bean (guar) [*Cyamopsis tetragonoloba* (L.) Taub.] is cultivated to a lesser extent. The research work done on different aspects of this crop is also meager. It is mainly grown for green vegetable, dry seeds and also as a forage crop.

1.1 Composition and uses

The green pods of cluster bean are as rich in food value as that of french bean

1.2 Composition of cluster bean green pod (per 100 g of edible portion)

S. No.	Name of Minerals/Vitamins	Quantity
1.	Moisture	81.0 %
2.	Carbohydrates	10.8 g
3.	Protein	3.2 g
4.	Fat	0.4 g
5.	Minerals	1.4 mg
6.	Thiamine	0.09 mg
7.	Riboflavin	0.09 mg
8.	Vitamin C	47 mg
9.	Vitamin A	316 mg

Aykroyed, (1963)

1.3 Properties

Cluster bean has several health benefits in both vegetable and powder form (guar gum). Guar gum has also been shown to be useful in weight loss and diabetes treatment. Guar pods are rich in soluble dietary fibre and lowers blood cholesterol levels. Guar gum is a common ingredient in fibre-rich drinks marketed as health drinks and weight-loss drinks. Low in calories, the cluster bean (guar) contains vitamin C, vitamin K, vitamin A, dietary fibre, folate, iron, manganese and potassium. The vitamin K is important for maintaining strong bones and proper development of foetus. The nutritional values per 100 gm of raw cluster bean is one of the most important and potential vegetable cum industrial crop grown for its tender

pods for vegetable purpose and for endospermic gum (30-35%)(Kumar and Singh, 2002).

1.4 Uses

The tender pods are used as vegetable and in the southern parts of India they are dehydrated and stored for use. It is a nutritious fodder for livestock and the seeds are also fed to the cattle. Besides, the crop can be used for soil improvement and as a medicine. The mucilaginous seed flour is valued as guar gum (galactomanan) and this gum is used in textile, paper, cosmetic and oil industries throughout the world and is a useful absorbent for explosive (Smith, 1976).

1.5 Origin

Guar is native to India where it is grown principally for its green fodder and for the pods that are used for food and feed. Guar was introduced into the United States from India in 1903. Production in the United States is centered in Texas, Oklahoma and Arizona, but it is also adapted to locations with more tropical climates, such as in Florida and Puerto Rico (Stephens, 1994). The crop is grown in India, Myanmar, Sri Lanka and Pakistan. Even in arid zones of the USA like Texas and Arizona it is being grown (Venkataratnam, 1973).

1.6 Taxonomy

Cluster bean or guar [*Cyamopsis tetragonoloba* (L.) Taub.]; (syn. *C. psoralioides* DC.) belongs to the tribe Galegae (Indigoferae) of Leguminosae family. The genus consists of three species, namely, *C. tetragonoloba*, *C. senegalensis* and *C. serrata*, out of which *C. tetragonoloba* ($2n=2x=14$) is the only member of economic importance (Smith, 1976).

1.7 History of cluster bean

Cluster bean is considered to be originated by domestication of the African wild species, *C. senegalensis* which appear to be the ancestor of the *C. tetragonoloba*. The domestication process could have been taken place in the dry areas of the north western region of the Indo-Pakistan Subcontinent (Hymowitz, 1972). It was cultivated as a minor crop in India during ancient times as a vegetable and feed for cattle. Guar was introduced in USA in 1903 for experimentation in the southwest region where the climate is hot and has long growing seasons to suit its adaptation (Hymowitz and Matlock, 1963). The objective was to use cluster bean as a soil improving legume and forage for cattle. Before World War II the carob (locust bean)

seed (*Ceratonia siliqua*) from Mediterranean was used to extract carob gum for extensive use in paper industry. During World War II the supply of imported carob seed from the Mediterranean region was cut off, as a result search for domestic source of galactomanan gum was initiated in USA by Institute of Paper Chemistry. This study revealed guar as an alternative source for galactomanan (Anderson, 1949). Further, studies were done on milling the guar seeds for gum production and application in the manufacturing of paper revealed the beneficial effects of guar gum on paper processing. This information helped in the adoption of guar gum in the different manufacturing process.

1.8 Production trend and international trade

Cluster bean, [*Cyamopsis tetragonoloba* (L.) Taub.] commonly known as Guar, is a drought and high temperature tolerant deep rooted summer annual legume of high social and economic significance. The qualities of the crop like high adaptation towards erratic rainfall, multiple industrial uses and its importance in cropping system for factors such as soil enrichment properties, low input requirement, etc have made the guar one of the most significant crops for farmers in arid areas in India. Guar is a native to the Indian subcontinent. The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab and to limited extent in Uttar Pradesh and Madhya Pradesh. The crop is also grown in other parts of the world, like, Australia, Brazil and South Africa. India is the largest producer of Guar and contributes 80 percent of total Guar production in the world. In India, Guar crop is cultivated mainly during Kharif season, with an annual production of around 2 million metric tons. Guar crop has experienced a remarkable journey from a traditional crop grown on marginal lands mainly for food, animal feed and fodder to a crop with various industrial usages. Guar gum is an important ingredient in producing food emulsifier, food additive, food thickener and other Guar gum products. The unique binding, thickening and emulsifying quality of guar gum powder obtained from guar seed has made it a much sought after product in international market. Guar is the source of a natural hydrocolloid, which is cold water soluble and form thick solution at low concentrations. The guar seed consists of three parts: the seed coat (14-17%), the endosperm (35-42%), and the germ (43-47%). It is from the endosperm that guar gum is derived, which is the prime marketable product of the plant. This spherical-shaped endosperm contains significant amounts of galactomanan gum (19

to 43% of the whole seed), which forms a viscous gel in cold water. Like other legumes, guar is an excellent soil-building crop with respect to availability of nitrogen. Root nodules contain nitrogen-fixing bacteria and crop residues, when ploughed under, improves yields of succeeding crops.

Guar is a crop of arid and semi-arid areas cultivated in north and northwest parts of India and east and south-eastern part of Pakistan. In Pakistan, Guar seed is mainly produced in Punjab and Sindh province with about 80 percent of total Guar acreage under irrigation. On the other hand production of Guar in India is mainly confined to arid zones of Rajasthan and parts of Gujarat, Haryana and Punjab. Guar in Rajasthan is mainly cultivated under rain-fed conditions (Yadav *et al.*, 2014).

1.9 World scenario

Guar is basically a crop that is cultivated mostly in the arid and semi arid areas as it is drought resistant. That is why the Southern Asian continent suits well to the cultivation of this crop especially the Indian subcontinent. The powder made after refining the gum obtained from the plant makes an important raw material in many industries. This powder has some unique characteristics like grease resistance, thickening agent, capacity to bind water, high viscosity and the capability to function in low temperatures which makes it a highly popular in those sectors. Among other by products of guar, guar gum powder is the main marketable commodity.

The world's total production of cluster bean is around 7.50 to 10.00 million tons at every year. The production list of guar is dominated by India as a leading producer of this crop. The consumption pattern of guar seeds is largely influenced by the demands from the petroleum industry of United States of America and the oil fields in the Middle East as the derivative products of these seeds are quite useful in the petroleum drilling industries. United States alone constitute to around 40 thousand tons of guar and its derivatives demand. Also, in rest of the world, the trend of consumption has increased with time that has lead to the introduction of this crop in many countries. The Major guar producing countries in the world are India, Pakistan, Sudan, USA, South Africa, Brazil, Malawi, Zaire and Australia. India leads the list of the major guar producing countries of the world contributing to around 75 to 80% in the world's total production of around 7.5 to 10 million tons. Pakistan follows India in the list with 10-15% share in the world's total produce. The most important by-product of this crop i.e. guar gum is obtained through the processing of endosperm of the seeds

of guar. This product is vastly produced in the countries such as USA, Germany, China, Italy, South Africa, and United Kingdom though these countries are not really indulged in the production of guar as a crop. (Anonymous, 2012). The other major producers of Guar are Pakistan, USA, South Africa, Malawi, Zaire and Sudan (Yadav *et al.*, 2014).

1.10 Indian scenario

India is the world's largest producer of guar in the world, which contributes to 80% of the total production. The average production of Guar seed in the country is 7.0 to 8.0 million tonnes and fluctuates largely from year to year based on rainfall pattern. India is also the largest producer of guar gum products.

India produces 6.0 million tons of guar annually i.e. the maximum level of production in the world. It contributes to around 80% share in the world's total production. In North Indian states like Rajasthan, Haryana, Gujarat and Punjab it is mainly cultivated for guar gum production and for forage, whereas in South India it is being cultivated for vegetable purpose. In Karnataka it is cultivated year round in limited area in northern districts like Dharwad, Belgaum, Bijapur, Haveri etc., for tender vegetable pods. From India, cluster bean is mainly exported to USA, Germany, Netherlands, Italy, UK, Japan and France value at Rs. 200 million rupees annually (Singh *et al.*, 2009).

Total production of Guar bean in India is estimated to have crossed 2.7 million metric tons during the agricultural year 2013-14 due to good weather conditions in the major Guar producing areas in India. With a moderate production of 250,000 metric tons in Pakistan which is another important guar producing area, the total global production of Guar bean is estimated to have crossed 3 million metric tons during 2013-14. Presently, India accounts for more than three-fourth (or nearly 80 percent) of the total world guar bean production. Thus, the total area of cluster bean in India is 4.25 million hectare and production is 2.41 million tonnes with productivity of 0.57 million tonnes/ha in 2014. The year 2002-03 was marked by a low production to the tune of 2 lakh tonnes due to severe drought whereas in 2011-12 the production rose to the level of 22 lakh tonnes due to good rainfall. The area under cultivation and the level of production has an increasing trend for guar crop in India. It is expected that the area under cultivation of guar in India in the year 2015 will be around 36 lakh

hectares and the production will reach to around 17 to 18 lakh metric tonnes (Yadav *et al.*, 2014).

The major cluster bean producing regions of this crop in India are Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Maharashtra, Karnataka, and Andhra Pradesh. Rajasthan can be termed as the largest guar producing state in the world as it dominates the Indian production scenario contributing to around 4.20 million tons of this crop i.e. over 70% of the total production in India. Haryana and Gujarat place themselves at the second and third positions regarding the production in India with 12% and 11% respectively. In Rajasthan, the districts where guar production is done are Churu, Bikaner, Jaisalmer, Barmer, Nagaur, Hanuman Garh, Jodhpur, Ganganagar, Jaipur, Sirohi, Dausa, Jhunjhunu and Sikar. The districts in Haryana indulged in the production of guar are Bhiwani, Gurgaon, Mahendragrh and Rewari and the districts in Gujarat are Kutch, Banaskantha, Mehsana, Sabarkantha, Vadodara and Ahmadabad.

India has been a dominant player in the context of guar and guar gum in the world market. The Arab traders introduced the crop in India and the place responded well to the crop's cause. It has been the major producer of guar seeds in the world. India's production contributes to 80% of the world's total production figuring up to 6 lakh tons. Rajasthan wholly retains the credit for India's position producing 70% of the production itself. But it has been observed that there is a lack of stability in India's performance due to the fluctuations in the rainfall level in the country. Guar is largely consumed as a vegetable in the Indian subcontinent. It is also used in making pickles. 25000 tons of the total production in the country constitutes to the domestic market. Guar gum has a vast range of industrial applications and the major share of demand comes from various industrial sectors only. India is the leading net exporter of guar seeds and guar gum. The country exports over 1.17 million tons of guar and its derivatives, which is comprised by 33000 tons of refined split guar gum, and 84000 tons of treated and pulverized guar gum. The net worth of the Indian exports is estimated over Rs 500 crores. The major importing countries of Indian guar products are United States of America (12000 tons), Germany (5200 tons), France (1800 tons), United Kingdom (1200 tons), South Africa (1000 tons), Netherlands, Italy and Japan (Anonymous, 2012).

Genetic diversity studies in a crop are important in management of genetic resources, identification of duplicate accessions in the germplasm collection and use of genetic resources in applied breeding programs.

Plant breeders have successfully improved the yield potential of most crops, which has resulted in higher production in last four decades, but further progress is not impressive. One of the main reasons for such a situation is the use of limited genetic diversity by the plant breeders who tend to use their working collection of highly adapted material (Evans, 1983; Upadhyaya *et al.*, 2006; 2011) or advanced breeding lines as parents and only a small proportion of the available germplasm has been used in national and international breeding programs. One of the main reasons for low use of germplasm in breeding programs is the lack of information on traits of economic importance which show high genotype x environment interaction, and require multi locational replicated evaluation to identify parents. Thus, the large variability in the germplasm instead of prompting more use has created a situation of not knowing where to begin (Upadhyaya *et al.*, 2005). The importance of diverse germplasm to generate new variability and to enhance the genetic yield potential and to stabilize it against various biotic and abiotic stresses has been well established (Singh, 1987; Upadhyaya *et al.*, 2009).

1.11 Diversity studies

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

1.12 Importance of diversity studies

Diversity is the foundation in which selection is practiced. Diversity studies in a crop are important for various aspects such as management of genetic resources, identification of duplicate accessions in the germplasm and in applied breeding programs. Various data have been used to analyze the genetic diversity in crops, including morphological, agronomical and ecogeographical traits. Most economic traits of the crop varieties are quantitative traits that are affected by the crop environment and also by genotype \times environment interaction. Traditionally phenotypic traits (Nozzolillo, 1985; De Leonardis *et al.*, 1996; Robertson *et al.*, 1997; Hassan, 2000; Javedi and Yamaguchi, 2004), hybridization success (Ladizinsky and Alder, 1976; Pundir and Vander Maesen, 1983; Pundir and Mengesha, 1995; Badami *et al.*, 1997) analysis of chromosome pairing in hybrids (Ladizinsky and Alder 1976; Ahmad, 1988), and the study of chromosomes structure (Ohri and Pal, 1991; Tayyar *et al.*, 1994; Ahmad, 2000) have been widely used methods for analysis of genomic relationships and the construction of phylogenies among *Cicer species*.

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

Morphological Characterization:

The preliminary characterization of the genotypes, morphological and agronomic traits of the plant are preferred, for being cheaper and easier to assess.

1.12.1 Genetic diversity

Genetic diversity, is the level of biodiversity, refers to the total number of genetic characteristics in the genetic makeup of a species. Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals. Genetic diversity plays a very important role in survival and adaptability of a species because when a species' environment changes, slight gene variations are necessary to produce changes in the organisms' anatomy that enables it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fit alleles (NBII, 2012).

1.12.2 Germplasm

Germplasm is a basic tool for any crop improvement programme. Natural variation represents a huge and largely untapped resource, which has been subjected to selection over millions of years of evolution, with both basic and practical value as well as the potential to break yield barriers of agricultural plants (Johal *et al.*, 2008; Tanksley and McCouch, 1997; Zamir, 2001). The variation available in the germplasm is utilized as a source of useful genes to improve the cultivars. As the importance of guar was realizing 1950's germplasm collection was initiated. The collection initially began from Maharashtra for vegetable varieties. Later the Pant Introduction Division of the Indian Agricultural Research Institute (IARI), New Delhi continued the collection and maintenance work. There are about 4,901 accessions in National Bureau of Plant Genetic Resources (NBPGR), New Delhi. These accessions have been catalogued based on the accession numbers and characterized for phenotypic traits like pubescence, days to 50% flowering, days to 50% maturity, plant height, branch number, total number of pods per plant, number of seeds per pod, seed

yield per plant, seed color, gum content and disease resistance under field conditions (Dabas, 2001). These studies have identified the accessions that can be donors to traits like dwarfing, branching/unbranched, pod length, pods per plant, seed size, days to maturity, gum content and disease resistance. Classical approaches using the donor cultivars as sources have led to the development of certain elite cultivars for cultivation in the previous decade. The development of elite cultivars and their widespread use in breeding programmes have reduced the utilization of available genetic resources. It has been estimated that for most crop species, less than 5 % of the biodiversity known to exist has been utilized in agriculture, particularly in the case of self-pollinated crops (Tanksley and McCouch, 1997). Much of the diversity present in living systems is probably adaptive (Johal *et al.*, 2008).

The assessment of genetic diversity using quantitative traits has been of prime importance in many contexts particularly in differentiating well-defined populations. The germplasm in a self-pollinated crop can be considered as heterogeneous sets of groups, since each group being homozygous within it. Selecting the parents for breeding program in such crops is critical because, the success of such programme depends upon the segregants of hybrid derivatives between the parents, particularly when the aim is to improve the quantitative characters like yield. To help the breeder in the process to identify the parents that nick better, several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives. Among them, Mahalanobis' generalized distance occupies a unique place and an efficient method to gauge the extent of diversity among genotypes, which quantify the difference among several quantitative traits.

1.12.3 Genetic variability

Genetic variability is a measure of the tendency of individual genotypes in a population to vary from one another. Variability is different from genetic diversity, which is the amount of variation seen in a particular population. The variability of a trait describes how much that trait tends to vary in response to environmental and genetic influences. Genetic variability in a population is important for biodiversity, because without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction (Sousa, 2012).

The determination of genetic variability and its partitioning into various components is necessary to have an insight into the genetic nature of yield and its components. Study of partitioning the total variability into heritable and environmental components had its beginning in the work of Johanson (1909), Nilson-Ehle (1909) and East (1916). Based on the study on non-segregating populations, Charles and Smith (1939), Powers (1942) separated genetic variance from total variance using estimate of environmental variance. The magnitude of variability and its genetic components are the most important aspects of breeding material. Hence, basic understanding of the genetic variability is a prerequisite for the planning of breeding programme. A great deal of information has been generated on genetic variability of various components of tomato. Generally, genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) are measured to study the variability.

Molecular Characterization:

Information regarding the extent and pattern of genetic variation in cluster bean is limited. Therefore, urgent efforts are required to improve yield and gum quality of cluster bean using conventional and biotechnological approaches. An assessment of the genetic diversity is an important first step to achieve this goal. Previous studies on characterization of cluster bean germplasm used phenotypic characters (Shabarish *et al.*, 2012) or qualitative traits (Pathak *et al.*, 2011).

Molecular marker is a sequence of DNA, which are located with a known position on the chromosome (Kumar, 1999), or a gene whose phenotypic expression is frequently easily discerned and used to detect an individual, or as a probe to mark a chromosomes, nucleus, or locus (King and Stansfield, 1990; Schulmann, 2007). Markers show polymorphism, which may arise due to alteration of nucleotide or mutation in the genome loci (Hartl and Clark, 1997) and make it possible to identify genetic differences between individual organisms or species (Collard *et al.*, 2005). Molecular markers are used in many different areas such as genetic mapping, paternal tests, detect mutant genes which are connected to hereditary diseases, cultivars identification, marker assisted breeding of crops, population history, epidemiology and food safety, population studies (Hartl and Jones, 2005). Collard *et al.* (2005) studies that genetic marker used to identify genetic variation among individual species or organisms. Schulmann (2007) studies that genetic marker used to construct

linkage maps and genetic diversity. Molecular marker also used in many different areas that are including detecting mutant genes, genetic mapping, epidemiology and for population studies (Hartl and Jones, 2005).

Recently the number of available markers in plants has increased dramatically with molecular biology techniques. Molecular markers are being widely used in various areas of plant breeding is an important tool for evaluating genetic diversity and determining cultivars identity (molecular fingerprinting). Establishment of a molecular marker and phenotypic assessment database of crop germplasm has helped breeders to trace down the origins and degrees of relatedness of many land races and cultivars. Considering the potential of molecular markers, the crop breeders extended their hands to use these to supplement other tools currently exploited in their crop breeding activities (Ajit *et al.*, 2013). A fragment of DNA that is associated with a certain location within the genome is called molecular marker (Randhawa, 2013).

Molecular marker assay variations at the level of nucleotide sequence of DNA are desirable for identification, germplasm protection and seed purity determination. Molecular markers have been developed for unambiguous identification of the cultivars. Polymerase chain Reaction (PCR) based molecular markers such as RAPD, SSR, STS, AFLP, ISSR have developed into powerful tools to analyze genetic relationship and genetic diversity (Williams *et al.*, 1990; Welsh and Mc Clelland, 1990). Today many plant breeders utilizing molecular markers to proof and identify desirable traits of important plants. Various plant researchers are using molecular markers to study genetic polymorphism and linkage maps construct (Schulmann, 2007). There are many advantages of molecular markers compared with morphological and molecular markers. For instance, when comparing morphological markers with molecular markers, expression of such markers is influenced both by dominant-recessive relationship and epistatic-pleiotropic interaction. The use of such markers has been criticized since the study of genetic variation among germplasm using morphological characteristics is labors and time consuming (Cooke, 1984). When comparing with molecular markers, their use is now limited due to large scale analysis and low level of genetic variation in a given species and subspecies (Rafinski and Babik, 2000).

1.13 Polymerase chain reaction (PCR)

PCR based markers involve in vitro amplification of particular DNA sequences with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme. The amplified DNA fragments are separated by electrophoresis and banding patterns are detected by different methods such as staining (using ethidium bromide dye) and autoradiography. With the advent of thermostable DNA polymerase the use of PCR in research and clinical laboratories has increased tremendously. PCR is extremely sensitive and operates at a very high speed. Its application for diverse purposes has opened up a multitude of new possibilities in the field of molecular biology (Saiki *et al.*, 1985; Saiki *et al.*, 1988). Various types of molecular markers are available and new methods are frequently being developed. Today, no method is ideal for all applications so scientist teams must weigh both pros and cons of methods when starting a new project (Harlt and Jones, 2005).

Molecular markers are grouped after their different abilities of showing homozygosity (dominant marker) or heterozygosity (co-dominant marker) (Hartl, 1988). The most commonly used dominant DNA marker for genetic diversity in plants are: Random Amplified Polymorphic DNA (RAPD; Williams *et al.*, 1990), DNA amplification fingerprinting (DAF; Caetano-Anolles *et al.*, 1991), arbitrarily primed polymerase chain reaction (AP-PCR; Welsh and McClelland, 1990), Inter simple sequence repeat (ISSR; Zietkiewicz *et al.*, 1994) and Amplified Fragment Length Polymorphisms (AFLP; Vos *et al.*, 1995).

1.14 Inter-simple sequence repeats (ISSR)

Inter-Simple Sequence Repeat (ISSR) involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. Inter-Simple Sequence Repeat usually 16-25 bp long as primers in a single primer PCR reaction targeting multiple genomic loci to amplify different sizes of inter-SSR sequences. The microsatellite repeats used as primer can be either dinucleotides or tri-nucleotides. ISSR markers are highly polymorphic and are used on genetic diversity, gene tagging, phylogeny, evolutionary biology and genome mapping studies (Reddy *et al.*, 2002). ISSR PCR is a technique, which overcomes the problems like high cost of AFLP, low reproducibility of RAPD,

and the flanking sequences to develop species specific primers for SSR polymorphism. ISSR segregate mostly as dominant markers following simple Mendelian inheritance. However, they have also been shown to segregate as co-dominant markers in some cases, thus enabling distinction between homozygote and heterozygote (Sankar and Moore, 2001). ISSR have been used since 1994 for a wide range of organisms (Zi tkiewicz *et al.*, 1994) in DNA fingerprinting, diversity analysis and genome mapping (Godwin *et al.*, 1997; Bornet and Branchard, 2001; De Vincente and Fulton, 2004). Among various molecular tools for genetic analysis, the inter-simple sequence repeat (ISSR), which involves PCR amplifications of DNA using a primer consisted of a microsatellite sequence anchored at 30 or 50 end by 2–4 arbitrary nucleotides, is a powerful tool for assessing genetic analysis of various plants, owing to the more rapidly, simply and inexpensively (Choudhary *et al.*, 2013). To afford abundant genetic information of the cluster bean for breeding, we aimed to estimate the genetic diversity of 30 genotypes of using ISSR marker.

Despite great industrial value, cluster bean has not been explored in detail which prompted us to undertake the present work with random molecular markers. This study represents genetic diversity estimation among 30 genotypes of cluster bean, using ISSR markers.

With the above information in view, the current research work was initiated with the objectives of collection, evaluation, morphological and molecular characterization of available indigenous germplasms so that they can be utilized for varietal improvement and commercial cultivation.

Keeping in view the importance of this crop, the present investigation entitled “Studies on Genetic Diversity and Variability in Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Genotypes Through Morphological and Molecular Characterization” while being under taking with the following objectives:

Objectives of the Study

The present studies are focused on the following objectives:

- To estimate the extent of genetic diversity in cluster bean germplasm.
- To evaluate the extent of genetic variability in the germplasm of cluster bean
- To study the contribution towards yield by correlation and path coefficient analysis.
- To identify potential genotypes of cluster bean on the basis of yield and yield attributes.
- To screen out the most desirable genotypes on the basis of morphological characterization.
- To investigate the most desirable genotypes on the basis of molecular characterization.

REVIEW OF LITERATURE

CHAPTER -II

Breeder of the any crop is primarily concerned with the improvement of both qualitative and quantitative plant characters. Hence, adequate knowledge of genetics of various traits is very essential in breeding programme for obtaining desired results in the generation. However, the success of a particular crop breeding depends on the extent and the magnitude of variability existing in the germplasm. At the same time, improvement is possible on the basis of heritable variation. Therefore, detailed information about genetic architecture of crop yield and its attributes should be the main concern.

The old world legume, guar [*Cyamopsis tetragonoloba* (L.) Taub.] is one such crop where studies on domestication have been negligible. Guar is an annual drought hardy leguminous crop cultivated mainly in semi-arid and arid tracts of India and Pakistan for the galactomanan gum extracted from its endosperm (Hanelt and IPK, 2001). Based on pertinent agronomic, botanical, historical and linguistic evidences, Hymowitz, (1972) proposed a working hypothesis on trans-domestication of guar. The trans-domestication concept explains the movement of a wild species from its indigenous area by man to another region where it subsequently is domesticated. It has been proposed that the drought tolerant African wild species, *C. senegalensis* Guill. et Perr., distributed in the semi-arid savannah zone of the Sahara from Senegal to Saudi Arabia, is the ancestor of the cultivated guar and it arrived on the Indo-Pakistan subcontinent as fodder for horses in Arab-Indian trade between the 9th to 13th Century A.D. The domestication of guar took place about 1,000 years ago in the dry areas of the north western region of Indo-Pakistan subcontinent. However, doubts have been raised over the hypothesis and *C. senegalensis* being guar's wild progenitor, as any form of *C. senegalensis* is absent in the subcontinent and no weedy forms have been reported from north western part of India till date (Ladizinsky, 1998).

A comprehensive understanding of the genetic variation available in the germplasm for different traits, their heritability and relationship with yield and extent of genetic diversity is important for any crop improvement program to be successful. Likewise the knowledge about genetic diversity in the available germplasm is very useful for plant breeders. It supports their decision on the selection of cross combinations from large sets of parent genotypes and is also helpful when they want to widen the genetic basis of a breeding program. Keeping in mind the above facts an investigation was carried out entitled “Studies on Genetic Diversity and Variability in Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Genotypes Through Morphological and Molecular Characterization”. Traditionally, morphological characters have been used for the identification of *Cyamopsis tetragonoloba* L.

2.1 Importance of genetic diversity

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

2.2 Germplasm collection and its uses

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

diversity and promoting its use. This has resulted in assemblage of large collections in national and international gene banks.

In the present study, the relevant literature pertaining to various aspects of horticultural and nutritive parameters of cluster bean are reviewed under the following heads:

2.3 Morphological Characterization:

The morpho-agronomic characterization provides information underlying conclusions on the genetic variability of the genotypes of the bank, identification of accessions maintained in duplicate (Valls, 2007), improvement of the data of identification and classification of accessions (Chiorato *et al.*, 2007) and support the regeneration and maintenance of the genetic integrity of genotypes. Among other information that can be obtained from the characterization of germplasm, the determination of the relative importance of the traits used to describe the genetic diversity (Rodrigues *et al.*, 2002, Chiorato *et al.*, 2005). Characterization of genetic resources is an important component of a crop improvement programme for their effective involvement in hybridization programmes. Later Anderson (1957) developed a technique to investigate the pattern of morphological variation in crop species, called as Metroglyph analysis. Metroglyph analysis is a method of studying variability in a large number of germplasm lines taken at a time based on the mean values of different traits.

A. Morphological Characters

2.3.1 Genetic diversity

2.3.2 Heritability and genetic advance

2.3.3 Coefficient of variability

2.3.4 Correlation coefficient

2.3.5 Path coefficient analysis

The literature pertaining to genetic diversity is as following:

2.3.1 Genetic diversity

For a successful plant breeding programme, genetic divergence is very much essential to classify the experimental material, based on the extent of similarity, into

close and divergent types. One of the potent techniques of assessing the genetic divergence is the D^2 statistic proposed by Mahalanobis (1936). This technique measures the forces of differentiation at two levels: intra cluster and inter-cluster levels, and thus helps in the selection of genetically divergent parents for exploitation in hybridization programs. Fisher (1936) suggested that Mahalanobis D^2 would be more useful when the number of groups happened to be more than two. Murthy and Pavate (1962), in taxonomic studies, suggested that D^2 analysis could be extended to the situations where overlapping species need to be discriminated, since the technique of Mahalanobis D^2 statistic has been applied widely to resolves genetic divergence at inter varietal, sub-species and species levels in several crops.

Genetic diversity refers to the variation at the level of heritable characters (polymorphism) and provides a mechanism for populations to adapt to their ever changing environment. The more variation, the higher the chance that at least some of the individuals will have an allelic variant that is suited for the new environment, and will produce offspring with the variant and will in turn reproduce and continue the population into subsequent generations. When a new allele appears in a population, it has the potential to change the genetic make-up of successive generations. Harmful mutations will likely not persist because the affected individual will either not survive, or will have limited reproductive success. However, some mutations may be passed on to successive generations because an organism with that allele is better equipped to survive in its environment, that is, it has a selective advantage. Those individuals that produce a greater number of offspring that survive are said to be more fit. Other mutations may have no effect on phenotype, and may persist simply by chance (genetic drift). It is the selective advantage that drives evolution, albeit momentarily, in one direction or another (Russell, 2003).

Mahalanobis technique is in the form of a generalized distance, which considers the variation produced by any character and their conjoint effect that it bears on other character. Mahalanobis also pointed out that D^2 would be remaining constant when sample were drawn from two different populations irrespective of the size of the representative sample. This indicates that D^2 provided a measure of actual magnitude of divergence between two individuals under comparison.

The assessment of genetic diversity using quantitative traits has been of prime importance in many contexts particularly in differentiating well defined populations.

The germplasm in a self-pollinated crop can be considered as heterogeneous sets of groups, since each group being homozygous within it. Selecting the parents for breeding program in such crops is critical because, the success of such program depends upon the segregants of hybrid derivatives between the parents, particularly when the aim is to improve the quantitative characters like yield.

To help the breeder in the process of identifying the parents, that need better, several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives. Among them, Mahalanobis generalized distance occupies a unique place and an efficient method to gauge the extent of diversity among genotypes, which quantify the differences among several quantitative traits.

Edang *et al.* (1971) stated that the clustering pattern could be utilized in choosing parents for cross combinations likely to generate the highest possible variability for various economic characters.

Gipson and Balakrishnan (1992) reported that pods per plant gave the highest contribution towards total divergence in cluster bean.

Smith *et al.* (1995) conducted average linkage cluster and principal component analyses, and reported the utility of these results in preservation and utilization of germplasm.

Saini *et al.* (1999) studied in 48 genotypes with a set of 11 characters contributing towards seed yield in cluster bean. Significant differences were observed for all the characters under study. Based on D^2 values, 48 genotypes were classified into nine clusters. The study also revealed that genetic divergence had less relevance to eco-geographical distribution of the varieties. Three genotypes viz., DSE 1J, Pusa Navbahar and HG 75 were most divergent.

Saini *et al.* (2001) showed that plant height contributed the most towards genetic divergence followed by grain yield per plant. So, it is encouraging that the divergence in the present materials due to these three characters will offer a good scope for improvement through rational selection.

Brahmi *et al.* (2004) reported high morphological diversity in guar. The present study attempts to analyze the genetic diversity in cultivated guar using allozyme polymorphism and compare it with reported morphological diversity. The morphological data of 108 selected accessions was subjected to UPGMA clustering. Fifty-five accessions were then picked randomly from the clusters generated. Tris-borate system (pH 8.3) was found to be most effective for detection of allozyme polymorphism in guar. Ten enzymes were selected for the study out of 18 systems tried, namely, malate dehydrogenase, malic enzyme, phosphogluco isomerase, glutamate oxaloacetate transaminase, esterase, peroxidase, alcohol dehydrogenase, glutamate dehydrogenase, glucose 6-phosphate dehydrogenase and shikimate dehydrogenase. A total of 20 loci were detected with 34 alleles. The number of polymorphic loci in different accessions was observed to be highly variable (0-9). An average of 1.27 allozyme per polymorphic locus was observed. The allele frequency data of the 55 accessions was used to determine genetic diversity parameters. The dendrogram generated on genetic distance showed three clusters in the distance range of 0–0.28. Most of the accessions were in the first clusters in a narrow distance range of 0–0.08. The second cluster consisted of improved varieties and accession from Pakistan. The third cluster of accessions was genetically more distant but there was no definite segregation of accessions according to the place of collection. The accessions from south west Rajasthan were scattered in all the three clusters showing higher variability in this region, also supported by higher morphological variation. The genetic distance was low among accessions of guar, indicating low diversity in India.

Henry *et al.* (2005) studied twenty-five cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes from different states were grown in three different environments, i.e. normal sown (E₁ and E₂ in 1991 and 1992, respectively) where the crop was sown at the onset of monsoon and late sown (E₃ in 1992) when the crop was sown in the first week of August, in Jodhpur, Rajasthan, India. Data were recorded for seed yield, days to maturity, plant height, branches per plant and pods per plant as well as gum and protein contents. Early maturing cultivars were grouped in separate clusters in both E₁ and E₂, while they were scattered in different clusters in E₃. In general, gum

content was higher in E₃ with the exception of cluster V, while protein content was higher in E₁ in all clusters. Analysis of diversity in all three environments grouped the cultivars into six clusters, with clusters V and VI comprising early genotypes only. CAZG-6, GAUG-14, JGC-19, RGC-986 and JG₂ recorded high gum content. These cultivars, except, GUAG-4, also recorded high protein content.

Singh *et al.* (2005) obtained maximum amount of heterosis in cross combinations involving the parents belonging to most divergent clusters.

Anandhi and Oommen (2006) reported that contribution of various characters towards the expression of genetic divergence indicated that yield per plant (50.98 per cent), number of pods per plant (38.42 per cent) and plant height (10.59 per cent) substantially contributed to the total divergence in the 29 genotypes of cluster bean. Contribution of other characters towards the total divergence was negligible.

Raghu *et al.* (2008) found the selection of accessions for hybridization to generate new gene combination should be based on genetic diversity rather than geographical diversity.

Pathak *et al.* (2009) explained the clustering between genetic diversity and geographical diversity was in agreement with Gipson and Balakrishnan (1992), Singh *et al.* (2003). The intra- cluster distance was ranged from 7.74-17.774(Table 2). The inter-cluster distance revealed that maximum divergence between cluster VII and VIII ($D^2=1640.054$) closely followed by cluster IV and VIII ($D^2= 1552.597$), cluster II and VIII ($D^2=1388.251$), cluster I and VI (1202.829), cluster I and VIII ($D^2=1183.916$) and cluster V and VIII ($D^2=1178.135$).

Singh *et al.* (2009) studied on clustering between genetic diversity and geographical diversity is in accordance with Gipson and Balakrishnan, (1992) in cluster bean.

Morris (2010) noticed low variation for seeds per pod, 100-seed weight, secondary branches per plant, pod length, pods per cluster and days to 50 per cent flowering in guar accessions.

Pervaiz and Rabbani (2010) reported on grouping of genotypes based on agromorphological characters is not always associated with their geographical origin.

Pathak et al. (2011) conducted a study for 7 qualitative traits on sixteen genotypes irrespective of their source were grouped in a single large cluster. 'HGS 26-01' was the most diverse genotype among the tested genotypes, further 'HGS 884' and 'HGS 02-20' were genetically diverse for the component traits and could be useful genetic resources. Genetic drift and selection in different environments have caused genetic diversity than the geographical distance as suggested by earlier studies in cluster bean (Singh et al. 2003; Henry and Mathur, 2008).

Girish et al. (2012) studied forty five genotypes of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] representing the broad spectrum of variation from various agro-climatic conditions of India was assessed for genetic divergence using Mahalanobis D^2 technique. The genetic material exhibited wide range of genetic divergence for all 20 characters investigated. All the genotypes were grouped into eight clusters.

Sultan et al. (2012) reported that the largest variation was found for days to maturity, plant height, pods per plant and cluster per plant in cluster bean accessions.

Sultan et al. (2012) stated according to the results of current work there are 101 guar accessions collected from diverse areas of Pakistan for estimate the extensive range of genetic diversity has been explored of guar germplasm in Pakistan.

Kumar et al. (2013) studied grouping of accessions based on morphological, phenological and reproductive characters is not always associated with their geographical origin guar accessions.

Manivannan and Anandakumar (2013) studied forty two cluster bean genotypes collected across the country were assessed for genetic divergence using Mahalanobis D^2 technique. The genetic material exhibited wide range of genetic diversity for all

the characters investigated and grouped into 15 different clusters. The maximum intra cluster distance was observed in cluster XIV indicated that genotypes are having diverse genetic architecture. The maximum inter-cluster distance was recorded between cluster VI and cluster XV, indicating the presence of wide range of variability among the genotypes of the cluster. The percent contribution towards genetic diversity was highest from 100 seed weight (44.48%) followed by seed yield per plant (26.25%).

Rai and Dharmatti (2013) worked on thirty one genotypes of cluster bean collected from various sources were assessed for genetic divergence using Mahalanobis D^2 technique. The genetic material exhibited wide range of genetic diversity for all the characters investigated and grouped into 3 different clusters. The maximum intra cluster distance was observed in cluster I indicated that genotypes are having diverse genetic architecture. The inter cluster distance was high between cluster II and cluster III, this indicated wide range of variability among clusters.

Kumar *et al.* (2014) reported thirty genotypes of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] were assessed for genetic divergence using Mahalanobis D^2 technique. These exhibited wide ranges of genetic diversity for all the characters and grouped into 5 different clusters. The maximum intra cluster distance was observed in cluster I indicated that genotypes under this group are diverse. Based on D^2 values, 30 genotypes were classified into five clusters. Maximum inter- cluster distance was between cluster I and cluster II and intra-cluster distance in cluster III, indicating the presence of genetic diversity among the genotypes between the cluster bean.

Verma (2014) depicted that all the French bean genotypes were grouped into five distinct clusters. An analysis of the percentage contribution of individual characters towards genetic diversity revealed that vitamin C content, number of pods/plant and dry matter content were the major characters contributing to genetic diversity in french bean.

Manivannan *et al.* (2015) found the clustering analysis based on various morphological traits assorted 42 guar genotypes into four main groups and six sub

clusters. Dendrogram based on hierarchical clustering grouped genotypes based on their morphological traits rather than geographic origin. Grouping of genotypes based on morphological traits is not always associated with their geographical origin. Outperformed genotypes based higher yield potential, 100-seed weight; pods per plant and early maturity are identified for guar variety development.

The literature pertaining to heritability and genetic advance is as following:

2.3.2 Heritability and genetic advance

Heritability is a useful measure for considering the ratio of genetic variance to the total variance and is generally represented in percentage. This is an index of transmissibility of characters from the parents to their off-springs and is a measure of genetic relationship between parents and progeny; hence, changing the characteristics of the population through selection can be predicted only from knowledge of the degree of correspondence between phenotypic and breeding values.

The degree to which the variability of quantitative character is transmitted to the progeny is referred as heritability.

Heritability measures the relative amount of the heritable portion of variability. Sometimes heritable variation is marked by non-heritable components. Hence it is necessary to classify the total heritability using genetic parameters into broad sense heritability and narrow sense heritability. Broad sense heritability is the ratio of genotypic variance to the total variance in non-segregating population (Hanson *et al.*, 1956).

The heritability value of a trait indicates the effectiveness of selection based on phenotypic expression. Quantitative characters are governed by more number of genes and further influenced by environment, of which a proportion observed value is heritable. Estimate of narrow sense heritability is not possible thus; by estimating broad sense heritability along with genetic gain is usually more useful in selecting the best individual (Johnson *et al.*, 1955a).

Genetic advance is the measure of improvement that can be achieved by practicing selection in a population. It is the ultimate goal of the plant breeder of have higher genetic advance in the material selected. In cluster bean, there are several reports available indicating high genetic advance for yield and yield contributing characters. However, some of the reports were contradicting the above findings, where genetic

advance was low for most of the characters except green pod breadth and green pod yield per plant.

Genetic advance is a product of heritability and infers the potentiality of selection intensity. Genetic advance, when considered along with heritability gives reasonable assessment of the resultant effects of selection in breeding populations (Johnson *et al.*, 1955).

Genetic advance is the measure of improvement that can be achieved by practicing selection in a population. Since the estimates of heritability give no indication of the amount of progress expected from the selection, they are most meaningful when accompanied by estimates of genetic advance. Genetic advance is affected by factors like intensity of selection, heritability and phenotypic variance. High genetic advance coupled with high heritability is an indication of more additive gene action (Panse, 1957).

Genetic advance is most useful estimate as it is the improvement in the phenotypic value in the new population in contrast to base population. According to Comstock and Robinson, (1952) expected genetic advance depends upon:

- I) The amount of genetic variability.
- II) The magnitude of masking effect of environment and interaction components of variability on genetic diversity.
- III) The intensity of selection.

Expected genetic advance in percentage of mean is the product of:

- I) Selection differential measured in terms of phenotypic standard deviation and
- II) The genetic coefficient of variability and square root of heritability ratio (Johnson *et al.*, 1955).

Since the genetic gain in a character is the product of the heritability and the selection differential expressed in unit of standard deviation, the heritability alone does not have much significance.

Johnson *et al.* (1955) suggested that high heritability combined with high genetic advance was indicative of additive gene action and selection based on these parameters would be more reliable.

Mital and Thomas (1969) studied the high range of expected genetic advance up to 83% for some of these characters like branch number and pod number.

Mitra *et al.* (2000a) depicted that high heritability for pod characters viz., pod length and number of seeds per pod.

Mitra *et al.* (2000b) determined the following non-hierarchical Euclidean analysis; all the 234 genotypes were grouped into 12 clusters with variable number of genotypes. The highest genetic divergence was observed between cluster V and IX, whereas cluster III and VIII were, closest one. On the basis of genetic divergence and mean performance eight diverse and superior genotypes, being exceptionally good for one or more traits and reasonable for others in comparison to checks, were selected: Old these genotypes may be useful in multiple crossing or diallel selective mating system to recover transgressive segregants.

Singh *et al.* (2001) reported that this may due to presence of both additive and non-additive gene action. To improve these characters, simple selection as well as exploitation of hybrid breeding would be useful. The similar types of results were found by some researchers (Singh *et al.*, 2002; Singh *et al.*, 2003; Chaudhary *et al.*, 2003; Singh *et al.*, 2005; Mahla and Kumar, 2006; Chaudhary and Shrimali, 2006).

Singh *et al.* (2001) found heritability estimates were high for all the characters studied. High heritability for pod yield and number of pods per plant.

Choudhary *et al.* (2003) and Singh *et al.* (2005) estimated the genetic advances were also moderate for these traits which indicated that phenotypic selection for improvement of these could be brought by simple methods. High estimates of heritability along with high genetic advances for seed yield, biological yield, test weight and plant height in cluster bean.

Saini *et al.* (2004) presented high heritability coupled with genetic advance as per cent of mean was recorded for viscosity, number of pods per plant, harvest index, days to maturity, seed yield per plant, plant height, leaf area index at 50 days after sowing, specific leaf area at 40 days after sowing, number of clusters per plant,

number of branches per plant and number of pods per cluster, which might be attributed to additive gene effects regulating their expression.

Further, Saini *et al.* (2005) found high heritability coupled with high genetic gain was observed for days to flowering, branches per plant, clusters per plant, pods per plant and plant height.

Singh *et al.* (2005) studied high heritability (broad sense) accompanied by high genetic advance was noticed for seed yield, biological yield, pods per plant, clusters per plant, days to flowering, branches per plant, plant height and days to maturity, indicating that genetic variation for these characters were primarily due to additive genetic effects.

Anandhi and Oommen (2007) reported high heritability coupled with high genetic advance was observed for plant height, number of pod clusters per plant, number of pods per cluster, pod weight, pod length, number of pods per plant and yield per plant indicating the presence of additive gene action in the expression of these characters.

Prakash *et al.* (2008) observed that high heritability coupled with genetic advance as percent of mean was observed for the characters i.e., number of pods per plant, seed yield per plant, leaf area index at 50 days after sowing, number of branches per plant, harvest index, number of clusters per plant, plant height, number of pods per cluster, days to maturity, specific leaf area at 40 days after sowing and viscosity indicating that simple selection program would be effective for genetic improvement of guar.

Nehru *et al.* (2009) suggested high heritability and high genetic advance as per cent of mean (GAM) attributable to highly additive gene effect was observed for cluster per plant, pods per plant, peduncle length, pod length, dry pod weight, seed per pod, number of seeds per plant, 100 seed weight and seed yield per plant in cowpea.

Idahosa *et al.* (2010) found high heritability estimates have been reported for days to 50% flowering, days to maturity, pod length, pod weight, seed per pod and 100 seed weight in cowpea.

Saini et al. (2010) reported that heritability is influenced by biometrical method, generation of hybrid, sample size or experimental material and environment. In the present study, high heritability associated with high genetic advance for different characters were indicative of dominance and epistatic effects. Thus, the traits like plant height, number of branches, pods per plant, clusters per plant and yield per plant recorded high heritability with high genetic advance over mean. These results are in conformity with those reported by Das *et al.*, (1973), Dabas *et al.*, (1982), Singh *et al.*, (2001) and Saini *et al.*, (2010).

Pathak et al. (2011) reported heritability ranged from 15% to carbohydrates to 81% in cash content. Similar observations were recorded by Chaudhary *et al.*, (2003) in cluster bean. The high value of heritability for ash content demonstrated that this least influences by environmental changes, moderated values for gum, fiber and protein suggested that to some extent environment influenced the expression of these traits.

Singh et al. (2011) estimated that high heritability were expressed by days to first flower, days to first picking, pod length and pods yield/ plant in hyacinth bean.

Shabarish et al. (2012) reported that high heritability coupled with high genetic gain in percentage was observed for pod yield per plant, number of pods per plant, days to 50% flowering, number of branches and plant height.

Shabarish et al. (2012) studied that high heritability with low genetic advance was shown for pods per cluster, number of seeds per pod and pod width. The results of present investigation suggests that selection based on plant height, number of branches, days to 50% flowering, clusters per plant and pod yield per plant might bring simultaneous improvement in vegetable pod yield of cluster bean.

Muthuselvi and Shanthi (2013) worked on fifty genotypes of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] to estimate variability, broad sense heritability and genetic advance for pod yield and related attributes High heritability coupled with high genetic advance was observed for plant height, number of branches per plant,

number of clusters per plant, number of pods per cluster, pod length, fresh pod yield per plant, dry pod yield per plant, seed yield per plant, 100 seed weight, days taken to maturity, crude protein and gum content.

Parmar *et al.* (2013) incorporated thirty genotypes of Dolichos bean (*Lablab purpureus*) were evaluated to study the genetic variability on yield, yield contributing and related characters viz. days to 50% flowering, days to first pod set, days to maturity, number of pods per cluster, pod length, width of pod, weight of 10 green pods, number of single podded clusters per plant, number of pods per plant and protein content. Days to 50% flowering, pod length, width of pod, weight of 10 green pods accounted for higher heritability and higher genetic advance which suggests the role of additive gene action in the expression of these characters and could be improved through selection.

Jitender *et al.* (2014) reported an investigation comprised 25 guar genotypes. The mean sums of squares due to genotypes were recorded to be highly significant for all the 18 characters studied, including morpho-physiological, biochemical characters and yield components and seed yield per plant. The studies revealed that characters like pods per plant, dry weight, plant height and proline content in leaves had high heritability and genetic advance as per cent of mean. Likewise germination/survival per cent had moderate genetic advance as per cent of mean with high heritability. However, 100 seed weight, branches per plant and leaf area index had high heritability associated with low genetic advance as per cent of mean.

Omvir and Singh (2015) found moderate to high heritability coupled with moderate to high expected genetic advance were observed for all studied traits. Number of seeds per pod, number of pods per plant, number of pods per cluster, number of clusters per plant, days to 50% flowering and days to maturity had positive and significant correlations with seed yield per plant.

The literature pertaining to coefficient of variability is as following:

2.3.3 Coefficient of variability

Genetic variability is a measure of the tendency of individual genotypes in a population to vary from one population to another. The determination of genetic variability and its partitioning into various components is necessary to have an insight into the genetic nature of yield and its components. Study of partitioning the total variability into heritable and environmental components had its beginning in the work of Johanson, (1909), Nilson-Ehle, (1909) and East, (1916). Based on the study on non-segregating populations, Charles and Smith, (1939), Powers, (1942) separated genetic variance from total variance using estimate of environmental variance. Sivasubramanian and Menon (1973) classified the review of literature on genotypic coefficient of variation and phenotypic coefficient of variation as low, medium and high values for getting uniformity in comparing different values.

The magnitude of variability present in a crop species is of utmost importance as it provides the basis for effective selection. The total variation i.e., phenotypic variation, present in a population arises due to genotypic and environmental effects. Phenotypic variability is the observable variation present in a character in a population: it includes both genotypic and environmental components of variation and as a result, its magnitude differs under different environmental conditions.

The knowledge of existing variability with respect to yield and yield attributing traits in the germplasm of a crop is the basic requirement in order to select the desirable types. The variability observed for a character is due to difference in the genetic constitution of the population as well as variation arising due to environmental condition. The coefficient of genetic variability and phenotypic variation are used as an aid in the plant breeding work. The concept of heritability is important to determine whether phenotypic difference observed among various individuals are due to genetic change or due to effect of environmental factors, heritability indicates the possibility and extent to which improvement can be brought about through selection.

Gipson *et al.* (1990) reported that higher the number of pods per cluster, higher will be the yield. In the present investigation, good possibility of selection for this character was revealed, as there were significant differences among varieties for number of pods per cluster. Number of pods per cluster varied from 5.13 to 11.10 with general mean of 7.09. The number of pods per plant directly influences yield. In

the present investigation highly significant differences were observed for this character. Vijay, (1988) and Gipson and Balakrishnan, (1990) have also reported similar results.

Mitra *et al.* (2000) studied two hundred thirty one germplasm-lines of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] along with three checks for seed yield and its components in augmented design. Pods per plant exhibited highest variability and contribution to divergence.

Singh *et al.* (2001) found a lot of variability for green pod yield and observed indicating huge scope for improvement in pod yield per plant. These findings are in close association with those of Mital *et al.*, (1969), Henry *et al.*, (1986) and Singh *et al.*, (2001).

Chaudhary *et al.* (2003) studied genetic variation and correlation analyses for 12 traits using 40 cluster bean genotypes grown in Durgapura, Rajasthan, India, during the Kharif season of 2001. Significant variation among the genotypes was observed for all the traits. High estimates of phenotypic and genotypic (GCV) coefficients of variation were recorded for all the characters except days to maturity, number of seeds per pod, 100-seed weight and pod length. Seed yield was positively correlated with number of clusters per plant, biological yield and harvest index, but was negatively correlated with plant height. The number of clusters per plant was positively correlated with harvest index. Biological yield showed positive association with days to maturity and seed yield per plant. Harvest index was positively associated with number of primary branches, and clusters and pods per plant. The number of clusters per plant, biological yield and harvest index were identified as the most important criteria in the selection for higher seed yield.

Saini *et al.* (2005) assessed variability, heritability and genetic advance for 12 traits in 40 genotypes of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] during Kharif 2003 at Durgapura, Navgoan and Tabiji in Rajasthan, India. The significant mean squares due to genotypes for all traits in all environments indicated the presence of enormous variability in the material studied. The maximum range of variation was

noted for branches per plant, biological yield, pods per plant, seed yield, clusters per plant and plant height.

Saini *et al.* (2005) reported that phenotypic and genotypic coefficients of variation were high for branches per plant, days to flowering, clusters per plant and pods per plant, and moderate for plant height and seed yield.

Singh *et al.* (2005) evaluated twenty-four advanced genotypes of cluster bean were reported to estimate the genetic variation. The analysis of variance indicated significant differences among genotypes for all the traits studied. Estimates of phenotypic and genotypic coefficients of variation were high for biological yield per plot, seed yield per plot, pod per plant, cluster per plant, days to flowering and branches per plant, and moderate for plant height and days to maturity. Thus, it may be concluded that a great amount of variability existed in the present material used and it would be desirable to give emphasis on biological yield per plot, clusters per plant, days to flowering, days to maturity, pods per plant, plant height and branches per plant for selection of high yielding types in cluster bean. On the basis of the above findings, the cultivars RGC-1017 and RGM-112 were selected and released for cultivation from the present material in 2001 and 2002, respectively.

Anandhi *et al.* (2007) studied on genetic variability and heritability in 29 varieties of cluster bean grown during *Kharif*, 2004. Variability was high for all the characters studied except number of seeds per pod and days to 50% flowering. High heritability coupled with high genetic advance was observed for plant height, number of pod clusters per plant, number of pods per cluster, pod weight, pod length, number of pods per plant and yield per plant indicating the presence of additive gene action in the expression of these characters. High estimates of genotypic coefficient of variation, heritability and genetic advance recorded for number of pod clusters per plant, number of pods per plant, pods per cluster and yield per plant suggest the scope for improvement of these characters through selection.

Prakash *et al.* (2008) studied on genetic parameters in fifty genotypes of Guar collected from Rajasthan, Gujarat, Haryana and Karnataka revealed high estimates of variation for number of pods per plant, harvest index, seed yield per plant, leaf area index at 50 days after sowing, number of branches per plant and number of clusters per plant.

Morris (2009) found both coefficient of variation and principal component analysis are useful parameters to determine an estimate of how much each trait contributes to its variation and have successfully been used to characterize regenerated cluster bean.

According to Saini *et al.* (2010) results indicated that there is highly significant variation among the genotypes for almost all the characters under study, i.e., plant height (cm), number of branches, days to 50% flowering, pods per plant, clusters per plant, pods per cluster, pod length (cm), number of seeds per pod, yield per plant (g), yield per hectare (t) and pod breadth (cm).

Arora *et al.* (2011) found considerable morphological variability among north Indian cluster bean landraces was identified which could be used as basis for genetic improvement programme. PCB-23, BR-112 and RGC-936 on account of better performance have been recommended for use in cluster bean improvement programme. Various economic characters needed to develop cluster bean ideotypes have been discussed.

Pathak *et al.* (2011) reported that, the analysis of variance showed wide range of genetic variability for all the characters studied suggesting that the present material of cluster bean was appropriate and hence suitable for further genetic analysis. The phenotypic coefficient of variation (PCV) was invariably higher than their corresponding genotype coefficient of variation (GCV) indicating that the apparent variability in these characters may not only be due to the genotypes but also environmental factors were influential in expressing these characters. Singh *et al.*, (2005) also observed similar trends for GCV and PCV in cluster bean. The PCV was higher for fat (24.1%), followed by fiber content (19.5%).

Shabarish *et al.* (2012) suggested a wide range of variability as recorded for several characters namely plant height (32.10-100.85), number of branches (0.00-9.09), pods per plant (75.50-185.00), clusters per plant (12.80-49.67), pods per plant (5.40-14.10), yield per plant (57.74-222.34) and for yield per hectare (9.02-19.29). Phenotypic and genotypic variance recorded high for plant height, pods per plant and yield per plant.

An investigation on variability for seed yield and its component characters was carried by **Malaghan *et al.* (2013)** on 67 cluster bean genotypes. The characters, *viz.*, seed yield, dry pod yield, number of dry pods per plant and number of cluster per plant recorded high estimates of genotypic and phenotypic coefficients of variation indicating ample scope for selection of genotypes from available germplasm for these traits.

Patil (2013) found the high phenotypic coefficient of variation for branches/plant (43.33) followed by leaf area (37.43), number of clusters/plant (36.69), seed yield/plant (27.30), dry pod yield/plant (21.55), plant height (20.09), number of pods/cluster (19.71) indicated scope for further improvement in these traits.

Kapoor (2014) studied seventy six cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes and analyzed for genetic variability, correlation and path coefficients. High estimates of GCV and PCV were observed for leaf weight, number of leaves per plant, number of branches per plant; stem girth, dry matter yield and green fodder yield suggesting that selection based on these characters would facilitate successful isolation of desirable types.

Omvir and Singh (2015) reported high degree of genetic variability during both seasons for seed yield per plant (g), 100-seed weight (g.), number of seeds per pod, number of pods per plant, number of pods per cluster, number of branches per plant, number of clusters per plant, plant height (cm.), number of days to 50% flowering and number of days to maturity.

The literature pertaining to correlation coefficient is as following:

2.3.4 Correlation coefficient

Correlations measures mutual association with regard to causation between to attributes. Knowledge of the nature and magnitude of interrelationship among yield and its components is of great interest in plant breeding. The statistics which measure the relationship and its extents, between two or more variable is known as Correlation coefficient. Correlation study provides information that selection for one character will result in progress for all positively correlated characters. Many of the characters are correlated, because of association positive or negative with other characters. As more variables are considered in correlation table, their indirect correlations become more complex. The original concept of correlation was presented by Galton, (1889), which was elaborated later by Fisher, (1918) and Wright, (1921).

Mitra *et al.* (2000) reported this trait was also significantly and positively correlated with seed yield which indicates its importance as selection criteria.

According to Saini *et al.* (2005) correlation analysis indicated the significant correlation of seed yield with pods per plant, harvest index, biological yield and clusters per plant. Seed yield had negative but desirable correlation with days to flowering, days to maturity and plant height.

Singh *et al.* (2005) found that seed yield per plot was significantly and positively correlated with biological yield per plot, clusters per plant, pods per plant and plant height, respectively.

Rai *et al.* (2008) studied on the number of seeds/pod and showed the positive correlation with seed length (0.422). The seed length also had positive correlation with seed width (0.371). In this investigation, pod yield/plant had maximum significant values for number of pods/plant (0.708) followed by seed length (0.413) and pod width (0.378) in hyacinth bean.

Raghuprakash et al. (2009) found the correlation studies on the positive and significant association of number of pods per plant, number of branches per plant, number of clusters per plant, number of seeds per pod, number of pods per cluster, harvest index, SCMR at 40 and 60 days after sowing with seed yield in guar.

Kumar et al. (2010) reported that the high significant correlation for pods per plant and harvest index at both genotypic and phenotypic levels with seed yield per plant and plant height, primary branch per plant, clusters per branch and days to maturity had direct positive effect on seed yield in mung bean.

Rai et al. (2010) found that the pod diameter had positive direct effect on yield per plant but its negative effect through number of seeds per pod and number of pods per plant made its association with yield per plant significantly negative.

Rakesh et al. (2011) obtained the plant height positive and highly significant association with number of vegetable pods per plant (0.567, 0.387) at both genotypic and phenotypic level.

Elshiekh et al. (2012) determined the set of sixteen guar genotypes was evaluated under rain-fed conditions. Data recorded at were used for partitioning the genotypic correlations between seed yield and five of its components into direct and indirect effects. Moreover, different selection indices were constructed using different combinations of these characters.

Kumar (2013) suggested that number of seeds per pod had positive correlations with pod length and plant height and these findings are found in green gram.

Saroj et al. (2013) found genotypic correlations were higher in magnitude than the phenotypic correlation indicting strong inherent relationship among the characters except few which could be due to modifying effects in the environment studied. Similar findings were reported in pigeon pea.

Bhatkotle (2014) estimated yield per hectare highly significant and positive correlation with plant height, pods per plant, pod length, pod breadth, fresh weight of ten pods, yield per plant and yield per plot at genotypic level and with plant height, pod length, yield per plant and yield per plot at phenotypic level.

Rai and Dharmatti (2014) reported that correlation positive associations of vegetable pod yield per hectare (t) with plant height (0.315, 0.309), pods per cluster (0.322, 0.298), pods per plant (0.443, 0.389) and pod yield per plant (0.905, 0.787) at genotypic and phenotypic levels respectively.

Shabarish and Dharmatti (2014) exhibited the pods per plant positive and significant correlation with vegetable pod yield per plant in cluster bean.

The literature pertaining to path coefficient analysis is as following:

2.3.5 Path coefficient analysis

The technique of path coefficient was originally developed by Wright, (1921). He defined the path coefficient as the ratio of the standard deviation of the effect to the total standard deviation when all the causes are constant, except the one in question. The variability of which is kept unchanged. Dewey and Lu, (1959) employed the path coefficient technique in cluster beans and french bean to establish the relative importance of plant height, fertility and plant size as the determinates of seed yield.

Arumugarangarajan et al. (2000) found that genotypic and phenotypic correlation and path coefficients in 28 diverse genotypes of cluster bean. The traits, clusters per plant, branches per plant, pods per cluster, plant height and pod yield per plant were positively associated with seed yield both at phenotypic and genotypic levels. These traits exhibited positive and significant interrelationship among themselves. The path analysis indicated a high magnitude of positive direct effect of clusters per plant on seed yield. Except for plant height, pod length and seeds per pod, other yield attributing traits showed positive direct effect on seed yield.

Hanchinamani (2004) carried out path coefficient analysis in 80 genotypes in cluster bean which revealed significant and positive association of vegetable pod yield per plant and seed yield per plant with number of branches per plant, number of leaves per plant, plant spread (East-West), number of clusters per plant, cluster length, number of seeds per pod, dry pod yield per plant and gum content of seed endosperm. Path analysis studies revealed high direct effects of plant height, number of leaves per plant, number of clusters per plant and dry pod yield per plant.

Singh *et al.* (2005) revealed that the path analysis of biological yield per plot had direct positive effect on seed yield per plot followed by days to maturity, pod length, pods per plant, 100 seed weight and plant height.

Dursun (2007) found that number of seeds per pod, the highest indirect effect on yield via wet pod weight. Percentages of direct effects on seed yield were 63.1%, 51.7% and 48.9%, respectively, for seed yield per plant, 1000 seed weight and plant height. In previous studies, it was reported that pod weight and seed number per pod had highest and positive direct effect on seed yield in french bean.

Lavanya *et al.* (2008) have also reported lack of correlation between geographic and genetic diversity in other legumes.

Rai *et al.* (2008) estimated path coefficient analysis and revealed that the importance characters such as number of pods per plant, pod length, pod width and seed length in selection of superior genotypes for pod yield/plant.

Punia *et al.* (2009) depicted a positive correlation between different genotypic varieties with respect to morpho-physiological characteristics using RAPD analysis as well as in cultivar identification was designated in cluster bean lines.

Raghuprakash *et al.* (2009) determined the path analysis and revealed that the number of pods per plant, SCMR at 40 DAS, number of seeds per pod and number of pods per cluster were important in formulating selection criteria for improvement of seed yield in guar.

Karasu and Oz (2010) found that the path coefficient analysis on seed yield per plant had the greatest direct effect (+0.668) on seed yield per hectare followed by 1000 seed weight (+0.185) and plant height (+0.301). Percentages of direct effects on seed yield per hectare were 63.1%, 51.7% and 48.9%, respectively for seed yield per plant, 1000 seed weight and plant height in french bean.

Malaghan *et al.* (2011) suggested the path analysis and revealed on ten dry pod seed weight, dry pods per plant and dry pod yield per plant had high direct effect on seed yield per plant, hence, these parameters are effective for selection to increase seed yield in cluster bean.

Elshiekh *et al.* (2012) found that correlation alone may not give complete information but when used in conjunction with path coefficient analysis will give a better measure of cause and effect relationship existing between different pairs of characters.

Girish *et al.* (2012) studied on the days to first flowering, days to fifty per cent flowering and days to pod maturity had negative and significant association at both genotypic and phenotypic correlations with pods per plant and vegetable pod yield.

Sultan *et al.* (2012) found that important agro morphological traits like greater yield potential, seeds per pod, pods per plant and early maturity, etc. served as a criterion to select promising guar genotypes. For the improvement of cultivated guar, it is dire need to use diverse collections with more variability for the purpose of variety development and for use in future breeding programs of cluster bean in Pakistan.

Saroj *et al.* (2013) reported that the path analysis study in pigeon pea and concluded that improvement in pod yield /ha could be bought by selection for component character like days to 50% flowering, pods per cluster, pod length, number of seeds per pod and pod yield per plant.

Bhatkotle (2014) studied on the results of path analysis and highlighted positive and direct effect of number of leaves, days to first flowering, clusters per plant, pods per cluster, pod length, yield per plant and yield per plot on estimated yield per hectare. Number of branches exhibited positive indirect effect through number of leaves, days to first flowering, clusters per plant, yield per plant and estimated yield per hectare. Fresh weight of ten pods showed positive indirect through number of leaves, days to first flowering, pods per cluster, pod length, yield per plant and yield per plot.

Rai and Dharmatti (2014) found the positive indirect effects on through days to 50% flowering (0.015), pods per cluster (0.035) and pod breadth (0.046). Phenotypic path coefficient shows that yield per plant had high positive direct effect (0.879) and strong degree of association (0.787) for pod yield per hectare. This direct effect is mainly due to the indirect effect of trait positively through plant height (0.043), pods per plant (0.023), pod length (0.025) and pod width (0.002).

Malaghan *et al.* (2015) report the path analysis study on ten dry pod seed weight, dry pods per plant and dry pod yield per plant had high direct effect on seed yield per plant, hence, these parameters are effective for selection to increase seed yield in cluster bean.

2.4 Molecular Characterization:

Traditionally, genetic variation is inferred by morphological/phenotypic variation or the growth response of the organism. Classical methods of establishing genetic diversity and /or relatedness among groups of plants relied upon phenotypic (observable) traits. However, these had two disadvantages: First, the quantitative traits are greatly influenced by environmental and genotype x environment interaction, and secondly the levels of polymorphism (allelic variation) that could be looked at are limited. These limitations were significantly overcome by deployment of environment-neutral biochemical makers (Isozyme) and protein electrophoresis and molecular markers that focus directly on the variation controlled by genes or on the genetic material (DNA itself). The higher resolution of molecular markers makes

them a valuable tool for finger printing, protection of breeders rights, facilitating appropriate choice of parents for breeding programmes, analyzing quantitative traits, detection of Quantitative Trait Loci (QTL), gene mapping, marker assisted selection, gene transfer, understanding evolutionary pathways and for the assessments of genetic diversity.

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

The applications of different techniques for genetic diversity analysis have been well reviewed (Malyshev and Karte, 1997; Newbury and Ford-Lloyd, 1997; Westman and Kresovich, 1997; Karp *et al.*, 1998). Some applications of diversity analysis using molecular marker tools includes, identifying areas of higher genetic diversity (Hamrich and Godt, 1990) and determining collection priorities and sampling strategies (Schoen and Brown, 1991).

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

Modern molecular techniques have been developed in order to meet the demands of the horticulture industry genetic variation, which range from morphological characterization to various DNA-based markers such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeats (SSR) (Crawford, 2000, Newton *et al.*, 2000, Martinez *et al.*, 2003, Fontaine *et al.*, 2004, Murtaza and Elect, 2006, Ferdousi Begum, 2013). Identification and characterization of germplasm is essential for the conservation and utilization of plant genetic resources (Suvakanta-Barik, 2006). Molecular markers are the best tools for germplasm characterization and determining genetic relationships. Markers based on differences in DNA sequences among individual genotype usually detect more polymorphism than morphological and protein-based markers and constitute a new generation of genetic markers (Botstein *et al.*, 1980; Tanksley *et al.*, 1989). Characterization of plant with the use of molecular markers is an ideal way to conserve plant genetic resources. Molecular characterization helps to determine the breeding behaviour of species, individual reproductive success and the existence of gene flow, the movement of alleles within and between populations of the same or related species, and its consequences. Molecular data improves the elucidation of phylogeny, and provide the basic knowledge for understanding taxonomy, domestication and evolution of plants (Nwakanma, 2003).

Molecular characterization is the most important tool of molecular breeding the, science-based multi-disciplinary approach used in food, feed and environmental risk/safety assessment of plants derived from modern biotechnology. The molecular characterization of these plants is used to gain an understanding of the genetic material introduced and expressed in them.

The purpose of molecular characterization is to inform the risk/safety assessment of plants derived from modern biotechnology. Such characterization provides knowledge at the molecular level of the inserted DNA within the plant genome 5, the insertion site and the expressed material (ribonucleic acid [RNA] and proteins), and may provide information on intended and possible unintended effects of the transformation. Molecular characterization of the genotypes contributes to a rigorous assessment of the potential impacts of transformation on the food, feed and environmental risk/safety of a recombinant-DNA plant. It assists in the prediction of

the phenotype and the genotype will ultimately determine whether the recombinant-DNA plant poses any risk/safety concerns.

Efforts are to be made to organize research programs on germplasm characterization, utilization, and molecular characterization. Superior cluster bean varieties were selected on the basis of morpho-physiological characters and subjected to DNA-based molecular marker analysis.

2.5 Inter-simple sequence repeats (ISSR) markers:

Microsatellites are usually more or less proportionally dispersed in the genome. However, regions with a greater abundance of these sequences have been found and are named as "SSR hot spots" (Bornet *et al.*, 2002, Bornet *et al.*, 2002 and Zietkiewicz, 1994). Such regions can serve as a source of ISSR markers.

The ISSR technology is based on the amplification of regions (100-3000 bp) between inversely oriented closely spaced microsatellites. Single primer (16-18 bp) consisting of several simple sequence repeats is used for amplification of these regions. Primers can be based on any SSR motif along with 5' or 3' anchored bases usually 2-4 bases which are arbitrary selective nucleotides. However, non anchored primers have also been used (Bornet *et al.*, 2002).

Inter simple sequence repeat (ISSR)-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. It is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology. This review provides an overview of the details of the technique and its application in genetics and plant breeding in a wide range of crop plants.

(B). Genotypic Characters:

2.5.1 Genetic diversity and phylogenetic analysis

2.5.2 Genome mapping

2.5.3 Number of amplified fragment

2.5.4 Polymorphic fragments

2.5.5 Polymorphism percentage

2.5.6. Monomorphic fragments

2.5.7. Monomorphism percentage

The literature pertaining to genetic diversity is as following:

2.5.1 Genetic diversity and phylogenetic analysis

Genetic diversity has been defined as the variety of alleles and genotypes present in a population and this is reflected in morphological, physiological and behavioural differences between individuals and populations (Frankham *et al.*, 2002). From a functional point of view, genetic diversity can be classified as neutral, deleterious or adaptive (Hedrick, 2001). Generally, neutral variants are used for conservation applications, but deleterious and adaptive variations are also important in the contexts of population survival and economically important traits in domestic plants. From a descriptive point of view, genetic information can refer to DNA sequences, individual genes, chromosomes or quantitative genetic variation. Since the beginning of the 1990s, the development of appropriate tools has resulted in a leading role for molecular markers in the characterization of genetic diversity.

ISSRs were more useful for the analysis of diversity in the genus *Eleusine* in terms of quality and quantity of data output as compared to RFLP and RAPD (Salimath *et al.*, 1995). Significantly, the efficiency of the technique was evident in characterization even at the varietal level of a species. For instance, three 5' anchored primers together could distinguish 20 cultivars of *Brassica napus* (Charters *et al.*, 1996). ISSR is the marker of choice for assessment of genetic diversity in cocoa (Charters and Wilkinson, 2000), gymnosperms such as Douglas fir and sugi (Tsumura *et al.*, 1996) and even fungi (Hantula *et al.*, 1996). In a study on white lupin it has been demonstrated that among 10 primers used any two were sufficient to distinguish all the 37 accessions studied (Gilbert *et al.*, 1999). Similarly, 4 primers were sufficient to distinguish 34 cultivars of potato (Prevost and Wilkinson, 1999) and 3 primers could distinguish 16 genotypes of redcurrant (Lanham and Brennan, 1998). The use of such highly informative primers lowers the cost, time and labour for diversity analysis.

ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species which include rice (Joshi *et*

al., 2000), wheat (Nagaoka and Ogihara, 1997), finger millet (Salimath *et al.*, 1995), *Vigna* (Ajibade *et al.*, 2000).

Maurya *et al.* (2000) carried out a survey of isozyme systems in a relatively little known legume, the guar (cluster bean), [*Cyamopsis tetragonoloba* (L.) Taub.] reported for the first time. The plant material included seven landrace accessions from India, six released cultivars from the United States, and two wild species populations of *C. senegalensis* and *C. serrata*. Starch gel electrophoresis protocols for seven isozyme systems, viz., MDH, IDH, PGD, ADH, PGI, GOT and ME were largely developed. The combination of cotyledons as the plant tissue, and Tris-HCl with ascorbic acid, PVP, PWP and 2-mercaptoethanol as the extraction buffer provided the best resolution of isozyme. Out of the four gel-electrode buffer systems attempted, the Histidine system was selected to study the patterns of diversity. All the cultivated species accessions were uniformly monomorphic. Two isozyme phenotypes were recorded for ADH in *C. senegalensis*, and for GOT in *C. serrata*.

Raina *et al.* (2001) reported that ISSRs (97% polymorphism) were found to be more efficient over RAPDs (90.3% polymorphism) in estimating genetic diversity in groundnut.

Vieira *et al.* (2001) studied morphological, chemical and genetic differences of 12 basil (*Ocimum gratissimum* L.) specimens to determine whether volatile oils and flavonoids could be used as taxonomical markers and to examine the correlation between RAPD and these chemical markers.

Kumar (2002) worked on 37 bands, 22 were found to be monomorphic, belonging to all genotypes of *C. tetragonoloba* along with wild species.

Reddy *et al.* (2002) suggested that inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify

mainly the inter SSR sequences of different sizes. The microsatellite repeats used as primers can be di-nucleotide, tri nucleotide, tetra nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta *et al.*, 1994; Meyer *et al.*, 1993; Wu *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994) (Figure1). The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD.

Singh *et al.* (2002) conducted PCA in guar germplasm based on 220 lines and reported that PNB, GAUG 9009, RGC1022 and Naveen were grouped into separate clusters. Thus, PCA highlights the character with maximum variability.

Bushehry (2003) evaluated genetic diversity in soybean as determined by RAPD and DAF markers. The relationships of several soybean cultivars were evaluated using simple matching coefficients, random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) markers.

Fall *et al.* (2003) found that RAPD is a powerful technique and its resolving power is several folds higher than morphological and protein markers. RAPD markers have been used for diversity analysis and other applications in a vast array of crops including cowpea.

Reist-Marti *et al.* (2003) considered between breed variation as much more important because the most valuable characteristics are probably those for which genes are fixed or at high frequencies within the population displaying these characteristics.

SanCristobal *et al.* (2003) is applied to breeds, genetic distance is a measure of distinctiveness at a given time, without reference to any model that has generated the differences but, in contrast, in the population genetics approach, genetic distance is an estimate of parameters of the model underlying the generation of differences observed.

In findings of Brahmi *et al.* (2004) worked on allozyme diversity in guar germplasm are in accordance with present work, who observed three clusters in the distance range of 0–0.28 with no exact separation of accessions according to their place of collection.

Fernandez *et al.* (2004) suggested with respect to the genetic management of a population, the strategy of maximizing gene diversity keeps levels of allelic diversity as high as strategies maximizing allelic diversity itself, but with a better control of inbreeding.

Ghafoor and Ahmad (2005) reported that characterization of germplasm using biochemical techniques has received attention because of the increasing recognition of germplasm resources in crop improvement and in selection of desirable genotypes to be used in breeding programmes.

Palomino *et al.* (2005) demonstrated that the RAPD technique can be applied for estimating the genetic variability among closely related guar genotypes and between species, which is otherwise difficult to detect by other techniques. RAPD markers have been used extensively to distinguish diverse crops, including pigeon pea from its wild relatives.

Wang *et al.* (2005), recently RAPD has been used for the estimation of genetic diversity in various endangered plant species.

Kafkas *et al.* (2006) reported a matrix correlation of 0.58 between ISSR and RAPD markers in their study.

Korpelainen *et al.* (2007) identified a fast and easy method for finding microsatellite markers that utilizes genome screening with inter-simple sequence repeat (ISSR) primers to detect microsatellite regions and to obtain sequence information flanking one side of the microsatellites and a restriction-ligation technique with a specific adaptor to allow sequence walking to obtain sequence information flanking the other side of the microsatellites. Two main alternatives of the method (with or without

cloning) are presented. We successfully utilized the method when identifying microsatellite markers for 21 bryophyte species. The proportion of polymorphic markers equaled 95%. We observed that microsatellites are commonly found within the sequenced ISSR amplification products (54% in the present study), in which case specific primers can be identified for the microsatellite without a further restriction-ligation step. It is evident that the DNA regions amplified by ISSR markers commonly represent microsatellite hotspots. We propose that the identified method and the knowledge of the common presence of additional microsatellite repeats within ISSR amplification products are especially attractive to researchers who conduct small-scale microsatellite identification, such as researchers in population genetics and conservation biology.

Agarwal *et al.* (2008) depicted that DNA based molecular markers have been useful in the evaluation of genetic diversity in many crop species.

Lavanya *et al.* (2008) stated that these five selected primers generated a total of 49 amplicons of which 39 were polymorphic and exhibited high degree of marker index ranging from 66.6% to 87.5% polymorphism in banding pattern (Table 2). Such degree of polymorphism in banding pattern has also been reported in *Vigna radiata*.

Kumar *et al.* (2009) suggested all the molecular markers including the bar-coding genes require PCR-based protocols except RFLP. The use of universal primers is important for retrospective species identification as they allow amplification across a wide taxonomic range governed by the PCR success rates, particularly for new or rarely studied plant species of arid region.

Punia *et al.* (2009a) reported that among all the genotypes, eighteen of the best genotypes were chosen for DNA extraction, optimization of PCR conditions, and genetic diversity studies using 37 random primers. A total of 381 random amplification fragments were obtained; number of amplifications ranging from 4 to 22 with an average of 10.29 amplified fragments per primer. Evaluation of RAPD data reveals a magnificent range (0.34–0.76) of genotypic similarity coefficients.

Punia *et al.* (2009b) reported that good quality genomic DNA was isolated from 34 independent cluster bean varieties and conditions were optimized for further amplification by PCR using random ten decamer operon primers. An evaluation was made for the application of RAPD as a genetic marker system in commercially important cluster bean varieties and a dendrogram was prepared. Various factors influencing the RAPD amplification were optimized and the data revealed that 50ng of template DNA, 1.5mM MgCl₂-ion concentration, 0.5U of *Taq* DNA polymerase and denaturing temperature of 94⁰C was found to be essential for reproducible RAPD banding pattern. Cluster bean cultivars revealed significant polymorphism with reference to RAPD markers showing authentic genotypic diversity among its races. Out of a total of seventeen operon primers employed; ten OPT primers showed amplified products in the size range of 50, 1200bp, yielding 34 polymorphic and 58 monomorphic reproducible bands with 72.7% polymorphism. Highest polymorphism was obtained in elite cultivars HG-75 and PNB.

Rahman *et al.* (2009) estimated molecular tools that have been developed for the characterization for biodiversity may allow classification of synonyms and detection of the origin of species and cultivars.

Thimmappaiah *et al.* (2009) reported ISSR markers represent the more efficient marker system because of their capacity to generate several informative bands within a single amplification reaction.

Abd El-Hady *et al.* (2010) studied genetic variations of seven *Vigna species* using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. Amplification of genomic DNA of the seven genotypes by RAPD analysis, five primers generated 64 fragments, of which 31 were polymorphic with an average of 12.8 bands/ primer. The amplified products varied in size from 2556 to 255 bp. Eleven selected ISSR primers produced 128 bands across seven genotypes of which 89 were polymorphic with an average of 11.64 / primer. The size of amplified bands ranged from 2838 to 264 bp. Similarity index values ranged from 0.913 to 0.120 (RAPD) 0.822 to 0.118 (ISSR) and 0.899 to 0.115 (RAPD and ISSR). The results indicated that both of the marker systems RAPD and ISSR, individually or

combined can be effectively used in determination of genetic relationship among *Vigna species*. It could be concluded that, the information of genetic similarities and diversity among *Vigna* genotypes are necessary for breeding programs.

Pathak et al. (2010) determined that the most distinct genotypes RGC 1002 and CAZG 6, exhibited the maximum genetic diversity of about 37%. The maximum genetic similarity up to 98% was observed between the genotypes RGC 2021 and CLBH 201. The RAPD markers used in the present study separated all the 32-cluster bean genotypes from each other and revealed considerable genetic diversity. On the basis of similarity coefficient, the most distinct genotypes viz., RGC 1038, CAZG 6, RGC 1002, RGC 1033, RGC 986, HGS 880, HGS 870, HGS 884 and RGC 1088 can be utilized in guar improvement programmes.

Rahman et al. (2010) found the fingerprint data lower polymorphism information contents (PIC-values) may be the result of closely related genotypes in beans.

Tang et al. (2010) suggested the proposition of the concept in 1980, various types of molecular markers such as RFLP, RAPD, AFLP, SSR, SNP among others have been developed in common beans.

Shinwari et al. (2011) depicted no relation between geographic location and genetic diversity in guar, other legumes and other species.

Razzak et al. (2012) programmed among 10 faba bean cultivar to assess the genetic diversity. Variability based on the DNA level was inspected through nine ISSR-PCR screening, which showed obvious differences among the various *Vicia faba* cvs. A total of 576 ISSR loci were detected and 398 (69.10%) of them were polymorphic, which represent a relatively high polymorphism level. Cluster analysis via ISSR markers separated three green large seeded cvs. (*V. faba* var. major) from dry small seeded cvs. (*V. faba* var. minor). The small seeded cvs. were further classified into two sub clusters according to two geographic locations. The first sub-cluster included dry small seeded cvs. grown under clay soil conditions (Abies location). However, the second sub cluster integrated the similar dry small seeded cvs. but were grown

under calcareous soil conditions (Fuka location). SDS-PAGE analysis of various faba bean leaf proteins reflected some variations among studied *V. faba* populations. The results clarified that ISSR markers and protein analysis were helpful to recognize genetic variation among faba bean cultivars.

Sabahelkheir (2012) performed a preliminary study concerning the comparison of *Cyamopsis tetragonoloba* by RAPD and HPLC analysis of cannabinoids. This study could be beneficial to the molecular biologists who are involved in the study of enhancement of Guar gum.

Sadeghi et al. (2012) observed that higher proportion of polymorphic bands reported that using RAPD (87.9%) than ISSR (76.1%) method. Mean PIC (polymorphism information content) for each of these marker systems (0.382 for RAPD and 0.379 for ISSR) suggested that both the marker systems were equally effective in determining polymorphism. Jaccard's pair wise similarity coefficients ranged from 0.16 to 0.69 (RAPD), 0.13 to 0.77 (ISSR) and 0.23 to 0.68 (combined RAPD and ISSR). These values showed the presence of high diversity at molecular level among the studied cultivars. Cluster analysis based on RAPD, ISSR and their combined data clearly discriminated the cultivars into different clusters. Result of principal component analysis was in agreement with cluster analysis with a few exceptions. The Mantel test revealed a highly significant correlation ($r=0.78$) between the two Jaccard's similarity matrices calculated using RAPD and ISSR marker systems. Therefore, these markers could be successfully used to assess genetic diversity with almost equal efficiency.

Sharma et al. (2012) combined the molecular genetics and phytochemical methods to investigate 35 cluster bean genotypes. The phenolic content in the methanolic extract of leaves of 35 different genotypes of [*Cyamopsis tetragonoloba* (L.) Taub.] were quantified by HPLC. Total phenolic contents in all cultivar were in the range of 78.5 to 204 μggfw^{-1} . Polyphenols such as sinnapic acid, cholorogenic acid, caffeic acid and gallic acids were detected. It can be observed that flavanoids like kaempferol and myricetin were showing variations among all cultivars. Out of forty RAPD primers selected twenty primers amplified 164 bands, 147 of which were polymorphic

(90.3%). Polymorphic band number varied from 3 (RP-3) to 12 (RP-44). Similarly ten selected ISSR primers produced 105 amplified products, 102 of which were polymorphic (97%). Polymorphic band no. varied from 5 (IS-14) to 17 (IS-7). The calculated discriminatory power values for both RAPD and ISSR markers ranged from 0.40 to 0.98 (RAPD) and 0.44 to 0.99 (ISSR) respectively. Genetic relationships were estimated using similarity coefficient (Jaccard's) values between cultivars; these varied from 0.38 to 0.91 for RAPD, and from 0.20 to 0.88 with ISSR. The value of correlation coefficient from Mantel correlation test $r = 0.69$, shows good correlation between RAPD and ISSR. The markers generated by RAPD and ISSR can provide practical information for the management of genetic resources and these results will also provide useful information for the molecular classification and breeding of new cluster bean varieties.

Studies were undertaken by **Ajit *et al.* (2013)** to evaluate the genetic diversity among fifteen genotypes of *Cyamopsis tetragonoloba* Taub. L by using RAPD technique. A total of three primers generated 19 amplicons with an average of 6.33 amplicons per primer. Of the total amplicons, 15 amplicons were found polymorphic and showed 78.94% polymorphism. Dendrogram constructed by cluster analysis of RAPD markers using Unweighted Pair Group Method of Arithmetic Averages (UPGMA) showed two major and four minor clusters. The maximum genetic similarity up to 98% was observed between the genotypes AVTG-2 and IVTG-8. Overall, the present study revealed the existence of considerable genetic variations among the genotypes.

Aswathnarayana *et al.* (2013) stated the amplification with RAPD primers and produced a total of 118 bands, out of which 103 were polymorphic. Out of the 13 primers used, OPQ-09 produced the highest number of bands (12). The average percentage polymorphism for RAPD markers was 87.63. Seven ISSR markers produced 64 bands out of which 50 were polymorphic. Among the ISSR primers used UBC-868 produced highest number of bands (13); the average percentage polymorphism for ISSR markers was 77.82. UPGMA tree was constructed using Jaccard's similarity for RAPD, ISSR and RAPD+ISSR data. RAPD and RAPD+ISSR showed separate clustering of landraces from commercial varieties. AMOVA showed the presence of higher variance within the populations than between the populations.

Mohanty (2013) found the analysis of the mutual genotypic relationships among twenty four genotypes of *P. tetragonolobus* through Mantel test found a significant correlation ($r = 0.839$) between similarity matrices of the results obtained from the use of the RAPD and ISSR molecular markers.

Patel et al. (2013) studied the present investigation to find out polymorphic molecular marker in 12 genotypes of cluster bean. Among different 40 RAPD primers, OPA 7, OPA 10 and OPA 12 revealed highest polymorphism (75%) while OPB 13 evinced the lowest polymorphism (37.5%) and 65 bands were polymorphic giving 58.55% polymorphism. The genetic similarities ranged from 0.52 to 0.88 and the average genetic similarity among these 12 genotypes was 0.70. This could be helpful in selection of diverse parents for breeding purpose though it needs confirmation of true association with the character in segregating generations.

Sharma and Sharma (2013) reported chemo profiling and molecular phylogenetic studies that have received considerable attention and used simultaneously in higher plants to characterize the plant species. Guar or cluster bean, (*Cyamopsis tetragonoloba* (L.) Taub) is a drought-tolerant annual legume crop. India is the world-leader for cluster bean production as it contributes 80% shares of its total production. The aim of the present work is to determine and evaluate the degree of polymorphism in cultivars grown in Rajasthan using RAPDs and to explore the correlation among RAPD and chemical markers in five varieties of guar RGC-936, 1002, 1003, 1031 and 1017 were taken. Phenolic acids such as sinapic acid, chlorogenic acid, caffeic acid and gallic acids were detected among all cultivars. Whereas flavanoids i.e. kaempferol and myricetin were showing variations among all cultivars. Polyphenols are powerful antioxidant and flavonoids play role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The phytochemical analysis of guar may expand its nutraceutical and pharmaceutical utilization and information from this study will be useful to breeding programmes for improving guar seed quality.

Patel et al. (2014) determined the investigation deal with the genomic DNA optimization and PCR standardization for RAPD analysis in cluster bean. The standard CTAB method for isolation of genomic DNA from cluster bean have their own limitation as it produces gel image with high contamination of yellowish, sticky and viscous matrix. The commonly used CTAB method was modified by giving Polyvinylpyrrolidone (PVP) treatments after grinding juvenile leaf. The yield of genomic DNA was increased in ranged from 100 ng/μl to 200 ng/μl of 0.1 g leaf tissue and the purity (ratio) was between 1.7-1.8 indicating minimal levels of contaminating metabolites. RAPD protocol was optimized after implementing various modifications.

Sharma et al. (2014) studied the on 35 genotypes of cluster bean which were collected from different states of India and analyzed using RAPD and ISSR markers. Further SCAR marker system was introduced in order to increase the reproducibility of the polymorphism and specificity. For this polymorphic (RP-3, 1000 bp; RP-19, 1250 bp and 1100 bp) and geographical specific bands (RP-9, 650 bp) from RAPD as well as genotype specific band (IS-8, 550 bp) from genotype RGC-1031 (Rajasthan) from ISSR were selected and converted into SCAR markers.

Sharma et al. (2014) reported the study, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers were used to estimate genetic diversity and relationships among 35 cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] genotypes. For ISSR analysis, 10 primers were used which produced 105 bands, 102 of which were polymorphic (97%). The size of amplified bands ranged from 450 to 3,500 bp. The efficiency of primers in generating sufficient information for genetic diversity analysis was computed using discriminatory power (Dj), which ranged from 0.40 to 0.98 for RAPDs and 0.44 to 0.99 for ISSRs. Jaccard similarity coefficients were used to estimate the genotypic association with each other, which varied from 0.38 to 0.91 for RAPDs and from 0.20 to 0.88 for ISSRs. Cluster analysis indicated that all 35 genotypes could be distinguished by both RAPD and ISSR markers. Both of the methods (RAPD and ISSR) showed significant correlation ($r=0.69$), implying their equal importance in cluster bean diversity analysis.

The literature pertaining to phylogenetic analysis is as following:

2.5.2 Phylogenetic analysis:

Phylogenetic analysis is the study of relationship among species or genes with the combination of molecular biology and mathematics. Most of the present phylogenetic analysis software and algorithms have limitations of low accuracy, restricting assumptions on size of the dataset, high time complexity, complex results which are difficult to interpret and several others which inhibits their widespread use by the researchers.

Phylogenetic analysis represent the evolutionary relationship provides a better understanding of the evolutionary process and the non-tree like events such as horizontal gene transfer, hybridization, recombination and homoplasy.

Fatokun *et al.* (1993) studied on the phylogenetic relationship and genetic diversity among and within *Vigna* species were first assessed by restriction fragment length polymorphism (RFLP) DNA markers.

Mimura *et al.* (2000) found the accession, PI-527686 was previously determined to be the wild form (or progenitor) of cultivated adzuki bean and the phylogeny supports this notion.

Choi *et al.* (2006) reported gene-derived markers across 15 legumes for assessing the phylogenetic relationships and genetic diversity among and within *Vigna* species.

Liwang *et al.* (2008) studied on the interspecific phylogenetic relationships and assess their genetic diversity, 48 accessions represent 12 *Vigna* species were selected, and 30 gene-derived markers from legumes were employed.

The literature pertaining to genome mapping is as following:

2.5.3 Genome mapping:

Over 25 linkage maps, mostly low density (markers on average every 10cM), have been developed for common bean (Kelly *et al.*, 2003; Miklas *et al.*, 2006). To maximize molecular polymorphism, the majority of mapping populations were derived from crosses between domesticated parents belonging to the Andean vs.

Middle American gene pools. For specialized purposes, some maps were developed by crossing parents within a gene pool but polymorphism was low.

A highly polymorphic core map utilizing a recombinant inbred population from the cross BAT93 x Jalo EEP558 (Nodari *et al.*, 1992) was developed to coalesce the mapping data (Freyre *et al.*, 1998). BAT93 is a breeding line from the Mesoamerican gene pool, and Jalo EEP558 is an Andean cultivar resulting from selection in a Brazilian landrace. The two parents show contrasting resistances to pathogens. Some 600 markers have been mapped directly in this population, including 71 RFLPs, 161 AFLPs, 158 RAPDs, 50 ISSRs, and 200 microsatellites (Freyre *et al.*, 1998; Papa and Gepts, 2003; González *et al.*, 2005; Blair *et al.*, 2003; Grisi *et al.*, 2007). Shared markers, principally RFLPs and sequence-tagged markers, are used to correlate linkage groups among the different maps. Recently, this map was greatly extended by the addition of 300 gene-based markers (McConnell *et al.*, 2007). These will be very useful to correlate the genetic map with the physical map.

ISSR markers are unmapped but can be used to saturate RFLP and SSR linkage maps. The RFLP map of barley was saturated with 60 ISSRs (referred as RAMPs in the study) which mapped to all chromosomes (Becker and Heun, 1995). Many of these markers are mapped in between clustered RFLPs, flanking RFLP clusters, at the tips of chromosomes and more importantly in areas of low RFLP marker density. In *Einkorn* wheat, however, the nine ISSR markers mapped at or close to the RFLP marker positions (Kojima *et al.*, 1998). ISSRs have also been used along with AFLP and RAPD markers in the mapping of Japanese and European larch genomes (Arcade *et al.*, 2000).

Nodari *et al.* (1992) suggested a highly polymorphic core map utilizing a recombinant inbred population from the cross BAT93 x Jalo EEP558.

Kaga *et al.* (2000) studied comparative molecular mapping in *Ceratotropis* species using interspecific crosses between adzuki bean (*Vigna angularis*) and rice bean (*Vigna umbellate*). They have developed a genetic linkage map with 86 F₂ derived plants from inter specific cross between adzuki bean (*Vigna angularis*) and rice bean (*Vigna umbellate*).

Blair et al. (2003) developed international common bean maps that have shown high polymorphic levels permitting their wide saturation. The polymorphic rate detected in the present map was approximately 4.2 times smaller (13.4% versus 56%) than that found on the map in french bean.

Kelly et al. (2003) reported over 25 linkage maps, mostly low density (markers on average every 10 cM), have been developed for common bean.

Garris et al. (2005) depicted high degree of similarity between different cereal and legume genomes in terms of gene content and gene order facilitates crop improvement and breeding for other crops as well.

Wright et al. (2005) recently reported on random genome-wide scans and identified genes positively associated with domestication and/or agronomic productivity in french bean.

Blair et al. (2006) obtained the total distance of the map and was estimated as 840.7 cM, approximately 68.6% of the value of 1226 cM of the core linkage map established by Freyre et al., (1998), and similar to the value of 869.5 cM in french bean.

Grisi et al. (2007) allowed us to relate the three linkage groups that obtained in this study with the chromosomes of the *P. vulgaris* species.

Ram et al. (2007) stated amongst these, SSRs and RFLPs are co-dominant in nature and their genetic map location on crop genome is publicly disclosed; in case of AFLP and RAPD, they produce random amplification and are largely dominant markers in nature.

Agarwal et al. (2008) found the AFLP technique generates fingerprints of DNA from any source, and without any prior knowledge of DNA sequence. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited greatly in genetic and physical mapping.

Schmutz *et al.* (2009) reported the second factor producing different genome sizes is the size of the repeat-rich, gene-poor, recombination-suppressed pericentromeres in the three genomes. In soybean, these are remarkably large, comprising nearly two-thirds of the total genome sequence.

Varshney *et al.* (2009) studied the genome sequences that can be leveraged to improve orphan crops. Many legumes are important regional food sources, but investment in genetic and genomic tools is limited—for example, common bean, pigeon pea, cowpea and lentil.

Ferreira *et al.* (2010) depicted the mapping population that consisted of 94 F₂ generation plants derived from a cross between the CNFC 7812 and CNFC 8056 lines, with different protein contents, 24% and 19% respectively. Seven hundred and fifty-two molecular markers were tested among the parents and four individuals from the segregants population. A total of 101 loci were used to develop the genetic map. The polymorphism rate was 8.3% and 23.2% for the microsatellite and RAPD markers, respectively. The sizes of the linkage groups ranged from 6.7 to 139.0 cM, presenting a mean of 49.4 ± 36.8 . The map length was 840.7 cM and the mean group length was 45.9 cM. The average distance between the framework loci was 16.1 cM in french bean.

Suzuki *et al.* (2012) determine the advancement in next generation genome sequencing (NGS) technologies have made it a realistic and viable proposition to develop new molecular markers based on the genome sequence data.

Ashfield *et al.* (2012) suggested the Pv04 cluster containing the Co-10 and Phg-ON genes has a homologous cluster in the soybean genome.

Deschamps *et al.* (2012) reported that marker to be powerful tools for wide ranging applications in many plant breeding areas, notably, identification and analysis of quantitative/qualitative trait loci (QTLs) and their positioning on linkage maps; cloning of genes for desirable traits based on the molecular linkage maps; gene

pyramiding and Marker-assisted selection (MAS), the determination and analysis of genetic diversity within germplasm and other plant collections and analysis of genome structures for several crop plants.

Vidigal (2013) studied the molecular marker g2303 which was linked to Co-10 and Phg-ON at a distance of 0.0 cM because of their physical linkage in a cis configuration; the Co-10 and Phg-ON resistance alleles are inherited together and can be monitored with great efficiency using g2303. The close linkage between the Co-10 and Phg-ON genes and prior evidence are consistent with the existence of a resistance gene cluster at one end of chromosome Pv04, which also contains the Co-3 locus and ANT resistance quantitative trait loci. These results will be very useful for breeding programs aimed at developing bean cultivars with ANT and ALS resistance using marker-assisted selection.

Schmutz (2014) assembled 473 Mb of the 587 Mb genome and genetically anchored 98% of this sequence in 11 chromosome-scale pseudomolecules. Compared the genome for the common bean against the soybean genome to find changes in soybean resulting from polyploidy. Using resequencing of 60 wild individuals and 100 landraces from the genetically differentiated Mesoamerican and Andean gene pools, we confirmed 2 independent domestications from genetic pools that diverged before human colonization. Less than 10% of the 74 Mb of sequence putatively involved in domestication was shared by the two domestication events. We identified a set of genes linked with increased leaf and seed size and combined these results with quantitative trait locus data from Mesoamerican cultivars. Genes affected by domestication may be useful for genomics-enabled crop improvement.

Sharma *et al.* (2014) suggested the first set of sequence-based SCAR markers in cluster bean which found more specific information using RAPD and ISSR profiles. One genotype specific SCAR-20 for RGC-1031 (tolerant genotype against *Macrophomina phaseolina*), could be used to prove identity of the genotype for improvement as well for its genetic purity assessment.

MATERIALS AND METHODS

CHAPTER -III

The present investigation entitled “Studies on Genetic Diversity and Variability in Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Genotypes Through Morphological and Molecular Characterization” was conducted at the Horticultural Research Farm-I, of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya Vihar, Rae Bareli Road, Lucknow, (U.P.) during the Rabi Season of 2012-2013 and 2013-2014). The studies on Molecular diversity of selected cluster bean genotypes by using 21 ISSR markers and molecular characterization of 30 genotypes accessions were carried out in the Department of Biotechnology at Babasaheb Bhimrao Ambedkar University, Vidya-Vihar, Rae Bareli Road, Lucknow. Further, the PCR amplification work was done at Central Soil Salinity Research Institute, Regional Research Station, Biotechnology Lab, Lucknow, (U.P.) during the year 2012-2013 and 2013-2014. The details methodology adopted in this investigation has been presented under the following heads:

3.1 Location and weather conditions

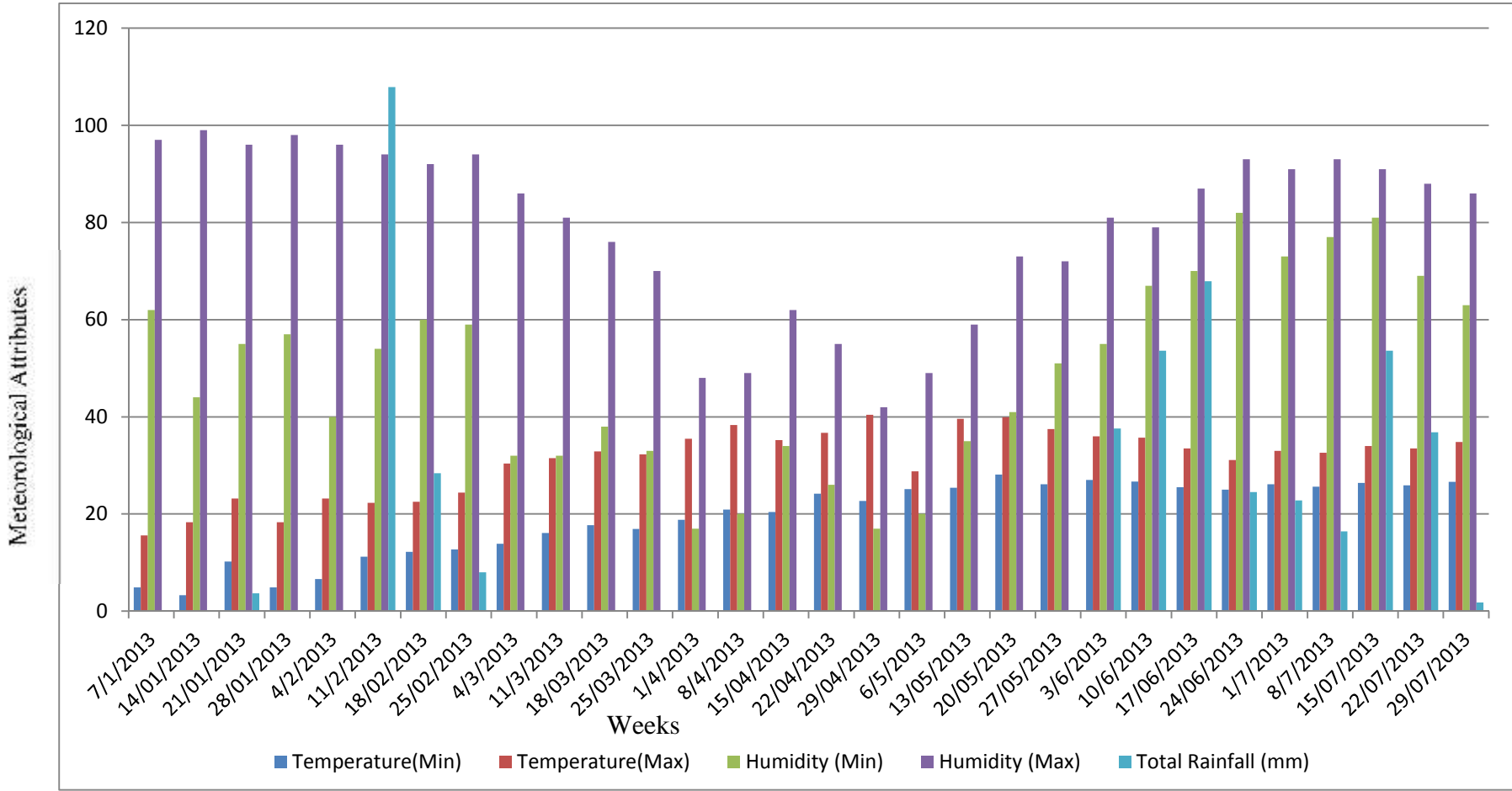
Geographically, Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya-Vihar, Rae Bareli Road, Lucknow is situated at 80⁰ 55' East longitude and 26⁰ 46' North latitude and 129 meter above MSL (mean sea level) and located approximate 5 km away from Chaudhary Charan Singh International Air-Port and 10 km away from the Lucknow Railway Station, Charbagh towards South-East on Lucknow, Rae Bareli Road, Lucknow. However, university is also well connected with Bijnor-Bangla Bazaar-Jail Road and Kanpur Road, Respectively. The climate of Lucknow is characterized by sub-tropical with hot, dry summer and cool winters. This region received an averages annual rainfall of 750 mm, which is distributed over a period of more than 100 days with peak period during July-August. It also received scattered showers during winter months. In general, the temperature ranges from 5.5 to 25°C. The coldest month is January, while the maximum temperature is observed during May-June. The averages relative humidity is 50-70% in different season of the

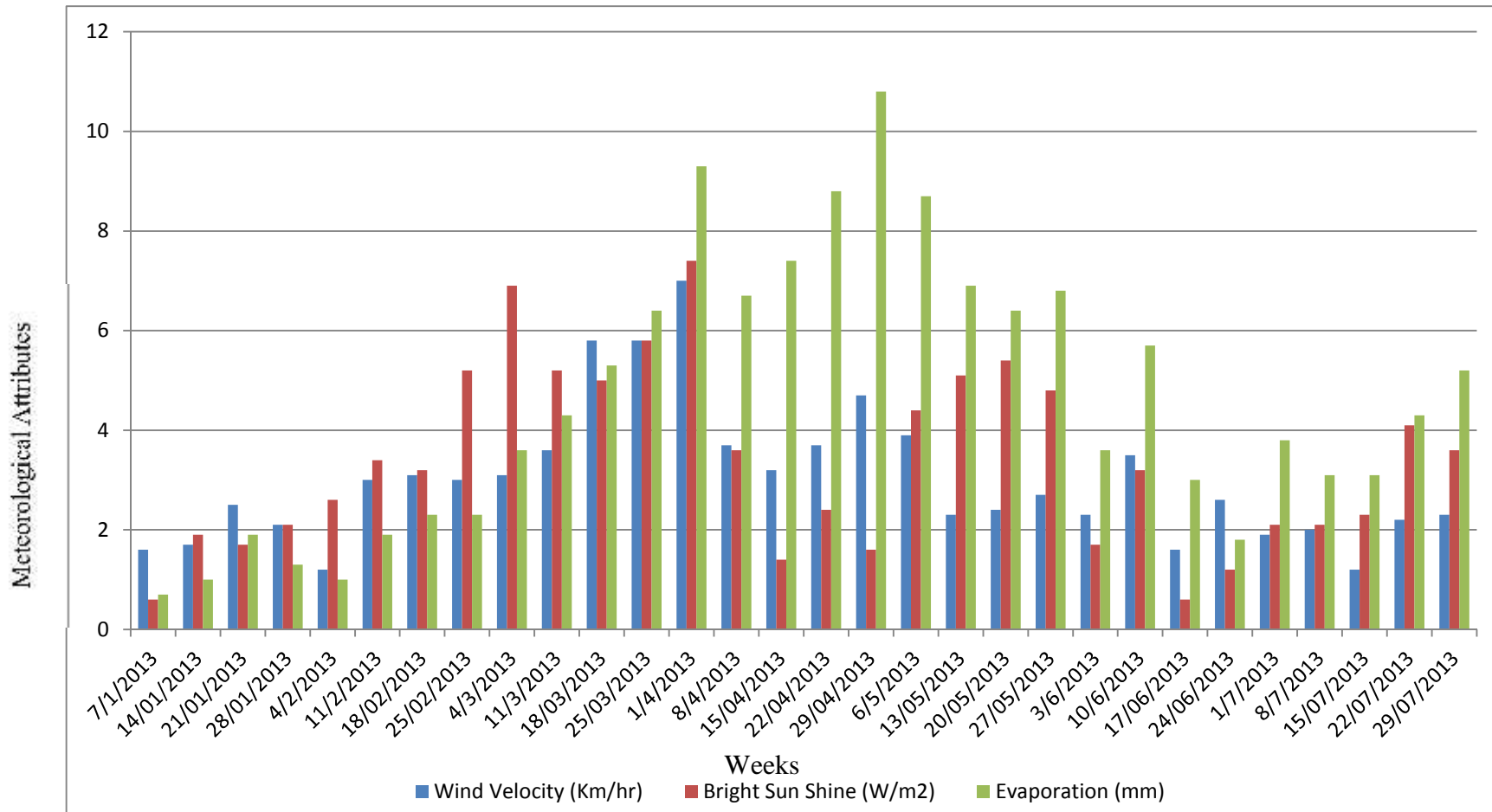
year. The weekly weather conditions prevailing during experimental period are given in Table- 3.1 and 3.2 and also shown in Graph- 3.1 and 3.2. The soil of the experiment site was a saline-alkaline, low in organic matter, nitrogen, phosphorus and also low potash in the soil.

**Table- 3.1 Weekly average meteorological data recorded during the experiment
(January 2013 to July 2013)**

Weeks	Temperature (°C)		Relative Humidity (%)		Total Rainfall (mm)	Wind Velocity (Km/hr)	Bright Sun Shine (W/m ²)	Evaporation (mm)
	Max	Min	Max	Min				
07/01/2013	15.6	4.90	97.00	62.00	0.00	1.60	0.60	0.70
14/01/2013	18.3	3.30	99.00	44.00	0.00	1.70	1.90	1.00
21/01/2013	23.20	10.20	96.00	55.00	3.70	2.50	1.70	1.90
28/01/2013	18.30	4.90	98.00	57.00	0.00	2.10	2.10	1.30
04/02/2013	23.20	6.60	96.00	40.00	0.00	1.20	2.60	1.00
11/02/2013	22.30	11.20	94.00	54.00	107.90	3.00	3.40	1.90
18/02/2013	22.50	12.20	92.00	60.00	28.40	3.10	3.20	2.30
25/02/2013	24.40	12.70	94.00	59.00	8.00	3.00	5.20	2.30
04/03/2013	30.40	13.90	86.00	32.00	0.00	3.10	6.90	3.60
11/03/2013	31.5	16.1	81.00	32.00	0.00	3.60	5.20	4.30
18/03/2013	32.90	17.70	76.00	38.00	0.20	5.80	5.00	5.30
25/03/2013	32.30	16.90	70.00	33.00	0.20	5.80	5.80	6.40
01/04/2013	35.50	18.80	48.00	17.00	0.00	7.00	7.40	9.30
08/04/2013	38.30	20.90	49.00	20.00	0.00	3.70	3.60	6.70
15/04/2013	35.20	20.40	62.00	34.00	0.00	3.20	1.40	7.40
22/04/2013	36.70	24.20	55.00	26.00	0.00	3.70	2.40	8.80
29/04/2013	40.40	22.70	42.00	17.00	0.00	4.70	1.60	10.80
06/05/2013	28.80	25.10	49.00	20.00	0.00	3.90	4.40	8.70
13/05/2013	39.60	25.40	59.00	35.00	0.00	2.30	5.10	6.90
20/05/2013	39.90	28.1	73.00	41.00	0.00	2.40	5.40	6.40
27/05/2013	37.50	26.10	72.00	51.00	0.00	2.70	4.80	6.8
03/06/2013	36.00	27.00	81.00	55.00	37.60	2.30	1.70	3.60
10/06/2013	35.70	26.70	79.00	67.00	53.60	3.50	3.20	5.70
17/06/2013	33.50	25.50	87.00	70.00	67.90	1.60	0.60	3.00
24/06/2013	31.10	25.00	93.00	82.00	24.50	2.60	1.20	1.80
01/07/2013	33.00	26.10	91.00	73.00	22.80	1.90	2.10	3.80
08/07/2013	32.60	25.60	93.00	77.00	16.40	2.00	2.10	3.10
15/07/2013	34.00	26.40	91.00	81.00	53.60	1.20	2.30	3.10
22/07/2013	33.50	25.90	88.00	69.00	36.80	2.20	4.10	4.30
29/07/2013	34.80	26.60	86.00	63.00	1.80	2.30	3.60	5.20

Source: ICAR-Indian Institute of Sugarcane Research, Dilkusha, Raebareli Road, Lucknow



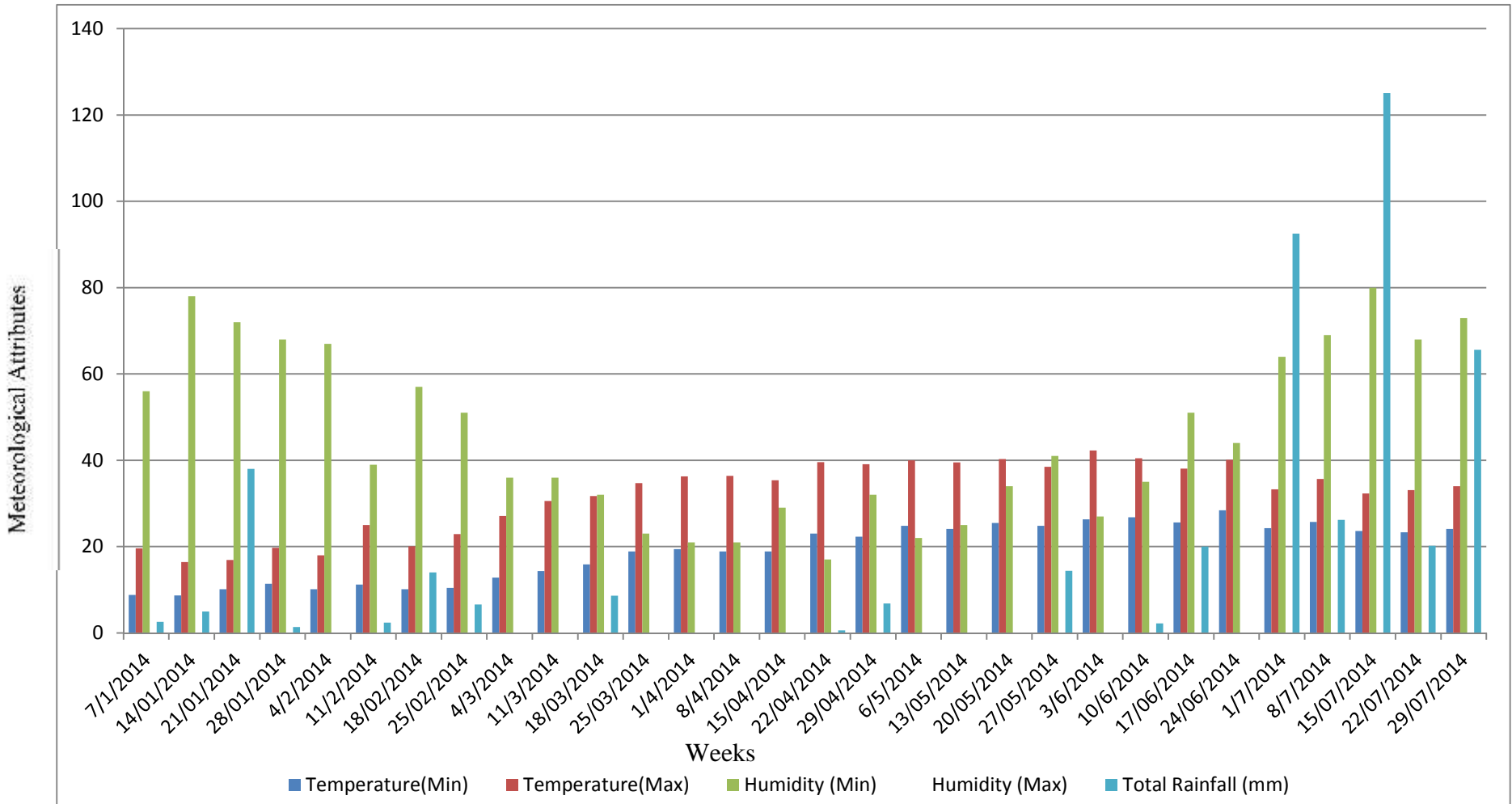


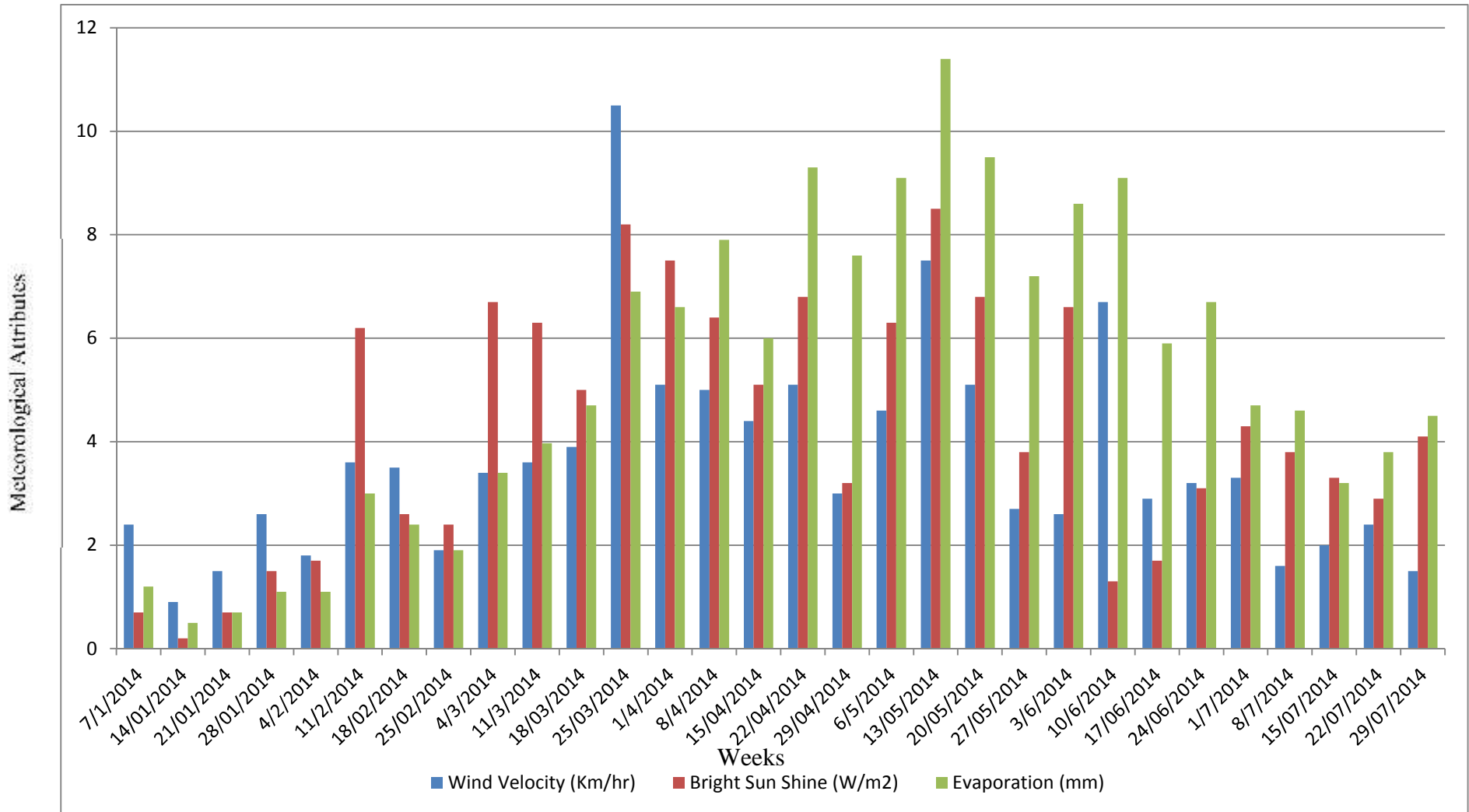
Graph-3.1 Weekly average meteorological data recorded during the experiment (January 2013 to July 2013)

**Table- 3.2 Weekly average meteorological data recorded during the experiment
(January 2014 to July 2014)**

Weeks	Temperature (°C)		Relative Humidity (%)		Total Rainfall (mm)	Wind Velocity (Km/hr)	Bright Sun Shine (W/m ²)	Evaporation (mm)
	Max	Min	Max	Min				
07/01/2014	19.60	8.80	95.00	56.00	2.60	2.40	0.70	1.20
14/01/2014	16.40	8.70	98.00	78.00	5.00	0.90	0.20	0.50
21/01/2014	16.90	10.10	97.00	72.00	38.00	1.50	0.70	0.70
28/01/2014	19.70	11.40	96.00	68.00	1.40	2.60	1.50	1.10
04/02/2014	18.00	10.10	94.00	67.00	0.00	1.80	1.70	1.10
11/02/2014	25.00	11.20	92.00	39.00	2.40	3.60	6.20	3.00
18/02/2014	20.10	10.10	91.00	57.00	14.00	3.50	2.60	2.40
25/02/2014	22.90	10.40	95.00	51.00	6.60	1.90	2.40	1.90
04/03/2014	27.10	12.80	86.00	36.00	0.00	3.40	6.70	3.40
11/03/2014	30.60	14.30	82.00	36.00	0.00	3.60	6.30	3.97
18/03/2014	31.70	15.90	77.00	32.00	8.60	3.90	5.00	4.70
25/03/2014	34.70	18.90	67.00	23.00	0.00	10.50	8.20	6.90
01/04/2014	36.30	19.40	62.00	21.00	0.00	5.10	7.50	6.60
08/04/2014	36.40	18.90	55.00	21.00	0.00	5.00	6.40	7.90
15/04/2014	35.40	18.90	57.00	29.00	0.00	4.40	5.10	6.00
22/04/2014	39.60	23.00	45.00	17.00	0.60	5.10	6.80	9.30
29/04/2014	39.10	22.30	58.00	32.00	6.80	3.00	3.20	7.60
06/05/2014	40.00	24.80	49.00	22.00	0.00	4.60	6.30	9.10
13/05/2014	39.50	24.10	49.00	25.00	0.00	7.50	8.50	11.40
20/05/2014	40.30	25.50	52.00	34.00	0.00	5.10	6.80	9.50
27/05/2014	38.50	24.80	71.00	41.00	14.40	2.70	3.80	7.20
03/06/2014	42.30	26.30	69.00	27.00	0.00	2.60	6.60	8.60
10/06/2014	40.50	26.80	65.00	35.00	2.20	6.70	1.30	9.10
17/06/2014	38.10	25.60	81.00	51.00	20.00	2.90	1.70	5.90
24/06/2014	40.10	28.40	66.00	44.00	0.00	3.20	3.10	6.70
01/07/2014	33.30	24.30	84.00	64.00	92.50	3.30	4.30	4.70
08/07/2014	35.70	25.70	86.00	69.00	26.20	1.60	3.80	4.60
15/07/2014	32.30	23.60	92.00	80.00	125.10	2.00	3.30	3.20
22/07/2014	33.10	23.30	89.00	68.00	20.20	2.40	2.90	3.80
29/07/2014	34.00	24.10	90.00	73.00	65.60	1.50	4.10	4.50

Source: ICAR-Indian Institute of Sugarcane Research, Dilkusha, Raebareli Road, Lucknow





Graph- 3.2 Weekly average meteorological data recorded during the experiment (January 2014 to July 2014)

3.2 Genotypes/lines

For present investigation 30 genotypes of cluster bean were carried out. Seeds of the genotypes were collected from the two places (especially, National Bureau Plant Genetic Resources (Indian Council of Agricultural Research), Regional Station, Jodhpur, India and Punjab Agricultural University, Ludhiana, India) and maintained at Horticulture Research Farm-I, of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Vidya-Vihar, Rae Bareilly Road, Lucknow, respectively. National Bureau Plant Genetic Resources (Indian Council of Agricultural Research), Regional Station, Jodhpur, India is geographically situated at (26°15' N 73°00' E, 23.5 M.S.L.) and was kept for 1 day for proper acclimatization of genotypes. The genotypes of cluster bean were sown during the last week of March 2013 and first week of March 2014.

3.3 Experimental Materials

3.3.1 Details of experiment

The experimental materials for the study comprised of different diverse germplasms of cluster bean were sown in normal condition and laid out in Randomized Block Design with three replications.

Table- 3.3 Name of 30 cluster bean genotypes and their collection sources

S. NO.	Genotypes/Variety	Symbol	Source
1.	IC- 258087	G ₁	NBPGR, RS, Jodhpur
2.	IC-258092	G ₂	NBPGR, RS, Jodhpur
3.	IC-28272	G ₃	NBPGR, RS, Jodhpur
4.	IC-311440	G ₄	NBPGR, RS, Jodhpur
5.	IC-311441	G ₅	NBPGR, RS, Jodhpur
6.	IC-325757	G ₆	NBPGR, RS, Jodhpur
7.	IC-329038	G ₇	NBPGR, RS, Jodhpur
8.	IC-369789	G ₈	NBPGR, RS, Jodhpur
9.	IC-369868	G ₉	NBPGR, RS, Jodhpur
10.	IC-370478	G ₁₀	NBPGR, RS, Jodhpur
11.	IC-370490	G ₁₁	NBPGR, RS, Jodhpur
12.	IC-373427	G ₁₂	NBPGR, RS, Jodhpur
13.	IC-373480	G ₁₃	NBPGR, RS, Jodhpur
14.	IC-402293	G ₁₄	NBPGR, RS, Jodhpur
15.	IC-415137	G ₁₅	NBPGR, RS, Jodhpur
16.	IC-415142	G ₁₆	NBPGR, RS, Jodhpur
17.	IC-415157	G ₁₇	NBPGR, RS, Jodhpur
18.	IC-415159	G ₁₈	NBPGR, RS, Jodhpur
19.	IC-421242	G ₁₉	NBPGR, RS, Jodhpur
20.	IC-421798	G ₂₀	NBPGR, RS, Jodhpur
21.	IC-421806	G ₂₁	NBPGR, RS, Jodhpur
22.	IC-421809	G ₂₂	NBPGR, RS, Jodhpur
23.	IC-421812	G ₂₃	NBPGR, RS, Jodhpur
24.	IC-421815	G ₂₄	NBPGR, RS, Jodhpur
25.	IC-421820	G ₂₅	NBPGR, RS, Jodhpur
26.	IC-421828	G ₂₆	NBPGR, RS, Jodhpur
27.	IC-421834	G ₂₇	NBPGR, RS, Jodhpur
28.	IC-421838	G ₂₈	NBPGR, RS, Jodhpur
29.	IC-421855	G ₂₉	NBPGR, RS, Jodhpur
30.	HG-365 (Durgajay × Hisar Local)	G ₃₀	PAU, Ludhiana

3.4 Methods

3.4.1 Layout plan

Experimental field was laid out in randomized block design with 30 genotypes/lines and replicated thrice. Each block was further subdivided into 30 unit plots. The thirty genotypes were allotted to the 30 unit plots of each block. The plots were raised by 15cm from the ground level to avoid water-logging, if occurred. The unit plot size was 2.70m X 1.20m, and the row-to-row and plant-to-plant spacing was 45cm and 30cm, respectively. Thus, all together, there were total 90 plots were made to accommodate thirty genotypes.

3.4.2 Details of layout

Design:	RBD
Number of genotypes/lines:	30
Replication:	3
Total plots:	90
Net plot size:	2.7 x 1.2 m
Gross plot size	3.00 x1.50m
Row to row distance:	45 cm
Plant to plant distance:	30 cm
Number of plants per row:	4
Number of rows per plot:	6
Number of plants per plot:	24
Total number of plants per plot:	2160

(A)



Plate (A) Showing collection of 29 cluster bean genotypes from NBPGR, Regional Station, Jodhpur, Rajasthan

(B)



Plate (B) Showing collection of another one genotype from PAU, Ludhiana, Punjab

Plate 1 (A) Showing collection of 29 cluster bean genotypes from NBPGR, Regional Station, Jodhpur, Rajasthan

(B) Showing collection of another one genotype from PAU, Ludhiana, Punjab

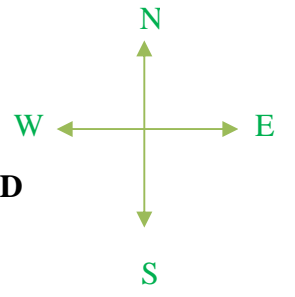
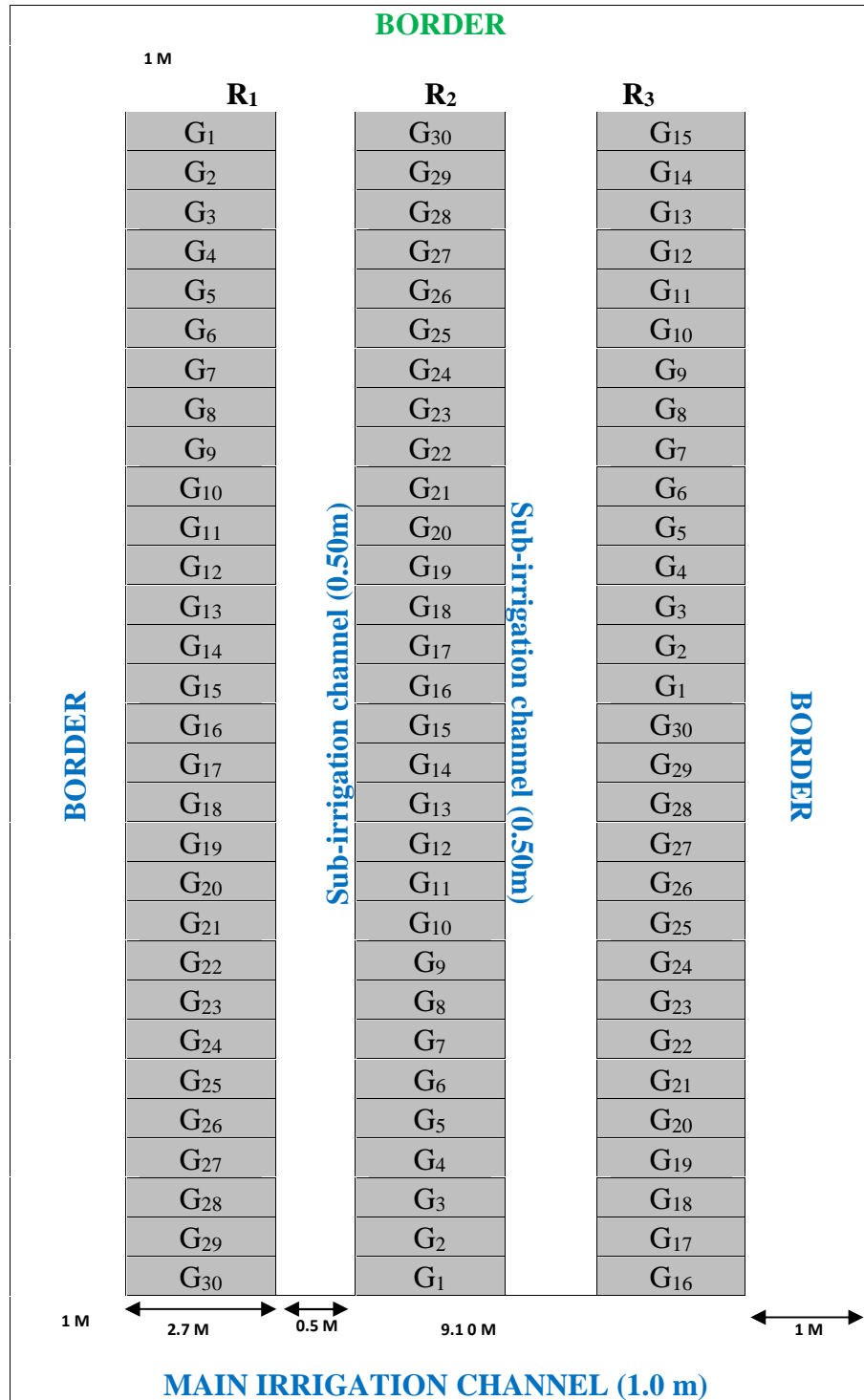


Figure 3.4.2 LAYOUT PLAN OF EXPERIMENTAL FIELD



38 M

11.1 M

**(B) Field view of layout plan and preparation of experimental field during 2013-
Plate 2 (A) Field view of layout plan and preparation of experimental field during
2012-13 (B) Field view of layout plan and preparation of experimental field during
2013-14**



(A) Field view of first year research trial during 2012-2013



(B) Field view of second year research trial during 2013-2014

Plate 3 (A) Field view of first year research trial during 2012-2013

(B) Field view of second year research trial during 2013-2014

3.4.3 Soil

It is a shallow rooted crop with surface feeding nature. The crop prefers well-drained sandy loam soil. It can tolerate saline and moderately alkaline soils with pH

ranging between 7.5 and 8.0 and in heavy soils bacterial nodulation is inhibited (Venkataratnam, 1973).

3.4.4 Soil analysis

The soil samples collected from different spots of the cluster bean field at the Horticulture Research Farm-I. The samples collected with the help of soil auger from 0-120 cm depth. The collected samples then mixed together, dried in air, powdered and again samples were mixed thoroughly. The representative samples, each of 5 g soil for each analysis were taken and the same were analyzed at the Laboratory of Central Soil Salinity Research Institute, Regional Research Station, Lucknow, Uttar Pradesh. Analysis of soil samples was taken plot wise for pH, organic carbon, available N₂O, P₂O₅ and K₂O are presented in the Table- 3.4.4.

Table- 3.4.4 Physico-chemical properties of soil and their measuring unit

Physico-chemical properties	Measuring unit (%/kg/ha/mol/L)
pH	8.53
Organic Carbon (%)	0.40
Available Nitrogen (kg/ha)	348.03
Available Phosphorus (kg/ha)	19.36
Available Potassium (kg/ha)	113.08

Source: Central Soil Salinity Research Institute, Regional Research Station, Lucknow

3.4.5 Climate

Cluster bean is a typical tropical vegetable crop preferring warm climate even though it can be grown in the subtropics during summer. The extent to which the crop can stand the heat is substantiated by the fact that it is grown during summer in arid zones of Rajasthan and Haryana, India where the temperature may go up to 44° C, average range being 30-40° C. However, the Kharif crop yields better because irrigation has been found to enhance bud production (Rogers and Stafford, 1976). It prefers long-day conditions for growth and short-day conditions for induction of flowering. Because of more or less uniform climate prevailing throughout the year in South India, it is grown round the year.

3.4.6 Soil preparation and sowing

The soil is thoroughly prepared by ploughing and pulverizing it. The seeds are either broadcast or line sown at a distance of 25 to 30 cm (Venkataratnam, 1973). A spacing of 45 cm between rows gave higher yield than 30 or 60 cm apart (Jain *et al.*, 1987). Depending upon spacing and method of sowing, i.e., broadcasting or line sowing, the seed rate varies from 15 to 40 kg/ha.

The crop is grown from seeds. The seeds are sown on ridges also. The planting distance is 45cm x15cm. About 30-40 kg of seeds is required for sowing one hectare. In northern plains of India the sowing is done in February-March for summer crop and in June-July or July-August for rainy season crop. Sowing in southern India can be done almost throughout the year but May-June sowing is advocated for obtaining the pods in the lean period (Chauhan and Mangal, 1993). The early crop of cluster bean is sown in February-March and the main crop is sown in June-July and even up to September-October in some areas.

3.4.7 Manures and fertilizers

Cluster bean is a legume vegetable crop, it responds well to the application of fertilizers. Approximate 150-200 quintal FYM are applied to the soil with 50 kg N₂O, 50-70 kg P₂O₅ and 50-70 kg K₂O per hectare at the time of land preparation. Only half quantity of N₂O is applied at the time of field preparation and the other half quantity is top dressed at the time of flowering (Parthasarathy, 1986).

3.4.8 Irrigation

Cluster bean is a hardy crop that comes up well under rainfed conditions. Flowering and pod development periods are the critical stages. Depending on the atmospheric conditions two or three protective irrigations may have to be given. For higher yield the crop should be irrigated at 7-10 days interval. The crop may not require irrigation during rainy season. The summer crop is irrigated once in 7-10 days (Nath *et. al.*, 1994).

3.4.9 Intercultural operation and irrigation

A light irrigation was given immediately after germination. As sowing was done in *Rabi* season and being a legumes crop there were need of frequent irrigation. Weeding and hoeing were done as whenever required.

3.4.10 Harvesting and yield

The immature tender green pods are harvested for vegetable purposes. In the early varieties, the first pod picking starts in 45-50 days and in late varieties, 70-90 days. Dry seeds are harvested when a large percentage of pods are full and most of the remaining will turn yellow. It is to be harvested before the lower pods dry enough to start shattering. The crop duration for the early type is about 80-90 days and for the late type is 135-145 days (Ram, 1998). A vegetable yield of 5-8 tonnes and seed yield of 0.6 to 1.0 t/ha are expected within crop duration of 120 days.

3.5 Observations recorded

Observations were recorded for the following quantitative and qualitative traits on five plants basis and averages were computed.

Morphological Characterization:

3.6 Phenotypic characters

3.7 Growth attributing traits

3.7.1 Plant height (cm)

The length of the main stem from ground level to the tip of the growing point at 45th day was measured in centimeter (cm).

3.7.2 Germination (%)

Hundred seeds in three replications were drawn at random from each treatment and the germination test was conducted using between paper methods as per ISTA procedure (Anonymous, 1999). The rolled paper towels were placed in a cabinet and seed germination maintained at a constant temperature of at 25 ±10⁰C and 95 percent relative humidity. The number of normal seedlings were counted at end of ninth day and expressed in percentage.

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds put for germination}} \times 100$$

3.7.3 Days taken for first flowering

The number of days taken from the days of germination to opening of first flower.

3.6.4 Days taken for 50% flowering

The number of days taken from the day of germination to opening of 50 per cent flowering.

3.7.5 Number of reproductive branches/plant

Total number of reproductive branches per plant at pod development stage was taken.

3.7.6 Pod breadth (cm)

The maximum breadth of pods measured using Vernier Calliper for five pods was averaged and recorded in centimeters (cm).

3.7.7 Pod length (cm)

The length (cm) of each selected pod was measured from the base to tip and average over 5 pods was computed.

3.7.8 Pod width (cm)

The width of the selected pods was measured in mm at the centre of pods by using Vernier Calipers and average of over 5 pods was computed.

3.7.9 Number of pods/plant

Number of pods produced on each tagged plants were counted.

3.7.10 Number of pods/cluster

Numbers of pods produced on pod bearing clusters on tagged plants in each Genotype were counted at every harvesting.

3.7.11 Numbers of clusters/plant

Numbers of clusters produced on plants of tagged plants in each genotype were counted on 90 days after sowing



Plate 7. Observations recorded on morphological characters of cluster bean

3.8 Yield and yield attributing traits

3.8.1 Number of branches/plant at maturity

Total number of branches on the primary branch of the main shoot of the plant was recorded at the time of harvest.

3.8.2 Number of seeds/pod

Five dry pods from each experimental plot were randomly picked and seeds were counted and average number of seeds per pods was worked out.

3.8.3 Pod yield/plant (g)

Total pod yield of individual plant recorded over the successive harvests was summed up and expressed in gram (g).

3.8.4 Pod yield/plot (kg)

The seed separated from each plot was weighed and seed yield per plot is expressed in kilograms by using electronic balance.

3.8.5 100- Seed weight (g)

One hundred seeds in three replications from each treatment were counted and weight was recorded by using electronic balance as per ISTA procedure (Anonymous, 1999). The average weight was expressed in gram.

3.8.7 Seed yield/plant (g)

Average seed weight of 5 randomly selected plants at 13 % moisture.

3.8.8 Pod yield (q/ha)

The seed yield obtained from the net plot area of all the treatments and replications were dried and subjected for size grading by using 11.75 (R) top screen and 4.75 (S) bottom screen (Anonymous, 2004). The care was taken to add the seed yield of five random plants which were harvested separately to the net plot seed yield before calculating the seed yield per ha. The average seed yield was computed and expressed in quintal per ha.

$$\text{Yield per hectare (q)} = \frac{\text{Yield per plot (kg)} \times 10,000 \text{ (m}^2\text{)}}{\text{Net area of the plot (m}^2\text{)}}$$

3.8.9 Days to maturity

Number of days from seed sowing until 75 % of plants reaching physiological maturity.

3.8.10 Flower colour

Flower colour is measured with the help of standard colour chart of Royal Horticultural Society colour chart (white, purple and purplish white).



Plate 8. Observations recorded on yield and yield attributing characters of cluster bean

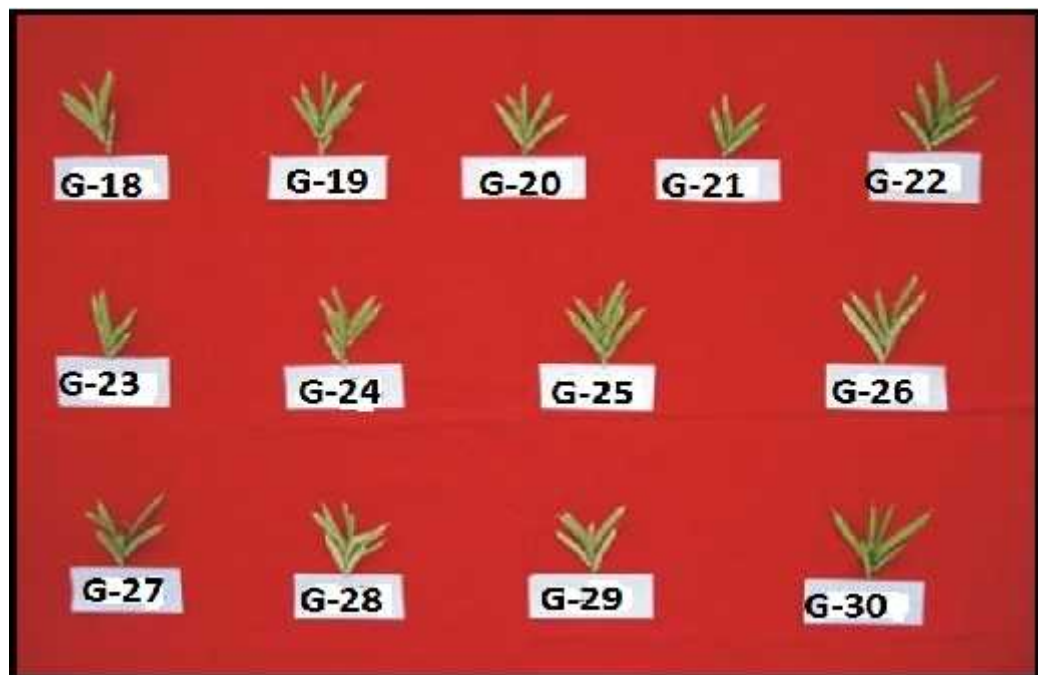
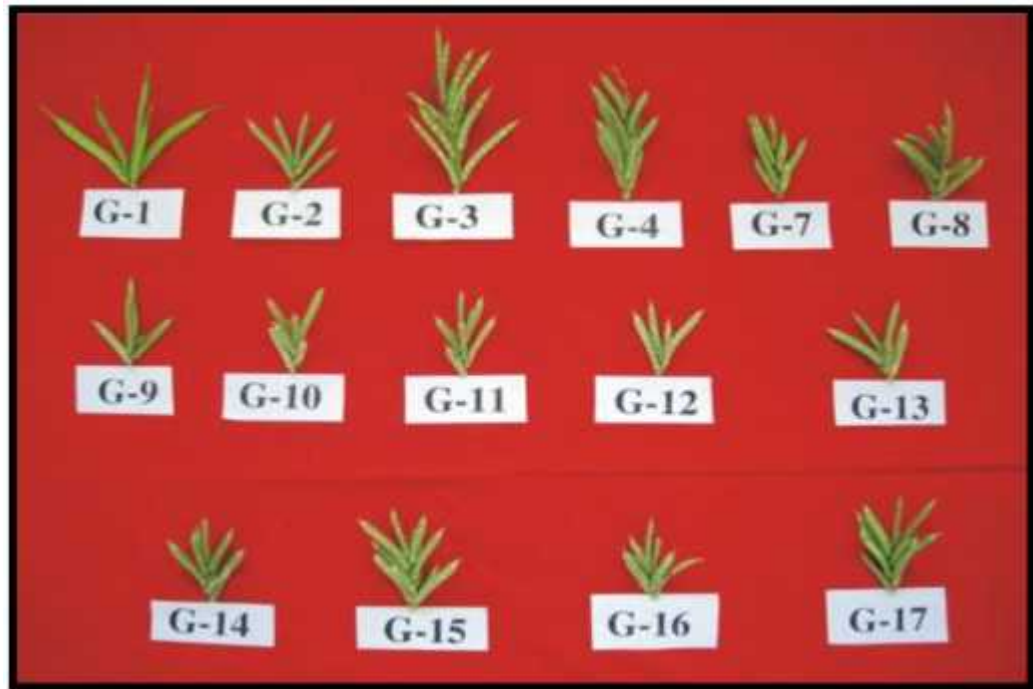


Plate 4. Variability in cluster characters of 30 genotypes in cluster bean

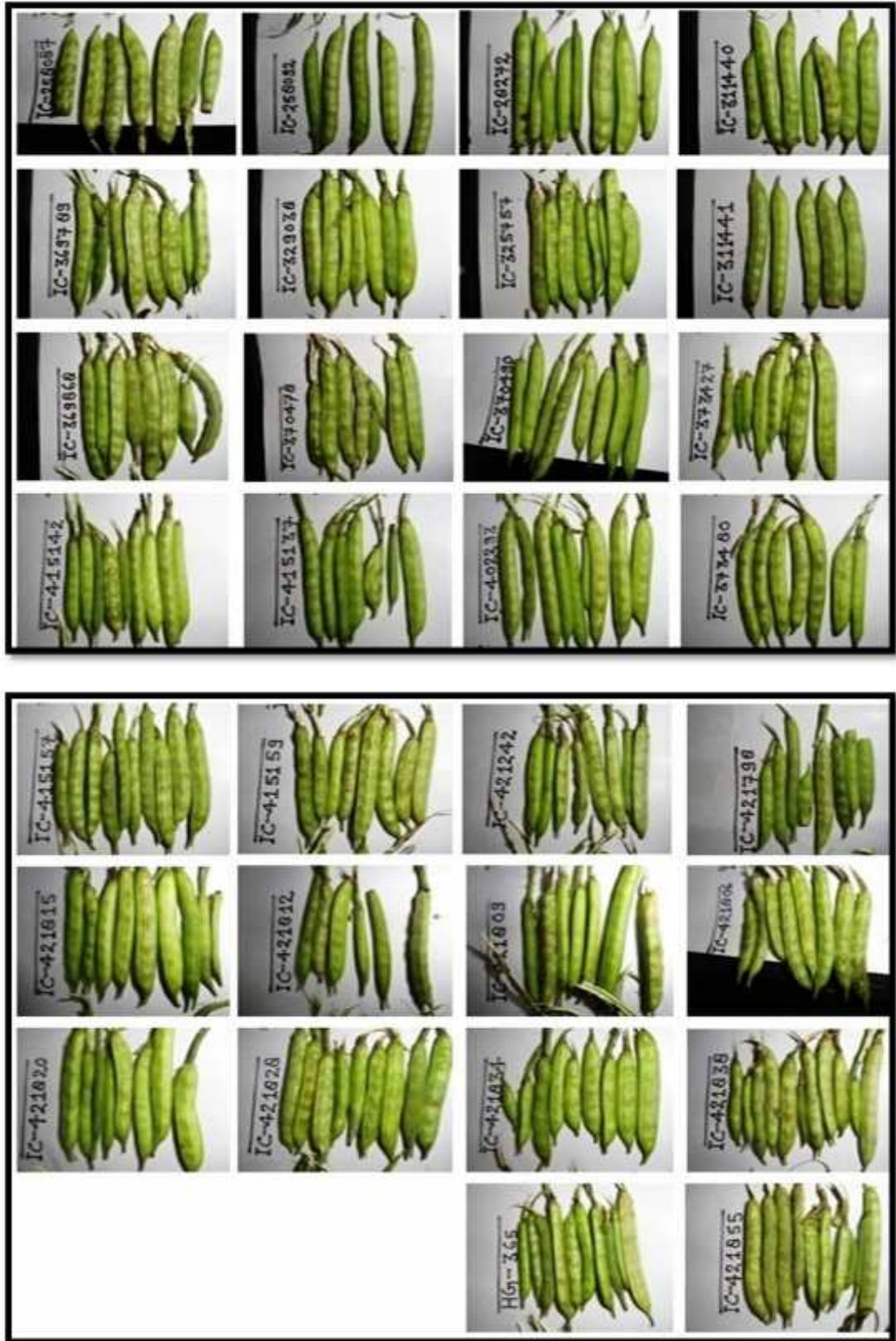


Plate 5. Variability in pod characters of 30 genotypes in cluster bean

Molecular Characterization

3.9 Molecular/genotypic characters

3.9.1 Genetic diversity

Genetic diversity has been defined as the variety of alleles and genotypes present in a population and this is reflected in morphological, physiological and behavioural differences between individuals and populations (Frankham *et al.*, 2002).

3.9.2 Phylogenetic analysis

Molecular phylogenetic analysis is the use of macromolecular sequences to reconstruct the evolutionary relationships between organisms. The extent of difference between homologous DNA, RNA, or protein sequences in different organisms is used as a measure of how much these organisms have diverged from one another evolutionarily.

3.9.3 Genome mapping

The legumes are remarkably well positioned in the genomic era. There are three essentially completed genome sequences in species related to large numbers of crops and forages. Extensive genetic and genomic tools have been developed for many crops and models. A great deal of work remains: to characterize more genes and traits, to better determine correspondences across the genomes, and to extend new genomic tools to orphan species. Some of the most critical work does not rely on new high-throughput sequencing or genomic technologies. This includes characterizing and managing germplasm collections and breeding lines in many species; developing mapping populations for various traits of interest in less studied species; working with indigenous farmers to ensure that the products of centuries of plant domestication are not lost; investigating protocols for hybrid seed production in various legumes; and working to maintain and develop understudied legumes for use in diverse, challenging growing environments around the globe. With energy supplies diminishing and higher nitrogen prices inevitably following, and the human population rising while the amount of arable land declines or degrades, legume

researchers now have both great opportunity and responsibility to help develop crops for a changing world.

3.9.4 Number of amplified fragment

There are the total numbers of amplified fragment produced by individual primer.

3.9.5 Polymorphic fragments

The bands which showed more variation during amplification by the primer.

3.9.6 Polymorphism percentage

This is calculated with the help of the following formula

$$\text{Polymorphism percentage} = \frac{\text{Number of polymorphic fragment}}{\text{Total fragment}} \times 100$$

3.9.7 Monomorphic fragments

The bands which showed lesser variation during amplification by the primer.

3.9.8 Monomorphism percentage

This is calculated with the help of the following formula

$$\text{Monomorphism percentage} = \frac{\text{Number of monomorphic fragment}}{\text{Total fragment}} \times 100$$

3.10 Plant material

The experiment was conducted in 2012-13 and 2013-14 and the 30 genotypes of cluster bean were selected from the available genetic materials from the National Bureau of Plant Genetic Resources, Regional Station, Jodhpur, Rajasthan and Punjab Agriculture University, Ludhiana, India.

3.11 Chemicals and solutions

Molecular biology grade chemicals used in the present study were obtained from B. R. Biochem Life Sciences Pvt. Ltd, New Delhi, India. PCR Master Mix for molecular analysis were obtained from Takara Biotechnology (Dalian) Co., Ltd. Taq Polymerase, Dextyribonucleotides, assay buffer, $MgCl_2$ and Agarose used for PCR analysis were also obtained from B. R. Biochem Life Sciences Pvt. Ltd, New Delhi, India. The primers used were obtained from SBS Genentech Company Limited, China. For all the molecular biology experiments, sterile double distilled water was used.

The standard extraction buffer comprises 2.5 % CTAB, 100 mM Tris HCL (pH 8), 20 mM EDTA (pH 8), 7 M NaCl, 0.5 % Polyvinylpyrrolidone (PVP), 0.2 % - mercapto ethanol (added to buffer just before use). Phenol: chloroform: iso-amyl alcohol (IAA) (25:24:1), 70 % alcohol and TE buffer consisting of 1mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0) were also used.

Procedure-

Protocol for genomic DNA isolation from plant leaves

Weigh 2 gm fresh plant leaves



Grind 2 gm of fresh plant tissue with the help of pastel and mortar



Add 500 μ l of CTAB extraction buffer



Transfer the CTAB/plant extract mix for about 15 minute at 55⁰ C in a recirculating water bath



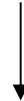
After incubate spin the CTAB/plant extract mix at 12000 RPM for 6 minute to spin down cell debris



Transfer the supernatant to clear microfuge tubes



To each tube add 250 µl of chloroform: Isoamyl alcohol (24:1) and mix the solution by inversion



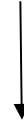
Again centrifuge the tubes at 12000 RPM for 2 minute



Transfer the upper aqueous phase to a clear microfuge tube



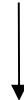
To each tube add 50 µl of 7.5 M ammonium acetate followed by 500 µl of ice cold absolute ethanol



Invert the tubes slowly several times to precipitate the DNA



Place the tubes at -20⁰ C for 1 hour to precipitate the DNA



Add 500 μ l of ice cold 70 % ethanol and slowly invert the tube



Again centrifuge the tubes at 12000 RPM for 2 minute

3.12 DNA isolation and purification

DNA was extracted from leaves of all the genotypes using the CTAB method of Doyle and Doyle (1990). The extracted DNA was purified through RNase treatment and tested on agarose gel for integrity. The juvenile fresh leaves (2 g) were taken from two week old seedling, washed with distilled water twice and dried well before grinding with a pestle and mortal with liquid nitrogen. Polyvinylpyrrolidone (PVP) treatment was given with 2 ml wash buffer (0.5 % PVP) to the grinding leaves. The grinded mixture was transferred to 2 ml micro centrifuge tubes and mixed thoroughly by gently inverting pellet so obtained was centrifuged at 5,000 rpm for 10 minutes at 4 °C. Supernatant was removed and pellets were washed with 100 μ l of 70% Ethanol. Pellets were air dried until the smell of ethanol was removed. Pellets were dissolved in 100 μ l of Tris-EDTA (TE) buffer at room temperature and were stored at 20 °C for further use.

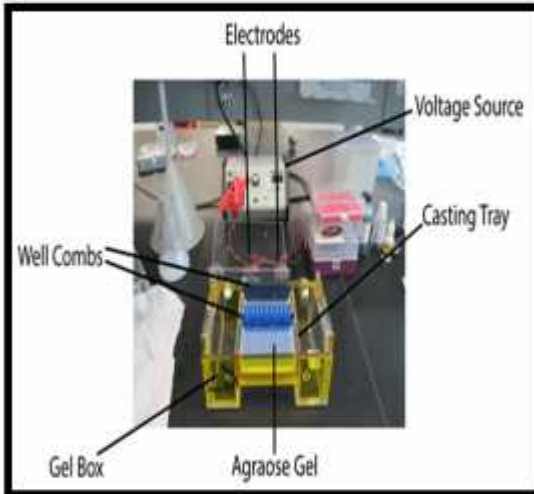


Plate 9. A view of laboratory work during DNA isolation and gel electrophoresis in the horticulture research lab and biotechnology lab

3.13 Amount and purity of genomic DNA

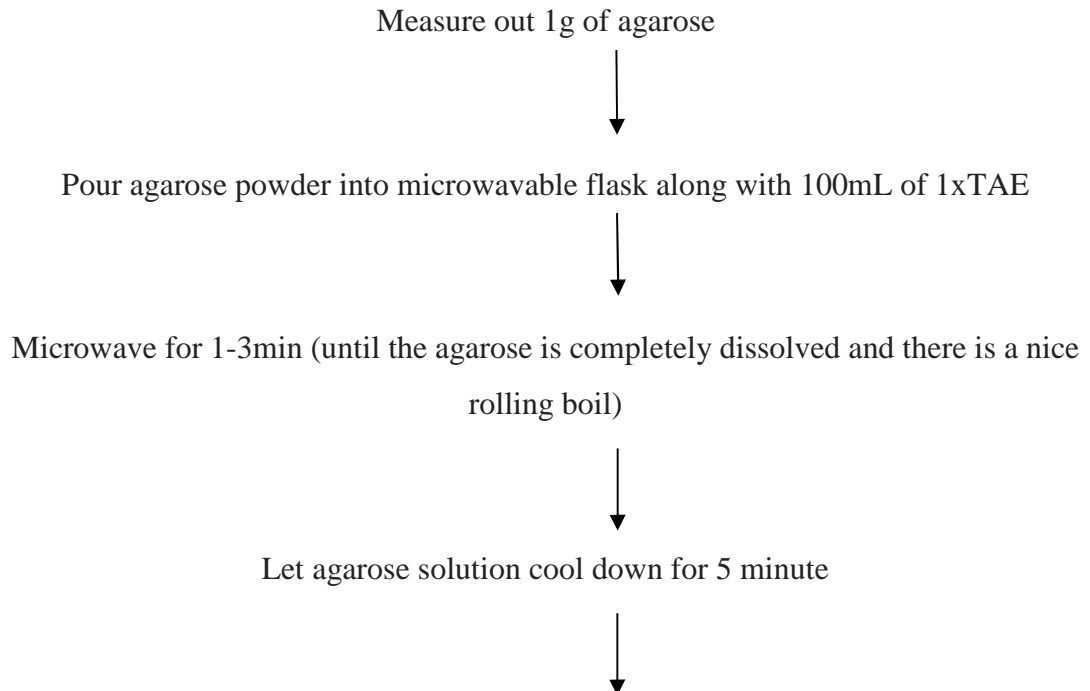
Purified DNA was quantified using Nano Drop (Nano Drop ND-1000 Version 3.1.1) spectrophotometer. After quantification, the DNA was diluted with TE buffer (Tris 10 mM and EDTA 1 mM, pH= 8.0) to a working concentration of 25 ng μl^{-1} for PCR analysis. The yield of genomic DNA per gram of leaf tissue extracted was measured using a nanodrop spectrophotometer. The purity of genomic DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA samples from the leaf tissues were electrophoresis on a 1.0 % agarose gel.

3.14 Agarose gel electrophoresis:

The amplified products were resolved on 1.0 % Agarose gel in 1 x TAE buffer at 100V for 90 min. The gel was stained in ethidium bromide. After electrophoresis the gel was carefully taken out of the casting tray and photograph were taken on a gel documentation system (Alphamager™ 2200).

Procedure:

Protocol for agarose gel electrophoresis analysis



Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 μ g/mL
(usually about 2-3 μ l of lab stock solution per 100mL gel)



Pour the agarose into a gel tray with the well comb in place



Place newly poured gel at 4°C for 10-15 minutes or let sit at room temperature for 20-30 minutes, until it has completely solidified



Add loading buffer to each of your digest samples



Once solidified, place the agarose gel into the gel box (electrophoresis unit)



Fill gel box with 50 ml 1xTAE (or TBE) until the gel is covered



Carefully load a molecular weight ladder into the first lane of the gel



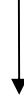
Carefully load your samples into the additional wells of the gel



Run the gel at 80-150V until the dye line is approximately 75-80% of the way down
the gel



Turn OFF power, disconnect the electrodes from the power source, and then carefully
remove the gel from the gel box



Place the gel into a container filled with 100 mL of TAE running buffer and 5µl of
EtBr, place on a rocker for 20-30 minutes, replace EtBr solution with water and
destain for 5 minutes



Using any device that has UV light, visualize your DNA fragments
(Gel documentation)

3.15 PCR amplification

Polymerase chain reaction was performed in a volume of 20 µl reaction mixture containing 0.5 unit Taq DNA polymerase (B. R. Biochem Life Sciences Pvt. Ltd), MgCl₂ (1.5 mM) (B. R. Biochem Life Sciences Pvt. Ltd), 200 mM each of dNTP, 0.2 µM of primer, and 50 ng of genomic DNA. Amplification was performed in PTC-384 programmable thermal cycler. After initial denaturation at 94 °C for 5 min, PCR was performed for 30-35 cycles consisting of a denaturation step for 1 min, followed by a gradient range of annealing temperatures 38°, 40°, 42°, 45°, and 50 °C for 1 min and extension at 72 °C for 2 min (Punia *et al.*, 2009); at the end of the reaction final extension period was appended (72°C) 10 min. Amplification products were separated on 1.0% agarose gels in TBE buffer and photographed using gel documentation system (Vilber Lourmat).

Procedure:

PCR amplification protocols for ISSR analysis of plants

Label two sets of tubes (1 set of 0.5 ml tubes for dilutions and 1 set of 0.2 ml PCR
tubes for reaction)



Dilute an aliquot of each DNA (use 1 μ L of stock to 99 μ l of ddH₂O) to obtain a final concentration of approx. 5-10 ng/ μ l



Aliquot 2 μ l of DNA to the appropriate tube



Make up the master PCR reaction mixture for the number of samples you have using the following table



Remember to include 1-2 extra for carry-over



Mix the master mix well and aliquot 23 μ l to each tube of DNA



Mix well and place in PCR machine with the following cycles



Establish an annealing temperature that produces bands or smears of bands



Use that temperature and systematically increase or decrease the concentration of MgCl₂ in the reaction mix



Take the best result from step 2 and systematically increase or decrease the volume of Taq polymerase



Plate 10. General view of laboratory work by using PCR analysis and gel documentation in CSSRI lab and BBAU, biotechnology lab

3.16 Statistical data analysis

The data collected on the quantitative characters were subjected to statistical analysis and following different statistical parameters were worked out.

3.16.1 Analysis of variance (ANOVA)

Analysis of variance was done separately for each character for all treatments. The analysis of variance for different characters was carried out by using the mean data in order to partition the variability due to different sources by following the method given by Panse and Sukhatme (1964). The model of analysis of variance is given below.

Source of Variance	D.F	SS	MSS	F Cal.
Replication	r-1	RSS	RSS/(r-1)	TMSS/EMSS
Treatments	t-1	TSS	TSS/(t-1)	
Error	(r-1)(t-1)	ESS	ESS/(r-1)(t-1)	
Total	(rt-1)	TSS		

Where,

t = Number of treatments (genotypes)

r = Number of replication

The standard error was calculated as,

$$SEm = \sqrt{EMSS/r}$$

The significance of treatments mean squares and replication mean squares were tested by comparing with error mean squares referring to 'F' table values at 5 and 1 per cent level of probabilities

$$\text{Grand mean} = \frac{\text{Grand total}}{N}$$

Where,

$N = \text{Total number of Treatments} \times \text{Replications}$

$$\text{Correction factor} = \frac{(\text{Grand total})^2}{N}$$

Total sum of squares = Sum square of each treatment - C.F.

$$\text{Treatment sum of squares} = \frac{\text{Sum of treatment squares}}{\text{Replication number (r)}} - \text{C.F.}$$

$$\text{Replications S.S.} = \frac{\text{Sum of replication square}}{\text{Treatment number (t)}} - \text{C.F.}$$

Error S.S. = Total S.S. - (Treatment S.S. + replication S.S.)

Mean sum of squares were obtained by dividing the sum of squares with the respective degrees of freedom.

$$\text{F. Variance ratio} = \frac{\text{Treatment MS}}{\text{Error MS}}$$

The significance of treatments was tested by 'F' test by comparing the calculated F value with tabulated F value at 5% level of significance for the corresponding degree of freedom. The analysis of variance was set up as follows:

$$\text{Standard Error/plot} = \sqrt{\text{Error MS}}$$

There, F. test was observed to be significant, comparison was further extended by testing difference of any 2 treatments means against critical difference (CD) at 5% level of significance.

$$\text{S.E. (Treatment means)} = \sqrt{\frac{\text{EMS}}{r}}$$

Coefficient of variation = SED x t values at 5%

$$= \sqrt{\frac{2\text{EMS}}{r}} \times t \text{ 5\% at Error df.}$$

3.16.2 Parameters of variability

Mean

The mean value of each character was determined by dividing the total number of corresponding number of observations.

$$\bar{X} = S_X/N$$

Where,

S_X is the summation of the variables

N is the number of observations.

Range

Lowest and highest values for each trait were recorded.

Standard error

S.E. of the mean was calculated with the help of error mean square from ANOVA.

$$\begin{aligned} \text{S.E. (Mean)} &= \sqrt{\frac{\sigma_e^2}{r}} \quad \text{or} \\ &= \sqrt{\frac{\text{Variance due to Error}}{\text{Number of replications}}} \end{aligned}$$

3.16.3 Components of variance

Phenotypic and genotypic components of variance were computed according to the formula given by Lush (1940).

The total variance of observations for a character in F_2 was considered as its phenotypic variance. The environmental variance for each character was estimated from the mean variance of the non-segregating parental populations.

Where, V_e is environmental variance

V_{p1} is the variance of parent 1

V_{p2} is the variance of parent 2

The genotypic variance was separated from the total phenotypic variance by subtracting the environmental variance as per the method of Weber and Moorthy (1952).

That is, $V_g = V_p - V_e$

Where,

V_g is the genotypic variance

V_p is the phenotypic (total) variance

V_e is the environmental variance

3.16.4 Coefficients of variation

The co-efficient of variability both at phenotypic and genotypic levels for all the characters were computed by applying the formula as suggested by Burton and De Vane (1953).

$$\text{Genotypic variance (} g^2) = \frac{\text{MS due to genotypes} - \text{MS due to error}}{r \text{ (replication)}}$$

$$\text{Phenotypic variance (} p^2) = g^2 + e^2 \text{ (MS due to error)}$$

3.16.5 Phenotypic and genotypic coefficient of variability

Phenotypic and genotypic coefficients of variability were calculated as per the method suggested by Burton and Devane (1953). The same is as follows.

$$\text{Genotypic co-efficient of variation (GCV)} \quad \text{GCV (\%)} = \frac{g}{X} \times 100$$

$$\text{Phenotypic co-efficient of variation (PCV)} \quad \text{PCV (\%)} = \frac{p}{X} \times 100$$

Where, X = General mean

p = Phenotypic standard deviation

g = Genotypic standard deviation

GCV and PCV were classified as suggested by Robinson *et al.* (1949) into low (0 - 10%), moderate (10.1% - 20%) and high (>20%).

3.16.6 Heritability (h^2)

Heritability in broad sense for all the characters was computed by the formula suggested by Lush (1945).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Where, h^2 = Heritability per cent (Broad sense)

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

Heritability was classified as suggested by Robinson *et al.* (1949) into low (0 - 30%), Moderate (30.1% - 60%) and High (>60%).

3.16.7 Genetic advance

To calculate the expected genetic advances the formula given by Lush (1949) and Johnson *et al.* (1955) was applied.

Genetic advance for each character was predicted by the formula given by Johnson *et al.* (1955).

$$GA = h^2 \times \sigma_p \times k$$

The expected GA as per cent of mean (GAM) was estimated as given below:

$$\text{Expected GAM (\%)} = \frac{GA}{X} \times 100$$

Where, h^2 = Heritability (Broad sense)

σ_p = Phenotypic standard deviation

K = Standardized selection differential which is 2.06 at 5% selection intensity (Lush, 1949) in large sample from normally distributed population.

GA = Genetic advance

X = General mean

3.16.8 Analysis of covariance

The analysis of covariance was done adopting the formula suggested by Federer (1955). Both the genotypic and phenotypic co-variance was calculated as given below:

$$\text{Genotypic co-variance } XY = \frac{\text{TMP} \times XY - \text{EMPXY}}{r}$$

$$\text{Error co-variance} = \text{EMPXY}$$

$$\text{Phenotypic covariance} - XY = \text{Genotypic co-variance } XY + \text{Error co-variance}$$

XY.

Where,

TMP = Treatment mean sum of products.

EMP = Error mean sum of products

r = Number of replication on XY being the two variables.

3.16.9 Estimation of correlation coefficient

The correlation co-efficient among all possible character combinations at phenotypic (rp) and genotypic (rg) level were estimated employing formula (Al-Jibouri *et al.*, 1958).

$$\text{Phenotypic correlation} = r_{xy}(p) = \frac{\text{COV } xy(p)}{V_x(p) \times V_y(p)}$$

$$\text{Genotypic correlation} = r_{xy}(g) = \frac{\text{COV } xy(g)}{V_x(g) \times V_y(g)}$$

The significance of 'r' values was tested at 5% and 1% from 'r' table at (V- 2) degrees of freedom.

Where,

V= Number of genotypes on which the observations were recorded.

3.16.10 Path coefficient analysis

Path co-efficient analysis suggested by Wright (1921), Dewey and Lu (1959) was carried out to know the direct and indirect effect of the morphological traits on plant yield. The following set of simultaneous equations were formed and solved for estimating various direct and indirect effects. The correlation 'cause' with effects was calculated by solving the following simultaneous equation.

$$r_{1y} = a + r_{12}b + r_{13}c + \dots + r_{1i}i$$

$$r_{2y} = a + r_{21}a + b + r_{23}c + \dots + r_{2i}i$$

$$r_{3y} = r_{31}a + r_{32}b + c + \dots + r_{3i}i$$

$$r_{1y} = r_{11}a + r_{12}b + r_{13}c + \dots + I$$

Where,

R_{1y} to r_{1y} = Co-efficient of correlation between causal factors 1 to I with dependent characters y.

R_{12} to r_{11} = Co-efficient of correlation among causal factors

a, b, c,.....i = Direct effects of characters 'a' to 'I' on the dependent character 'y'

Residual effect (R) was computed as follows.

$$\text{Residual effect (R)} = 1 - \bar{O}a^2 + b^2 + c^2 + \dots i^2 + 2abr_{12} + 2acr_{13} + \dots$$

3.16.11 Genetic divergence

One of the potent techniques of assessing the genetic divergence is the D^2 statistic proposed by Mahalanobis (1936). This technique measures the forces of differentiation at two levels: intra cluster and inter-cluster levels, and thus helps in the selection of genetically divergent parents for exploitation in hybridization programs. The D^2 analysis was carried out using the data recorded on germplasm.

Mahalanobis generalized distance (D^2) between any two populations is given by the formula

$$D^2 = S_{ij}$$

\bar{i}

\bar{j}

Where, D^2 = Square of generalized distance l_{ij} = Reciprocal of the common dispersal index

$$\bar{i} = \mu_{i1} - \mu_{i2}$$

$$\bar{j} = \mu_{j1} - \mu_{j2}$$

μ = General mean

The next commencing chapter deals with the findings of the investigation.

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

3.16.12 Clustering D^2 values

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

Intra and inter-cluster distance:

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

$$\text{Square of intra cluster distance} = \frac{SD_{ij}^2}{N}$$

Where,

SD_{i2} = Sum of distances between all possible combinations of the entries included in the cluster

N = Number of all possible combinations

$$\text{Square of inter-cluster distance} = \frac{SD_{ij}^2}{n_{ij}}$$

Where,

SD_{ij}^2 = Sum of distances between all possible combinations (n_{ij}) of the entries included in the cluster

n_i = Number of entries in the cluster i

n_j = Number of entries in the cluster j

3.17 Molecular data analysis

3.18 Analysis of ISSR data

A similarity matrix was computed for ISSR data using Jaccard's coefficient by the software package PAST (Hammer *et al.*, 2001).

A dendrogram was obtained by Unweighted Pair-Group Average (UPGMA) method using the similarity matrix by Jaccard's similarity coefficient (Jaccard, 1908) to determine the relatedness of 30 genotypes under study.

Population genetic analysis (POPGENE Yet 1.32) software was used to determine the values of Nei's genetic diversity (Nei's 1978), Shannon's information index, total genetic diversity among populations (H_t), genetic diversity within population (H_s) and mean coefficient of gene differentiation (G_{ST}) for two populations, i.e. landraces and commercial varieties.

3.19 Nei's genetic diversity

A method is presented by which the gene diversity (heterozygosity) of a subdivided population can be analyzed into its components, i.e., the gene diversities within and between subpopulations.

Nei's (1978) defined the coefficient of gene diversity G_{ST} as,

$$G_{ST} = \frac{D_{ST}}{H_s + D_{ST}}$$

The Dice algorithm is corresponding to that of Nei and Li (1979) as under:

$$\text{Similarity (F)} = \frac{2N_{ab}}{(N_a + N_b)}$$

Where,

Na = Number of scored fragments of individual 'a'

Nb = Number of scored fragments detected in individual 'b' and

Nab = Number of shared fragments between individual 'a' and 'b'.

3.20 Shannon information index

Shannon diversity index are statistics used to summarize the diversity of a population in which each member belongs to a unique group. For example, in ecology the groups are typically species Shannon's information index, total genetic diversity among populations (H_t), genetic diversity within population (H_s) and mean coefficient of gene differentiation (G_{st}) for two populations, i.e. landraces and commercial varieties. Shannon diversity index is calculated with following formula

$$H = - \sum_{i=1}^s (P_i * \ln P_i)$$

Where,

H = the Shannon diversity index

P_i = fraction of the entire population made up of species i

S = numbers of species encountered

= sum from species 1 to species S

3.21 Analysis of molecular variance (AMOVA)

AMOVA is a statistical model for the molecular variation in a single species, typically biological. The name and model are inspired by ANOVA. The method was developed by Laurent Excoffier, Peter Smouse and Joseph Quattro at Rutgers University in 1992.

Analysis of Molecular Variance (AMOVA) is a method for studying molecular variation within a species. The analysis of molecular variance (AMOVA) was used to study the patterns and degree of relatedness revealed by Multidimensional scaling and the Clustering dendrogram.

ISSR data were also subjected to Analysis of Molecular Variance (AMOVA) at individual and population level, using GenALEX software (Peakall and Smouse, 2006). Φ_{ST} represents the correlation between random genetic accessions within a group relative to random accessions from the population at large.

AMOVA is calculating with the help of following formula.

$$p_{jig} = p + a_g + b_{ig} + c_{jig}$$

General design for hierarchical analysis of molecular variance (AMOVA)

S. No.	Source of variance	Df	SS	MSS	Estimated variance	%
1.	Among population					
2.	Within population					
3.	Total					

3.22 Polymorphic information content (PIC)

Polymorphic Information Content (PIC) for dominant marker system was calculated according to Roldan-Ruiz *et al.* (2000) as-

$$PIC_i = 2f_i(1 - f_i)$$

Where, PIC_i is polymorphic information content of marker 'i',

f_i is frequency of the amplified allele (band present),

And $(1 - f_i)$ is frequency of the null allele (band absent).

PCI was averaged for all the fragments for each primer.

3.23 Resolving power (Rp)

Resolving power (Rp) for each primer was calculated according to Prevost and Wilkinson (1999) as-

$$RP = I_b$$

Where, I_b is the informativeness of the bands scored which can be calculated as-

$$I_b = 1 - (2 \times [0.5 - p])$$

Where 'p' is the proportion of 30 genotypes containing bands.

EXPERIMENTAL FINDINGS

CHAPTER -IV

The experimental results obtained from the present investigation entitled “Studies on Genetic Diversity and Variability in Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Genotypes Through Morphological and Molecular Characterization” are presented under the following heads.

Morphological Characterization:

4.1 Analysis of variance

4.2 Mean performance and range variation

4.3 Coefficient of variability

4.4 Heritability and expected genetic advance in percent of mean

4.5 Phenotypic and genotypic correlation coefficient

4.6 Path coefficient analysis

4.7 Genetic divergence

4.1 Analysis of variance

The analysis of variance was carried out for 19 characters i.e., plant height (cm), germination (%), days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant, pod breadth (cm), pod length (cm), pod width (cm), number of pods/plant, number of pods/cluster, number of clusters /plant, number of branches/plant at maturity, number of seeds/pod, pod yield/plant(g), pod yield/plot(kg), 100-seed weight (g), seed yield/plant (g), pod yield (q/ha) and days to maturity. The analysis of variance (ANOVA) is appended in Table- 4.1, 4.2 and 4.3. The mean sum of square due to treatment was highly significant for all the characters under study. The data were recorded on 120th days at the crop harvest stage for different characters from the experiments conducted in 2012-2013 and 2013-2014 was subjected to analysis of variance to test the significance of difference among 30 genotypes of cluster bean for different characters is present in Table- 4.1, 4.2 and 4.3 for 120th days and crop harvest stage. The differences due to genotypes were highly

significant for all the characters under study. The differences due to replications were not significant. Mean of yield and its contributing characters for 30 genotypes of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] have been presented in Table- 4.4 and 4.5 with also in Graph 4.4 and 4.5 for 120th days and crop harvest stage.

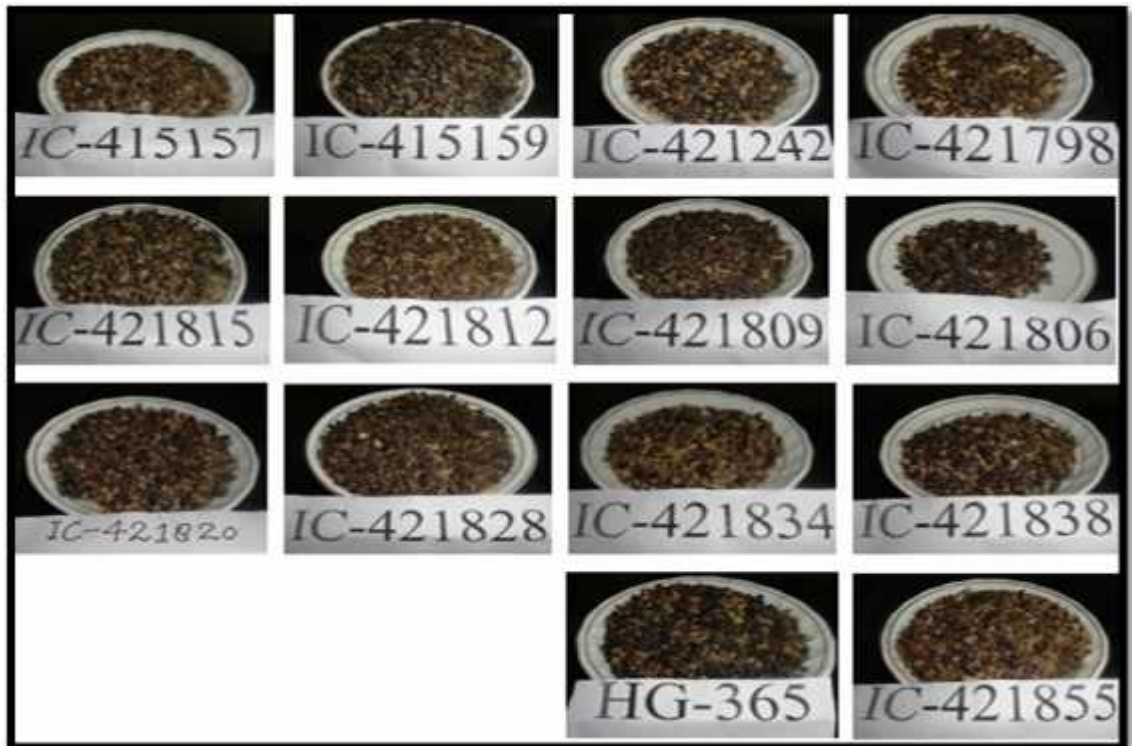
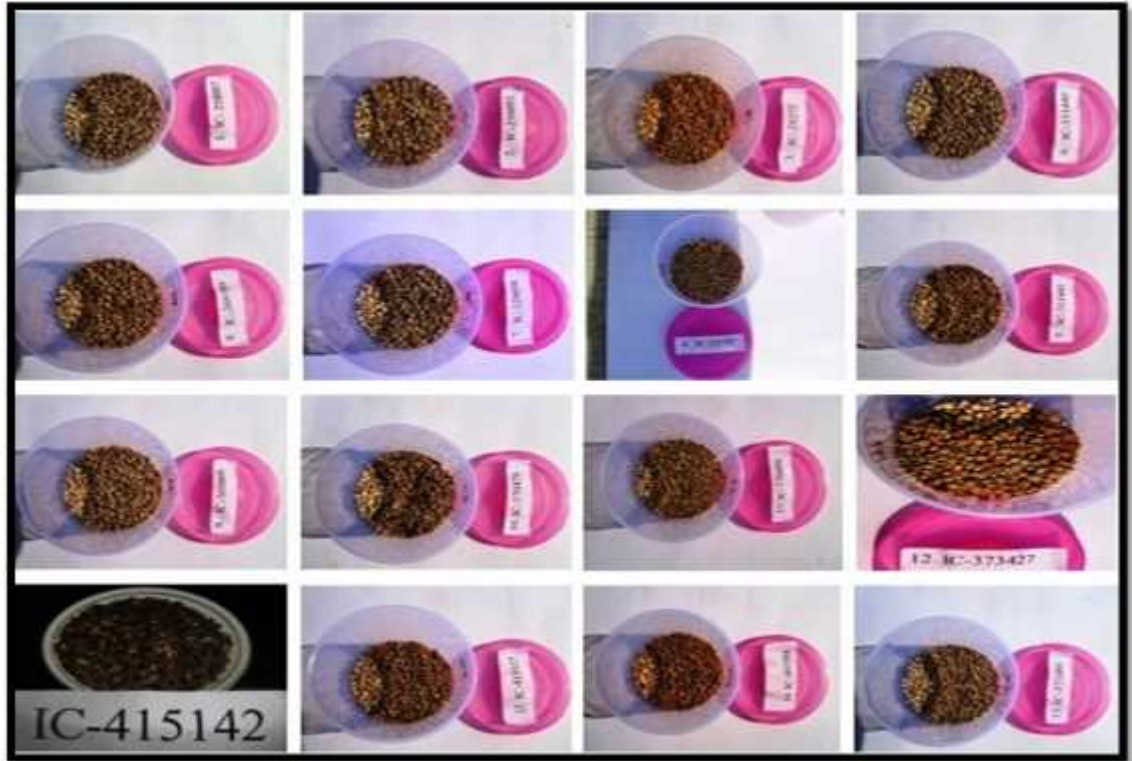


Plate 6. Variability in seed characters of 30 genotypes in cluster bean

Table- 4.1 Analysis of variance (mean sum of squares) for different growth, yield and yield attributing characters in cluster bean genotypes 2012-2013

S. No.	Source of variation/characters	Replication	Treatments	Error	SEm±	CD at 5%
	Degrees of freedom	2	29	58		
1.	Plant height (cm)	33.43	518.34**	36.36	3.67	7.50
2.	Germination (%)	22.59	30.07**	6.36	3.68	7.52
3.	Days taken for first flowering	5.90	3.33**	1.42	0.974	1.99
4.	Days taken for 50% flowering	5.88	5.43**	0.879	0.765	1.56
5.	Number of reproductive branches/plant	0.911	3.43**	0.314	0.457	0.934
6.	Pod breath (cm)	2.16	0.182*	0.136	0.301	0.616
7.	Pod length (cm)	0.160	0.733*	0.178	0.344	0.703
8.	Pod width (cm)	0.708	0.0097*	0.113	0.275	0.562
9.	Number pods/plant	20.28	188.27**	15.44	3.20	6.55
10.	Number of pods /cluster	0.118	71.75**	0.614	0.640	1.30

11.	Number of clusters /plant	2.73	77.77**	1.26	0.919	1.88
12.	Number of branches /plant at maturity	2.61	1.31*	0.283	0.434	0.887
13.	Number of seed /pod	0.433	0.629*	0.182	0.348	0.711
14.	Pod yield /plant (g)	123.31	462.54**	12.12	2.84	5.80
15.	Pod yield /plot (kg)	0.0056	0.025*	0.0023	0.00396	8.09
16.	100- Seed weight (g)	0.045	0.309*	0.089	0.243	0.496
17.	Seed yield/plant (g)	2.37	2.77**	0.333	0.471	0.963
18.	Days to maturity	34.31	28.02**	2.19	1.20	2.45
19.	Pod yield (q/ha)	36.75	98.87**	32.71	4.67	9.55

****Significant at 5% and *Significant at 1% level of significance**

Table- 4.2 Analysis of variance (mean sum of squares) for different growth, yield and yield attributing characters in cluster bean genotypes 2013-2014

S. No.	Source of variation/characters	Replication	Treatments	Error	SEm±	CD at 5%
	Degrees of freedom	2	29	58		
1.	Plant height (cm)	13.06	485.43**	20.29	3.67	7.50
2.	Germination (%)	90.84	20.16**	20.29	3.68	7.52
3.	Days taken for first flowering	3.72	1.91*	2.72	1.34	2.74
4.	Days taken for 50% flowering	0.382	3.67**	3.09	1.43	2.92
5.	Number of reproductive branches/plant	0.363	3.57**	0.488	0.570	1.16
6.	Pod breath (cm)	0.778	0.177*	0.300	0.447	0.914
7.	Pod length (cm)	0.466	0.740*	0.501	0.578	1.18
8.	Pod width (cm)	1.88	0.104*	1.01	0.822	1.68

9.	Number pods/plant	15.46	118.47**	44.82	5.46	11.16
10.	Number of pods /cluster	2.05	47.23**	1.14	0.875	1.78
11.	Number of clusters /plant	0.361	58.18**	0.223	0.385	0.787
12.	Number of branches /plant at maturity	0.00024	1.24*	0.00012	0.00917	0.018
13.	Number of seed /pod	0.099	0.671*	0.168	0.335	0.685
14.	Pod yield /plant (g)	12.53	459.63**	11.04	2.71	5.54
15.	Pod yield /plot (kg)	0.014	0.023*	0.0041	0.052	0.106
16.	100- Seed weight (g)	0.097	0.349**	0.078	0.229	0.468
17.	Seed yield/plant (g)	0.633	2.76**	0.214	0.378	0.773
18.	Days to maturity	2.31	26.54**	1.09	0.852	1.74
19.	Pod yield (q/ha)	56.06	94.09**	11.96	2.82	5.76

****Significant at 5% and *Significant at 1% level of significance**

Table- 4.3 Analysis of variance (mean sum of squares) for different growth, yield and yield attributing characters in cluster bean genotypes 2012-2013 and 2013-2014

S. No.	Source of variation/characters	Replication	Treatments	Error	SEm±	CD at 5%
	Degrees of freedom	2	29	58		
1.	Plant height (cm)	40.62	1002.91**	28.32	3.07	6.27
2.	Germination (%)	98.56	35.21**	13.33	2.10	4.29
3.	Days taken for first flowering	8.28	3.59**	2.07	0.831	1.69
4.	Days taken for 50% flowering	3.84	7.65**	1.98	0.813	1.66
5.	Number of reproductive branches/plant	1.13	6.34**	0.401	0.365	0.746
6.	Pod breath (cm)	2.29	0.337*	0.218	0.269	0.550
7.	Pod length (cm)	0.468	1.46*	0.340	0.336	0.687
8.	Pod width (cm)	2.11	0.189*	0.563	0.433	0.885
9.	Number of pods/plant	30.50	287.02	30.13	3.16	6.46
10.	Number of pods /cluster	1.39	117.41**	0.881	0.542	1.10

11.	Number of clusters /plant	2.26	134.24**	0.745	0.498	1.01
12.	Number of branches /plant at maturity	1.29	2.48*	0.141	0.217	0.443
13.	Number of seed /pod	0.368	1.26*	0.175	0.241	0.492
14.	Pod yield /plant (g)	104.25	911.74**	11.59	1.96	4.01
15.	Pod yield /plot (kg)	0.0016	0.047*	0.0032	0.032	0.065
16.	100- Seed weight (g)	0.116	0.645*	0.084	0.167	0.341
17.	Seed yield/plant (g)	2.69	5.34**	0.273	0.302	0.617
18.	Days to maturity	27.25	52.31**	1.63	0.738	1.50
19.	Pod yield (q/ha)	47.12	192.08**	22.35	2.72	5.56

****Significant at 5% and *Significant at 1% level of significance**

4.2 Mean performance and range variation

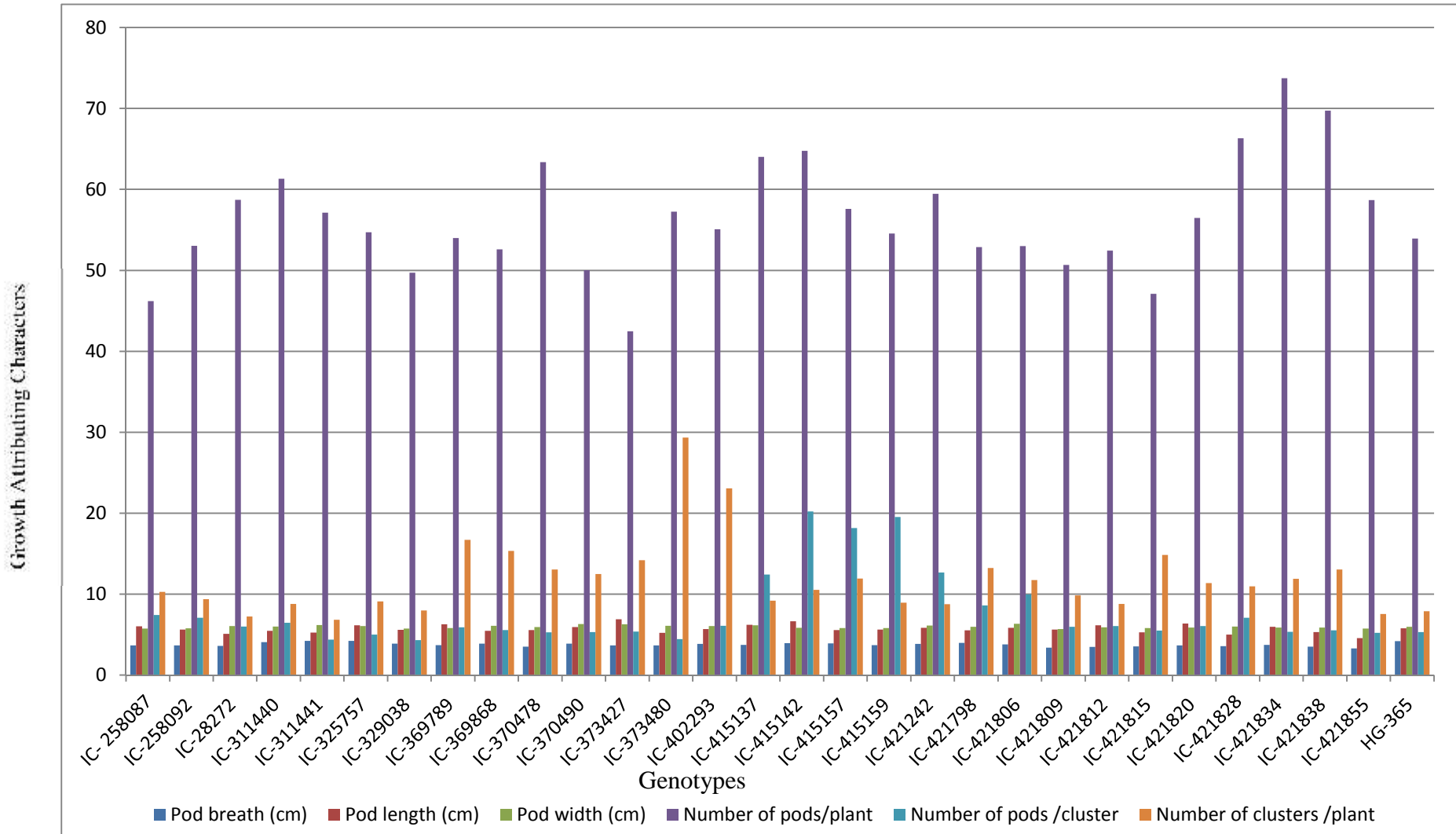
The mean, range, coefficient of variance, standard error and critical difference of variability for 11 growth attributing characters and 8 yield and yield attributing characters are present in Table (4.4 and 4.5), respectively. A wide range of variation was observed for almost all the traits in the material studied. The best lines with respect to growth and yield contributing characters have shown in plates.

Table- 4.4 Mean performance of growth attributing characters in 30 genotypes of cluster bean

Characters Genotypes	Plant height (cm)	Germination (%)	Days taken for first flowering	Days taken for 50% flowering	Number of reproductive branches/plant	Pod breath (cm)	Pod length (cm)	Pod width (cm)	Number of pods/plant	Number of pods /cluster	Number of clusters /plant
IC- 258087	68.5	87.67	21.95	33.99	4.91	3.65	6.01	5.73	46.21	7.41	10.27
IC-258092	73.3	90.53	22.71	33.87	5.12	3.67	5.61	5.76	53.03	7.08	9.39
IC-28272	74.21	95.54	22.85	34.86	4.83	3.6	5.1	6.07	58.72	5.98	7.25
IC-311440	71.49	93.94	20.46	32.7	4.62	4.07	5.45	5.99	61.31	6.45	8.8
IC-311441	75.58	92.69	20.61	32.34	4.13	4.22	5.26	6.17	57.12	4.38	6.83
IC-325757	78.51	90.73	21.07	33.76	5.38	4.21	6.14	6.04	54.72	4.99	9.09
IC-329038	82.92	88.5	21.26	33.14	4.51	3.88	5.59	5.75	49.71	4.33	7.99
IC-369789	97.63	89.72	21.69	32.2	5.43	3.68	6.26	5.82	53.99	5.91	16.7
IC-369868	69.32	88.64	23.28	32.43	4.7	3.87	5.46	6.09	52.58	5.56	15.34
IC-370478	104.57	95.94	22.87	33.74	6.08	3.5	5.57	5.94	63.36	5.28	13.05
IC-370490	74.02	93.36	22.83	34.38	4.35	3.89	5.92	6.29	50.03	5.31	12.48
IC-373427	79.07	89.26	22.63	33.84	5.54	3.67	6.88	6.26	42.47	5.37	14.19
IC-373480	73.81	89.44	22.97	33.49	6.45	3.65	5.21	6.08	57.25	4.44	29.33
IC-402293	86.95	92.5	21.6	31.4	5.64	3.86	5.69	6.07	55.07	6.08	23.06
IC-415137	107.69	95.44	22.72	32.97	7.12	3.73	6.2	6.16	64.02	12.41	9.18
IC-415142	86.3	95.7	22.77	33.02	6.99	3.95	6.66	5.85	64.76	20.22	10.52
IC-415157	64.05	91.42	22.68	33	6.69	3.92	5.57	5.81	57.59	18.16	11.93
IC-415159	67.8	87.24	23.22	32.35	6.98	3.71	5.63	5.82	54.56	19.54	8.93
IC-421242	80.84	93.3	23.83	33.18	7.75	3.86	5.84	6.12	59.47	12.67	8.75
IC-421798	98.86	90.77	22.96	32.55	7.19	3.96	5.52	5.96	52.88	8.61	13.22
IC-421806	89.36	89.38	22.57	32.01	6.39	3.79	5.85	6.34	53	9.98	11.75
IC-421809	92.68	91.92	22.39	33.66	6.08	3.37	5.61	5.68	50.67	5.97	9.83

IC-421812	77.14	93.99	22.81	35.33	6.94	3.48	6.14	5.89	52.44	6.06	8.8
IC-421815	75.08	91.13	22.3	34.96	6.84	3.53	5.28	5.8	47.11	5.51	14.84
IC-421820	71.11	91.98	21.97	34.58	5.4	3.66	6.35	5.86	56.47	6.05	11.37
IC-421828	70.01	90.99	22.84	36.34	6.41	3.57	4.99	5.99	66.31	7.08	10.97
IC-421834	55.71	92.78	22.43	35.34	5.18	3.74	5.96	5.88	73.74	5.35	11.89
IC-421838	66.5	93.57	22.64	34.33	4.59	3.52	5.31	5.86	69.72	5.52	13.04
IC-421855	96.67	91.86	22.47	32.98	6.4	3.29	4.57	5.75	58.68	5.21	7.54
HG-365	97.55	89.38	22.8	33.83	7.17	4.19	5.77	5.97	53.94	5.3	7.89
F-value	**	**	**	*	*	*	*	**	**	**	*
GM	80.24	91.64	22.4	33.55	5.86	3.75	5.71	5.95	56.36	7.74	11.8
Range	55.71-107.69	87.24-95.94	20.46-23.83	31.40-36.34	4.13-7.75	3.29-4.22	4.57-6.66	5.68-6.34	42.47-73.74	4.33-20.22	6.83-29.33
SEm±	3.07	2.1	0.831	0.813	0.365	0.269	0.336	0.433	3.16	0.542	0.498
CD (p= .05)	6.27	4.29	1.69	1.66	0.746	0.55	0.687	0.885	6.46	1.1	1.01

* Significance at 5% level; ** Significance at 1% level



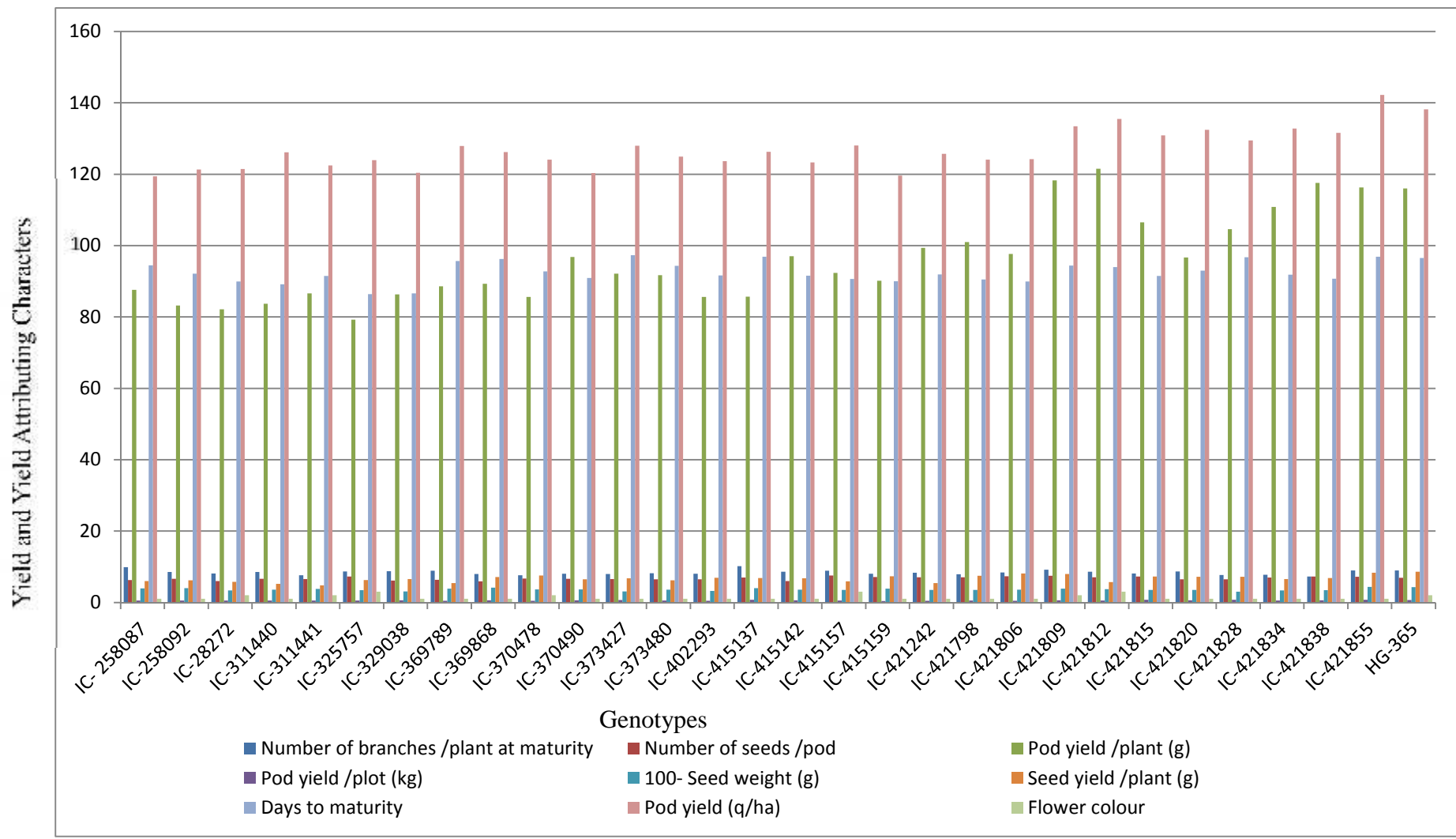
Graph- 4.4 Mean performance of growth attributing characters in 30 genotypes of cluster bean

Table- 4.5 Mean performance of yield and yield attributing characters in 30 genotypes of cluster bean

Characters Genotypes	Number of branches /plant at maturity	Number of seeds /pod	Pod yield /plant (g)	Pod yield /plot (kg)	100- Seed weight (g)	Seed yield /plant (g)	Days to maturity	Pod yield (q/ha)
IC- 258087	9.88	6.29	87.61	0.52	3.92	5.97	94.5	119.4
IC-258092	8.53	6.66	83.2	0.55	4	6.18	92.1	121.32
IC-28272	8.15	5.96	82.14	0.51	3.38	5.77	89.93	121.44
IC-311440	8.53	6.62	83.7	0.56	3.58	5.24	89.15	126.12
IC-311441	7.6	6.55	86.57	0.52	3.82	4.79	91.47	122.42
IC-325757	8.69	7.3	79.23	0.52	3.47	6.25	86.42	123.96
IC-329038	8.78	6.16	86.33	0.58	3.12	6.59	86.6	120.41
IC-369789	8.91	6.34	88.58	0.48	3.87	5.42	95.67	127.92
IC-369868	7.94	5.95	89.28	0.61	4.16	7.09	96.25	126.2
IC-370478	7.62	6.72	85.58	0.5	3.66	7.53	92.77	124.09
IC-370490	8.07	6.64	96.8	0.59	3.67	6.5	90.92	120.35
IC-373427	8	6.57	92.12	0.66	3.11	6.74	97.27	127.99
IC-373480	8.2	6.48	91.67	0.54	3.55	6.17	94.32	124.92
IC-402293	8.02	6.49	85.61	0.48	3.24	6.94	91.62	123.65
IC-415137	10.17	7.01	85.67	0.72	4	6.81	96.88	126.28
IC-415142	8.62	5.96	97.02	0.64	3.56	6.78	91.55	123.29
IC-415157	8.89	7.52	92.31	0.56	3.51	5.95	90.65	128.07
IC-415159	8.02	7.12	90.14	0.39	3.84	7.36	90	119.61
IC-421242	8.31	7.03	99.35	0.48	3.53	5.42	91.9	125.71
IC-421798	7.93	7.03	100.96	0.51	3.52	7.48	90.52	124.11
IC-421806	8.38	7.37	97.65	0.49	3.57	8.15	89.95	124.22
IC-421809	9.16	7.51	118.24	0.59	3.89	7.99	94.43	133.41

IC-421812	8.59	7.03	121.49	0.54	3.73	5.69	94	135.48
IC-421815	8.13	7.27	106.51	0.72	3.5	7.25	91.52	130.91
IC-421820	8.66	6.5	96.69	0.59	3.49	7.19	93	132.44
IC-421828	7.72	6.49	104.58	0.76	3	7.17	96.7	129.49
IC-421834	7.77	6.96	110.8	0.57	3.39	6.55	91.82	132.77
IC-421838	7.25	7.24	117.54	0.64	3.46	6.85	90.72	131.59
IC-421855	8.94	7.23	116.31	0.75	4.33	8.34	96.9	142.23
HG-365	8.99	6.93	115.97	0.66	4.29	8.64	96.52	138.15
F-value	*	*	*	**	*	*	**	**
GM	8.41	6.76	96.32	0.57	3.63	6.69	92.53	126.93
Range	7.25-10.17	5.95-7.52	79.23-121.49	0.39-0.76	3.00-4.33	4.79-8.64	86.42-97.27	119.40-142.23
SEm±	0.217	0.241	1.96	0.032	0.167	0.302	0.738	2.72
CD (p= .05)	0.443	0.492	4.01	0.065	0.341	0.617	1.5	5.56

* Significance at 5% level; ** Significance at 1% level



Graph- 4.5 Mean performance of yield and yield attributing characters in 30 genotypes of cluster bean

4.2.1 Plant height (cm)

The treatment mean for plant height ranged from 55.71 to 107.69 with minimum in genotype IC-421834 and maximum in genotype IC-415137. The grand mean reported was 80.24 ± 3.07 .

4.2.2 Germination (%)

The treatment mean for germination percent ranged from 87.24 to 95.94 with minimum in genotype IC-415159 and maximum in genotype IC-370478 with grand mean of 91.64 ± 2.10 , ranged from 87.24 % in genotype IC-415159 to 95.94% in genotype IC-370478.

4.2.3 Days taken for first flowering

The treatment mean for the days taken for first flowering ranged from 20.46 to 23.83 with minimum days taken for first flowering in genotype IC-311440 followed by IC-311441 (20.61), IC-325757 (21.07) and IC-329038 (21.26). The genotype IC-421242 (23.83) showed maximum days taken for first flowering with grand mean 22.40 ± 0.831 .

4.2.4 Days taken for 50% flowering

The maximum days taken for 50% flowering was recorded in genotype IC-421828 (36.43) and minimum days taken for 50% flowering in genotype IC-402293 (31.40), with grand mean 33.55 ± 0.813 .

4.2.5 Number of reproductive branches/plant

The maximum number of reproductive branches /plant was recorded in genotype IC-421242 (7.75) followed by IC-421798 (7.19). The genotype IC-311441 (4.13) showed minimum number of reproductive branches /plant with grand mean 5.86 ± 0.365 .

4.2.6 Pod breadth (cm)

The treatment mean for pod breadth ranged from (3.29 to 4.22) with minimum pod breadth in genotype IC-421855 (3.29 cm) followed by IC-421809 (3.37 cm), IC-421812 (3.48 cm), IC-421838 (3.52 cm) and IC-421815 (3.53 cm). The genotype IC-421815 (4.22 cm) showed maximum pod breadth with grand mean 3.75 ± 0.269 .

4.2.7 Pod length (cm)

The treatment mean for pod length ranged from 4.57 to 6.66 with minimum pod length in IC-421855 (4.57 cm), followed by IC-421828 (4.99 cm), IC-28272 (5.10 cm), IC-373480 (5.21 cm), IC-311441 (5.26 cm) and IC-421815 (5.28 cm). The genotype IC-415142 (6.66 cm) showed maximum pod length with grand mean 5.71 ± 0.336 .

4.2.8 Pod width (cm)

The maximum pod width was recorded in genotype IC-421806 (6.34 cm) and minimum pod width in genotype IC-421809 (5.68 cm) with grand mean 5.95 ± 0.433 .

4.2.9 Number of pods/plant

The maximum and minimum number of pods /plant was recorded in genotype IC-421834 (73.74) and IC-373427 (42.47), respectively with grand mean 56.36 ± 3.16 .

4.2.10 Number of pods /cluster

The maximum and minimum number of pods/cluster was recorded in genotype IC-415142 (20.22) and IC-329038 (4.33), respectively with grand mean 7.74 ± 0.542 .

4.2.11- Number of clusters /plant

The treatment mean for number of clusters/plant ranged from 6.83 to 29.33 with minimum number of clusters/plant in genotype IC-311441 (6.83) followed by IC-28272 (7.25), IC-421855 (7.54), HG-365 (7.89) and IC-329038 (7.99). The genotype IC-373480 (29.33) showed maximum number of clusters/plant with grand mean 11.80 ± 0.498 .

4.2.12 Number of branches /plant at maturity

The maximum number of branches /plant at maturity was recorded in genotype IC-415137 (10.17) followed by IC-258087 (9.88). The genotype IC-421838 (7.25) showed minimum number of branches /plant at maturity with grand mean 8.41 ± 0.217 .

4.2.13 Number of seeds/pod

The treatment mean for number of seeds/pod ranged from (5.95 to 7.52) with minimum number of seeds/pod in genotype IC-369868 (5.95) followed by IC-28272

(5.96), IC-415142 (5.96) and IC-329038 (4.38). The genotype IC-415157 (7.52) showed maximum number of seeds/pod with grand mean 6.76 ± 0.241 .

4.2.14 Pod yield/plant (g)

The maximum pod yield/plant was recorded in genotype IC-421812 (121.49 g) and minimum in genotype IC-325757 (79.23 g) followed by IC-28272 (82.14 g) with grand mean 96.32 ± 1.96 .

4.2.15 Pod yield/plot (kg)

The maximum pod yield/plot was recorded in genotype IC-421828 (0.76 kg) followed by IC-421855 (0.75 kg) and minimum in genotype IC-415159 (0.39 kg) followed by IC-421242 (0.48 kg) and IC-369789 (0.48 kg) with grand mean 0.57 ± 0.032 .

4.2.16 100-seed weight (g)

The maximum and minimum 100-seed weight was recorded in genotype IC-421855 (4.33 g) and genotype IC-421828 (3.00), respectively with grand mean 3.62 ± 0.167 .

4.2.17 Seed yield/plant (g)

The maximum seed yield/plant was recorded in genotype HG-365 (8.64 g) and minimum in genotype IC-311441 (4.79 g) followed by IC-311440 (5.24 g) with grand mean 6.69 ± 0.302 .

4.2.18 Days to maturity

The maximum days to maturity was recorded in genotype IC-373427 (97.27) followed by IC-461855 (96.90) and minimum in genotype IC-325757 (86.42) followed by IC-329038 (86.60) with grand mean 92.53 ± 0.738 .

4.2.19 Pod yield (q/ha)

The treatment mean for pod yield ranged from (119.40 to 142.23 q/ha). The minimum pod yield was recorded in genotype IC-258087 (119.40 q/ha) followed by IC-415159 (119.61 q/ha), IC-370490 (120.35 q/ha), IC-329038 (120.41 q/ha), IC-258092 (121.32 q/ha) and IC-28272 (121.44 q/ha). The genotype IC-421855 (142.23 q/ha) showed maximum pod yield with grand mean 126.93 ± 2.72 .

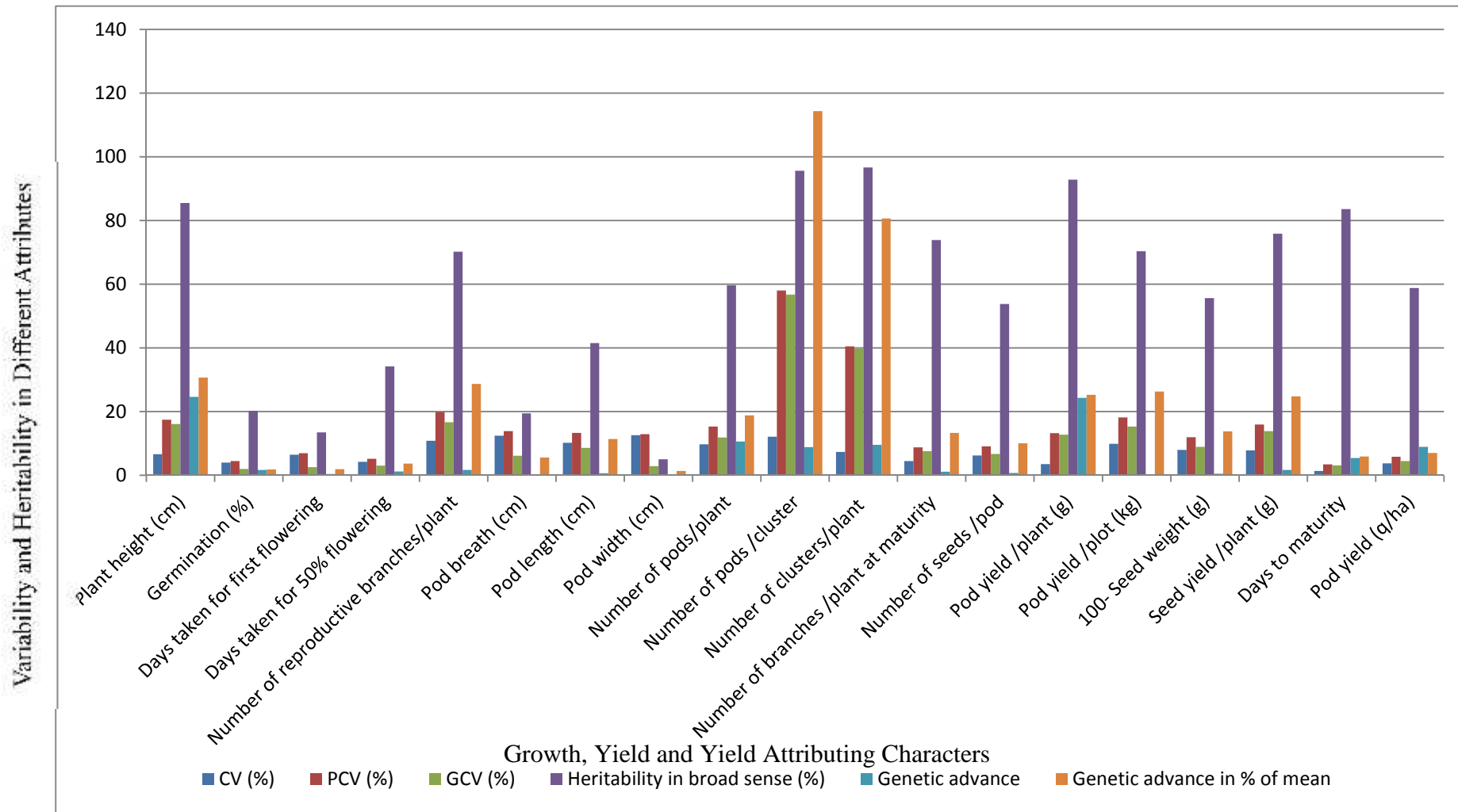
4.3 Coefficient of variation

4.3.1 Phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation (GCV and PCV) for 11 growth characters and 8 yield and yield attributing characters revealed that all the horticultural parameters had significant range of phenotypic and genotypic coefficient of variation are presented in Table- 4.6 with also in Graph- 4.6. In morphological and yield contributing characters, the highest phenotypic coefficient of variation was observed for number of pods/cluster (58.05) followed by number of clusters/plant (40.47) and lowest for days to maturity (3.41) followed by germination % (4.46%).

Table- 4.6 Mean, SEM±, range, coefficient of variability, heritability in broad sense, genetic advance and genetic advance in percent of mean with different characters in 30 genotypes of cluster bean

Characters	General Mean	SEM±	Range		Co-efficient of variation			Heritability in broad sense (%)	Genetic advance (GA)	Genetic advance in % of mean
			Min.	Max.	CV (%)	PCV (%)	GCV (%)			
Plant height (cm)	80.24	3.07	55.71	107.69	6.63	17.42	16.11	85.50	24.62	30.68
Germination (%)	91.64	2.10	87.24	95.94	3.98	4.46	2.00	20.20	1.70	1.85
Days taken for first flowering	22.40	0.831	20.46	23.83	6.42	6.91	2.54	13.50	0.43	1.91
Days taken for 50% flowering	33.55	0.813	31.40	36.34	4.20	5.18	3.03	34.20	1.22	3.63
Number of reproductive branches/plant	5.86	0.365	4.13	7.75	10.80	19.81	16.61	70.20	1.68	28.66
Pod breath (cm)	3.75	0.269	3.29	4.22	12.43	13.85	6.09	19.40	0.21	5.60
Pod length (cm)	5.71	0.336	4.57	6.66	10.20	13.34	8.60	41.50	0.65	11.38
Pod width (cm)	5.95	0.433	5.68	6.34	12.60	12.93	2.88	5.00	0.08	1.34
Number of pods/plant	56.36	3.16	42.47	73.74	9.73	15.33	11.84	59.70	10.62	18.84
Number of pods /cluster	7.74	0.452	4.33	20.22	12.13	58.05	56.77	95.60	8.85	114.34
Number of clusters/plant	11.80	0.498	6.83	29.33	7.31	40.47	39.80	96.70	9.52	80.67
Number of branches /plant at maturity	8.41	0.217	7.25	10.17	4.47	8.77	7.54	73.90	1.12	13.31
Number of seeds /pod	6.76	0.241	5.95	7.52	6.19	9.11	6.68	53.80	0.68	10.05
Pod yield /plant (g)	96.32	1.96	79.23	121.49	3.53	13.21	12.72	92.80	24.33	25.25
Pod yield /plot (kg)	0.57	0.032	0.39	0.76	9.89	18.20	15.27	70.40	0.15	26.31
100- Seed weight (g)	3.63	0.167	3.00	4.33	7.96	11.96	8.92	55.60	0.50	13.77
Seed yield /plant (g)	6.69	0.302	4.79	8.64	7.81	15.91	13.86	75.90	1.66	24.81
Days to maturity	92.53	0.738	86.42	97.27	1.38	3.41	3.12	83.60	5.44	5.87
Pod yield (q/ha)	126.93	2.72	119.40	142.23	3.72	5.80	4.45	58.80	8.92	7.02



Graph- 4.6 Mean, SE(m)±, range, coefficient of variability, heritability in broad sense, genetic advance and genetic advance in percent of mean with different characters in 30 genotypes of cluster bean

4.3.2 Genotypic coefficient of variation

The genotypic coefficient of variation for germination% (2.00%) followed by days taken for first flowering (2.54%) were the minimum while it was observed to be the highest genotypic coefficient of variation was observed for number of pods/cluster (56.77) followed by number of clusters/plant (39.80) in morphological and yield contributing characters.

It was manifested from the data that the phenotypic coefficient of variation for all the characters (morphological, yield and yield attributing characters) was higher than the corresponding genotypic coefficient of variation are presented in Table- 4.6 with also in Graph- 4.6.

The largest variation was found for days to maturity, plant height, pods per plant and number of clusters per plant, these results were in agreement with the findings of Sultan *et al.* (2012).

Comparatively, low variation was noticed for seeds per pod, 100-seed weight, secondary branches per plant, pod length, pods per cluster and days to 50 per cent flowering, these results were in agreement with the findings of Morris (2010), Sultan *et al.* (2012) and Gopalakrishnan *et al.* (2011). Observed variability found among guar accessions can be probably attributed to the genetic differences and the environment, in which these accessions were grown.

The characters like 100-seed weight, plant height, green pod yield/plot, green pod yield/plant and green pod yield (q/ha), showed high genotypic coefficient variation and phenotypic coefficient variation with genetic advance, indicating there by that selections based on phenotypic performance could be effective for improvement of these characters, these results were in accordance with the findings of Jay Prakash *et al.* (2015).

4.4 Heritability and expected genetic advance in percent of mean

In the present study, high heritability coupled with high genetic advance as percent over mean was recorded for all the growth characters viz., number of clusters/plant, number of pods/cluster, pod yield/plant (g), plant height (cm) and seed yield/plant (g). The characters like number of pods/plant, 100-seed weight (g),

number of branches/plant at maturity and pod length (cm) had shown high heritability values with moderate genetic advance as percent over mean indicating predominance of additive gene component. Thus, there is ample scope for improving of these characters through direct selection.

The estimates for heritability in broad sense for different characters are presented in Table- 4.6 with also in Graph- 4.6. The heritability estimates for morphological and yield characters ranged from (5.00%) for pod width (cm) to (96.70%) for number of clusters/plant. High heritability was recorded for number of clusters/plant (96.70%) followed by number of pods/plant (95.60%), pod yield/plant (92.80%), plant height (85.50%) and days to maturity (83.60%).

Heritability estimates were high for all the characters except 100-seed weight (g), plant height (cm) and number of pods/plant, whereas, in the second year 100-seed weight (g), plant height (cm), moisture (%), number of pods/plant, green pod yield (q/ha) and number of leaves/plant attained lower heritability values, these results were in confirmation with Prakash and Ram (2014).

4.4.1 Expected genetic advance

Expected genetic advance and genetic gain for morphological and yield characters. Genetic advance at 5% selection intensity and its estimates as percentage of mean for various morphological and yield characters were calculated and presented in Table- 9, respectively. The data recorded revealed maximum genetic advance was observed for plant height (cm) (24.62) with 30.68% advance high over the mean and minimum for pod width (0.08) with 1.34% genetic advance over the mean are also presented in Table- 4.6 with also in Graph- 4.6.

With respect to yield and yield component parameters the characters like pods/plant (g), days to maturity, seed yield/plant (g), number of branches/plant at maturity and pod yield (q/ha) recorded high heritability coupled with moderate to high genetic advance over mean. This indicates existence of additive components for these traits. Thus, there is ample scope for direct selection for these characters. The characters like pod breadth (cm), days taken for 50% flowering, days taken for first flowering, germination% and pod width (cm) recorded high heritability coupled with low

genetic advance over mean. This was the indicative of dominant and epistatic effects for these characters.

4.5 Correlation coefficient

Genotypic and phenotypic correlation coefficient between yield and yield contributing characters were estimates and results are presented in Table- 4.7, respectively. In general, the results revealed that the estimates of genotypic correlation coefficients are higher than the phenotypic correlation for almost under study. The results obtained from the genotypic and phenotypic correlations are presented characters wise here as under.

Table- 4.7 Estimates of genotypic and phenotypic correlation coefficients of different characters in cluster bean

Characters	Plant height (cm)	Germination (%)	Days taken for first flowering	Days taken for 50% flowering	Number of reproductive branches/plant	Pod breadth (cm)	Pod length (cm)	Pod width (cm)	Number of pods/plant	Number of pods /cluster	Number of clusters /plant	Number of branches /plant at maturity	Number of seeds /pod	Pod yield /plant (g)	Pod yield /plot (kg)	100-seed weight (g)	Seed yield /plant (g)	Days to maturity	Pod yield (q/ha)
1	G	0.254	0.063	-0.435*	0.430*	-0.061	0.09	0.091	-0.112	-0.029	-0.035	0.359*	0.11	0.023	0.096	0.328*	0.412*	0.282	0.148
	P	0.123	-0.045	-0.248*	0.312*	-0.032	0.045	0.028	-0.087	-0.015	-0.031	0.265*	0.103	0.024	0.096	0.235	0.368*	0.243	0.135
2	G	rg	-0.191	0.276*	0.046	-0.145	0.013	0.157	0.735**	0.068	-0.241	-0.135	0.012	0.11	0.155	-0.178*	-0.282*	-0.084	0.151
	P		0.051	0.096	0.013	0.028	0.015	0.01	0.289*	0.027	-0.095	-0.098	-0.087	0.068	0.118	-0.056	-0.086	-0.032	0.094
3	G		rg	0.352*	0.811**	-0.481*	-0.012	0.152	0.108	0.499*	0.13	-0.15	0.203	0.476*	0.085	0.115	0.429*	0.550**	0.135
	P			0.045	0.212*	-0.03	-0.042	0.051	0.087	0.167	0.087	-0.07	0.142	0.183	0.038	0.043	0.118	0.218*	0.092
4	G			rg	-0.025	-0.479*	-0.046	-0.21	0.233	-0.314*	-0.21	-0.156	0.016	0.456*	0.488*	-0.311*	-0.019	0.121	0.335*
	P				0.048	0.058	-0.048	0.178	0.18	-0.161	-0.125	-0.062	0.028	0.282*	0.289*	-0.053	-0.006	0.092	0.183
5	G				rg	-0.158	0.085	-0.08	0.055	0.567**	0.038	0.219	0.467**	0.400*	0.1	0.13	0.403*	0.286	0.319*
	P					-0.085	0.056	-0.031	0.037	0.443*	0.035	0.19	0.243	0.312*	0.091	0.106	0.352*	0.211	0.308*
6	G					rg	0.236	0.390*	-0.045	0.144	-0.127	-0.039	-0.167	-0.431*	-0.257	-0.037	-0.315*	-0.441*	-0.380*
	P						0.163	0.477*	0.156	0.085	-0.043	-0.106	-0.135	-0.212	-0.125	-0.025	-0.191	-0.172	-0.204
7	G						rg	0.172	-0.224*	0.254*	-0.007	0.295*	-0.132	-0.151	-0.147	-0.135	-0.135	0.056	-0.149
	P							0.073	-0.187	0.185	-0.015	0.164	-0.066	-0.108	-0.054	-0.049	-0.086	0.037	-0.082
8	G							rg	-0.05	-0.11	0.183	-0.292*	-0.105	-0.288*	-0.08	-0.204	-0.107	0.017	-0.284*
	P								0.290*	-0.032	0.054	-0.075	0.054	-0.086	0.018	-0.056	-0.052	0.015	-0.028
9	G								rg	0.178	-0.079	-0.271*	0.052	0.167	0.141	-0.13	-0.056	-0.049	0.203*
	P									0.107	-0.052	-0.135	0.042	0.145	0.093	-0.04	-0.048	-0.033	0.146
10	G									rg	-0.176	0.202*	0.149	-0.101	-0.179	0.031	0.006	-0.142	-0.246*
	P										-0.166	0.174	0.102	-0.083	-0.129	0.032	0.025	-0.124	-0.187
11	G										rg	-0.252	-0.165	-0.115	-0.122	-0.238*	0.009	0.185	-0.099
	P											-0.213*	-0.132	-0.109	-0.091	-0.164	0.035	0.168	-0.098
12	G											rg	0.07	-0.08	0.151	0.457*	0.034	0.258*	0.155

	P												0.078	-0.059	0.185	0.339*	0.128	0.185	0.065	
13	G												rg	0.488*	0.053	0.154	0.346*	-0.132	0.435*	
	P													0.332*	0.097	0.125	0.294*	-0.043	0.309*	
14	G													rg	0.442*	0.17	0.473*	0.339*	0.805**	
	P														0.397*	0.168	0.456*	0.335*	0.607**	
15	G														rg	0.063	0.376*	0.501**	0.570**	
	P															0.289	0.359*	0.451*	0.560**	
16	G															rg	0.222	0.431*	0.282	
	P																0.244	0.388*	0.438*	
17	G																rg	0.285	0.423*	
	P																	0.278	0.457*	
18	G																	rg	0.517**	
	p																			0.473**
																				rg

***and** indicate significant of values at P=0.05 and 0.01, respectively**

4.6 Genotypic correlation coefficient

4.6.1 Plant height (cm)

Plant height exhibited significant and positive correlation with number of reproductive branches/plant (0.430), number of branches/plant at maturity (0.359), 100-seed weight (0.328) and seed yield/plant (0.412).

4.6.2 Germination (%)

Germination % exhibited significant and positive correlation with days taken for 50% flowering (0.276) and number of pods/plant and negative correlation with 100-seed weight (-0.178) and seed yield/plant (-0.282).

4.6.3 Days taken for first flowering

Days taken for first flowering exhibited significant and positive correlation with number of reproductive branches/plant (0.811), number of pods/cluster (0.499), pod yield/plant (0.476), seed yield/plant (0.429) and days to maturity(0.550) and moderate negative correlation with pod breadth (-0.481).

4.6.4 Days taken for 50% flowering

Days taken for 50% flowering exhibited significant and positive correlation with pod yield/plant (0.456), pod yield/plot (0.488) and pod yield per hectare (0.335) and moderately negative correlation with pod breadth (-0.479), number of pods/cluster (-0.314) and 100-seed weight (-0.311).

4.6.5 Number of reproductive branches/plant

Number of reproductive branches/plant exhibited significant and positive correlation with number of pods/cluster (0.567), number of seeds/pod (0.467), pod yield/plant (0.400) and seed yield/plant (0.403).

4.6.6 Pod breadth (cm)

Pod breadth exhibited significant and positive correlation with pod width (0.390) and moderate negative correlation with pod yield/plant (-0.431), seed yield/plant (0.315), days to maturity (0.441) and pod yield (-0.380).

4.6.7 Pod length (cm)

Pod length showed positive correlation of 0.254 and 0.295 with number of pods/cluster and number of branches/plant at maturity and negative correlation of -0.224 with number of pods/plant.

4.6.8 Pod width (cm)

Pod width exhibited significant low negative correlation with number of branches/plant at maturity (-0.292), pod yield/plant (-0.288) and pod yield per hectare (-0.284).

4.6.9 Number of pods/plant

Number of pods/plant exhibited negative correlation with number of branches/plant at maturity (-0.271) and positive correlation with pod yield per hectare (0.203).

4.6.10 Number of pods /cluster

Number of pods/cluster is positive correlated with number of branches/plant at maturity (0.202) and negative correlated with pod yield per hectare (-0.246).

4.6.11 Number of clusters /plant

Number of clusters/plant exhibited significant negative correlation with 100 seed weight (-0.238).

4.6.12 Number of branches/plant at maturity

Number of branches/plant at maturity exhibited positive correlation with 100 seed weight (0.457) and days to maturity (0.258).

4.6.13 Number of seeds/pod

Number of seeds/pod exhibited positive correlation with seed yield/plant (0.346) and pod yield per hectare (0.435).

4.6.14 Pod yield/plant (g)

Pod yield/plant exhibited high significant and positive correlation with seed yield/plant (0.473), days to maturity (0.339) and pod yield per hectare (0.805).

4.6.15 Pod yield/plot (kg)

Pod yield/plot exhibited high significant and positive correlation with seed yield/plant (0.376), days to maturity (0.501) and pod yield per hectare (0.570).

4.6. 16 100-seed weight (g)

100-seed weight exhibited significant and positive correlation with days to maturity (0.431).

4.6.17 Seed yield/plant (g)

Seed yield/plant exhibited significant and positive correlation with pod yield per hectare (0.423).

4.6.18 Days to maturity

Days to maturity exhibited highly significant and positive correlation with pod yield per hectare (0.517).

4.7 Phenotypic correlation coefficient

A perusal of data on phenotypic correlation coefficient for morphological and yield attributing characters has been presented in Table- 4.7.

4.7.1 Plant height

Exhibited significant and positive correlation with number of reproductive branches/plant (0.312), number of branches/plant at maturity (0.265) and seed yield/plant (0.368) and negative correlation with days taken for 50% flowering (-0.248) at phenotypic level.

4.7.2 Germination %

Exhibited significant and positive correlation with number of pods/plant (0.289) at phenotypic level.

4.7.3 Days taken for first flowering

Exhibited significant and positive correlation with pod breadth (0.212) and days to maturity (0.218) at phenotypic level.

4.7.3 Days taken for 50% flowering

Exhibited significant and positive correlation with pod yield/plant (0.282) and pod yield/plot (0.289).

4.7.4 Number of reproductive branches/plant

Exhibited significant and positive correlation with number of pods/cluster (0.443), pod yield/plant (0.312), seed yield/plant (0.352) and pod yield (0.308).

4.7.5 Pod breadth

Exhibited significant and positive correlation with pod width (0.477) and negative correlation with pod yield/plant (-0.212).

4.7.6 Number of branches/plant at maturity

Exhibited significant and positive correlation with 100 seed weight (0.339) at phenotypic level.

4.7.7 Number of seeds/pod

Exhibited significant positive correlation with seed yield/plant (0.294) and pod yield per hectare (0.339).

4.7.8 Pods yield/plant

Exhibited high significant and positive correlation with pod yield (0.607), seed yield/plant (0.456) and days to maturity (0.335) at phenotypic level.

4.7.9 100-seed weight

Exhibited significant and positive correlation with days to maturity (0.388) and pod yield per hectare (0.438).

4.7.10 Seed yield/plant

Seed yield/plant is significantly and positively correlated with pod yield per hectare (0.457).

4.7.11 Days to maturity

Days to maturity is positively and significantly correlated with pod yield hectare (0.473).

4.8 Path analysis

The correlation coefficient would only indicate the relationship of independent variable without specifying cause and effect relationship. Using path analysis, it is possible to resolve the correlations, which will provide direct and indirect contribution of different morphological and yield and yield contributing characters.

In case of path coefficient analysis, pod yield per hectare was considered as the dependent variable for 11 morphological characters, while remaining 8 yield and

yield attributing characters were considered as independent variables. For morphological characters, the phenotypic and genotypic path analysis representing the direct and indirect effects of different characters has been presented in Table- 4.8 and yield contributing characters also presented in Table- 4.8, respectively.

Table- 4.8 Estimates genotypic and phenotypic path coefficient effect of different characters in cluster bean

Characters		Plant height (cm)	Germination (%)	Days taken for first flowering	Days taken for 50% flowering	Number of reproductive branches/plant	Pod breath (cm)	Pod length (cm)	Pod width (cm)	Number of pods/plant	Number of pods /cluster	Number of clusters /plant	Number of branches /plant at maturity	Number of seeds /pod	Pod yield /plant (g)	Pod yield /plot (kg)	100- seed weight (g)	Seed yield /plant (g)	Days to maturity
1	G	5.527	-0.966**	0.197	-0.463*	-2.220**	-0.029	-0.023	-0.086	-0.255	-0.042	-0.02	0.06	0.175	0.043	0.151	-0.186	-1.412**	0.148
	P	-0.076	0.009	0.045	0.0012	0.026	0.011	0.034	0.007	-0.009	0.011	0.032	0.042	0.013	0.016	0.009	0.019	0.022	0.135
2	G	1.403	-3.807**	-0.596**	0.294*	-0.240*	-0.068	0.008	-0.08	1.668**	0.097	-0.149	-0.023	0.026	0.202	0.245*	0.101	0.966**	0.151
	P	-0.008	0.048	0.009	-0.015	0.018	0.007	-0.011	0.029	0.023	-0.009	0.008	0.01	-0.009	0.026	0.021	-0.009	-0.028	0.094
3	G	0.349	0.726	3.126**	0.375*	-4.189**	-0.227	-0.009	-0.077	0.246	0.716**	0.081	-0.025	0.322*	0.875**	0.133	-0.068	-1.470**	0.135
	P	0.011	-0.006	-0.098	0.005	0.025	0.009	0.015	0.007	0.025	-0.029	-0.005	0.012	0.048	0.062	0.011	0.004	0.031	0.092
4	G	-2.404	-1.052	1.101	1.065**	0.128	-0.226*	-0.012	0.107	0.527**	-0.451*	-0.13	-0.026	0.039	0.839**	0.770*	0.176	0.085	0.335*
	P	0.007	0.005	-0.004	-0.082	0.016	0.021	-0.004	0.005	0.014	0.056	0.062	0.007	0.003	0.108	0.058	-0.008	0.018	0.183
5	G	2.377	-0.177	2.536	-0.026	-5.163**	-0.074	0.028	0.043	0.124	0.814**	0.023	0.037	0.742**	0.734**	0.158	-0.074	-1.380**	0.319*
	P	-0.008	0.011	0.084	-0.005	0.093	0.019	0.01	0.058	0.126	-0.078	-0.016	0.023	0.037	0.119	0.012	0.019	0.015	0.308*
6	G	-0.334	0.552	-1.503	-0.51	0.814	0.472*	0.06	-0.199	-0.102	0.207*	-0.078	-0.009	-0.265*	-0.792**	-0.406*	0.021	1.081**	-0.380*
	P	0.012	0.008	0.023	0.019	-0.008	-0.023	0.018	0.009	0.013	-0.015	0.006	0.008	-0.109	-0.081	-0.025	-0.009	-0.028	-0.204
7	G	0.497	-0.049	0.036	-0.069	-0.437	0.112	0.255*	-0.088	-0.508**	0.364*	-0.015	0.05	-0.21	-0.277*	-0.232	0.077	0.464*	-0.149
	P	-0.007	0.005	0.009	0.11	0.028	-0.035	0.038	0.008	-0.015	-0.033	-0.02	0.018	-0.01	-0.041	-0.011	-0.011	-0.017	-0.082
8	G	0.504	-0.599	0.474	-0.223	0.412	0.184	0.044	-0.510**	-0.113	-0.157	0.113	-0.051	-0.167	-0.530**	-0.126	0.116	0.367*	-0.284
	P	-0.005	0.008	-0.006	-0.009	-0.006	-0.02	0.011	0.029	0.039	0.009	0.007	-0.006	0.042	0.011	-0.033	0.01	-0.008	-0.028
9	G	-0.621	-2.8	0.339	0.248	-0.282	-0.061	-0.057	0.025	2.268**	0.255*	-0.059	-0.046	0.082	0.308*	0.223	0.074	0.19	0.203
	P	0.005	0.012	-0.007	-0.017	0.128	0.035	-0.012	0.009	0.179	-0.086	0.048	0.019	0.01	0.048	0.017	-0.008	-0.015	0.146
10	G	-0.161	-0.258	1.56	-0.335	-2.929	0.078	0.065	0.056	0.403	1.436**	-0.109	0.034	0.237*	-0.186	-0.282*	-0.018	-0.053	-0.246
	P	0.009	0.005	-0.025	0.015	0.041	0.238	0.008	0.01	-0.176	0.046	0.068	0.015	-0.032	-0.026	0.011	0.015	-0.014	-0.187

11	G	-0.182	0.918	0.408	-0.223	-0.194	-0.06	-0.032	-0.093	-0.18	-0.253	0.619**	-0.042	-0.262*	-0.212*	-0.193	0.135	-0.03	-0.099
	P	0.006	-0.008	-0.006	0.009	0.038	0.025	0.018	0.035	-0.039	0.029	-0.047	0.012	-0.018	-0.041	-0.019	-0.015	0.029	-0.098
12	G	1.984	0.514	-0.468	-0.166	-1.13	-0.048	0.075	0.259	-0.615	0.29	-0.156	0.168	0.111	-0.147	0.238*	-0.259*	-0.117	0.155
	P	-0.009	-0.011	0.019	0.025	0.018	0.015	0.021	0.008	-0.011	-0.029	0.063	0.089	0.012	-0.022	0.018	0.032	0.039	0.065
13	G	0.608	-0.057	0.634	0.017	-2.41	-0.079	-0.034	0.056	0.117	0.214	-0.102	0.036	1.590**	0.896**	0.083	-0.087	-1.186**	0.435*
	P	-0.012	-0.006	-0.015	-0.009	0.023	0.021	-0.007	0.016	0.238	-0.098	0.035	0.038	0.152	0.127	0.013	0.011	0.016	0.309*
14	G	0.128	-0.418	1.489	0.486	-2.063	-0.203	-0.039	0.147	0.379	-0.145	-0.071	-0.013	0.775	1.838**	0.697* *	-0.096	-1.621**	0.805**
	P	-0.007	0.009	-0.01	-0.014	0.029	0.019	-0.011	0.008	0.089	0.365	0.119	0.045	0.05	0.382*	0.079	0.016	0.059	0.607**
15	G	0.53	-0.591	0.264	0.52	-0.518	-0.121	-0.038	0.041	0.321	-0.257	-0.086	0.025	0.095	0.813	1.577* *	-0.045	-1.289**	0.570**
	P	-0.01	0.012	-0.011	-0.014	0.015	0.028	-0.008	0.009	0.023	0.159	0.293	0.068	0.01	0.151	0.2	0.02	0.058	0.560**
16	G	1.815	0.676	0.36	-0.331	-0.67	-0.018	-0.035	0.134	-0.295	0.048	-0.147	0.077	0.245	0.313	0.068	-0.566**	-0.761**	0.282
	P	-0.013	-0.007	-0.008	0.01	0.014	0.005	-0.006	0.012	-0.009	0.078	0.088	0.185	0.018	0.064	0.042	0.094	0.062	0.438*
17	G	2.277	1.073	1.341	-0.02	-2.079	-0.149	-0.035	0.055	-0.126	0.089	0.037	0.006	0.55	0.87	0.593	-0.126	-3.427**	0.423*
	P	-0.01	-0.005	-0.037	0.014	0.027	0.009	-0.008	0.01	-0.049	-0.136	0.096	0.045	0.048	0.174	0.072	0.023	0.086	0.457*
18	G	1.557	0.319	1.719	0.128	-1.477	-0.208	0.014	-0.038	-0.111	-0.205	0.112	0.043	-0.176	0.623	0.79	-0.244	-0.978	0.517**
	p	-0.018	-0.009	-0.038	-0.005	0.02	0.029	0.01	0.015	-0.008	0.135	-0.146	0.089	-0.007	0.128	0.09	0.037	0.169	0.473*

and* indicate significant of values at P=0.05 and 0.01, respectively

Residual value: (Genotypic - 0.8951 and phenotypic - 0.5084)

Bold figures indicate direct effects

4.9 Genotypic path coefficient analysis for pod yield (q/ha)

4.9.1 Plant height (cm)

Plant height despite its positive effect (5.527) had strong positive association with pod yield (q/ha) ($r_G=0.148$). This is mainly because of its high indirect negative effects through number of reproductive branches/plant (-2.220), seed yield/plant (-1.412), germination% (-0.966) and days taken for 50% flowering (-0.463).

4.9.2 Germination (%)

Germination % despite its negative effect (-3.807) had strong positive association with pod yield q/ha ($r_G=0.151$). This is mainly because of its high indirect positive effects through number of pods/plant (1.668), seed yield/plant (0.966), days taken for 50% flowering (0.294) and pods yield/plot (0.245). The negative indirect effects of germination% *via* days taken for first flowering (-0.596) and number of reproductive branches/plant (-0.240) failed to alter positive association.

4.9.3 Days taken for first flowering

Days taken for first flowering exhibited positive direct effect on yield (3.126) and positively associated with pod yield (q/ha) ($r_G=0.135$). This is mainly because of its positive indirect effects through pod yield/plant (0.875), number of pods/cluster (0.716), days taken for 50% flowering (0.375) and number of seeds/pod (0.322). The negative indirect effects were through number of reproductive branches/plant (-4.189) and seed yield/plant (-1.470).

4.9.4 Days taken for 50% flowering

Days taken for 50% flowering exhibited positive direct effects on yield (1.065) and positively associated with pod yield (q/ha) ($r_G=0.335$). This is mainly because of its positive indirect effects through pods number of pods/plant (0.527, pod yield/plant (0.839) and pod yield/plot (0.770). The negative indirect effects were through pod breadth (-0.226) and number of pods/cluster (-0.451).

4.9.5 Number of reproductive branches/plant

Number of reproductive branches/plant despite its high negative direct effect (-5.163) had positive association with pod yield (q/ha) ($r_G=0.319$). This was mainly because of its positive indirect effects through number of pods/cluster (0.814),

number of seeds/pod (0.742) and pod yield/plant (0.734). The negative indirect effects were seen through seed yield/plant (- 1.380).

4.9.6 Pod breadth (cm)

Pod breadth exhibited positive direct effect (0.472) but had negative association with pod yield (q/ha) ($rG=-0.380$). The positive indirect effects were through seed yield/plant (1.081) and number of pods/cluster (0.207). The negative indirect effects were seen through number of seeds/pod (-0.265), pod yield/plant (-0.792) and pod yield/plot (-0.406).

4.9.7 Pod length (cm)

Pod length exhibited positive direct effect (0.255) but had negative association with pod yield (q/ha) ($rG=-0.149$). The positive indirect effects were through number of pods/cluster (0.364) and seed yield/plant (0.464). The negative indirect effects were seen through number of pods/plant (-0.508) and pod yield/plant (-0.277).

4.9.8 Pod width (cm)

Pod width exhibited negative direct effect (0.510) and also had negative association with pod yield (q/ha) ($rG=-0.284$). The positive indirect effects were through seed yield/plant (0.367). The negative indirect effects were seen through pod yield/plant (-0.530).

4.9.9 Number of pods/plant

Number of pods per plant exhibited positive direct effect (2.268) and also had positive association with pod yield (q/ha) ($rG=0.203$). The positive indirect effects were through number of pods/cluster (0.255) and pod yield/plant (0.308).

4.9.10 Number of pods/cluster

Number of pods per cluster exhibited positive direct effects (1.436) but had negative association with pod yield (q/ha) ($rG=-0.246$). The positive indirect effects were through number of seeds/pod (0.237). The negative indirect effects were through pod yield/pot (-0.282).

4.9.11 Number of clusters/plant

Number of clusters/plant exhibited positive direct effect (0.619) and had negative association with pod yield (q/ha) ($rG=-0.099$). The negative indirect effects were seen through number of seeds/pod (-0.262) and pod yield/plant (-0.212).

4.9.12 Number of branches/plant at maturity

Number of branches/plant at maturity exhibited positive direct effects (0.168) and also had positive association with pod yield (q/ha) ($r_G=0.155$). The positive indirect effects were through pod yield/plot (0.238) and negative indirect effects were seen through 100-seed weight (-0.259).

4.9.13 Number of seeds/pod

Number of seeds/pod shown positive direct effect (1.590) and positive association ($r_G=0.435$) on pod yield (q/ha). The positive indirect effects were through pod yield/plot (0.896). The negative indirect effects were due to seed yield/plant (-1.186).

4.9.14 Pod yield/plant (g)

Pod yield/plant exhibited positive direct effect (1.838) and also had positive association with pod yield (q/ha) ($r_G=0.805$). The positive indirect effects were through pod yield/plot (0.697). The negative indirect effects were through seed yield/plant (-1.621).

5.9.15 Pod yield/plot (kg)

Pod yield/plot exhibited positive direct effect (1.577) and also had positive association with pod yield (q/ha) ($r_G=0.570$). The negative indirect effects were through seed yield/plant (-1.289).

4.9.16 100 seed weight (g)

100-seed weight exhibited negative direct effect (-0.566) but also had positive association with pod yield (q/ha) ($r_G=0.282$). The negative indirect effects were through seed yield/plant (-0.761).

4.9.17 Seed yield/plant (g)

Seed yield/plant exhibited negative direct effect (-3.427) but also had positive association with days to maturity ($r_G=0.423$). The negative indirect effects was none. Positive direct effect for number of seeds/pod on seed yield/plant.

4.10 Phenotypic path coefficient analysis for pod yield (q/ha)

All the 19 characters of pod yield and its contributing characters were subjected to phenotypic path coefficient analysis by considering pod yield per hectare as dependent variable on other independent variable.

Critical reviews of path coefficient analysis at phenotypic level represent direct and indirect effect on morphological and yield and yield contributing characters (Table-4.8).

4.10.1 Plant height

It had negative direct effect (-0.076) on pod yield but has got moderate positive association ($r_P=0.135$). This was mainly because of positive indirect effect on yield via days taken for first flowering (0.045), pod length (0.034) and number of branches/plant at maturity (0.042). Its negative indirect effect on pod yield via number of pods per plant (-0.009).

4.10.2 Germination %

It despite its positive effect (0.048) had strong positive association with pod yield q/ha ($r_P=0.094$). This is mainly because of its high indirect positive effects through pod yield/plant (0.026), pod yield/plot (0.021) and pod width (0.029). The negative indirect effects of germination% via days taken for 50% flowering (-0.015) and seed yield/plant (-0.028) failed to alter positive association.

4.10.3 Days taken for first flowering

It had negative direct effect (-0.098) on pod yield and had positive association ($r_G=0.092$). This was mainly because of higher positive indirect effect of trait on yield via number of seeds/pod (0.048), pod yield/plant (0.062) and seed yield/plant (0.031).

4.10.4 Days taken for 50% flowering

It had negative direct phenotypic effect (-0.082) on pod yield and had positive association ($r_G=0.183$). This was mainly because of higher positive indirect effect of trait on yield via number of pods/cluster (0.056), number of clusters/plant (0.062), pod yield/plant (0.108) and pod yield/plot (0.058).

4.10.5 Number of reproductive branches/plant

It had exhibited high positive direct effect on pod yield (0.093) had also positive association ($r_P=0.308$). However positive indirect effect of pod width (0.058), number of pods/plant (0.126) and pod yield/plant (0.119). The negative indirect effects were through number of clusters/plant (-0.016).

4.10.6 Pod breadth

It exhibited negative direct effect (-0.023) but had negative association with pod yield q/ha ($rP=-0.204$). The positive indirect effects were through pod length (0.018) and number of pods/plant (0.013). The negative indirect effects were seen through number of seeds/pod (-0.109) and pod yield/plant (-0.081).

4.10.7 Pod length

It exhibited positive direct effect (0.038) but had negative association with pod yield q/ha ($rP=-0.082$). The positive indirect effects were through number of branches/plant at maturity (0.018). The negative indirect effects were seen through number of pods/cluster (-0.033) and pod yield/plant (-0.041).

4.10.8 Pod width

It found positive direct effect (0.029) with negative association ($rP=-0.028$) for pod yield q/ha. This positive effect of trait had indirect effect through number of pods/plant (0.039) and number of seeds/pod (0.042). The negative indirect effects were seen through pod yield/plot (-0.033).

4.10.9 Number of pods/plant

It had strong positive direct effect (0.179) with strong positive association ($rP=0.146$) on pod yield q/ha. This positive direct effect is due to indirect effect of trait through number of clusters/plant (0.048), number of branches/plant at maturity (0.019) and pod yield/plant (0.048) and also through negative indirect effect of number of pods/cluster (-0.086) and seed yield/plant (-0.015).

4.10.10 Number of pods/cluster

It had positive direct effect on pod yield q/ha of moderate magnitude (0.046) but found negative association ($rP=-0.187$). The trait had indirect positive effect through number of clusters/plant (0.064) and number of branches/plant at maturity (0.015). Trait also had indirect negative effect through number of seeds/pod (-0.032) and pod yield/plant (-0.26).

4.10.11 Number of clusters/plant

It had negative direct effect on pod yield q/ha (-0.047) with positive association ($rP=-0.098$). This direct negative effect is influenced due to negative indirect effect of number of seeds/pod (-0.018) and pod yield/plant (-0.041) and pod

yield/plot (-0.019). Its positive indirect effect through number of branches/plant at maturity (0.012) and seed yield/plant (0.029).

4.10.12 Number of branches/plant at maturity

It exhibited positive direct effects (0.089) and also had positive association with pod yield q/ha ($rP=0.065$). The positive indirect effects were through 100-seed weight (0.032) and seed yield/plant (0.039). The negative indirect effects were seen through pod yield/plant (-0.022).

4.10.13 Number of seeds/pod

It had positive direct effect on pod yield q/ha (0.152) and also had positive association ($rP=0.309$). This is mainly due to positive indirect effect of trait through pod yield/plant (0.127), pods per plant and seed yield/plant (0.016). The negative indirect effect was none.

4.10.14 Pod yield/plant

It had high positive direct effect (0.382) and strong degree of association ($rP=0.607$) for pod yield q/ha. This direct effect is mainly due to the indirect effect of trait positively through pod yield/plot (0.079) and seed yield/plant (0.059) and 100-seed weight (0.016). The negative indirect effect was none.

4.10.15 Pod yield/plot

It exhibited positive direct effect (0.200) and also had strong positive association with pod yield q/ha ($rP=0.570$). The positive indirect effects were through 100-seed weight (0.020) and seed yield/plant (0.058). The negative indirect effect was none.

4.10.16 100-seed weight

It exhibited positive direct effect (0.094) and also had positive association with pod yield q/ha ($rP=0.438$). The positive indirect effects were through seed yield/plant (0.062).

4.10.17 Seed yield/plant

It exhibited positive direct effect (0.086) and also had positive association with days to maturity ($rP=0.457$). The negative indirect effects were none.

In both morphological and yield and yield contributing characters, the residual effect at genotypic level was less compared to the effect of phenotypic level.

So, it may be concluded from these findings that correlation alone may not give complete information but when used in conjunction with path coefficient analysis will give a better measure of cause and effect relationship existing between different pairs of characters.

4.11 Genetic divergence

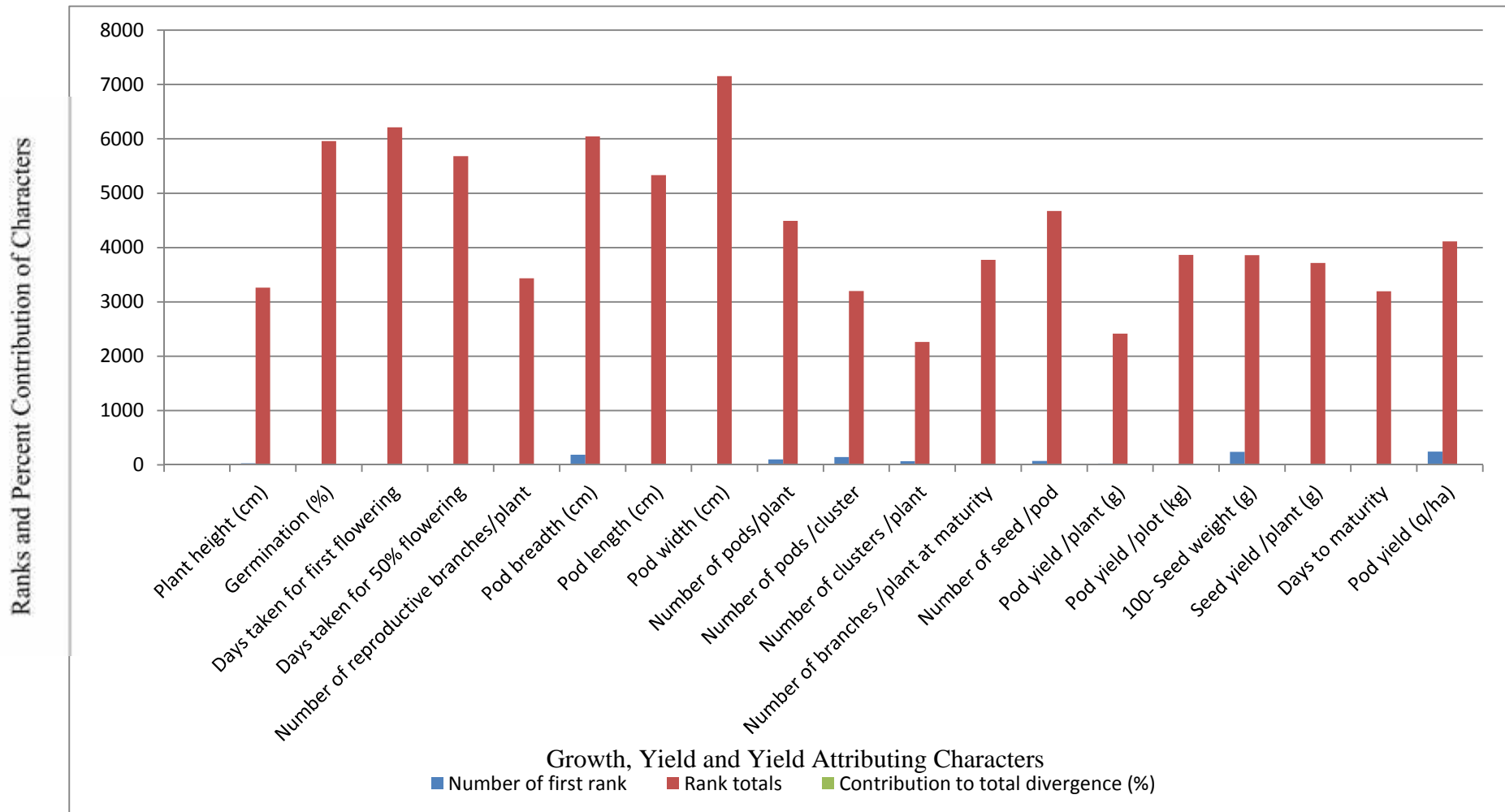
Thirty cluster bean genotypes were evaluated for 19 characters and the data obtained was subjected to D^2 statistics to assess the genetic divergence. Only 5 clusters were constructed using Toucher's method.

4.12 Relative contribution of different characters towards divergence

The relative contribution of different characters for genetic divergence (D^2) is given in Table- 4.9 with Graph- 4.9. Pod yield (q/ha) (20.66%) contributed maximum to the genetic diversity among the genotypes followed by 100-seed weight (g) (20.49%), pod breadth (cm) (16.07%), number of pods/cluster (12.33%), number of pods per plant (8.42%), number of seeds/pod (6.04%) and number of clusters/plant (5.87). While there was lowest contribution found from plant height (cm) (2.47%), pod yield/plant (g) (1.96%), number of reproductive branches/plant (1.87%) and pod yield/plot (kg) (2.37%).

Table- 4.9 Percent contribution of different characters of the total divergence in 30 genotypes of cluster bean

S. No.	Characters	Number of first rank	Rank totals	Contribution to total divergence (%)
1.	Plant height (cm)	29	3260	2.47
2.	Germination (%)	6	5961	0.51
3.	Days taken for first flowering	5	6214	0.43
4.	Days taken for 50% flowering	2	5681	0.17
5.	Number of reproductive branches/plant	22	3432	1.87
6.	Pod breadth (cm)	189	6047	16.07
7.	Pod length (cm)	5	5331	0.43
8.	Pod width (cm)	1	7157	0.09
9.	Number of pods/plant	99	4489	8.42
10.	Number of pods /cluster	145	3200	12.33
11.	Number of clusters /plant	69	2265	5.87
12.	Number of branches /plant at maturity	9	3772	0.77
13.	Number of seed /pod	71	4673	6.04
14.	Pod yield /plant (g)	23	2417	1.96
15.	Pod yield /plot (kg)	17	3864	1.45
16.	100- Seed weight (g)	241	3861	20.49
17.	Seed yield /plant (g)	0.00	3718	0.00
18.	Days to maturity	0.00	3193	0.00
19.	Pod yield (q/ha)	243	4115	20.66
	Total	1176.00		100.00



Graph- 4.9 Percent contribution of different characters of the total divergence in 30 genotypes of cluster bean

4.13 Classification of cluster bean genotypes (D^2 and D value)

By adopting Toucher's method, the 30 genotypes were grouped into five clusters by treating estimated D^2 values as the square of the generalized distance. The distribution pattern of entries into various clusters is given in the Table- 4.10. Cluster I was the largest cluster having 8 genotypes followed by cluster II and cluster IV with six genotype each. Intra and inter-cluster average D^2 values are presented in Table- 4.11 with also in Graph- 4.11. The computed D^2 values for 30 genotypes had wide range showing high genetic divergence among the genotypes. Among the 5 clusters, cluster I with 8 genotypes followed by cluster II and cluster IV with 6 genotypes each showed maximum intra-cluster diversity. The maximum intra-cluster distance was found in cluster V ($D^2=6.625$) closely followed by cluster II (3.813) and cluster III (3.526). Based on distance between clusters (inter-cluster), the maximum divergence was observed between cluster I and cluster II ($D^2=8.235$), followed by cluster II and cluster IV ($D^2=6.552$) and the least inter-cluster distance was found between cluster IV and cluster V ($D^2=3.773$).

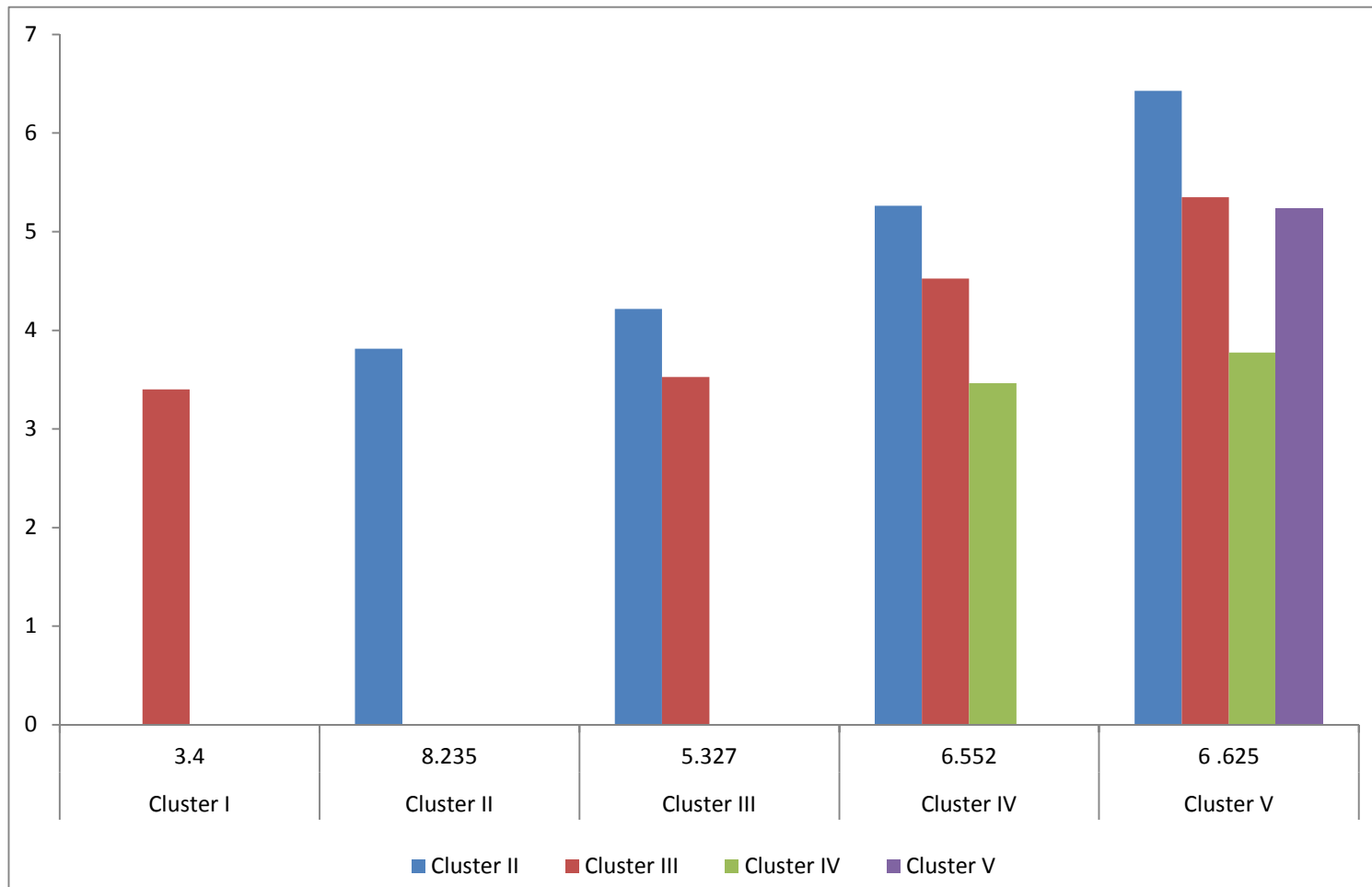
Maximum amount of heterosis is generally expected in cross combinations involving the parents belonging to most divergent clusters. Grouping of accessions based on morphological, phenomonal and reproductive characters is not always associated with their geographical origin.

Table- 4.10 Grouping of cluster bean genotypes based on D² values

S. No.	Cluster number	Number of individual/genotypes	Name of the genotypes
1.	I	1, 2, 3, 4, 5, 8, 9, 11	IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789, IC-369868, IC-370490.
2.	II	6, 7, 12, 13, 14, 25	IC-325757, IC-329038, IC-373427, IC-373480, IC-402293, IC-421820.
3.	III	22, 23, 24, 26, 27	IC-421809, IC-421812, IC-421815, IC-421828, IC-421834.
4.	IV	10, 15, 16, 17, 18, 19	IC-370478, IC-415137, IC-415142, IC-415157, IC-415159, IC-421242.
5.	V	20, 21, 28, 29, 30	IC-421798, IC-421806, IC-421838, IC-421855, HG-365.

Table- 4.11 Average intra and inter cluster D² and D values in 30 genotypes of cluster bean

S. No.	Cluster distances	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
1.	Cluster I	3.400	8.235	5.327	6.552	6.625
2.	Cluster II		3.813	4.218	5.263	6.428
3.	Cluster III			3.526	4.525	5.348
4.	Cluster IV				3.464	3.773
5.	Cluster V					5.243



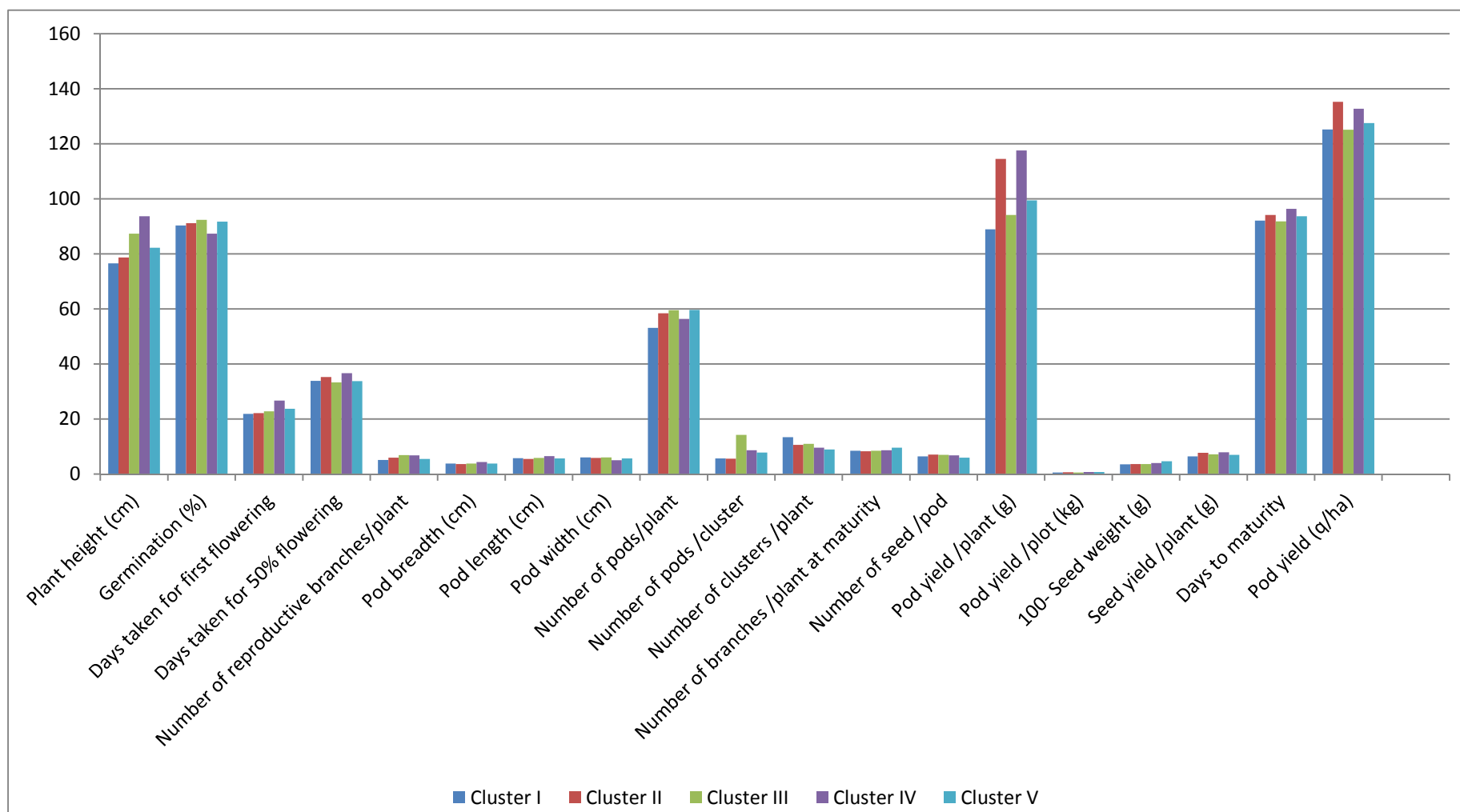
Graph- 4.11 Average intra and inter cluster D^2 and D values in 30 genotypes of cluster bean

4.14 Mean performance of characters in clusters

Cluster means were calculated in all clusters for all 19 characters and presented in Table- 4.12 with also in Graph- 4.12

Table- 4.12 Cluster mean values for 19 biometrical characters in 30 genotypes of cluster bean

S. No.	Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
	Characters					
1.	Plant height (cm)	76.58	78.70	87.38	93.71	82.25
2.	Germination (%)	90.32	91.21	92.43	87.32	91.75
3.	Days taken for first flowering	21.83	22.14	22.77	26.65	23.67
4.	Days taken for 50% flowering	33.90	35.29	33.28	36.67	33.78
5.	Number of reproductive branches/plant	5.10	5.98	6.88	6.82	5.52
6.	Pod breadth (cm)	3.83	3.62	3.82	4.34	3.76
7.	Pod length (cm)	5.80	5.45	5.86	6.48	5.65
8.	Pod width (cm)	6.02	5.86	6.03	4.98	5.63
9.	Number of pods/plant	53.10	58.38	59.55	56.38	59.65
10.	Number of pods /cluster	5.63	5.60	14.23	8.66	7.78
11.	Number of clusters /plant	13.41	10.63	11.01	9.55	8.89
12.	Number of branches /plant at maturity	8.43	8.25	8.44	8.62	9.58
13.	Number of seed /pod	6.41	7.04	6.94	6.74	5.92
14.	Pod yield /plant (g)	88.94	114.50	94.18	117.56	99.45
15.	Pod yield /plot (kg)	0.55	0.66	0.54	0.70	0.74
16.	100- Seed weight (g)	3.54	3.65	3.62	3.95	4.68
17.	Seed yield /plant (g)	6.37	7.69	7.14	7.86	6.95
18.	Days to maturity	92.15	94.13	91.83	96.34	93.72
19.	Pod yield (q/ha)	125.21	135.32	125.10	132.73	127.58



Graph- 4.12 Cluster mean values for 19 biometrical characters in 30 genotypes of cluster bean

4.14.1 Plant height

The maximum cluster mean for plant height at 90 days after sowing was recorded in cluster IV (93.71 cm) followed by cluster III (87.38 cm) and the minimum cluster mean values for plant height was recorded in cluster I (76.58 cm).

4.14.2 Germination (%)

The lowest cluster mean was observed for germination % in cluster IV (87.32%) and the highest was observed in cluster III (92.43%) followed by cluster V (91.75%).

4.14.3 Days taken for first flowering

The lowest cluster mean for days to first flowering was observed in cluster I (21.83) followed by cluster II (22.14) and the highest was observed in cluster IV (26.65).

5.14.4 Days taken for 50% flowering

The lowest cluster mean for days to 50% flowering was observed in cluster III (33.28%) and the highest was observed in cluster IV (36.67%).

4.14.5 Number of reproductive branches/plant

The minimum cluster mean for number of reproductive branches/plant was observed in cluster I (5.10) and maximum number of reproductive branches/plant was observed in cluster III (6.88).

4.14.6 Pod breadth (cm)

The maximum cluster mean value for pod breadth was observed in cluster IV (4.34 cm) followed by cluster I (3.83 cm). The minimum value for the character was observed in cluster II (3.62 cm).

4.14.7 Pod length

The highest cluster mean for pod length was observed in cluster IV (6.48 cm) followed by cluster III (5.86 cm) and the least was observed in cluster II (5.45 cm).

4.14.8 Pod width

The lowest cluster mean for pod width was observed in cluster IV (4.98 cm) followed by cluster V (5.63 cm) and the highest was observed in cluster III (6.03 cm).

4.14.9 Number of pods/plant

The highest cluster mean for number of pods/plant was observed in cluster V (59.65) followed by cluster III (59.55) and least in cluster I (53.10).

4.14.10 Number of pods /cluster

The cluster mean for number of pods/cluster was maximum in cluster III (14.23) followed by cluster IV (8.66) and the minimum was observed in cluster II (5.60).

4.14.11 Number of clusters /plant

The number of clusters/plant was maximum in cluster I (13.41) and cluster III (11.01) and the minimum was observed in cluster V (8.89).

4.14.12 Number of branches/plant at maturity

The number of branches/plant at maturity was maximum in cluster V (9.58) followed by cluster IV (8.62) and the minimum was observed in cluster II (8.25).

4.14.13 Number of seed /pod

The highest cluster mean for number of seed/pod was observed in cluster II (7.04) and lowest was observed in cluster V (5.92).

4.14.14 Pod yield /plant (g)

The pod yield/plant was maximum in cluster IV (117.56 g) followed by cluster II (114.50 g) and the minimum was observed in cluster I (88.94 g).

4.14.15 Pod yield /plot (kg)

The highest cluster mean for pod yield/plot was observed cluster V (0.74 kg) followed by cluster IV (0.70 kg) and least in cluster III (0.54 kg).

4.14.16 100- Seed weight (g)

The 100-seed weight was maximum in cluster V (4.68 g) followed by cluster IV (3.95 g) and the minimum was observed in cluster I (3.54 g).

4.14.17 Seed yield /plant (g)

The highest cluster mean for seed yield/plant was observed in IV (7.86 g) followed by cluster II (7.69 g) and least in cluster I (6.37 g).

4.14.18 Days to maturity

The days to maturity was maximum in cluster IV (96.34) followed by cluster II (94.13) and the minimum was observed in cluster III (91.83).

4.14.19 Pod yield (q/ha)

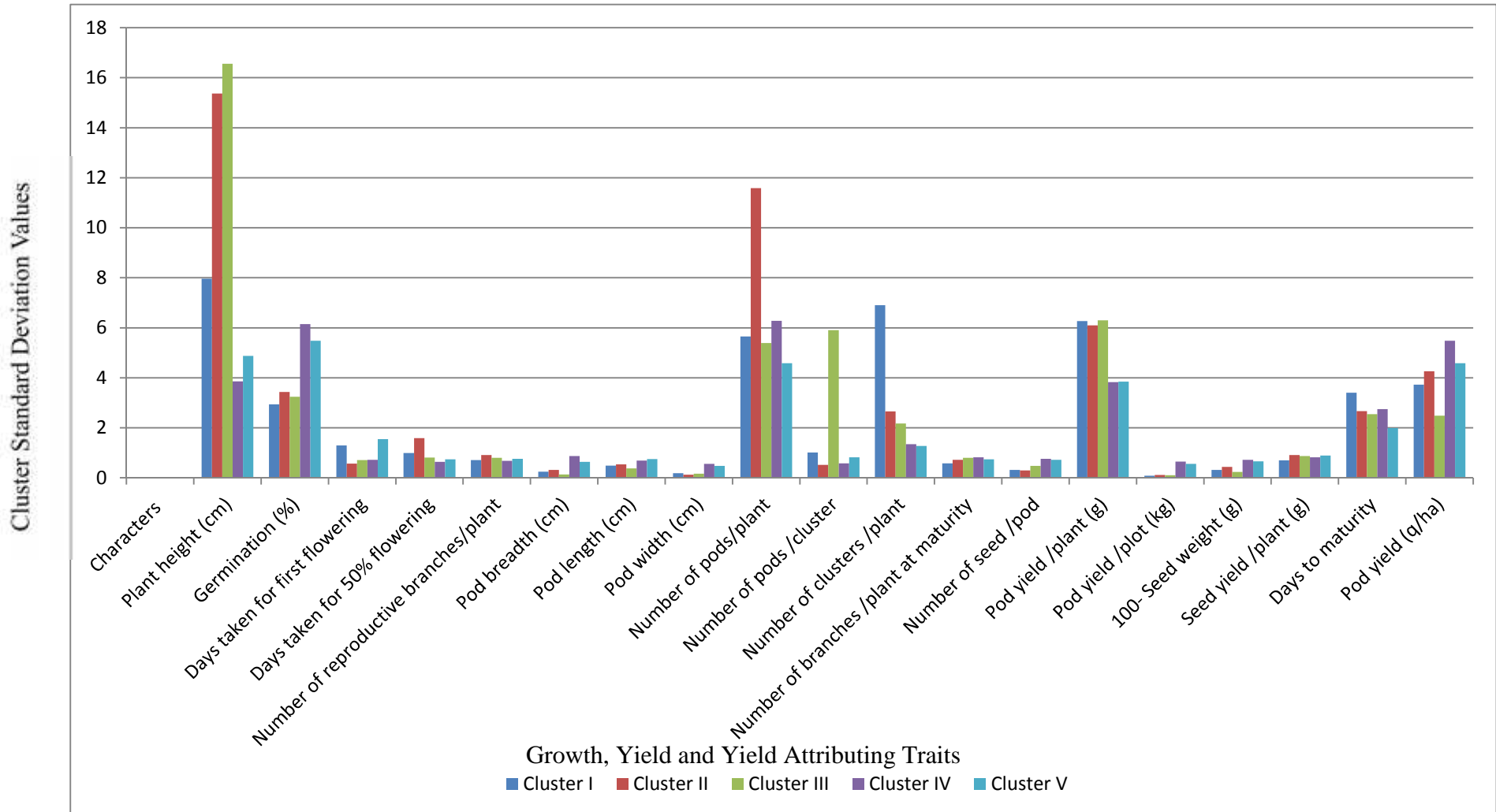
The highest cluster mean for pod yield was observed in cluster II (135.32 q/ha) followed by cluster IV (132.73 q/ha) and lowest in cluster III (125.10 q/ha).

4.15 Standard deviation performance of characters in clusters

Cluster standard deviation were calculated in all clusters for all 19 characters and presented in Table- 4.13 with also in Graph- 4.13.

Table- 4.13 Cluster standard deviation values for 19 biometrical characters in 30 genotypes of cluster bean

S. No.	Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
	Characters					
1.	Plant height (cm)	7.96	15.37	16.56	3.86	4.88
2.	Germination (%)	2.94	3.43	3.24	6.15	5.48
3.	Days taken for first flowering	1.30	0.57	0.71	0.72	1.55
4.	Days taken for 50% flowering	0.99	1.59	0.81	0.64	0.74
5.	Number of reproductive branches/plant	0.71	0.91	0.80	0.68	0.76
6.	Pod breadth (cm)	0.25	0.32	0.14	0.87	0.64
7.	Pod length (cm)	0.49	0.54	0.38	0.69	0.75
8.	Pod width (cm)	0.19	0.13	0.17	0.56	0.48
9.	Number of pods/plant	5.65	11.59	5.39	6.28	4.58
10.	Number of pods /cluster	1.01	0.52	5.91	0.58	0.82
11.	Number of clusters /plant	6.90	2.66	2.17	1.35	1.28
12.	Number of branches /plant at maturity	0.58	0.72	0.80	0.82	0.74
13.	Number of seed /pod	0.32	0.30	0.48	0.76	0.72
14.	Pod yield /plant (g)	6.27	6.10	6.30	3.83	3.85
15.	Pod yield /plot (kg)	0.08	0.11	0.10	0.65	0.56
16.	100- Seed weight (g)	0.32	0.44	0.24	0.72	0.66
17.	Seed yield /plant (g)	0.70	0.91	0.87	0.82	0.89
18.	Days to maturity	3.40	2.67	2.55	2.75	1.99
19.	Pod yield (q/ha)	3.73	4.26	2.49	5.48	4.58



Graph- 4.13 Cluster standard deviation values for 19 biometrical characters in 30 genotypes of cluster bean

4.15.1 Plant height

The maximum cluster standard deviation for plant height at 90 days after sowing was recorded in cluster III (16.56 cm) followed by cluster II (15.37 cm) and the minimum cluster deviation for plant height was recorded in cluster IV (3.86 cm).

5.15.2 Germination (%)

The lowest cluster standard deviation was observed for germination % in cluster I (2.94%) and the highest was observed in cluster IV (6.15%) followed by cluster V (5.48%).

4.15.3 Days taken for first flowering

The lowest cluster standard deviation for days to first flowering was observed in cluster II (0.57) followed by cluster III (0.57) and the highest was observed in cluster V (1.55).

4.15.4 Days taken for 50% flowering

The lowest cluster standard deviation for days to 50% flowering was observed in cluster IV (0.64) and the highest was observed in cluster II (1.59).

4.15.5 Number of reproductive branches/plant

The minimum cluster standard deviation for number of reproductive branches/plant was observed in cluster IV (0.68) and maximum number of reproductive branches/plant was observed in cluster II (0.91).

4.15.6 Pod breadth (cm)

The maximum cluster standard deviation values for pod breadth was observed in cluster IV (0.87 cm) followed by cluster (0.64 cm). The minimum value for the character was observed in cluster III (0.14 cm).

4.15.7 Pod length

The highest cluster standard deviation for pod length was observed in cluster V (0.75 cm) followed by cluster IV (0.69 cm) and the least was observed in cluster III (0.38 cm).

4.15.8 Pod width

The lowest cluster standard deviation for pod width was observed in cluster II (0.13 cm) followed by cluster III (0.17 cm) and the highest was observed in cluster IV (0.56 cm).

4.15.9 Number of pods/plant

The highest cluster standard deviation for number of pods per plant was observed in cluster II (11.59) followed by cluster IV (6.59) and least in cluster V (4.58).

4.15.10 Number of pods /cluster

The cluster standard deviation for number of pods/cluster was maximum in cluster III (5.91) followed by clusters I (1.01) and the minimum was observed in cluster II (0.52).

4.15.11 Number of clusters /plant

The value of cluster standard deviation for number of clusters/plant was maximum in cluster I (6.90) and cluster II (2.66) and the minimum was observed in cluster V (1.28).

4.15.12 Number of branches/plant at maturity

The value of cluster standard deviation for number of branches/plant at maturity was maximum in cluster IV (0.82) followed by cluster III (0.80) and the minimum was observed in cluster I (0.58).

4.15.13 Number of seed /pod

The highest cluster standard deviation for number of seed/pod was observed in cluster IV (0.76) and lowest was observed in cluster II (0.30).

4.15.14 Pod yield /plant (g)

The value of cluster standard deviation for pod yield/plant was maximum in cluster III (6.30 g) followed by cluster I (6.27 g) and the minimum was observed in cluster IV (3.83 g).

4.15.15 Pod yield /plot (kg)

The highest cluster standard deviation for pod yield/plot was observed cluster IV (0.65 kg) followed by cluster V (0.56 kg) and least in cluster I (0.08 kg).

4.15.16 100- Seed weight (g)

The value of cluster standard deviation for 100-seed weight was maximum in cluster IV (0.72 g) followed by clusters V (0.66 g) and the minimum was observed in cluster III (0.24 g).

4.15.17 Seed yield /plant (g)

The highest cluster standard deviation for seed yield/plant was observed in II (0.91 g) followed by cluster V (0.89 g) and least in cluster I (0.70 g).

4.15.18 Days to maturity

The value of cluster standard deviation for days to maturity was maximum in cluster I (3.40) followed by cluster IV (2.75) and the minimum was observed in cluster V (1.99).

4.15.19 Pod yield (q/ha)

The highest cluster standard deviation for pod yield was observed in cluster IV (5.48 q/ha) followed by cluster II (4.26 q/ha) and lowest in cluster III (2.49 q/ha).

Molecular Characterization:

4.16 Analysis of ISSR marker data

A similarity matrix was computed for ISSR data using Jaccard's coefficient by the software package PAST (Hammer *et al.*, 2001).

A dendrogram was obtained by Unweighted Pair-Group Average (UPGMA) method using the similarity matrix by Jaccard's similarity coefficient (Jaccard, 1908) to determine the relatedness of 30 genotypes under study.

Population genetic analysis (POPGENE Yet 1.32) software was used to determine the values of Nei's genetic diversity (Nei's 1978), Shannon's information index, total genetic diversity among populations (H_t), genetic diversity within population (H_s) and mean coefficient of gene differentiation (G_{st}) for two populations, i.e. landraces and commercial varieties.

ISSR data were also subjected to Analysis of Molecular Variance (AMOVA) at individual and population level, using GenALEX software (Peakall and Smouse, 2006).

4.17 Annealing temperature

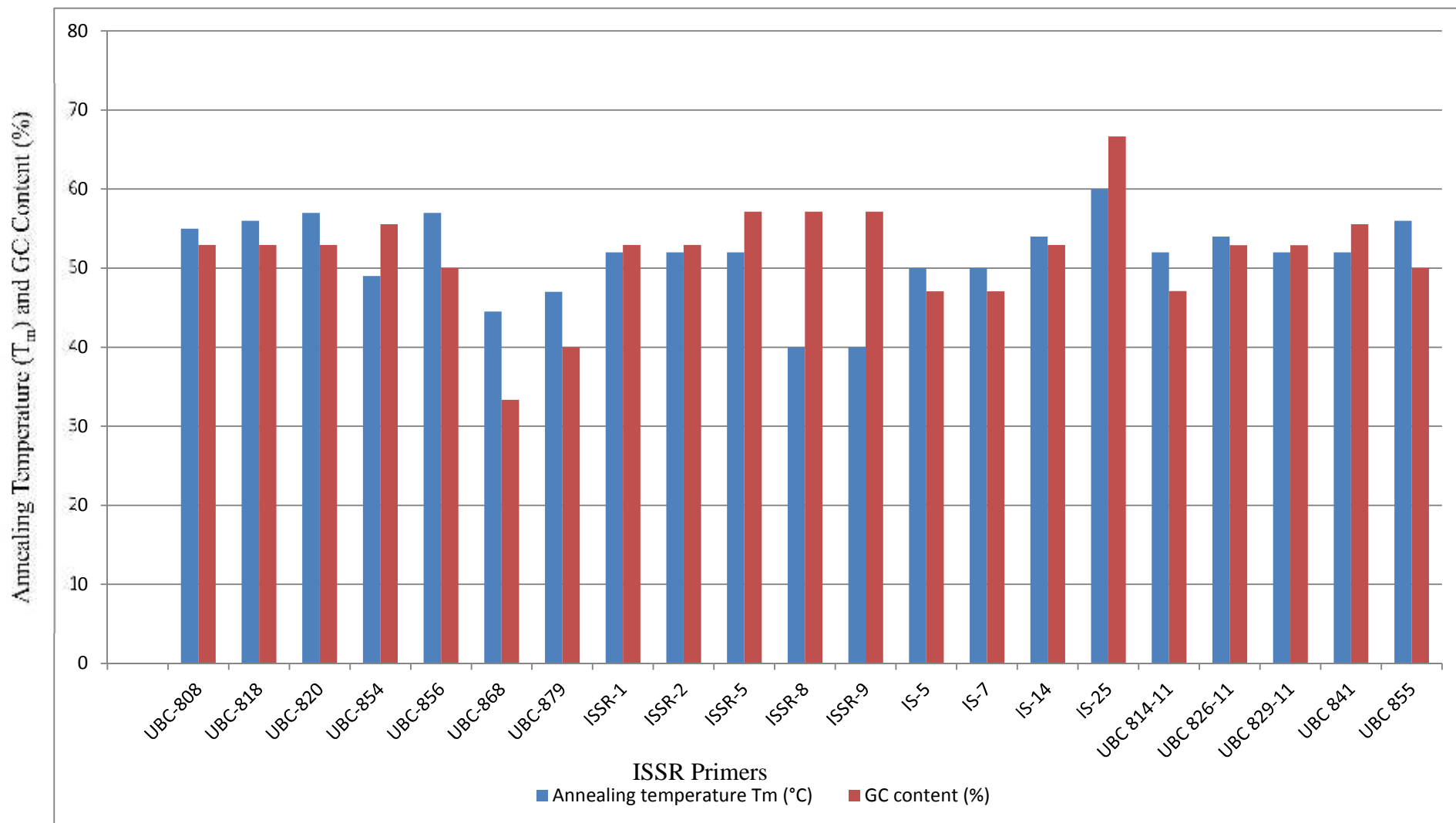
Among 21 ISSR primers, the highest annealing temperature exhibited in IS-25 (60%) primer and lowest annealing temperature exhibited by ISSR-8 (40%) and ISSR-9 (40%) primer with average annealing temperature of (51.50%) (Table- 4.14) with also in Graph- 4.14.

4.18 G C content (%)

Among 21 ISSR primers, the maximum GC content was found in IS-25 (66.67%) primer and the minimum GC content exhibited in UBC-868 (33.33%) primer with average GC content of (51.75%) (Table- 4.14) with also in Graph- 4.14.

Table- 4.14 Details of the 21 ISSR primers and their sequence, annealing temperature and GC content used in the present study

S. No.	Primer code	Sequence of the primer (5'- 3')	Annealing temperature Tm (°C) (%)	GC content (%)
1.	UBC-808	AGAGAGAGAGAGAGAGC	55.00	52.94
2.	UBC-818	CACACACACACACACAG	56.00	52.94
3.	UBC-820	GTGTGTGTGTGTGTGTC	57.00	52.94
4.	UBC-854	TCTCTCTCTCTCTCRG	49.00	55.56
5.	UBC-856	ACACACACACACACACYA	57.00	50.00
6.	UBC-868	GAAGAAGAAGAAGAAGAA	44.50	33.33
7.	UBC-879	CTTCACTTCACTTCA	47.00	40.00
8.	ISSR-1	GAGAGAGAGAGAGAGAC	52.00	52.94
9.	ISSR-2	CACACACACACACACAC	52.00	52.94
10.	ISSR-5	CACACACACACAGG	52.00	57.14
11.	ISSR-8	GAGAGAGAGAGACC	40.00	57.14
12.	ISSR-9	GTGTGTGTGTGTGCC	40.00	57.14
13.	IS-5	AGAGAGAGAGAGAGAGT	50.00	47.06
14.	IS-7	GAGAGAGAGAGAGAGAT	50.00	47.06
15.	IS-14	GTGTGTGTGTGTGTGTC	54.00	52.94
16.	IS-25	AGCAGCAGCAGCAGCAGC	60.00	66.67
17.	UBC 814-11	CTCTCTCTCTCTCAT	52.00	47.10
18.	UBC 826-11	ACACACACACACACACC	54.00	52.90
19.	UBC 829-11	TGTGTGTGTGTGTGTGT	52.00	52.90
20.	UBC 841	GAGAGAGAGAGAGAGAYC	52.00	55.56
21.	UBC 855	ACACACACACACACACYT	56.00	50.00
		General Mean	51.50%	51.75%



Graph- 4.14 Details of the 21 ISSR primers, annealing temperature and their GC content used in the present study

4.19 Genetic diversity studied in germplasm

Molecular diversity has been studied on the basis of molecular characterization present in the 30 genotypes of cluster bean.

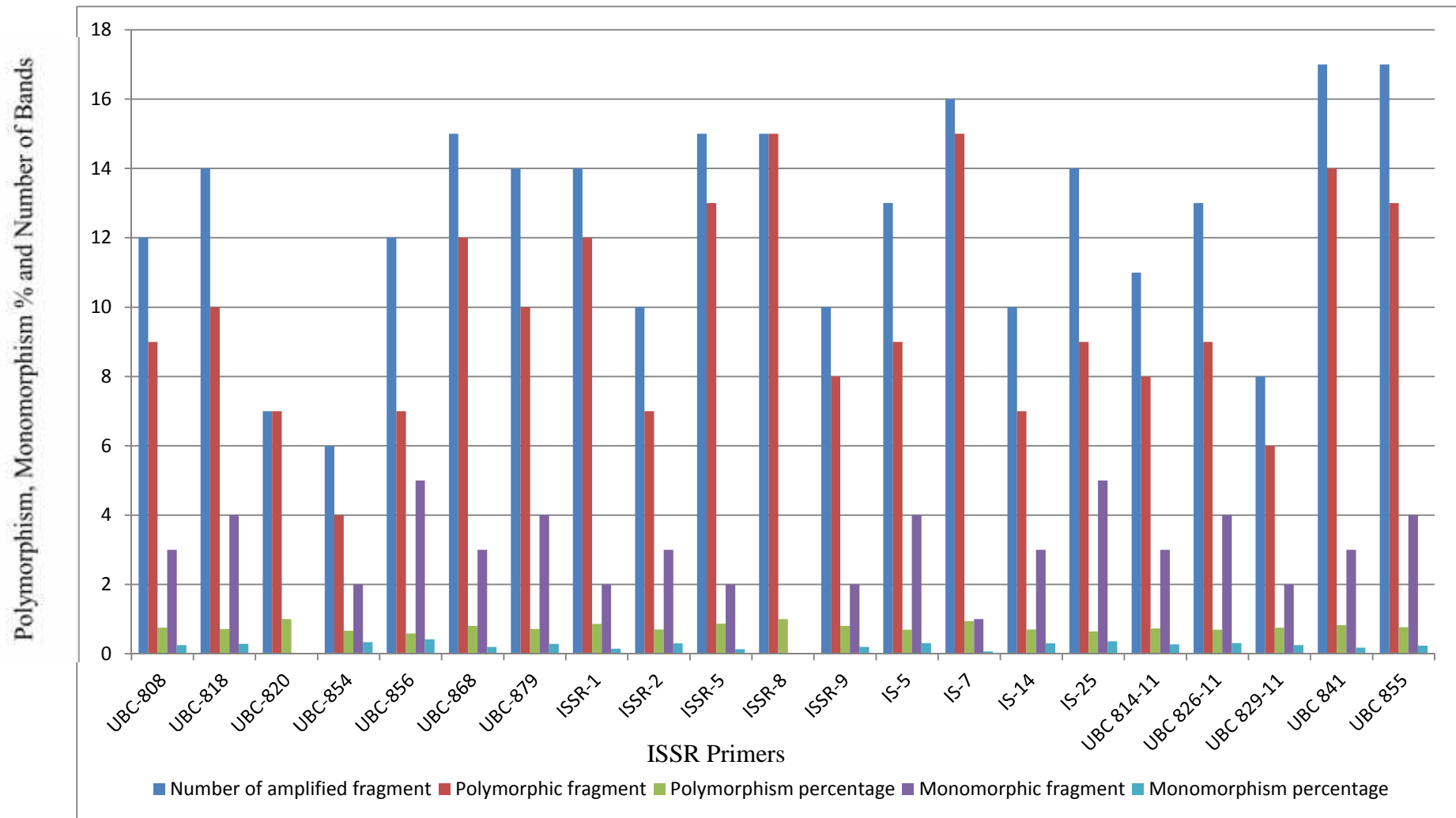
To investigate the genetic diversity and relationships within and among 30 cluster bean germplasm, we tested 21 ISSR primers, 8 of which revealed reproducible polymorphic patterns and were used for further analysis. Two main aspects of genetic diversity, marker informativeness (polymorphic and overall efficiency of informative fragment detection) and marker performance (overall efficacy of a primer set used in determining polymorphism level, genetic diversity, and discriminatory power) were evaluated (Table- 4.15) with also in Graph- 4.15.

4.20 Marker Informativeness

Marker informativeness of the 21 ISSR primers was analyzed using several parameters. There are 263 total fragments generated across all germplasm, 204 (77.57%) were polymorphic and 59 (22.43%) were monomorphic. The number of polymorphic fragments per primer ranged from four (markers UBC-854) to fifteen (ISSR-5 and IS-7 marker), with an average of 9.71. The percentage polymorphism ranged from 58.33% (UBC-856) to 100% (UBC-820 and ISSR-8), with an average of 77.06%. The range of frequencies of polymorphic fragments for a given primer across all germplasm was 0.25-0.98, with an average of 0.046. A large proportion (28.3%) had frequencies in the range of 0.12-0.15 (Table- 4.15) with also in Graph- 4.15.

Table- 4.15 Details of ISSR primers showing number of band and percentage of polymorphism generated from 30 genotypes of cluster bean

S. No.	Primer Name	Sequence (5'-3')	Number of amplified fragment	Polymorphic fragment	Polymorphism percentage	Monomorphic fragment	Monomorphism percentage
1.	UBC-808	AGAGAGAGAGAGAGAGC	12	9	75.00%	3	25.00%
2.	UBC-818	CACACACACACACACAG	14	10	71.43%	4	28.57%
3.	UBC-820	GTGTGTGTGTGTGTGTC	7	7	100.00%	0	0.00%
4.	UBC-854	TCTCTCTCTCTCTCRG	6	4	66.67%	2	33.33%
5.	UBC-856	ACACACACACACACACYA	12	7	58.33%	5	41.66%
6.	UBC-868	GAAGAAGAAGAAGAAGAA	15	12	80.00%	3	20.00%
7.	UBC-879	CTTCACTTCACTTCA	14	10	71.43%	4	28.57%
8.	ISSR-1	GAGAGAGAGAGAGAGAC	14	12	85.71%	2	14.28%
9.	ISSR-2	CACACACACACACACAC	10	7	70.00%	3	30.00%
10.	ISSR-5	CACACACACACAGG	15	13	86.67%	2	13.33%
11.	ISSR-8	GAGAGAGAGAGACC	15	15	100.00%	0	0.00%
12.	ISSR-9	GTGTGTGTGTGTGTC	10	8	80.00%	2	20.00%
13.	IS-5	AGAGAGAGAGAGAGAGT	13	9	69.23%	4	30.76%
14.	IS-7	GAGAGAGAGAGAGAGAT	16	15	93.75%	1	6.25%
15.	IS-14	GTGTGTGTGTGTGTGTC	10	7	70.00%	3	30.00%
16.	IS-25	AGCAGCAGCAGCAGCAGC	14	9	64.29%	5	35.71%
17.	UBC 814-11	CTCTCTCTCTCTCAT	11	8	72.73%	3	27.27%
18.	UBC 826-11	ACACACACACACACACC	13	9	69.23%	4	30.76%
19.	UBC 829-11	TGTGTGTGTGTGTGTGT	8	6	75.00%	2	25.00%
20.	UBC 841	GAGAGAGAGAGAGAGAYC	17	14	82.35%	3	17.64%
21.	UBC 855	ACACACACACACACACYT	17	13	76.47%	4	23.52%
		Total	263.00	204.00	1618.29%	59.00	481.65%
		General Mean	12.52	9.71	77.06%	2.80	22.93%



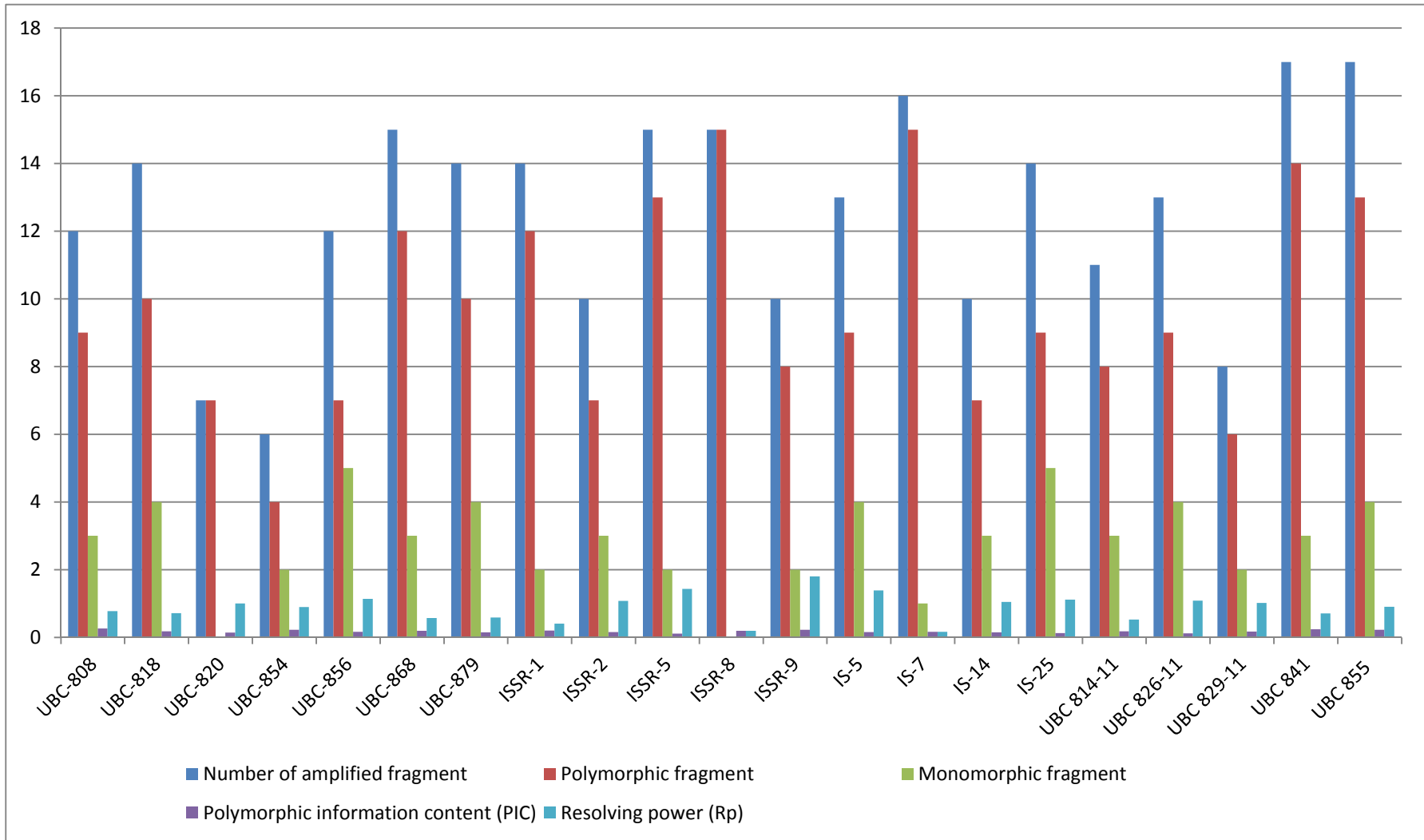
Graph- 4.15 Details of ISSR primers showing number of band and percentage of polymorphism generated from 30 genotypes of cluster bean

4.21 Marker Performance

Information on the genetic profile of each accession was used to assess the marker performance by evaluating the PIC and resolving power. The range of PIC for the 204 polymorphic fragments was 0.110-0.258. The highest PIC value (0.258) was observed for primer UBC-808 and the lowest (0.110) for primers ISSR-5. The average was 0.176. The resolving power, a feature that indicates the discriminatory potential of the primer. The values of resolving powers for ISSR marker was ranged between 0.406 (ISSR-1) and 1.800 (ISSR-9). The average was 0.881 (Table- 4.16).

Table- 4.16 Performance of different ISSR primer with their polymorphic information content and resolving power

Primer Name	Name of sequence (5'- 3')	Number of amplified fragment	Polymorphic fragment	Monomorphic fragment	Polymorphic information content (PIC)	Resolving power (Rp)
UBC-808	AGAGAGAGAGAGAGAGC	12	9	3	0.258	0.774
UBC-818	CACACACACACACACAG	14	10	4	0.179	0.716
UBC-820	GTGTGTGTGTGTGTGTC	7	7	0	0.143	1.001
UBC-854	TCTCTCTCTCTCTCTCRG	6	4	2	0.224	0.896
UBC-856	ACACACACACACACACYA	12	7	5	0.162	1.134
UBC-868	GAAGAAGAAGAAGAAGAA	15	12	3	0.190	0.570
UBC-879	CTTCACTTCACTTCA	14	10	4	0.146	0.584
ISSR-1	GAGAGAGAGAGAGAGAC	14	12	2	0.203	0.406
ISSR-2	CACACACACACACACAC	10	7	3	0.154	1.078
ISSR-5	CACACACACACAGG	15	13	2	0.110	1.430
ISSR-8	GAGAGAGAGAGACC	15	15	0	0.193	0.193
ISSR-9	GTGTGTGTGTGTGCC	10	8	2	0.225	1.800
IS-5	AGAGAGAGAGAGAGAGT	13	9	4	0.154	1.386
IS-7	GAGAGAGAGAGAGAGAT	16	15	1	0.165	0.165
IS-14	GTGTGTGTGTGTGTGTC	10	7	3	0.149	1.043
IS-25	AGCAGCAGCAGCAGCAGC	14	9	5	0.124	1.116
UBC 814-11	CTCTCTCTCTCTCTCAT	11	8	3	0.174	0.522
UBC 826-11	ACACACACACACACACC	13	9	4	0.120	1.080
UBC 829-11	TGTGTGTGTGTGTGTGT	8	6	2	0.169	1.014
UBC 841	GAGAGAGAGAGAGAGAYC	17	14	3	0.235	0.705
UBC 855	ACACACACACACACACYT	17	13	4	0.226	0.904
	Total	263.00	204.00	59.00	3.703	18.517
	General Mean	12.52	9.71	2.80	0.176	0.881



Graph- 4.16 Performance of different ISSR primer with their polymorphic information content and resolving power

4.22 Molecular genetic diversity

A set of 21 ISSR markers was used for analysis of genetic diversity among 30 genotypes of cluster bean. The Genetic diversity and phylogenetic analysis, genome mapping, number of amplified fragment, polymorphic fragments, polymorphism percentage, monomorphic fragments and monomorphism percentage obtained for each primer are shown in (Table- 4.15) with also in Graph- 4.15.

(B). Molecular/Genotypic Characters:

4.23.1 Genetic diversity and phylogenetic analysis

Genetic diversity among 30 genotypes of cluster bean analyzed on following basis describe in this study. The primers UBC-841, UBC-855, ISSR-5 and ISSR-8 produced highest bands as compared to UBC-856 and UBC-829-11 primers produce lowest bands. The primers IS-5 and UBC-814-11 produced 13 and 11 bands respectively. The primer ISSR-2 produced unclear bands; the primer ISSR-9 did not produce any band. There are 21 primers used and produced a total of 263 bands out of which 204 were polymorphic and 59 were monomorphic. The highest bands were observed in genotypes namely, IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789 and IC-369868 and the lowest band were observed in genotypes IC-421809, IC-421812 and IC-421815. Three unique bands were observed in genotypes number IC-370490 with 2 bands in primer UBC-856 of about 320 bp and 350bp and a single band with primer UBC-868 of about 275 bp (Table- 4.15) with also in Graph- 4.15. The genotypes from the same geographical region were also grouped into different clusters. The different genotypes may have whole or partial common pedigrees and may have been subjected to the same selection during their breeding but are still distinguishable from each other on the basis of RAPD profiles. Bisht *et al.* (1998), Manivannan *et al.* (1998) and Lavanya *et al.* (2008) have also reported lack of correlation between geographic and genetic diversity in other legumes. RAPD markers have been used for the identification of cultivars and the genetic relationships among cultivars of other leguminous crops including *Phaseolus vulgaris* (Skroch *et al.*, 1992), *V. unguiculata* (Mignouna *et al.*, 1998), *V. angularis* (Yee *et al.*, 1999) and *V. radiata* (Lakanpaul *et al.*, 2000; Lavanya *et al.*, 2008). The

present study has revealed that there is wide genetic base in *C. tetragonoloba* genotypes for crop improvement. The gel image of this character has been described in Figure- 4.23.1.

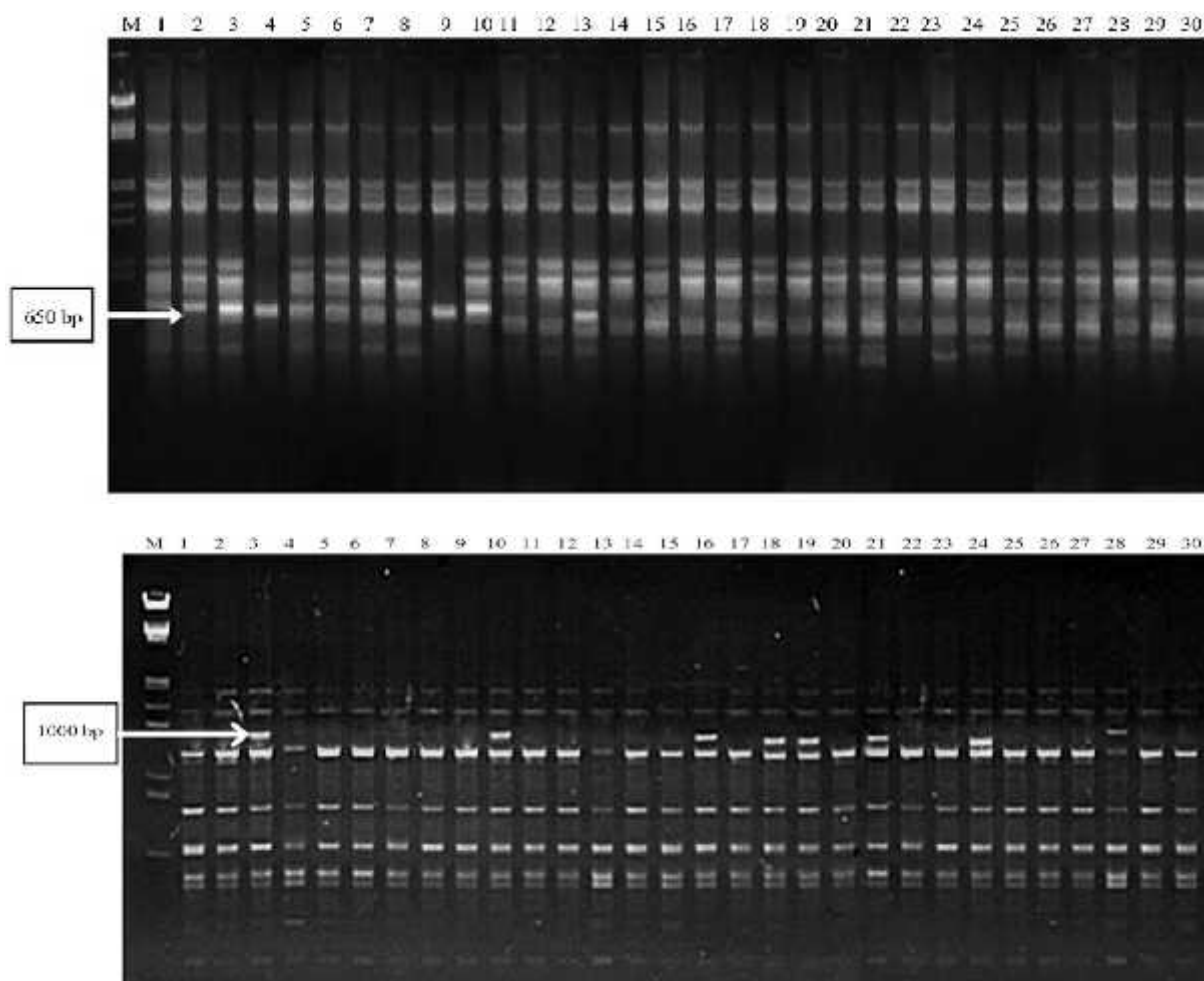


Figure- 4.23.1 ISSR profiling of 30 cluster bean genotypes with primer ISSR-5 and ISSR-8 digested at 650 bp and 1000 bp respectively

4.23.2 Phylogenetic analysis

This is the study of evolutionary relationships. Phylogenetic analysis is the means of inferring or estimating these relationships. Phylogenetic analysis from DNA fragment data classified 30 investigated cluster bean germplasm into five clusters. The first cluster contained eight germplasm (IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789, IC-369868 and IC-370490); second cluster contained six germplasm (from IC-325757, IC-329038, IC-373427, IC-373480, IC-402293 and IC-421820.), third cluster contained five germplasm (IC-421809, IC-421812, IC-421815, IC-421828 and IC-421834), fourth cluster contained six germplasm also (IC-370478, IC-415137, IC-415142, IC-415157, IC-415159 and IC-421242) and fifth cluster contained also five germplasm. The result from morphological and molecular observation was consistent with those from phylogenetic analysis. The results demonstrated that phylogenetic analysis with morphological and molecular examination may provide a more complete approach to classify germplasm or to examine misidentified germplasm in a plant germplasm collection.

The assessment of phylogenetic relationships and genetic diversity of various DNA markers have been developed from different *Vigna* species, there are neither a common nor a sufficient set of DNA markers available for evaluation of germplasm applicable to all *Vigna* species. These results are in accordance with the findings of Simon *et al.* (2007). The phylogenetic tree has been shown in Figure- 4.23.2.

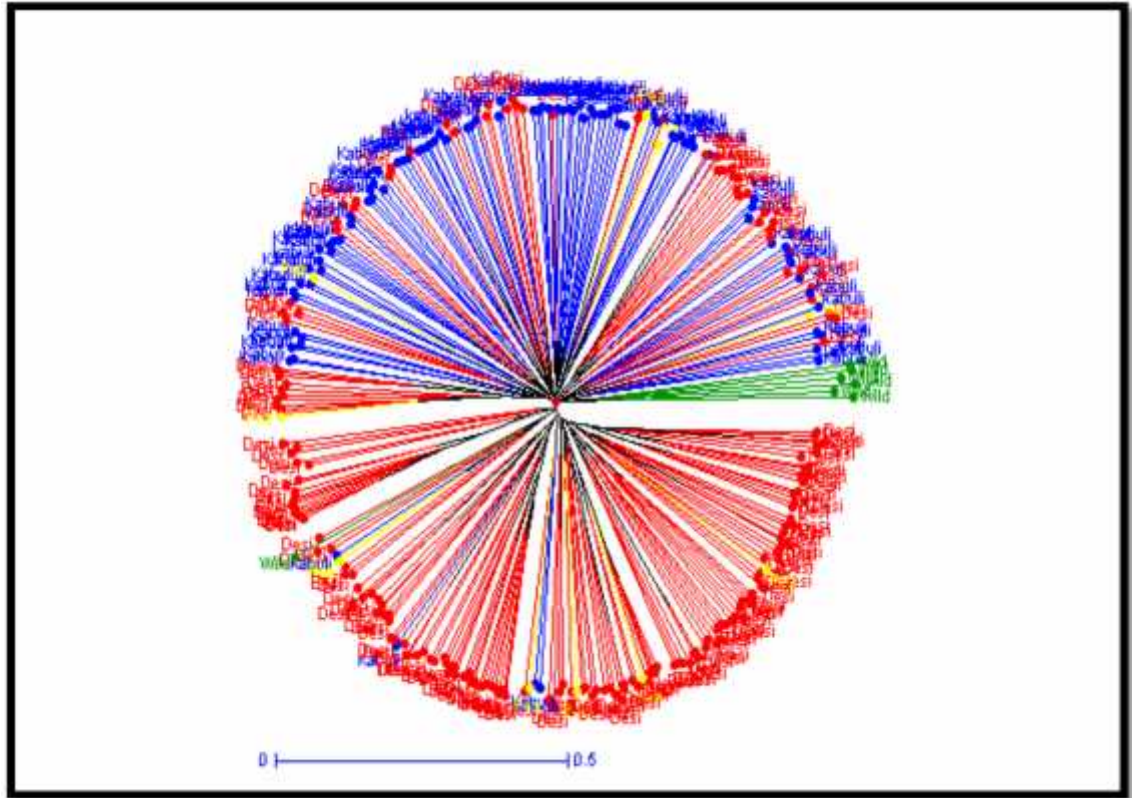


Figure- 4.23.2 Unweighted phylogenetic tree based on the simple matching dissimilarity matrix of 21 ISSR markers genotyped across the cluster bean genotypes

4.23.3 Genome mapping

Guar is strictly a self-pollinated crop and diploid legume with chromosome number $(2n) = 14$ belongs to the family Fabaceae. The genome size of cluster bean is approximately 2.45 Giga Bases/Centimeter. Cross pollination is strictly prohibited due to the cleistogamous nature of flowers. One of the major goals of plant breeders is to develop genotypes with high yield potential and the ability to maintain the yield across environments. With the development of molecular markers, breeders have a complimentary tool to traditional selection and markers linked to variation in a trait of interest which could be used to assist the breeding programs.

The availability of DNA marker based maps for the genomes of many crops facilitated mapping of QTLs of interest and marker-assisted selection (Winter and Kahl, 1995). QTL mapping analysis has provided an effective approach for locating

and subsequently manipulating the QTLs associated with different quantitative traits in plants (Rachid *et al.*, 2004).

Total 29 linkage groups, each containing more than 2 markers were constructed with one phenotypic and 21 ISSR markers. The total map size was 1520 cM and the average distance between markers was 8.5 cM. Although the present is not fully saturated, it may facilitate gene tagging, QTL mapping and further useful gene transfer for adzuki bean breeding.

4.23.4 Number of amplified fragment

The values of number of amplified fragment for ISSR marker was ranged between 6 (UBC-854) and 17 (UBC-841), 17 (UBC-855) respectively. The average number of amplified fragment in this study was 12.52 (Table- 4.15) with also in Graph- 4.15.

4.23.5 Polymorphic fragments

The values of polymorphic fragment for ISSR marker was ranged between 4 (UBC-854) and 15 (ISSR-8), 15 (IS-7) respectively. The average polymorphic fragment in this study was 9.71 (Table- 4.15) with also in Graph- 4.15.

4.23.6 Polymorphism percentage

The values of polymorphism percentage for ISSR marker was ranged between 58.33% (UBC-856) and 100% (UBC-820), 100% (ISSR-8) respectively. The average polymorphism percentage in this study was 77.06% (Table- 4.15) with also in Graph- 4.15.

4.23.7 Monomorphic fragments

The values of monomorphic fragment for ISSR marker was ranged between 1 (IS-7) and 5 (UBC-856), 5 (IS-25) respectively. The average monomorphic fragment in this study was 2.80 (Table- 4.15) with also in Graph- 4.15.

4.23.8 Monomorphism percentage

The values of monomorphism percentage for ISSR marker was ranged between 6.25% (IS-7) and 41.66% (UBC-856). The average monomorphism percentage in this study was 22.93% (Table- 4.15) with also in Graph- 4.15.

4.24 Diversity Analysis

4.25 Cluster analysis

The UPGMA dendrogram was constructed from a similarity matrix based on Jaccard's similarity coefficient values. The dendrogram (Figure- 4.25) from ISSR data showed five major cluster at lower level of similarity and ten minor clusters at lower level of similarity. The major cluster possessed seven sub clusters show the Gel profiling of genomic DNA in thirty diverse genotypes of cluster bean (Figure- 4.25.1). Similarly, the Gel profiling of ISSR-PCR amplification produced by UBC-879 primer (Figure- 4.25.2). Whereas figure 4.25.3 and figure 4.25.4 show the ISSR fingerprint patterns produced with UBC-841 and UBC-855 primers. The dendrogram clearly differentiate between genotypes.

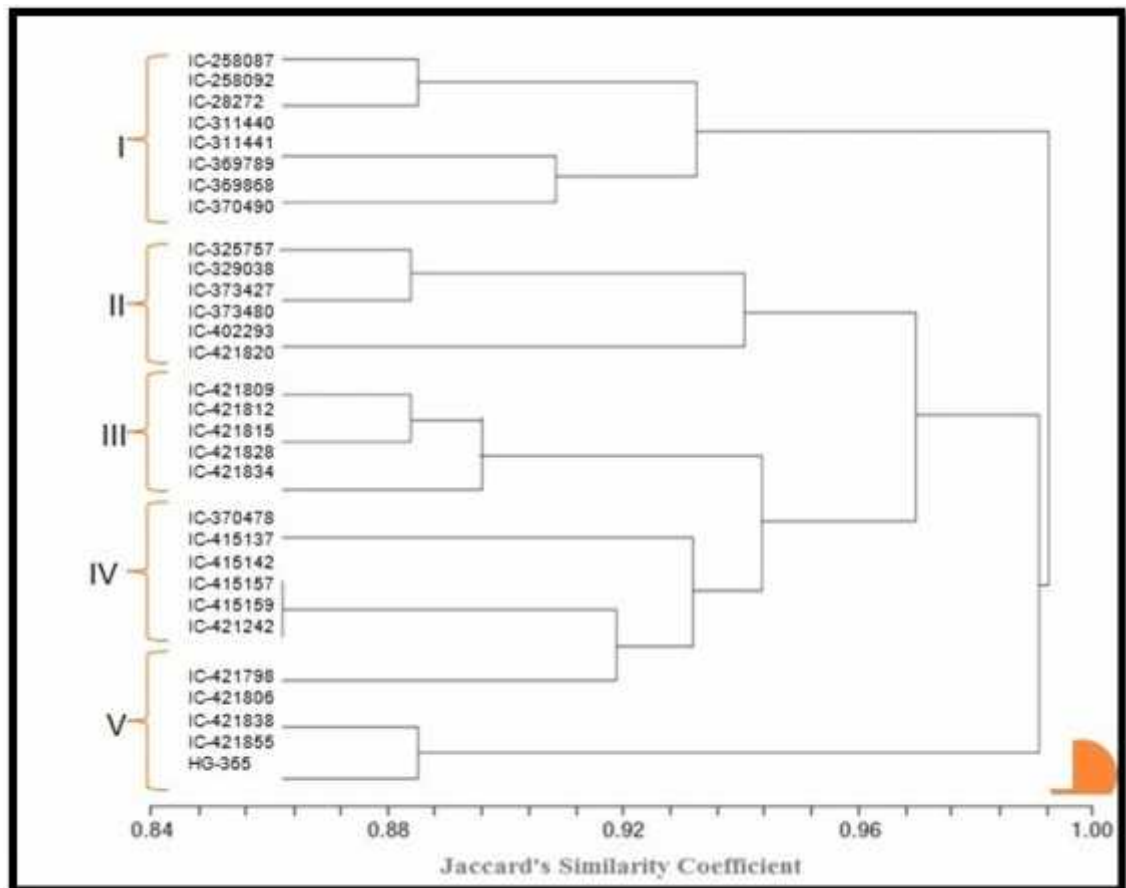


Figure- 4.25 UPGMA Dendrogram indicating the genetic relationship among 30 genotypes of cluster bean by using cluster analysis

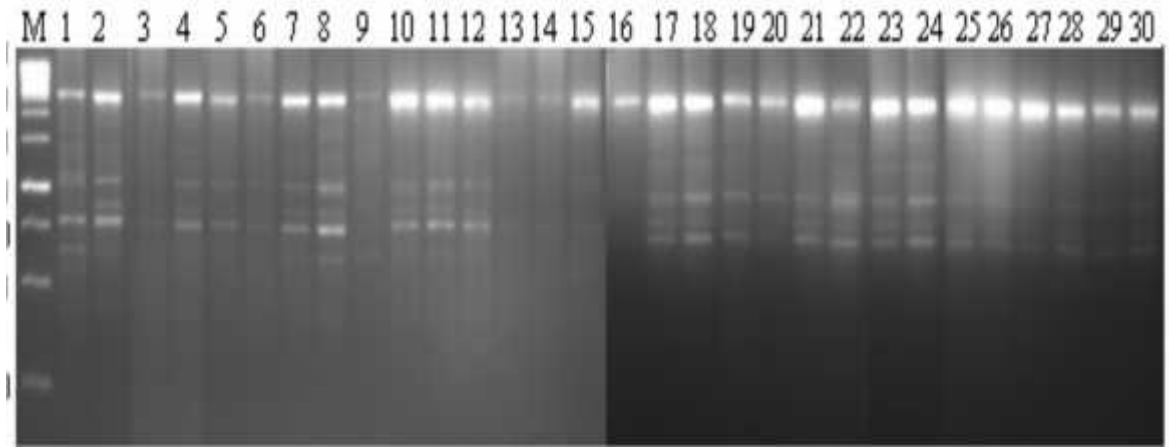


Figure- 4.25.1 Gel profiling of genomic DNA in thirty diverse genotypes of cluster bean

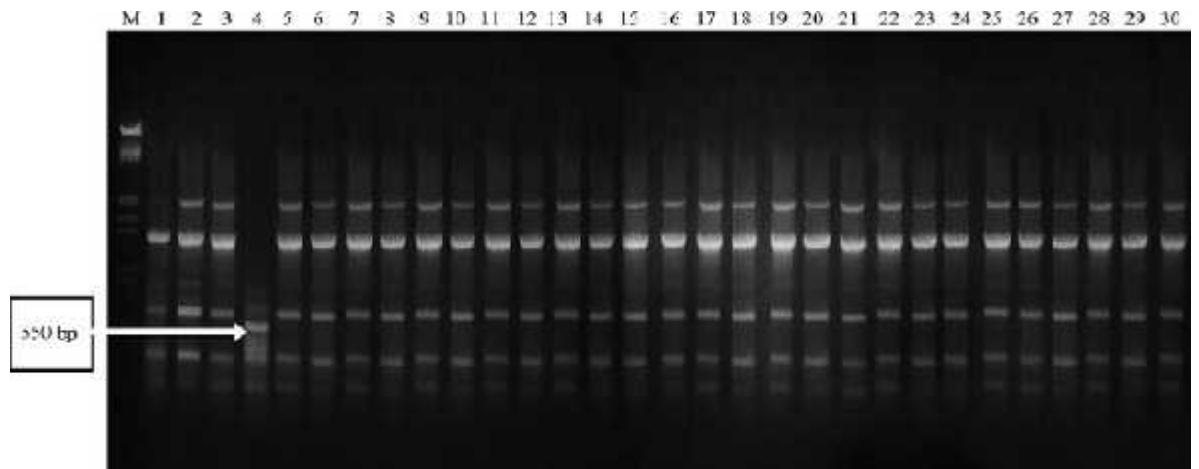


Figure- 4.25.2 Gel profiling of 30 cluster bean genotypes with ISSR primer UBC-879 digested at 550 bp

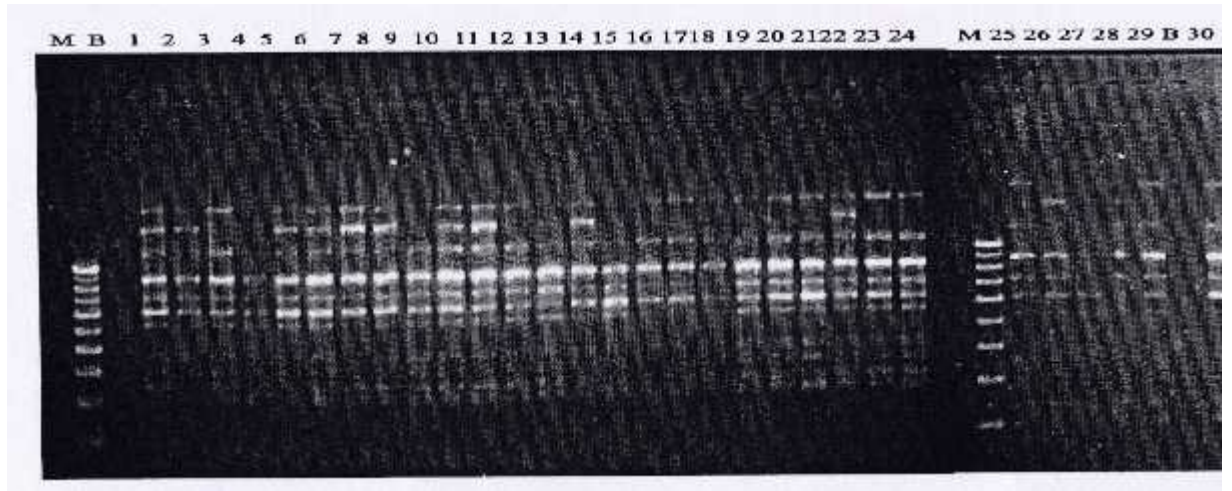


Figure- 4.25.3 Ethidium bromide stained agarose gel showing ISSR profile from UBC-841

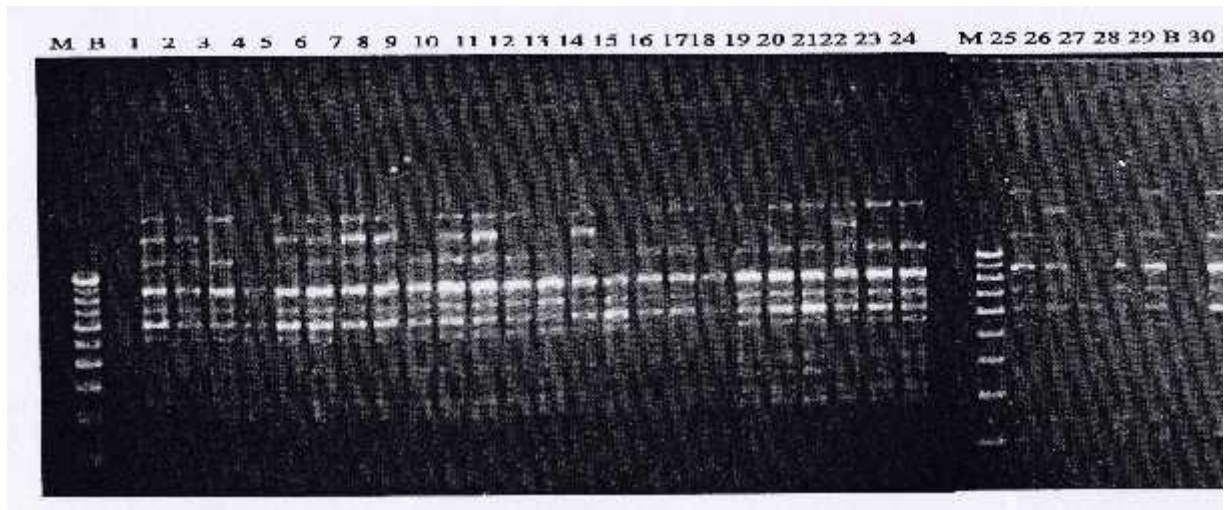


Figure- 4.25.4 Ethidium bromide stained agarose gel showing ISSR profile from UBC-855

4.26 Shannon's diversity index

Shannon diversity index are statistics used to summarize the diversity of a population in which each member belongs to a unique group. For example, in ecology the groups are typically species Shannon's information index, total genetic diversity

among populations (H_t), genetic diversity within population (H_s) and mean coefficient of gene differentiation (G_{st}) for two populations, i.e. landraces and commercial varieties. Shannon's Information Index is a commonly used index to characterize gene diversity in a population.

The observed number of alleles, effective number of alleles, Nei's genetic diversity and Shannon's information index for cluster bean genotypes using 21 ISSR markers were found to be 1.78120 ± 0.4167 , 1.4627 ± 0.3844 , 0.2671 ± 0.1939 and 0.3988 ± 0.268 , respectively. The value of total genotype diversity among population (H_t) was 0.2639 ± 0.0378 whereas diversity within population (H_s) was found to be 0.253 ± 0.035 . Mean coefficient of gene differentiation (G_{st}) value was 0.041 and the estimated gene flow in the population was 11.549 (Table- 4.17).

Table- 4.17 A comparative list of genetic variability factors across the accessions using ISSR primers

		Observed number of alleles	Effective number of alleles	Nei's gene diversity	Shannon's Information index	Ht	Hs	Gst	Estimate of gene flow
ISSR	Mean	1.7812	1.4627	0.267	0.3988	0.2639	0.253	0.041	11.549
	S.D.	0.4167	0.3844	0.1939	0.2681	0.0378	0.035		

4.27 Polymorphic information content (PIC)

The relative informativeness of each marker can be evaluated on the basis of its polymorphic information content (PIC) value. PIC value of marker reveals the discriminating power of markers. PIC values for ISSR marker was ranged between 0.110 (ISSR-5) to 0.258 (UBC-808). The average PIC value in this study was 0.176 (Table- 4.16) with also in Graph- 4.16.

Similar estimates of PIC values were observed in case of earlier microsatellite studies in chickpea (Geleta *et al.*, 2006, Taran *et al.*, 2007). Gupta *et al.* (2003) reported increased PIC with greater number of markers.

4.28 Resolving power (Rp)

Resolving power of a marker gives a moderate idea about the number of genotypes that could be resolved by that marker (Prevost *et al.*, 1999). The values of resolving powers for ISSR marker was ranged between 0.406 (ISSR-1) and 1.800 (ISSR-9). The average Rp value in this study was 0.881 (Table- 4.16) with also in Graph- 4.16.

However, in cowpea (*Vigna unguiculata* (L.) Walp.) landraces, more polymorphic loci were detected with ISSR than with RAPD fingerprinting (Ghalmi *et al.*, 2010).

4.29 Analysis of molecular genetics variance (AMOVA)

AMOVA was used to analyze variation among and within the populations. The distribution of molecular genetic variation among and within the 30 genotypes of cluster bean was estimated by AMOVA. AMOVA revealed that molecular variances were 8 percent of the total variance was among the subpopulations, while 92 percent was among individuals within the populations respectively. The same trend was observed when the AMOVA estimated based on one cluster bean types in germplasm set. The estimated variance based on ISSR marker data was 0.608 (among the population) and 6.783 (within the population). The average estimated variance was 3.965 (Table- 4.18).

Table 4.18 Analysis of Molecular variance (AMOVA) based on ISSR marker data within and among genotypes (level of significance based on 999 iteration steps)

Primers	Source of variance	Estimated variance	Percentage (%)	pt	P(rand>= data)
ISSR	Among population	0.608	8 %	0.082	0.001
	Within population	6.783	92%		
Total		7.931	100%		

DISCUSSION

CHAPTER -V

Leguminous vegetables have been cultivated for more than 6000 years in different parts of the world. Legumes for human consumption constitute about 5% of total cultivated crops (Shanmugasundaram, 1990). Leguminous vegetable serves as a major source of protein in a diet. To meet the situation of increasing rate of population there is a need to identify and popularize new crop species yielding multiple useful products having food, industrial, forage and nutritional value. Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], one such legume crop mainly grown for grain in North India, whereas in South India it is grown for its tender pods for vegetable purpose and foliage as green fodder (Bhatti and Sial, 1971). Cluster bean also used as green manure which fixes atmospheric nitrogen (50-60 kg/ha) and adds organic matter to soil (Lal, 1985). Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] is an extensively cultivated pulse and vegetable crop in India. It is important pulse and vegetable around the world in respect to area, production, productivity, availability and utility. The present investigations were carried out by keeping all the information in mind. The results of experiment would help in selection of suitable genotypes on the basis of following objectives which were taken in the study.

1. To estimate the extent of genetic diversity in cluster bean germplasm.
2. To evaluate the extent of genetic variability in the germplasm of cluster bean
3. To study the contribution towards yield by correlation and path coefficient analysis.
4. To identify potential genotypes of cluster bean on the basis of yield and yield attributes.
5. To screen out the most desirable genotypes on the basis of morphological characterization.
6. To investigate the most desirable genotypes on the basis of molecular characterization.

It must be clear that to apply appropriate methodology for achieving the desired target. Before initiating any improvement programme one must build up a germplasm pool that will serve as a gene complex reservoir for different characters and consequently provide better source of variability, which forms the base for improvement. Therefore, an attempt has been made in the present investigation to study the genetic variability in the available cluster bean genotypes.

For systematic improvement of any crop, genetic variability information is essential to formulate appropriate breeding programmes. Thorough evaluation and utilization of germplasm needed to understand the genetic architecture of the germplasm, intern improvement of productivity in the field crops (Allard and Bradshaw, 1964). Estimation of genetic variability parameters is the foremost step to be adopted in the source population when the breeding programme is aimed at improving economically important traits. The success of a crop improvement programme depends on the ability of the breeder to define and assemble the required genetic variability and select for yield indirectly through yield associated and highly heritable characters after eliminating the environmental component of phenotypic variation (Mather, 1949).

When several genotypes are involved in a breeding programme, it is impossible for the breeder to evaluate each and every genotype in the field for yield and other yield attributing characters. Therefore it is necessary to estimate the association of seed yield with other characters, so that at the first stage, a few selections could be made based on visual judgment of the characters correlated with yield and in the next stage one or two promising ones could be finally chosen based on actual yield potential of those few lines selected in the first stage. Correlation depicts the sum total of direct and indirect effects of independent characters on the dependent character. Path analysis is required to partition the correlation into direct and indirect effects so as to get an accurate picture of the association of characters. In view of this, correlation and path analysis were studied.

Success of crop improvement programmes depends largely on the extent of variability present in the germplasm stock for the traits for which the improvement is aimed at. The knowledge of genetic variability and association of various characters

are essential in planning the breeding programmes. With this perspective, in the present study, 30 genotypes of cluster bean were evaluated for green vegetable pod yield along with its contributing characters. The proportion of variability, broad sense heritability and genetic advance over mean was estimated. The association among the characters and their direct and indirect effect on yield and genetic divergence were studied.

The present studies were carried out with thirty genotypes of cluster bean. These genotypes were evaluated for some important characters i.e. plant height (cm), germination (%), days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant, pod breadth (cm), pod length (cm), pod width (cm), number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, number of seed/pod, pod yield/plant (g), pod yield/plot (kg), 100-seed weight (g), seed yield/plant (g), days to maturity and pod yield (q/ha). The parameters of variability such as coefficient of variation, heritability, genetic advance correlation, path analysis and genetic divergence was computed so, as to help breeders to select desirable genotypes for different characters.

To estimates of different statistical parameters such as variability, heritability, genetic advance correlation coefficient, path analysis and genetic divergence helps in deciding the best possible breeding approach to be employed for the improvement of yield and yield contributing characters in cluster bean. Salient findings from these investigations are discussed under the following heads:

Morphological Characterization

5.1 Coefficient of variation

The relative values of phenotypic variance, genotypic variance and coefficient of variance (PCV and GCV) give an idea about the magnitude of variability present in a population, among morphological characters. In morphological and yield characters, the highest phenotypic coefficient of variation was observed for number of pods/cluster (58.05) followed by number of clusters/plant (40.47) and lowest for days to maturity (3.41days) followed by germination% (4.46%) and the genotypic coefficient of variation for germination% (2.00%) followed by days taken for first

flowering (2.54) were the minimum, while it was observed to be the highest genotypic coefficient of variation was recorded for number of pods/cluster (56.77) followed by number of clusters/plant (39.80) in morphological and yield characters. The results get support from the findings of (Johanson *et al.*, 1955 and Singh, 1999). In the present studies the information obtained show that the estimates of PCV were higher than the GCV meaning there by that the apparent variation was not only due to genotypes but also environment influenced. These mentioned characters, having higher range of variation like that, pod yield (q/ha), pod yield/plant, germination%, plant height and number of seed yield/plant have better scope of improvement through selection characters such as pod yield/plant, pod yield/plot and pod yield(q/ha). Showed the moderate value of GCV and PCV, which is considered sufficient to make an effective selection.

In the present studies the GCV and PCV estimates revealed that appreciable variation for characters like 100-seed weight, pod yield (q/ha), plant height, pod yield/plant and pod yield/plot which suggest important of these characters in the improvement programme.

With the help of GCV alone it is a possible to determine the amount of variation that is heritable. The relative degree to which a character is transmitted from parent to offspring is indicated by the estimates of heritability, if character showed high heritability value, it indicates that there is influence for environment in its expression. Accordingly, such traits have an option to be improved by adopting simple selection methods. Heritability estimates along with genetic advance are normally help full in predicting the gain under selection than heritability estimate alone. Hence, both heritability and genetic advance were determined to get a clear picture of the scope for improvement in various characters though selection.

Genotypic and phenotypic correlation were work out the degree of association of different component characters with pod yield (q/ha) and path coefficient analysis was done to provide information on the case of this association (Wright, 1921; Dewey and Lu, 1959).

In the present studies, analysis of variance indicated highly significant differences for all characters studied. Although breeding programme depends up on variability

present, but absolute variability in different characters cannot be the decisive factor for deciding as to which characters is showing the highest degree of variability.

The quantification of available variability was therefore, carried out for morphological and yield contributing characters in order to identify desirable genotypes based on performance and to select potential donors for various characters that may be used for hybridization programme to obtain useful recombination and to create additional genetic variability.

5.2 Heritability

In the present investigation, the characters like number of pods/plant, 100-seed weight (g), number of branches/plant at maturity and pod length (cm) had shown high heritability values and moderate genetic advance as percent over mean indicating predominance of additive gene component, while pod length, days taken for 50% flowering, germination% and pod breadth exhibited medium heritability estimates. Thus, there is ample scope for improving of these characters through direct selection. These observations were in accordance with the earlier findings of Liang and Waltre (1969). It is suggested that highly heritable nature of variability for these characters and effectiveness of selection.

Broad sense heritability the portion of genetic advance to the phenotypic variance is improvement parameters in breeding and genetics. The heritability estimates together with expected genetic gain is usually more reliable than either these of two parameters alone in predicting the resultant effects of selecting the best individuals by (Johanson *et al.*, 1955). High heritability accompanied with high genetic advance indicates that most likely the heritability is due to additive gene effects and selection may be effective, from this point of view number of clusters/plant, number of pods/cluster, pod yield/plant (g), plant height (cm) and seed yield/plant (g) possessed greater estimates of genetic advance as percent of mean coupled with high amount of heritability indicating these traits are governed by additive gene action and continued selection would be help in modifying the mean performance of the population. Similar observations were recorded by (Johanson *et al.*, 1955).

High heritability coupled with moderate genetic advance were expressed by the characters like number of pods/plant, 100-seed weight (g), number of branches/plant

at maturity and pod length (cm). It indicates equal contribution of additive and non-additive gene for these traits. The characters, like pod width, number of seeds/pod and number of branches/ plant at maturity single. Showed low heritability accompanied with low genetic advance medicating non-additive gene action for expression of these traits. These confirm the findings of Burton (1952), that GCV together with heritability estimates would give a better picture of genetic advance under selection. Variability and heritability studies provide information regarding the possibility of improvement in different traits and they do not indicate any kind of relationship between traits of interest. Therefore, knowledge regarding the association of different characters among themselves and with other important traits like yield is essential for indirect selection and improvement of important characters. These results corroborate the findings of Arumugarangarajan *et al.* (2000) and Singh *et al.* (2002).

The variability and high heritability along with high genetic advance expressed by the above mentioned characters indicated that genotypes could be evaluated in multi location trials and selected as donors for these characters or used as parents in hybridization programme.

5.3 Genetic advance

The data recorded revealed that the maximum genetic advance was observed for plant height (cm) (24.62) with 30.68 % advance high over the mean and minimum for pod width (0.08) with 1.34 % genetic advance over the mean. The characters like pods/plant (g), days to maturity, seed yield/plant (g), number of branches/plant at maturity and pod yield (q/ha) recorded high genetic advance over mean.

It has been suggested that characters with high heritability coupled with high genetic advance would response to selection better than with high heritability and low genetic advance were reported by Johanson *et al.* (1955).

5.4 Correlation coefficient

Study of correlation coefficient provides better understanding of yield component that helps the plant breeder during selection. A positive correlation between desirable characters is favorable to the plant breeder because it helps in simultaneously improvement in both the characters. A negative correlation, on other hands, will under the simultaneously expression of both characters with high values.

The correlation studies showed higher estimates of genotypic correlation coefficient than the corresponding phenotypic correlation coefficient such findings were also reported by (Saha *et al.*, 1990) and Kumarswamy (1990), which showed that the apparent due to favorable influence of improvement.

In the present investigation, pod yield exhibited positive and significant association with all the characters viz., plant height (cm), germination (%), days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant, pod breadth (cm), pod length (cm), pod width (cm), number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, number of seed/pod, pod yield/plant (g), pod yield/plot (kg), 100-seed weight (g), seed yield/plant (g), days to maturity and pod yield (q/ha). Thus it may be deduced that the selection based on these traits either in combination or alone would be beneficial to identify the genotypes having better yield potential. This is in agreement with findings of Kumarswamy (1990) and Rai *et al.* (2004) it had a also non-significant positive correlation with green pod length, single green pod weight and number of seeds/pod as reported by (Shah *et al.*, 1986) and Singh *et al.* (2000), and negative significant correlation coefficient with germination%, pod length, number of pods/plant, number of pods/cluster and number of clusters/plant.

Pod yield (q/ha) showed positive and significant correlation with plant height, days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant at maturity, pod breadth, pod length, number of pods/cluster and number of clusters/plant. Hence, pod yield/plant and pod yield/plot showed highly significant and positive association with pod yield at genotypic correlation coefficient level. Breeder can rely upon all these nineteen characters in selecting plant with high yield in cluster bean.

Pod width showed non-significant negative correlation with all the morphological characters at both the level. Non-significant negative correlation with yield and most of the yield contributing characters suggest that pod width is an undesirable character and for improvement in yield attention should be given for selecting of genotypes having pod yield/plant. Similar findings have been also reported by Kumarswamy (1990).

In this present study the characters like plant height, germination%, days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant, pod breadth, number of reproductive branches/plant, 100-seed weight, seed yield/plant and days to maturity are significant and positive correlation on pod yield. The character like pod yield/plant is highly significant and positive correlation on pod yield at phenotypic correlation coefficient level.

Pod yield had significant and positive correlation with number of pods yield/plant and pod yield/plot this is accordance with the results of (Rai *et al.*, 2004) for quality characters also, the correlation studies showed higher estimates of genotypic correlation coefficient than corresponding phenotypic correlation coefficient. The significantly positive associations suggest that selection should be oriented towards higher content of seed yield/plant, pod yield/plot and thus ultimately resulting in higher pod yield content. These results get support from the findings of Kumarswamy 1998

5.5 Path analysis

Correlation studies alone are not sufficient to ascertain a clear association among the characters as more variables are considered in the correlation. Path coefficient has been therefore, suggested by various characters and helps in understanding the direct and indirect contribution of various independent variables towards dependent variables (for morphological and yield contributing characters).

The results of the path coefficient analysis indicated the direct and indirect effects of the 19 morphological and yield contributing characters on the pod yield (q/ha).

The highest direct effect on pod yield was contributed to plant height. Further, the pod yield is directly influenced the days taken for first flowering, days taken for 50% flowering, number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, number of seeds/pod, pod yield/plant and pod yield/plot showed positive direct effects on the pod yield (q/ha) at genotypic path coefficient analysis indicated that these characters are important in the selection of breeding programme. Path coefficient analysis for morphological traits were also studied by Baswana *et al.* (1980) and Biju *et al.* (2001) in Indian bean, Rai *et al.* (2001) in french bean and Patil *et al.* (1989) in cowpea.

The highest direct effect on pod yield was contributed to germination%, number of pods/plant and pod yield/plant. Further, pod yield is directly influenced the number of reproductive branches/plant, pod length, pod width, number of pods/cluster, number of branches/plant at maturity, number of seeds/pod, pod yield/plot, 100-seed weight and seed yield/plant showed positive direct effects on pod yield (q/ha) at phenotypic path coefficient analysis indicated that these characters are important in the selection of breeding programme. These results are in accordance with the findings of Saini *et al.* (2010).

Among the quality parameters, the highest direct effect on green pod yield (q/ha) was contributed by protein content followed by total sugars and moisture content indirectly influenced by the green pod yield through total sugars content and protein content. This is in consonance with the results of Dev and Rastogi (1999) in pea.

5.6 Genetic divergence

The Success of a breeding programme depends upon the selection of parents. It has been found that the progenies derived from crossing divergent parents give divergent and useful progenies. The D^2 analysis proposed by Mahalanobis (1928) has been reported to be an effective tool to assess the genetic divergence. Such an analysis eventually helps to choose desirable parents for recombination breeding and thus results in the development of superior varieties. Quantification of genetic diversity existing within and between groups of germplasm is important and particularly useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. D^2 Statistics is a unique method for disseminating populations considering a set of parameters together rather than interning from indices based upon morphological similarities: eco-geographical diversity and phylogenetic relationships.

5.6.1 Generalized distance

Based on D^2 values 30 cluster bean genotypes were grouped into 5 clusters, 8 genotypes in cluster I and six genotypes each in clusters II and IV and five genotypes each in clusters III and V. The formation of solitary clusters may be due to total isolation preventing the gene flow or intensive human selection for diverse adaptive complexes. The intra cluster distance varies from 3.400 in clusters I and to the

maximum distance in cluster II (8.235). This reveals that presence of divergence in cluster I. The inter cluster D^2 values also ranged from 3.464 between cluster IV and cluster III, 4.218 between cluster III and V and of maximum distance of 6.625 between cluster I and cluster IV. Selection of genotypes is recommended from the clusters showing higher inter cluster distance (Cluster III and V) also with higher pod yield as parents in recombination breeding programmes for obtaining desirable segregants.

5.6.2 Contribution of characters towards divergence

Among 19 characters studied, the most important character contributing to maximum divergence was pod yield per hectare followed by other main responsible characters like 100-seed weight (g), pod breadth (cm), number of pods/cluster, number of pods per plant, number of seeds/pod and number of clusters/plant (5.87). These results were supported by Singh *et al.* (2003) and Henry *et al.* (1984); pods per cluster and pods per plant contribution are in accordance with Gipson and Balakrishnan (1992), contribution of plant height in accordance with Hanchinamani (2004).

Molecular Characterization

5.7 Genetic diversity

Genetic diversity among 30 genotypes of cluster bean analyzed on following basis describe in this study. The primers UBC-841, UBC-855, ISSR-5 and ISSR-8 produced highest bands as compared to UBC-856 and UBC-829-11 primers produce lowest bands (Table- 4.15). The primers IS-5 and UBC-814-11 produced 13 and 11 bands respectively. The primer ISSR-2 produced unclear bands; the primer ISSR-9 did not produce any band. There are 21 primers used and produced a total of 263 bands out of which 204 were polymorphic and 59 were monomorphic. The highest bands were observed in genotypes namely, IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789 and IC-369868 and the lowest band were observed in genotypes IC-421809, IC-421812 and IC-421815. Three unique bands were observed in genotypes number IC-370490 with 2 bands in primer UBC-856 of about 320 bp and 350bp and a single band with primer UBC-868 of about 275 bp.

RAPD and ISSR marker generated good diversity for the present set of cluster bean genotypes. Earlier studies also showed a large genetic variation among different genotypes of cluster bean using the RAPD (Punia *et al.*, 2009; Pathak *et al.*, 2010).

5.8 Cluster analysis

The UPGMA dendrogram was constructed from a similarity matrix based on Jaccard's similarity coefficient values. The dendrogram (Figure- 4.25) from ISSR data showed five major cluster at lower level of similarity and ten minor clusters at lower level of similarity. The major cluster possessed seven sub clusters. Figure- 4.25.1 shows the Gel profiling of genomic DNA in thirty diverse genotypes of cluster bean. Figure- 4.25.2 shows the Gel profiling of ISSR-PCR amplification produced by UBC-879 primer. Figure- 4.25.3 and Figure- 4.25.4 show the ISSR fingerprint patterns produced with UBC-841 and UBC-855 primers. The dendrogram clearly differentiate between genotypes.

The results of the present ISSR analysis were similar but not identical to our earlier SSR study of the same set of germplasm (Choudhary *et al.*, 2012). The differences may be attributed to the different numbers of loci analyzed and to differences in the nature of the marker systems analyzed, reinforcing the importance of the number and nature of the loci examined and their overall coverage of the genome in obtaining reliable estimates of the genetic diversity and relationships among the germplasm. Understanding the genetic diversity and genetic relationships in germplasm collections is critical to crop improvement programmes.

The use of molecular markers enables cluster bean breeders to connect the gene action underlying a specific phenotype with the distinct regions of the genome in which the gene reside. Molecular markers allow the direct selection for genotypes, thereby providing a more efficient means of selection. Molecular markers provide an opportunity to identify and isolate the gene relating to character by map-based cloning. Genetically diverse line provides ample opportunity to create a favourable gene combination and probability of producing a unique genotype increases in proportion to the number of genes by which the parents differ. Genetic diversity

analysis of cluster bean germplasm can provide practical information for selection of parental material and thus, assist in forecasting breeding strategies.

Therefore, the ISSR markers represent the more efficient marker system because of their capacity to generate several informative bands within a single amplification reaction. Similar results have been reported in cashew by Thimmappaiah *et al.* (2009). In fact, ISSR exhibits a higher capacity to reveal polymorphisms and greater potential to determine intra and inter-genomic diversity than other arbitrary primer methods such as RAPD (Zietkiewicz *et al.*, 1994). Thus, the genetic variation obtained from RAPD and ISSR analysis could be useful for selecting parents to generate the necessary cluster bean populations for genomic mapping and breeding purposes.

5.9 Analysis of molecular genetics variance (AMOVA)

AMOVA was used to analyze variation among and within the populations. The distribution of molecular genetic variation among and within the 30 genotypes of cluster bean was estimated by AMOVA. AMOVA revealed that molecular variances were 8 per cent of the total variance was among the subpopulations, while 92 per cent was among individuals within the populations respectively. The same trend was observed when the AMOVA estimated based on one cluster bean types in germplasm set in Table- 4.18. The estimated variance based on ISSR marker data was 0.608 (among the population) and 6.783 (within the population). The average estimated variance was 3.965.

AMOVA was used to analyze variation among and within the populations. Molecular variances were 8% and 92% among and within the population. These results are in accordance with the findings of Aswathnarayana *et al.* (2013).

SUMMARY AND CONCLUSION

CHAPTER- VI

The present investigation entitled “Studies on Genetic Diversity and Variability in Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Genotypes Through Morphological and Molecular Characterization.” were conducted at the Horticulture Research Farm-I, of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rae Bareilly Road, Lucknow, (U.P.), India during Rabi season of 2012-13 and 2013-14. The objectives of present investigation were to study variability in the morphological and yield and yield attributing characters. The present study is focused on the following objectives:

1. To estimate the extent of genetic diversity in cluster bean germplasm.
2. To evaluate the extent of genetic variability in the germplasm of cluster bean.
3. To study the contribution towards yield by correlation and path coefficient analysis.
4. To identify potential genotypes of cluster bean on the basis of yield and yield attributes.
5. To screen out the most desirable genotypes on the basis of morphological characterization.
6. To investigate the most desirable genotypes on the basis of molecular characterization.

To determine direct and indirect effect for this study, thirty genotypes were grown with recommended cultural practices in randomized block design with three replications.

The observations recorded on 20 characters i.e., plant height (cm), germination (%), days taken for first flowering, days taken for 50% flowering, number of reproductive branches/plant, pod breadth (cm), pod length (cm), pod width (cm), number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, number of seeds/pod, pod yield/plant (g), pod yield/plot

(kg), 100-seed weight (g), seed yield/plant (g), days to maturity, pod yield (q/ha) and flower colour. The data were statistically analyzed for different variability parameters.

The study was carried out during 2012-2013 and 2013-2014 at the Horticulture Research Farm-I, of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Vidya-Vihar, Rae-Bareilly Road, Lucknow, (U.P.), India. The salient experimental findings of the present investigation are summarized below:

6.1 Morphological Characterization

The analysis of variance revealed that sufficient variation existed among genotypes for all the characters under study.

1. The considerable wide range of variation was observed in germplasm for different characters like plant height (cm), germination%, number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, pod yield/plant (g), pod yield/plot (kg), seed yield/plant (g), days to maturity and pod yield (q/ha).
2. High magnitude of GCV and PCV was observed for number of pods/cluster (56.77) followed by number of clusters/plant (39.80) and number of pods/cluster (58.05) followed by number of clusters/plant (40.47).
3. Highest estimates of heritability in broad sense along with high genetic advance as percent of means were recorded for number of clusters/plant, number of pods/cluster, pod yield/plant (g), plant height (cm) and seed yield/plant (g). High heritability along with moderate genetic advance were expressed by the characters like number of pods/plant, 100-seed weight (g), number of branches/plant at maturity and pod length (cm).
4. Highest genetic advance over mean was observed in the characters like pods/plant (g), days to maturity, seed yield/plant (g), number of branches/plant at maturity and pod yield (q/ha) recorded high genetic advance over mean.
5. Correlation studies revealed that pod yield exhibited highly significant and positive association with pod yield/plant and pod yield/plot at genotypic correlation

coefficient level and the character like pod yield/plant is highly significant and positive correlation on pod yield at phenotypic correlation coefficient level.

6. The genotype IC-421855 (121.49 g) produced high yield on the basis of pod yield/plant (g) in both the year. The genotypes IC-421828 (0.76 kg) produced high yield followed by IC-421855 (0.75 kg) on the basis of pod yield/plot (kg). The genotypes HG-365 (8.64 g) produced high yield on the basis of seed yield/plant (g). The genotypes IC-421855 (142.23 q/ha) produced high yield followed by HG-365 (138.15 q/ha), IC-421809 (135.48 q/ha) and IC-421806 (133.41 q/ha) on the basis of pod yield (q/ha).

7. Path analysis identified characters such as days taken for first flowering, days taken for 50% flowering, number of pods/plant, number of pods/cluster, number of clusters/plant, number of branches/plant at maturity, number of seeds/pod, pod yield/plant and pod yield/plot showed significant and positive direct effects on the pod yield (q/ha) at genotypic path coefficient analysis.

8. Path analysis identified characters such as number of reproductive branches/plant, pod length, pod width, number of pods/cluster, number of branches/plant at maturity, number of seeds/pod, pod yield/plot, 100-seed weight and seed yield/plant showed significant and positive direct effects on pod yield (q/ha) at phenotypic path coefficient analysis.

9. Thus present path analysis study in cluster bean, it may be concluded that improvement in pod yield/ha could be brought by selection for component characters like days to 50% flowering, pods/cluster, pod length, number of seeds/pod and pod yield/plant.

10. The most important characters contributing to maximum genetic divergence was pod yield per hectare followed by other main responsible characters like 100-seed weight (g), pod breadth (cm), number of pods/cluster, number of pods per plant, number of seeds/pod and number of clusters/plant (5.87).

6.2 Molecular Characterization

Morphological and molecular characterization of germplasm and identification of genetically diverse attributes specific sources are important for enhanced utilization of cluster bean genetic resources in breeding improved cultivars.

Hence, the current study was undertaken to understand the morphological and molecular genetic diversity in cluster bean germplasm, to identify trait specific germplasm and the ISSR markers associated with phenotypic variation.

For the molecular characterization of cluster bean germplasm, 21 ISSR markers were used. The results are summarized below.

1. Genetic diversity among 30 germplasm of cluster bean analyzed on following basis describe in this study. The primers UBC-841, UBC-855, ISSR-5 and ISSR-8 produced highest bands as compared to UBC-856 and UBC-829-11 primers produce lowest bands.
2. The phylogenetic study was done in 30 germplasm of cluster bean. The phylogenetic tree was constructed on the basis of phylogenetic analysis. A dendrogram is generated with the help of UPGMA Jaccard coefficient analysis. The dendrogram possess five major cluster and seven sub cluster.
3. The genome size of cluster bean is approximately 2.45 Giga Bases/Centimeter. Cross pollination is strictly prohibited due to the cleistogamous nature of flowers. There are 30 major maps and 90 sub maps of cluster bean construct in the present study.
4. The values of number of amplified fragment for ISSR marker was ranged between 6 (UBC-854) and 17 (UBC-841), 17 (UBC-855) respectively. The average number of amplified fragment in this study was 12.52.
5. The values of polymorphic fragment for ISSR marker was ranged between 4 (UBC-854) and 15 (ISSR-8), 15 (IS-7), respectively. The average polymorphic fragment in this study was 9.71.
6. The values of polymorphism percentage for ISSR marker was ranged between 58.33% (UBC-856) and 100% (UBC-820), 100% (ISSR-8) respectively. The average polymorphism percentage in this study was 77.06%.
7. The values of monomorphic fragment for ISSR marker was ranged between 1 (IS-7) and 5 (UBC-856), 5 (IS-25), respectively. The average monomorphic fragment in this study was 2.80.

8. The values of monomorphism percentage for ISSR marker was ranged between 6.25% (IS-7) and 41.66% (UBC-856). The average monomorphism percentage in this study was 22.93%.

In summary, the cluster bean germplasm is genetically diverse and possesses potential variation for morphological and yield attributing characters and hence could be extensively evaluated for greater exploitation for use in breeding programs. The superior trait specific germplasm identified could be utilized in breeding programs to improve traits and to widen the genetic base of cluster bean cultivars. Marker trait associations identified in this study using ISSR markers and association mapping approach the first effort in this crop, and will provide important information to the research community for further QTL identification, to identify candidate genes and gene cloning that underlie QTLs in cluster bean.

CONCLUSION

Leguminous vegetable serves as a major source of protein in a diet. Tender pods of cluster bean are rich in minerals like calcium, phosphorus, iron, vitamin A and C. Immature pods are dried and fried like potato chips and can be cooked like french bean. The gum from seeds has usages in many industries, viz., textile, paper, pharmaceutical, food, dairy, explosives, etc. Because of its highly drought tolerating property, much of its area is concentrated in states like Rajasthan, Gujarat and Haryana. In Karnataka, though large extent of area is under arid and semi-arid experiencing frequent drought. Moreover cluster bean cultivation has not been exploited for vegetable purpose. The principle objective of the present investigation was to study and highlight genetic diversity and variability in the cluster bean genotypes on morphological and molecular basis, to know the association between different phenotypic and genotypic characters. To facilitate investigation of above objective in cluster bean genotypes obtained from National Bureau Plant Genetic Resources (Indian Council of Agricultural Research), Regional Station, Jodhpur and Punjab Agricultural University, Ludhiana. The study was carried out during Rabi season of 2012-2013 and 2013-2014. The materials were grown in the field with Randomized Block Design. The data obtained for 19 characters were subjected to statistical analysis for elucidating the information on genetic diversity and variability existing for different component characters of pod yield.

6.3 Morphological Characterization

The morpho-agronomic characterization provides information underlying conclusions on the genetic variability of the genotypes of the bank, identification of accessions maintained in duplicate (Valls, 2007), improvement of the data of identification and classification of accessions (Chiorato *et al.*, 2007) and support the regeneration and maintenance of the genetic integrity of genotypes. Among other information that can be obtained from the characterization of germplasm, the determination of the relative importance of the traits used to describe the genetic diversity (Rodrigues *et al.*, 2002, Chiorato *et al.*, 2005). Characterization of genetic

resources is an important component of a crop improvement programme for their effective involvement in hybridization programmes.

Success of crop improvement programmes depends largely on the extent of variability present in the germplasm stock for the traits for which the improvement is aimed at. The knowledge of genetic variability and association of various characters are essential in planning the breeding programmes. With this perspective, in the present study, 30 accessions of cluster bean were evaluated for green vegetable pod yield along with its contributing characters. The proportion of variability, broad sense heritability, and genetic advance over mean was estimated. The association among the characters and their direct and indirect effect on yield and genetic divergence were studied.

Further, the genetic variability obtained in guar genotypes highlights the potential persisting in the germplasm for crop improvement. This wide variability in guar, undoubtedly, is only a proportion of the variation that exists, and further evaluation is warranted to use and conserve these potentially valuable genetic resources. Breeding strategies need to exploit existing variation within the guar germplasm for widening the genetic base. Eventually, the results of the present study can be used for varietal/genotype identification and parental selection, and will be helpful in augmentation of the guar improvement programme.

Coefficient of variability, estimates of heritability and genetic advance as percent of mean, as well as correlation coefficients among yield attributes of cluster bean observed in the present study substantially reveal the great scope for improvement in many of the desirable economic traits of the crop.

Morphological characters i.e., number of pods/plant, pod yield/plant (g), pod yield/plot (kg), seed yield/plant (g), pod yield (q/ha), number of seeds/pod and 100-seed weight (g) are more reliable for varietal characterization.

Finally, based on the number of pods/plant, pod yield/plant (g), pod yield/plot (kg), seed yield/plant (g), pod yield (q/ha), number of seeds/pod and 100-seed weight (g) for higher yielder genotype IC-421834, IC-421855, IC-421828, IC-421855, HG-365, IC-421809 and IC-421806 were identified to be the superior genotypes in both the year.

Most desirable genotypes screen out on the basis of morphological characterization in this study are IC-421834, IC-421855, IC-421828, IC-421855, HG-365, IC-421809 and IC-421806.

6.4 Molecular Characterization

Molecular diversity has been studied on the basis of molecular characterization present in the 30 genotypes of cluster bean. Molecular diversity has been studied on the basis of molecular characterization present in the 30 genotypes of cluster bean. A set of 21 ISSR markers (Table 2) was used for analysis of genetic diversity among 30 germplasm of cluster bean. The Genetic diversity and phylogenetic analysis, genome mapping, number of amplified fragment, polymorphic fragments, polymorphism percentage, monomorphic fragments and monomorphism percentage obtained for each primer. Analysis of genetic relationships in crop species, which provides information on planning the crosses between two diverse varieties, is the preliminary requirement for crop improvement strategy. The present set of cluster bean genotypes showed greater genetic diversity on the basis of random primers. However, the efforts of estimating genetic diversity among the present set of genotypes are not sufficient as only cultivated genotypes were included. Co-dominant markers such as SSRs are required for more information of genetic diversity of cluster bean by including more number of genotypes.

To investigate the genetic diversity and relationships within and among 30 cluster bean germplasm, we tested 21 ISSR primers, 8 of which revealed reproducible polymorphic patterns and were used for further analysis.

The estimated variance based on ISSR marker data was 0.608 (among the population) and 6.783 (within the population). The average estimated variance was 3.965. The observed number of alleles, effective number of alleles, Nei's genetic diversity and Shannon's information index for cluster bean genotypes using 21 ISSR markers were found to be 1.78120 ± 0.4167 , 1.4627 ± 0.3844 , 0.2671 ± 0.1939 and 0.3988 ± 0.268 , respectively. The value of total genotype diversity among population (H_t) was 0.2639 ± 0.0378 whereas diversity within population (H_s) was found to be

0.253+0.035. Mean coefficient of gene differentiation (G_{st}) value was 0.041 and the estimated gene flow in the population was 11.549.

Diversity on the basis of molecular characterization in the present study was successfully categorized in the different *C. tetragonoloba* genotypes into various groups and also established the relatedness among them based on genetic diversity. Molecular markers are useful for genotyping accessions. This study does not distinguish all the genotypes individually, but provides important information in the form of unique alleles/protein marker for certain genotypes. Significant variability was obtained at the inter-species level using PCR analysis as well as ISSR markers were found to be more informative.

Conclusively, this study clearly indicates that ISSR proved to be an efficient marker system for studying genetic diversity and relationships among members of the germplasm characterization. It will serve as an important consideration for efficient rationalization and utilization of the germplasm characterization, providing a basis for future cluster bean crop variety identification, conservation and management. The promising germplasm identified through this study will serve as useful resources for functional and comparative genomics, mapping and cloning genes, and in applied breeding for enhancing the genetic potential of the cluster bean.

The cultivars with dissimilar DNA profiles provided useful information for selection of parents to develop new cluster bean hybrids.

Molecular markers are more efficient in characterization of cluster bean genotypes and are classified in to five distinct clusters as studied.

However, the most desirable genotypes investigate on the basis of molecular characterization in this study are IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789 and IC-369868.

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Appendix-I: Analysis of variance for plant height (cm)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	81.25	40.625	1.434	
2.	Treatment	29	29084.39	1002.910	35.404	2.045
3.	Error	58	1642.966	28.327		
4.	Total	89	30808.966			

General Mean = 80.24 SE (m) \pm = 3.07 CD = 6.27

Result: - Significant at 5 % level

Appendix-II: Analysis of variance for germination (%)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	197.124	98.562	7.389	
2.	Treatment	29	1021.322	35.218	2.640	2.045
3.	Error	58	773.662	13.339		
4.	Total	89	1992.108			

General Mean = 91.64 SE (m) \pm = 2.10 CD = 4.29

Result: - Significant at 5 % level

Appendix-III: Analysis of variance for days taken for first flowering

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	16.57	8.285	3.993	
2.	Treatment	29	104.255	3.595	1.733	2.045
3.	Error	58	120.292	2.074		
4.	Total	89	241.117			

General Mean = 22.40 SE (m) \pm = 0.831 CD = 1.69

Result: - Non-significant at 5% level

Appendix-IV: Analysis of variance for days taken for 50% flowering

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	7.686	3.843	1.935	
2.	Treatment	29	221.937	7.653	3.853	2.045
3.	Error	58	115.188	1.986		
4.	Total	89	344.811			

General Mean = 33.55 SE (m) \pm = 0.813 CD = 1.66

Result: - Significant at 5 % level

Appendix-V: Analysis of variance for number of reproductive branches/plant

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	2.268	1.134	2.824	
2.	Treatment	29	184.092	6.348	15.817	2.045
3.	Error	58	23.258	0.401		
4.	Total	89	209.618			

General Mean = 5.86 SE (m) \pm = 0.365 CD = 0.746

Result: - Significant at 5 % level

Appendix-VI: Analysis of variance for pod breadth (cm)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	4.598	2.299	10.539	
2.	Treatment	29	9.773	0.337	1.544	2.045
3.	Error	58	12.644	0.218		
4.	Total	89	27.015			

General Mean = 3.75 SE (m) \pm = 0.269 CD = 0.550

Result: - Significant at 5 % level

Appendix-VII: Analysis of variance for pod length (cm)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	0.936	0.468	1.377	
2.	Treatment	29	42.398	1.462	4.299	2.045
3.	Error	58	19.72	0.340		
4.	Total	89	63.054			

General Mean = 5.71 SE (m) \pm = 0.336 CD = 0.687

Result: - Significant at 5 % level

Appendix-VIII: Analysis of variance for pod width (cm)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	4.238	2.119	3.578	
2.	Treatment	29	5.481	0.189	0.336	2.045
3.	Error	58	32.654	0.563		
4.	Total	89	42.373			

General Mean = 5.95 SE (m) \pm = 0.433 CD = 0.885

Result: - Significant at 5 % level

Appendix-IX: Analysis of variance for number of pods/plant

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	61.00	30.500	1.012	
2.	Treatment	29	8323.754	287.026	9.524	2.045
3.	Error	58	1747.83	30.135		
4.	Total	89	10132.584			

General Mean = 56.36 SE (m) \pm = 3.16 CD = 6.46

Result: - Significant at 5 % level

Appendix-X: Analysis of variance for number of pods /cluster

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	2.792	1.396	1.584	
2.	Treatment	29	3404.977	117.413	133.176	2.045
3.	Error	58	51.098	0.881		
4.	Total	89	3458.867			

General Mean = 7.74 SE (m) \pm = 0.452 CD = 1.10

Result: - Significant at 5 % level

Appendix-XI: Analysis of variance for number of clusters /plant

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	4.522	2.261	3.033	
2.	Treatment	29	3893.076	134.244	180.029	2.045
3.	Error	58	43.21	0.745		
4.	Total	89	3940.808			

General Mean = 11.80 SE (m) \pm = 0.498 CD = 1.01

Result: - Significant at 5 % level

Appendix-XII: Analysis of variance for number of branches /plant at maturity

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	2.588	1.294	9.117	
2.	Treatment	29	72.152	2.488	17.527	2.045
3.	Error	58	8.178	0.141		
4.	Total	89	82.918			

General Mean = 8.41 SE (m) \pm = 0.217 CD = 0.443

Result: - Significant at 5 % level

Appendix-XIII: Analysis of variance for number of seed /pod

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	0.736	0.368	2.098	
2.	Treatment	29	36.627	1.263	7.192	2.045
3.	Error	58	10.15	0.175		
4.	Total	89	47.513			

General Mean = 6.76 SE (m) \pm = 0.241 CD = 0.492

Result: - Significant at 5 % level

Appendix-XIV: Analysis of variance for pod yield /plant (g)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	208.50	104.250	8.994	
2.	Treatment	29	26440.663	911.747	78.663	2.045
3.	Error	58	672.22	11.590		
4.	Total	89	27321.383			

General Mean = 96.32 SE (m) \pm = 1.96 CD = 4.01

Result: - Significant at 5 % level

Appendix-XV: Analysis of variance for pod yield /plot (kg)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	0.00328	0.00164	0.506	
2.	Treatment	29	1.363	0.047	14.687	2.045
3.	Error	58	0.185	0.0032		
4.	Total	89	1.551			

General Mean = 0.57 SE (m) \pm = 0.032 CD = 0.065

Result: - Significant at 5 % level

Appendix-XVI: Analysis of variance for 100- Seed weight (g)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	0.232	0.116	1.381	
2.	Treatment	29	18.705	0.645	7.679	2.045
3.	Error	58	4.872	0.084		
4.	Total	89	23.809			

General Mean = 3.63 SE (m) \pm = 0.167 CD = 0.341

Result: - Significant at 5 % level

Appendix-XVII: Analysis of variance for seed yield /plant (g)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	5.386	2.693	9.835	
2.	Treatment	29	155.063	5.347	19.529	2.045
3.	Error	58	15.834	0.273		
4.	Total	89	176.283			

General Mean = 6.69 SE (m) \pm = 0.302 CD = 0.617

Result: - Significant at 5 % level

Appendix-XVIII: Analysis of variance for days to maturity

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	54.50	27.250	16.677	
2.	Treatment	29	1517.077	52.313	32.015	2.045
3.	Error	58	94.714	1.633		
4.	Total	89	1666.291			

General Mean = 92.35 SE (m) \pm = 0.738 CD = 1.50

Result: - Significant at 5 % level

Appendix-XIX: Analysis of variance for pod yield (q/ha)

S. No.	Source of Variance	D.F.	S.S.	M.S.S.	F (Calculated)	F (Tabulated)
1.	Replication	2	94.25	47.125	2.018	
2.	Treatment	29	5570.32	192.080	8.592	2.045
3.	Error	58	1296.532	22.354		
4.	Total	89	6961.102			

General Mean = 126.93 SE (m) \pm = 2.72 CD = 5.56

Result: - Significant at 5 % level