

**Morpho-molecular characterization of Common bean
(*Phaseolus vulgaris* L.) germplasm**

Syed Zainab Kashani
(MSH-2020-332)



**Division of Vegetable Science
Faculty of Horticulture
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir**

2023

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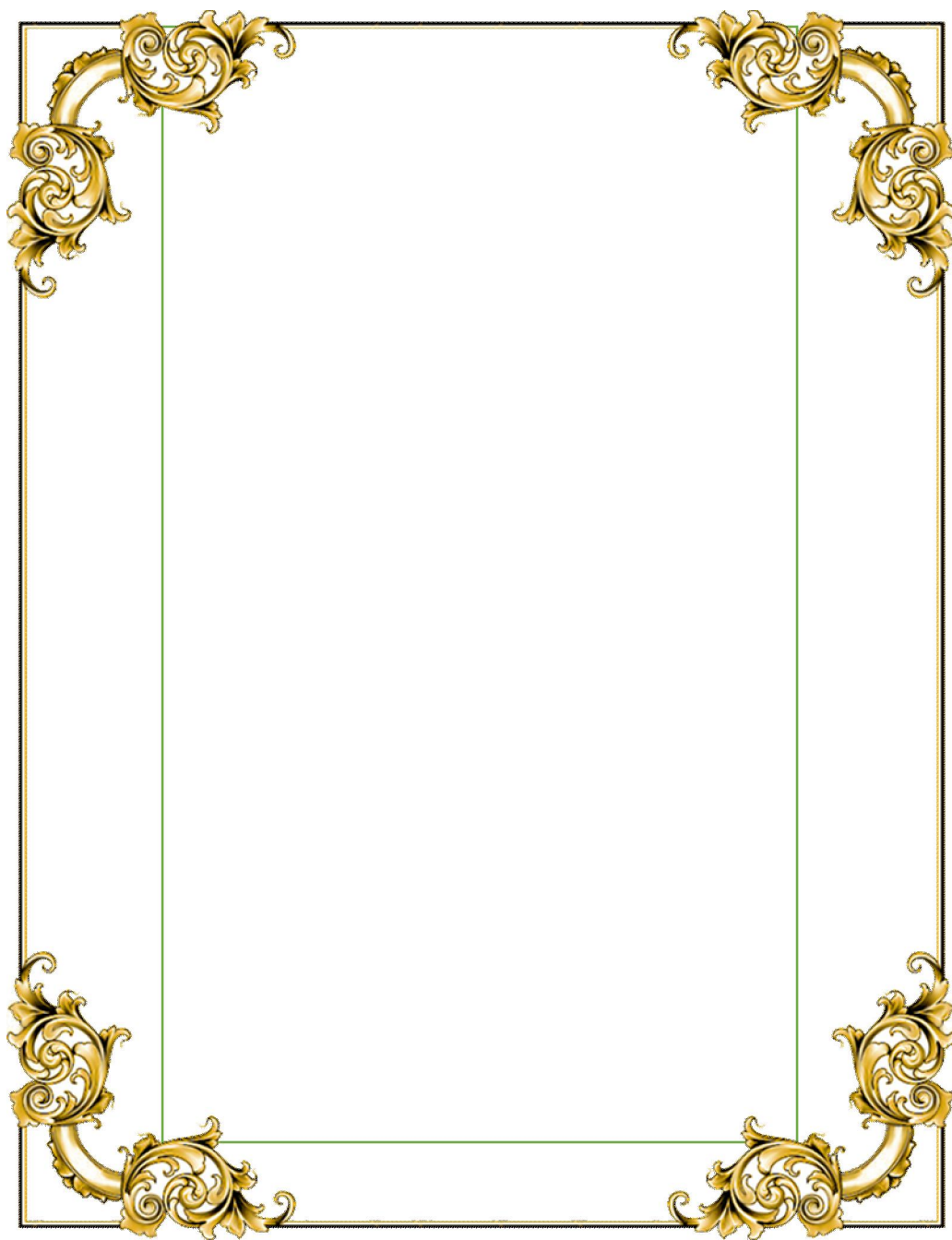
Thesis

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2023**



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Vegetable Science

Certificate – I

This is to certify that the thesis entitled, “**Morpho-molecular characterization of Common bean (*Phaseolus vulgaris* L.) germplasm**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Horticulture (Vegetable Science)**, to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Syed Zainab Kashani (Regd. No. MSH-2020-332)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

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ABSTRACT

The Common bean (*Phaseolus vulgaris* L.) also known as the French bean, Haricot bean, Snap bean, garden bean, navy bean, faras bean, and Kidney bean with a 473Mb genome size is one of the most important leguminous vegetable crops, grown throughout the world for its green pods. Genetic resources enable plant breeders to create novel plant gene combinations and select crop varieties more suited to the needs of a diverse horticulture system. In the present study, 48 test entries along with 3 checks were evaluated in an augmented block design for yield and yield component traits. The mean values of days to flowering, days to marketable maturity, days to pod initiation, hundred seed weight (g), number of pods per plant, number of seeds per pod, pod length (cm), pod thickness (mm), pod weight (g), pod yield per hectare (q), pod yield per plant (q), seed yield per plant (g), and seed yield per hectare (q) were 51.88, 68.51, 60.69, 29.13 g, 27.62, 5.1, 11.13 cm, 9.47 mm, 4.49 g, 304.61 q, 121.85 g, 38.17 g, 9.543 q respectively. The Analysis of variance revealed a significant mean sum of squares for all traits for different sources of variation. The Block effect (unadjusted) and the treatment effects (adjusted as well as unadjusted) were significant for all the traits. Similarly, the effects due to checks and varieties were significant. However, the adjusted block effects were non-significant for all traits indicating the homogeneity of evaluation blocks. Similarly, the mean square due to checks v/s varieties was significant for all the traits except pod weight,

indicating thereby that the test entries were significantly different from checks except for the pod weight. The genotypes namely WB1306, SB106, SB167, WB469, SB156.A and SB152 were found to be the most promising vegetable-type beans. Morphological diversity analysis grouped the genotypes into 2 major clusters where Cluster I contained 21 genotypes and Cluster II contained 30 genotypes. Correlation coefficients revealed that the Pod yield per hectare exhibited a significant positive association with pod length, pod thickness, pod weight, number of pods per plant, number of seeds per pod, and seed yield per plant. A negative and significant correlation was found between days to flowering and seed yield per plant, number of seeds per pod, and hundred seed weight. The analysis of genotypic data of 11 markers led to the detection of 54 alleles ranging from 1-8 with an average of 4.90 alleles per locus. The markers *BM152* and *PVBR45-A* exhibited the highest PIC value of (0.867) and (0.862) respectively. On the basis of SSR analysis, 51 genotypes were grouped into 3 clusters where Cluster I contained 19 genotypes, cluster II contained 21 genotypes, and cluster III contained 11 genotypes. The genotypes WB1306 and SB107 were found to be the most diverse with a dissimilarity index of 0.93 which also showed separate clustering for morphological diversity.

Keywords: Common bean, augmented, correlation coefficients, PIC value

Signature of student

Dated: _____

Signature of Major Advisor

Dated: _____

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Chapter 1

INTRODUCTION

The Common bean (*Phaseolus vulgaris* L.) also known as the French bean, Haricot bean, Snap bean, garden bean, navy bean, faras bean and Kidney bean with a 473Mb genome size is one of the most important leguminous vegetable crop, grown throughout the world for its green pods as well as dry beans (Kansal, 2016). It belongs to the family Fabaceae and is mainly a self-pollinating diploid ($2n= 2x= 22$) having its primary center of origin in southern Mexico and Central America with Peruvian, Ecuadorian and Bolivian areas as secondary centers (Vavilov, 1951). Beans are cultivated in several climates in the tropics, sub-tropics and temperate zone and in several regions, it is a staple food crop. However, despite worldwide distribution, it is being exclusively cultivated in many areas of America, Europe, Africa and Asia (Wortmann *et al.*, 2006).

The Common bean is a high-quality commercial vegetable crop grown in India's North Western Himalayan region. It is the premier legume crop in Jammu and Kashmir state known for its high quality and flavor and is widely grown in Rajouri, Poonch, Doda, Kishtwar, Bhaderwah, Budgam, Baramullah, Shopian, Kulgam, Qazigund, Ramban and other areas (Bhat *et al.*, 2017). As a vegetable crop, the French bean is grown in Kashmir over an area of 3.81 thousand hectares with an annual production of 55.25 thousand metric tonnes and productivity of 14.51 Mt^{-1} (Anonymous, 2018). Further its cultivation can be exploited to a great extent under the climatic conditions of Kashmir and during summer, it can be sent to distant parts of the country where it finds a ready market. As a result, it can be a reliable source of revenue for hill farmers.

Beans are a key source of proteins, with high content of lysine and methionine (green bean has 6.1% protein). Pods contain pectic substances such as calcium pectate 9-15 *per cent* on a dry basis. As the pods mature, the carotenes, thiamine and riboflavin decrease while ascorbic acid, copper, phosphorus and

protein increase. The green pods contain a substance that lowers the blood sugar level and is somewhat diuretic. It is employed to treat diabetes. It has been referred to as “poor man’s meat” by the World Food Organization as it can solve malnutrition and hunger-related problems for the poor of the world (Anonymous, 1992; Beebe *et al.*, 2000; Tryphone and Msolla, 2010) and thus are becoming more popular as a meat substitute among low-income families in developing countries (Iranga *et al.*, 1985).

The Common bean is thought to have been domesticated from two origins, one in Middle America and the other in South America, resulting in two distinct gene pools, Mesoamerican and Andean, which may be identified on morphological, physiological and molecular levels (including phaseolin seed protein, isozyme and DNA polymorphisms) (Gepts *et al.*, 1986; Koenig and Gepts 1989; Singh *et al.*, 1991a; Lynch *et al.*, 1992; Tohme *et al.*, 1996). The Mesoamerican gene pool extends from Mexico through Central America and in to Columbia and Venezuela, whereas the Andean gene pool is found in Southern Peru, Chile, Bolivia and Argentina (McClellan *et al.*, 2004). The Mesoamerican gene pool includes the races Durango, Jalisco and Mesoamerica whereas the Andean gene pool includes the races Chile, Nueva Granada and Peru, based on the agro-ecological and morphological facts confirmed by the molecular evidence. However, more recently, Bitochi *et al.* carried out a study examining the nucleotide diversity at 5 gene loci of a large sample representing the whole geographic spread of the wild forms of this species to settle the ongoing argument over the origins of the common bean. Their findings unequivocally prove that common beans originated in Mesoamerica and that a bottleneck occurred while the Andean gene pool was forming. There are noticeable phenotypic distinctions between Andean and Mesoamerican gene pools. Andean types feature larger leaves with ovate or lanceolate central leaflets and lanceolate or triangular bracteoles, longer internodes in the main stem, larger seeds, a stripless standard on the white flower and a central pod beak position. On the other hand,

Mesoamerican types have striped standards and colored flowers, a placental pod beak position and cordate or ovate bracteoles. The association between morphological traits and molecular markers is due to the autogamous reproductive system of the common bean and geographic isolation (Singh *et al.*, 1991b).

Characterization of the genetic diversity in the available germplasm provides essential information for its conservation, management of gene banks and utilization in genetic breeding program to fulfill the demand for future food security (Arunga *et al.*, 2015). Parents must belong to separate genetic clusters for the crossing program to be effective, hence it is essential to know the genetic diversity of the existing genotypes before carrying out any hybridization study (Gyang *et al.*, 2017). The use of morphological and agronomic traits to estimate genetic diversity is common, however, they are influenced by the environment and developmental stage and do not correctly reflect genetic relatedness between accessions. To overcome these problems, molecular markers represent a potential tool for effectively characterizing genetic diversity and aiding in managing plant resources (Blair *et al.*, 2006; Blair *et al.*, 2011; Grisi *et al.*, 2007; Laurentin H, 2009). When closely related to genes of interest, these DNA molecular markers can be utilized to select favorable allele/s in marker-assisted breeding programs (Okii *et al.*, 2014). Genetic diversity in common beans has been studied using various molecular markers such as allozymes (Singh *et al.*, 1991b; Santaella *et al.*, 2002); Amplified Fragment Length Polymorphism, AFLP (Lioi *et al.*, 2005; Svetleva *et al.*, 2006); Random Amplified Polymorphism, RAPD (Ocampo *et al.*, 2005; Martins *et al.*, 2006; Marrotti *et al.*, 2007); Restriction Fragment Length Polymorphism, RFLP (Nodari *et al.*, 1992); Inter Simple Sequence Repeats, ISSR (Svetleva *et al.*, 2006; Marrotti *et al.*, 2007) and Simple sequence Repeats, SSR (Okii *et al.*, 2014; Asfaw *et al.*, 2009). SSRs have various advantages for genetic fingerprinting over other DNA molecular markers, including being highly polymorphic and reproducible, the enormous extent of allelic diversity, frequently co-dominant, strong discriminatory power specific PCR-based assay and

randomly and widely distributed in the genome (Powell *et al.*, 1996; Pejic *et al.*, 1998). Moreover, these markers are more closely connected with the genes of known function (Ellis JR and Burke JM, 2007). Therefore, the present study will help us to determine the genetic diversity of common bean genotypes using SSR markers for use in the present and future breeding schemes and conservation programs. This data would help in understanding the genetic link between genotypes and provide basic information for parental selection of common bean breeding material.

Objectives of research:

1. Evaluation of common bean germplasm for different morphological traits and selection of a set of most promising diverse genotypes/vegetable type beans.
2. Molecular characterization of common bean germplasm using SSR markers.

Chapter 2

REVIEW OF LITERATURE

Common bean is an important legume crop grown worldwide for its fresh pods and dry seeds. Research has been carried out on various aspects of common beans. The available literature related to various aspects of the present investigation has been discussed below.

Legumes constitute the third largest family of higher plants with 20,000 species, second in agricultural importance based on area and total production (Graham and Vance, 2003). They are also important constituents in diets because they are good sources of protein (>20%), which helps to supplement cereal diets (Galvan *et al.*, 2001). Among pulses, the common bean (*Phaseolus vulgaris* L.) is the most widely distributed and consumed legume species of the genus *Phaseolus*, where the common bean (*P. Vulgaris*) accounts for more than 90 percent of the cultivated crop worldwide and is by far the most widely consumed grain legume in the world.

French bean is an important source of carbohydrates (61.4 %), proteins (20 - 28.5 %) and mineral matter (3.2 – 5.0 %). It has a significant amount of fiber and supplies minerals like iron, potassium, phosphorus, magnesium, copper, etc. Hence, it is very popular among people due to its nutritional quality and higher biological efficiency. It also possesses medicinal properties that are useful in controlling diabetes and certain cardiac problems and is a good natural cure for bladder burn. It has both carminative and reparative properties against constipation and diarrhea respectively (Duke, 1981)

2.1 Origin and Distribution

The common bean was domesticated ~8000 years ago in Central Mexico and South America. These were individual events and resulted in two major genetic pools, the Mesoamerican covering from northern Mexico to Colombia and the Andean, extending from Peru to Argentina (Gepts, 1998). The red beans from

both genetic pools were brought to the Iberian peninsula from America about 500 years ago and distributed to the rest of Europe, Africa and Asia shortly after (Angioi *et al.*, 2009; Santalla *et al.*, 2010).

There exist ten important gene pools in *Phaseolus* species, (Singh *et al.*, 1991) in the world, where six of them belong to the Central American and four to the South American centers. French bean is the most widely cultivated bean species in these areas and contributes to 90 percent of the total bean production. Out of a total of 70 wild *Phaseolus* species of American origin, only five namely, common bean (*P. vulgaris* L), yardlong bean (*P. polyanthus*), scarlet runner bean (*P. coccineus*), tepary bean (*P. acutifolius*) and lima bean (*P. lunatus*) have been domesticated.

The two major domestication events, Middle America (possibly west-central Mexico) and southern Andes (Andean) diverged into three races within each of the two major domesticated gene pools (Singh *et al.*, 1991). The Middle American gene consisting of races Durango, Jalisco and Mesoamerica is represented by the medium and small-seeded pinto, pink, black, white and some snap beans. The Andean gene pool, consisting of races Nueva Granada, Peru and Chile, is represented by the large-seeded kidney beans

The common bean is distributed in a wide geographic area that extends from around 52° north latitude to 32° south latitude. Bean crops can be seen in fields from sea level up to 3000 and above MSL. Beans have been adapted to high areas of the tropics and temperate zones, beans are also grown in the humid tropics, in the semi-arid tropics and even in cold climate regions.

French bean was introduced to India in the 17th century. It is also known by many names as kidney bean, common bean, field bean, garden bean, faras bean, bush bean, navy bean, haricot bean, pinto bean, string bean, marrow bean, snap bean, dry bean, etc. The large dry seed type varieties are called 'Rajma' in India. In India, it is mainly grown in Himachal Pradesh, Jammu and Kashmir,

Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu.

2.2 Taxonomy and Morphology

French bean belongs to the family *Fabaceae*. It is an annual herb with an erect and bushy growth (20-60 cm tall) or twining with stems of 2-3 m long with a tap root and nitrogenous nodules. Although the germinating bean has a tap root, adventitious roots emerge 1-2 days after germination, to dominate the tap root which remains 10-15 cm long (Duke, 1981).

French bean consists of bush and pole types. The bush type grows with erect leafy clumps reaching 1 m (3.25 ft) tall, while the pole type need support or trellising and grows up to 4 m (13 ft) long. Plants have trifoliolate compound leaves with oval to rhombic leaflets, each up to 16cm (9.25 in) long, which are pubescent. The flower color will be white, yellow, violet, or red in loose, open unbranched clusters (raceme) that are shorter than the leaves and develop into linear round to slightly flattened pods up to 15 cm.

2.2.1 Morphological diversity of Common bean genotype for pod and seed quality characters

In the preliminary characterization of the genotypes, morphological and agronomic traits of the plant are preferred for being cheaper and easier to assess. The morphological description provides information underlying conclusions on the genetic variability of the genotypes and identification of accessions maintained in duplicate (Valls, 2007). For any efficient plant improvement program, the existence of sufficient genetic variability in the population is a prerequisite and selection is perhaps the most important activity in all plant breeding programs which are made based on morphological characterization. Johannsen (1903) gave the concept of heritable and nonheritable variation, he demonstrated in beans that variation within the pure lines was environmental (non-heritable) while between the lines was heritable. Vavilov (1951) was the first

to realize that a wide range of variability in any crop provides better chances of selecting the desirable types.

Common bean is consumed as fresh pods as well as dry seeds. While dry bean market classes are based on seed characteristics and horticultural traits, fresh pod market classes are based on pod characteristics and plant type (Myers and Baggett, 1999). Beans comprise a group of common beans selected for succulent pods, with reduced fiber content.

Froussion (1970) observed a wide range of variability for plant height, days to flowering, pod length, seed shape and seed weight while studying 100 varieties of common beans. Similarly, while studying ten varieties of French beans, Seth *et al.* (1972) reported significant differences for days to flowering, primary branch number, average pod weight, pod number per plant and green pod yield per plant. A wide range of variations in several primary branches per plant, pod number per plant and green pod yield per plant were observed. A high genetic coefficient of variation was shown for primary branch number, average pod length and green-pod yield per plant.

Pande *et al.* (1975) observed a wide range of variability for plant weight, primary and secondary branches and pods per plant of French bean. Heritability estimates were high for plant height, primary and secondary branches and pod weight. Plant weight, days to flower, primary branches, pod length and pods per plant accounted for about 74 percent of the variability in the yield.

Guzhov *et al.* (1981) studied variability for 18 quantitative characters in French bean and reported that the number of nodes on the main stem, plant height, seeds per pod and 100-seed weight was the least variable. Similarly, Ustimenko *et al.* (1981) studied variability for twelve characters in seven *Phaseolus vulgaris* varieties and reported considerable phenotypic and genotypic variation for most characters. In general, phenotypic variation was greater than phenotypic variation

except for plant height which showed the highest phenotypic variation but a relatively low phenotypic variation.

Joshi and Mehra (1984) studied 42 geographically diverse genotypes of French bean and reported a wide range of variability for plant height, number of pods per plant, days to flowering, days to maturity and yield per plant in French bean. Similarly, Shah *et al.* (1986) in a study of 25 varieties of French beans reported variability in plant height, number of pods per plant, pod length, number of primary branches and yield per plant.

Saha *et al.* (1990) reported wide variability in relation to the general mean and range of the characters for pod yield per plant. The phenotypic and genotypic coefficients of variation were high for seeds per pod, pod yield per plant and pods per plant. While Narsinghani and Saxena (1991) reported the highest genotypic coefficient of variation for plant height, followed by 100 seed weight but lowest for days to maturity and days to 50 percent flowering. Similarly, Mishra and Dash (1991) evaluated eleven genotypes of French beans and reported greater variability for plant height, yield and pod length than for days to maturity and pod girth.

Patil *et al.* (1993) studied the phenotypic and genotypic variabilities of nine characters in 18 varieties. A wide range of variation was observed for the characters such as days to flowering, plant height, number of pods per plant, 100 seed weight and yield per plant, it was low for seeds per pod. The genotypic coefficient of variability was high for 100 seed weight and plant height while it was low for days to flowering, days to maturity and protein percent.

Vasic *et al.* (1997) examined plant height, first pod length, number of pods per plant and yield per plant in 14 Yugoslav genotypes of French beans. Similarly, Korla *et al.* (1997) reported high heritability and genetic advance for 50 percent flowering while studying twenty-one bush bean genotypes. They also reported a

wide range of variation for number of pods per plant, pod yield per plant and number of seeds per plant.

Jasmine and Azhakiamaavalan (1998) evaluated 24 French bean cultivars of diverse origins and observed a wide range of variations in plant height, growth period, pods per plant and seed yield. Similarly, Deepak et al. (1999) evaluated thirty exotic as well as indigenous accessions of French bean which exhibited a wide range of phenotypic variation among the number of pods per plant, pod length, plant height and pod yield per plant. Arya *et al.* (1999) also studied twenty-five indigenous and exotic genotypes of French bean and reported that the highest variances were observed for green pod yield per plant followed by pods per plant, 100-seed weight, seed vigor and plant height.

Azizi *et al.* (2001) experimented to investigate the genetic diversity of morphological traits in 121 genotypes of Lima, red and pinto beans and to determine the factors affecting the interaction between characters by multivariate analysis. High variability was observed among genotypes for all the characters studied. The phenotypic coefficient of variability was greater than the genotypic ones for all traits. Days to maturity exhibited the lowest variability.

Amini *et al.* (2002) cited the highest variability among seed number per plant, seed yield, pod weight, number of filled pods, biological yield, plant height and number of pods on the main stem. Similarly, Singh *et al.* (2002) observed significant variability in seed protein content in 88 common bean germplasm which can be used as a genetic resource for the improvement of seed protein content in common beans.

Kapila *et al.* (2002) evaluated twenty-nine genotypes of beans and observed wide variability for eight quantitative traits *viz.*, plant height, pods per plant, pod length, seeds per pod, seed yield per plant, biological yield per plant, 100 seed weight and days to maturity. Similarly, Rai *et al.* (2004) studied twenty-seven genotypes of French bean and reported a high range of variability for all the

characters. Pod yield per plant, number of pods per plant, pod weight and seed weight showed high estimates of the genotypic coefficient of variation.

Rai *et al.* (2006) evaluated fifty-two genotypes of French bean and observed a wide range of phenotypic variations for pod yield per plant, number of pods per plant, seed weight and pod weight. Similarly, Dursun (2007) evaluated twenty-one genotypes of French bean and observed significant variability in yield, leaf area, pod length, pod width, pod number per plant, fresh pod weight, seed number per pod and 1000-seed weight.

Sabokdast and Khyalparast (2008) studied thirty common bean cultivars and observed significant variability in varieties in terms of the number of seeds per pod, pod weight, number of pods per plant, seed number per plant, 100 seed weight, pod length, biological yield, days to flowering and maturity.

Corte *et al.* (2010) investigated the genetic control of seed morphological traits and their correlations with grain yield in common beans. Plant grain yield and seed morphological traits were found to correlate. Results showed that the seed morphological traits were controlled by a complex of genes. High and negative correlations among seed length and thickness with grain yield suggested greater grain yield in bean plants with smaller seeds.

The study of the associations between morpho-agronomic traits of common bean (*Phaseolus vulgaris L.*) was carried out in twenty cultivars of common bean and data on seed yield and twenty-one morpho-agronomic characters were recorded. The correlation analysis demonstrated that seed yield is highly correlated with harvest index, seed number per plant, seed number per pod, pod number per plant, seed length, strawweight, 50 percent flowering, 50 percent pod set, pod length and 100 seed weight. Path analysis indicated that among primary traits, seed number per plant and harvest index had a high positive direct effect on the seed yield. Cluster analysis separated the characters into four distinct groups. Hence, the study results proved that seed number per plant, pod number

per plant, harvest index, seed number per pod, pod weight and pod length were the variables most closely related to seed yield (Sadeghi *et al.*, 2011).

Szilagyi *et al.* (2011) experimented with the evaluation of genetic diversity in twenty common bean (*Phaseolus vulgaris L.*) morpho-agronomic traits. For the morphological study, observations were made on several characteristics viz., seed size, 100 seed weight, seed color, number of pods per plant, number of seeds per pod, number of seeds per plant and seed yield per plant. The cluster analysis based on morphological traits separated genotypes into 2 main groups according to seed size (100 seeds weight), type of growth habit, maturity and seed yield per plant. The first large group with small to medium-sized seeds (100 seeds weight - 18.0 to 37.0 g) and the second group with large seeds (100 seeds weight; 40.0 to 46.0 g), where the accessions with higher 100 seed weight can be used in the breeding program.

Pandey *et al.* (2013) evaluated Twenty genotypes including a few released cultivars of French bean (*Phaseolus vulgaris L.*) to determine genetic variability, heritability, genetic advance as per cent of mean, correlations among yield and economically important traits and direct and indirect effects of these traits on overall yield. A high PCV (34.20) and GCV (34.03) were recorded for several pods per plant followed by green pod yield, pod weight and pedicel length. A significant positive correlation between green pod yield and economic traits like the number of pods per plant, pod length, individual pod weight and the number of seeds per pod were estimated. This indicates that improvement in these traits could lead to an increase in pod yield. The traits like plant height, number of pods per plant, pod length and individual pod weight were important dependable yield contributing traits and need while formulating selection strategies for the genetic improvement of French bean.

Stoilova *et al.* (2013) conducted a study on the morphological variability among the fifteen Portuguese and fifteen Bulgarian landraces. These were evaluated for 16 morphological characters. A considerable morphological

variation was found among genotypes. The majority of landraces had white seeds in color but some had also cream, purple and white with red colors around the hilum. The predominant seed shape was long. The components that mainly determined yield were the number of pods and the number of seeds per plant. The highest value for these traits was found in one Portuguese accession (No. PH2) and one Bulgarian accession (No 99E0128).

Boros *et al.* (2014) carried out a study to analyze the morphological, phenological and agronomical variability among common bean accessions from the NBPGR, A considerable genotypic variation for the number of seeds per plant, number of pods per plant and weight of seeds per plant were found. Bean accessions differed significantly in terms of thousand seeds weight (TSW). The cluster analysis allowed the identification of five groups of bean accessions. The accessions in cluster 1 and cluster 2 were of the highest usefulness for breeding purposes as there observed a considerable variance in number of seeds per pod and the number of seeds per plant.

Gangadhar *et al.* (2014) experimented on diversity in morphological characters in sixty-six French bean accessions and different morphological characters were studied. Among the characters studied, days to fifty per cent flowering (43.86%) contributed the maximum to the total genetic diversity among the genotypes followed by hundred seed weight (22.33%), green pod yield (11.79%), pod weight (11.04%) and pod length (6.71%). Through cluster analysis, two clusters were made, where cluster 1 included the 48 accessions which differed in 100 seed weight. The genotypes belonging to cluster I had maximum inter-cluster distance and were found to be genetically more divergent and these genotypes could be used in the hybridization program.

Gangappa *et al.* (2014) carried out a study on diversity among the sixty-six common bean accessions The Genetic diversity among the genotypes was worked out by using Mahalanobis D2 statistics. Based on genetic distance, these genotypes were broadly grouped into two clusters. Cluster II was the largest,

consisting of 48 genotypes, while cluster I contained 18 genotypes. Among the different characters studied, hundred seed weight (22.33%), green pod yield (11.79%), pod weight (11.04%) and pod length (6.71%) exhibited maximum intra-cluster distance (122.419) in cluster 1. The genotypes belonging to the cluster with maximum inter-cluster distance were genetically more divergent and these genotypes could be used in the hybridization program.

Kumar *et al.* (2014) conducted work on the genetic diversity of French bean in 29 genotypes collected from different parts of India especially the vegetable type (green pod). The morphological characters studied were, the number of pods per plant, pod length, pod width, pod thickness, seeds per pod, seed length, seed width, seed thickness and hundred seed weight and the data was recorded. The high CV was observed for pods per plant, per cent pod set per cluster, pod length and 100 seeds weight. The line IVFB-1 exhibited the maximum number of pods per plant, while, the maximum per cent pod set per cluster was recorded in genotypes DWDFB-1. The pod length and width were the highest in Arka Anoop. The lines VRFBB-2 and RCMFB-18 showed maximum pod thickness. The line RCMFB-66 exhibited the maximum seed length, seed width and seed thickness.

Okii *et al.* (2014) studied the genetic diversity of common bean in Uganda, using the available collection consisting of 284 bean accessions. The Shannon Weaver index (H), which measures morphological variance, ranged from 0.47 to 0.58, with an overall mean of 0.56 ± 0.19 , indicating moderate genetic diversity. Using principal component analysis (PCA), the germplasm was clustered into three major groups (G1, G2, G3). Growth habits, pod cross-section, pod curvature, hypocotyl color, days until flowering, node number on the main stem, number of flower buds and 100 seeds were the primary genetic differences across the genotypes.

Scarano *et al.* (2014) conducted a study among the 25 common bean landraces for the selected twenty-six characters. All the landraces could be

distinguished by at least one character. Taking into account the polymorphic characters, forty-one different IPGRI morphological phenotypes (out of a total of 65) were present. Considering the number of IPGRI phenotypic classes that were covered, a trait that displayed remarkable variability was the dry pod color. Large differences were also observed for the dry seed color and shape and the most frequent bean color was white.

Singh *et al.* (2014) conducted a study on eighteen French bean varieties for twenty-one morphological traits, especially for the seeds. The varieties *viz.*, Pusa Himalaya showed circular to elliptic, Kentucky Wonder and Swarna Lata showed elliptic and rest 15 varieties showed kidney-shaped seeds. However, none of the varieties had a round seed shape. The size of the seed varied from small to very large seeds. The results suggested the characterization of these varieties for breeding French bean varieties for particular traits.

Sofi *et al.* (2014) analyzed Fifty diverse germplasm accessions (local and exotic) representing different growth habits for 12 seed morphological and physical characteristics namely seed color, seed brilliance, seed shape, seed coat pattern, dry seed weight, soaked seed weight, seed length, seed breadth, seed coat proportion. The CV was highest for dry seed weight (3.056) and coat proportion (1.221). However, CV was very low for seed length and seed breadth owing to low variation in these traits. The accessions with high dry seed weight can be used for future breeding purposes.

Sultan *et al.* (2014) conducted a study on a total of 80 diverse germplasm accessions of common bean (*Phaseolus vulgaris L.*) for the seed quality characters. The variability was observed in seed color (red color was predominant), shape (cuboid, kidney and oval), size (small, medium and large) and 100 seed weight. There were significant variations in seed length, width and 100 seed weight being highly significant in the latter case. A highly positive correlation was observed between 100 seed weight, seed length and width. Grid maps generated through DIVA-GIS software indicated diverse accessions of

common beans in terms of seed size and weight. Conservation of this remarkable genetic diversity was recommended for future propagation in breeding.

Das (2015) conducted an experimental study to evaluate the performance of fourteen different bush-type French bean genotypes for seed yield. From the observations recorded and the analysis made, it's been revealed that the genotype with relatively bolder seeds with more number of seeds per pod and higher bearing capacity per plant generally gives higher seed yield. Among the genotypes under study, Arka Suvidha was the best one as it produced the highest seed yield (2180.92 kg/ha) and relatively good plant vigor and a fairly high seed vigor index (2944.38).

Arunga *et al.* (2015) conducted an experiment on the genetic diversity analysis of 36 French bean accessions based on 20 morpho-agronomic characters in Kenya. The accessions varied significantly ($P \leq 0.05$) with respect to pod and seed characters. A significant ($P \leq 0.05$) interaction between accession and season for pod characters was observed. The cluster analysis based on seed color and number of pods per plant split the clusters into four groups. The first cluster was dominated by low pod diameter ($< 6.5\text{mm}$) and the second cluster was a mixture of large and small pod diameter accessions.

Morojele and Mbewe (2015) worked on diversity analysis in forty-two French bean accessions, distinguished using 17 morphological characters. Ten principal components were generated from 17 characters, of which the first three components constituted 54.57 percent of the total variation and were considered for analysis. The characters in the first principal component were plant height, growth habit and seed pattern. The characters in the second principal component include number of flowers per node, the number of locules per pod, seed color and leaflet length. Along with the third principal component, the pod colors and flower colors were used for the cultivar separation. In the cluster analysis, three groups were made.

Prakash *et al.* (2015) studied Genotypic variability and heritability in 25 genotypes of French bean for various quantitative and quality traits. The material showed a wide range of variability for green pod yield per plot (91.50-248.09 q/ha), green pod yield per plant (54.91-148.87 g), plant height (34.47-128.21 cm), green pod width, single green pod weight. The genotypic, phenotypic variances and heritability were high for 100seed weight (43.11-43.22 g). The characters like 100 seed weight, plant height, green pod yield per plot, green pod yield per plant and green pod yield (q/ha) showed high genotypic and phenotypic co-efficient of variation with genetic advance, which indicates that selections based on phenotypic performance could be effective for improvement of these characters.

Phenotypic diversity among Croatian common bean (*Phaseolus vulgaris L.*) landraces was assessed by analyzing twelve qualitative and six quantitative traits in 338 accessions collected from all production areas in Croatia. Out of 12 qualitative morphological traits, pod position, seed coat color, seed pattern, seed brilliance and seed shape (longitudinal) were the most polymorphic among accessions having the highest Shannon's information index and discriminating power (Vidak *et al.*, 2015).

Haralayya *et al.* (2017) conducted the genetic diversity analysis in 36 genotypes for thirteen yield and yield-attributing traits and grouped them into six clusters through Mahalanobis' D2 analysis. Cluster I was the biggest with 19 genotypes followed by cluster VI, cluster II and III. Among the traits studied, green pod yield contributed the maximum to the diversity (40.17 %). The genotype such as IIHR-103 recorded the highest green pod yield of 165 g per plant, while it recorded an average number of pods of 23.85. It is desirable to select genotypes from clusters showing high inter-cluster distance (clusters V and VI) for the further crop improvement program.

Loko *et al.* (2018) conducted a study to determine the usefulness of the central region's bean germplasm for breeding programs, 57 accessions were gathered from 23 villages. Following a classification based on the morphological

characteristics of the seeds, these accessions were assessed in an experimental field at the Faculty of Sciences and Technology of Dassa using 30 morphological attributes (18 qualitative and 12 quantitative). These accessions were divided into 8 morphotypes based on the variation in seed morphology. However, 9 morphological types were obtained with cluster analysis based on the UPGMA classification method using qualitative variables, whereas in Principal Component Analysis (PCA) they were gathered into 4 clusters using quantitative variables. The accessions in cluster 1 (11 accessions) were identified as possessing the highest values in quantitative traits like days to maturity and number of pods per plant. While accessions in cluster 2 (3 accessions) had the lowest number of days to flowering and the highest pod length, the accessions in cluster 3 (23 accessions) presented the highest 100- seed weight. The correlation coefficient of 100-seed weight was positive and significant ($p \leq 0.001$) with leaf length, pod length, pod width and seeds length while it was negatively correlated ($p \leq 0.05$) with days to flowering, pod length and the number of seeds per pod.

Ekbic and Hasancaoglu (2019) conducted a study for morphological and molecular characterization of 33 common bean genotypes collected from Ordu province Turkey. The genetic relationship among the local common bean genotypes was also identified. The 3 phenological and 22 morphological characteristics were investigated. The first flower and the first beans were formed 43.42 and 48.55 days after sowing time respectively.

Jan *et al.* (2021) carried out a study to characterize 109 common bean genotypes collected from different hotspots for morphological traits viz., plant (growth habit, growth type and twining habit); leaf (color, size and shape); flower (color, stripping on outer petals); pod (shape in relation to suture, the shape of the cross-section, shape of distal part and stringness), seed color and shape) traits and quantitative morphological traits (seed weight, length and breadth). The preliminary analysis of traits data showed wide variation for different morphological traits. Genotypes of Mesoamerican origin carrying “S” type

phaseolin was smaller in size than the genotypes of Andean origin carrying “T” phaseolin which tend to have larger seed.

2.2.2 Heritability and genetic advance

In any crop improvement operation, heritability and genetic advance estimations aid in selecting the best breeding methods. Heritability and genetic advance are highly dependent on genetic variation, therefore the higher the heritable variation, the greater the chance that the traits may be fixed via the selection process. To determine whether the observed variance for a specific trait is completely genetically determined or is impacted by environmental factors, heritability studies are therefore crucial. Since heritability in a broad sense may not give precise estimates of gene action, it is desirable to work out along with genetic advance as suggested by Ramanujam and Trimula Char (1968).

Lal and Padda (1972) observed a high value of heritability and genetic advance for pod length and seed yield per plant. In terms of pod output per plant, moderate heritability and strong genetic advance were found. The heritability of pods per plant was the lowest, but the genetic advance was moderate. While Agarwal and Singh (1973) noted high heritability estimates and anticipated genetic advance for plant pod number and seed weight. Sharma et al. (1977) noted high and low heritability estimates for internodal length and days to first picking in French bean genotypes.

Seth *et al.* (1972) observed a high genetic coefficient of variation for primary branch number, average pod length and green pod yield per plant among 10 varieties. All characters except average pod yield showed high heritability, while as for primary branch number, average pod weight and green pod yield per plant high expected genetic advance for found.

Joshi and Mehra (1984) studied forty-two geographically diverse genotypes of French bean (*Phaseolus vulgaris L.*) and reported that the heritability

estimates and expected genetic advance were high for 100-seed weight, plant height, number of pods per plant, pod length and yield per plant.

Mishra and Dash (1991) studied 11 genotypes of *Phaseolus vulgaris* and reported very high heritability for yield and pod girth and also high genetic advance was reported by these two characters. Tripathy *et al.* (1994) also reported a high value for heritability and genetic advance for yield per plant, pod length and pods per plant on 5 characters in seven genotypes of French bean.

Singh *et al.* (1994) demonstrated strong heritability and high genetic progress for yield per plant, pod length and number of pods per plant while working with five characters in seven genotypes of French bean. Similar to this, Nandi *et al.* (1996) observed moderately high genetic advance and heritability for green pod yield per plant and number of pods per plant when working with 26 genotypes of French bean.

According to Lenka *et al.* (1998) in a field experiment with advanced brown-seeded pole French bean mutants, pod weight had the highest heritability, whereas pods per plant and green pod yield per plant exhibited fairly high heritability and genetic advance. Paredes *et al.* (1998) assessed the heritability and genetic gain for three quantitative traits. According to the findings, heritable features include seed output, 100 seed weight and days to maturity.

In forty-eight genotypes of French bean, Dahiya *et al.* (2000) found high genetic advance together with high heritability for traits including days to first flowering, days to 50% flowering, days to pod initiation, plant height and primary and secondary branches per plant. Similar to this, Rafi and Nath (2004) evaluated 31 French bean genotypes and found high heritability and genetic advancement for all yield-contributing variables, including plant height, number of pods per plant, pod length, number of seeds per plant and 20 seed weight.

Rai *et al.* (2004) evaluated twenty-seven genotypes of French bean and observed high heritability and genetic advance for the number of pods per plant, pod weight, seed weight and pod yield per plant.

Rai *et al.* (2006) evaluated fifty-two genotypes of French bean and observed a wide range of genotypic variation along with high heritability and high genetic advance for pod yield per plant, number of pods per plant, seed weight and pod weight. Rai *et al.* (2007) evaluated fifty-two genotypes of French bean and observed a wide range of genotypic variation along with high heritability and high genetic advance for pod yield per plant, number of pods per plant, seed weight and pod weight.

Santhi *et al.* (2015) evaluated 10 bush-type accessions of French bean from Nilgiris for their performance at different elevations for yield and quality traits. Observations on yield and pod traits were recorded. The highest GCV was observed for plant height, number of branches, number of leaves, pod length, pod weight and yield per plant. The highest PCV was observed for plant height, number of branches, number of leaves, days for flowering, pod number, pod length, pod diameter, average weight and average pod yield. The highest PCV and GCV were observed in plant height, number of branches, number of leaves, pod length, pod weight and yield per plant. Hence, these traits can be used in selection.

Jhanavi *et al.* (2016) investigated variability and genetic diversity studies in 36 French bean (*Phaseolus vulgaris L.*) genotypes for 27 morphological characters. Moderate genotypic and phenotypic co-efficient of variations were observed for pod length, pod flesh thickness, number of pods per cluster, number of pods per plant, the weight of ten pods and dry matter content of pods and roots. The high heritability along with high genetic advance was recorded for the plant height, pod width, number of pods per plant, average pod weight and yield per plant. Correlation studies revealed that total yield per plant was positively and significantly ($p=0.01$) associated with all the characters except days to first flowering, days to 50 per cent flowering, days to first pod picking and pod width.

Path analysis studies revealed direct effects on number of clusters per plant, the number of pods per cluster weight of ten pods and the total yield of pods per plant. Mahalanobis D2 analysis grouped 36 genotypes into five clusters. Hybridization studies can be planned by involving genotypes belonging to cluster II and cluster V as they had high inter-cluster analysis. The high-yielding genotypes such as IIHR-62, Arka Arjun, IIHR-53, IIHR-232 and Arka Anoop had desirable quality characters.

2.2.3 Correlation Coefficient analysis

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines component characters on which selection can be based for improvement in yield. The basic concept of correlation was first developed by Galton (1889) and later it was elaborated by Fisher (1918, 1936) and Wright (1921) in the improvement of plant breeding programs. Thus, the efficiency of selection can be improved by estimating the relative degree of association between different pairs of characters based on significant correlation coefficient values between yield and other economic trait. This association includes both genotypic and environmental influences, genotypic correlation coefficient provides a real association between two characters and is highly useful in selection (Johnson *et al.*, 1995).

Lal and Padda (1972) observed a positive correlation of fruit set with pod length, number of pods per plant, seed yield per plant and protein. Pods per plant showed a positive and highly significant correlation with pod yield per plant and seed yield per plant. Correlation between pods per plant with pod length and protein content of green pods were positive and non-significant. A positive but non – significant correlation was observed between pod length and pods per plant. Pod length had a positive and significant correlation with pod yield per plant.

Agarwal and Singh (1973) reported a significant correlation of days to flowering, days to maturity, pods per plant, seeds per pod and 100-seed weight

with seed yield per plant. They further found a positive correlation of days to flowering with days to maturity and pods per plant with seeds per pod. A negative association was found between number of pods and 100-seed weight and between pod length and pod yield.

Shetter *et al.* (1975) observed a positive and significant correlation between pod yield and the number of pods per plant. While as Pande *et al.* (1975) observed that days to maturity were not associated with any yield character suggesting that simultaneous improvement of yield and this character could be obtained through selection. Natrajan and Arumugam (1981) also evaluated 20 genotypes for pod yield and 7 yield components. The plant height, number of branches per plant, number of pods per plant, pod length, number of seeds per pod and weight of seeds per pod showed a highly significant positive correlation with green pod yield.

Singh and Saini (1985) reported that yield was positively and significantly correlated with days to flowering, maturity, height, branch number and pod cluster. Similarly, Shah *et al.* (1986) reported a significant positive correlation between pods per plant and pod yield per plant, plant height, number of primary branches and pod width and also between pod length and pod width, plant height and number of primary branches. The pod yield per plant was positively correlated with the number of primary branches, pod length and pods per plant at the phenotypic and genotypic levels.

Santos *et al.* (1986) studied the correlation in *Phaseolus vulgaris* and observed that the number of pods per plant was closely correlated with yield per plant. Similarly, Saha *et al.* (1990) collected and evaluated nine elite varieties of French bean and found that the pod yield per plant was positively and significantly correlated with plant height, pod number per plant and weight, days to flowering were significantly and positively correlated with plant height and pods per plant.

Narsinghani and Saxena (1991) evaluated a diverse collection of French bean and reported that days to maturity was positively and significantly associated with plant height and pods per plant, but observed a significant negative association with pod length and 100 seed weight. Singh (1993) also recorded a positive and significant correlation of green pod yield per plant with plant height, seed yield and seeds per pod and a negative correlation with 100 seed weight. Similarly, Mishra *et al.* (1996) studied 22 genotypes of French bean and reported that pod yield per plant had a significant and positive correlation with number of pods per plant, pod weight, pod length and seeds per pod.

Thakur (1997) studied 54 *Phaseolus vulgaris* cultivars and reported that days to 50 per cent flowering, days to first picking, pods per cluster, pods per plant, pod girth, plant height and harvest index were positively correlated with pod yield. Similarly, Chand and Chand (1998) studied the relative degrees of association among different quantitative characters of French bean. The correlation study indicated that phenotypically yield was positively and significantly correlated with pods per plant, seeds per pod and 100 seed weight but at the genotypic level, the association was highest for pods per plant.

Arya *et al.* (1999) conducted a study on 25 indigenous and exotic genotypes of French bean and reported a positive and significant correlation of green pod yield with pods per plant, branches per plant, plant height, seed vigor and 100-seed weight. There showed a strong positive association of plant height and branches with pod number per plant.

Agsakall and Olgun (2001) reported that the coefficient of correlation between days to flowering and yield, pod number and yield, 100 seed weight and yield were found to be highly significant. Rai *et al.* (2004) also studied the correlation coefficient and reported that the pod yield per plant was positively and significantly correlated with pod weight and pod length.

Shukla *et al.* (2006) conducted a study on French bean and reported that a positive association was recorded between seed yield and the number of seeds per pod, the number of pods per plant, pod length and the number of days to maturity, whereas the negative association was recorded between seed yield and plant height. Also, the correlation analysis showed that the number of days to maturity, number of seeds per pod, pod length and number of pods per plant have a positive contribution to crop yield. Similarly, Lopez and Ligarreto (2006) also studied correlations among yield and its components and found that the number of pods per plant was the most important characteristic in the yield determination of common bean.

Dursun (2007) conducted a study on 21 French bean genotypes and reported that the correlations between pod number per plant and yield, fresh pod weight and yield and seed number per pod and yield were found to be significant and positive.

Sabokdast and Khyalparast (2008) while working on 30 common bean cultivars observed that the grain yield had a positive and significant genotypic correlation with the number of seeds per pod, pod weight, number of pods per plant, biological yield, days to flowering and maturity.

Vidyakar *et al.* (2017) investigated the common bean diversity for morphological characters, especially for seeds with twenty French bean genotypes, where the observations on 13 quality characters were taken. The characters like plant height, a number of seeds per plant, harvest index and seed yield per plant exhibited high Genotypic co-efficient of variance (GCV) and phenotypic co-efficient of variance (PCV), heritability and genetic advance. The cluster analysis made five clusters. The genetically more divergent genotypes for seed quality characters present in clusters III and V may be utilized as parents in future hybridization programs to produce high yielding in terms of number of seeds per plant. The grain yield exhibited a positive and significant correlation with biological yield per plant, harvest index and test weight.

2.3 Molecular markers in French bean

Leguminous pulses are mostly harvested for their dry seeds, so understanding genetic variety is crucial when choosing parents for a crop development program. To properly use germplasm in breeding programs, it is crucial to characterize it using molecular marker techniques. These techniques provide quantitative estimates of genetic diversity. Traditional plant breeding goes through several stages, from the cross-pollination of distinct species to the hybridization of diverse species to get around the challenges provided by merging species from different families.

However, genetic advancement in pulse crops is slow because of several limitations in conventional breeding techniques. To increase the effectiveness of conventional breeding, researchers from around the world are now using molecular markers in all of the major crops. Chickpeas, pigeon peas and certain other pulses have seen an acceleration in the development of molecular markers and other genomic sources. Marker-assisted selection has been used in pulse breeding programs, but progress has been extremely slow and has only been applied to a small number of pulse crops, like chickpeas and common beans.

Despite ongoing breeding efforts using traditional techniques, significant genetic improvement in pulse yield and productivity could not be achieved. High genotype and environment interactions on the expression of critical quantitative traits, which result in a gradual increase in genetic improvement for yield, are the main barrier to pulse breeding for quantitative traits. Utilizing molecular markers in traditional breeding for agricultural development is one method for the selection of suitable genotypes.

A variety of methods, including isozyme (Singh *et al.*, 1991b), allozyme (Lioi *et al.*, 2005; Svetleva *et al.*, 2006), morphological (Singh *et al.*, 1991a) and based on phaseolin, a protein found in beans, have been used to study the genetic diversity in beans (Gepts, 1998) to accurately calculate the diversity among the

many bean accessions. It is found that DNA markers are preferable to morphological features since they can distinguish between accessions even when they have similar morphologies and can be used to identify more polymorphism loci than isozymes and the seed protein phaseolin (Beebe *et al.*, 2001). The optimal molecular marker for analyzing common bean diversity should be highly polymorphic, evenly dispersed throughout the genome and offer co-dominant, accurate and reproducible data which can be generated in high throughput and cost-effective manner. Different DNA markers, including RFLP (Nodari *et al.*, 1992), RAPD (Ocampo *et al.*, 2005), AFLP (Lioi *et al.*, 2005) and SSRs (Kandemir *et al.*, 2010), have been employed in the genetic diversity analysis of beans. Each marker has advantages and disadvantages of its own.

2.3.1 Different markers studies in French bean

2.3.1.1 RFLP in French bean

In a study conducted by Viviana *et al.* (1994), where eighty-five accessions of common bean, were examined for restriction fragment length polymorphism (RFLPs), Twelve probes were employed for the hybridization after genomic DNA underwent restriction digestion using EcoRI. Within each gene pool, accessions were divided into two main groups and the level of genetic diversity ($HI = 0.38$), which was discovered to be twice as high, was determined by analyzing isozymes as a marker. When compared to isozymes, genetic diversity within races ($H_r = 0.27$) was five times greater.

Furthermore, because they cover a larger portion of the genome and exhibit significant levels of polymorphism, RFLP markers were discovered to be superior to isozymes. The first molecular linkage map of the common bean was created using RFLP (Gepts *et al.*, 1993). The drawback of RFLP is that it is time-consuming, expensive and labor-intensive.

2.3.1.2 RAPD in French bean

The advantage of Random Amplified Polymorphic DNA (RAPD) markers as a rapid, cost-effective tool for the indirect selection of economic traits was immediately recognized (Kelly and Miklas, 1998). The RAPDs have been deployed extensively in germplasm identification and characterization. RAPD markers overcome the limited number and undesirable pleiotropic effects of many morphological markers as well as, the limited number and difficulties associated with the tissue and development-dependent isozyme markers.

Sroch *et al.* (1998) constructed a core collection for the *Phaseolus vulgaris* germplasm collection at CIAT (Centro Internationale de Agricultura Tropical, Cali, Colombia) to enable more thorough examination and utilization of the more than 24000 accessions held in the collection. Based on 224 RAPDs, a comparison between a random sample of 90 Mexican *P. vulgaris* accessions from the core collection and a random sample of 90 Mexican accessions from the reserve collection was made (random amplified polymorphic DNAs). All of the abovementioned accessions' growth habits, primary seed coat colors, seed weights and collection site altitudes were documented for the marker-derived genetic distance matrices. Based on marker data, no discernible variations between the core and reserve samples were discovered. There were also no discernible changes between the core and reserve samples when it came to the primary seed coat color, growth pattern, or the state of Mexico. The means and variances for seed weight and site altitude did not differ much either. Thus, the enrichment of diversity was not identified in the core sample. The outcomes, however, suggested that the core's genetic makeup would reflect the CIAT's reserve collection of common bean.

Duarte *et al.* (1999) studied the genetic divergence of 27 cultivars of the common bean (*Phaseolus vulgaris* L.) using RAPD markers. It was also evaluated whether this divergence was related to various indirect variability estimates found in the field experiments. The Sorensen Dice similarity coefficient was used to

calculate genetic distances, which were then shown in a dendrogram using the UPGMA method and projected into two-dimensional centers. Within these centers, genetic diversity was also estimated using RAPD markers. Based on morpho-agronomical features, it was also possible to assess the link between estimates of genetic distances derived inside RAPD markers and estimates of Mahalanobis' generalized distances, specific combining ability and mid-parent heterosis. RAPD markers worked well for classifying cultivars into domestication centers. The grouping obtained and the proposed classification of races, however, only had a strong agreement for the Middle American domestication center. These markers produced genetic divergence that was similar to that offered by morpho agronomical characteristics. However, the specific combining ability and mid-parent heterosis expressed in crosses were not well predicted by RAPD markers.

Beebe *et al.* (2000) studied a collection of 269 landraces of common bean (*Phaseolus vulgaris L.*) using correspondence analysis of random amplified polymorphic DNA (RAPD) data to ascertain the genetic makeup of the Middle American gene pool for the cultivated bean. One hundred eighty landraces originated in Mexico, the remainder in Central America and secondary centers of diversity within the Americas and two checks were studied. One hundred eighty landraces originated in Mexico, the remainder in Central America and secondary centers of diversity within the Americas and two checks were studied. Groups were created that, to some extent, matched the races that had previously been identified by morphological and agroecological criteria. However, tropical small-seeded Race M was divided into two groups: one largely Mexican one, which had the majority of small-seeded black beans with an upright plant habit; and a Central American one, which contained landraces with a variety of seed colors. Except for the cream- and purple-seeded accessions from Brazil, the majority of non-black small-seeded germplasm of the Race M phenotype from secondary centers was grouped with the Central American landraces. Races D and J could be distinguished and within races D and J, further distinctions relating to geographic

origin could be found. A genetic group made up primarily of the more commercial Race D landraces predominated in the western portion of the Mexican highland plateau. Another Race D group, which was also distinct morphologically, was centered near the eastern end of the neovolcanic axis. Climbing bean accessions found in Guatemalan germplasm that did not associate with any of the previously recognized races were treated as belonging to a separate race. Thus, it was discovered that the germplasm of the common bean in Middle America was more diverse than previously believed and more complex than had been assumed.

Duran *et al.* (2005) studied the genetic relationship among Caribbean landraces. The 54 red-mottled or large-seeded bean landraces and cultivars from the Caribbean (16 from the Dominican Republic, 14 from Haiti, 1 from Jamaica and 23 from Puerto Rico) were compared with 11 Andean bean lines from other regions for their morphological traits, phenological traits, phaseolin status and RAPD banding patterns. By using the entire clustering method, distances were calculated and dendrograms for morphological, phenological, and/or molecular traits were created to assess the evolutionary relationships among the lines. morphologically, the landraces were divided into two clusters: one with Mesoamerican traits, which included all of the red-mottled lines from Haiti and three landraces from the Dominican Republic collected close to the Haitian border and the other with Andean traits, which included all of the lines from Puerto Rico and the remaining lines from the Dominican Republic. Three groups were identified using RAPD and Phaseolin polymorphisms: one group matched the genotypes with Mesoamerican morphologies, another to those with Andean morphologies and a third group had Andean phenotypes but was close to the Mesoamerican group, indicating possible introgression between the gene pools. The assignment of genotypes to a Mesoamerican or Andean gene pool was made possible by the agreement between phaseolin variability and other genetic and morphological data. Phaseolin variability agreed with other molecular and

morphological data allowing the assignment of genotypes to a Mesoamerican or Andean gene pool.

Szilagyi *et al.* (2011) examined twenty common bean accessions for the diversity analysis using eleven randomly chosen RAPD primers, only four of which produced polymorphism that was satisfactory for identifying the bean cultivars. Only 29 of the 56 bands that these four primers generated (51.78%) were polymorphic. The genotypes were divided into two clusters using the dendrogram created using the RAPD data. According to the RAPD study, genotypes can be employed in breeding programs.

Razvi *et al.* (2013) carried out the molecular characterization of thirteen common bean genotypes using random amplified polymorphic DNA (RAPD) markers. Seven out of the initial 15 primers were chosen after being tested, yielding a total of 65 amplification products, 63 of which (96.625) were polymorphic, showing moderate levels of polymorphism. The genotypes were genetically similar to one another by 43%. The genotypes were divided into three groups using cluster analysis, with seven, five and one genotype in each of clusters I, II and III, respectively. The genotypes SKUA-R-21 and SKUA-R-19 had the highest similarity index (82.35) based dice similarity coefficient, whereas genotypes PBG-29 and SKUA-R-01 had the lowest (27.72).

Kumar *et al.* (2014) investigated genetic diversity in twenty-nine bean genotypes gathered from geographically diverse origins, specifically for the sort of vegetable. 29 polymorphic RAPD primers were employed for the diversity investigation, yielding 186 fragments with a range of 190–2780 bp, of which only 94 were polymorphic. The percentage of polymorphism varied from 27-87.5%. It was discovered that the types VRFBB-2 and Arka Komal were the most diversified of all and they also demonstrated superiority for a few morphological traits. Consequently, the study found that RAPD analysis can be a useful technique for determining the genetic diversity of French bean and the outcomes can be applied to crop development.

2.3.1.3 AFLP in French bean

AFLP is highly polymorphic and shows considerable reproducibility within a laboratory. Because of AFLP's efficiency and dependability, it is being used more frequently in diversification studies, phylogeny, genomic linkage mapping and variety identification (Tohme *et al.*, 1996; Papa and Gepts, 2003). According to Vos *et al.* (1995), AFLP is a high multiplex PCR-based system that has the potential to produce a significant number of polymorphic loci (Powell *et al.*, 1996).

In a study, eight AFLP primer pairs were used to analyze forty-four Indian common bean accessions, yielding 820 products, 698 of which were polymorphic. The accessions were found to differ greatly in terms of the polymorphic information content. Seven significant clusters were found using UPGMA cluster analysis. The aforementioned data from the study of genetic diversity revealed that the Indian common bean accessions had moderate to high genetic diversity analysis and may be employed in breeding programs (Kumar *et al.*, 2008)

The study on genetic diversity in eleven common bean genotypes using AFLP markers was carried out by Akbult *et al.* (2013). The AFLP amplification products were scored using the NTSYS-pc program and the Dice coefficient approach generated the similarity matrix for all genotypes. From 0.178 to 0.713, the similarity coefficients were measured. These coefficient values led to the formation of two major clusters. The findings indicate that the gathered bean genotypes accurately reflect *Phaseolus vulgaris's* genetic diversity, supporting the materials' varied provenances and their potential as a source of variation for breeding programs.

2.3.1.4 ISSR marker in French bean

Thirty common bean accessions were utilized in a study to examine genetic diversity using ISSR markers. From 10 primer pairs, the markers amplified 82 polymorphic bands. Following Jaccard's distance analysis, four

groups were formed based on the diverse data collected. Despite the bean's self-pollinating tendency, which accounts for their remarkable genetic homogeneity, the study found that the ISSR markers could distinguish the bean accessions at their genetic level (Ortega *et al.*, 2014).

2.3.1.5 SSRs in French bean

SSRs are more prevalent DNA markers that are employed most frequently in common bean and many other crops. These tandem repeating DNA sequences are among the most varied forms identified in all so far studied organisms. These markers are used to identify a particular region (locus) in the genome and for genome analysis. They are highly informative and are employed to investigate the genetic diversity of various crops and their wild relatives (Cabral *et al.*, 2010). Common bean SSR markers have also been developed and they have also been employed in a variety of ways, including the construction of PCR-based genetic maps and the identification of genetic variability (Blair *et al.*, 2003). Various common bean core collections have been assessed using microsatellites, which may make it possible to identify the sources of diversity needed for adaptability to various environmental conditions. SSRs are currently the preferred markers for assessing genetic diversity in plant types, including common beans, primarily because of the high polymorphism they present as well as the capacity for and ease of interpreting data.

Twelve microsatellites were used to estimate the genetic diversity of the bean genotypes in Turkey, where 10 SSR markers revealed polymorphic loci. Based on the number of alleles produced, the SSR loci PVGLND5, PVMEIG, PVag001 and PVag004 were discovered to be the most informative. Due to the common bean's propensity for inbreeding, the SSR loci revealed poor heterozygosity within genotypes. The dendrogram was constructed using UPGMA software based on the SSR data and produced two significant clusters. Turkish green bean lines can be selected using this information (Sarkamis *et al.*, 2009).

Cabral *et al.* (2010) conducted the genetic diversity analysis of 57 dry bean accessions using 16 microsatellite primers. Thirteen of these primers—which resulted in 29 polymorphic alleles—were discovered to be polymorphic. Primer BM141, which had four alleles, had the most alleles per locus than any other locus. For the loci BM212 and BM141, the polymorphic information content ranged from 0.11 to 0.51. The accessions Carioca and E22 were found to differ in most ways (1.0). The commercial cultivars were found to have a somewhat narrow genetic basis, but the local genotypes exhibited a relatively high genetic diversity. Therefore, it is necessary to use the local landrace in breeding.

Hegay *et al.* (2012) conducted a study on genetic diversity analysis was conducted on 28 common beans (*Phaseolus vulgaris L.*) accessions with nine microsatellite markers. The number of alleles per microsatellite locus ranged from two to four and there was a total of 24 alleles. The observed heterozygosity of each accession over all loci ranged from 0 to 0.11 (with an average of 0.01), while the expected average heterozygosity was 0.05, which reflected the self-pollinating breeding behavior of common beans. The molecular variance also showed that differences between accessions accounted for 94.71 percent of the overall variation.

Cardoso *et al.* (2012) estimated the extent of genetic diversity in 172 lineages and cultivars of the common bean by integrating five tests of value for cultivation and use (VCU) that were conducted over the last eight years by the breeding program of Embrapa Arroz e Feijão in Brazil. Nine multilocus genotyping systems composed of 36 fluorescent microsatellite markers distributed across 11 different chromosomes of the common bean were used, of which 24 were polymorphic in all trials. One hundred and eighty-seven alleles were identified, with an average of 7.79 alleles per locus and an average gene diversity of 0.65. The combined probability of identity for all loci was 1.32×10^{-16} . Lineages that are more genetically divergent between the selection cycles were identified, allowing the breeding program to develop a crossbreed between elite

genotypes with a low degree of genetic relatedness. HE values ranged from 0.31 to 0.63, with a large reduction in the genetic base over successive selection cycles. The test showed a significant degree of differentiation ($F_{ST} = 0.159$).

Khaidizar *et al.* (2012) assessed the genetic diversity of 38 common beans (*Phaseolus vulgaris L.*) landraces and 12 registered cultivars grown in Turkey with 30 SSR marker systems. Based on the SSR markers, two significant clusters were created. The bulk of the commercial cultivars and landrace accessions were clustered into the second group. Smaller numbers of commercial cultivars and landrace accessions were grouped into the first group, however. There was a lot of variety within the genotypes gathered from the North-East Anatolia Region and registered cultivars.

Kumar *et al.* (2014) estimated the genetic diversity of 115 common bean genotypes using 17 microsatellite markers, including the released varieties, landraces and exotic collections. According to a dendrogram based on UPGMA analysis, the majority of released varieties fall into a single cluster because they have a weaker genetic foundation than native landraces and exotic germplasm. The Mantel test revealed a significant association between the morphological genetic distance and the microsatellite genetic distance ($r = 0.876$).

Scarano *et al.* (2014) investigated the morphological and genetic diversity in 25 common bean populations cultivated in the Campania region (Southern Italy). They analyzed 26 qualitative and 11 quantitative traits following the IPGRI descriptors. Furthermore, 10 SSRs were employed to examine genetic polymorphism, differentiation and population structure. Molecular and morphological data distinguished all the landraces under investigation. A considerable phenotypic diversity among landraces was observed for many characters, including some related to agronomical performance. All the SSRs were polymorphic at the molecular level, with an average of 8.5 alleles per locus. Moreover, the vast majority of the landraces (92%) displayed intra-varietal differences. Their work indicated the presence of a wide-ranging variation among

and within cultivated common bean landraces. Moreover, it provided evidence that the implementation of measures for their on-farm conservation, management and promotion should be useful also to preserve genetic variability.

Sharma and Singh, 2014 conducted a study to evaluate the diversity among the local bean varieties in Himachal Pradesh. In the current study, 30 microsatellite markers were used to analyze five common bean varieties for polymorphism. Only 18 of the 30 utilized SSRs exhibited polymorphism and amplified a total of 51 alleles. With a mean value of 0.40, the polymorphic information content varied from 0.15 to 0.75. The variants were divided into three major groups with a similarity coefficient of 43%. The current results demonstrated the utility of microsatellite markers as a tool for determining the genetic diversity of common bean varieties and creating appropriate DNA fingerprints of those varieties. The results could be exploited by the plant breeders for the establishment of enhanced quality commercial classes of common bean.

Arunga *et al.* (2015) conducted a study to evaluate the genetic diversity in a set of thirty-six Kenyan French bean accessions, based on 26 SSR primer pairs, where only 18 SSR primers were found to be polymorphic. The polymorphic SSR primers yielded an average of 2.17 alleles per SSR locus with a PIC value range of 0.17 to 0.41. The low observed mean heterozygosity was due to the French bean's predominately self-pollinating nature. The impact of intense selection for pod quality can be attributable to the small amount of genotypic variation discovered. According to a phylogeny based on the useful SSR loci, there are three main clusters, each of which corresponds to a certain pod diameter class. The use of DNA-based markers in this evaluation of the germplasm allows for the best features of marker types to be utilized in the identification of French bean varieties and the use of molecular markers in breeding.

Immaculee *et al.* (2015) conducted a diversity analysis on 64 French bean genotypes of (*Phaseolus vulgaris L.*) utilizing fifteen microsatellites. Ten of the fifteen SSR primers used were polymorphic and five were monomorphic. Two

main clusters were found using the clustering of SSR marker data, which also revealed significant genetic variation between genotypes. All exotic collections, landraces, local types and released varieties were grouped in the same cluster with some indigenous collections, whereas cluster A comprised only indigenous collections.

Maras *et al.* (2016) conducted a study to assess the genetic diversity of seventy-one common bean accessions by using 13 microsatellites. The average number of alleles per microsatellite was 5.8 and ranged from 3 to 16 alleles. In the UPGMA dendrogram, a very distinct separation of accessions into two groups was seen. The larger represented Andean gene pool had 40 accessions (56% of the total), whereas the Mesoamerican gene pool was made up of 31 accessions (44% of the total). A sub-group of 16 climbing accessions and 24 bush accessions was divided within the larger Andean cluster in the UPGMA dendrogram. The lack of string in the climber's pods indicates that this subgroup consists of snap beans farmed exclusively for their fresh pods. To implement a breeding program, analysis of the genetic relationships between accessions and their classification into respective gene pools offered crucial information.

Buah *et al.* 2017 assembled 100 common bean accessions from SW Uganda and assayed them with 6 DNA simple sequence repeat (SSR) markers. A total of 41 alleles were detected giving an overall average gene diversity of 0.299 (30%) in both districts. The accessions clustered into two major gene pools i.e., Mesoamerican and Andean. Within each gene pool, there was evidence of clonal populations suggesting the wide distribution of certain accessions. Kabale district had a higher average gene diversity (38%) compared to the Kisoro district (22%). Detection of Andean phaseolin in a clone set of the Mesoamerican gene pool suggests introgression between the two groups.

Gyang *et al.* (2017) conducted a study to determine the genetic diversity of 40 common bean genotypes collected from different regions in Kenya using five SSR markers. With an average of 0.60 and 4.5 alleles per locus, the

polymorphism information content ranged from 0.48 to 0.74. The 40 genotypes were divided into two large clusters by the UPGMA cluster analysis and each major cluster contained a subcluster that represented the genotypes from each region. The SSR markers' principal component analysis (PCA) revealed 28.79 per cent of the genetic variance. When compared to variation across populations, an examination of molecular variance showed that genetic variation within populations is significantly higher (87%) than that between populations. This study showed that the genotypes of common beans grown in Kenya exhibit significant genetic variation and it can serve as the basis for future breeding initiatives aimed at creating hybrids with desirable features.

Chapter 3

MATERIALS AND METHODS

The present study entitled “**Morpho-molecular characterization of common bean (*Phaseolus vulgaris L.*) germplasm**” was conducted during the year 2021-2022. The materials and the methodologies adopted in the present study are presented below under appropriate headings.

3.1 Morphological characterization of common bean landraces collected from different hotspots regions of Jammu and Kashmir

3.1.1 Location and Experimental site

The present investigation was carried out at the research farm of the Division of Genetics and Plant Breeding, Faculty of Agriculture, SKUAST-Kashmir, Wadura campus, Sopore. The experimental site is situated at a height of 1584m above mean sea level, between 33-37⁰ N latitude and 72-80⁰ E longitude. The trial was conducted for morphological and molecular characterization of common bean germplasm.

3.1.2 Seed material

The study included 51 genotypes of common bean (*Phaseolus vulgaris L.*), mostly from the north-western Himalayan regions of Jammu and Kashmir and some exotic genotypes. Local landraces from Jammu province (Poonch, Rajouri, Kishtawar, Doda and Baderwah), Kashmir province's (Kupwara, Baramullah, Anantnag and Bandipora) and several exotic germplasm lines that were already in our possession at Wadura, SKUAST-Kashmir, were among the lines. The checks included Shalimar Rajmash 1 (SR1, variety released by SKUAST-K), E10 and Local Baramulla (Lcl Bml) (Promising genotypes). Due to the invariably admixed and heterogeneous nature of the lines collected from farmers, they were first purified to ensure uniform datasets within each accession and to exclude any off-types that did not adhere to the general characteristics of that landrace. These genotypes were evaluated for morphological, yield and pod quality parameters.

The origin and codes of each of the genotypes that were chosen are listed in Table 1 along with their genotypes.

Table 1: List of 51 genotypes along with their site of origin used in the present investigation

| S.no | Genotype code | Site of collection | Origin |
|-------------|----------------------|---------------------------|---------------|
| 1 | NBPGR-1 | NBPGR, Shimla | Exotic |
| 2 | EC-21753 | Unknown | Exotic |
| 3 | NBPGR-2 | NBPGR, Shimla | Exotic |
| 4 | EC-385256 | Unknown | Exotic |
| 5 | EC-394659 | Unknown | Exotic |
| 6 | NBPGR-3 | NBPGR, Shimla | Exotic |
| 7 | IC-326603 | Not known | Exotic |
| 8 | IC-326619 | Not known | Exotic |
| 9 | IC-326854 | Not known | Exotic |
| 10 | IC-328438 | Not known | Exotic |
| 11 | WB1306 | Baramulla | Indigenous |
| 12 | WB832 | Baramulla | Indigenous |
| 13 | WB665 | Bandipora | Indigenous |
| 14 | WB1151 | Baramulla | Indigenous |
| 15 | WB1199 | Baramulla | Indigenous |
| 16 | WB192 | Not known | Indigenous |
| 17 | WB603 | Not known | Exotic |
| 18 | WB469 | Norway | Exotic |
| 19 | WB1187 | Baramulla | Indigenous |
| 20 | E1 VENYL | Not known | Indigenous |
| 21 | E2 | Not known | Indigenous |
| 22 | SB123 | Not known | Indigenous |
| 23 | SB107 | Akingam, Anantnag | Indigenous |

Contd....

Table1: contd...

| S. No. | Genotype code | Site of collection | Origin |
|---------------|----------------------|---|---------------|
| 24 | SB151 | Bandipora | Indigenous |
| 25 | SB106 | Akingam, Anantnag | Indigenous |
| 26 | SB19 | NBPGR, Shimla | Exotic |
| 27 | SB166 | Not known | Indigenous |
| 28 | SB183.1 | Kupwara | Indigenous |
| 29 | WB1189 | Baramulla | Indigenous |
| 30 | SB155.1 | Bandipora | Indigenous |
| 31 | SB152 | Bandipora | Indigenous |
| 32 | SB111 | Badasgam, Anantnag | Indigenous |
| 33 | SB165 | Watergam, Baramulla | Indigenous |
| 34 | SB169 | Baramulla | Indigenous |
| 35 | SB149 | Bandipora | Indigenous |
| 36 | SB128 | Not known | Indigenous |
| 37 | SB116.1` | Not known | Indigenous |
| 38 | SB131 | Chilibalera, Doda, Baderwah, Kishtiwari | Indigenous |
| 39 | SB167 | Watergam, Baramulla | Indigenous |
| 40 | SB162 | Watergam, Baramulla | Indigenous |
| 41 | SB142.1 | Padder, Kishtiwari | Indigenous |
| 42 | SB181.1 | Kupwara | Indigenous |
| 43 | SB117.2 | Akingam, Anantnag | Indigenous |
| 44 | SB156.A | Bandipora | Indigenous |
| 45 | SB136 | Sartingil, Doda, Baderwah, Kishtiwari | Indigenous |
| 46 | SB159 | Haspilot Thanamandi, Poonch Rajouri | Indigenous |
| 47 | SB121.1 | Larnoo, Anantnag | Indigenous |
| 48 | SB158 | Not known | Indigenous |
| 49 | E10 | Not known | Indigenous |
| 50 | SR1 | Local | Indigenous |
| 51 | Lcl Bml | Baramulla | Indigenous |

3.1.3 Experimental setup

All 51 common bean genotypes were evaluated in Augmented Block Design (ABD) at the Division of Genetics and Plant Breeding research farm, Faculty of Agriculture, SKUAST-Kashmir, Wadura Campus, Sopore during the year 2021. Each genotype was sown in two rows of 2 m each, with a 40 cm gap between the rows and a 10 cm gap between plants. Throughout the cropping cycle, plots were kept free of weeds, diseases, insects and pests. Standard agronomic procedures were used in both years to ensure regular crop growth. Five plants from each genotype were chosen to collect the data.

3.1.4 Data recording on morphological traits

To study the magnitude of genetic variability and level of genetic divergence in the germplasm of 51 lines, data were recorded on various qualitative and quantitative morphological characteristics/traits like plant growth characteristics, leaf characteristics, flower characteristics, pod characteristics and seed characteristics. Observations as per descriptor PPV and FRA (2007) were recorded at the proper developmental stage of plant growth and morphological characters. A description of these traits is given below:

Table 2: Descriptor traits, codes and stages of observation of 51 French bean

| S. No. | Descriptor | Code | Stage of observation |
|---------------|-----------------------------------|----------------------------------|--------------------------------|
| 1 | Use category | | Mid Pod fill to maturity |
| | Dry | 1 | |
| | Snap | 2 | |
| | Green shelled | 3 | |
| 2 | Popping | 4 | Peak flowering |
| | Growth Habit/ Plant Growth | | |
| | Determinate Bush | G1 | |
| | Indeterminate Bush | G2 | |
| 3 | Indeterminate prostate | G3 | Peak flowering |
| | Determinate climbing | G4 | |
| | Growth type | | |
| 4 | Erect | 1 | Peak flowering |
| | Semi-erect | 2 | |
| | Spreading | 3 | |
| 5 | Leaf colour | | Fully expanded trifoliate leaf |
| | Green | 1 | |
| 6 | Purple | 2 | Peak flowering |
| | Leaf shape | | |
| | Cordate | 1 | |
| | Ovate | 2 | |
| 7 | Rhombohedra | 3 | Peak flowering |
| | Hastate | 4 | |
| | Colour of standard flower | | |
| | White | 1 | |
| 8 | White with tones | 2 | Peak flowering |
| | Pink | 3 | |
| | Lilac | 4 | |
| | Light Purple | 5 | |
| | Dark purple | 6 | |
| | 9 | Pod shape (cross-section) | |
| Elliptical | | 1 | |
| Cordate | | 2 | |
| Eight | | 3 | |
| Circle | | 4 | |
| 10 | Ovate | 5 | |

Contd....

Table2: contd....

| S. No. | Descriptor | Code | Stage of observation |
|-------------|---------------------------------------|------|------------------------|
| 8 | Pod colour | | Mid-pod filling stage |
| | Dark purple | 1 | |
| | Carmin red | 2 | |
| | Purple stripe green | 3 | |
| | Carmin striped green | 4 | |
| | Pale red striped green | 5 | |
| | Dark Pink | 6 | |
| | Normal green | 7 | |
| | Shiny green | 8 | |
| | Dull green | 9 | |
| | Golden yellow | 10 | |
| Pale yellow | 11 | | |
| Others | 99 | | |
| 9 | Pod Stringiness | | Fully grown green pods |
| | Present | 1 | |
| | Absent | 2 | |
| 10 | Pod Degree of Curvature | | Fully Grown green pods |
| | Absent | 1 | |
| | Slight | 2 | |
| | Medium | 3 | |
| | Strong | 4 | |
| Very Strong | 5 | | |
| 11 | The shape of the Pod curvature | | Fully grown green pods |
| | Concave | 1 | |
| | Plane | 2 | |
| | Convex | 3 | |
| 12 | Leaf size | | Peak flowering stage |
| | Small | 1 | |
| | Medium | 2 | |
| Large | 3 | | |
| 13 | The intensity of leaf colour | | Peak flowering |
| | Light | 1 | |
| | Dark | 2 | |

Contd....

Table2: contd....

| S. No. | Descriptor | Code | Stage of observation |
|----------------------|---------------------------------|------|-----------------------------|
| 14 | Pod shape of Distal Part | | |
| | Acute | 1 | Fully grown green pod stage |
| | Acute to truncate | 2 | |
| Truncate | 3 | | |
| 15 | Pod Pigmentation | | |
| | Present | 1 | Fully grown green pods |
| Absent | 2 | | |
| 16 | Seed testa colour | | |
| | Dark red | 1 | Mature seed stage |
| | Cream or grey white | 2 | |
| | Black | 3 | |
| | White | 4 | |
| | Purple | 5 | |
| Any others (specify) | 6 | | |
| 17 | Seed testa variegation | | |
| | Present | 1 | Mature seed stage |
| Absent | 2 | | |
| 18 | Seed shape | | |
| | Round | 1 | At harvest after drying |
| | Oval | 2 | |
| | Cuboid | 3 | |
| | Kidney | 4 | |
| | Truncate | 5 | |
| Cylindrical | 6 | | |
| 19 | Plant twining habit | | |
| | Vinyl | 1 | Peak flowering |
| Non-vinyl | 2 | | |

3.1.4.1 Qualitative characteristics:

3.1.4.2 Plant characteristics

1. **Plant growth type:** Erect, semi-erect and spreading characteristics of plant growth were recorded at the peak flowering stage.
2. **Plant growth habit:** Determinate or indeterminate growth habit was recorded at the peak flowering stage.
3. **Plant twining habit:** Vinyl or non-vinyl characteristics was recorded at the peak flowering stage.

3.1.4.3 Leaf characteristics:

1. **Intensity of green color:** Light or dark characteristics were recorded at the peak flowering stage.
2. **Shape of central leaflet:** Cordate, ovate, rhombo-hedric and hastate characteristics were recorded at the peak flowering stage.
3. **Size (at terminal leaflet of the first flowering node):** Small, medium and large characteristics were recorded at the peak flowering stage.

3.1.4.4 Flower characteristics

1. **Colour of standard petals:** White, yellow, pink and violet and purple characteristics were recorded at the peak flowering stage.

3.1.4.5 Pod characteristics

1. **Degree of curvature:** Absent, medium and strong characteristics were recorded at the fully grown green pod stage.
2. **Shape of cross-section (through seed):** Cordate, circular, eight-shaped, oval characteristics were recorded at the fully grown green pod stage.
3. **Shape of distal part:** Acute, acute to truncate and truncate characteristics were recorded at the fully grown green pod stage

4. **Colour:** Pale green, green and purple characteristics were recorded at the fully grown green pod stage.
5. **Stringiness:** Absence or presence characteristics were recorded at the fully grown green pod stage.
6. **Pigmentation on the pod:** Absence or presence characteristics were recorded at the fully grown green pod stage.
7. **Shape (in relation to suture):** Concave, S-shaped and convex characteristics were recorded at the fully grown green pod stage.

3.1.4.6 Seed characteristics

1. **Shape:** Circular, elliptical, circular to elliptical, kidney-shaped characteristics were recorded at the mature seed stage.
2. **Testa color:** White, brown, red, dark red, black, yellow and many more colors were recorded at the mature seed stage.
3. **Testa variegation:** Absence or presence characteristics were recorded at the mature seed stage.

3.1.5 Quantitative characteristics:

3.1.5.1 Days to flower initiation

The days to the appearance of first flower set on the chosen plants was recorded from the date of sowing and the average was worked out.

3.1.5.2 Days to pod initiation:

Days to pod set was measured by counting the number of days taken from sowing to the time when pods attained a length of 2 cm.

3.1.5.3 Days to marketable maturity

Days to marketable maturity were measured by counting the number of days taken from the date of sowing till the pods are full bright, green, fleshy and the seeds are small and green.

3.1.5.4 Pod length (cm)

Pod length (cm) is the average length of all pods excluding beak and pedicel from selected plants and was calculated in centimeters using a scale.

3.1.5.5 Pod thickness (mm)

Pod thickness (mm) is the diameter of the pod or distance between pod sides at the level of the second and the third seed from the apex and was calculated in millimeters using a vernier calliper.

3.1.5.6 Number of pods per plant

The number of pods per plant was counted and recorded when the flowering was completed.

3.1.5.7 Average pod weight (g)

The pods used for measuring the pod length and thickness were weighed and the average pod weight of 5 plants was worked out.

3.1.5.8 Pod yield per plant (g)

The green pods harvested from each tagged plant were weighed for each picking and pooled to determine the total green pod yield per plant in grams and the average of five plants was worked out.

3.1.5.9 Pod yield per hectare (q)

The green pods harvested from each genotype were weighed and pooled to determine the total pod yield per hectare in quintals.

3.1.5.10 Number of seeds per pod

The number of seeds was counted from five randomly selected pods in each tagged plant and the average was calculated.

3.1.5.11 Hundred seed weight (g)

Hundred-seed weight of five randomly drawn samples of sun-dried seeds from each experimental plot was weighed in grams and averaged. The value so obtained was expressed in grams (g).

3.1.5.12 Seed yield per plant (g)

The yield of seed obtained from selected plants from each experimental plot was weighed and averaged to a single plant basis in grams.

3.1.5.13 Seed yield per hectare (q)

The seed yield per hectare was calculated in all genotypes and expressed in quintals per hectare.

3.2 Statistical and biometrical analysis

The data recorded during the present investigation was subjected to the following statistical and biometrical analysis.

3.2.1 Field experiment-Genetic variability for morphological, yield and seed-traits

Utilizing an augmented block design, a field evaluation was conducted (Federer, 1956). Two types of treatments make up enhanced designs: checks, standard treatments and new, or augmented treatments (Federer, 1956). The design takes into account that checks are fixed effects whereas new entries are random effects. The new entries are not replicated in most cases, especially when working with huge data sets. However, the checks are replicated to act as points of reference. Both the checks and new entries are randomly distributed among the blocks. Federer (2001) provided for statistical analysis of such designs wherein the random effects of new entries are used to account for various sources of variation. Augmented block design (ABD)

$$Y_{ij} = \mu + \beta_j + C_j + \tau_i + \sigma_{ij},$$

Where,

Y_{ij} = overall mean

β_j = effect of jth block

C_j = effect of checks in jth block

τ_i = effect of ith treatment

σ_{ij} = random variation of ith treatment in jth block

In augmented designs, the checks to be used are usually the varieties that have been under cultivation for a large time and as such serve to monitor the progress. Other genotypes may also be used as checks for acting as baselines for diverse purposes. However, a new variety could be the best standard for yield trials. The checks used in the design are assumed to be treatments of Randomized Block Design for estimating experimental error. There are certain basic prerequisites for the efficient use of augmented designs such as:

1. The minimum number of degrees of freedom for error in the ANOVA should be 12, with the error degree of freedom calculated as $(R-1)(C-1)$, where, R is the number of blocks and C is the number of checks in each block. In the present study error degree of freedom was 14.
2. The minimum number of blocks in augmented block design should be $r \geq [10 / (C-1)] + 1$. Therefore, the minimum number of blocks with three checks is $[10 / 2] + 1 = 6$. In the present study, 51 lines were evaluated in 8 blocks with the checks being replicated in each block.
3. Each block in the augmented block design should have at least $C+1$ plots. With 3 checks it is 4 plots for the present study.

The augmented design also provides estimates of standard errors of difference between different sources of variation (Table 3).

Table 3: Formulae for calculating standard errors for comparison

| STANDARD ERROR | FORMULA |
|---|---|
| Difference between two check varieties (Sc) | $\sqrt{\text{MSE}/R}$ |
| Difference between adjusted means of two Testentries in the same block (Sb) | $\sqrt{2\text{MSE}}$ |
| Difference between adjusted means of two testentries in different blocks (Sv) | $\sqrt{2 (C + 1) \text{MSE} / C}$ |
| Difference between adjusted test entry and check mean (Svc) | $\sqrt{\{(R + 1) (C + 1) \text{MSE}\} / R.C}$ |

where,

MSE is the error mean square estimated by the design at (R-1) (C-1) degrees of freedom

R is the number of blocks or the replicates of a check.

C is the number of checks used, and

3.2.2 Genotypic variance

The genotypic variance was calculated using the method suggested by Johnson *et al.* (1955) as follows.

$$\sigma^2 g = \frac{\text{MSG} - \text{ME}}{r}$$

Where,

$\sigma^2 g$ = genotypic variance,

MSG = mean sum of squares for the genotypes,

and ME = error mean sum of squares

3.2.4 Phenotypic variance

Phenotypic variance was calculated as per the procedure given by Johnson *et al.* (1955).

$$\hat{\sigma}^2 p = \hat{\sigma}^2 g + \hat{\sigma}^2 e$$

Where,

$\hat{\sigma}^2 p$ = Phenotypic variance

$\hat{\sigma}^2 g$ = genotypic variance and

$\hat{\sigma}^2 e$ = error variance

3.2.5 Phenotypic and genotypic co-efficient of variation

The magnitude of phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) existing in a trait was worked out by the formula given by Burton (1952):

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

Where,

$\hat{\sigma}^2 p$ = Phenotypic variance and

\bar{X} = grand mean of the trait studied

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

Where,

$\hat{\sigma}^2 g$ = Genotypic variance and

\bar{X} = grand mean of the trait studied

PCV and GCV were categorized as low, moderate and high by following Sivasubramanian and Menon (1973) as follows:

| | | |
|-----------|---|----------|
| 0 – 10% | : | Low |
| 10 – 20% | : | Moderate |
| Above 20% | : | High |

Heritability (broad sense)

It was estimated as per the procedure presented by Burton and Devane (1953) and Johnson *et al.* (1955),

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Where,

h^2 = Estimate of heritability in broad sense,

σ^2_g = Genotypic variance, and

σ^2_p = Phenotypic variance

The heritability percentage was categorized as low, moderate and high as suggested by Robinson *et al.* (1949):

0-30% : Low

30-60% : Moderate

60% and above: High

3.2.3 Genetic advance

Genetic advance at 5 per cent selection intensity was worked out using the formula given by Lush (1949) and Johnson *et al.* (1955).

$$G.A. = \frac{\sigma^2_g}{\sigma^2_p} \times (\sigma^2_p)^{0.5} \times K$$

Where,

G.A. = genetic advance,

σ^2_g = genotypic variance of the trait,

σ^2_p = phenotypic variance of the trait,

and K = selection differential and value of K at 5 per cent selection intensity being 2.06

The GA as per cent of mean was categorised as low, moderate and high by following Johnson *et al.* (1955).

| | | |
|-----------|---|----------|
| 0-10% | : | Low |
| 10-20% | : | Moderate |
| Above 20% | : | High |

Heritability (bs), expected genetic gain, ANOVA, components of variability, phenotypic and genotypic coefficient of variation, cluster analysis and Pearson's correlation coefficients were estimated by using R – Studio software.

3.3 Molecular Characterisation of genotypes using SSR markers

3.3.1 Collection of leaf material

Young, fresh, disease and insect-free leaves from all fifty-one genotypes were used for DNA extraction. Leaf samples at the 2-3 leaf stage (seedling stage) were collected in sterile plastic bags and placed in ice containers to protect them from heat, air and light exposure while transferring from the field to the laboratory. The samples were then weighed and packed in sterile bags and stored in a deep freezer at -80⁰C till DNA extraction and SSR marker studies were carried out at Molecular Biology Laboratory, Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, Sopore.

3.3.2 Genomic DNA Isolation

The genomic DNA was extracted from all the plant samples by the Cetyl Tri methyl Ammonium Bromide (CTAB) method (Saghai-Marooof *et al.* 1984), a widely used protocol for the isolation of genomic DNA from plants.

3.3.3 Plant genomic DNA isolation protocol

The different steps that were followed for genomic DNA extraction are as under:

- Step 1:** Two to three grams of leaf sample was crushed in 500 microlitres of CTAB (Cetyl Trimethyl Ammonium Bromide) extraction buffer, 100 microlitres of PVP (Polyvinylpyrrolidone) and 100 microlitres of 2% β - mercaptoethanol using pre-chilled mortar and pestle.
- Step 2:** After proper crushing, the sample was transferred into a 2 ml Eppendorf tube and incubated for 1 hour in a water bath at 62^o-65^oC by immersing three fourth of the tube in water. Occasional mixing of the tube contents was done by gently inverting the tubes after 5-10 minute intervals.
- Step 3:** Equal amount of chloroform: isoamyl alcohol (24:1 i.e., 24ml chloroform and 1 ml isoamyl alcohol) was added to tubes and samples were mixed thoroughly by gently inverting the tubes several times followed by centrifugation for 10 minutes at 10,000 rpm to separate the phases.
- Step 4:** The upper aqueous phase after the centrifugation was pipetted out in a pre-sterilized 1.5ml Eppendorf tube without disturbing the interface.
- Step 5:** Equal volume of ice-cold isopropanol was added to tubes to precipitate the DNA and kept in a -20^oC refrigerator for 1-1.5 hours to precipitate the DNA.
- Step 6:** Tubes were centrifuged for 5 minutes at 10, 000 rpm after which pellets were formed at the base of the tube
- Step 7:** Pellets were then washed with 300-400 microlitre of 70% ethanol and centrifuged at 5000 rpm for 5 minutes after which the supernatant was discarded and the DNA pellet at bottom of the tube was dried at room temperature.

Step 8: After drying, the DNA was subsequently dissolved in 40- 80 μ l TE (Tris EDTA) buffer (pH 8.0).

3.3.4 DNA Quantification:

The concentration and purity of DNA were checked by Agarose gel electrophoresis. The various steps followed for DNA Quantification are as under:

Step 1: Agarose gel of 0.8 per cent was prepared by dissolving 0.8 g of agarose in 100 ml of 1 X TAE electrophoresis buffer (Tris base-45 Mm, Acetic acid -45mM and EDTA- 1mM).

Step 2: The mixture was heated in the microwave for 2 minutes till the agarose dissolved completely. i.e., when the solution became transparent and clear. It was allowed to cool for a couple of minutes and then stained with ethidium bromide (5 μ l) of buffer and stirred for some time.

Step 3: It was then poured into the casting tray with combs in it and allowed to solidify at room temperature for 20-25 minutes.

Step 4: Remove the combs and then start loading the DNA (5 μ l) to estimate the concentration of DNA. Before loading the DNA is stained with Bromophenol. The DNA samples were loaded into wells with the help of a micropipette.

Step 5: A marker of known concentration was also loaded with the DNA samples.

Step 6: The electrophoresis was carried out at 75V for 2 hours.

Step 7: The gel was then visualized under the Gel Doc electrophoresis system.

Step 8: The intensity of DNA bands was compared with that of a standard marker; hence, the DNA concentration of each sample was ascertained. The quality of DNA samples was indexed based on

whether DNA formed a single high molecular weight band (good quality) or a smear (degraded/ poor quality).

3.3.5 Selection of SSR primers

For the selection of SSR markers, various earlier publications were referred (Blair *et al.*, 2003; Blair *et al.*, 2006; Galeano *et al.*, 2012; Blair *et al.*, 2012; Garzon and Blair 2014). SSR markers were chosen based on many criteria such as mapped position on the chromosome (one marker from each arm of 11 chromosomes), marker with high PIC value (>0.5), number of alleles detected by the SSR marker in previous research (ideally > 5) were selected (Figure 1). The mapping positions of these markers have been presented in the form of figures (Figure 2 a and 2 b). The details of the primer sequence of 11 markers used during the present study are given in (Table 4)

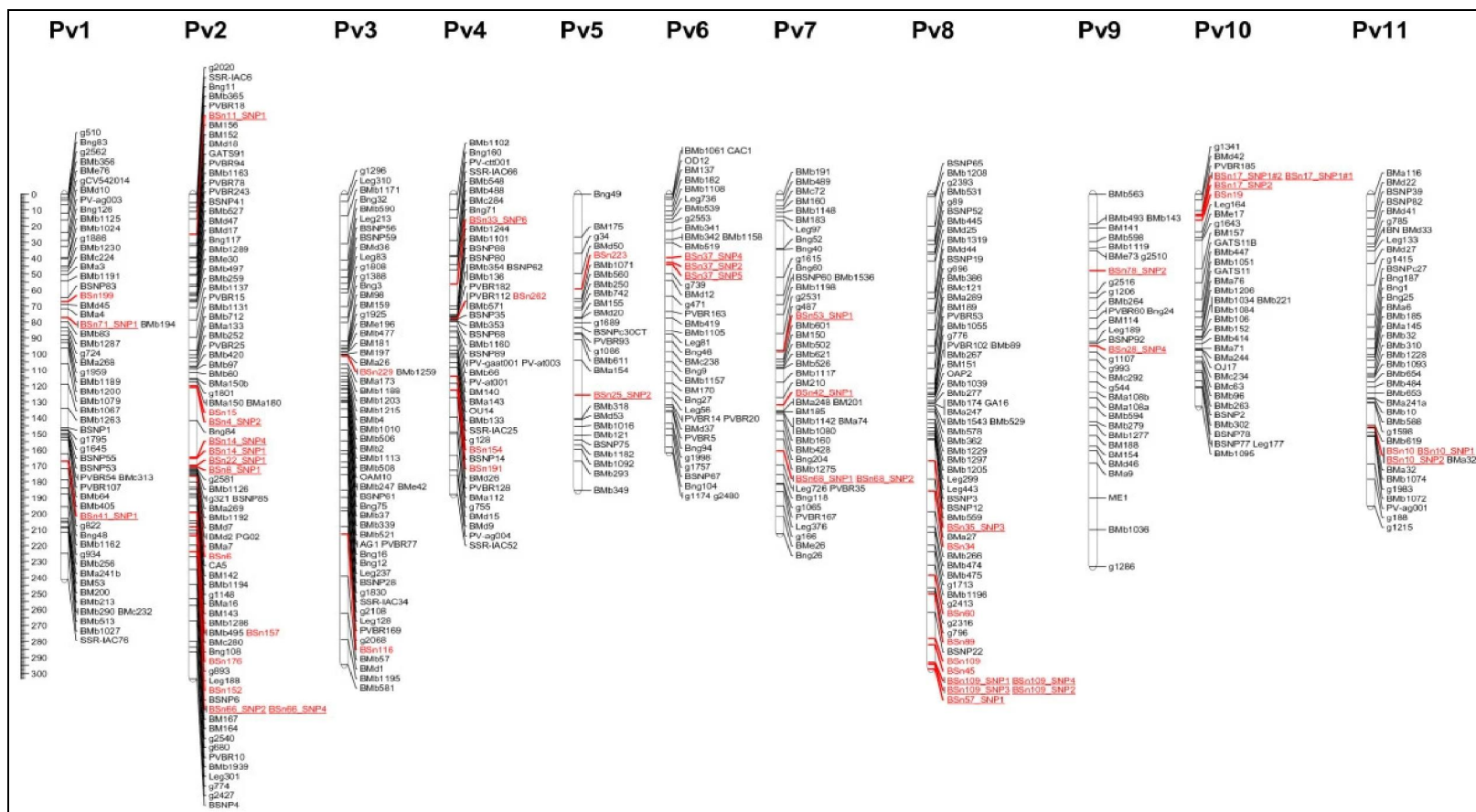


Fig. 1: Representative genetic linkage map of common bean showing distribution of markers on different linkage groups. The map was used for selecting markers for the present study

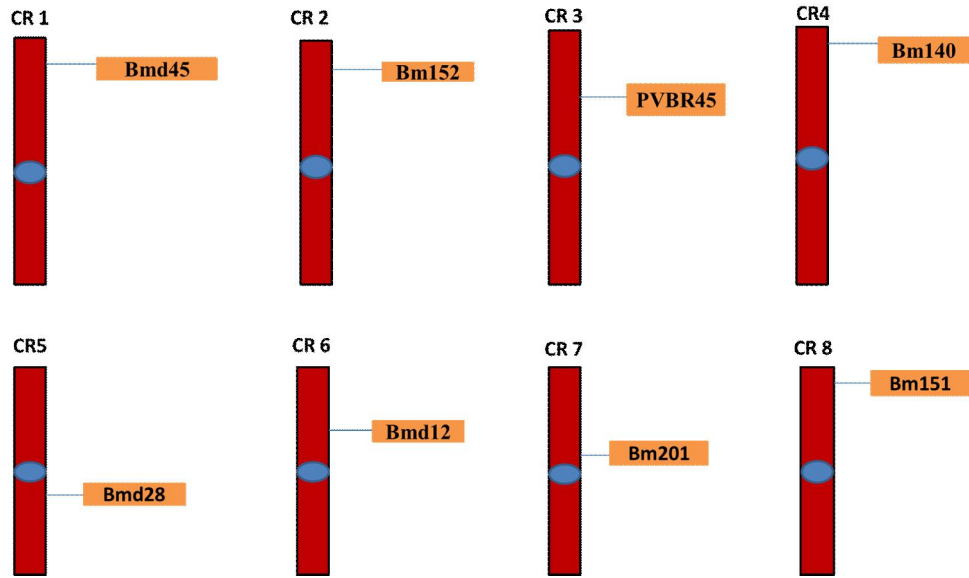


Fig. 2a: Framework Linkage map of common bean showing different markers used during present study

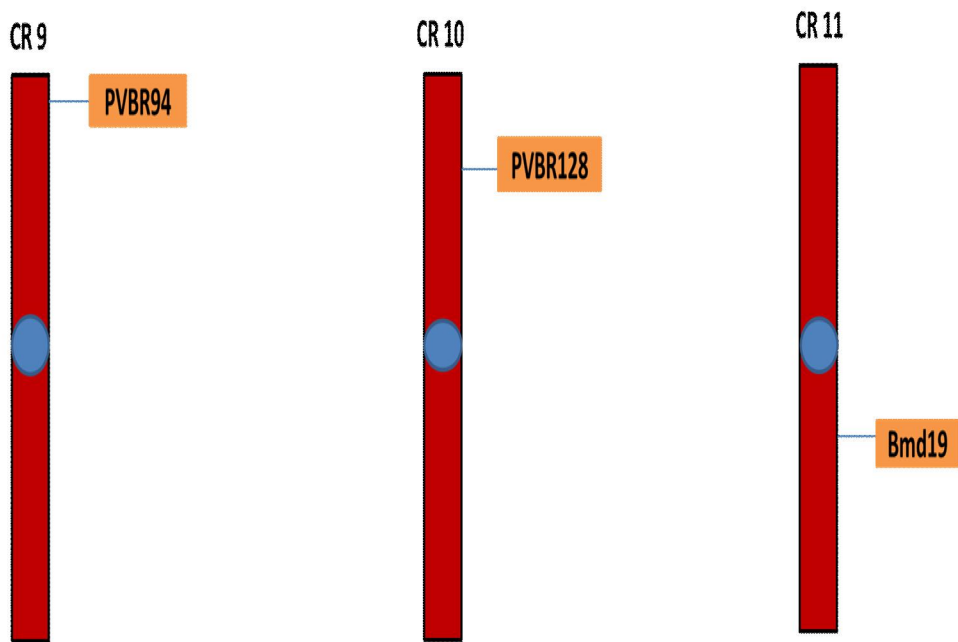


Fig. 2b: Framework Linkage map of common bean showing different markers used during present study

Table 4: List of 11 SSR markers along with their details for marker genotyping work on 51 common bean lines

| S. No | Marker | F/R | Sequence | Linkage group | Base pairs |
|-------|----------------|-----|---------------------------|---------------|------------|
| 1 | <i>BM152</i> | F | AAGAGGAGGTCGAAACCTTAAATCG | 2 | 25 |
| | | R | CCGGGACTTGCCAGAAGAAC | | 20 |
| 2 | <i>PVBR45</i> | F | CGATTGAACGCACTCTACGA | 3 | 20 |
| | | R | GAGGCTGGTTCCTTCAAACA | | 20 |
| 3 | <i>BM140</i> | F | TGCACAACACACATTTAGTGAC | 4 | 22 |
| | | R | CCTACCAAGATTGATTTATGGG | | 22 |
| 4 | <i>BMd28</i> | F | TGCATCAACTTTAGGAGCTTG | 5 | 21 |
| | | R | TCTTGTCTTATCAGCAGGTGGA | | 22 |
| 5 | <i>BMd12</i> | F | CATCAACAAGGACAGCCTCA | 6 | 20 |
| | | R | GCAGCTGGCGGGTAAAACAG | | 20 |
| 6 | <i>BM201</i> | F | TGGTGCTACAGACTTGATGG | 7 | 20 |
| | | R | TGTCACCTCTCTCCTCCAAT | | 20 |
| 7 | <i>BM151</i> | F | CACAACAAGAAAGACCTCCT | 8 | 20 |
| | | R | TTATGTATTAGACCACATTACTTCC | | 25 |
| 8 | <i>PVBR94</i> | F | ACGACGGAGAGAGAGGTTGA | 9 | 20 |
| | | R | CCGTGTTCTTCTGTCTGTG | | 20 |
| 9 | <i>BMd19</i> | F | GCCAACCACATTCTTCCCTAC | 11 | 21 |
| | | R | GGAAGCGAGGCAGTTATCTTT | | 21 |
| 10 | <i>BMd45</i> | F | GGTTGGGAAGCCTCATAACAG | 1 | 20 |
| | | R | ATCTTCGACCCACCTTGCT | | 20 |
| 11 | <i>PVBR128</i> | F | GAAGAGCTCTCATCGCAACG | 10 | 20 |
| | | R | CTAGCTCCCTCCCCTCGTAA | | 20 |

3.3.6 PCR standardization and amplification

3.3.6.1 Components used for PCR Reactions

For performing Polymerase Chain Reaction, the constituents of PCR reaction mixture along with their volume is indicated in Table 5:

Table 5: Constituents of PCR reaction mixture

| Constituents | Volume |
|------------------------------|-----------------------------|
| NFW (Nuclease Free Water) | 7.8 μ l |
| PCR buffer (10 X) | 2 μ l |
| MgCl ₂ (2.5 mM) | 2 μ l |
| dNTPs (10 mM) | 2 μ l |
| Forward primer (5 pmol) | 2 μ l |
| Reverse primer (5 pmol) | 2 μ l |
| Taq Polymerase (5U/ μ l) | 0.2 μ l |
| DNA sample | 2 μ l |
| Total volume | 20 μl |

3.3.6.2 PCR Amplification Program

PCR tubes containing all the PCR mixture constituents and DNA template were thoroughly mixed and subjected to the thermal profile given in Table-6. The amplification reaction was carried out in a gradient master cycler. An initial denaturation step of 3 minutes was programmed in the thermocycler, followed by a loop of 35 cycles each consisting of denaturation (at 94°C for 35 seconds), annealing (at 52-67° C for 30 seconds), elongation (at 72°C for 1 minute) and the final extension was performed (at 72 °C for 15 minutes).

Table 6: Thermal profiles used for DNA amplification

| Step | Temperature (°C) | Time | Number of cycles |
|----------------------|------------------|------------|------------------|
| Initial denaturation | 94 | 3 minutes | 1 |
| Denaturation | 94 | 35 seconds | } 35 |
| Annealing | 52-67 | 30 seconds | |
| Elongation | 72 | 1 minute | |
| Final Extension | 72 | 15 minutes | 1 |

3.3.6.3 Polyacrylamide Gel (PAGE) Electrophoresis of amplified PCR product:

Denaturing Polyacrylamide gel is an extremely helpful tool for detecting microsatellite markers and STS markers. Polyacrylamide gels are chemically cross-linked gels formed by acrylamide polymerization with a cross-linked substance, typically N, N'-methylene bisacrylamide (Bis). Polymerization begins with free radical formation, which is typically carried out with ammonium per sulphate as the initiator and N, N, N', N'-tetramethylene diamine (TEMED) as the catalyst. The concentration of acrylamide in the polymerization procedure can determine the length of the chain. The acrylamide solution was created in a 38:2 ratio. It was prepared by combining 38 grams of acrylamide and 2 grams of Bis-acrylamide to a final volume of 100 ml of double distilled water. As a result, we generated a 40% acrylamide stock solution from which we prepared our working solution. A 10% acrylamide solution was created by combining 25 ml of 40% acrylamide stock with 10 ml of 10x TBE, to which 19.6 gram of urea was added and the final volume was increased to 100 ml.

Polyacrylamide gel casting procedures: The glass plates were carefully rinsed before being cleaned with 70% ethanol. Spacers were put between two glass plates, which were then sealed from the bottom using sealing tape or clamps.

Approximately 20 ml of 10% working acrylamide solution was collected, 200 l of APS and 20 l of TEMED were added and the solution was immediately employed for gel casting with matching combs. The combs were removed from the glass plates after the gel polymerized. The wells were cleansed with distilled water before being placed in the electrophoresis machine. In each well, 12 ul of material with 6x loading dye were dispensed. The gel was allowed to run on 100 volts for 3 hours followed by silver staining to visualise the bands.

3.3.6.4 Scoring of SSR allele profile

The SSR allele sizes were determined by the position of bands relative to the DNA ladder. The bands were scored based on relative mobility in gel and the allele difference was determined according to their fragment size (bp) corresponding to the 100 bp standard marker (Thermo-Scientific). The banding patterns of all genotypes against every primer were compared. The accessions that did not show any amplification were scored as missing values if amplification was not repeated and as null alleles if the amplification was repeated 2-3 times.

3.2.6.5 Statistical analysis of molecular characterization

SSR based analysis was carried out using GeneA1Ex 6.41 (Peakall and Smouse 2006) and scoring was done as base pair scoring and binary scoring in which bands were scored as '1' (for presence) and '0' (absence) (Yeh, 1999). The SSR marker genotypic data was used to calculate genetic indices to estimate total number of alleles (Na), frequency, range of alleles. All these analysis helped to compare genetic diversity parameters between common bean genotypes. Cluster analysis of genotypes was performed using UPGMA (un-weighted pair group method and arithmetic average) clustering method to study the relationships among the genotypes (Mir *et al.* 2021).

The genetic dissimilarity coefficients were used to construct a neighbor joining (NJ) dendrogram using software program DARwin version 6 (Perrier *et al.* 2003). The Principal Coordinate analysis (PCoA) was done using software

program DARwin ver. 6 (Perrier and Jacquemoud-Collet 2006). The polymorphic information content (PIC) was also calculated by using formula of Nei and Li (1979):

$$PIC = 1 - \sum (f_{ij})^2$$

Where, f_{ij} is the frequency of the j th allele for the i th locus summed across all alleles for the locus.



Plate 1: Common bean field SKUAST-Wadura during the year 2021

Chapter-4

EXPERIMENTAL FINDINGS

The present study entitled “**Morpho-molecular characterization of common bean (*Phaseolus vulgaris* L.) germplasm**” was undertaken to have in-depth elucidation of a pattern of variation in the 51 common bean genotypes (including local and exotic genotypes) for 17 qualitative and 13 quantitative traits drawn from descriptors developed by CIAT (International Centre for Tropical Agriculture), USDA (United States Department of Agriculture) and PPVFRA (Protection of Plant Varieties and Farmers’ Rights Authority). The findings pertaining to various aspects of the investigation are presented under following headings:

- 4.1 Evaluation of common bean germplasm for different morphological traits and selection of a set of most promising diverse genotypes/vegetable-type beans.
- 4.2 Molecular characterization of common bean germplasm using SSR markers.

4.1 Evaluation of common bean germplasm for different morphological traits and selection of a set of most promising diverse genotypes/vegetable-type beans.

Common bean genotypes collected from different hot-spot regions of Jammu and Kashmir were evaluated for various qualitative (plant, flower, leaf, pod and seed) and quantitative traits including (days to flowering, days to pod initiation, days to marketable maturity, number of pods per plant, pod length, pod thickness, pod weight, pod yield per plant, pod yield per hectare, hundred seed weight, number of seeds per pod, seed yield per plant, seed yield per hectare). The data collected showed wide variation in the plant, flower, leaf, pod and seed characteristics (Table 7). Results obtained from the evaluation of these traits are presented in the following sub-headings.

4.1.1 Plant growth characteristics

4.1.1.1 Use category

Out of 51 genotypes, 33 (64.7%) genotypes were snap/vegetable type whereas 18 (35.29%) genotypes were dry/pulse-type.

4.1.1.2 Plant growth type

Out of 51 genotypes, 31 (60.7%) genotypes were found to be spreading, 15 (29.4%) were found erect and only 5 (9.8%) were found semi-erect.

4.1.1.3 Plant growth habit

Out of 51 genotypes, 41 (80.4%) genotypes were indeterminate types and 10 (19.6%) genotypes were determinate.

4.1.1.4 Plant twining habit

Two types of twining habits in 51 genotypes were observed (vinyl/non-vinyl). Plants with vinyl twining habit produced vines used for climbing, non-vinyl did not produce vines. It was found that 40 (78.4%) genotypes had vinyl twining habits and 11 (21.5%) genotypes had non-vinyl twining habits.

4.1.2 Flower colour

The material used in the present study was analysed for flower colour. Good variation in flower colour was recorded (Plate-3). Pink, white, yellow, violet and purple flowers were observed. However, the white flower colour was predominant and found in 28 (54.9%) genotypes, followed by the pink flower colour in 11 (21.5%) genotypes. Violet flower colour was observed in 7 (13.7%) genotypes, 4 (7.8%) genotypes had yellow-coloured flowers and only one genotype (WB469) had a purple-coloured flower.

4.1.3 Leaf characteristics

4.1.3.1 Shape of central leaflet

With respect to leaf shape two classes were found (Cordate and Ovate). The cordate shape was most predominant and was found in 32 (62.7%) genotypes while the ovate shape was observed in 19 (37.2%) genotypes.

4.1.3.2 Leaf size (at terminal leaflet of the first flowering node)

Leaf size showed a significant variation and three classes of leaf size were observed (large, medium and small). Out of 51 genotypes, 33 (64.7%) genotypes had medium leaves, 13 (25.4%) genotypes had small leaves and only 5 (9.8%) genotypes had larger leaves.

4.1.3.3 Intensity of green colour

With respect to leaf colour, green, dark green and purple coloured leaves were observed. Dark green leaf colour was found to be predominant as 40 (78.4%) genotypes had this leaf colour followed by green leaf colour which was found in 9 (17.6%) genotypes while only one genotype (WB469) had a purple-coloured leaf.

4.1.4 Pod characteristics

4.1.4.1 Pod colour

With respect to pod colour three classes were found (Green, pale green and purple). Pods of green colour were found to be dominant as 43 (84.3%) genotypes had this pod colour followed by 7 (13.7%) genotypes having pale green coloured pods whereas, only one genotype (1.9%) had purple-coloured pods.

4.1.4.2 Pod shape (in relation to suture)

The pod shape (in relation to the suture) was found to be concave or convex. The pods having a concave shape in relation to suture were most commonly found in 42 (82.3%) genotypes, convex pod shape was seen in 2

(3.9%) genotypes. However, in 7 (13.7%) genotypes neither convex nor concave shape was seen, they were found to be straight.

4.1.4.3 Pod cross-section (through seed)

The shape of the cross-section of pods was seen through seeds. The shape of the cross-section (through seed) was found to be cordate, elliptical, ovate and circular (Plate-4). The cordate shape was found to be predominant in 21 (41.1%) genotypes followed by the ovate shape found in 19 (37.2%) genotypes. The elliptical shape was observed in 6 (11.7%) genotypes while the circular shape was seen only in 5 (9.8%) genotypes.

4.1.4.4 Shape of the distal part of pods

The shape of the distal part of the pods was found to be acute, acute to truncate and truncate (Plate-5). The acute to truncate shape was predominant and found in 38 (74.5%) genotypes. In 11 (21.5%) genotypes the shape of the distal part was found to be acute; in only 2 (3.9%) genotypes, it was truncated in shape.

4.1.4.5 Pod stringiness

When the pods were broken at the full green mature stage it was found that 32 (62.7%) genotypes possessed strings while 19 (37.2%) genotypes showed the absence of strings in pods.

4.1.4.6 Pod degree of curvature

Out of 51 genotypes, the medium degree of curvature was observed in 27 (52.9%) genotypes followed by a strong degree of curvature in 9 (17.6%) genotypes. The less degree of curvature in pods was seen in 8 (15.68%) genotypes whereas, the degree of curvature was absent in 7 (13.7%) genotypes.

4.1.4.7 Pod pigmentation

The pod pigmentation was seen in 5 (9.8%) genotypes while no pigmentation was seen in 46 (90.1%) genotypes (Plate-6)

4.1.5 Seed characteristics

4.1.5.1 Seed shape

Seeds of circular, kidney, cuboidal, circular to elliptical and elliptical shapes were observed, however circular to elliptical, cuboidal and kidney shapes were most predominant. 20 (39.2%) genotypes had circular to elliptical shapes. 14 (27.3%) genotypes exhibited the cuboidal form. Kidney shape was seen in 10 (19.6%) genotypes, followed by circular shape in 5 (9.8%) genotypes. Only 2 (3.9%) genotypes have the elliptical shape as their most common form.

4.1.5.2 Seed pigmentation

Out of 51 genotypes, seed pigmentation was observed in 8 (15.6%) while the pigmentation was absent in 43 (84.3%) genotypes.

4.1.5.3 Seed testa colour

Variation was noticed in seed testa colour (Plate-10). In red seed colour, different shades of red colour were observed. Six (11.7%) genotypes were having dark red coloured seeds, 6 (5.8%) genotypes had Canadian red coloured seeds, 3 (5.8%) genotypes had red coloured seeds, while as 2 (3.9%) genotypes had red-painted lady seeds. Cream-coloured seeds were observed in 9 (17.6%) genotypes. In 2 (3.9%) genotypes painted lady seeds were seen. The light-yellow seeds were observed in 2 (3.9%) genotypes. Similarly, different shades were seen in brown colour. In 6 (11.7%) genotypes dark brown coloured seeds were seen. The light brown coloured seeds were seen in 5 (9.8%) genotypes. 3 (5.8%) genotypes had painted brown seeds. Black, cranberry, cannellini, navy, olive, sapphire blue, yellow, grey northern and light grey seeds were seen in 1.98% of each genotype.

Table 7: Qualitative trait analysis of 51 common bean landraces studied during the present study

| S. No. | Variable | Observations | No. of accessions |
|--------|---|-------------------|-------------------|
| 1. | Use category | Snap/ vegetable | 33 |
| | | Dry/pulse | 18 |
| 2. | Growth type | Erect | 15 |
| | | Semi erect | 5 |
| | | Spreading | 31 |
| 3. | Plant growth habit | Indeterminate | 41 |
| | | Determinate | 10 |
| 4. | Plant twining habit | Vinyl | 40 |
| | | Non-vinyl | 11 |
| 5. | Flower colour | White | 28 |
| | | Pink | 11 |
| | | Violet | 7 |
| | | Purple | 1 |
| | | Yellow | 4 |
| 6. | Leaf shape | Cordate | 32 |
| | | Ovate | 19 |
| 7. | Leaf colour | Dark green | 40 |
| | | Green | 9 |
| | | Purple | 1 |
| 8. | Leaf Size | Medium | 33 |
| | | Small | 13 |
| | | Large | 5 |
| 9. | Pod shape of cross-section through seed | Ovate | 19 |
| | | Cordate | 21 |
| | | Elliptical | 6 |
| | | Circular | 5 |
| 10. | Pod shape in relation to suture | Concave | 42 |
| | | Convex | 2 |
| | | Absent | 7 |
| 11. | Pod shape of distal part | Acute | 11 |
| | | Acute to truncate | 38 |
| | | Truncate | 2 |
| 12. | Pod curvature | Medium | 27 |
| | | Strong | 9 |
| | | Absent | 7 |
| | | Less | 8 |
| 13. | Pod stringiness | Absent | 32 |
| | | Present | 19 |

Contd....

Table 7: contd.....

| S. No. | Variable | Observations | No. of accessions |
|---------------|-------------------|---|---|
| 14. | Pod pigmentation | Present Absent | 5 46 |
| 15. | Pod colour | Green Dull green Purple | 43 7 1 |
| 16. | Seed Pigmentation | Present Absent | 8 43 |
| 17. | Seed shape | Circular Circular to elliptical Cuboidal Kidney Elliptical | 5 20 14 10 2 |
| 18. | Seed testa colour | Black Canadian red Cannellini Cranberry Creamish Dark brown Dark red Great Northern Light brown Light grey Light yellow Navy Olive Painted brown Red painted lady Red Sapphire blue Yellow Painted lady | 1 3 1 1 9 6 6 1 5 1 2 1 1 3 2 3 1 1 2 |

Table 8: Genotypes identified as vegetable type and dry bean type

| S. No. | Genotype | Vegetable/ dry type |
|---------------|-----------------|----------------------------|
| 1 | NBPGR-1 | Vegetable type |
| 2 | EC-21753 | Vegetable type |
| 3 | NBPGR-2 | Dry type |
| 4 | EC-385256 | Vegetable type |
| 5 | EC-394659 | Vegetable type |
| 6 | NBPGR-3 | Dry type |
| 7 | IC-326603 | Vegetable type |
| 8 | IC-326619 | Vegetable type |
| 9 | IC-326854 | Dry type |
| 10 | IC-328438 | Vegetable type |
| 11 | WB1306 | Vegetable type |
| 12 | WB832 | Dry type |
| 13 | WB665 | Dry type |
| 14 | WB1151 | Dry type |
| 15 | WB1199 | Dry type |
| 16 | WB192 | Vegetable type |
| 17 | WB603 | Dry type |
| 18 | WB469 | Vegetable type |
| 19 | WB1187 | Dry type |
| 20 | E1 VENYL | Dry type |
| 21 | E2 | Dry type |
| 22 | SB123 | Dry type |
| 23 | SB107 | Vegetable type |
| 24 | SB151 | Vegetable type |
| 25 | SB106 | Vegetable type |
| 26 | SB19 | Vegetable type |
| 27 | SB166 | Vegetable type |

Contd....

Table 8: contd...

| S. No. | Genotype | Vegetable/ dry type |
|---------------|-----------------|----------------------------|
| 28 | SB183.1 | Vegetable type |
| 29 | WB1189 | Vegetable type |
| 30 | SB155.1 | Vegetable type |
| 31 | SB152 | Vegetable type |
| 32 | SB111 | Dry type |
| 33 | SB165 | Vegetable type |
| 34 | SB169 | Dry type |
| 35 | SB149 | Vegetable type |
| 36 | SB128 | Dry type |
| 37 | SB116.1` | Vegetable type |
| 38 | SB131 | Vegetable type |
| 39 | SB167 | Vegetable type |
| 40 | SB162 | Vegetable type |
| 41 | SB142.1 | Vegetable type |
| 42 | SB181.1 | Vegetable type |
| 43 | SB117.2 | Vegetable type |
| 44 | SB156.A | Dry type |
| 45 | SB136 | Vegetable type |
| 46 | SB159 | Vegetable type |
| 47 | SB121.1 | Vegetable type |
| 48 | SB158 | Dry type |
| 49 | E10 | Vegetable type |
| 50 | SR1 | Dry type |
| 51 | Lcl Bml | Vegetable type |



Plate 2: Representative diversity in leaf shape and size for 51 common bean genotypes



Plate 3: Representative diversity in flower colour for 51 common bean genotypes



Plate 4: Representative diversity in pod cross-section (through seed) in 51 common bean genotypes



Plate 5: Representative diversity in pod shape of distal part in 51 common bean genotypes



Plate 6: Representative diversity in pod pigmentation in 51 common bean genotypes



Plate 7: Representative diversity in Pod colour in 51 common bean genotypes



Plate 8: Representative diversity in pod length in 51 common bean genotypes

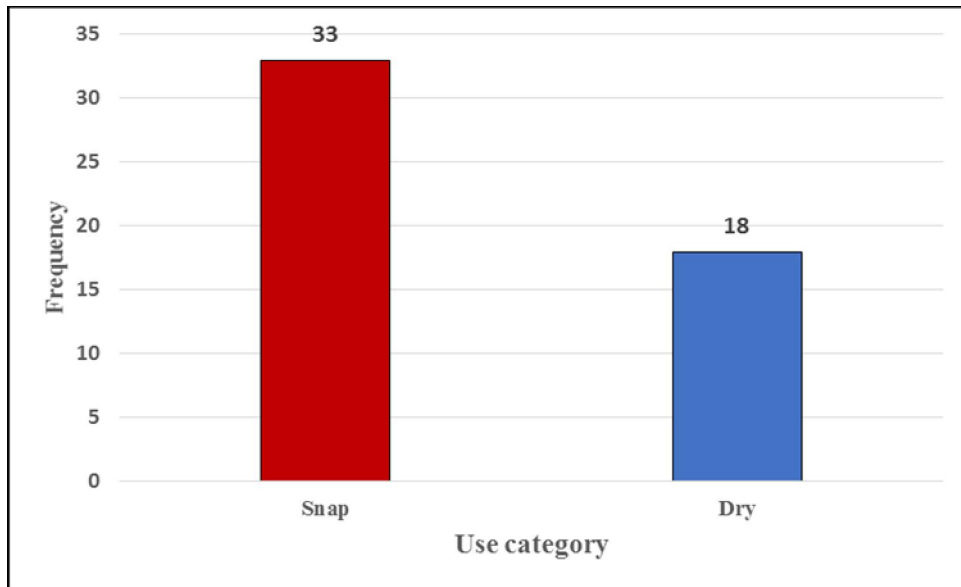


Plate 9: Representative diversity in use category (Snap/dry) of 51 common bean genotypes

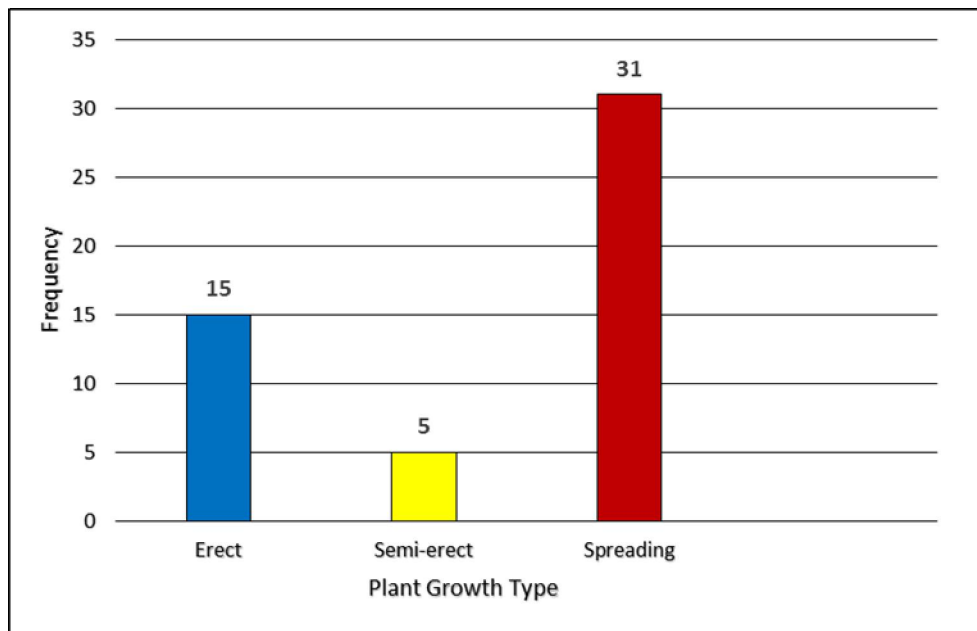


Plate 10: Representative diversity in seed testa colour and shape in 51 common bean genotypes

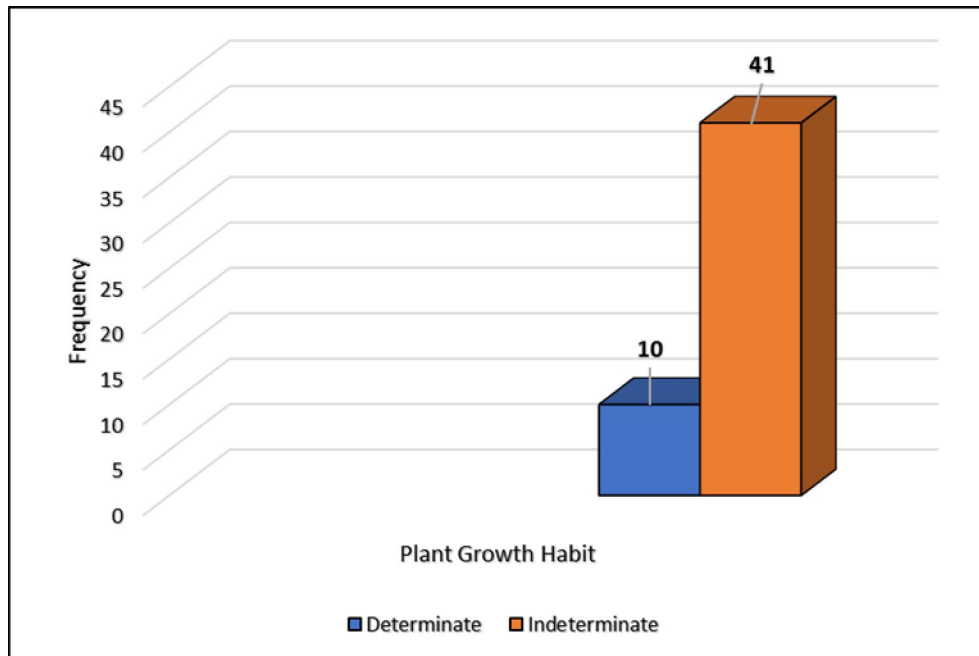
4.1.2 Graphical representation of different Qualitative Parameters



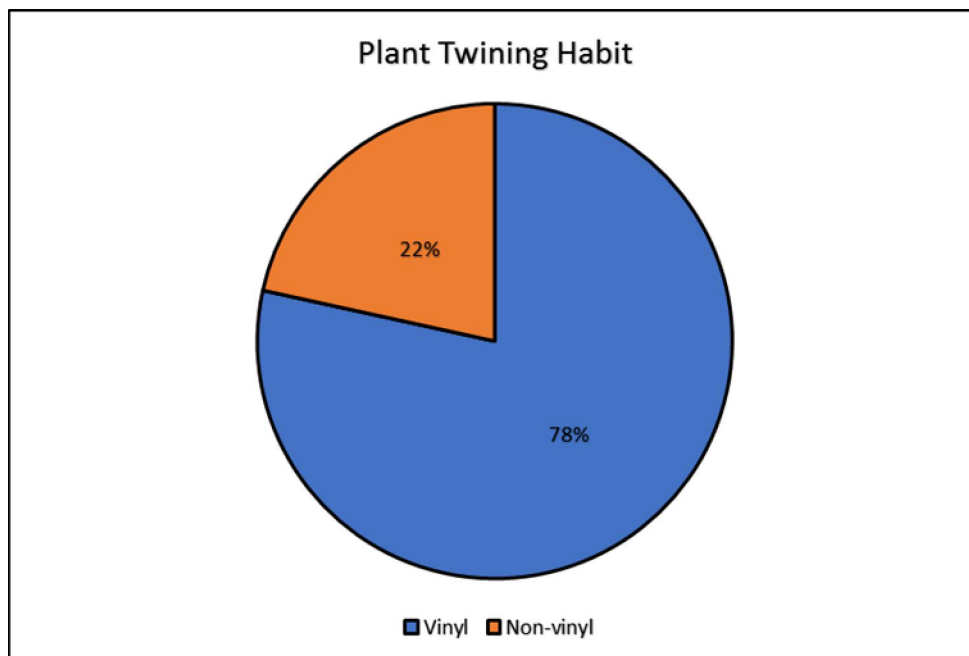
4.1.2 (a) Frequency distribution of use category



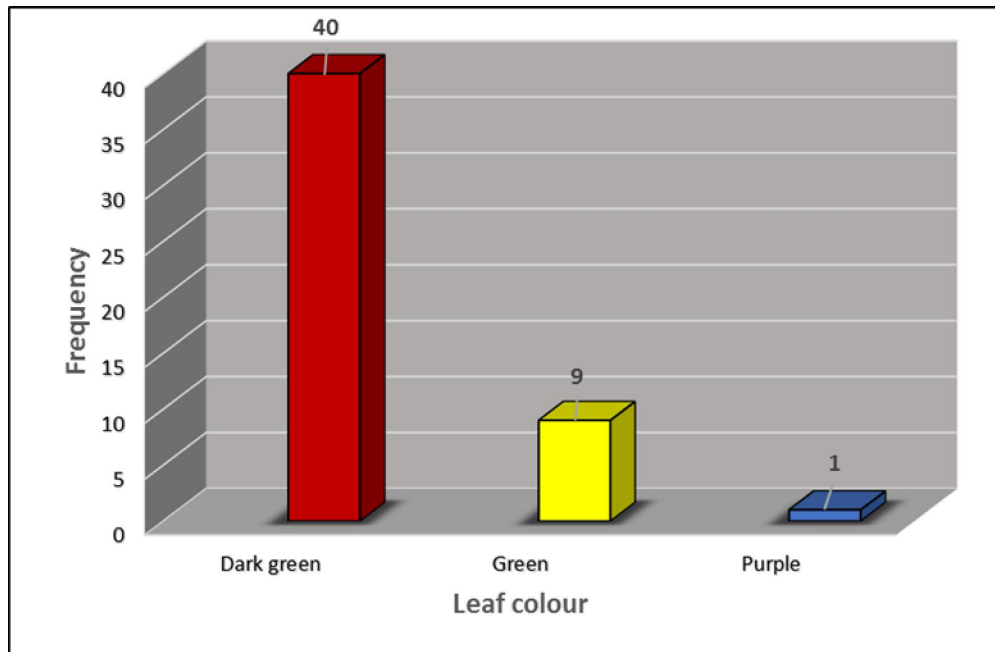
4.1.2 (b) Frequency distribution of Plant growth type



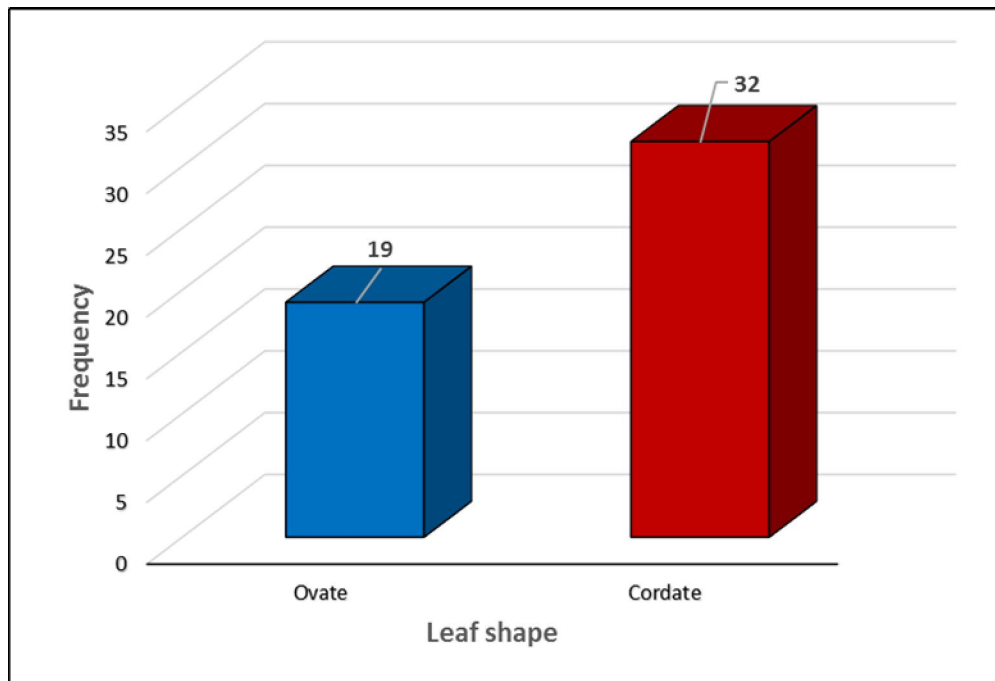
4.1.2 (c) Frequency distribution of Plant Growth Habit



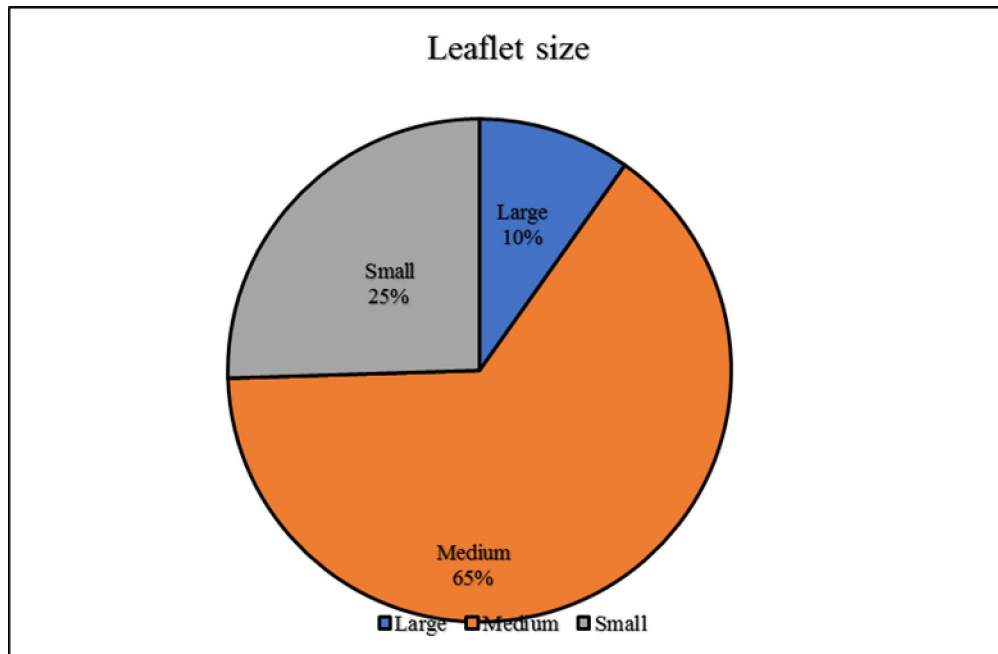
4.1.2 (d) Frequency distribution of Plant Twining Habit



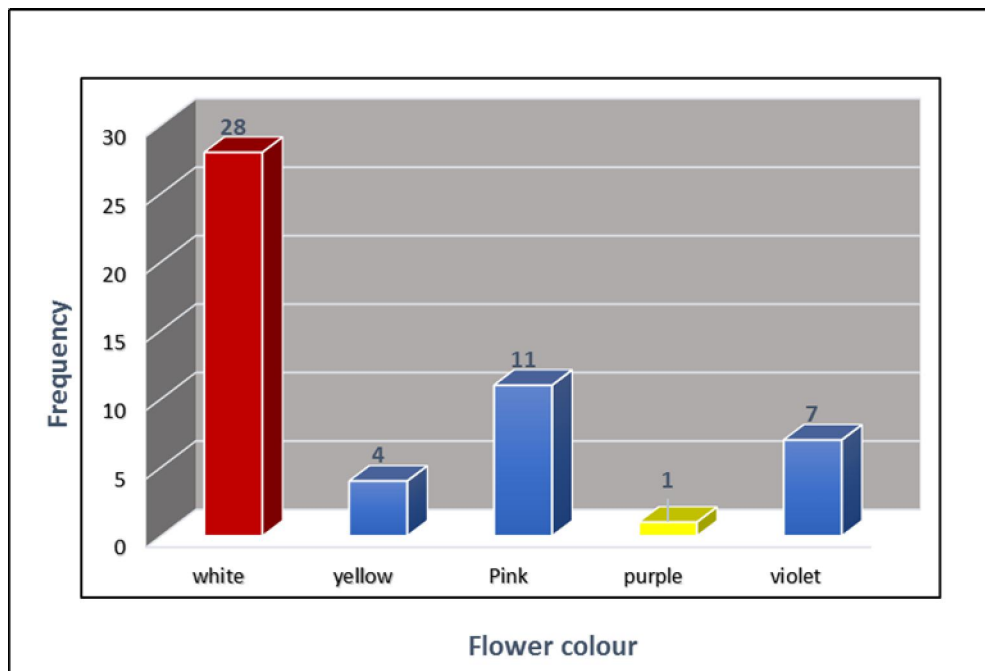
4.1.2 (e) Frequency distribution of Leaf colour



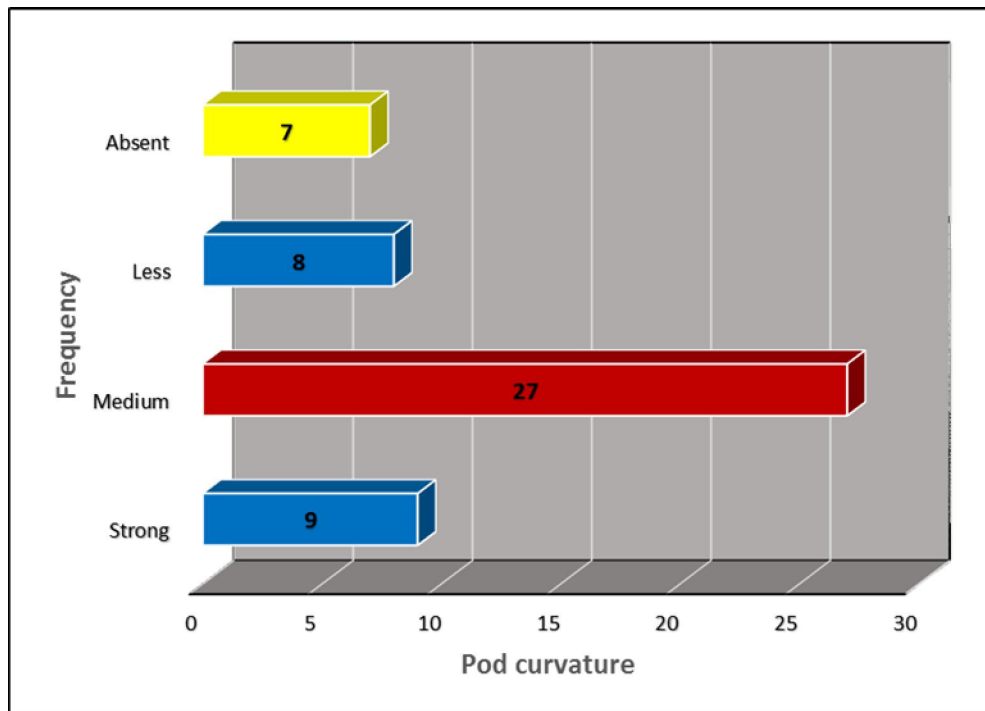
4.1.2 (f) Frequency distribution of Leaf shape



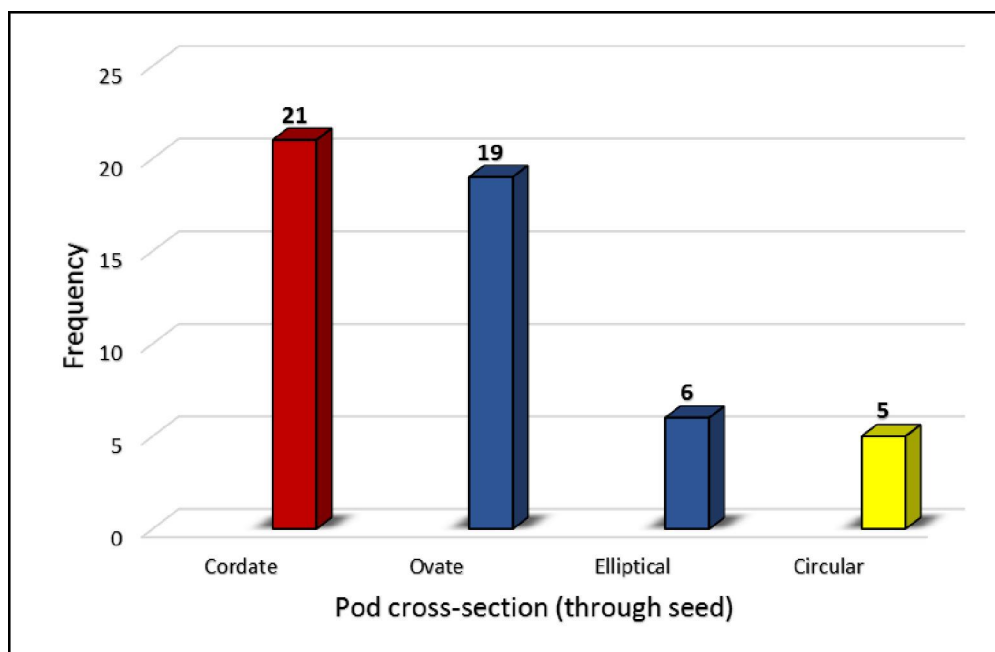
4.1.2 (g) Frequency distribution of Leaf size



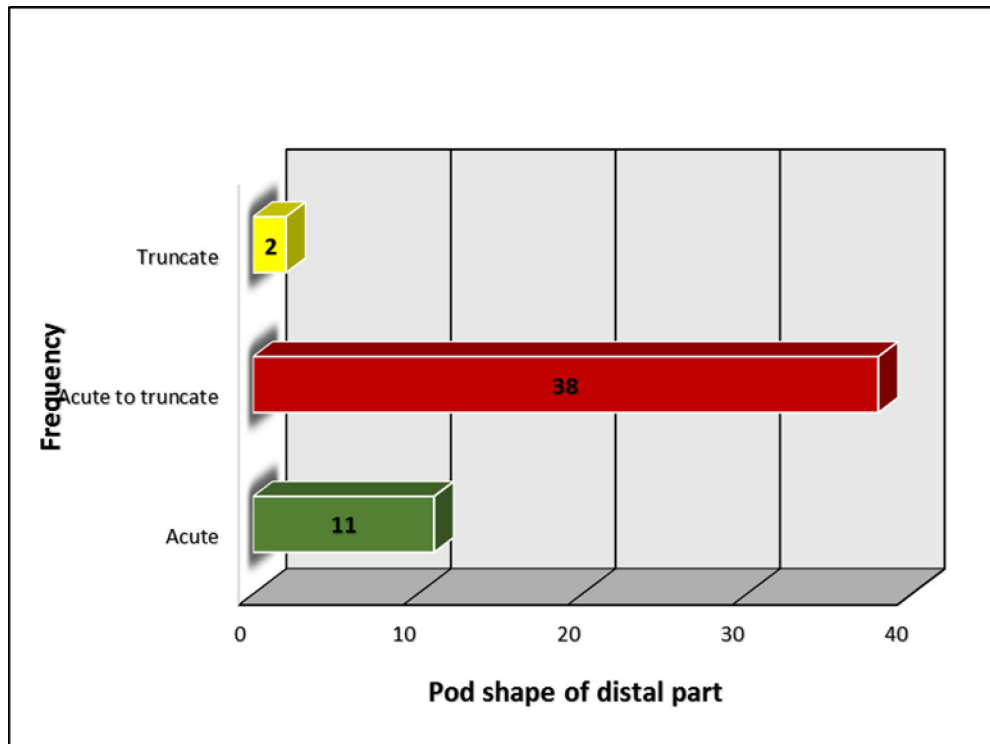
4.1.2 (h) Frequency distribution of Flower colour



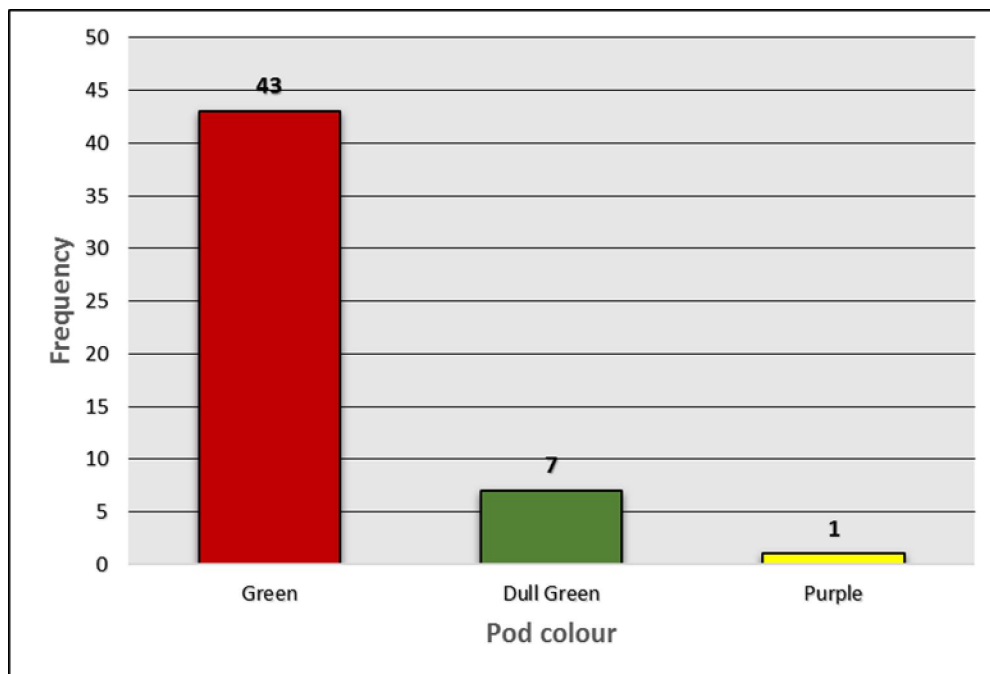
4.1.2 (i) Frequency distribution of Pod curvature



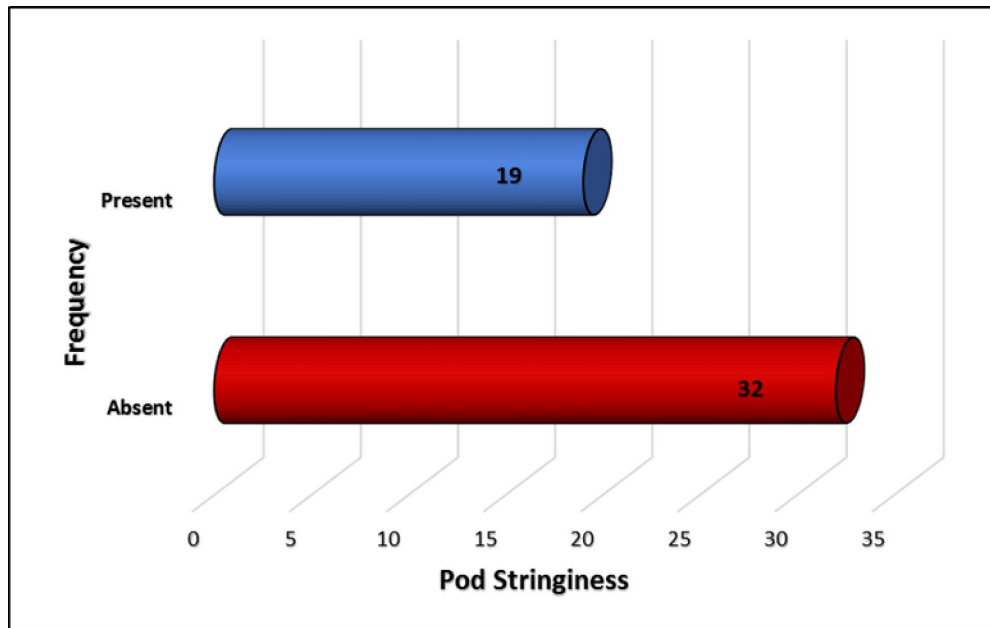
4.1.2 (j) Frequency distribution of Pod cross- section (through seed)



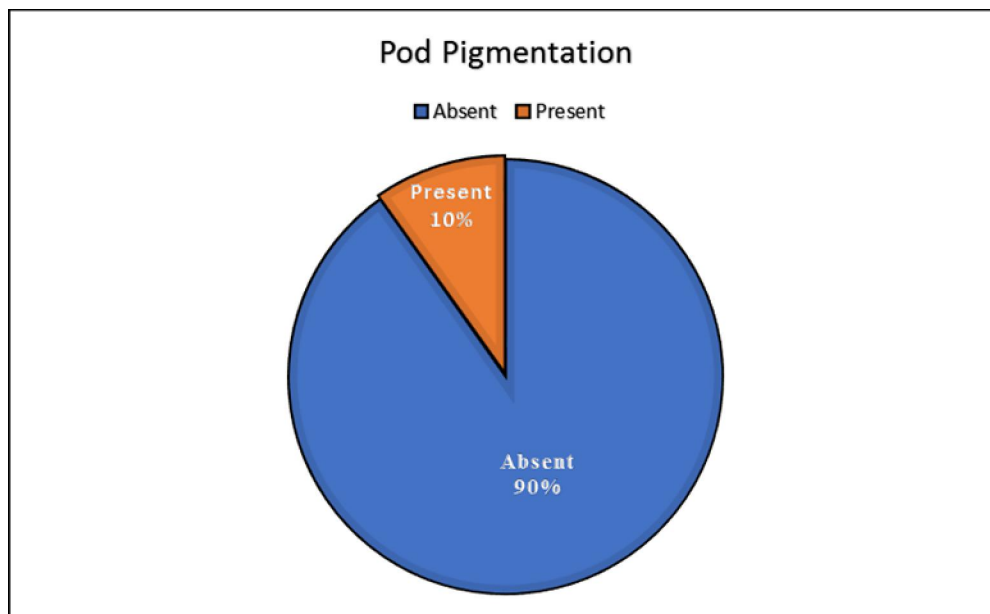
4.1.2 (k) Frequency distribution of Pod shape of distal part



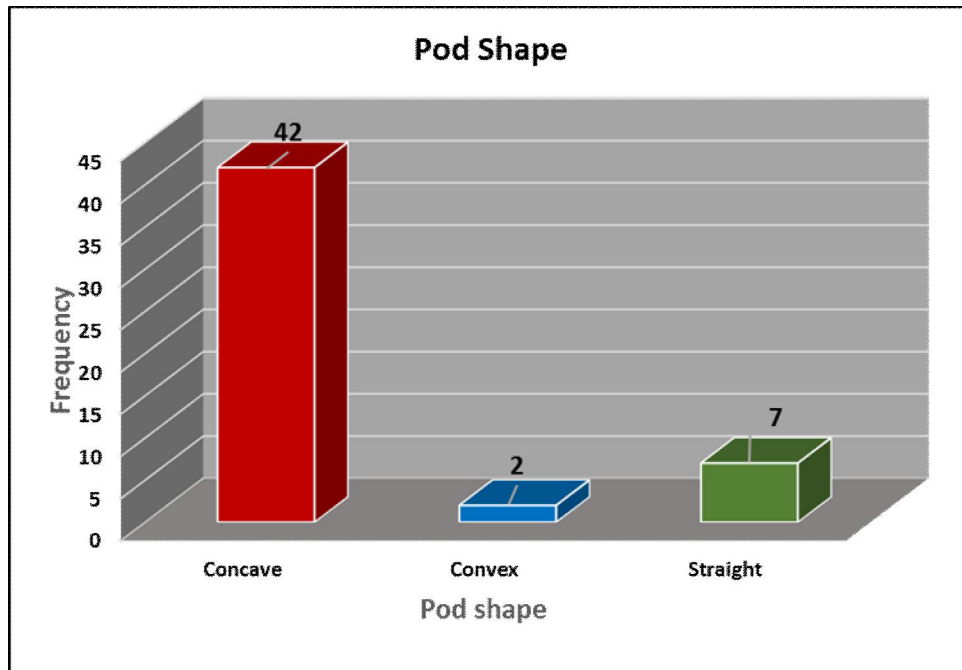
4.1.2 (l) Frequency distribution of Pod colour



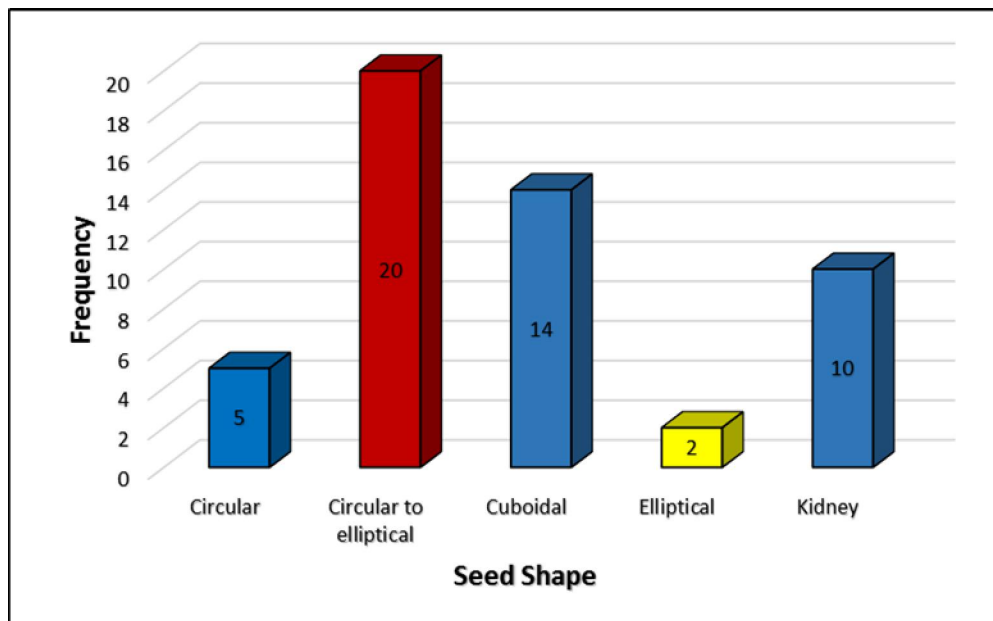
4.1.2 (m) Frequency distribution of Pod stringiness



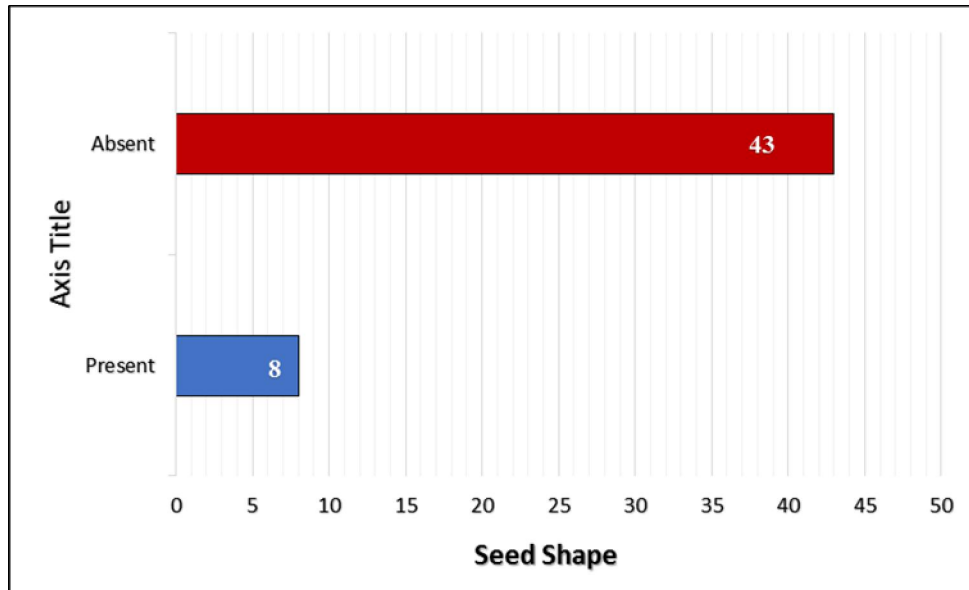
4.1.2 (n) Frequency distribution of Pod Pigmentation



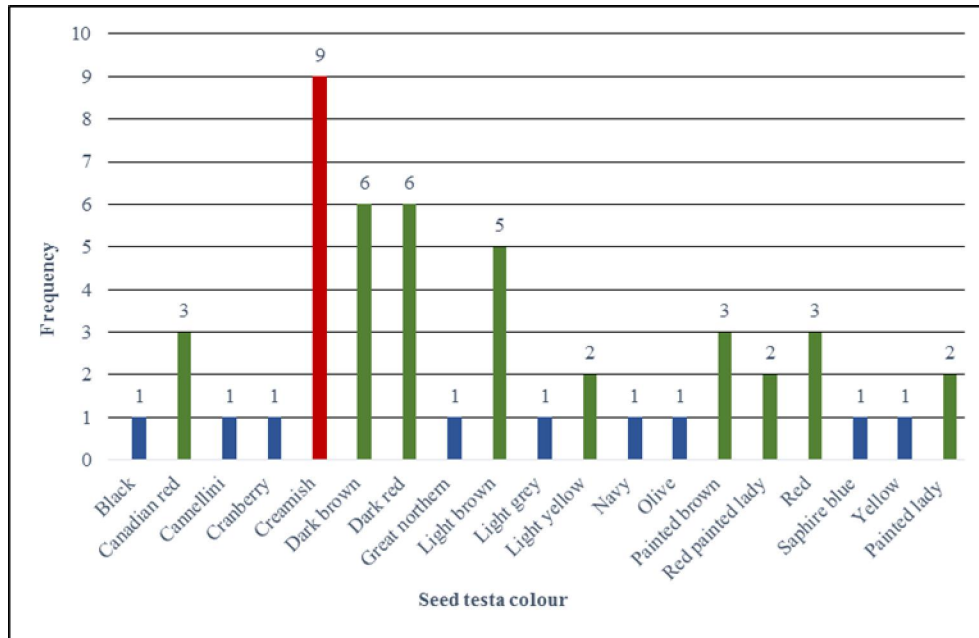
4.1.2 (o) Frequency distribution of Pod shape



4.1.2 (p) Frequency distribution of Seed shape



4.1.2 (q) Frequency distribution of Seed shape



4.1.2 (r) Frequency distribution of Seed testa colour

4.1.3 Descriptive statistics for morphological, yield and pod quality traits

The data pertaining to descriptive statistics of morphological, yield and seed quality traits in 51 genotypes of common bean is presented in Table 9. The trait-wise data of minimum, maximum, mean and standard deviation are presented below under appropriate headings.

4.1.3.1 Days to flower initiation

The mean value of days to flower initiation was 51.88 days with minimum and maximum values of 41.96 and 61.96 days respectively. The standard deviation was 5.27

4.1.3.2 Days to pod initiation

The mean value of days to pod initiation was 60.69 days with minimum and maximum values of 47.71 and 72.04 days respectively. The standard deviation was 5.75

4.1.3.3 Days to marketable pod maturity

The mean value of days to marketable maturity was 68.51 days with minimum and maximum values of 56 and 80 respectively. The standard deviation was 5.8

4.1.3.4 Number of pods per plant

The mean number of pods per plant recorded was 27.62 with minimum and maximum values of 6.2 and 60 respectively. The standard deviation was 13.1

4.1.3.5 Pod length (cm)

The mean pod length observed was 11.31 cm with minimum and maximum values of 7.8 and 14.8 cm respectively. The standard deviation was 1.74

4.1.3.6 Pod thickness (mm)

The mean pod thickness observed was 9.47 mm with minimum and maximum values of 6.4 and 11.4 mm respectively. The standard deviation was 1.08

4.1.3.7 Pod weight (g)

The mean pod weight observed was 4.49 g with minimum and maximum values of 3.01 and 5.83 respectively. The standard deviation was 0.59

4.1.3.8 Pod yield per plant (g)

The mean value of the number of pods per plant was 121.85 g with minimum and maximum values of 28 and 253.76 g respectively. The standard deviation was 55.53

4.1.3.9 Pod yield per hectare (q)

The mean value of pod yield per hectare was 304.61 with minimum and maximum values of 70 and 634.69 q/ ha respectively. The standard deviation was 138.83.

4.1.3.10 Number of seeds per pod

The mean value of the number of seeds per pod was 5.1 with minimum and maximum values of 3.5 and 7.5 respectively. The standard deviation was 1

4.1.3.11 Weight of 100 seeds in (g)

The mean value of 100 seed weight was 29.1 g with minimum and maximum values of 16.59 and 57.41 g respectively. The standard deviation was 8.87

4.1.3.12 Seed yield per plant (g)

The mean value of seed yield per plant was 38.17 g with minimum and maximum values of 10.08 and 88.5 g respectively. The standard deviation was 17.24

4.1.3.13 Seed yield per hectare (q)

The mean value of seed yield per hectare was 9.543 q with minimum and maximum values of 2.52 and 22.1 q respectively. The standard deviation was 4.31

Table 9: Descriptive statistics of morphological, yield and pod quality traits

| Trait | Min | Max | Mean | Std.Deviation |
|--------------|------------|------------|-------------|----------------------|
| DF | 41.96 | 61.96 | 51.88 | 5.27 |
| DPI | 47.71 | 72.04 | 60.69 | 5.75 |
| DMM | 56 | 80 | 68.51 | 5.8 |
| NPP | 6.2 | 60 | 27.62 | 13.1 |
| PL | 7.8 | 14.8 | 11.31 | 1.74 |
| PT | 6.4 | 11.4 | 9.47 | 1.08 |
| PW | 3.01 | 5.83 | 4.49 | 0.59 |
| PYPP | 28 | 253.76 | 121.85 | 55.53 |
| PYPH | 70 | 634.39 | 304.61 | 138.83 |
| NSPP | 3.5 | 7.5 | 5.1 | 1 |
| HSW | 16.59 | 57.41 | 29.13 | 8.87 |
| SYPP | 10.08 | 88.5 | 38.17 | 17.24 |
| SYPH | 2.52 | 22.12 | 9.54 | 4.31 |

DF= Days to flowering; **DPI**= Days to pod initiation; **DMM**= Days to marketable maturity; **NPP**= Number of pods per plant; **PL**= Pod length (cm); **PT**= Pod thickness (mm); **PW** = Pod weight (g); **PYPP**= Pod yield per plant (g); **PYPH** = Pod yield per hectare; **NSPP**= Number of seeds per pod; **HSW**= Hundred seed weight (g); **SYPP**= Seed yield per plant (g); **SYPH**= Seed yield per hectare (q)

4.1.4 Analysis of variance (ANOVA) for various Quantitative traits in common bean

In the present study, 48 test entries along with 3 checks were evaluated in an augmented block design (ABD) for 13 quantitative traits (DF= Days to flowering; DPI= Days to pod initiation; DMM= Days to marketable maturity; NPP= Number of pods per plant; PL= Pod length (cm); PT= Pod thickness (mm); PW = Pod weight (g); PYPP= Pod yield per plant (g); PYPH = Pod yield per hectare; NSPP= Number of seeds per pod; HSW= Hundred seed weight (g); SYPP= Seed yield per plant (g); SYPH= Seed yield per hectare (q)). The analysis of variance revealed the significant mean sum of squares for all the traits for different sources of variation (Table 10). It was found that the block ignoring treatments was highly significant for all the traits. However, the blocks-eliminating treatments were non-significant for all the traits indicating the homogeneity of the evaluation blocks. The treatment ignoring blocks and treatment eliminating blocks were highly significant for all the traits. Also, the test and check effect was found to be highly significant for all the traits. However, the test vs check effect was found to be highly significant for all the traits except for the trait days to pod initiation and Pod weight where it was found significant and non-significant respectively indicating that the test entries were significantly different from the checks.

Table 10: ANOVA of Augmented block design for 13 quantitative common bean traits

| Source of variation | Df | DF | DMM | DPI | HSW | NPPP | NSPP | PL (cm) | PT (mm) | PW (g) | PYPH (q) | PYPP(g) | SYPH (q) | SYPP (g) |
|--|----|---------|---------|---------|----------|-----------|---------|---------|---------|--------|------------|------------|------------|-----------|
| Block (ignoring Treatments) | 7 | 36.44** | 58.62** | 52.81** | 87.1 ** | 204.32** | 0.67** | 2.64** | 1.04** | 0.41** | 26505.21** | 4240.83** | 1285.56** | 205.69** |
| Blocks (eliminating Treatments) | 7 | 0.04 | 9.3 | 0.14 | 0.11 | 1.6 | 1.5 | 4.7 | 7.8 | 0.0024 | 31.55 | 5.05 | 2.28 | 0.36 |
| Treatment (ignoring Blocks) | 50 | 29.65** | 34.01** | 33.12** | 86.35** | 249.23** | 1.28** | 3.39** | 1.36** | 0.35** | 29398 ** | 4703.68** | 2533.95** | 405.43** |
| Treatment (eliminating Blocks) | 50 | 24.55** | 25.8 ** | 25.75** | 74.17** | 220.62** | 1.18** | 3.02** | 1.22** | 0.29** | 25691.69** | 4110.67** | 2354.29** | 376.69** |
| Test entries | 47 | 29.73** | 35.74** | 34.14** | 82.11** | 165.77** | 1.02** | 3.17** | 1.2 ** | 0.36** | 18249.9 ** | 2919.98** | 1871.33** | 299.41** |
| Checks | 2 | 32.04** | 8 ** | 24.67** | 130.38** | 308.48** | 2.67** | 6.49** | 3.83** | 0.39** | 29598.05** | 4735.69** | 13286.73** | 2125.88** |
| Test vs Checks | 1 | 21.01** | 4.34 ** | 2.25 * | 197.59** | 4053.02** | 10.67** | 7.68** | 3.78** | 0.01 | 552958.5** | 88473.36** | 12171.84** | 1947.49** |
| Error | 14 | 0.04 | 1.3 | 0.33 | 0.06 | 1.8 | 8.8 | 8.6 | 1 | 0.01 | 127.19 | 20.35 | 1.14 | 0.18 |

ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$

DF= Days to flowering; **DPI**= Days to pod initiation; **DMM**= Days to marketable maturity; **NPP**= Number of pods per plant; **PL**= Pod length (cm); **PT**= Pod thickness (mm); **PW** = Pod weight (g); **PYPP**= Pod yield per plant (g); **PYPH** = Pod yield per hectare; **NSPP**= Number of seeds per pod; **HSW**= Hundred seed weight (g); **SYPP**= Seed yield per plant (g); **SYPH**= Seed yield per hectare (q)

Table 11: Standard Error mean for 13 quantitative traits in common bean (*Phaseolus vulgaris* L.)

| Source | Standard Error (S.E) | | | | | | | | | | | | |
|---|----------------------|-----|------|------|-----|------|-----|-----|------|-------|------|------|------|
| | DF | DMM | DPI | HSW | NPP | NSPP | PL | PT | PW | PYPH | PYPP | SYPH | SYP |
| Control Treatment Means | 0.1 | 6.2 | 0.29 | 0.12 | 2.5 | 2 | 1.9 | 4.8 | 0.05 | 5.64 | 2.26 | 0.53 | 0.21 |
| Two Test Treatments (Same Block) | 0.29 | 1.7 | 0.82 | 0.35 | 7 | 5.6 | 5.4 | 1.4 | 0.15 | 15.95 | 6.38 | 1.51 | 0.6 |
| Two Test Treatments (Different Blocks) | 0.22 | 2 | 0.94 | 0.41 | 8.1 | 6.5 | 6.3 | 1.6 | 0.17 | 18.42 | 7.37 | 1.74 | 0.7 |
| A Test Treatment and a Control Treatment | 0.25 | 1.5 | 0.71 | 0.31 | 6.1 | 4.8 | 4.7 | 1.2 | 0.13 | 13.81 | 5.52 | 1.31 | 0.52 |

DF= Days to flowering; **DMM**= Days to marketable maturity; **DPI**= Days to Pod initiation; **HSW**= Hundred seed weight; **NPPP**= Number of pods per plant; **NSPP**= Number of seeds per pod; **PL**= Pod length; **PT**= Pod thickness; **PW**= Pod weight; **PYPH**= Pod yield per hectare; **PYPP**= Pod yield per plant; **SYPH**= Seed yield per hectare; **SYPP**= Seed yield per pod

4.1.5 Standard error for various quantitative traits of common bean genotypes (Table 11)

4.1.5.1 Days to flower initiation

The standard error was 0.1 for two control treatments, 0.29 for two test treatments (same block), 0.22 for two test treatments (different blocks) and 0.25 for a test treatment and a control Treatment.

4.1.5.2 Days to Marketable Maturity

The standard error was 6.2 for two control treatments, 1.7 for two test treatments (same block), 2 for two test treatments (different blocks) and 1.5 for a test treatment and a control Treatment.

4.1.5.3 Days to Pod Initiation

The standard error was 0.29 for two control treatments, 0.82 for two test treatments (same block), 0.94 for two test treatments (different blocks) and 0.71 for a test treatment and a control treatment.

4.1.5.4 Hundred Seed weight (g)

The standard error was 0.12 for two control treatments, 0.35 for two test treatments (same block), 0.41 for two test treatments (different blocks) and 0.31 for a test treatment and a control treatment.

4.1.5.5 Number of pods per plant

The standard error was 2.5 for two control treatments, 7 for two test treatments (same block), 8.1 for two test treatments (different blocks) and 6.1 for a test treatment and a control treatment.

4.1.5.6 Number of Seeds per pod

The standard error was 2 for two control treatments, 5.6 for two test treatments (same block), 6.5 for two test treatments (different blocks) and 4.8 for a test treatment and a control treatment.

4.1.5.7 Pod length (cm)

The standard error was 1.9 for two control treatments, 5.4 for two test treatments (same block), 6.3 for two test treatments (different blocks) and 4.7 for a test treatment and a control treatment.

4.1.5.8 Pod thickness (mm)

The standard error was 4.8 for two control treatments, 1.4 for two test treatments (same block), 1.6 for two test treatments (different blocks) and 1.2 for a test treatment and a control treatment.

4.1.5.9 Pod weight (g)

The standard error was 0.05 for two control treatments, 0.15 for two test treatments (same block), 0.17 for two test treatments (different blocks) and 0.13 for a test treatment and a control treatment.

4.1.5.10 Pod yield per plant (g)

The standard error was 2.26 for two control treatments, 6.38 for two test treatments (same block), 7.37 for two test treatments (different blocks) and 5.52 for a test treatment and a control treatment.

4.1.5.11 Pod yield per hectare (q)

The standard error was 5.64 for two control treatments, 15.95 for two test treatments (same block), 18.42 for two test treatments (different blocks) and 13.81 for a test treatment and a control treatment.

4.1.5.12 Seed yield per hectare (q)

The standard error was 0.53 for Two control treatments, 1.51 for two test treatments (same block), 1.74 for two test treatments (different blocks) and 1.31 for a test treatment and a control treatment.

4.1.5.13 Seed yield per plant (g)

The standard error was 0.21 for two control treatments, 0.6 for two test treatments (same block), 0.7 for two test treatments (different blocks) and 0.52 for a test treatment and a control treatment.

Table 12: Critical Difference (5%) for 13 Quantitative traits of common bean

| Source | CD (5%) | | | | | | | | | | | | |
|--|---------|-----|------|------|-----|------|-----|-----|------|-------|-------|------|------|
| | DF | DMM | DPM | HSW | NPP | NSPP | PL | PT | PW | PYPH | PYPP | SYPH | SYPP |
| Control Treatment Means | 0.22 | 1.3 | 0.62 | 0.27 | 5.3 | 4.2 | 4.1 | 1 | 0.11 | 12.09 | 4.84 | 1.14 | 0.46 |
| Two Test Treatments (Same Block) | 0.62 | 3.7 | 1.75 | 0.76 | 1.5 | 1.2 | 1.2 | 2.9 | 0.31 | 34.21 | 13.68 | 3.23 | 1.29 |
| Two Test Treatments (Different Blocks) | 0.71 | 4.3 | 2.02 | 0.87 | 1.7 | 1.4 | 1.3 | 3.4 | 0.26 | 39.5 | 15.8 | 3.73 | 1.49 |
| A Test Treatment and a Control Treatment | 0.54 | 3.2 | 1.52 | 0.66 | 1.3 | 1 | 1 | 2.5 | 0.27 | 29.62 | 11.85 | 2.8 | 1.12 |

DF= Days to flowering; **DMM**= Days to marketable maturity; **DPI**= Days to Pod initiation; **HSW**= Hundred seed weight; **NPPP**= Number of pods per plant; **NSPP**= Number of seeds per pod; **PL**= Pod length; **PT**= Pod thickness; **PW**= Pod weight; **PYPH**= Pod yield per hectare; **PYPP**= Pod yield per plant; **SYPH**= Seed yield per hectare; **SYPP**= Seed yield per pod

Table 13: Estimates of mean, range, phenotypic variance, genotypic variance, phenotypic and genotypic coefficients of variation for various quantitative traits in common bean (*Phaseolus vulgaris L.*)

| Trait | Mean | PV | GV | PCV | GCV | h² (%) | GAM |
|-----------------|-------------|-----------|-----------|------------|------------|--------------------------|------------|
| DF | 51.88 | 29.69 | 29.20 | 10.76 | 10.5 | 89.5 | 21.65 |
| DPI | 60.69 | 34.14 | 33.81 | 9.63 | 9.58 | 92.7 | 19.67 |
| DMM | 68.51 | 35.74 | 34.92 | 8.73 | 8.41 | 87.25 | 18 |
| NPP | 27.62 | 165.77 | 165.75 | 46.62 | 46.60 | 99.8 | 96.17 |
| PL (cm) | 11.31 | 3.21 | 3.17 | 15.74 | 15.71 | 99.5 | 32.47 |
| PT (mm) | 9.47 | 1.25 | 1.20 | 11.59 | 11.58 | 99.8 | 23.91 |
| PW (g) | 4.49 | 0.36 | 0.35 | 13.3 | 13.1 | 97.03 | 26.63 |
| PYPP (g) | 121.85 | 2919.98 | 2899.63 | 44.35 | 44.19 | 99.3 | 90.85 |
| NSPP | 5.1 | 1.02 | 1.0 | 19.76 | 19.75 | 99.96 | 40.77 |
| HSW (g) | 29.13 | 82.11 | 82.05 | 31.11 | 31.10 | 99.92 | 64.13 |
| SYPP (g) | 38.17 | 299.41 | 299.23 | 45.33 | 45.32 | 99.94 | 93.46 |

DF= Days to flowering; **DMM**= Days to marketable maturity; **DPI**= Days to Pod initiation; **HSW**= Hundred seed weight; **NPP**= Number of pods per plant; **NSPP**= Number of seeds per pod; **PL**= Pod length; **PT**= Pod thickness; **PW**= Pod weight; **PYPH**= Pod yield per hectare; **PYPP**= Pod yield per plant; **SYPH**= Seed yield per hectare; **SYPP**= Seed yield per pod

4.1.6 Variability and genetic components of variation in common bean

Since the phenotypic variation roughly represents the magnitude of divergence present among different genotypes and cannot differentiate between genetic and environmental effects, it is necessary to divide the phenotypic or observed variation into heritable (variation due to genotype) and environmental components for which different biometrical and genetical methods were employed. The estimates of mean, range, phenotypic and genotypic variance, as well as phenotypic and genotypic coefficients of variance, are shown in Table 13.

The estimations of phenotypic and genotypic coefficients of variation are more accurate estimates of genetic variation and by comparing the coefficients of genotypic and phenotypic variation, one can determine the relative amount of variation for various characters.

The degree to which a character may be passed down from parent to offspring is typically assessed using heritability, a measure of the genetic relationship between parent and progeny. Estimating the genetic advance from selection requires an understanding of the heritability of the character. The result of heritability and percentage of genetic advance is also present in Table 13. A certain trait is selected based on its phenotype, which is produced by the interaction of a person's genotype and environment. Genetic advance gives a good idea of actual position (Jonson *et al.*, 1955). Comparative performance of all the genotypes for various traits has been described as follows.

4.1.6.1 Days to flower initiation

The genotypes under study possessed a sufficient amount of variability for this character. Days to 50% flower had an overall mean of 51.88. The phenotypic and genotypic variance for this trait were 29.69 and 29.20 respectively. The phenotypic and genotypic coefficient of variation was found to be 10.76 and 10.5 respectively. A very high heritability (89.5%) along with a high genetic advance as a per cent of mean (21.65) was observed for this trait.

4.1.6.2 Days to Pod initiation

The genotypes under study showed an ample amount of variability for this character. Days to Pod initiation had an overall mean of 60.69. The phenotypic and genotypic variance for this trait were 34.14 and 33.81 respectively. The phenotypic and genotypic coefficient of variation was found to be 9.63 and 9.58 respectively. A very high heritability (92.7 %) along with a high genetic advance as a per cent of mean (19.67) was observed for this trait.

4.1.6.3 Days to marketable maturity

The genotypes under study possessed a sufficient amount of variability for this character. Days to marketable maturity showed an overall mean of 68.51. The phenotypic and genotypic variance for this trait were 35.74 and 35.92 respectively. The phenotypic and genotypic coefficient of variation was found to be 8.73 and 8.41 respectively. A very high heritability (87.25%) along with a moderate genetic advance as a per cent of mean (18) was observed for this trait.

4.1.6.4 Number of pods per plant

The genotypes under study showed a considerable amount of variation for this trait. The number of pods per plant showed an overall mean of 27.62. The phenotypic and genotypic variance for this trait were 165.77 and 165.75 respectively. The phenotypic and genotypic coefficient of variation was found to be 46.62 and 46.60 respectively. A very high heritability (99.8%) along with a high genetic advance as a per cent of mean (96.17) was observed for this trait.

4.1.6.5 Pod length (cm)

There were significant differences among all the genotypes for this character. Pod length showed an overall mean of 11.31. The phenotypic and genotypic variance for this trait were 3.21 and 3.17 respectively. The phenotypic and genotypic coefficient of variation was found to be 15.74 and 15.71 respectively. High heritability (99.5%) coupled with a high genetic advance as a per cent of mean (32.47) was observed for this trait.

4.1.6.6 Pod thickness (mm)

The genotypes under study possessed a good amount of variability for this character. Pod thickness showed an overall mean of 9.47. The phenotypic and genotypic variance for this trait were 1.25 and 1.20 respectively. The phenotypic and genotypic coefficient of variation was found to be 11.59 and 11.58 respectively. High heritability (99.8%) coupled with a high genetic advance as a per cent of mean (23.91) was observed for this trait.

4.1.6.7 Pod weight (g)

Pod weight showed an overall mean of 4.49. The phenotypic and genotypic variance for this trait were 0.36 and 0.35 respectively. The phenotypic and genotypic coefficient of variation was found to be 13.3 and 13.1 respectively. High heritability (97.03%) along with a very high genetic advance as a per cent of mean (26.63) was observed for this trait.

4.1.6.8 Pod yield per plant (g)

The genotypes under study possessed a good amount of variability for this character with an overall mean of 121.85. The phenotypic and genotypic variance for this trait were 2919.98 and 2899.63 respectively. The phenotypic and genotypic coefficient of variation was found to be 44.35 and 44.19 respectively. High heritability (99.3%) and a very high genetic advance as a per cent of mean (90.85) were observed for this trait.

4.1.6.9 Number of seeds per pod

The number of seeds per pod showed an overall mean of 5.1. The phenotypic and genotypic variance for this trait were 1.02 and 1.0 respectively. The phenotypic and genotypic coefficient of variation was found to be 19.76 and 19.75 respectively. High heritability (99.96%) and a high genetic advance as a per cent of mean (40.77) were observed for this trait.

4.1.6.10 Hundred seed weight (g)

The genotypes under study possessed a good amount of variability for this character with an overall mean of 29.13. The phenotypic and genotypic variance for this trait were 82.11 and 82.05 respectively. The phenotypic and genotypic coefficient of variation was found to be 31.11 and 31.10 respectively. A very high heritability (99.92%) along with a high genetic advance of 2.08 was observed for this trait.

4.1.6.11 Seed yield per plant (g)

The seed yield per plant showed an overall mean of 38.17. The phenotypic and genotypic variance for this trait were 299.41 and 299.23 respectively. The phenotypic and genotypic coefficient of variation was found to be 45.33 and 45.32 respectively. A very high heritability (99.94 %) along with a high genetic advance as a per cent of mean (93.46) was observed for this trait.

4.1.7 Coefficient of variability

The genotypic and phenotypic coefficients of variation are the simplest measures of variability. The relative level of variability in a genetic population is shown by the relative values of different types of coefficients. As a result, the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was calculated. The phenotypic coefficients of variation were somewhat greater than the genotypic coefficients of variation for all the traits, indicating that variation in these traits is attributed to both genetic and environmental factors. According to Sivasubramanian and Madhavamenon (1973), the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was classified as low (less than 10%), moderate (10-20%) and high (more than 20%). The genotypic coefficient of variation was lowest for days to marketable maturity (8.41) and highest for number of pods per plant. (46.60). The phenotypic coefficient of variation was lowest for days to marketable maturity (8.73) and highest for number of pods per plant (46.62).

4.1.7.1 Heritability (h^2_{bs}) and genetic advance as per cent of mean (GAM)

Estimates of heritability in the broad sense (h^2_{bs}) for 11 characters in common bean genotypes are shown in Table 13. The estimates of heritability in broad sense (h^2_{bs}) ranged from 87.25 per cent (days to marketable maturity) to 99.96 per cent (number of pods per plant). The genetic gain was expressed as per cent of mean. It ranged from 18 (days to marketable maturity) to 96.17 (number of pods per plant). The high estimates of heritability (>60%) coupled with high genetic advance as per cent of mean were observed for the majority of characters suggesting that these characters are governed by additive gene effects to a greater extent and improvement of these traits would be effective through selection.

4.1.8 Correlation coefficient

The degree of correlation of plant traits has historically been useful for selection in plant breeding. The existence of an association between different characters is usually determined by the existence of a correlation between them. For this aim, it is necessary to understand the genetic correlation between various traits, which may provide information about the correlated response to selection. Pearson's correlation coefficients were calculated using variances and covariances to determine the association between various characters and their relationship with an economically desirable trait i.e., pod yield per plant. Table 14 shows the correlation matrix for 11 quantitative traits and is mentioned under appropriate headings. Figure 3 depicts the heat map of Pearson's correlation trait association.

4.1.8.1 Positive and Significant correlation

In the present study, the number of days to flower initiation was significantly and positively correlated with the number of days to maturity (0.93) and days to Pod initiation (0.96). Number of days to maturity showed a significant and positive correlation with Days to Pod initiation (0.98). Significant and positive correlations were also seen in the case of number of pods per plant with

pod length (0.58), pod weight (0.38), pod thickness (0.42,) and pod yield per plant (0.95). The pod yield per plant also showed a significant and positive correlation with seed yield per plant (0.35), pod length (0.66), pod weight (0.41) and pod thickness (0.56). Also, the pod thickness showed a positive and significant correlation with seed yield per plant (0.29), no. of seeds per pod (0.29), pod length (0.58) and pod weight (0.47). Similarly, a positive and significant correlation was found between pod weight and pod length (0.74), hundred seed weight and seed yield per plant (0.39).

4.1.8.2 Negative and Significant Correlation

In the present study, number of days to flower initiation showed a negative and significant correlation with seed yield per plant (-0.48), hundred seed weight (-0.29), pod length (-0.28) and pod weight (0.31). Similarly, days to pod initiation also showed a negative and significant correlation with seed yield per plant (-0.51), pod length (-0.30), pod weight (-0.40) and pod thickness (-0.28). A negative and significant correlation was found between days to marketable maturity and seed yield per plant (-0.52), pod length (-0.30) and pod weight (-0.43). The number of pods per plant showed a negative and significant correlation with the Hundred seed weight (0.30).

4.1.9 Identification of most promising/ best vegetable type genotypes

The perusal of Table 15 indicated that the genotypes WB1306 (days to flower initiation, days to pod initiation and number of seeds per pod), SB106 (pod length, pod weight, hundred seed weight), SB167 (days to marketable maturity and number of pods per plant), WB469 (pod thickness), SB156. A (pod yield per plant) and SB152 (seed yield per plant) were found as the most promising genotypes for different yield-related traits.

Table 14: Correlation matrix for morphological traits in Common bean

| S. No. | Parameters | PW (g) | PL (cm) | PT (mm) | NPP | DF | DPI | DMM | PYPP(g) | NSPP | HSW | SYPP (g) |
|--------|-----------------|----------|----------|-----------|-----------|-----------|------------|-----------|-----------|----------|----------|----------|
| 1 | PW (g) | 1 | | | | | | | | | | |
| 2 | PL (cm) | 0.736*** | 1 | | | | | | | | | |
| 3 | PT (mm) | 0.467*** | 0.581*** | 1 | | | | | | | | |
| 4 | NPP | 0.381** | 0.584*** | 0.418 | 1 | | | | | | | |
| 5 | DF | -0.306* | -0.282* | -0.220 ns | -0.026 ns | 1 | | | | | | |
| 6 | DPI | -0.40** | -0.301* | -0.278* | -0.072 ns | 0.955*** | 1 | | | | | |
| 7 | DMM | -0.431** | -0.297* | -0.270 ns | -0.089 ns | 0.926*** | 0.984*** | 1 | | | | |
| 8 | PYPP (g) | 0.406** | 0.663** | 0.562*** | 0.950*** | -0.124 ns | -0.167 ns | -0.180 ns | 1 | | | |
| 9 | NSPP | 0.223 ns | 0.252 | 0.293* | -0.04 ns | -0.186 ns | -0.236 ns | -0.191 ns | 0.054 ns | 1 | | |
| 10 | HSW (g) | 0.02 ns | -0.028 | 0.01 ns | -0.301* | -0.289* | -0.254 ns | -0.271 ns | -0.168 ns | 0.131 ns | 1 | |
| 11 | SYPP (g) | 0.09 ns | 0.174 | 0.29* | 0.20 ns | -0.48*** | -0.510 *** | -0.518*** | 0.345* | 0.225 ns | 0.386 ** | 1 |

ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$

DF= Days to flowering; **DMM**= Days to marketable maturity; **DPI**= Days to Pod initiation; **HSW**= Hundred seed weight; **NPP**= Number of pods per plant; **NSPP**= Number of seeds per pod; **PL**= Pod length; **PT**= Pod thickness; **PW**= Pod weight; **PYPP**= Pod yield per plant; **SYPP**= Seed yield per pod

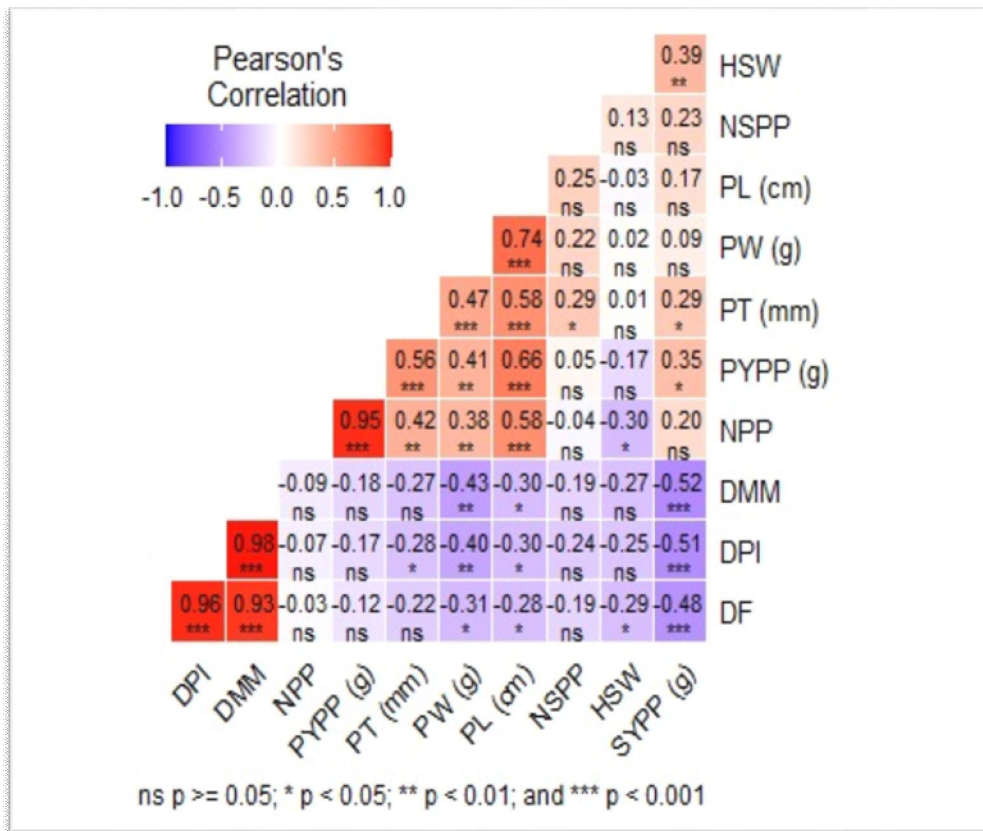


Fig. 3: Heat map showing Pearson's correlation between morphological traits

Table 15: Best Snap/Vegetable type genotypes in respect of various yield and yield attributing traits in Common bean (*Phaseolus vulgaris* L.) based on performance

| S.No. | Characters | Best genotypes |
|-------|-----------------------------|---|
| 1. | Days to flower initiation | WB1306 (43.29), IC-328438 (44.29), SB142.1 (44.96), WB469 (45.96), NBPGR-1 (45.96) |
| 2. | Days to pod initiation | WB1306 (49.04), IC-328438 (52.04), SB162 (54.04), WB192 (54.71), SKUA-44 (55.04) |
| 3. | Days to marketable maturity | SB167 (56), WB192 (60), IC-328438 (61), WB1306 (62), SB162 (65) |
| 4. | Number of pods per plant | SB167 (60), SB152 (53), SB156.A (52), SB159 (50.2), SB181.1 (45) |
| 5. | Pod length (cm) | SB106 (14.8cm), SB136 (14.68cm), WB469 (14.5cm), SB142.1 (13.83cm), SB152 (13.5cm) |
| 6. | Pod thickness (mm) | WB469 (10.77mm), SB19 (10.5mm), SB117.2 (10.4mm), SB181.1 (10.3mm), IC-326603 (10.17mm) |
| 7. | Pod weight (g) | SB106 (5.21g), WB469 (5.19g), SB151 (4.92g), SB156.A (4.89g), SB19 (4.87g) |
| 8. | Pod yield per plant (g) | SB156.A (253.76g), SB159 (213.92g), SB152 (211.43g), SB181.1 (197.44g), SB167 (186.78g) |
| 9. | Number of seeds per pod | WB1306 (7.5), IC-326619 (7), SB107 (6.6), SB117.2 (6.5), EC-385256 (6.5) |
| 10. | Hundred seed weight (g) | SB106 (37.24g), SB166 (35.5g), SB107 (34.97g), WB192 (34.9g), IC-326619 (32.13g) |
| 11. | Seed yield per plant (g) | SB152 (75.62g), SB107 (66.47g), SB166 (65.45g), WB1306 (59.09g), WB1189 S(58.19g) |

4.1.8 Clustering pattern based on morphological data:

Knowledge of the genetic diversity of a crop and its quantitative assessment usually helps a plant breeder in choosing desirable parents for the breeding programme. Geographic diversity in crop plants very often fails to convey information about genetic divergence. Therefore, it is worthwhile to use a suitable tool like D^2 statistics (Mahalanobis, 1936) as a quantitative measure of genetic divergence.

4.1.8.1 Distribution of genotypes into different clusters

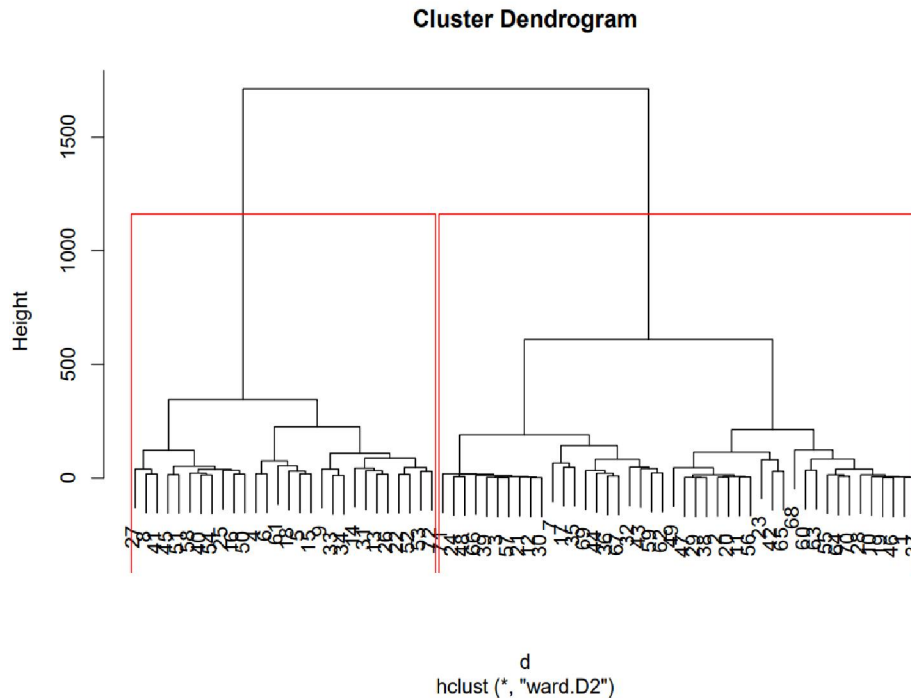
Based on the performance of genotypes, fifty-one Common bean genotypes were grouped into two clusters (Table 16). The cluster dendrogram indicated that the maximum number of genotypes fell in cluster II (30). Cluster I consisted of 21 genotypes i.e, WB603, EC-385256, SB106, WB1189, SB111, Lcl Bml, SB149, WB1199, IC-326854, SB152, EC-21753, SB167, WB1306, NBPGR-1, IC-326619, EC-394659, E1VENYL, E2, IC-326603, WB192, SB165. Cluster II consisted of 30 common bean genotypes i.e, SB159, WB1151, SR1, NBPGR-2, IC-328438, SB123, SB156.A, SB183.1, SB107, SB169, WB1187, SB166, SB116.1, SB162, SB121.1, SB158, WB665, SB19, E10, SB117.2, SB131, SB142.1, SB128, SB181.1, SB136, WB469, NBPGR-3, WB832, SB155.1, SB151. The formation of different clusters with a variable number of entries in each cluster indicated diversity among genotypes. The genotypes collected from different agro-climatic regions were found to be scattered in different clusters, which suggested that a pattern of clustering of accessions was independent of their geographic origin.

4.1.8.2 Cluster means

The data presented in Table 17 showed that cluster means for different traits had considerable differences between the clusters. The two clusters had in general shown medium mean performance for most of the characters under study.

Cluster means for different characters (Table-17) revealed that Cluster-I having 21 genotypes expressed a mean pod length, pod thickness, pod weight and hundred seed weight of 11.26 cm, 9.56 mm, 4.49 g and 30.62 g respectively. The mean number of pods per plant and the number of seeds per pod observed were 17.73 and 5.17 respectively. It took 50.67 days for the flowering, 59.35 and 67.17 days for pod initiation and marketable maturity respectively with average pod yield plant⁻¹ and pod yield hectare⁻¹ of 77.73 g and 194.33 q respectively. The seed yield plant⁻¹ and seed yield hectare⁻¹ were 26.52 g and 6.63 q respectively.

The Cluster-II having maximum (30) genotypes expressed a mean pod length, pod thickness, pod weight and hundred seed weight of 11.02 cm, 9.62 mm, 4.50 g and 26.59 g respectively. The mean number of pods per plant and the number of seeds per pod observed were 41.07 and 4.69 respectively. It took 53.15 days for the flowering, 61.70 and 69.59 days for pod initiation and marketable maturity respectively with average pod yield plant⁻¹ and pod yield hectare⁻¹ of 183.30 g and 458.27 q respectively. The seed yield plant⁻¹ and seed yield hectare⁻¹ were 50.58 g and 12.64 q respectively.



27= WB603, 8= EC-385256, 41= SB106, 45=WB1189, 51= SB111, 58, 40, 54, 25, 16, 50, 4, 6= C3 (Lcl Bml), 61= SB149, 18 =WB1199, 5= IC-326854, 15= SB152, 9= EC-21753, 33= SB167, 34= WB1306, 14= NBPGR-1, 31= IC-326619, 13= EC-394659, 26= E1VENYL, 22= E2, 52= IC-326603, 53= WB192, 72= SB165, 71=SB159, 24= WB1151, 48, 66, 39, 3, 57, 21, 12, 30= C2 (SR1), 7= NBPGR-2, 17= IC-328438 35= SB123, 69= SB156.A, 44= SB183.1, 36= SB107, 67= SB169, 32= WB1187, 43= SB166, 59= SB116.1, 62= SB162, 49= SB121.1, 47, 29, 38, 2, 20, 11, 56, 23= C1 (E10), 42= SB19, 65= WB665, 68=SB117.2, 60= SB131, 63= SB142.1, 55= SB128, 64= SB181.1, 70= SB136, 28= WB469, 10= NBPGR-3, 19= WB832, 46= SB155.1, 1= SB158, 37= SB151

Fig. 4: Distribution of Common bean genotypes into different clusters as depicted by cluster dendrogram (Morphological analysis)

Table 16: Distribution of Common bean (*Phaseolus vulgaris* L.) genotypes into clusters based on morphological data

| Cluster | Total number of genotypes in the cluster | Name of the genotypes included in different clusters |
|---------|--|--|
| I | 21 | WB603 (27), EC-385256 (8), SB106 (41), WB1189 (45), SB111 (51), Lcl Bml (58, 40, 54, 25, 16, 50, 4, 6), SB149 (61), WB1199 (18), IC-326854 (5), SB152 (15), EC-21753 (9), SB167 (33), WB1306 (34), NBPGR-1 (14), IC-326619 (31), EC-394659 (13), E1VENYL (26), E2 (22), IC-326603 (52), WB192 (53), SB165 (72). |
| II | 30 | SB159 (71), WB1151 (24), SR1 (48, 66, 39, 3, 57, 21, 12, 30), NBPGR-2 (7), IC-328438 (17), SB123 (35), SB156.A (69), SB183.1 (44), SB107 (36), SB169 (67), WB1187 (32), SB166 (43), SB116.1 (59), SB162 (62), SB121.1 (49), E10 (47, 29, 38, 2, 20, 11, 56, 23), SB19 (42), WB665 (65), SB117.2 (68), SB131 (60), SB142.1 (63), SB128 (55), SB181.1 (64), SB136 (70), WB469 (28), NBPGR-3 (10), WB832 (19), SB155.1 (46), SB158 (1), SB151 (37). |

Table 17: Cluster means for various yield characters in different clusters of Common bean genotypes (Tocher Method)

| S.no | Cluster | DF | DPI | DMM | NPP | PL (cm) | PT (mm) | PW (g) | PYPP (g) | PYPH (q) | NSPP | HSW (g) | SYPP (g) | SYPH (q) |
|------|---------|-------|-------|-------|-------|------------|------------|-----------|-------------|-------------|------|------------|-------------|-------------|
| 1. | I | 50.67 | 59.35 | 67.17 | 17.73 | 11.26 | 9.56 | 4.49 | 77.73 | 194.33 | 5.17 | 30.62 | 26.52 | 6.63 |
| 2. | II | 53.15 | 61.70 | 69.59 | 41.07 | 11.02 | 9.62 | 4.50 | 183.30 | 458.27 | 4.69 | 26.59 | 50.58 | 12.64 |

DF= Days to flowering; **DMM**= Days to marketable maturity; **DPI**= Days to Pod initiation; **HSW**= Hundred seed weight; **NPPP**= Number of pods per plant; **NSPP**= Number of seeds per pod; **PL**= Pod length; **PT**= Pod thickness; **PW**= Pod weight; **PYPP**= Pod yield per plant; **SYPP**= Seed yield per pod

4.2 Molecular characterization of Common bean (*Phaseolus vulgaris* L.) germplasm using SSR markers

4.2.1 SSR marker analysis

Clear DNA bands of the genotypes obtained in this study are depicted in Plate-11. Table- 18 presents the summarized data of 11 chromosome-specific SSR markers used for the identification and evaluation of the genetic diversity of 51 Common bean genotypes. In the entire germplasm set, all of the primers displayed clear and consistent amplification profiles. A total of 54 alleles were amplified by 15 polymorphic SSR loci and the number of alleles ranged from 1 to 8 with an average of 4.9 alleles per locus. The highest number of alleles i.e, 8 were amplified by *PVBR45a*. Plate-12 (a) and 12 (b) depicts the banding pattern of Common bean genotypes based on different SSR markers).

4.2.2 Polymorphic information content (PIC)

The PIC values provide an estimate of the discriminating power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of those alleles in the genotypes. The data pertaining to polymorphic information content (PIC) values and the number of alleles detected for each of the 11 polymorphic SSR markers are presented in Table-18. PIC value ranged from 0 (*Bmd45a*, *Bmd45b*, *PVBR128a*, *PVBR128b*, *Bmd12b* and *PVBR45b*) to 0.87(*Bm152*) with an average value of 0.519 across 51 Common bean genotypes. Therefore, the markers *Bm152*, *PVBR45a* and *Bmd12a* were identified as the most potential markers for genetic diversity studies in Common bean.

Table 18: Allelic variation and the polymorphic information content (PIC) for SSR loci across 51 selected Common bean (*Phaseolus vulgaris* L.) genotypes

| S.No | SSR markers | Number of alleles amplified | PIC value |
|------|------------------------------------|-----------------------------|--------------|
| 1 | <i>Bm152</i> | 6 | 0.87 |
| 2 | <i>PVBR45a</i> <i>PVBR45b</i> | 8 1 | 0.862 0 |
| 3 | <i>Bm140</i> | 7 | 0.341 |
| 4 | <i>Bmd28</i> | 7 | 0.513 |
| 5 | <i>Bmd12a</i> <i>Bmd12b</i> | 5 1 | 0.730 0 |
| 6 | <i>Bm201</i> | 2 | 0.498 |
| 7 | <i>Bm151</i> | 6 | 0.718 |
| 8 | <i>Pvbr94</i> | 2 | 0.498 |
| 9 | <i>Bmd19</i> | 5 | 0.685 |
| 10 | <i>Bmd45a</i> <i>Bmd45b</i> | 1 1 | 0 0 |
| 11 | <i>PVBR128a</i> <i>PVBR128b</i> | 1 1 | 0 0 |
| | Total | 54 | 5.715 |
| | Average | 4.909 | 0.519 |
| | Range | 1-8 | |

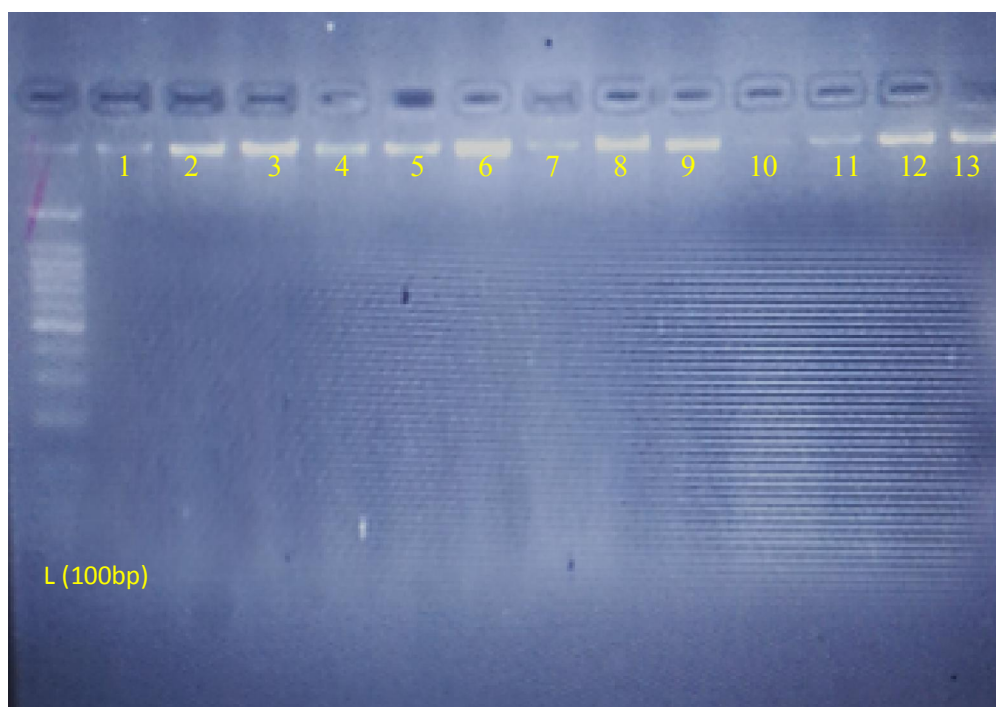


Plate 11: Representative image of DNA isolated from common bean genotypes on agarose gel

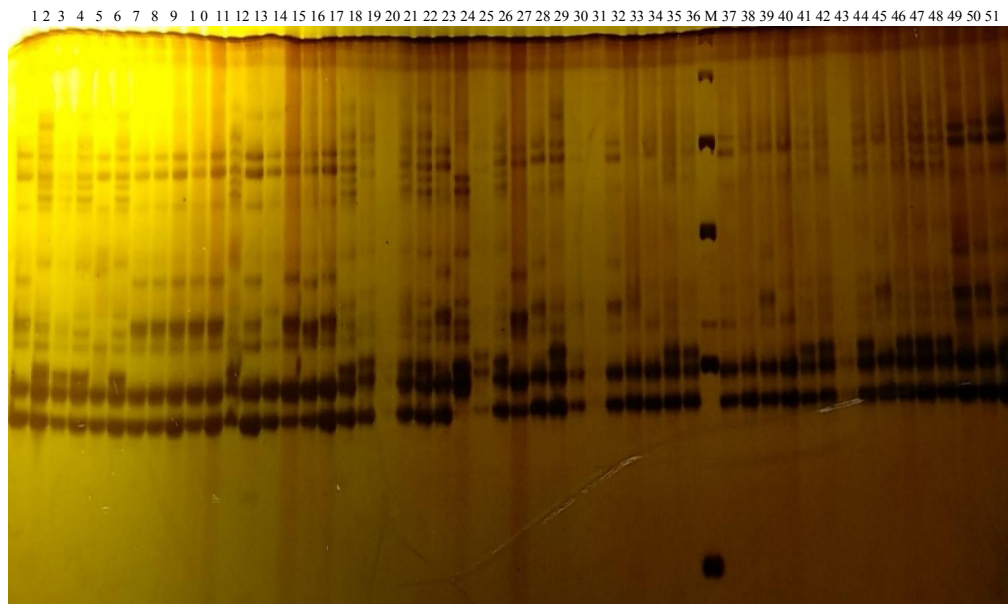


Plate 12(a): Representative gel image of SSR marker *Bm152* showing PCR amplification profile and alleles in 51 common bean genotypes. The serial number 1-51 represents different common bean genotypes while M= 100 bp ladder

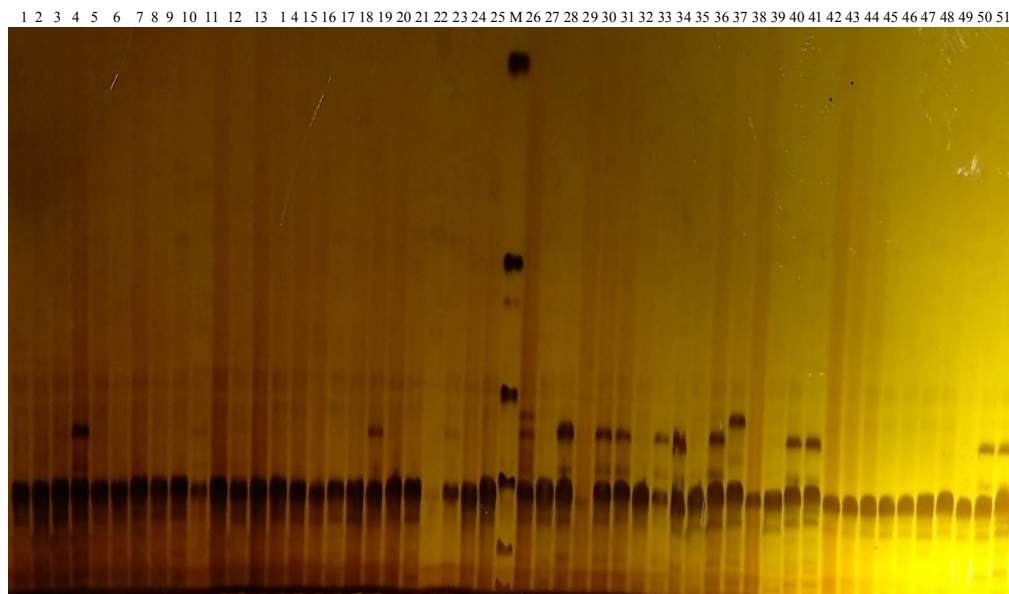


Plate 12(b): Representative PAGE gel image of SSR marker *PVBR45a* showing PCR amplification profile and alleles in 51 common bean genotypes. The serial number 1-51 represents different common bean genotypes while M= 100 bp ladder

4.2.3 Cluster analysis

Allelic diversity data was utilized to generate a dendrogram by using a distance matrix revealing the genetic relationship among all common bean genotypes (Fig-4). From the distance matrix, an unweighted Neighbor-Joining tree (UNJ) was constructed as shown in figure 5. Dendrograms are an effective way of summarizing microsatellite data and can reveal relationships including individuals with identical genotypes. The cluster dendrogram classified 51 genotypes into 3 major clusters (Table 19). Cluster II was further divided into two sub-clusters, i.e Iia and Iib. Cluster I consisted of 19 genotypes namely WB1151, WB665, SB158, E10, SB121.1, SB166, WB832, IC-328438, WB1189, E2, IC-326854, SR1, WB1187, SB123, WB1306, SB159, SB136, SB169, WB192. Cluster II contained maximum genotypes (21) with sub-cluster Iia consisting of 11 genotypes namely E1 VENYL, WB1199, NBPGR1, SB111, NBPGR3, EC-21753, Lcl Bml, EC-394659, WB603, NBPGR2, SB152 and sub-cluster Iib containing 10 genotypes namely IC-326619, IC-326603, EC-385256, SB128, SB151, SB155.1, SB183.1, SB107, SB19, SB106. The cluster III had 11 genotypes, SB181.1, SB116.1, SB167, SB162, SB131, SB156.A, SB117.2, SB142.1, SB165, WB469, SB149. Principal Coordinate Analysis (PCoA) also separated the 51 genotypes into 3 distinct groups, which was consistent with the results of the cluster analysis (Fig-6). The genotypes belonging to Cluster I were mainly distributed in the quadrant I of the resulting plot. The genotypes belonging to Cluster II were mainly distributed in the quadrant III and IV of the resulting plot whereas the genotypes of cluster III were mainly concentrated in the quadrant IV of the resulting plot.

4.2.4 Dissimilarity matrix based on Jaccards dissimilarity coefficient

The genetic distance matrix was generated from the allelic data using the simple matching method as a dissimilarity index. The 51 common bean genotypes showed dissimilarity coefficient values ranging from 0.00 to 0.93. The highest dissimilarity value of 0.93 was observed between genotypes WB1306 and SB107

falling in cluster I and sub-cluster IIb of cluster II respectively, IC-326854 and E1 VENYL falling in cluster I and sub-cluster IIa of cluster II respectively and SB123 and SB116.1 falling in cluster I and Cluster III respectively. The minimum dissimilarity 0.00 was observed between genotypes EC-394659 and Lcl Bml falling in sub-cluster Ia of Cluster II, followed by WB1151 and WB 665 with a value of 0.26 falling in cluster I. Table 20 shows dissimilarity matrix obtained from the molecular data.

Table 19: Distribution of Common bean genotypes into three clusters based on SSR marker analysis of 51 genotypes

| Cluster | Sub-cluster | Total number of genotypes in the cluster | Name of the genotypes included in different clusters |
|---------|-------------|--|---|
| I | | 19 | WB1151(14), WB665 (13), SB158 (48), E10 (49), SB121.1(47), SB166 (27), WB832 (12), IC-328438 (10), WB1189 (29), E2 (21), IC-326854 (9), SR1(50), WB1187 (19), SB123 (22), WB1306 (11), SB159 (46), SB136 (45), SB169 (34), WB192 (16) |
| II | IIa | 11 | E1 VENYL (20), WB1199 (15), NBPGR-1 (1), SB111 (32), NBPGR-3 (6), EC-21753 (2), Lcl Bml (51), EC-394659 (5), WB603 (17), NBPGR-2 (3), SB152 (31) |
| | IIb | 10 | IC-326619 (8), IC-326603 (7), EC-385256 (4), SB128 (36), SB151(24), SB155.1(30), SB183.1 (28), SB107 (23), SB19 (26), SB106 (25) |
| III | | 11 | SB181.1 (42), SB116.1 (37), SB167 (39), SB162 (40), SB131 (38), SB156.A (44), SB117.2 (43), SB142.1 (41), SB165 (33), WB469 (18), SB149 (35) |

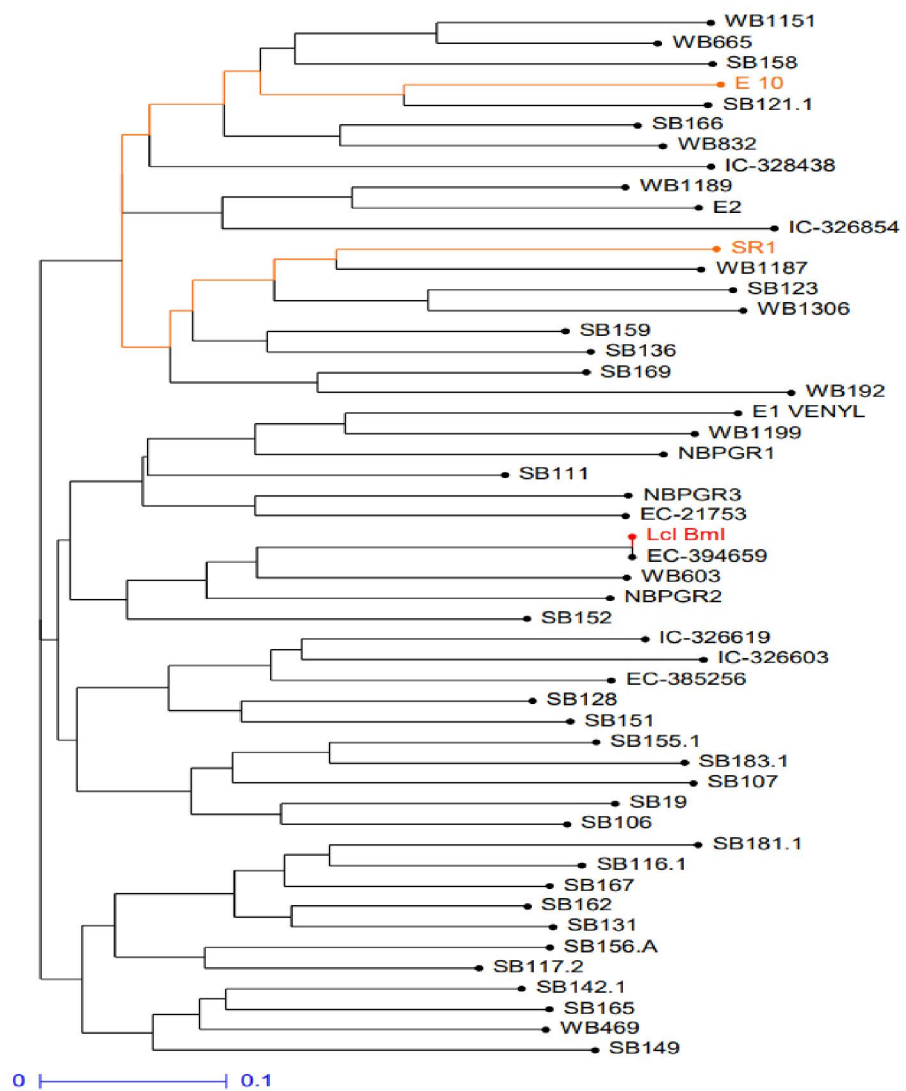


Fig. 5: Distribution of common bean genotypes into different clusters as depicted by cluster dendrogram (Molecular analysis)

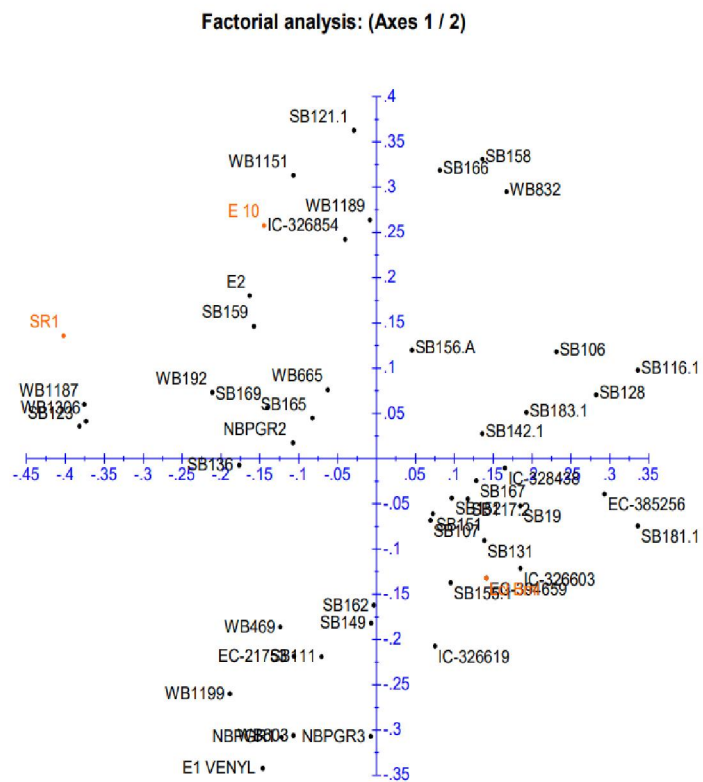


Fig. 7: Principal coordinate analysis (PCoA) of common bean germplasm

Table 20: Dissimilarity matrix

| Units | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|-------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 2 | 0.466667 | | | | | | | | | | | | | | | | | |
| 3 | 0.666667 | 0.666667 | | | | | | | | | | | | | | | | |
| 4 | 0.6 | 0.733333 | 0.6 | | | | | | | | | | | | | | | |
| 5 | 0.6 | 0.533333 | 0.4 | 0.533333 | | | | | | | | | | | | | | |
| 6 | 0.533333 | 0.4 | 0.466667 | 0.466667 | 0.533333 | | | | | | | | | | | | | |
| 7 | 0.466667 | 0.666667 | 0.666667 | 0.666667 | 0.4 | 0.666667 | 0.466667 | | | | | | | | | | | |
| 8 | 0.533333 | 0.666667 | 0.466667 | 0.4 | 0.466667 | 0.466667 | 0.4 | | | | | | | | | | | |
| 9 | 0.733333 | 0.733333 | 0.533333 | 0.466667 | 0.6 | 0.733333 | 0.733333 | 0.6 | | | | | | | | | | |
| 10 | 0.733333 | 0.6 | 0.733333 | 0.6 | 0.533333 | 0.733333 | 0.6 | 0.533333 | 0.733333 | | | | | | | | | |
| 11 | 0.6 | 0.733333 | 0.6 | 0.733333 | 0.733333 | 0.733333 | 0.666667 | 0.533333 | 0.533333 | 0.8 | | | | | | | | |
| 12 | 0.8 | 0.666667 | 0.466667 | 0.6 | 0.533333 | 0.8 | 0.666667 | 0.666667 | 0.533333 | 0.466667 | 0.733333 | | | | | | | |
| 13 | 0.6 | 0.733333 | 0.466667 | 0.6 | 0.6 | 0.666667 | 0.666667 | 0.6 | 0.733333 | 0.533333 | 0.533333 | 0.466667 | | | | | | |
| 14 | 0.8 | 0.8 | 0.466667 | 0.733333 | 0.666667 | 0.733333 | 0.733333 | 0.8 | 0.6 | 0.6 | 0.466667 | 0.266667 | | | | | | |
| 15 | 0.4 | 0.6 | 0.733333 | 0.666667 | 0.8 | 0.6 | 0.666667 | 0.6 | 0.8 | 0.733333 | 0.533333 | 0.866667 | 0.6 | 0.733333 | | | | |
| 16 | 0.533333 | 0.733333 | 0.666667 | 0.8 | 0.866667 | 0.666667 | 0.666667 | 0.733333 | 0.733333 | 0.6 | 0.6 | 0.666667 | 0.533333 | 0.6 | 0.533333 | | | |
| 17 | 0.533333 | 0.533333 | 0.533333 | 0.666667 | 0.4 | 0.533333 | 0.666667 | 0.466667 | 0.8 | 0.466667 | 0.466667 | 0.666667 | 0.533333 | 0.666667 | 0.533333 | 0.8 | | |
| 18 | 0.466667 | 0.4 | 0.6 | 0.666667 | 0.533333 | 0.6 | 0.666667 | 0.533333 | 0.666667 | 0.666667 | 0.6 | 0.733333 | 0.6 | 0.666667 | 0.466667 | 0.666667 | 0.533333 | |
| 19 | 0.666667 | 0.533333 | 0.666667 | 0.866667 | 0.733333 | 0.666667 | 0.8 | 0.8 | 0.666667 | 0.666667 | 0.6 | 0.8 | 0.666667 | 0.533333 | 0.666667 | 0.533333 | 0.666667 | 0.466667 |
| 20 | 0.533333 | 0.6 | 0.666667 | 0.8 | 0.6 | 0.6 | 0.733333 | 0.666667 | 0.933333 | 0.666667 | 0.666667 | 0.866667 | 0.6 | 0.666667 | 0.4 | 0.6 | 0.4 | 0.533333 |
| 21 | 0.666667 | 0.533333 | 0.6 | 0.8 | 0.6 | 0.6 | 0.866667 | 0.866667 | 0.6 | 0.733333 | 0.666667 | 0.6 | 0.666667 | 0.866667 | 0.666667 | 0.733333 | 0.666667 | 0.733333 |
| 22 | 0.666667 | 0.6 | 0.666667 | 0.8 | 0.733333 | 0.6 | 0.8 | 0.666667 | 0.733333 | 0.666667 | 0.333333 | 0.666667 | 0.6 | 0.666667 | 0.666667 | 0.666667 | 0.466667 | 0.733333 |
| 23 | 0.733333 | 0.466667 | 0.733333 | 0.666667 | 0.666667 | 0.533333 | 0.8 | 0.866667 | 0.866667 | 0.6 | 0.933333 | 0.533333 | 0.733333 | 0.733333 | 0.666667 | 0.533333 | 0.666667 | 0.666667 |
| 24 | 0.466667 | 0.6 | 0.733333 | 0.466667 | 0.666667 | 0.6 | 0.466667 | 0.466667 | 0.733333 | 0.6 | 0.666667 | 0.666667 | 0.666667 | 0.733333 | 0.6 | 0.733333 | 0.533333 | 0.6 |
| 25 | 0.8 | 0.466667 | 0.666667 | 0.533333 | 0.466667 | 0.6 | 0.666667 | 0.733333 | 0.733333 | 0.533333 | 0.8 | 0.4 | 0.666667 | 0.6 | 0.8 | 0.8 | 0.666667 | 0.733333 |
| 26 | 0.666667 | 0.533333 | 0.733333 | 0.6 | 0.6 | 0.533333 | 0.533333 | 0.466667 | 0.866667 | 0.466667 | 0.733333 | 0.533333 | 0.666667 | 0.733333 | 0.6 | 0.8 | 0.533333 | 0.666667 |
| 27 | 0.733333 | 0.8 | 0.533333 | 0.6 | 0.6 | 0.8 | 0.666667 | 0.733333 | 0.533333 | 0.666667 | 0.6 | 0.333333 | 0.533333 | 0.466667 | 0.733333 | 0.533333 | 0.8 | 0.666667 |
| 28 | 0.666667 | 0.666667 | 0.866667 | 0.533333 | 0.6 | 0.6 | 0.6 | 0.733333 | 0.733333 | 0.666667 | 0.733333 | 0.6 | 0.733333 | 0.733333 | 0.8 | 0.866667 | 0.666667 | 0.866667 |
| 29 | 0.733333 | 0.6 | 0.6 | 0.666667 | 0.666667 | 0.733333 | 0.733333 | 0.733333 | 0.466667 | 0.8 | 0.6 | 0.466667 | 0.733333 | 0.6 | 0.8 | 0.733333 | 0.8 | 0.666667 |
| 30 | 0.533333 | 0.6 | 0.733333 | 0.533333 | 0.6 | 0.466667 | 0.6 | 0.733333 | 0.866667 | 0.666667 | 0.8 | 0.666667 | 0.6 | 0.733333 | 0.733333 | 0.666667 | 0.6 | 0.666667 |
| 31 | 0.533333 | 0.6 | 0.466667 | 0.666667 | 0.466667 | 0.666667 | 0.533333 | 0.4 | 0.666667 | 0.533333 | 0.733333 | 0.533333 | 0.733333 | 0.666667 | 0.666667 | 0.666667 | 0.533333 | 0.466667 |
| 32 | 0.466667 | 0.466667 | 0.6 | 0.6 | 0.733333 | 0.4 | 0.6 | 0.466667 | 0.8 | 0.733333 | 0.533333 | 0.733333 | 0.666667 | 0.8 | 0.4 | 0.733333 | 0.466667 | 0.4 |
| 33 | 0.6 | 0.466667 | 0.6 | 0.666667 | 0.6 | 0.533333 | 0.8 | 0.733333 | 0.533333 | 0.8 | 0.6 | 0.666667 | 0.666667 | 0.6 | 0.666667 | 0.733333 | 0.733333 | 0.4 |
| 34 | 0.6 | 0.6 | 0.6 | 0.6 | 0.8 | 0.666667 | 0.6 | 0.666667 | 0.733333 | 0.666667 | 0.466667 | 0.533333 | 0.4 | 0.533333 | 0.6 | 0.4 | 0.6 | 0.6 |
| 35 | 0.6 | 0.533333 | 0.6 | 0.6 | 0.466667 | 0.533333 | 0.8 | 0.666667 | 0.533333 | 0.6 | 0.6 | 0.733333 | 0.733333 | 0.733333 | 0.6 | 0.666667 | 0.466667 | 0.4 |
| 36 | 0.666667 | 0.733333 | 0.733333 | 0.333333 | 0.666667 | 0.6 | 0.533333 | 0.533333 | 0.733333 | 0.666667 | 0.8 | 0.533333 | 0.6 | 0.666667 | 0.733333 | 0.8 | 0.733333 | 0.6 |
| 37 | 0.733333 | 0.733333 | 0.666667 | 0.4 | 0.6 | 0.733333 | 0.6 | 0.666667 | 0.6 | 0.466667 | 0.866667 | 0.533333 | 0.6 | 0.533333 | 0.733333 | 0.666667 | 0.8 | 0.533333 |
| 38 | 0.6 | 0.733333 | 0.6 | 0.466667 | 0.533333 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.666667 | 0.666667 | 0.6 | 0.533333 | 0.666667 | 0.6 | 0.733333 | 0.6 | 0.533333 |
| 39 | 0.666667 | 0.533333 | 0.6 | 0.6 | 0.666667 | 0.6 | 0.666667 | 0.733333 | 0.733333 | 0.6 | 0.733333 | 0.6 | 0.533333 | 0.533333 | 0.533333 | 0.6 | 0.666667 | 0.4 |
| 40 | 0.533333 | 0.6 | 0.533333 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.6 | 0.733333 | 0.733333 | 0.533333 | 0.666667 | 0.466667 | 0.6 | 0.466667 | 0.733333 | 0.466667 | 0.4 |
| 41 | 0.6 | 0.533333 | 0.533333 | 0.6 | 0.533333 | 0.6 | 0.666667 | 0.666667 | 0.733333 | 0.6 | 0.8 | 0.466667 | 0.466667 | 0.533333 | 0.666667 | 0.666667 | 0.666667 | 0.333333 |
| 42 | 0.733333 | 0.8 | 0.8 | 0.466667 | 0.733333 | 0.733333 | 0.533333 | 0.666667 | 0.733333 | 0.6 | 0.8 | 0.6 | 0.6 | 0.733333 | 0.533333 | 0.666667 | 0.733333 | 0.666667 |
| 43 | 0.666667 | 0.466667 | 0.533333 | 0.533333 | 0.466667 | 0.533333 | 0.666667 | 0.6 | 0.6 | 0.6 | 0.733333 | 0.6 | 0.666667 | 0.666667 | 0.533333 | 0.733333 | 0.6 | 0.466667 |
| 44 | 0.733333 | 0.6 | 0.6 | 0.466667 | 0.6 | 0.733333 | 0.6 | 0.6 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.666667 | 0.666667 | 0.666667 | 0.6 | 0.6 | 0.466667 |
| 45 | 0.6 | 0.666667 | 0.4 | 0.6 | 0.666667 | 0.466667 | 0.733333 | 0.6 | 0.666667 | 0.866667 | 0.533333 | 0.6 | 0.466667 | 0.6 | 0.666667 | 0.666667 | 0.666667 | 0.533333 |
| 46 | 0.666667 | 0.533333 | 0.466667 | 0.6 | 0.666667 | 0.533333 | 0.8 | 0.733333 | 0.6 | 0.8 | 0.6 | 0.533333 | 0.6 | 0.6 | 0.6 | 0.6 | 0.733333 | 0.466667 |
| 47 | 0.8 | 0.733333 | 0.666667 | 0.666667 | 0.733333 | 0.866667 | 0.666667 | 0.666667 | 0.6 | 0.533333 | 0.6 | 0.533333 | 0.6 | 0.4 | 0.666667 | 0.6 | 0.8 | 0.6 |
| 48 | 0.8 | 0.8 | 0.666667 | 0.6 | 0.733333 | 0.733333 | 0.666667 | 0.666667 | 0.6 | 0.666667 | 0.666667 | 0.466667 | 0.466667 | 0.4 | 0.8 | 0.8 | 0.733333 | 0.8 |
| 49 | 0.733333 | 0.8 | 0.733333 | 0.6 | 0.8 | 0.8 | 0.6 | 0.666667 | 0.666667 | 0.666667 | 0.466667 | 0.6 | 0.466667 | 0.6 | 0.666667 | 0.666667 | 0.733333 | 0.666667 |
| 50 | 0.666667 | 0.533333 | 0.533333 | 0.8 | 0.733333 | 0.666667 | 0.8 | 0.733333 | 0.6 | 0.733333 | 0.466667 | 0.666667 | 0.666667 | 0.533333 | 0.533333 | 0.666667 | 0.666667 | 0.466667 |
| 51 | 0.6 | 0.533333 | 0.4 | 0.533333 | 0 | 0.533333 | 0.666667 | 0.466667 | 0.6 | 0.533333 | 0.733333 | 0.533333 | 0.6 | 0.666667 | 0.8 | 0.866667 | 0.4 | 0.533333 |

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Contd....

Table 20: contd....

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|----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
| 2 | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | |
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| 16 | | | | | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | | | | | | |
| 20 | 0.6 | | | | | | | | | | | | | | | | |
| 21 | 0.4 | 0.733333 | | | | | | | | | | | | | | | |
| 22 | 0.4 | 0.6 | 0.466667 | | | | | | | | | | | | | | |
| 23 | 0.666667 | 0.6 | 0.533333 | 0.6 | | | | | | | | | | | | | |
| 24 | 0.666667 | 0.6 | 0.533333 | 0.6 | 0.733333 | | | | | | | | | | | | |
| 25 | 0.733333 | 0.666667 | 0.466667 | 0.6 | 0.4 | 0.533333 | | | | | | | | | | | |
| 26 | 0.8 | 0.6 | 0.666667 | 0.6 | 0.533333 | 0.466667 | 0.333333 | | | | | | | | | | |
| 27 | 0.733333 | 0.8 | 0.6 | 0.666667 | 0.666667 | 0.6 | 0.333333 | 0.533333 | | | | | | | | | |
| 28 | 0.666667 | 0.8 | 0.4 | 0.6 | 0.533333 | 0.533333 | 0.4 | 0.466667 | 0.666667 | | | | | | | | |
| 29 | 0.466667 | 0.933333 | 0.333333 | 0.6 | 0.666667 | 0.533333 | 0.466667 | 0.733333 | 0.533333 | 0.4 | | | | | | | |
| 30 | 0.533333 | 0.666667 | 0.466667 | 0.533333 | 0.4 | 0.466667 | 0.466667 | 0.533333 | 0.666667 | 0.333333 | 0.6 | | | | | | |
| 31 | 0.6 | 0.6 | 0.6 | 0.733333 | 0.733333 | 0.333333 | 0.533333 | 0.4 | 0.533333 | 0.666667 | 0.466667 | 0.6 | | | | | |
| 32 | 0.6 | 0.6 | 0.666667 | 0.533333 | 0.6 | 0.4 | 0.666667 | 0.466667 | 0.733333 | 0.6 | 0.533333 | 0.466667 | 0.4 | | | | |
| 33 | 0.533333 | 0.8 | 0.466667 | 0.6 | 0.533333 | 0.6 | 0.6 | 0.733333 | 0.6 | 0.6 | 0.4 | 0.533333 | 0.533333 | 0.333333 | | | |
| 34 | 0.466667 | 0.6 | 0.666667 | 0.466667 | 0.6 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.6 | 0.466667 | 0.666667 | 0.466667 | 0.6 | | | |
| 35 | 0.533333 | 0.666667 | 0.6 | 0.733333 | 0.6 | 0.733333 | 0.6 | 0.733333 | 0.533333 | 0.666667 | 0.6 | 0.533333 | 0.6 | 0.533333 | 0.6 | 0.666667 | |
| 36 | 0.733333 | 0.733333 | 0.666667 | 0.733333 | 0.6 | 0.333333 | 0.4 | 0.466667 | 0.4 | 0.466667 | 0.533333 | 0.4 | 0.466667 | 0.466667 | 0.533333 | 0.533333 | 0.6 |
| 37 | 0.733333 | 0.666667 | 0.733333 | 0.933333 | 0.666667 | 0.533333 | 0.466667 | 0.6 | 0.466667 | 0.666667 | 0.6 | 0.6 | 0.466667 | 0.6 | 0.533333 | 0.6 | 0.533333 |
| 38 | 0.666667 | 0.533333 | 0.8 | 0.666667 | 0.733333 | 0.466667 | 0.6 | 0.666667 | 0.6 | 0.666667 | 0.6 | 0.533333 | 0.533333 | 0.466667 | 0.533333 | 0.466667 | 0.6 |
| 39 | 0.666667 | 0.666667 | 0.733333 | 0.866667 | 0.533333 | 0.733333 | 0.533333 | 0.666667 | 0.6 | 0.666667 | 0.533333 | 0.533333 | 0.6 | 0.4 | 0.4 | 0.533333 | 0.466667 |
| 40 | 0.6 | 0.466667 | 0.733333 | 0.666667 | 0.733333 | 0.6 | 0.6 | 0.733333 | 0.6 | 0.666667 | 0.533333 | 0.533333 | 0.6 | 0.4 | 0.533333 | 0.466667 | 0.4 |
| 41 | 0.666667 | 0.8 | 0.666667 | 0.8 | 0.466667 | 0.666667 | 0.533333 | 0.533333 | 0.466667 | 0.666667 | 0.6 | 0.466667 | 0.466667 | 0.466667 | 0.333333 | 0.6 | 0.466667 |
| 42 | 0.866667 | 0.6 | 0.866667 | 0.933333 | 0.6 | 0.666667 | 0.6 | 0.6 | 0.666667 | 0.533333 | 0.6 | 0.6 | 0.666667 | 0.6 | 0.733333 | 0.533333 | 0.6 |
| 43 | 0.6 | 0.666667 | 0.666667 | 0.8 | 0.6 | 0.533333 | 0.466667 | 0.666667 | 0.6 | 0.6 | 0.333333 | 0.6 | 0.4 | 0.4 | 0.4 | 0.6 | 0.466667 |
| 44 | 0.6 | 0.8 | 0.733333 | 0.733333 | 0.666667 | 0.533333 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.466667 | 0.666667 | 0.466667 | 0.666667 | 0.6 | 0.466667 | 0.6 |
| 45 | 0.466667 | 0.666667 | 0.6 | 0.466667 | 0.666667 | 0.6 | 0.666667 | 0.733333 | 0.533333 | 0.733333 | 0.533333 | 0.466667 | 0.6 | 0.4 | 0.533333 | 0.333333 | 0.533333 |
| 46 | 0.466667 | 0.8 | 0.466667 | 0.466667 | 0.466667 | 0.533333 | 0.533333 | 0.666667 | 0.4 | 0.733333 | 0.466667 | 0.533333 | 0.6 | 0.466667 | 0.4 | 0.533333 | 0.533333 |
| 47 | 0.533333 | 0.8 | 0.666667 | 0.666667 | 0.8 | 0.6 | 0.6 | 0.466667 | 0.4 | 0.733333 | 0.6 | 0.866667 | 0.533333 | 0.733333 | 0.666667 | 0.6 | 0.8 |
| 48 | 0.8 | 0.866667 | 0.533333 | 0.666667 | 0.733333 | 0.533333 | 0.533333 | 0.466667 | 0.466667 | 0.533333 | 0.466667 | 0.666667 | 0.6 | 0.6 | 0.533333 | 0.666667 | 0.866667 |
| 49 | 0.466667 | 0.666667 | 0.733333 | 0.466667 | 0.866667 | 0.6 | 0.6 | 0.6 | 0.466667 | 0.6 | 0.6 | 0.733333 | 0.733333 | 0.666667 | 0.733333 | 0.4 | 0.866667 |
| 50 | 0.4 | 0.666667 | 0.533333 | 0.466667 | 0.733333 | 0.666667 | 0.733333 | 0.733333 | 0.666667 | 0.8 | 0.533333 | 0.8 | 0.6 | 0.533333 | 0.466667 | 0.6 | 0.733333 |
| | 0.733333 | 0.6 | 0.6 | 0.733333 | 0.666667 | 0.666667 | 0.466667 | 0.6 | 0.6 | 0.6 | 0.666667 | 0.6 | 0.466667 | 0.733333 | 0.6 | 0.8 | 0.466667 |

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Contd....

Table 20: contd....

| | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
|----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 2 | | | | | | | | | | | | | | | |
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| 34 | | | | | | | | | | | | | | | |
| 35 | | | | | | | | | | | | | | | |
| 36 | | | | | | | | | | | | | | | |
| 37 | 0.333333 | | | | | | | | | | | | | | |
| 38 | 0.4 | 0.266667 | | | | | | | | | | | | | |
| 39 | 0.533333 | 0.266667 | 0.4 | | | | | | | | | | | | |
| 40 | 0.466667 | 0.466667 | 0.266667 | 0.266667 | | | | | | | | | | | |
| 41 | 0.4 | 0.466667 | 0.6 | 0.333333 | 0.466667 | | | | | | | | | | |
| 42 | 0.466667 | 0.333333 | 0.4 | 0.4 | 0.4 | 0.6 | | | | | | | | | |
| 43 | 0.466667 | 0.4 | 0.4 | 0.266667 | 0.333333 | 0.466667 | 0.466667 | | | | | | | | |
| 44 | 0.466667 | 0.466667 | 0.6 | 0.466667 | 0.666667 | 0.533333 | 0.6 | 0.333333 | | | | | | | |
| 45 | 0.466667 | 0.666667 | 0.4 | 0.6 | 0.333333 | 0.533333 | 0.666667 | 0.533333 | 0.6 | | | | | | |
| 46 | 0.466667 | 0.6 | 0.533333 | 0.533333 | 0.533333 | 0.4 | 0.8 | 0.466667 | 0.466667 | 0.333333 | | | | | |
| 47 | 0.533333 | 0.466667 | 0.666667 | 0.6 | 0.733333 | 0.533333 | 0.733333 | 0.6 | 0.4 | 0.733333 | 0.466667 | | | | |
| 48 | 0.466667 | 0.533333 | 0.6 | 0.533333 | 0.666667 | 0.533333 | 0.666667 | 0.6 | 0.6 | 0.666667 | 0.6 | 0.4 | | | |
| 49 | 0.533333 | 0.666667 | 0.6 | 0.733333 | 0.6 | 0.733333 | 0.666667 | 0.666667 | 0.533333 | 0.533333 | 0.6 | 0.333333 | 0.533333 | | |
| 50 | 0.8 | 0.8 | 0.8 | 0.666667 | 0.733333 | 0.666667 | 0.933333 | 0.533333 | 0.466667 | 0.533333 | 0.4 | 0.466667 | 0.666667 | 0.466667 | |
| | 0.666667 | 0.6 | 0.533333 | 0.666667 | 0.6 | 0.533333 | 0.733333 | 0.466667 | 0.6 | 0.666667 | 0.666667 | 0.733333 | 0.733333 | 0.8 | 0.733333 |



Plate 13: Molecular lab work at Wadura.

Chapter-5

DISCUSSION

The Common bean is a significant niche crop in the Kashmir valley and a crucial part of subsistence farming, including low-input marginal farmers. There is growing evidence about the livelihood and health benefits of this crop and is sometimes designated as a nearly complete food. Due to its great quality, nutritional balance and higher biological efficiency, it is extremely popular among people. It is a natural remedy for bladder burn and aids in the management of diabetes and heart issues. It has both carminative and reparative properties against constipation and diarrhea. The common bean is a significant source of minerals (3.2–5.0%), proteins (17.5–28.5%) and carbohydrates (61.4%). It also contains Vitamin C and pro-Vitamin A. It has a significant amount of fiber and supplies minerals like Iron, Potassium, Phosphorus, Magnesium, Copper, etc. Common bean is a feasible alternative in the face of the impending threat of climate change, where there will be changes in cropping patterns, particularly for crops with greater water and nutrient demands, as it performs well even under low input systems, enriches the soil and enhances soil health. However, the progress of promoting the common bean as a crop that could compete with vegetables has been largely hampered by little progress in breeding high-yielding varieties due to which the yields are hovering at low levels in most of the countries, especially in sub-Saharan Africa where it is a substantial staple crop.

The delayed progress in breeding high-yielding varieties that can be used in both productive and low-input systems is partly due to the narrow genetic base accessible in breeders' stocks (Kelly *et al.*, 1998). There is a need to have genetically diverse germplasm for yield, quality and resilience. The identification of agronomically superior genotypes is determined by the limits of diversity in usable genetic stocks. Common bean is a crucial part of the farming system in the state of Jammu & Kashmir and the valley of Kashmir in particular, where it is

invariably grown as an intercrop with maize and other crops based on system suitability and agronomic compatibility.

Traditional landraces of the Common bean, in particular, have persisted in our farming system in Kashmir despite competition from modern varieties and imports of seeds from neighboring states to meet demand. Such persistence is attributed to cultural values and characteristic quality parameters of common bean landraces and as such, there is a need to harness such advantage. Identifying trait-based sources of genes for enhancing common beans for yield, quality and resilience calls for extensive characterization of genetic resources available in the state and the national and international gene banks. The current study aimed to identify the most diverse genotypes that could be used in breeding programs as the base material for varietal development through selection schemes or as parents in hybridization programs to create desirable variability that could be used for varietal development in segregating generations.

For the effective management and use of germplasm, it is crucial to understand the nature and magnitude of variability as well as the genetic relationships between traits by analyzing the genetic diversity in large collections conserved in gene banks. The simplified phenotypic approach has been found useful for preliminary characterization and discrimination of accessions to identify the level of genetic diversity (Szilagyi *et al.*, 2011). It aids in the analysis of population diversity and generates precise information on the nature and degree of genetic variability among prospective parents for the selection of potential parents for hybridization programs. In the present study, 48 genotypes and 3 checks were characterized for 13 quantitative and 17 qualitative traits.

The results of the present study entitled “**Morpho-molecular characterization of common bean (*Phaseolus vulgaris L.*) germplasm**” are discussed in light of available literature under appropriate headings.

5.1 Pattern of Variation and Diversity for Morphological, Yield and Pod Quality Traits.

5.1.1 Frequency distribution for Qualitative Traits

The results pertaining to the morphological characterization of germplasm are presented in Table 6. A perusal of the table reveals that among various morphological traits the genotypes could be grouped into two distinct classes for Plant growth habit, Plant twining habit, Pod stringiness, Pod pigmentation, Shape of the central leaflet and Seed Pigmentation, whereas for the rest of the traits the germplasm can be grouped into more than 2 classes. The frequency distribution of the common bean for the traits revealed the highest number of classes (19) recorded for seed testa colour, (5) classes for seed shape and flower colour, (4) classes for pod curvature and pod shape of a cross-section through seed, (3) for growth type, leaf colour, leaf size, pod shape (in relation to suture), pod colour, pod shape of distal part. The highest frequency of 90.01% was recorded for pod pigmentation at maturity followed by 84.3% for pod colour and seed pigmentation and 82.3% for pod shape in relation to suture. Sofi *et al.* (2011) also reported that the exotic common bean genotypes could be classified into various groups on the basis of morphological descriptors. Rana *et al.* (2015) have done a detailed analysis of the pattern of variation in bean diversity in Indian gene banks and reported wide variability in morphological and quantitative traits. Neupane *et al.* (2005) also examined 100 accessions of local and foreign bean germplasm obtained from diverse places and analyzed for agro-morphological features.

The majority of genotypes used were snap type (64.7%) on account of the present study for the selection of most promising vegetable type genotypes. The majority of the genotypes (80.4%) were indeterminate types and the rest were determinate (19.6%).

Singh (1989) discovered that all four growth patterns (upright bush, prostrate non-climbing, semi-climbing and climbing) are represented in cultivated

landraces in both Middle and South American beans, but in varying amounts. Furthermore, seed size varied greatly across both locations, with South American variants being larger on average. There seems to be a correlation between climate and growth habitat in both regions. For example, upright bush types are more common in relatively warmer lower altitudes in both centers of domestication, prostrate non-climbing and semi-climbing types are more common in semi-arid or drier areas at intermediate or higher altitudes and climbing beans are more common in cool and wet high lands.

The plant growth habit was discovered to be diverse, ranging from bushy to aggressive climbing type. The dominance of one type of growth habit is related to both ecological adaptability and the cropping strategy used. Most exotic lines and lines obtained from national gene banks were bush types, whereas most locally collected lines, particularly small-seeded lines, were pole types. This is largely owing to the fact that in states such as Jammu and Kashmir, beans are often intercropped with maize and other crops based on fitness and agronomic compatibility, for which climbing varieties are favoured, whereas bush types are preferred in plains where beans are produced as a mono-crop. Climbing beans have a longer life cycle, better productivity and late maturity, whereas bush beans have early maturity, a short plant and low productivity (Rana *et al.*, 2015). These findings are consistent with those of Garcia *et al.* (1997), who found that increasing climbing ability increases the time required to reach physiological maturity and that plants with indeterminate growth habits are more productive than plants with determinate growth habits.

Most of the genotypes (62.7%) had cordate leaf shape followed by ovate shape found in the rest (37.2%) genotypes. The medium leaf size was predominant and found in (64.7%) of genotypes followed by small and large leaf sizes found in 25.4% and 9.8%, respectively. The flower colour was white in 54.9%, pink in 21.5%, violet in 13.7%, yellow in 7.8% and purple in only 1% of

genotypes. The intensity of leaf colour was dark green in 78.4%, green in 17.6% and only one genotype had a purple-colored leaf.

Pod Shape (cross-section through seed) was cordate (41.1%), ovate (37.2%), elliptical (11.7%) and circular in 9.8% of genotypes. The pods were normal green in colour in 84.3%, pale green in 13.7% and purple in 1.9% of genotypes. Pod pigmentation was present in 9.8% and absent in 90.1% of genotypes. The pod stringiness was present in 62.7% and absent in 37.2% of genotypes. Curvature was absent in 13.7%, strong in 17.6%, less in 15.68% and medium in 52.9% of genotypes. For shape of curvature of pod, 82.3% were concave, 3.9% were convex and 13.7% of genotypes were straight. For the shape of distal part of pods, 74.5% were acute to truncate, 21.5% were acute and 3.9% of genotypes were truncate in shape. Similar findings were reported by Arunga *et al.* (2015)

Variation was noticed in seed testa colour. In the red seed colour, different shades of red colour were observed. In 11.7% of genotypes, seeds were dark red in colour, 5.8% of genotypes had canadian red-coloured seeds, seeds of 5.8% genotypes were red in colour and 3.9% genotypes had red-painted lady seeds. Cream-coloured seeds were observed in 17.6% of genotypes. In 3.9% of genotypes painted lady seeds were seen. The light-yellow seeds were observed in 3.9% genotypes. Similarly, different shades were seen in brown colour. In 11.7% genotypes dark brown coloured seeds were seen. The light brown coloured seeds were seen in 9.8% of genotypes and 5.8% of genotypes had painted brown seeds. Black, cranberry, cannellini, navy, olive, sapphire blue, yellow, grey northern and light grey seeds were seen in 1.98% of each genotype. Seeds were cuboidal to elliptical in 39.2%, cuboid in 27.3%, circular in 9.8% and elliptical in only 3.9% of genotypes. Seed pigmentation was present in 15.6% and absent in 84.35% of genotypes. Seed traits are more important among different traits as they have been found to be the major determinants of the commercial acceptability of varieties (Park *et al.*, 2000; Bisht and Sharma, 2014; Rana *et al.*, 2014). Hornakova *et al.* (2003) also found

significant diversity in plant and seed traits for 33 morphological and agronomic traits. However, several of the accessions were identical in terms of these traits. There was significant variation in seed coat color, coat pattern and shape. The preference for seed color was seen to vary by location. Kidney-shaped red, maroon, pink and yellow beans are popular among bean consumers and producers in India (Sharma and Rana 2006; Rana *et al.*, 2015). Similar findings on seed color preference have been reported in various parts of the world (Trutmann *et al.*, 1996; Wortmann *et al.*, 1998; Fonseca *et al.*, 2007). This implies that seed color genes may have been transferred between gene pool groups via hybridization, inter-gene pool introgression and substantial selection for diverse preferences by producers and consumers worldwide. Aside from seed color, the accessions had substantially more variety in seed shape and size (100-seed weight=3.5-96.3 g).

Okii *et al.* (2014) evaluated 284 accessions of common bean using 22 descriptors and found that different classes of descriptor traits were observed in different proportions that indicated wide variability for trait classes in French bean. In French bean, similar results have been reported in local and exotic accessions by Sofi *et al.* (2011) in 42 accessions, Sofi *et al.* (2014) in 300 accessions, Rana *et al.* (2015) in 4724 accessions and Jan *et al.* (2021) in 109 accessions of French bean originating from diverse sources across the world that are present in different gene banks of India. The in-depth characterization of germplasm resources is of paramount importance for creating a credible database for trait-based core set development. It is also an important prerequisite for the registration of genetic resources for long-term storage. The frequency distribution obtained for 17 qualitative traits showed the presence of a substantial range of variability as has also been reported in the Andean and Mesoamerican gene pools of French beans (Singh *et al.* 1991a; Bitocchi *et al.* 2012; De-La Fuente *et al.* 2013).

5.1.2 Frequency distribution for Morphological, Yield and Pod traits

The frequency distribution reported for 13 quantitative features revealed a wide range of variability. Despite the presence of extreme values on the lower and upper ends of the scale in a few genotypes, the majority of genotypes converged into a rather narrow range.

The mean value of days to flowering was 51.88 days with minimum and maximum values of 41.96 and 61.96 days respectively. The mean value of days to pod initiation was 60.69 days with minimum and maximum values of 47.71 and 72.04 days respectively. The mean value of days to marketable maturity was 68.51 days with minimum and maximum values of 56 and 80 respectively. Similar results were obtained by (Saba *et al.* 2017 and Sofi *et al.* 2020).

Among six quantitative traits recorded on pods, pod length had a mean value of 11.31 cm with minimum and maximum values of 7.8 and 14.8 cm respectively. Pod thickness had a mean value of 9.47 mm with minimum and maximum values of 6.4 and 11.4 mm respectively. Pod weight had a mean value of 4.49 with minimum and maximum values of 3.01 and 5.83 respectively. The number of pods per plant had a mean value of 27.62 with minimum and maximum values of 6.2 and 60 respectively. Pod yield per plant had a mean value of 121.85 g with minimum and maximum values of 28 and 253.76 g respectively. Similarly, pod yield per hectare had a mean value of 304.61 with minimum and maximum values of 70 and 634.39 q respectively. Kumar, A. *et al.*, 2014., Saba *et al.* 2017, Sofi *et al.* 2017., Loko *et al.* 2018 and Heema and D.K Rana. 2020 also reported substantial variability in pod traits in French bean.

Similar results were reported by Sofi *et al.* (2014). Razvi (2011) also found significant variability in yield and yield component variables among 75 germplasm lines. He reported wide range for almost all the traits evaluated except for the number of branches/plants and number of pods/ plant. Santalla *et al.*

(2004) investigated 31 Spanish runner bean landraces, finding substantial differences in major agronomical characteristics except for seeds per pod.

The mean value of seed yield per plant was 29.13 g with minimum and maximum values of 10.08 and 88.5 g respectively. The mean value of seed yield per hectare was 9.54 q with minimum and maximum values of 2.52 and 22.12 q respectively. The mean value of 100 seed weight was 29.13 g with the minimum and maximum values of 16.59 and 57.41 g respectively. The mean value of the number of seeds per pod was 5.1 with minimum and maximum values of 3.5 and 7.5 respectively. Similar findings were reported by Sofi *et al.* 2011 and Sofi *et al.* 2020).

A large number of studies have been done wherein the natural variation for seed physical parameters has been studied earlier (Sofi *et al.*, 2014). Similar findings have been found in common bean gene pools from the Andes and Mesoamerica (Rana *et al.*, 2015). These findings were consistent with those of other writers who reported substantial variation in seed shape and size in bean germplasm (Rodino *et al.*, 2003; Rai *et al.*, 2006; Rodino *et al.*, 2009; Cabral *et al.*, 2010; Lioi *et al.*, 2012). Singh (1989) and Singh *et al.* (1991a) have provided high taxonomic value for seed size, color and shape in comparison to the vegetative properties of the plant in common bean. Seed physical features such as size, shape, color and coat pattern are essential parameters that influence consumer acceptability of common bean and have thus garnered a lot of attention in all breeding programs. Red-colored beans with kidney or cuboid shapes are more popular in Kashmir region.

Although descriptor qualities are useful in determining the pattern of variation in a germplasm collection, the variances are mostly determined by customer preferences. However, from a breeding standpoint, yield criteria are more essential because they define the boundaries of advancement that can be achieved through appropriate breeding procedures and determine the competitive capabilities of modern varieties in comparison to traditional landraces and

farmers' varieties. This is seen from the fact that, despite the fact that the varietal profile of common bean in Kashmir is driven by the cultural persistence of landraces, new varieties have made major inroads into the farming system, eventually replacing the old types.

5.1.3 Descriptive statistics for Morphological, Yield and Pod traits

In this study, Common bean genotypes showed a wide range of variability for most of the morphological, growth, yield attributes and quality characters. The estimates of mean values from table-1 provided in the Appendix revealed that no genotype was superior for all the characters under study.

The mean values of days to flowering, days to pod initiation and days to marketable maturity were 51.88, 60.69 and 68.51 days respectively. The range of these traits was 41.96-61.96, 47.71-72.04 and 56-80 days respectively. For Days to flowering, the highest value was recorded for EC-394659 and SB107 (61.96) followed by SB183.1(60.96) while the lowest value was recorded in WB1151 (41.96). Days to pod initiation has the highest value recorded for EC-394659 (72.04) followed by SB107 (70.04) while the lowest value was recorded in WB1151 (47.71). For Days to marketable maturity highest value was recorded in SB107 (80) followed by SB183.1 (79) while the lowest value was recorded in WB832 and SB167 (56).

The mean values for the number of pods per plant, pod length (cm), pod thickness (mm), Pod weight (g), Pod yield per plant (g) and pod yield per hectare (q) were 27.62, 11.31 cm, 9.47 mm, 4.49 g, 121.85 g and 304.61 q respectively. The range of these traits was 6.2-60, 7.8-14.8 cm, 6.4-11.4 mm, 3.01-5.83 g, 28-253.76g and 70-634.39 q respectively. The number of pods per plant had the highest value recorded in SB167 (60) followed by SB152 (53) and SB156.A (52) while the lowest value was recorded in EC-394659 (8). In case of pod length, the highest value was recorded in SB106 (14.8 cm) followed by WB603 (14.7 cm) while the lowest value was recorded in SB165 (7.8 cm). For pod thickness, the

highest value was recorded in WB1151 (11.4 mm) followed by SB123 (11.2 mm) and WB1187 (10.99 mm) while the lowest value was recorded in SB167 (6.4 mm). Pod weight was recorded highest in WB1187 (5.83 g) followed by SB128 (5.58 g) and SB136 (5.27 g) while the lowest value was recorded in SB165 (3.01 g). Pod yield per plant and pod yield per hectare had the highest value recorded in SB156.A (253.76 g and 634.39 q), followed by SB159 (213.92 g and 534.8 g) and SB152 (211.43 g and 528.57 g) while the lowest value was recorded for NBPGR-2 (28 g and 70 q).

For the number of seeds per pod, the highest value was recorded in WB1306 (7.5) followed by IC-326619 and E1 VENYL (7) while the lowest value was recorded in WB832 and SB121.1 (3.5). For 100 seed weight, the highest value was recorded for NBPGR-3 (57.41 g) followed by WB1187 (51.85 g) and WB1151 (51.74 g) while the lowest value was recorded in SB162 (16.59 g). In seed yield per plant and seed yield per hectare, the highest value was recorded for WB1151 (88.5 g and 22.1 q) followed by SB152 (75.62 g and 18.9 q) and SB107 (66.47 g and 16.6 q) while the lowest value was recorded for NBPGR-1 (10.08 g and 2.51 q) (Table I) (Appendix 1).

The perusal of Table 15 indicated that the among vegetable type genotypes, WB1306 (days to flower initiation, days to pod initiation and number of seeds per pod), SB106 (pod length, pod weight, hundred seed weight), SB167 (days to marketable maturity and number of pods per plant), WB469 (pod thickness), SB156. A (pod yield per plant) and SB152 (seed yield per plant) were found as the most promising vegetable type genotypes for different yield-related traits.

In the current study, a substantial number of genotypes were found to perform significantly better than checks for several parameters. The substantial amount of variation recorded for maturity and yield traits provides us with a desirable working pool of genotypes that might be used for trait-based genetic enhancement of common bean. Razvi (2011); Blair *et al.* (2010); Sofi *et al.* (2014);

Rana *et al.* (2014); Rana *et al.* (2015) and Abdollahi *et al.* (2016) also reported substantial variability in maturity and yield traits of common bean. The results reported by Razvi (2011); Sofi *et al.* (2014), Rana *et al.* (2014) and Saba *et al.* (2017) are more relevant in support of the present study as the material used for these studies used a substantial genetic diversity from the state of Jammu and Kashmir.

5.1.4 ANOVA/ Variability parameters for Morphological, Yield and Pod traits

The materials in the present investigation were analyzed through augmented block design and the analysis revealed a significant mean sum of squares for all the traits for different sources of variation. The Block effect (unadjusted) i.e., the block ignoring treatments and the treatment effect (adjusted as well as unadjusted) were significant for all the traits indicating that the blocks were heterogeneous. Similarly, the effects due to checks and test entries were also significant. However, the adjusted block (i.e., Block eliminating treatment) effects were non-significant for all the traits indicating homogeneity of the evaluation blocks or experimental units. Similarly, the mean square due to checks vs test entries was significant for all the traits except the pod weight indicating that the test entries were significantly different from the checks except for pod weight. Similar results were reported by Saba *et al.* 2017.

Despite the fact that only three (3) checks were employed, they were carefully chosen to reflect a diversified trait expression in terms of morphological, yield and pod quality traits, as well as belonging to different pedigrees. Several research studies have shown similar results (Sofi *et al.*, 2014; Rana *et al.*, 2015). When dealing with huge germplasm sets, it is proposed that a specific field design be used to derive useful information of choice. The method provides adequate information on the extent of variability in the materials and can also help delineate differences among the test entries, among checks and between checks and test entries.

5.1.5 Coefficient of variability, heritability and genetic advance (as per cent of mean)

The phenotypic variability reflected by the range values is not a reliable indicator of variability since it comprises genotypic, environmental and genotype environmental interaction components and does not reveal which character exhibits the greatest degree of genetic variability (heritable part). Because the crop's phenotype is influenced by additive gene effect (heritable), dominance (non-heritable), epistasis (no allelic interaction) and GxE interaction, the observed variability must be divided into the phenotypic coefficient of variation and genotypic coefficient of variation, which ultimately indicates the extent of genetic variability present for various traits.

The estimates of phenotypic and genotypic coefficients of variation of all the characters studied are presented in (Table 13). In general, the phenotypic and genotypic coefficients of variation were almost similar with slightly higher phenotypic coefficients of variation, which indicates the role of environment in the expression of traits under observation. This was in agreement with the study of (Sofi *et al.*, 2011; Saba *et al.*, 2017; Razvi *et al.*, 2018; Gupta *et al.*, 2021).

It is evident from the data that the number of pods per plant (46.60, 46.62), pod yield per plant (44.35, 44.19), hundred seed weight (31.11, 31.10), seed yield per plant (45.33, 45.32) recorded high phenotypic and genotypic coefficients of variation respectively, indicating that genotypes had a broad genetic base for these characters. The rest of the traits such as the number of days to flowering (10.76, 10.5), number of days to pod initiation (9.63, 9.58), number of days marketable maturity (8.73, 8.41), pod length (15.71, 15.74), pod thickness (11.58, 11.59), pod weight (13.1, 13.1), number of seeds per pod (19.75, 19.76) showed low to moderate phenotypic and genotypic coefficients of variation. This was in conformity with the findings of Sofi *et al.*, 2011., Rana *et al.*, 2015., Kassa *et al.*, 2019., Yohannes *et al.*, 2020., Gupta *et al.*, 2021.

Since phenotypic and genotypic coefficients of variation do not give a true picture of the extent of inheritance of the character, therefore, the heritability of a character can be relied upon as it enables the breeder to decide the extent of selection pressure to be applied under a particular environment, which separates out the environmental influence from the total variability. The estimation of heritability has a greater role to play in determining the effectiveness of the selection of a character provided it is considered in conjunction with the predicted genetic advance as suggested by Panse and Sukhatme (1957) and Johnson *et al.* (1955) because the heritability is influenced by bio-metrical method, generation of hybrid, the sample size of experimental material and environment. Furthermore, the progress in selection is also directly proportional to the amount of genetic gain. Therefore, the effect of selection is realized more quickly in those characters which have high heritability as well as high genetic gain. The relative amount of the heritable portion of variation was, therefore, estimated with the help of heritability estimates and genetic advance.

When high heritability is accompanied by high genetic gain, it indicates additive gene effects and selection may be effective. High heritability with low GAM indicates the predominance of non-additive gene action where high heritability is exhibited due to the favorable influence of environment rather than genotype and the selection for such traits may not be rewarding. Low heritability with high GAM is governed by additive gene effects in which low heritability is exhibited due to favorable environmental effects and the selection may be effective in such cases. Low heritability coupled with low GAM indicates that character is highly influenced by environmental effects and selection would be ineffective.

Heritability (b.s.) was high for all the characters and ranged from 87.25 to 99.96 per cent indicating that the characters are less influenced by environmental effects and the characters are effectively transmitted to the progeny, suggesting a major role of the genetic constitution in the expression of a character and thus

selection based on phenotypic expression could be relied upon. This is in accordance with the findings of Sofi *et al.*, 2011 for days to flowering, days to marketable maturity, no. of pods per plant, pod length, no. of seeds per pod and seed yield per plant., Razvi *et al.*, 2011 for hundred seed weight., Jhanavi *et al.*, 2018 for pod thickness., Singh *et al.*, 2013 for pod yield per plant., Rai *et al.*, 2006 for pod weight and Shah *et al.*, 2021 for days to pod initiation. High heritability values for these traits indicate that the variation observed was mainly under genetic control and was less influenced by the environment.

High estimate of heritability along with high genetic gain (per cent of mean) is more reliable than heritability alone for predicting the effect of selection (Johnson *et al.*, 1955). The characters *viz.*, Days to flowering, days to pod initiation, number of pods per plant, pod length, pod thickness, pod weight, pod yield per plant, number of seeds per pod, hundred seed weight and seed yield per plant showed the high estimates of heritability coupled with high genetic advance as per cent of mean (GAM), indicating the preponderance of additive gene action in control of these traits. This suggests that real progress in improvement through selection could be made for yield. These results are in conformity with several workers *viz* Sofi *et al.*, 2011 for days to flowering, no. of pods per plant, pod length, no. of seeds per pod, hundred seed weight and seed yield per plant., Shah *et al.*, 2021 for days to pod initiation, pod weight and pod yield per plant and Jhanavi *et al.*, 2018 for pod thickness. However, days to marketable maturity showed high heritability coupled with moderate genetic advance as per cent of the mean.

Pod yield is an important character, which decides the commercial viability of the hybrid/variety. Thus, the trait deserves the highest priority in any breeding program. High heritability along with high genetic advance as per cent of mean for this trait suggested the possibility of selecting high-yielding cultivars from the present collection.

5.1.6 Correlation coefficient:

Increment in pod yield is one of the prime objectives of all breeding programs; however, using pod yield per se as a selection criterion for crop improvement in terms of yield is difficult due to the complexity of its nature as well as low heritability; and therefore, the realized gains for yield have not been very encouraging by direct selection for yield. Therefore, there has been great emphasis across all breeding programs to study the nature of relationship and trait associations between yield and other traits which are relatively less complex and have comparatively better heritability for use as indirect selection criteria. The concept of correlation was first given by Galton in 1889 with the basic premise that the knowledge of nature and magnitude of associations among components of economic worth can help improve the efficiency of selection by making possible use of suitable traits or a combination of traits. The phenotypic correlation among traits reflects the observed relationship between traits arising out of both genetic and environmental factors; while as the genotypic correlations arise from linkage and pleiotropy. The basic criteria for using a trait or set of traits for indirect selection are high heritability of the trait and desirable correlations with yield. The knowledge of trait associations in common bean breeding materials is essential for a variety of reasons: it enables us to perceive the diversity of breeding materials and identify the traits through which a bean plant is able to grow successfully in a given ecological condition with optimum productivity and to avoid characters that have little or no breeding value.

The economically important trait i.e., pod yield per plant exhibited positive and significant association with pod length, pod weight, pod thickness, no. of pods per plant and seed yield per plant which suggested that rational improvement in yield is possible through simultaneous selection for these component characters. It depicted a positive but non-significant association with the number of seeds per pod. These results are in conformity with the findings of Lyngdoh *et al.*, 2018 for the number of pods/plant on pod yield; Devi *et al.*, 2015

for pods/plant and pod weight on pod yield., Rai *et al.*, 2010., for pods/plant on pod yield., Shah *et al.*, 2021., for pod length/ pod thickness, no. of pods/ plant, pod weight on pod yield., Singh *et al.*, 2018 for seed yield per plant on pod yield.

However, it showed a negative non-significant correlation with the number of days to flowering, number of days to first pod initiation, number of days to marketable maturity and hundred seed weight which clearly indicated the independent nature of these characters and selection for fruit yield based on these characters might not be reliable. These results are in conformity with the findings of Verma *et al.*, 2014 for days to flowering on pod yield., Rai *et al.*, 2010 for hundred seed weight on pod yield. and Haralayya *et al.*, 2015 for days to pod set and days to maturity on pod yield.

5.1.7 Genetic diversity

As an initial step to developing high-yielding and superior-quality varieties, it is imperative to evaluate a large number of existing genotypes for their diversity. These genotypes must have been selected on the basis of their *per se* performance from diverse sources. Superior genotypes are selected and used as parents in hybridization programmes. However, the selection of superior parents from a large number of genotypes is a difficult task to perform. Genetic divergence analysis among genotypes is helpful to screen the genetically diverse parents that are likely to produce high heterotic effects among crosses and also generate a large spectrum of variability during the segregation and recombination of genes at heterozygous polygenic blocks. The multivariate technique using D^2 statistics (Mahalanobis, 1928) is a powerful tool for quantifying the degree of divergence among the genotypes. This would help to identify putative parents for executing an effective breeding strategy to obtain a high heterotic response and transgressive segregants. Estimating genetic divergence helps reduce the large data of genotypes to manageable proportions. It is assumed that the parents showing wide genetic divergence are best suited for being used in the hybridization program. In the process of formulating the common bean

improvement program through hybridization and creating variability for the improvement of yield and other desirable traits, it is essential to understand the nature and degree of genetic divergence present in the available germplasm.

Based on the multivariate analysis performed on 13 quantitative traits, all 51 accessions were grouped into two major clusters, cluster I and cluster II with each cluster containing different number of accessions. Cluster I contained 21 accessions and cluster II contained maximum (30) number of genotypes. The mean value of genotypes grouped into each cluster showed that accessions in Cluster II had the highest mean value for days to flowering (53.15), days to pod initiation (61.70), days to marketable maturity (69.59), number of pods per plant (41.07), pod thickness (9.62 mm), pod weight (4.50 g), pod yield per plant (183.30 g), pod yield per hectare (458.27 q), seed yield per plant (50.58 g) and seed yield per hectare (12.64 q), while the genotypes grouped in cluster I showed the highest mean value for pod length (11.26 cm), number of seeds per pod (5.17) and hundred seed weight (30.62 g). The results are in conformity with the findings of Piergovanni *et al.*, 2000., Boros *et al.*, 2014., Gangappa *et al.*, 2014., Haralayya D *et.al.*, 2017., Loko *et al.*, 2018).

It is clear from the above discussion that tremendous potential exists for converging the elite allelic resources present in the present set of common bean genotypes through a systematic breeding and selection approach so as to recover high-yielding recombinants, with good quality characteristics. Selection of the parents for hybridization should be done from different clusters having wide distances and those selected parents should have high *per se* performance for the traits contributing maximum towards divergence (Singh *et al.*, 1996).

5.2 Diversity study at molecular level

5.2.1 Diversity analysis:

Molecular markers or DNA-based markers are of various types, with SSRs (Simple Sequence Repeats) being the most popular due to multiple advantages such as abundant polymorphism, co-dominance, locus specificity, multi-allelic and high reproducibility. SSRs, or microsatellites, are short tandem repeats of DNA sequences found throughout an organism's genome that are considered more reliable due to their ability to provide very consistent profiles (McCouch *et al.*, 1997). SSR technique has been successfully applied in various taxonomic and genetic diversity studies. Due to the abundance and coverage of SSR markers of whole genome, reliable estimates of relationships among cultivars can be obtained.

In this study an attempt was made to evaluate genetic diversity among 51 common bean genotypes using a total of 11 SSR primers. Among the selected 11 SSR primers, a total of 9 markers, amplified alleles across the fifty-one genotypes with varying degrees of polymorphism and revealed clear and consistent amplification profiles in the entire germplasm set. A total of 54 alleles were amplified by 15 polymorphic SSR loci and the number of alleles ranged from 1 to 8 with an average of 4.909 alleles per locus. The obtained number of alleles per loci is comparable with other authors *viz* Mall *et al.*, 2014., Sharma *et al.*, 2014., Avican, O *et al.*, 2022 and the average number of alleles per loci was comparable with Gyang *et al.*, 2017 with 4.5 alleles per locus. The highest number of alleles i.e, 8 were amplified by *PVBR45a*. The variation in the number of alleles produced by SSR markers demonstrates heterozygosity in different alleles at a given locus in which the heterozygosity could reflect greatly the state of genetic variability.

The PIC value provides an estimate of the discriminating power of markers by taking into account not only the number of alleles at a locus but also

the relative frequencies of these alleles. Lower PIC value might be a result of closely related genotypes and vice-versa. The PIC value obtained in the present study varied from 0 (*Bmd45a*, *Bmd45b*, *PVBR128a*, *PVBR128b*, *Bmd12b* and *PVBR45b*) to 0.87 (*Bm152*) with an average value of 0.519 across 51 common bean genotypes. indicating that SSR markers are highly informative. Our results were in accordance with (Sharma and Singh, 2014, Mall *et al.*, 2014., Saba 2016. and Ozkan *et al.*, 2022).

Dendrograms are efficient means of summarizing microsatellite data and can reveal relationships including individuals with identical genotypes. The cluster dendrogram classified 51 genotypes into 3 clusters (Table-19). The results were in accordance with (Okii *et al.*, 2014., Arunga *et al.*, 2015., Ozkan. G *et al.*, 2022) and almost comparable to (Immaculee *et al.*, 2015 and Gyang *et al.*, 2017) where only 2 clusters were formed.

The dendrogram shows that there is a considerable amount of genetic variation among the accessions in relation to SSR primers studied. Some of the accessions having the same geographic origin were distributed in different sub-clusters suggesting their genetic diversity. The fifty-one common genotypes (Table-20) showed dissimilarity coefficient value ranging from 0.00 to 0.93. The highest dissimilarity value of 0.93 was observed between genotypes WB1306 and SB107 falling in cluster I and sub-cluster IIb of cluster II respectively, IC-326854 and E1 VENYL falling in cluster I and sub-cluster IIa of cluster II respectively and SB123 and SB116.1 falling in cluster I and Cluster III respectively. The minimum dissimilarity 0.00 was observed between genotypes EC-394659 and Lcl Bml falling in sub-cluster Ia of Cluster II, followed by WB1151 and WB 665 with a value of 0.26 falling in cluster I. The PCoA could also distribute the genotypes in all four quadrants. The assessment of genetic distance using dissimilarity matrix was conducted by Gyang *et al.*, 2017.

SSR markers proved to be very informative in the assessment of genetic variation. The analysis with SSR markers disclosed wide variation within

common bean genotypes. It was retrieved suitable for use because of its ability to generate reproducible polymorphic bands. The present findings further strengthened previous reports that SSR markers can be used effectively to estimate genetic differences among genotypes and are in agreement with the results of (Sharma and Singh, 2014., Mall. N *et al.*, 2014., Okii et al., 2014. and Ozkan. G *et al.*, 2022) which the studied genotypes were discriminated according to the gene pool level by molecular-based cluster analysis. Moreover, recent studies revealed the potential of SSRs in providing intra-specific diversity within the genus.

The genotypes showing extreme behavior with respect to the morphological traits under study were grouped into different clusters through molecular analysis as well i.e, these genotypes were found to be in different clusters under both cases indicating their genetic divergence. This can be attributed to different lineage of the genotypes or due to the large geographical separation that led to the accumulation of detectable genetic differences.

The morphological characterization, by producing fewer groups, indicate that the characters under study had less variation in the 51 collected genotypes; while the molecular characterization allowed a more accurate estimation of genetic variation attributed to SSR markers which are environmentally neutral. On the other hand, by not expressing genetic variations restricted by the unique assessment environment (Boada-Higuera *et al.*, 2010), morphological characterizations can lose accuracy in estimating the variation by confusing both the genetic effects and those of the genotype x environment interaction as suggested by Sultan (2000), which indicates that due to the phenotypic plasticity, a genotype can alter its development and physiology according to the environmental conditions where it grows. In the present study, the morphological characterization detected less diversity than the molecular one and generated different clustering patterns. However, molecular analysis with additional SSR markers and with greater genomic coverage could reveal genetic diversity

accurately and also unambiguously differentiate the genotypes with identical allelic patterns as revealed by the set of primers used in this study. Further, by maximizing the number of genotypes from different agroecological regions along with the use of a higher number of polymorphic SSR markers, an improved estimation of genetic diversity could be worked out.

Chapter 6

SUMMARY AND CONCLUSION

The present study entitled “**Morpho-molecular characterization of common bean (*Phaseolus vulgaris L.*) germplasm**” was conducted during Kharif 2021 at the research farm of the Division of Genetics and Plant Breeding, Faculty of Agriculture, SKUAST-Kashmir, Wadura campus, Sopore. The Laboratory work was conducted at Molecular Biology Laboratory, Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, Sopore. The study included 51 genotypes of common bean (*Phaseolus vulgaris L.*), mostly from the north-western Himalayan regions of Jammu and Kashmir and some exotic genotypes, Local landraces from Jammu province (Poonch, Rajouri, Kishtawar, Doda and Baderwah), Kashmir province's (Kupwara, Baramullah, Anantnag and Bandipora) and several exotic germplasm lines that were already in our possession at Wadura, SKUAST-Kashmir. The genotypes were evaluated in Augmented Block Design (ABD) with 8 blocks and each block contained 6 treatments and 3 checks. The genetic diversity was assessed using morphological characters and SSR markers. Genetic divergence studies through morphological and molecular markers are vital tools in providing useful information on genetic diversity at both inter-specific and intra-specific levels. Genetic variability revealed through morphological and molecular approaches help in germplasm conservation and maintenance, it is also important as it provides the basis for successful breeding programs.

The morphological descriptors used were drawn from USDA, CIAT and PPVFRA manuals to have an in-depth characterization of the materials. For the morphological divergence study, the genotypes were evaluated for 17 qualitative (leaf, pod, flower, seed) and 13 quantitative traits. The quantitative traits studied were: days to flowering, days to pod initiation, days to marketable maturity, number of pods per plant, pod length (cm), pod thickness (mm), pod weight (g), number of seeds

per pod, hundred seed weight (g), pod yield per plant (g), pod yield per hectare (q), seed yield per plant (g) and seed yield per hectare (q).

In the materials under the current investigation, out of 51 genotypes, 33 were found vegetable type. Most of the lines (60.7%) were bush types; however, a sizeable number of climbing types were also incorporated. The climbing types were purposefully included in line with the feedback from the farmers through participatory rural appraisal about the agronomic compatibility of these genotypes in maize-bean intercropping. Other descriptor traits also had sizeable data distribution among different classes, as the materials were carefully drawn from the germplasm bank to represent the diversity of market classes in relation to growth habit, colour, shape, length and visual appearance of the pod.

Analysis of variance showed that mean square values were highly significant for all the characters for different sources of variation. The maximum range was recorded for pod yield per hectare (q), pod yield per plant (g), seed yield per plant (g), seed yield per hectare (q), number of pods per plant and hundred seed weight (g) while the lowest range was observed for pod weight (g), number of seeds per pod and pod thickness (mm). For the rest of the traits, the range was moderate. Based on the standard errors of difference, it could be observed that there was wide variation among the genotypes for the traits studied. A sizeable number of genotypes was found to have desirable attributes in respect of these traits as compared to checks.

On the basis of mean performance of vegetable-type genotypes, the maximum pod yield per plant and pod yield per hectare respectively, was recorded in SB156.A (253.76 g, 634.39 q), SB159 (213.92 g, 534.8 q), SB152 (211.43 g, 528.57 q), SB181.1 (197.44 g, 493.61 q) and SB167 (186.78 g, 466.94 q).

The phenotypic and genotypic coefficients of variation ranged from 8.73-46.62 and 8.41-46.60 respectively. However, there was a narrow difference

between the magnitude of phenotypic and genotypic coefficients of variation for all the characters studied, indicating low environmental effect on the expression of these characters, which implies that phenotypic variability is a reliable measure of genotypic variability. High phenotypic coefficient of variation was exhibited by number of pods per plant (46.62), seed yield per plant (45.33), pod yield per plant (44.35) and hundred seed weight (31.11) while the moderate phenotypic coefficient of variation was observed for number of seeds per pod (19.75), pod weight (13.3), pod thickness (11.59) and days to flowering (10.76). Maximum genotypic coefficient of variation was observed in number of pods per plant (46.60), seed yield per plant (45.32), pod yield per plant (44.19) and hundred seed weight (31.10) while the moderate phenotypic coefficient of variation was observed for number of seeds per pod (19.75), pod weight (13.1), pod thickness (11.58) and days to flowering (10.5).

High estimate of heritability along with high genetic gain (per cent of mean) is more reliable than heritability alone for predicting the effect of selection. The characters *viz*, days to flowering, days to pod initiation, number of pods per plant, pod length (cm), pod thickness (mm), pod weight (g), pod yield per plant (g), number of seeds per pod, hundred seed weight (g), seed yield per plant (g) revealed high estimates of heritability coupled with high genetic advance as per cent of mean (GAM), indicating the preponderance of additive gene action in control of these traits.

In the present investigation, correlation coefficients revealed that the economically important trait i.e., pod yield per plant exhibited positive and significant association with pod length, pod weight, pod thickness, number of pods per plant and seed yield per plant which suggested that rational improvement in yield is possible through simultaneous selection for these component characters. It depicted a positive but non-significant association with the number of seeds per pod. However, it showed a negative non-significant correlation with the number of days to flowering, number of days to first pod initiation, number of

days to marketable maturity and hundred seed weight which clearly indicated the independent nature of these characters and selection for fruit yield based on these characters might not be reliable.

Clustering based on 13 morphological traits allowed the grouping of fifty-one common bean genotypes into 2 clusters. The formation of different clusters with a variable number of entries in each cluster indicated diversity among genotypes. The genotypes from different agro-ecological zones were found to be scattered in different clusters, which suggested that a pattern of clustering of accessions was independent of their geographical origin. The cluster dendrogram indicated that the maximum number of genotypes fell in cluster II (30) while Cluster I consisted of 21 genotypes.

For molecular diversity analysis, 11 SSR markers were amplified through Polymerase Chain Reaction. The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix. The PIC value obtained in the present study varied from 0 (*Bmd45a*, *Bmd45b*, *PVBR128a*, *PVBR128b*, *Bmd12b* and *PVBR45b*) to 0.87 (*Bm152*) with an average value of 0.519 across 51 common bean genotypes. indicating that SSR markers are highly informative. Among the selected 11 SSR primers, a total of 9 markers, amplified alleles across the fifty-one genotypes with varying degrees of polymorphism and revealed clear and consistent amplification profiles in the entire germplasm set. A total of 54 alleles were amplified by 15 polymorphic SSR loci and the number of alleles ranged from 1 to 8 with an average of 4.909 alleles per locus.

Cluster analysis of genotypes using binary data generated by microsatellite markers was conducted. The 51 common bean genotypes were classified into 3 clusters and showed dissimilarity coefficient value ranging from 0.00 to 0.93. The Principal Coordinate Analysis (PCoA) also separated the 51 genotypes into 3 distinct groups, which was consistent with the results of the cluster analysis. The highest dissimilarity value of 0.93 was observed between genotypes WB1306 and SB107 falling in cluster I and sub-cluster IIb of cluster II respectively, IC-326854

and E1 VENYL falling in cluster I and sub-cluster IIa of cluster II respectively and SB123 and SB116.1 falling in cluster I and Cluster III respectively. The minimum dissimilarity 0.00 was observed between genotypes EC-394659 and Lcl Bml falling in sub-cluster Ia of Cluster II, followed by WB1151 and WB 665 with a value of 0.26 falling in cluster I. The genotypes which exhibited extreme behaviour with respect to the morphological traits under study were grouped into different clusters through molecular analysis as well i.e., these genotypes were found to be in different clusters under both cases indicating their genetic divergence.

Based on the findings of the present investigation, the following conclusions could be drawn:

- The study revealed enough genetic variability among the 51 genotypes at morphological and molecular level.
- Analysis of variance indicated significant variation existed among genotypes for various characters under study.
- Correlation studies indicated that characters like pod length, pod thickness, pod weight, number of pods per plant, pod yield per plant and pod yield per hectare should be considered for improving quantitative traits in common bean.
- On the basis of mean performance of vegetable type genotypes, WB1306, SB106, SB167, WB469 SB156. A and SB152 were identified as the most promising vegetable type genotypes for different yield-related traits.
- Morphological cluster analysis revealed considerable genetic diversity and grouped genotypes into 2 different clusters.

- Selection of parents from different clusters showing best performance can be used in hybridization programme for development of high yielding varieties.
- SSR markers showed genetic variability in the studied common bean genotypes and are powerful tools for estimating genetic dissimilarities and diversity. The genetic relationships presented among the genotypes is helpful for future breeding programmes through selection of genetically diverse parents.
- A total of 54 alleles were detected with an average 4.909 alleles per loci. The maximum number of alleles were 8 in primer *PVBR45a*. The polymorphic information content value ranged from 0 (*Bmd45a*, *Bmd45b*, *PVBR128a*, *PVBR128b*, *Bmd12b* and *PVBR45b*) to 0.87 (*Bm152*) with an average value of 0.519. SSR primers *Bm152*, *PVBR45a*, *Bmd28* were the most polymorphic with PIC value of 0.87 for *Bm152*, 0.862 for *PVBR45a* and 0.730 for *Bmd12a*.
- SSR based clustering classified 51 genotypes into 3 clusters and showed considerable diversity among the genotypes. The genotypes which exhibited extreme behaviour with respect to the morphological traits under study were grouped into different clusters through molecular analysis as well i.e, these genotypes were found to be in different clusters under both cases indicating their genetic divergence.
- Molecular analysis with additional SSR markers and with greater genomic coverage could help analyse genetic diversity accurately and also help to unambiguously differentiate these genotypes with identical allelic patterns.

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Appendix I

| S.no | Genotype name | DF | DPI | DMM | NPP | PL (cm) | PT (mm) | PW (g) | PYPP (g) | PYPH (q) | HSW (g) | NSPP | SYPP (g) | SYPH (q) |
|------|---------------|-------|-------|-----|-------|---------|---------|--------|----------|----------|---------|------|----------|----------|
| 1 | E10© | 51 | 60.88 | 68 | 48.6 | 9.67 | 9.14 | 4.29 | 208.37 | 520.93 | 24.61 | 4 | 47.85 | 11.962 |
| 2 | IC-326603 | 50.29 | 56.04 | 65 | 17.34 | 10.75 | 10.17 | 4.5 | 78.49 | 196.22 | 31.03 | 6 | 32.49 | 8.124 |
| 3 | IC-326619 | 47.29 | 55.04 | 62 | 20.34 | 11.6 | 9.87 | 3.92 | 80.13 | 200.33 | 32.13 | 7 | 45.89 | 11.473 |
| 4 | IC-326854 | 54.29 | 62.04 | 70 | 14 | 8.67 | 7.49 | 3.44 | 48.69 | 121.72 | 19.31 | 6 | 16.46 | 4.116 |
| 5 | IC-328483 | 44.29 | 52.04 | 61 | 22.34 | 9.2 | 8.78 | 4.03 | 90.39 | 225.97 | 29.16 | 5.33 | 34.91 | 8.726 |
| 6 | WB1306 | 43.29 | 49.04 | 57 | 30.61 | 11.08 | 8.35 | 4.63 | 141.92 | 354.8 | 25.74 | 7.5 | 59.09 | 14.774 |
| 7 | WB832 | 45.29 | 54.04 | 62 | 10.4 | 10.98 | 8.3 | 4.6 | 48.44 | 121.1 | 36.56 | 3.5 | 13.63 | 3.408 |
| 8 | WB665 | 49.96 | 56.71 | 64 | 18.67 | 10.58 | 9.7 | 4.43 | 82.48 | 206.19 | 19.06 | 5 | 17.52 | 4.379 |
| 9 | WB1151 | 41.96 | 47.71 | 56 | 38 | 12.9 | 11.4 | 5 | 189.96 | 474.9 | 51.74 | 4.5 | 88.5 | 22.12 |
| 10 | WB1199 | 50.96 | 60.71 | 69 | 36 | 10.6 | 9.6 | 4.44 | 159.78 | 399.45 | 22.14 | 4 | 31.8 | 7.949 |
| 11 | WB192 | 48.96 | 54.71 | 61 | 24 | 9.33 | 8.8 | 4.17 | 99.9 | 249.75 | 34.9 | 4 | 33.24 | 8.31 |
| 12 | SR1© | 52.87 | 59.38 | 69 | 43 | 11.43 | 10.44 | 4.73 | 203.23 | 508.07 | 30.32 | 5 | 65.2 | 16.29 |
| 13 | WB603 | 53.96 | 61.71 | 70 | 15 | 14.7 | 10.53 | 5.24 | 78.33 | 195.83 | 36.84 | 6.5 | 35.66 | 8.915 |
| 14 | WB469 | 45.96 | 55.71 | 65 | 22.5 | 14.5 | 10.77 | 5.19 | 116.58 | 291.45 | 27.16 | 5.5 | 33.46 | 8.365 |
| 15 | WB1187 | 43.96 | 54.04 | 60 | 12.4 | 14 | 10.99 | 5.83 | 73 | 182.5 | 51.85 | 5 | 32.21 | 8.052 |
| 16 | E1 VENYL | 46.96 | 56.04 | 62 | 35.5 | 12.5 | 9.6 | 4.9 | 174.2 | 435.49 | 23.79 | 7 | 59.07 | 14.768 |
| 17 | E2 | 51.96 | 61.04 | 69 | 13 | 12.3 | 9.4 | 4.89 | 64.27 | 160.67 | 46.6 | 5 | 30.35 | 7.587 |
| 18 | SB123 | 53.96 | 64.04 | 72 | 14.5 | 12.8 | 11.2 | 4.98 | 65.64 | 164.09 | 39.07 | 5 | 28.38 | 7.094 |
| 19 | SB107 | 61.96 | 71.04 | 78 | 28.8 | 11 | 10 | 4.66 | 134.59 | 336.47 | 34.97 | 6.6 | 66.47 | 16.617 |
| 20 | SB151 | 59.96 | 68.04 | 74 | 32 | 12.75 | 8.6 | 4.92 | 157.76 | 394.39 | 31.37 | 5.5 | 55.21 | 13.803 |
| 21 | SB106 | 50.96 | 60.04 | 69 | 17.67 | 14.8 | 9.2 | 5.21 | 91.59 | 228.98 | 37.24 | 4.66 | 30.75 | 7.688 |
| 22 | SB19 | 54.96 | 66.04 | 75 | 23.2 | 12.6 | 10.5 | 4.87 | 112.7 | 281.74 | 20.87 | 5 | 24.24 | 6.061 |
| 23 | Lcl Bml © | 55 | 62.88 | 70 | 36.2 | 10.88 | 10.2 | 4.53 | 163.9 | 409.74 | 22.53 | 4 | 32.62 | 8.154 |
| 24 | SB166 | 51.96 | 61.04 | 68 | 41 | 10.2 | 9.7 | 4.41 | 180.81 | 452.02 | 35.5 | 4.5 | 65.45 | 16.362 |
| 25 | SB183.1 | 60.96 | 70.04 | 77 | 39 | 10.8 | 9.2 | 4.48 | 174.68 | 436.69 | 28.34 | 4 | 44.2 | 11.05 |
| 26 | WB1189 | 52.96 | 62.04 | 70 | 33 | 11.5 | 8.8 | 4.6 | 151.64 | 379.09 | 29.43 | 6 | 58.19 | 14.549 |

Contd....

Appendix I: contd...

| S.no | Genotype name | DF | DPI | DMM | NPP | PL (cm) | PT (mm) | PW (g) | PYPP (g) | PYPH (q) | HSW (g) | NSPP | SYPP (g) | SYPH (q) |
|-------------|----------------------|-----------|------------|------------|------------|----------------|----------------|---------------|-----------------|-----------------|----------------|-------------|-----------------|-----------------|
| 27 | SB155.1 | 54.96 | 63.04 | 71 | 21 | 11.4 | 7.9 | 4.59 | 96.05 | 240.13 | 25.04 | 5 | 26.35 | 6.586 |
| 28 | SB152 | 49.96 | 63.71 | 72 | 53 | 13.5 | 9.2 | 3.99 | 211.43 | 528.57 | 28.53 | 5 | 75.62 | 18.904 |
| 29 | SB111 | 44.96 | 53.71 | 62 | 19 | 13.2 | 10.6 | 4.78 | 90.78 | 226.94 | 35.03 | 6 | 39.86 | 9.965 |
| 30 | SB165 | 56.96 | 63.71 | 70 | 31.6 | 7.8 | 7.91 | 3.01 | 95.07 | 237.68 | 18.14 | 4.3 | 24.58 | 6.146 |
| 31 | SB169 | 42.96 | 49.71 | 56 | 18 | 11.33 | 10.19 | 4.68 | 84.2 | 210.49 | 27.29 | 4 | 19.54 | 4.886 |
| 32 | SB149 | 56.96 | 65.71 | 73 | 26 | 8.1 | 9.7 | 3.11 | 80.82 | 202.04 | 20.65 | 4.5 | 24.09 | 6.021 |
| 33 | SB128 | 44.96 | 55.71 | 63 | 16 | 12.8 | 10.3 | 5.58 | 89.24 | 223.09 | 37.51 | 5 | 29.91 | 7.478 |
| 34 | NBPGR-2 | 45.96 | 57.04 | 66 | 8 | 11.79 | 9.34 | 4.79 | 38.07 | 95.17 | 30.46 | 4 | 10.08 | 2.519 |
| 35 | SB116.1 | 55.96 | 63.04 | 71 | 19.34 | 11.2 | 7.3 | 4.51 | 85.97 | 214.92 | 25.28 | 6 | 29.23 | 7.307 |
| 36 | SB131 | 55.96 | 64.04 | 71 | 37 | 10.1 | 10.16 | 4.35 | 160.58 | 401.44 | 27.06 | 5 | 50.06 | 12.515 |
| 37 | SB167 | 53.96 | 62.04 | 69 | 60 | 11.8 | 6.4 | 3.1 | 186.78 | 466.94 | 16.8 | 4 | 40.41 | 10.102 |
| 38 | SB162 | 47.96 | 54.04 | 63 | 14 | 11 | 9.1 | 4.55 | 62.18 | 155.44 | 16.59 | 6 | 13.78 | 3.445 |
| 39 | SB142.1 | 44.96 | 56.04 | 66 | 40.6 | 13.83 | 8.9 | 4.03 | 163.43 | 408.56 | 24.54 | 4.5 | 44.82 | 11.206 |
| 40 | SB181.1 | 54.96 | 64.04 | 72 | 45 | 10.5 | 10.3 | 4.39 | 197.44 | 493.61 | 20.42 | 4 | 36.74 | 9.185 |
| 41 | SB117.2 | 56.96 | 67.38 | 76 | 34 | 11 | 10.4 | 4.7 | 160.18 | 400.44 | 25.09 | 6.5 | 55.39 | 13.847 |
| 42 | SB156.A | 54.96 | 63.38 | 70 | 52 | 12 | 9.5 | 4.89 | 253.76 | 634.39 | 23.85 | 4.5 | 55.73 | 13.932 |
| 43 | SB136 | 50.96 | 62.38 | 70 | 29.25 | 14.68 | 9.9 | 5.27 | 154.76 | 386.9 | 30.53 | 5.3 | 47.27 | 11.818 |
| 44 | SB159 | 55.96 | 64.38 | 72 | 50.2 | 9.5 | 7.1 | 4.27 | 213.92 | 534.8 | 24.05 | 4 | 48.23 | 12.057 |
| 45 | EC-21753 | 50.96 | 64.04 | 71 | 15.34 | 8.62 | 9.08 | 3.33 | 50.9 | 127.26 | 23.55 | 6 | 21.87 | 5.469 |
| 46 | SB121.1 | 57.96 | 70.38 | 78 | 37.4 | 9.9 | 9.1 | 4.44 | 166.26 | 415.66 | 26.53 | 3.5 | 34.67 | 8.668 |
| 47 | SB158 | 53.96 | 64.38 | 72 | 16.34 | 9.8 | 10.6 | 4.38 | 72.83 | 182.07 | 25.44 | 6 | 24.9 | 6.225 |
| 48 | NBPGR-2 | 55.96 | 63.04 | 72 | 6.2 | 10.83 | 10.09 | 4.56 | 28 | 70 | 28.71 | 6 | 11 | 2.749 |
| 49 | EC-385256 | 59.96 | 68.04 | 79 | 33.3 | 9.25 | 9.15 | 4.1 | 136.53 | 341.33 | 19.85 | 6.5 | 42.9 | 10.726 |
| 50 | EC-394659 | 61.96 | 72.04 | 80 | 28 | 9.1 | 9.18 | 3.96 | 110.83 | 277.07 | 24.97 | 4 | 28.13 | 7.032 |
| 51 | NBPGR-3 | 48.96 | 58.04 | 66 | 11 | 11.75 | 10.8 | 4.81 | 52.69 | 131.72 | 57.41 | 4.5 | 28.71 | 7.177 |

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr Mamta Pathak, Principal Olericulturist, Division of Vegetable Science, PAU, Ludhiana**, during Viva-Voce examination held on 23-02-2023 have been incorporated in the manuscript entitled, **“Morpho-molecular characterization of Common bean (*Phaseolus vulgaris* L.) germplasm”** submitted by **Ms. Syed Zainab Kashani (Regd. No. MSH-2020-332)**

(Dr. Feroz Ahmad Parry)
Chairman
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