Studies on phenotypic and molecular polymorphism in rice bean (*Vigna umbellata*)

THESIS

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(Plant Breeding and Genetics)

By

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2009

CERTIFICATE – I

This is to certify that the thesis entitled, ***"Studies on phenotypic and molecular polymorphism in rice bean (Vigna umbellata)"*** *submitted in partial fulfillment of the requirement for the degree of* ***MASTER OF SCIENCE IN AGRICULTURE (Plant breeding and Genetics)*** *of the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by* ***Ms. Aparna Pandey*** *under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.*

No part of the thesis has been submitted for any other degree or diploma (Certificate awarded etc.) or has been published/published part has been fully acknowledged. She has duly acknowledged all the assistance and help received during the course of investigations.

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CERTIFICATE – II

This is to certify that the thesis entitled ***"Studies on phenotypic and molecular polymorphism in rice bean (Vigna umbellata)"*** *submitted by* ***Ms. Aparna Pandey*** *to the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, in partial fulfillment of the requirements for the degree of* ***Master Of Science in Agriculture (Plant breeding and Genetics)*** *has been, after evaluation, approved by the External Examiner and by the Student's Advisory Committee after an oral examination on the same.*

Place: Jabalpur

Date: ..03./03./2010

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ABBREVIATIONS

%	:	Per cent
√	:	square root
∑	:	summation
µl	:	Micro liter
µM	:	Micro molar
AFLP	:	Amplified fragment length polymorphism
bp	:	Base pair
cM	:	Centi morgan
cm	:	Centi meter
CTAB	:	Cetyl trimethyl ammonium bromide
DNA	:	Deoxyribo nucleic acid
df	:	Degree of freedom
et al.,	:	And others
EDTA	:	Ethylene diamine tetra-acetate
hrs.	:	Hours
ISSR	:	Inter simple sequence repeat
Kb	:	Kilo base pair
M	:	Molar
mA	:	Mili ampere
MAS	:	Marker assisted selection
mg	:	Mili gram
min.	:	Minute
ml	:	mili liter
mM	:	Mili molar
mmt	:	Million metric tonne
ng	:	Neno gram
nm	:	Neno meter
O.D.	:	Optical density
PCR	:	Polymerase chain reaction
Q./ha	:	Quintal per hectare
QTL	:	Quantitative trait loci

RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
rpm	:	Rotation per minute
sec.	:	Second
TAE	:	Tris base acetic acid glacial EDTA
v/v	:	Volume by volume
w/v	:	Weight by volume
g	:	Gram
Viz	:	Namely
PCV	:	Phenotypic coefficient of variation
GCv	:	Genotypic coefficient of variation
GA	:	Genetic advance
R	:	Residual effect
h	:	Heritability
P_{KY}	:	Direct effect

INTRODUCTION

INTRODUCTION

Increase in global land degradation over the years due to agricultural activities and increasing global population, there is pressure on agriculture to increase food production. Rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi, previously *Phaseolus calcaratus*) is an annual vine legume which is little known, little researched and little exploited. It is regarded as a minor food and fodder crop and is often grown as intercrop or mixed crop with maize (*Zea mays*), sorghum (*Sorghum bicolor*) and cowpea (*V. unguiculata*) as well as a sole crop in uplands in a very limited area. Like the other Asiatic *Vigna* species, rice bean is a fairly short-lived warm-season annual. Grown mainly for dried pulse, it is also important as fodder, green manure and vegetable. In the past it was widely grown as lowland crop on residual soil water after the harvest of long-season rice, but it has been displaced to a great extent where shorter duration rice varieties are grown. Rice bean grows well on a range of soils. It establishes rapidly and has the potential to produce large amounts of nutritious animal fodder and high quality grain.

The cultivated Asiatic *Vigna* species belong to the subgenus Ceratotropis, a fairly distinct and homogeneous group, largely restricted to Asia, which has a chromosome number of 2n =22 (except *V. glabrescens*, 2n = 44). Rice bean plays an important role in human, animal and soil health improvement. All varieties seem to be good sources of protein; essential amino acids, essential fatty acids and minerals (Mohan and Janardhanan, 1994), and the dried seeds make an excellent addition to a cereal based diet.

In India, rice been is found as both wild as well as cultivated forms. It is grown in West Bengal, Assam, Manipur, Madhya Pradesh and Haryana. The plant is also used as green manure, cover crop and biological barrier. By virtue of high production potential (35 tons/ha), it is now attracting attention as leguminous crop during Kharif lean period. It can be successfully grown under extreme environmental condition i.e. excessive moisture, low rainfall and poor soils. As a rotation crop, it enriches soil in nitrogen content. This provides subsistence to the farmers with food supply through out growing season for animal population. It has been projected that the requirement for green fodder would be 1060 million tones by 2010 AD for the projected livestock population of more than 530 million.

Rice bean is a highly nutritious, palatable fodder. It is nutritionally richer in proteins, calcium and phosphorus as compared to cowpea and black gram. The approximate biochemical analysis of fodder at flowering stage reveals 24% dry matter, 14.5% crude protein, 32.1% crude fiber, 1.0% fat, 41.6% N-free extract, 10.8% ash, 1.2% calcium and 0.4% phosphorus.

Selection is said to be effective if the population is genetically diverse and have proper knowledge about the degree of association of yield and various components affecting it. Path analysis helps in determining the direct and indirect effect of the character on yield and thus helps to improve yield by selecting strongly correlated traits.

Molecular technique is being used as a tool to detect the extent and structure of genetic variation applying molecular markers, providing insights into the diversity of crop varieties and potential contributions represented by their wild relatives. Microsatellite markers have become the DNA markers of choice for a wide range of application in genetic mapping and genome analysis, genotype identification and variety protection.

Inter Simple Sequence Repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers are simpler, reproducible and show greater polymorphism as compared to other marker systems. ISSR is an alternative method of simple sequence repeat (SSR) where the repeated sequences are used as primer to produce polymorphism. Inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) is a PCR based DNA markers used for identification, evolutionary studies, genetic diversity studies and plant genome analysis.

To asses the variability at phenotypic, genic and molecular level in rice bean, present study has been undertaken with the following objectives

1. To determine the nature and amount of genetic variability for forage yield and its components.
2. To estimate correlation and path analysis for fodder yield and its components.
3. To detect the genetic diversity among the germplasm by RAPD and ISSR markers.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Vigna umbellata (Thunb.) Ohwi and Ohashi commonly known as rice bean or climbing mountain bean is under-exploited tropical legume. It is used as vegetable, folk medicine and fodder in Southeast Asian countries (Wu *et al.*, 2001). Among the various centers of diversity of cultivated rice bean has been reported in India, China, Malaysia, Myanmar and Thailand (Tomooka *et al.*, 2002).

It establishes rapidly and has the potential to produce large amounts of nutritious animal fodder and high quality grain. Being a native species and high local adaptability it can be grown well in the less fertile, exhausted, degraded marginal land without much efforts and input (Joshi, 2002). It is a minor pulse crop with remarkable drought tolerance capacity and feasibility for cultivation in marginal soils.

2.1 Taxonomy

Kingdom	Plantae	Subfamily	Faboideae
Division	Magnoliophyta	Genus	*Vigna*
Class	Magnoliopsida	Subgenus	*Ceratotropis*
Order	Fabales	Tribe	Phaseoleae
Family	Fabaceae		

2.2 Origin and Distribution

Rice bean is native to south and south-east Asia. In India, the crop is mainly found in the Western-Eastern Ghats and the NE Himalayas including Assam, Meghalaya, Mizoram and Manipur. It is also grown in the sub-temperate western Himalaya in the Uttaranchal and Himachal Pradesh hills.

Rice bean show considerable diversity and its four types have bean recognized viz., (1) Glabes, which has smooth stems and leaves; (2) Major, which has larger flowers; (3) Rumbaiya, which has shorter stems; and, (4) Gracilis, a wild type which is found in northeastern India, Burma, Thailand, Laos and Vietnam.

2.3 Morphology

Rice bean is an annual, erect to semi-erect or viny and twig crop in habit. The leaves are pinnately trifoliate with short hairs. Inflorescence raceme, flowers are borne in clusters and are self-fertile but some natural out crossing also occurs. Flowers are golden yellow in colour. Pod attaches to the peduncle downward and has eight to twelve seeds per pod. Seed is slender and hilum is protruding. Propagation is done by the seed and germination is hypogeal. The plant is photoperiod sensitive and the reproductive phase covers mid-November to February.

2.4 Nutritive value and use

Rice bean (*Vigna umbellata*), in spite of its grain yield potential comparable to major pulse crops and excellent nutritional qualities. The seed contains 14 to 24 per cent of protein, total carbohydrate 60.7-65.4 per cent, fat 0.6-1.2 per cent, calcium 142-257 mg/100 gm, phosphorus 301–480mg/100g, vitamin A 30 iu, thiamine 0.39-0.57mg/100g, niacin 2.2-2.4g/100g and riboflavin 0.08–0.21mg/100g. It is richer than cowpea and black gram in protein, calcium and phosphorus. Dry seed is boiled and eaten with rice. It is also used to make Indian sweets.

2.2 Genetic variability

Genetic variability is the scope for selection in any crop-breeding programme. Variability is the rule of nature, it would be worthy to quote that variability is an appraisal guiding a breeder to look forward for improving a crop species.

Patel *et al.* (1977) observed variability in 5 varieties of fodder cowpea and found that green forage and dry matter yield showed significant differences.

Thakur (1990) observed phenotypic and genotypic coefficient of variations in various genotypes of forage soybean. The high estimates for PCV and GCV showed for leaf area per plant, plant height and number of branches. However, low estimates for PCV and GCV showed for days to harvest.

Backiyarani and Nadarajan (1995) reported variability in thirty four cowpea genotypes and found highest genotypic and phenotypic coefficient of variation for leaf area followed by leaf breadth.

Mishra *et al.* (1995) observed genetic variability in ten germplasm lines of rice bean. Moderate to high genotypic coefficients of variation were observed for all the traits except seeds per pod.

Das *et al.* (1997) derived information on variation from data on twelve yield components in sixty three rice bean (*V. umbellata*) lines and found high phenotypic and genotypic coefficient of variation for seed yield per plant, effective nodes per plant, pods per plant, days to 50% flowering, pod maturity, 100 seed weight and plant height.

Singh *et al.* (1997) evaluated high genetic variability in thirty two rice bean cultivars for plant height, pods per plant, 1000 seed weight and yield per plant.

Borah and Khan (2000) observed genetic variability in sixty cowpea genotypes. High phenotypic and genotypic coefficient of variation were observed for number of branches and leaves, leaf stem ratio, dry fodder yield and green fodder yield suggesting the available potential for these characters in fodder cowpea.

Chauhan *et al.* (2003) recorded genotypic and phenotypic coefficient of variation in eighteen forage cowpea genotypes. For yield components, .The additive gene effects were significant for plant height, pod per plant, plant stand, and 100-seed weight.

Lokesh *et al.* (2003) Indicated that seed yield could be increased in early flowering, early maturing and short rice bean plants showing short pod length, low test weight, high cluster number/plant, high pod number/cluster, high pod number/plant and high seed number/plant.

Singh *et al.* (2005) observed the presence of genetic variability among the forty four genotypes of mung bean and rice bean for 100 seed weight, total number of pods per plant and number of seeds per pod.

Manohar *et al.* (2005) evaluated fifteen rice bean cultivars for association of green forage yield and its components .Green forage yield/plant was significantly and positively correlated with grain yield/plant, pod number/plant, branch number/plant, plant height, node number/plant, test weight and dry matter yield/plant. Grain yield/plant, plant height and test weight showed very high and positive effect on green forage yield, while dry matter yield showed a negative direct effect. However, the indirect effect of dry matter yield via grain yield, plant height and test weight was very high.

Bhattacharya *et al.* (2005) evaluated fifteen rice bean (*Vigna umbellata*) cultivars for 11 yield and growth related characters High phenotypic and genotypic variances were observed for forage yield, dry matter yield, pod number and plant height. Phenotypic and genotypic coefficients of variation were high in pod number/plant, grain yield, crude protein, green forage yield and dry matter yield. High heritability and high genetic advance was observed for green forage yield, plant height and pod number/plant, suggesting that these characters are governed by additive gene action.

Sarkar *et al.* (2007) studied the association between yield and various yield contributing components plant height, number of branches per plant, days to 50% flowering, clusters per plant, pods per cluster, pods per plant, pod length, seeds per pod, test weight and days to maturity. There were significant differences among the genotypes for all characters in rice bean.

Sahu *et al.* (2007) in a study upon rice bean (*Vigna umbellata*) cultivars of diverse origins showed significant differences for 8 yield component traits and yield correlation of other traits with yield was greatly influenced by indirect positive effect via pods per plant and seeds per pod. Thus, these rice bean cultivars possessed large genetic diversity in multivariate traits.

Sarkar and Mukherjee (2007) observed genetic variability in eleven quantitative characters in eighteen genotypes of rice bean. The PCV values were higher than the GCV values. The difference between GCV and PCV values were smaller for plant height, days to 50% flowering, pods per plant and days to maturity.

Jhariya (2007) studied the genetic variability, correlation coefficient analysis and path analysis for fodder yield in 21 genotypes of rice bean. The characteristics protein yield per plant, green fodder per day, fiber yield per day, crude fiber percent and number of nodules per plant had positive direct effect on yield.

2.3 Heritability and genetic advance

Heritability is a property not only of a character but also of the population and of the environmental circumstance to which the individuals are subjected. The value of the heritability depends on the magnitude of all the components of variance a change in any one of these will affect it. Lush (1940) carried out the term broad sense heritability for the genetic variance to total variance. Robinson (1949) carried out the term narrow sense heritability for the ratio of additive genetic variance to phenotypic variance.

Kumar and Mishra (1981) estimated that broad sense heritability for dry fodder yield and green fodder yield in cowpea were found 21.3% and 36.4% respectively

Thaware *et al.* (1991) estimated different component of forage yield in cowpea for green forage yield, leaf area, number of nodules, leaf stem ratio which showed high values for heritability and genetic advance.

Borah and Khan (2000) observed high heritability and high genetic advance as a percentage of mean for the characters i.e. number of branches, number of leaves, leaf stem ratio, dry matter yield, green fodder yield, plant height. The crude protein content, days to 50% flowering, stem girth and leaf area exhibited low genetic advance and high heritability estimates.

Tyagi *et al.* (2002) determined heritability and genetic advance in 5 cowpea cultivars (TCS-85, TCS-76, ARL-25, ECS-89, and TCS-39). High heritability was recorded for green fodder yield per plant, number of days to 50% flowering, plant height and dry fodder yield per plant. Genetic advance was higher for green fodder yield, plant height and days to 50% flowering.

Mehta *et al.* (2007) observed twenty eight strains of soybean for fodder potential and for their desirable traits. High heritability with high genetic advance were recorded for leaf area while, high heritability with low genetic advance could be recorded for stem girth and crude protein per cent.

Sarkar and Mukerjee (2007) observed habitability in eleven quantitative characters for eighteen genotypes of rice bean. The broad sense heritability for plant height (94.9%), days to 50% flowering (95.70%), pods per plant (98.60%) and days to maturity (93.70%) were high. The habitability were moderate to high for almost all the traits except branches per plant, seeds per pod and 100 seed weight.

2.4 Correlation analysis

Correlation refers to the degree and direction of association between two or more than two variables. The original concept of correlation was presented by Galton (1888), which was elaborated later by Fisher (1918).

Kumar (1979) in fodder cowpea reported that dry fodder yield per plant was significantly correlated with days to flowering, number of primary branches and green fodder yield per plant. Correlation between days to flowering and dry matter yield per plant was negative at phenotypic and genotypic level.

Mishra *et al.* (1995) studied inter relationship between yield and its seven components in ten germplasm seed yield was significantly and positively associated only with pods per plant.

Singh *et al.* (1997) observed correlation in thirty two rice bean cultivars and found that pods per plant, seed weight and pod length were positively correlated with seed yield. Plant height and days to maturity were negatively correlated with seed yield.

Lokesh *et al.* (2003) studied correlation of morphological traits and quality parameters in seventy nine genotypes of rice bean. Morphological characteristics cluster per plant, pods per cluster, pods per plant and seed yield per plant showed positive and highly significant correlation with different quality parameters (mineral, carbohydrates and protein content). However, days to flowering, days to maturity, plant height, pod length and test weight showed negative and highly significant relationship with different quality characteristics.

Mehta *et al.* (2007) reported correlation in twenty eight varieties of fodder soybean in fifteen fodder potential traits. Green fodder yield per plant had positive and significant association with GFY per day, DFY per day, protein yield per day, total protein yield, dry weight of nodules, number of nodules, plant height, leaf area whereas significant negative association was noted in days to harvest.

2.5 Path Coefficient Analysis

The concept of path analysis was originally developed by Wright (1921), which was firstly used by Dewey and Lu (1959).

Kumar and Mishra (1981) observed six forage yield components in fifty varieties of cowpea and found that dry matter yield followed by plant height had the highest positive effect on green forage yield.

Borah and Khan (1999) analyzed path coefficient for fodder yield and its component traits in 60 genotypes of fodder cowpea and found that the highest positive direct effect on green fodder yield was exhibited by dry matter yield followed by leaf stem ratio and plant height. The indirect effects of number of leaves, leaflet width, days to 50% flowering, dry weight of leaves and dry weight of stem on green fodder yield through dry matter yield were also appreciably high.

Lokesh *et al.* (2003) studied seventy nine genotypes of rice bean including rice bean cultivar RBL-6 and mung bean cultivar Asha as controls for different morphological and quality traits contributing to grain yield in rice bean. High direct and positive effects were recorded for days to flowering, clusters per plant, pods per cluster, 1000 grain weight, and pod length on seed yield per plant.

Nigude *et al.* (2004) studied forty five indigenous and exotic genotypes of cowpea. Biomass (dry weight) at harvesting recorded the highest positive direct effect followed by harvest index and days to 50% flowering on grain yield per plant. The number of pods per plant and number of seeds per pod had negative direct effect on seed yield.

Patil *et al.* (2004) reported that among the biomass partitioning characters, the number of compound leaves per plant, leaf area per plant, number of inflorescences per plant, number of days to first pod maturity and dry weight per plant had positive direct effect on seed yield per plant at both phenotypic and genotypic levels.

Mehta *et al.* (2007) studied fifteen fodder potential and desirable traits in twenty eight varieties of forage soybean and found that high positive direct effect on green fodder yield per plant for dry fodder yield per plant, dry fodder yield per day, plant height, protein yield, number of primary branches, protein yield per day. While days to harvest showed higher negative direct effect on green fodder yield followed by leaf stem ratio, leaf area, number of nodules and dry weight of nodules.

2.6 Genetic divergence

Ramanujam *et al.* (1974) studied Genetic divergence in 35 populations (10 parents and 25 F_1's) of mung bean using D^2 and canonical analyses. The ten parents formed as many as eight separate clusters, suggesting that the genetic divergence between them was quite substantial. The parent BR-2 was highly divergent from all the other entries. They observed that flowering time, maturity, seed density and seed size (100-seed weight) contributed substantially to the divergence. Canonical analysis supported the divergence pattern obtained by D^2 analysis and the contribution of different characters to genetic divergence. The relationship between genetic divergence (D^2) and heterosis was also evaluated. In general, there was fair agreement between the extent of heterosis and the genetic divergence between the parents.

Chattopadhyay *et al.* (2005) On the basis of evaluation of 100 accession of pigeonpea (Arhar) revealed that Plant height and seeds/pod contributed positively and directly, whereas contribution of 100 seed weight was negative. While measuring their diversity, genotypes were grouped under three clusters.

Cluster I was the biggest and cluster uncontained two germplasm which gave around 3500 kg/ha seed yield in around 270 days. Very high inter-and intra cluster distances.

Prakash *et al.* (2007) studied genetic diversity of 36 exotic and indigenous mung bean genotypes. The genotypes were grouped into clusters following Tocher's method and relative contribution of characters towards divergence was estimated using canonical analysis.

Sandhu *et al.* (2006) evaluated genetic diversity in 90 genotypes of chickpea in three environments using Mahalanobis D^2 Statistic. The genotypes were grouped into ten clusters; three of which were more genetically divergent than the others. Common genotypes were sorted out within a cluster for combination of environments and pooled data over environments.

Sunil *et al.* (2009) measured nine characters contributing to seed yield on 20 accessions of horse gram [*Macrotyloma uniflorum* (Lam.) Verd]. and subjected to genetic divergence analysis using Mahalanobis statistic and mapping using DIVA-GIS. The accessions were collected from North Coastal and Rayalaseema eco-geographical regions of Andhra Pradesh. Based on D^2 values, they grouped the genotypes into five clusters. Genetic diversity was not related to eco-geographical distribution. The greatest inter-cluster distance separated clusters II and V, followed by clusters IV, and V, III and IV. Entries in clusters V and II appeared suitable as parents for horse gram improvement. The Rayalaseema region is the source of useful variation for days to flowering, maturity and yield.

2. 7 Molecular markers and diversity analysis

The molecular markers have become an essential tool in the selection programs of pulse crops. Developing of molecular marker required several steps. At first, the nature of desired trait has to be analyzed, followed by mapping of the population which has been then developed and evaluated in the field or in a laboratory. The same lines are to be analyzed by DNA techniques and based on field / laboratory analysis and DNA tests putative markers are identified.

The DNA based marker had several advantages over morphological and biochemical marker such as highly polymorphic, have no pleotropic and epistatic effect, enable to employ non-destructive method, independent of environmental stress, independent on growth and developmental stages and provide easy access, high reproducibility and high genetic resolution (Jain *et al.*, 1994).

With the advent of molecular markers, a new generation of markers has been introduced over the last two decades, which have revolutionized the entire scenario of biological sciences. DNA-based molecular markers act as versatile tools and had found their own position in various fields like taxonomy, physiology, embryology, genetic engineering, etc. They are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis (Joshi *et al.*, 1999).

Markers are characters whose inheritance pattern can be followed, at the morphological, biochemical (protein and isozyme) or DNA level (molecular marker) these characters are called markers as they are being used to obtain direct-indirect information about the genetics of other traits of interest under investigation (Jana, 2000).

DNA based genetic markers had forever changed the practice of genetics. In the 20 years since that discovery many different types of DNA based genetic markers have been used for the construction of genetic maps, for the analysis of genetic diversity, trait mapping as well as for applied diagnostic purposes. A bewildering array of acronyms, such as RFLP, SSR, AFLP, RAPD, AP-PCR, ISSR, ESP, SSLP, DAS and SCAR describe these methodologies.

2.7.1 Random Amplified Polymorphic DNA (RAPD)

Welsh and McClelland (1990) used PCR to randomly amplify anonymous segments of nuclear DNA with an identical pair of primers 8-10 bp in length. RAPD polymorphism could occur due to base substitution at the primer binding site or due to indels in the regions between the sites.

McDonald *et al.* (1993) amplified DNA fragments from genomic DNA using RAPD and polymerase chain reaction (PCR) they examined the differences in the size of amplified products via electrophoresis.

Weising *et al.* (1995) showed that the RAPD technique used to study population genetic variation in plants and fungi has also been proven reliable in several taxonomic studies of higher plants.

Dangi *et al.* (2004) generated RAPD markers through PCR amplifications of random genomic DNA segments using 10-15 base pairs of arbitrary sequence. RAPD method however, was unable to distinguish heterozygote from homozygote due to its dominant nature.

2.7.2 Inter Simple Sequence Repeat (ISSR)

ISSR are the primers based on microsatellites utilized to amplify inter-SSR DNA sequences. An unlimited number of primers can be synthesized for various combinations of di-, tri-, tetra- and pentanucleotides [(4)3 = 64, (4)4 = 256] etc. with an anchor made up of a few bases and can be exploited for a broad range of applications in plant species.

Tautz (1989) described ISSR markers as one of the most efficient markers. The strategy has several benefits over other procedures (isozymes, RFLPs, and RAPDs). It permits detection of polymorphism in microsatellite loci without previous knowledge of the DNA sequence.

Hauntula *et al.* (1996) illustrated ISSR technique as more useful for the study of diversity in genus in terms of quality and quantity of data inputs as compared to RFLP and RAPD. Significantly the efficiency of technique is evident in characterization even at varietals level of a species.

2.8 Molecular diversity

Smith *et al.* (1997) calculated marker index for RAPD and ISSR markers in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes of cowpea.

Maciel *et al.* (2004) evaluated the variability among 15 cultivars and 18 landraces of common bean (*Phaseolus vulgaris* L.), using fifteen RAPD oligonucleotide primers in PCR reactions showing diversity among the cultivars.

Ajibade *et al.* (2004) investigated the utility of DNA polymorphisms generated by ISSR to distinguish taxa within the genus *Vigna*. Nineteen primers, most containing either AGA or CA repeat, generated amplification products that differed among the taxa examined. The ISSR polymorphisms produced by 15 of these primers were very effective for distinguishing taxa at the species level or below.

Ba *et al.* (2004) using RAPD characterized genetic variation in domesticated cowpea and its wild progenitor. A total of 28 primers generated 202 RAPD bands. More than hundred bands were polymorphic among the domesticated compared to 181 among wild / weedy cowpea accessions.

Marotti *et al.* (2005) used RAPD, ISSR and a semi-random PCR system to analyze the genetic diversity of 16 Italian common bean landraces. A higher proportion of polymorphic bands were observed using ISSR (85%) and semi-random (90%) primers as compared to RAPD (69%) method.

Dikshit *et al.* (2007) genetically differentiated seventy genotypes belonging to 7 wild and cultivated *Vigna* species using RAPD, universal rice primer (URP) and SSR markers.

Fang *et al.* (2007) studied 87 cowpea accessions and revealed that they shared a minimum of 86% genetic similarity. PCA showed clustering of breeding lines by program origin, indicating lack of genetic diversity compared to potential diversity.

Muthusamy *et al.* (2009) evaluated the genetic variation among 10 landraces of rice bean using RAPD and ISSR markers. Among these markers, RAPD primers generated 987 amplification products of which 719 were polymorphic and ISSR markers produced 479 amplification products, out of which 296 were polymorphic.

2.9 Phenotypic and molecular polymorphism analysis

Lepse *et al.* (2005) investigated that DNA isolation from the cucumber seeds was developed, were evaluated for homogeneity by RAPDs. Five lines were selected for submission in the Latvian Gene Bank of Cultivated Plants based both on molecular and field evaluation data.

MATERIAL AND METHODS

Materials and Methods

3.1 Experimental materials and other details

3.1.1 Experimental material

Comprised of 94 genotypes along with a standard check (Bidhan -1) the list of accessions and check is given at Table 3.1.

Table 3.1 List of genotypes

Sl. №.	Genotypes	Sl. №.	Genotypes
1.	BFRB-3	48.	JRB06-9-1
2.	BFRB-3-1	49.	JRB06-11
3.	KRB-86	50.	JRB06-11-1
4.	KRB-86-1	51.	JRB06-13
5.	KRB-99	52.	JRB06-13-1
6.	KRB-99-1	53.	JRB06-10
7.	BFRB-5	54.	JRB06-114
8.	BFRB-5-1	55.	JRB04-1
9.	BFRB-6	56.	JRB04-2
10.	BFRB-6-1	57.	JRB07-1
11.	BFRB-7	58.	JRB07-1-1
12.	BFRB-7-1	59.	JRB07-2
13.	KRB-102	60.	JRB07-2-1
14.	KRB-136	61.	JRB07-2-2
15.	KRB-136-1	62.	JRB07-3
16.	KRB-167	63.	JRB07-3-1
17.	Bidhan-1	64.	JRB07-4
18.	Bidhan-1-1	65.	JRB07-11
19.	BFRB-8	66.	JRB07-11-1
20.	BFRB-8-1	67.	JRB07-13
21.	JRB-06	68.	JRB07-33

22.	JRB-06-1	69.	JRB07-33-1
23.	BFRB-9	70.	JRB07-33-2
24.	KRB-19	71.	JRB07-34-1
25.	KRB-19-1	72.	JRB07- 35-1
26.	BFRB-10	73.	JRB07-35-2
27.	KRB-128	74.	JRB07-35-3
28.	KRB-235	75.	JRB07-36-1
29.	KRB-239	76.	JRB07-39-1
30.	KRB-239-1	77.	JRB07-39-2
31.	KRB-239-2	78.	JRB07-43-1
32.	JRB06-1-1	79.	JRB07-43-2
33.	JRB06- 2	80.	JRB07-43-3
34.	JRB06-2-1	81.	JRB07-43-4
35.	JRB06-3	82.	JRB07-48-1
36.	JRB06-4	83.	JRB07-49-1
37.	JRB06-4-1	84.	JRB07-50-1
38.	JRB06-5-1	85.	JRB07-50-2
39.	JRB06-5-2	86.	JRB07-54-1
40.	JRB06-6	87.	JRB07-54-2
41.	JRB06-6-1	88.	JRB07-54-3
42.	JRB06-7	89.	JRB07-1-1
43.	JRB06-7-1	90.	JRB07-28-1
44.	JRB06-8	91.	JRB07-28-2
45.	JRB06-8-1	92.	JRB07-22
46.	JRB06-8-2	93.	JRB07-22-1
47.	JRB06-9	94.	JRB07-22-2

3.1.2 Experimental site and location

The field experiment of present investigation was carried out during kharif season 2008-09 at the Seed Breeding Farm, National Seed Project Unit, Department Of Plant Breeding and Genetics, JNKVV, Jabalpur (M. P.). Jabalpur is situated at 411.78 meters above sea level 23*.9"N latitude and 79*.58"E longitudes.

3.1.3 Climate and season

The climate of Jabalpur is typically semi-humid and sub-tropical with severe winter and summer. Jabalpur is traditionally rice-wheat crop zone of M.P. under "Kymore Plateau and Satpura Hills Agro-Climatic Zone". The average rainfall on this region is 1258.4mm, which is mostly received between mid-Junes to end of September with occasional showers in other months.

As per national bureau of soil science and land planning of ICAR this area belongs to agro-ecological sub region on 10.1 (sub-humid dry eco-region). The soil of experimental areas was clay loam with uniform topography. The data related to weekly maximum and minimum temperature number of rainy days, sunshine hours and evaporation meteorological observatory Krishi Nagar, JNKVV, Jabalpur (M.P.). (Appendix 1)

3.1.4 Experimental methods:

The experiment was conducted in Randomized Complete Block Design (RCBD) with two replications during July 2008 - February 2009. All the 94 entries were planted in 4x3m plots. The row to row spacing was kept 30 cm and plant to plant 5 cm. Fertilizer doses of 20kg N, 60kg P_2O_5 and 20kg K_2O per hectare were applied. The standard agronomic practices and plant protection measure were adopted for normal crop growth as per recommendations.

3.2 Observations recorded:

Five competitive plants were randomly selected from each plot from each replication for all the 94 treatments and observations were taken on single plant basis as per the standard procedures listed below. However, days to 50% flowering was recorded on plot basis.

3.2.1 Days to 50% flowering

It was measured in days at which 50% of the plants of the plot attained flowering from date of sowing in each block.

3.2.2 Plant height (cm)

The height of plants was measured in centimeters (cm) by Meter scale from the base of plants to tip of the main stem of plant at the time of harvest.

3.2.3 Number of branches per plant

The total numbers of branches per plant were counted from randomly tagged plants at the time of harvest.

3.2.4 Number of leaves per plant

The total numbers of leaves per plant were counted from randomly tagged plants at the time of harvest.

3.2.5 Leaf stem ratio

Leaf stem ratio was calculated by using the formula.

$$\text{Leaf stem ratio} = \frac{\text{Weight of fresh green leaves (g)}}{\text{Weight of fresh green stems (g)}}$$

3.2.6 Green fodder yield per plant (g)

Green fodder yield was recorded in gram by weighing whole plant after harvesting at 50% flowering.

3.2.7 Green fodder yield per day (g)

$$\text{Green fodder yield per day} = \frac{\text{Green fodder yield per plant (g)}}{\text{Number of days to harvest}}$$

3.2.8 Dry fodder yield per plant (g)

The sample of freshly harvested plants were air dried followed by oven drying at 60°C for three days to obtain constant dry weight. With the help of dry matter %, dry fodder yield per plant was calculated for each genotype.

3.2.9 Dry fodder yield per day (g)

$$\text{Dry fodder yield per day} = \frac{\text{Dry fodder yield per plant (g)}}{\text{Number of days to harvest}}$$

3.2.10 Crude protein percentage

The fodder crude protein percentage was estimated at 50% flowering stage by Kjeldahl Digestion and Distillation Method as given by (AOAC, 1980). It provides the estimation of the feed coming from the protein and non-protein nitrogenous compounds. The percentage of nitrogen (N_2) is then expressed in terms of crude protein:

$$N\% = \frac{\text{Normality of } H_2SO_4 \times \text{volume of } H_2SO_4 \times 0.014}{\text{Weight of samples}} \times 100$$

3.2.11 Crude fiber percentage

Crude fiber refers to the residue of a feed that is insoluble after successive, boiling with dilute acid and alkali. This method was originally proposed at the Weende Experiment Station (VanSoest, 1963). Hence it is known as Weende method of determination of crude fiber is the portion of total carbohydrate of a food that is resistant to acid and alkali treatment.

$$\% \text{ of crude fiber} = \frac{W1 - W2}{W} \times 100$$

W = Weight of the samples

W1 = Weight of crucible before ashing

W2 = Weight of crucible after ashing

W1- W2 = Crude fiber

3.3 Statistical methodology

The data obtained was subjected to the following statistical analysis.

3.3.1 Mean

It was calculated by using following formula:

$$\text{Mean } (\bar{X}) = \frac{\Sigma X}{N}$$

Where,

ΣX = the sum of all the observation

N = number of observation

3.3.2 Analysis of variance (ANOVA)

The data for different characters were statistically analyzed on the basis of model described by Panse and Sukhatme (1967).

The working out the standard error for composition of the means an ANOVA table is prepared for RCBD design.

ANOVA for RCBD:

Source of variation	d.f.	Sum of Square	Mean Sum of Square	F Value
Replication	r-1	R.S.S.	R.M.S.	R.M.S./E.M.S.
Treatment	t-1	T_T.S.S.	T_T.M.S.	T_T.M.S./E.M.S.
Error	(r-1)(t-1)	E.S.S.	E.M.S.	
Total	rt-1			

Where,

r = Number of replications

t = Number of treatments

RSS = Replication sums of square

ESS = Error sums of square

TSS = Treatment sums square

RMS = Replication mean sums square

EMS = Error mean sums of square

TMS = Treatment mean sums square

A significant value of F-test indicates that the test entries differ, significantly among themselves, which require computing CD.

$$CV = \sqrt{\frac{E.M.S}{G.M.}X100}$$

$$SE(d) = \sqrt{\frac{2E.M.S}{r}}$$

CD = t (0.05) x SE(d)

Where,

CV= Coefficient of variance

EMS = Error mean sum of square

SE(d) = Standard error of difference

GM = Grand total

CD = Critical difference

t (0.01) = t-value at 1% probability level

3.3.3 Genetic variability

Genotypic and phenotypic coefficient of variance (expressed in %) were calculated by using the formula given by Burton (1952)

$$\text{Genotypic variance } (i^2g) = \frac{T.M.S. - E.M.S.}{r}$$

Where,

TMS = Treatment mean sum of square

EMS = Error mean sum of square

r = Number of replication

$$\text{Environment variance } (i^2e) = \frac{E.M.S.}{r}$$

Where,

EMS = Error mean sum of square

$$\text{Phenotypic variance } (i^2p) = i^2g + i^2e$$

Where,

(i^2g) = genotypic variance

(i^2e) = environmental variance

3.3.4 Heritability estimates (h^2)

Heritability in broad sense was calculated using the formula proposed by Hanson *et al.* (1956)

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

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

h^2 (b) = Heritability expressed in %

3.3.5 Genetic advance (GA)

The GA was calculated as per following formula (Jhonson *et al.*, 1955)

$$GA = h \times \sigma p \times k$$

Where,

GA = Genetic advance

H = Heritability

σp = phenotypic standard deviation

K = selection intensity at 5% level of selection i. e. (2.06)

3.3.6.1 Genotypic and phenotypic correlation coefficient

All correlation coefficient were calculated at genotypic and phenotypic levels using the formula suggested by Miller *et al.* (1958). Genotypic and phenotypic correlation coefficient was computed by substituting corresponding variance and covariance for all positive character combinations.

$$rxy = \frac{Cov(x_i y_j)}{\sqrt{(\text{var}.x_i)(\text{var}.y_j)}}$$

Where,

rxy = coefficient of correlation between character Xi and Yj

cov(xiyj)= covariance of characters Xi and Yj

(var.xi)= variance of character Yj

(var.yj) = variance of character Xi

$$\text{Phenotypic r (XiYj)} = \frac{\text{Phenotypic cov.}(X_i\ Y_j)}{\sqrt{VP(X_i).VP(Y_j)}}$$

Where,

VP (X_i) =phenotypic variance of character Xi

Vp (Y_j) = phenotypic variance of character Yj

3.3.6.2 Testing of correlation for significance

To test the significance of correlation, *t*-test was performed and the computed *t* value was compared with the tabulated value at n-2 degree of freedom at 5% and 1% level, where 'n' denoted the number of entries tested (Singh and Chaudhary, 1977)

$$tc = \frac{|r|}{\sqrt{1 - r^2}} \quad \text{at n-2 degree of freedom}$$

'tc' = calculated value of 't'

r = estimation value of correlation coefficient

n = number of observation

3.3.7 Path coefficient analysis

Path analysis is simple standardized partial coefficient and is helpful to detect the direct and indirect effects of the independent variable on dependent variable especially on yield. It permits separation of correlation into components of direct and indirect effects.

Wright (1921) develops the method for calculating the path coefficient analysis and later it was modified by Deway and Lu (1959).

r_{1y} = p_{1y} + $r_{12}\,p_{2y}$+ $r_{13}\,p_{3y}$ + ----------------- $r_{1y}\,p_{1y}$

r_{2y} = $r_{2y}\,p_{1y}$+ p_{2y} + $r_{23y}\,p_{3y}$ + ----------------- $r_{21y}\,p_{1y}$

rky = $r_{ki}\,p_{1y}$ + $r_{k-1}\,p_{2y}$ + $r_k^3\,p_{3y}$ + ---------------- p_{ky}

r_{xky} = $r_{xk1}\,p_{1y}$+ $r_{xk2}\,p_{2y}$ + $r_{xk3}\,p_{3y}$ + ---------------- p_{xky}

r_{xky} = Coefficient of correlation between independent character

P_{1y} to P3=Direct effect of character 1 to 3 character y

3.7.1 Direct effect

The direct effect were calculated as follows

$$Pky = \sum_{i=1}^{K} C_{kirk}Y$$

3.7.2 Indirect effect

Indirect effect of any independent traits on the dependent one (yield) via other independent traits are computed by multiplying the direct effects (Pxy) of that independent variables with the corresponding correlation coefficient follows

$$K^{th} \text{ trait via } (n-1) = rk\,(n-1)\,P\,(n-1)\,Y$$

3.7.3 Residual effect

Residual effect was obtained as per formula given below

$$R = \sqrt{1 - Edirij}$$

di= direct effect of character

rij = correlation coefficient of ith character with jth character

3.8 Genetic Divergence

D^2 was calculated as suggested by Mahalanobis (1936)

$$D^2 = b_1d_1 + b_2d_2 \ldots\ldots\ldots\ldots\ldots\ldots + bpdp$$

A measure for group distance based on multiple characters with x_1 x_2 x_3 .... x_p as the multiple measurements available on each individual and d_1 d_2 ....dp as $x^1{}_1$-$x^2{}_2$..............x^1p-x^2p respectively, being the difference in the means of two populations. The 'p' values are to be estimated so that the ration of variance between the populations to the variance within the populations is maximized. Taking variance and covariance under consideration, the D^2 values may be estimated by this formula:

$$D^2 = \Sigma\, Wij\, \Sigma\, (x^1i - x^2i)\,(x^1j - x^2j)$$

Where, Wij is the inverse of estimated variance and covariance matrix.

3.8.1 Steps to Estimate D^2 Values

Collection of data: Data are controlled considering "V" populations and "P" characters which have been estimated on each individual.

Test of significance: According to the Wilk's criteria, a simultaneous test of difference between mean values of number of correlated variance is done (Rao, 1948)

Using pivotal condensation method, the determinant of error and error + variety was calculated as follows:

$$\Lambda = \left|\frac{W}{S}\right| = \left|\frac{Determinant\ of\ error\ matrix}{Determinant\ of\ error + variety\ matrix}\right|$$

$$v\ (state) = -m\ \log_e = -\frac{n}{2}\ (p+q+1)\ \log_e$$

$$\text{Where } m = \frac{n}{2}\ (p+q+1)$$

p = Number of variance or characters

q = Degree of freedom for population

n = Degree of freedom for error + variety

e = constant (2.1783)

V (state) is distributed as x^2 with p_q degree of freedom

The tabulated value of x^2 (chi square) for p_q degree of freedom at 5% level was compared with above value of x^2 for testing the significance.

3.8.2 Transformation of correlation values

The transformation was done using the pivotal condensation method. The correlated variables were first transformed into uncorrelated ones and D^2 values were calculated.

3.8.3 Computation of D^2 values

The D^2 values obtained for a pair of population and were taken as the calculated values of x^2 tested against the tabulated value of x^2 at p where, "p" is the number of characters.

3.8.4 Contribution of different characters towards divergence

Among all combinations, each character is ranked on the basis of di = Yij - Yik values. Rank I is given to the highest mean difference.

3.8.5 Grouping of varieties into various clusters by Tocher's Method

Testing D^2 as the square of generalized distance, all genotypes were grouped into a number of clusters, according to the method described by Tocher's and Rao (1952) the criterion in clustering by this method is that any two genotypes belonging to the same cluster, should at least on an average, show a similar D^2 than those belonging to other clusters. In other words, if genotypes V_1 and V_2 are close together and V_3 is distant from both of them as shown by their generalized distances, the V_1 and V_2 from one cluster, the average D^2 values In all possible combination of genotypes on one cluster with those in the other were computed and its square root was those in the represent the statistical distance between the two clusters.

3.8.6 Average intra cluster distance

The following formula is used for measuring the intra cluster distance:

$$\text{Intra-cluster Distance} = \sum \frac{Di^2}{n}$$

Where, $\sum Di^2/n$ is the sum of distance between all possible combinations (n) of the populations included in the cluster.

3.8.7 Average inter cluster distance

First of all, the distances between clusters were estimated, and then clusters were taken one by one and from with their other clusters are calculated.

3.4 Method for molecular analysis

3.4.1 Plant Materials

Plant material consisting of germplasm of rice bean was obtained from the Seed Breeding Farm, National Seed Project Unit, Development of Plant Breeding and Genetics, JNKVV, Jabalpur (M.P.) during Kharif. Out of 94 genotypes, 38 were selected on the basis of diversity analysis at phenotypic level consisting better fodder and crude protein yield (Table 3.2).

Leaf samples of rice bean genotypes were selected on random basis after 25-30 days of sowing. Leaves were stored at -80°C for further molecular analysis.

Table 3.2 List of germplasm

Sl. №.	Genotype	Original accession №. from Table 3.1
1	BFRB-3	1
2	BFRB-3-1	2
3	BFRB-6	9
4	KRB-167	16
5	Bidhan-1	17
6	Bidhan-1-1	18
7	BFRB-8	19
8	BFRB-8-1	20
9	JRB-06	21
10	JRB-06-1	22
11	BFRB-9	23
12	KRB-19	24
13	KRB-19-1	25
14	JRB06-3	35
15	JRB06-8-1	45
16	JRB06-8-2	46
17	JRB06-9	47
18	JRB06-9-1	48
19	JRB06-11	49
20	JRB06-13	51
21	JRB06-13-1	52
22	JRB06-10	53
23	JRB06-114	54
24	JRB04-1	55
25	JRB04-2	56
26	JRB07-1	57
27	JRB07-1-1	58

28	JRB07-33-1	69
29	JRB07-33-2	70
30	JRB07-34-1	71
31	JRB07- 35-1	72
32	JRB07-35-2	73
33	JRB07-35-3	74
34	JRB07-36-1	75
35	JRB07-39-1	76
36	JRB07-54-2	87
37	JRB07-54-3	88
38	JRB07-1-1	89

3.4.2 Methods

3.4.2.1 DNA Isolation

The technique of DNA isolation relied upon the fact that nucleic acid would form suitable complex with detergent acetyl trimethyl ammonium bromide (CTAB) under high salt concentration and when the concentration was reduced to 0.4M NaCl the CTAB-NA complex would precipitate. Genomic DNA was isolated using Saghai-Maroof *et al.* (1984) method with suitable modifications. The method described below gave a good quality and quantity of DNA.

1. 2g of leaf sample was weighed and was homogenized in liquid nitrogen using a pre-chilled pestle and a mortar.
2. The fine powder was transferred to a 50ml Oakridge tube and 10ml of DNA extraction buffer (preheated to 65°C) was added and mixed thoroughly.
3. The samples were incubated in a water bath at 65°C for one hour. Meanwhile, the samples were frequently mixed after every 10 min to ensure complete and even extraction.
4. The samples were then removed from water bath and cooled to room temperature.

5. To it, was added an equal volumes of Phenol: chloroform: isoamyl alcohol (25:24:1) v/v and mixed thoroughly but gently for not less than 5 minutes.

6. The mixture was then centrifuged for about 15 min at 8000 rpm at room temperature.

7. Supernatant so obtained was transferred to a fresh tube.

8. Than it, was added an equal volumes of chloroform: isoamyl alcohol (24:1) v/v and mixed thoroughly but gently for not less than 5 minutes.

9. The mixture was then centrifuged for about 15 minutes at 8000 rpm at room temperature.

10. Supernatant so obtained was transferred to a fresh tube.

11. An equal volume of isopropanol was added to the tube which had supernatant, mixed gently by inverting and kept for 10 minutes undisturbed.

12. The DNA precipitate was then spool out using 1ml cut tips and transferred to a 1.5ml microcentrifuge tube.

13. DNA was again pelleted by centrifugation at 8000 rpm for 5 minutes.

14. The supernatant was now discarded and pellet was washed with 70% ethanol.

15. The pellet was dried up at room temperature and dissolved in 200 µl of TE buffer for further use.

Table 3.3: Composition of DNA Extraction buffer

Sl. №.	Chemicals	Concentration
1.	Tris HCl (pH 8.0)	100 mM
2.	EDTA (pH 8.0)	20 mM
3.	NaCl	1.4 M
4.	CTAB	2 %
5.	β-mercaptoethanol*	0.2 %

The solution was made without β-mercaptoethanol on a magnetic stirrer to avoid foaming. β-mercaptoethanol was added when solution has cooled to room temperature.

3.4.2.2 DNA purification

The purification of DNA was done in order to remove the impurities like RNA, proteins and polysaccharides. These are considered as important inhibitors in DNA amplification during PCR.

1. 5µl of RNAase (5mg/ml) was added to DNA extract, mixed well and incubated at 37°C for 30 min.
2. This was followed by the addition of equal volumes of chloroform: isoamyl alcohol (24:1) v/v and mixed vigorously.
3. The above mixture was centrifuged at 14000 rpm for 10 min.
4. Supernatant was transferred to a fresh microcentrifuge tube and 1/10 volume of 3M sodium acetate (pH 5.4) was added followed by further addition of two volumes of pre-chilled ethanol mixed gently for DNA precipitation.
5. The precipitated DNA was pelleted by centrifugation at 12000 rpm for 5 min.
6. The pellet was dried at room temperature to completely remove ethanol and was then dissolved in 100µl of TE buffer and stored at -20°C for further use.

3.4.2.3 Quantification of DNA

Isolated DNA was quantified by measuring the absorbance at 260nm and 280nm on a UV-spectrophotometer. 50 µg/ml concentration of double stranded DNA showed an absorbance of 1 at 260nm. Concentration of DNA samples was calculated using following formula:

$$\frac{O.D._{260\,nm} \times 50\ \mu g\ DNA/ml \times \text{Dilution factor}}{1000}$$

3.4.2.4 Dilution of DNA

The quantified DNA was diluted properly according to the DNA present in each sample for PCR amplification in sterile double distilled water. Dilutions were carried out according to the following formula (Edward, 2000).

$$\text{Dilution} = \frac{\text{Required concentration of DNA (ng/µl) X Total volume required (µl)}}{\text{Available concentration of DNA (ng/µl)}}$$

3.4.3 Polymorphism study among germplasm by RAPD markers

The amplification of genomic DNA was done by using 10 random oligonucleotide (10-mer) primers; fifteen primers were used for assessing the polymorphism among 38 selected lines (Table 3.4). Procedure for amplification of DNA was followed as per protocol described by Williams *et al.* (1990). The components and their concentration used in the RAPD-PCR reaction were prepared as per Table 3.5.

Table 3.4 Sequence of random operon primers used in the study

Sl. №.	Code	5' to 3' sequence (GC %)
1.	OPC–9	CTCACCGTCC (70%)
2.	OPC–10	TGTCTGGGTG (70%)
3.	OPC–11	AAAGCTGCGG (60%)
4.	OPC–13	AAGCCTCGTC (60%)
5.	OPC–15	GACGGATCAG (60%)
6.	OPC–16	CACACTCCAG (60%)
7.	OPC–19	GTTGCCAGCC (70%)
8.	OPH–20	GGGAGACATC (60%)
9.	OPC–12	TGTCATCCCC (60%)
10.	OPC–14	TGCGTGCTTG (60%)
11.	OPC–17	TTCCCCCCAG (70%)
12.	OPC–18	TGAGTGGGTG (60%)
13.	OPC–20	ACTTCGCCAC (60%)
14.	OPH–17	CACTCTCCTC (60%)
15.	OPH–19	CTGACCAGCC (70%)

Table 3.5 List of components for RAPD – PCR

Sl. №.	Components	Concentration
1.	PCR buffer	1X
2.	$MgCl_2$	2.5mM
3.	dNTPs	1mM
4.	Primer	50 ng
5.	*Taq* Polymerase	1 unit
6.	DD H_2O	-
7.	DNA	25 ng

3.4.3.1 PCR conditions for RAPD primers

The amplification of DNA was done in "ThermoHybaid Px2" programmable thermal cycler as program given in Table 3.5.

Table 3.6 Temperature profile used in PCR amplification for RAPD

Steps	Temperature	Duration	Cycles	Activity
1.	$94^{o}C$	5 min	1	Initial Denaturation
2.	$94^{o}C$	1 min	45	Denaturation
3.	$36^{o}C$	1 min		Annealing
4.	$72^{o}C$	2 min		Extension
5.	$72^{o}C$	7 min	1	Final Extension
6.	$4^{o}C$	1 hrs		Storage

3.4.3.2 Electrophoretic analysis of RAPD – PCR products

The products of RAPD-PCR were analyzed on 1.5% (w/v) agarose gel to generate RAPD fragments, and later stained with EtBr. Stained gels were visualized with UV-transilluminator and documented by 'Syngene' Multigenius Bioimaging Gel Documentation System. The molecular weight of bands was estimated using a wide range (50-10,000bp) 1kb DNA ladder.

Method

1. Gel casting plates were prepared by sealing the ends with rubber stoppers and kept on a perfectly horizontal leveled plate form.
2. Agarose was melted in 1X TAE buffer and was allowed to down to temperature of about 45 - 50°C.
3. Ethidium bromide was added to it @ 5μg/ml.
4. Melted agarose was then poured into the gel casting plate and a comb of desired size and number was fixed on one end of the plate
5. Gel was allowed to solidify at room temperature.
6. After the solidification the casted gel was placed on the electrophoretic assembly and submerged with 1X TAE buffer
7. The comb was carefully removed from gel without disturbing the wells
8. The DNA samples were mixed with loading dye at a concentration of 1X before carefully loaded on to the wells.
9. The cathode and anode were connected to power pack and the current was adjusted to 20mA
10. The negatively charged DNA moved towards the anode and separated according to their molecular weight and size. The power was turned off when the tracking dye reached about 3/4 distance of the gel.

Solutions

Table 3.7 Composition of 50X TAE Electrophoresis buffer

Sl. №.	Components	Concentration
1.	Tris base	242 gm
2.	Glacial Acetic acid	57.1 ml
3.	0.5 M EDTA (pH 8.0)	100 ml

Volume was made up to 1lit by distilled water. The solution was autoclaved.

Table 3.8 Composition of Loading dye / Tracking dye (6X)

Sl. №.	Components	Concentration
1.	Bromophenol blue	0.25 %
2.	Xylene cyanol	0.25 %
3.	Sucrose in water	40 % w/v

3.4.4 Polymorphism study among germplasm by ISSR marker

Polymorphism was detected using repeat-anchored primer (ISSRs) that amplifies between simple sequences repeats (SSRs). ISSRs are codominant markers, target multiple loci in the genome and are easily and economically assayed by PCR (Zietkiewicz *et al.*, 1994). ISSRs are among the widely used markers for genotypic analysis and molecular mapping in plants (Table 3.9).

During the present study **20** ISSR markers were detected by the use of repeat anchored primers that amplify between SSRs. These dominant markers targeted multiple loci in the genome. ISSR markers amplify by single primer due to their even distribution in the genome.

Table 3.9 Sequences of ISSR primers used

Sl. №.	UBC Primers	5' to 3' primer sequence	GC%	bp
1.	885	ACTCGTACTAGAGAGAGAGAGAG	48%	23
2.	880	GGAGAGGAGAGGAGA	60%	15
3.	821	GTGTGTGTGTGTGTGTC	53%	17
4.	853	TCTCTCTCTCTCTCTCAGA	47%	19
5.	851	GTGTGTGTGTGTGTGTCTG	53%	19
6.	843	CTCTCTCTCTCTCTCTAGA	47%	19
7.	859	TGTGTGTGTGTGTGTGAGC	53%	19
8.	862	AGCAGCAGCAGCAGCAGC	67%	18
9.	846	CACACACACACACACAAGT	47%	19
10.	867	GGCGGCGGCGGCGGCGGC	100%	18
11.	873	GACAGACAGACAGACA	60%	16
12.	884	ACTCGTACTAGAGAGAGAGAGAG	48%	23

13.	807	AGAGAGAGAGAGAGAGT	47%	17
14.	808	AGAGAGAGAGAGAGAGC	53%	17
15.	810	GAGAGAGAGAGAGAGAT	47%	17
16.	811	GAGAGAGAGAGAGAGAC	53%	17
17.	814	CTCTCTCTCTCTCTCTA	47%	17
18.	816	CACACACACACACACAT	47%	17
19.	820	GTGTGTGTGTGTGTGTC	53%	17
20.	824	TCTCTCTCTCTCTCTCG	53%	17

Table 3.10 List of components used for ISSR PCR

Sl. №.	Components	Concentration
1.	PCR buffer	1X
2.	$MgCl_2$	2.5 mM
3.	dNTPs	1mM
4.	Primer	10 pmol
5.	*Taq* Polymerase	1 unit
6.	DD H_2O	-
7.	DNA	50 ng

3.4.4.1 PCR condition for ISSR markers

PCR conditions were standardized with different parameters viz. initial denaturation, denaturation, annealing, extension and final extension using ThermoHybaid Px2 PCR machine. The standard conditions provided during the run are as follows (Table 3.11)

Table 3.11 Temperature profile used in PCR amplification for ISSR

Steps	Temperature	Duration	Cycles	Activity
1	94°C	6 min	1	Initial Denaturation
2	94°C	1 min	40	Denaturation
3	56°C	1 min		Annealing
4	72°C	2 min		Extension
5	72°C	10 min	1	Final Extension
6	4°C	1 hrs		Storage

3.4.4.2 Electrophoretic analysis of ISSR-PCR products

The PCR products for ISSR were analyzed on 1.5% (w/v) agarose gel to generate ISSR fragments, and later stained with EtBr. Stained gels were visualized with UV-Transilluminator and documented by Syngene Multigenius Bioimaging Gel Documentation System. The molecular weight of bands was estimated using a wide range 1kb (50-10,000 bp) DNA ladder.

RESULTS

RESULTS

he results of the study on rice bean are presented in the following rder.

.1 Analysis of variance for green forage yield and its components

.2 Mean performance of green forage and its components

..3 Estimation of different parameters of genetic variability

.3.1 Genotypic, phenotypic coefficient of variation between different characters

4.3.2 Heritability estimates (h^2)

4.3.3 Genetic advance (G_A)

4.4 Genotypic and phenotypic correlation coefficient between different characters

4.5 Path coefficient analysis

4.6 Genetic divergence

4.7 Molecular analysis

4.8 Comparison between phenotypic and molecular marker polymorphism.

4.1 Analysis of variance

A variation leads to observational differences in individuals for a particular trait. Amount of variability which has been observed among 94 cultivars of rice bean for all the traits is shown in the Table 4.1a and 4.1b.

The analysis of variance was carried out for all the eleven traits i.e. days to 50% flowering, plant height, number of branches per plant, number of leaves per plant, leaf stem ratio, green fodder yield per plant (g), green fodder yield per day (g), dry fodder yield per plant (g), dry fodder yield per day (g), crude

Table4.1 (a) Analysis of variance for fodder yield and its components

Source of variation	Degree of freedom	Mean sum of squares					
		Days to 50% flowering	Plant height (cm)	No. of branches per plant	No. of leaves per plant	Leaf stem ratio	Green fodder yield/plant
Replication	1	0.13	23.29	3.06	196.08	1.62	0.52
Treatments	93	1022.34	1126.84	3.73	378.41	1.84	1619.53
Error	93	1.02	12.73	0.71	213.24	1.70	0.65

Table 4.1(b) Analysis of variance for fodder yield and its components

Source of variation	Degree of freedom	Mean sum of squares				
		Green fodder yield per day	Dry fodder yield per plant	Dry fodder yield per day	Crude protein (%)	Crude fiber (%)
Replication	1	196.18	0.53	0.001	0.002	0.62
Treatments	93	96.30	37.54	0.01	2.49	14.86
Error	93	97.10	0.36	0.0001	0.005	0.52

protein percent, crude fibre per cent. Observations reveal that the variance due to the varieties was highly significant at 0.01 and 0.05 probability level for almost all the traits under study. It indicates that there was considerable genetic variability in the germplasm. However, variance due to replication was not significant. It suggests that the experimental plots were homogenous and there were differences due to soil heterogeneity.

4.2 Mean performance of green forage and its components

The mean performance of 94 rice bean accessions for all the traits under study is presented in appendix 2.

4.2.1 Days to 50% flowering

Days to 50% flowering ranged between 61 to 154 days with the mean performance of 106.23 days. The accession JRB07-54-1 took maximum 154 days to flower 50% followed by KRB-235 (152) and KRB-239 (151). On the other hand, JRB06-9 recorded least (61) days to 50% flower proceeded by JRB06-9-1 (64).

4.2.2 Plant height (cm)

Plant height per plant ranged from 50.95 to 154.70 cm with an average of 108.57 cm. Accessions JRB06-5-2 was found to be the shortest with 50.95cm height in contrast to BFRB-3, the tallest one with 154.70cm.

4.2.3 Number of branches per plant

The maximum number of branches per plant ranged between 2.0 to 7.5, Accessions JRB07-33 and JRB07-33-1 (7.5) branched maximum, while accessions JRB-06-1, KRB-239-2, JRB06-9-1, JRB04-2, JRB07-1-1 and JRB07-36-1 proliferated as low as only 2.0 branches per plant.

4.2.4 Number of leaves per plant

Number of leaves per plant ranged within 10.0 to 41.5 with the mean value of 24.47. The highest number of leaves per plant was recorded in the accession JRB07-1-1 (41.50) followed by KRB-99 and KRB-136-1 (38.5). Minimum numbers of leaves (10.0) were recorded for accession JRB07-50-1.

4.2.5. Leaf stem ratio

Among all the 94 accessions, leaf stem ratio ranged from 0.62 to 1.50 with a mean of 1.05. Maximum leaf stem ratio was assessed for the accession BFRB-5-2 (1.50) and minimum for KRB-19 (0.62).

4.2.6. Green fodder yield per plant (g)

Green fodder yield per plant ranged from 83.10 to 170.25g with an overall average of 126.86g. The highest value was recorded for the accession JRB07-54-3 (170.25g) followed by JRB06-13 (169.95g) and JRB-06 (163.45g). Minimum value of green fodder yield per plant was recorded by the accession KRB-86 (83.10g).

4.2.7. Green fodder yield per day (g)

Maximum green fodder yield per day was observed from accession JRB06-9-1 (1.78g) and minimum for KRB-86 (0.57g) with an average of 2.22g.

4.2.8. Dry fodder yield per plant (g)

The highest value of dry fodder yield per plant was recorded for the accession JRB06-2-1 (17.46) followed by BFRB-8-1 (32.31g) and JRB07-11-1 (32.29g). The minimum value was seen in the accession JRB07-28-1 (17.46g). Mean for all the accessions was 24.72g.

4.2.9. Dry fodder yield per day (g)

Dry fodder yield per day ranged from 0.12 to 0.33g with the mean value of 0.20g. The highest value of dry fodder yield per day was recorded for BFRB-8-1 followed by JRB-06 and JRB07-54-3 (0.315g) and JRB07-54-2 (0.305g). The minimum dry fodder yield per day was recorded for JRB06-11.

4.2.10. Crude protein (%)

In case of crude protein percent average was 15.02 and it varied in between 13.31% to 18.21%. The minimum crude protein percent was recorded for accession BFRB-5 while, maximum for JRB-06-1.

4.2.11. Crude fibre (%)

Maximum crude fibre 39.46% was observed for accession JRB07-54-3 and minimum 28.45% for KRB-86 with a mean value of 34.02%.

4.3 Estimation of different parameters of genetic variability

Genetic parameters for eleven traits of rice bean are presented in Table 4.2.

4.3.1 Coefficient of variation

4.3.1.1 Genotypic coefficient of variation

It is apparent from the Table 4.2 that the genotypic coefficient of variation (gcv) ranged from 7.42% (crude protein percent) to 37.14% (number of leaves per plant) among eleven different traits under study.

High gcv was observed for number of leaves per plant (37.14) followed by number of branches per plant (33.61), green fodder yield per day (28.36), dry fodder yield per day (25.76), leaf Stem Ratio (25.31). The lowest value of genotypic coefficient of variation was recorded for crude protein percent (7.42) followed by crude fibre per cent (7.88), dry fodder yield per plant (17.44), days to 50% flowering (21.27), plant height (21.74) and green fodder yield per plant (22.42).

4.3.1.2 Phenotypic coefficient of variation

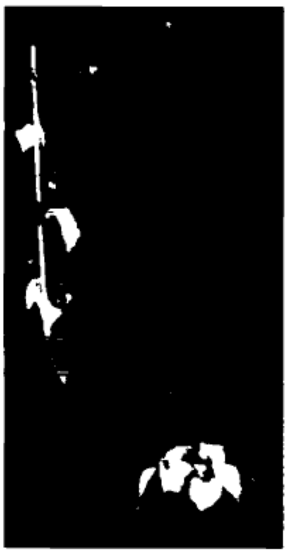
It is evident from the Table 4.2 that the phenotypic coefficient of variation ranged from 7.44% (crude protein percent) to 70.29% (number of leaves per plant) for different traits under study.

General Overview

Bud

Flower

Tendil



Pod

Plate-1 : General overview of Rice bean
(Vigna umbellate)

Table 4.2 Genetic parameters of variation for fodder yield and its components in rice bean

Characters	Range		Mean	Variances		Coefficient of variance		Heritability (h^2) %	Genetic advance (GA)	GA % of mean at 5% level
	Min.	Max.		Phenotypic	Genotypic	PCV	GCV			
Days to 50% flow	61.00	154.00	106.23	511.68	510.66	21.29	21.27	49.00	27.88	23.05
Plant height (cm)	50.95	154.70	108.57	569.78	557.05	21.98	21.74	80.52	33.29	26.40
Branches / plant	2.00	7.50	3.65	2.23	1.51	40.87	33.61	67.60	2.08	56.95
Leaves / plant	10.00	41.50	24.47	295.83	82.58	70.29	37.14	57.08	11.21	46.38
Leaf stem ratio	0.61	1.50	1.05	1.77	0.07	26.92	25.31	71.61	33.51	35.20
Green fodder yield/plant (g)	83.10	170.25	126.86	810.10	809.44	22.44	22.42	63.65	45.66	35.77
Green fodder yield/day (g)	0.57	1.78	2.22	96.70	0.39	44.19	28.36	51.80	0.48	43.34
Dry fodder yield/plant (g)	17.46	32.32	24.72	18.95	18.59	17.61	17.44	68.37	7.65	31.39
Dry fodder yield/day (g)	0.12	0.33	0.20	0.003	0.003	25.88	25.76	49.80	0.07	37.64
Crude protein (%)	13.31	18.21	15.02	1.25	1.24	7.44	7.42	80.88	2.12	14.51
Crude fiber (%)	28.45	39.46	34.02	7.70	7.17	8.15	7.88	92.83	8.70	25.92

High phenotypic coefficient of variation was recorded for the characters number of leaves per plant (70.29) followed by green fodder yield per day (44.19), number of branches per plant (40.87), leaf stem ratio (26.92), dry fodder yield per day (25.88), green fodder yield per plant (22.44), plant height (21.98), days to 50% flowering (21.29), dry fodder yield per plant (17.61) and crude fibre percent (8.15). Lowest phenotypic coefficient of variation was recorded for crude protein percent (7.44).

4.3.2 Habitability Estimates (h^2)

Heritability in broad sense for each trait was estimated to measure relative magnitude of genotypic and phenotypic variability and to judge the masking influence of environment. High and moderate heritability estimates were recorded for all the traits (Table 4.2). Broad sense heritability was estimated for all the traits under study.

The heritability estimates ranged between 49.00 percent (days to 50% flowering) to 92.83 percent (crude fibre percent). Highest estimates were recorded for crude fibre percent (92.83) followed by crude protein percent (80.88), plant height (80.52), leaf stem ratio (71.61). Whereas, moderate estimates were recorded for dry fodder yield per plant (68.37), number of branches per plant (67.60), green fodder yield per plant (63.65), number of leaves per plant (57.08), green fodder yield per day (51.86). However, low estimate of heritability were recorded for dry fodder yield per day (49.80) and days to 50% flowering (49.00).

4.3.3 Genetic advance as percentage of mean

The estimates of genetic advance as percentage of mean for all the eleven traits studied are depicted in Table 4.2.

The estimates of genetic advance as percentage of mean ranged between 14.51% for character crude protein percent to

56.95% for number of branches per plant with selection intensity, of five percent.

The estimates of genetic advance as percentage of mean have been found to be high for number of branches per plant (56.95) followed by number of leaves per plant (46.38), green fodder yield per day (43.34), dry fodder yield per day (37.64), green fodder yield per plant (35.77) and leaf stem ratio (35.20), while, genetic advance was moderate for dry fodder yield per plant (31.39), plant height (26.40) and crude fibre percent (25.92) and low for days to 50% flowering (23.04) and crude protein percent (14.51).

The high heritability estimates coupled with high genetic advance were observed for the trait crude fibre percent, crude protein percent and leaf stem ratio. High heritability with moderate genetic advance was observed for dry fodder yield per plant and plant height. Moderate heritability with high genetic advance was recorded for number of leaves per plant and green fodder yield per day. While, moderate heritability with low genetic advance was observed for days to 50% flowering. High heritability estimates with low genetic advance was observed for the trait crude fibre percent.

4.4 Correlation coefficient

The correlation coefficient is the index of association between two variables. This has been worked out in all possible combinations at genotypic and phenotypic level and is presented in Table 4.3.

The results revealed that the estimates of genotypic correlation coefficient were higher than the phenotypic correlation coefficient for almost all the characters studied except plant height with number of branches, days to 50% flowering with leaf stem ratio, number of branches per plant with number of leaves per plant, leaf stem ratio, plant height with green fodder yield per day, leaf stem ratio with dry fodder yield per day, crude protein percent with dry fodder yield per day and crude fibre percent.

4.4.1Genotypic correlation coefficient

4.4.1.1 Days to 50% flowering

Days to 50% flowering showed significant positive association with plant height (0.3500). Whereas, it had significant negative association with number of branches per plant (-0.7560), green fodder yield per day (-2.6770), dry fodder yield per plant (0.8350), leaf stem ratio (-0.3155), crude protein percent (0.2576).

4.4.1.2 Plant height (cm)

Plant height exhibited significant positive association with dry fodder yield per plant (0.3490), dry fodder yield per day (0.7980), green fodder yield per plant (0.2027), whereas, it had significant negative association with number of branches per plant (-0.3480), leaf stem ratio (-0.7020), green fodder yield per day (-0.3280), number of leaves per plant (-0.3369).

4.4.1.3 Number of branches per plant

Number of branches showed significant positive association with dry fodder yield per plant (0.3960), crude fibre percent (0.2274), whereas, it had negative association with green fodder yield per day (-2.6416), dry fodder yield per day (-0.6150), green fodder yield per plant (-0.2230).

4.4.1.4 Number of leaves per plant

Number of leaves performed significant positive association with leaf stem ratio (0.4440), green fodder yield per day (0.6830), dry fodder yield per day (0.2214), it had significant negative association with green fodder yield per plant (-0.2265).

Table 4.3 Genotypic (G) and phenotypic (P) correlation coefficient of fodder yield and its components of rice bean

Characters		Plant height (cm)	Branches per plant	Leaves per plant	Leaf stem ratio	Green fodder yield/day	Dry fodder yield/ plant	Dry fodder yield/day	Crude protein (%)	Crude fibre (%)	Green fodder yield/plant
Day to 50% flowering	G	0.3500**	-0.7560**	0.2120	-0.3155*	-2.6770**	-0.8350**	0.1642	-0.2576*	0.1798	-0.1883
	P	0.3458**	-0.641**	-0.1080	-0.6210**	0.1695	-0.8240**	0.1632	-0.2568	0.1750	-0.1880
Plant height (cm)	G		-0.3480**	-0.3369*	-0.7020**	-1.0328**	0.3490**	0.7980**	-0.1970	-0.1055	0.2027*
	P		-0.4610**	-0.1863	0.1130	0.6230**	0.3390	0.7850**	-0.1941	-0.984**	0.2001
Branches per plant	G			-0.1739	-0.1117	-2.6416**	0.3960**	-0.6150**	-0.1137	0.2274*	-0.2230*
	P			-0.5110**	-0.8260**	0.1572	0.2590	-0.5220**	-0.9160	0.1849	-0.1710
Leaves per plant	G				0.4440**	0.6830**	-0.1983	0.2214*	0.2280	0.1202	-0.2265*
	P				0.3130	-0.2080	-0.1055	0.1174	0.0920	0.6330**	-0.1212
Leaf stem ratio	G					1.4133**	-0.5633**	-0.2257	-0.5909**	-0.0912	-0.4121**
	P					-0.5290**	-0.1146	-0.4420**	-0.1161	-0.0154	-0.0811
Green fodder yield/day (g)	G						0.6148**	1.5853**	0.8100**	0.0321	0.3033
	P						-0.2980	-0.1008	-0.4960**	0.0037	-0.1780
Dry fodder yield/plant (g)	G							0.2475*	0.1455	0.0812**	0.1609
	P							0.2459	0.1438	0.0749**	0.1595
Dry fodder yield/day (g)	G								0.7400**	-0.3720	0.1594
	P								0.8100**	-0.3600	0.1585
Crude protein (%)	G									-0.0580	0.3530**
	P									-0.0690	0.3520**
Crude fiber (%)	G										0.8610**
	P										0.8480**

4.4.1.5 Leaf stem ratio

Leaf stem ratio showed significant positive association with green fodder yield per day (0.4133), it had significant negative association with dry fodder yield per plant (-0.5633), crude protein percent (-0.5909), green fodder yield per plant (-0.4121)

4.4.1.6 Green fodder yield per day (g)

Green fodder yield per day showed significant positive association with dry fodder yield per plant (0.6148), dry fodder yield per plant (0.5833), crude protein percent (0.8100).

4.4.1.7 Dry fodder yield per plant (g)

Dry fodder yield per plant performed significant positive association with crude fibre percent (0.8120), dry fodder yield per day (0.2475).

4.4.1.8 Dry fodder yield per day (g)

Dry fodder yield per day exhibited positive significant association with crude protein percent (0.7400).

4.4.1.9 Crude protein (%)

Crude protein percent performed positive significant association with green fodder yield per plant (0.3530).

4.4.1.10 Crude fibre (%)

Crude fibre percent showed significant positive association with green fodder yield per plant (0.8610).

4.4.2 Phenotypic correlation coefficient

4.4.2.1 Days to 50% flowering

Days to 50% flowering showed significant positive correlation with plant height (0.3458). However, days to 50% flowering exhibited significant negative correlation with number of branches per plant (-0.641), leaf stem ratio (-0.621) and dry fodder yield per plant (-0.824).

4.4.2.2 Plant height (cm)

Plant height performed significant positive correlation with green fodder yield per day (0.623), dry fodder yield per day (0.785). Significant negative correlation of plant height was observed with number of branches per plant (-0.461), crude fibre percent (-0.984).

4.4.2.3 Number of branches per plant

Number of branches showed significant negative correlation with number of leaves per plant (-0.511), leaf stem ratio (-0.826), dry fodder yield per day (-0.522).

4.4.2.4 Number of leaves per plant

The degree of association of number of leaves per plant with crude fibre percent (0.633) was significant and positive.

4.4.2.5 Leaf stem ratio

Leaf stem ratio exhibited significant negative association with green fodder yield per day (-0.529), dry fodder yield per day (-0.442).

4.4.2.6 Green fodder yield per day (g)

Green fodder yield per day showed significant negative correlation with crude protein percent (-0.4960).

4.4.2.7 Dry fodder yield per plant (g)

Dry fodder yield per plant showed significant positive correlation with crude fibre percent (0.749).

4.4.2.8 Dry fodder yield per day (g)

Dry fodder yield per day performed significant positive correlation with crude protein percent (0.810)

4.4.2.9 Crude protein percent

Crude protein percent exhibited significant positive correlation with green fodder yield per day (0.352).

4.4.2.10 Crude fibre percent

Crude fibre percent exhibited significant positive correlation with green fodder yield per plant (0.848).

4.5 Path Coefficient analysis

Path coefficient analysis was carried out for green fodder yield per plant and its components. The estimates of path coefficient are provided in Table 4.4.

Genotypic path coefficient

4.5.1 Days to 50% flowering

Days to 50% flowering exhibited negative direct effect (-49.8529) on green fodder per plant and observed the maximum positive indirect effect on green fodder yield per plant through green fodder yield per day (133.4577), leaf stem ratio (15.7310), crude protein percent (12.8418), dry fodder yield per plant (4.1631), number of branches per plant (3.7664), number of leaves per plant (1.0575), However, its negative indirect effect was shown through plant height (-17.4485) and crude fibre per cent (8.9637), dry fodder yield per day (-8.1866).

4.5.2 Plant height (cm)

Plant height exhibited positive direct effect (10.1734) on green fodder yield per plant. The maximum positive indirect effect on green fodder yield per plant was observed through days to 50% flowering (3.5607), dry fodder yield per day (0.8121), dry fodder yield per plant (0.3554). However, its negative indirect effect on green fodder yield per plant was shown through green fodder yield per day (-10.5075), number of leaves per plant (-3.4279), crude protein percent (-2.0039), crude fibre percent (-1.0733), leaf stem ratio (-0.7145), number of branches per plant (-0.3542).

Table 4.4 Genotypic (G) phenotypic (P) path coefficient sowing direct and indirect effects of different contributing characters on fodder yield (g) in rice bean

Characters		Days to 50% fl.	Plant height (cm)	Branches/ plant	Leaves/plant	Leaf stem ratio	Green fodder yield/plant	Dry fodder yield/plant	Dry fodder yield/day	Crude protein (%)	Crude fiber (%)
Day to 50% flowering	G	-0.3869	-0.1338	0.0248	0.0042	0.0240	-0.0656	0.0319	-0.0631	0.0993	-0.0677
	P	**-49.8529**	-17.4485	3.7664	1.0575	15.7310	133.4577	4.1631	-8.1866	12.8418	-8.9637
Plant height (cm)	G	0.1062	**0.3071**	-0.0142	-0.0572	0.0035	0.0191	0.0104	0.0241	-0.0596	-0.0302
	P	3.5607	**10.1734**	-0.3542	-3.4279	0.7145	-10.5075	0.3554	0.8121	-2.0039	-1.0733
Branches per plant	G	0.0053	0.0038	**-0.0829**	0.0042	0.0069	-0.0130	-0.0021	0.0043	0.0076	-0.0153
	P	1.4217	0.6522	**-18.8178**	3.2725	2.1023	49.7094	-0.7450	1.1579	2.1396	-4.2791
Leaves per plant	G	0.0010	0.0181	0.0050	**-0.0971**	-0.0030	0.0020	0.0102	-0.0114	-0.0009	-0.0001
	P	-0.5972	-9.4870	-4.8964	**28.1556**	12.5016	1.9222	-5.5823	6.2350	0.6415	3.3838
Leaf stem ratio	G	0.0058	-0.0010	0.0077	-0.0029	**0.0928**	0.0049	0.0100	0.0041	0.0108	0.0014
	P	23.3830	5.2041	8.2785	-32.9031	**-74.1027**	-104.7257	41.7395	16.7233	43.7883	6.7549
Green fodder yield/day	G	0.0092	0.0034	0.0086	-0.0011	-0.0029	**0.0545**	-0.0016	-0.0055	-0.0027	0.0002
	P	6.8297	0.6350	6.7394	-0.1742	-3.6055	**-2.5512**	-1.5686	-4.0445	-2.0665	-0.0820
Dry fodder yield/plant	G	-0.0059	0.0024	0.0010	-0.0076	-0.0083	-0.0021	**0.0720**	0.0177	0.0104	-0.0054
	P	2.4613	-1.0296	-1.1669	5.8436	16.6016	-18.1218	**-23.4738**	-7.2947	-4.2873	2.3945
Dry fodder yield/day	G	0.0320	0.0154	-0.0103	0.0230	-0.0087	-0.0198	0.0483	**0.1963**	0.0016	-0.0071
	P	-0.7912	-0.3846	0.2964	1.0669	1.0876	-7.6378	-1.1924	**-4.8178**	0.0355	0.1794
Crude protein (%)	G	0.0076	0.0058	0.0027	-0.0003	0.0035	0.0015	0.0043	-0.0002	**-0.0297**	0.0002
	P	13.1306	10.0407	5.7959	-1.1615	30.1214	-41.2896	-7.4148	-0.3751	**-50.9742**	0.2943
Crude fiber (%)	G	0.0376	-0.0211	0.0397	0.0136	-0.0033	0.0008	-0.0160	-0.0077	0.0015	**0.2148**
	P	0.2660	-0.1561	0.3364	0.1778	-0.1348	0.0475	-0.1202	-0.0551	-0.0085	**1.4793**

4.5.3 Number of branches per plant

Number of branches showed negative direct effect (-18.8178) on green fodder yield per plant and reported the maximum positive indirect effect on green fodder yield per plant through green fodder yield day (49.7094), number of leaves per plant (3.2725), crude protein percent (2.1396), leaf stem ratio (2.1023), dry fodder yield per day (1.1579), dry fodder yield per day (0.0043). However, its negative indirect effect was shown through green fodder yield per day (-0.0130), crude fibre percent (-0.0153).

4.5.4 Number of leaves per plant

Number of leaves exhibited positive direct effect (28.1556) on green fodder yield per plant and observed maximum positive indirect effect on green fodder yield per plant through leaf stem ratio (12.5016), dry fodder yield per day (6.2350), crude fibre percent (3.3838), green fodder yield per day (1.9222), crude protein percent (0.6415). However, its negative indirect effect was shown through plant height (-9.4870), dry fodder yield per plant (-5.5823), number of branches per plant (-4.8964), days to 50 % flowering (-0.5972).

4.5.5 Leaf stem ratio

Leaf stem ratio performed negative direct effect (-74.1027) on green fodder yield per plant and maximum positive indirect effect was shown through crude protein percent (43.7883), dry fodder yield per plant (41.7395), days to 50% flowering (23.3830), number of branches per plant (8.2785), crude fibre percent (6.7549), plant height (5.2041), whereas, its negative indirect effect was shown through green fodder yield per day (-104.7257), number of leaves per plant (-32.9031), dry fodder yield per day (-16.7283).

4.5.6 Green fodder yield per day (g)

The direct effect of green fodder yield per day was negative (-2.5512) on green fodder yield per plant and maximum positive indirect effect was shown through days to 50% flowering (6.8297), number of branches per plant (6.7394), plant height (2.6350). However, negative indirect effect was shown through dry fodder yield per day (-4.0445), leaf stem ratio (-3.6055), crude protein percent (-2.0665), dry fodder yield per plant (-1.5686), number of leaves per plant (-0.1742), crude fibre percent (-0.0820).

4.5.7 Dry fodder yield per plant (g)

Dry fodder yield per plant showed negative direct effect (-29.4238) on green fodder yield per plant and positive indirect effect was shown through leaf stem ratio (16.6016), number of leaves per plant (5.8436), days to 50% flowering (2.4613), crude fibre percent (2.3945). However, its negative indirect effect was shown by green fodder yield per day (-18.1218), dry fodder yield per day (-7.2947), crude protein percent (-4.2873), number of branches per plant (-1.1669), plant height (-1.0296).

4.5.8 Dry fodder yield per day

Dry fodder yield per day performed negative direct effect (-4.8178) on green fodder yield per plant and maximum positive indirect effect was shown through leaf stem ratio (1.0876), number of branches per plant (0.2964), crude fibre percent (0.1794). However, its negative indirect effect was shown through green fodder yield per day (-7.6378), dry fodder yield per day (-1.1924), number of leaves per plant (-1.0669), days to 50% flowering (-0.7912), plant height (-0.3846), crude protein percent (-0.0355).

4.5.9 Crude protein percent

Crude protein percent exhibited negative direct effect (-50.9742) on green fodder yield per plant and maximum positive indirect effect was shown by leaf stem ratio (30.1214), days to 50% flowering (13.1306), plant height (10.0407), number of branches per plant (5.7959), crude fibre percent (0.2943). However, negative indirect effect was shown through green fodder yield per day (-41.2896), dry fodder yield per plant (-7.4148), number of leaves per plant (-1.1615), dry fodder yield per day (-0.3751).

4.5.10 Crude fibre per cent

Crude fibre percent performed positive direct effect (1.4793) on green fodder yield per plant. Maximum positive indirect effect was shown through number of branches per plant (0.3364), days to 50% flowering (0.2660), number of leaves per plant (0.1778), green fodder yield per day (0.0475). However, negative indirect effect was shown through plant height (-0.1561), leaf stem ratio (-0.1348), dry fodder yield per plant (-0.1202), dry fodder yield per day (-0.0551), crude protein percent (-0.0085).

4.6Genetic divergence

4.6.1Mahalanobis generalized distance (D^2)

For the determination of genetic divergence among 94 accessions of rice bean Wilk's ^ criterion value (30732.58) was found highly significant. The V statistics value (5647.99) was also highly significant at 185 degree of freedom when all the traits were considered simultaneously.

4.6.2 Mahalanobis's D^2 values

Pivotal condensation method was used to transform correlated means of all the traits under study in to standard uncorrelated means. The statistical distance (Mahalanobis's D^2) between the pairs of 94 accessions of rice bean, were obtained between the pairs of corresponding uncorrelated values of any two accessions considered together.

4.6.3 Contribution of individual trait towards genetic divergence

The percentage contributions towards genetic divergence by all the 11 characters under study are presented in Table 4.5.

All eleven characters under study contributed towards genetic divergence. The highest contribution percentage of towards genetic divergence was found from green fodder yield per plant (56.69%) followed by days to 50% flowering (22.72%), crude protein percentage (13.29%), dry fodder yield per day (4.60%), dry fodder yield per plant (1.17%), plant height (1.12%), crude fiber percentage (0.32%), number of branches per plant (0.05%), number of leaf per plant and leaf stem ratio (0.02%) while, green fodder yield per day gave minimum contribution (0.001%) towards genetic divergence.

4.6.4 Grouping of accessions in various clusters

94 accessions and one standard check Bidhan-1 were grouped in to eight clusters based on divergence analysis. Distributions of accessions according to clusters are given in Table 4.6 and Fig 1 is showing the clustering by Tochar's method.

Table 4.5 Contribution of traits towards divergence in rice bean genotypes

S.No.	Characters	Number of times raised first	Percentage contribution toward divergence (%)
1.	Days to 50% flowering	993	22.72%
2.	Plant height (cm)	49	1.12%
3.	Branches per plant	2	0.05%
4.	Leaves per plant	1	0.02%
5.	Leaf stem ratio	1	0.02%
6.	Green fodder yield per days (g)	0	0.001%
7.	Dry fodder yield per plant (g)	51	1.17%
8.	Dry fodder yield per day (g)	201	4.60%
9.	Crude protein %	581	13.29%
10.	Crude fibre %	14	0.32%
11.	Green fodder yield per plant (g)	2478	56.69%

Table 4.6 Distribution of genotypes in different clusters

Cluster	Number of genotypes in cluster	Names of genotypes in corresponding cluster
1 (I)	6	KRB-102, KRB-136, KRB-239-1, JRB07-50-1, JRB07-50-2, BFRB-10
2 (II)	4	KRB-86, KRB-86-1, JRB07-28-2, JRB07-28-1
3 (III)	5	KRB-235, KRB-239, JRB04-1-1, BFRB-6, BFRB-5-2
4 (IV)	33	BFRB-6-1, BFRB-7, BFRB-5-1, BFRB-5, KRB-99-1, JRB07-13, KRB-99, JRB07-48-1, JRB07-49-1, JRB07-43-4, JRB-7-2-1, JRB07-43-3, JR06-4-1, KRB-239-2, JRB07-43-1, JR-06-01-1, JRB07-11, BFRB-7-1, JRB07-22, JRB7-2-2, JR06-6-1, JRB07-11-1, JR06-3, JRB07-39-2,JRB07-43-2,JR06-4,JR06-6.JRB07-22-1,JR06-5-2, JR06-11, JRB-7-3-1, KRB-19-1, JR06-5-1
5 (V)	33	Bidhan-1-1, BFRB-8, BFRB-3-1, BFRB-3, JRB07-33, JRB-6, BFRB-8-1, JR04-1, JR04-02, JRO6-8, KRB-136-1, JR07-1, JR06-8-2, JRB07-36-1, JR06-8-1, JR06-114, JRB07-34-1, JRB07-33-2,JR07-33-1,JRB07-35-1,JRB07-35-2,JRB07-35-3,KRB-107, Bidhan-1, JR-06-2-1, JR-06-10, JR-06-2, JRB07-39-1, KRB-128, JR-06-7-1, JR-06-7, JR06-9-1, JR06-9
6 (VI)	5	BFRB-9,KRB-19,JRB07-54-2,JRB07-54-1,JRB-54-3
7 (VII)	4	JR06-13, JR06-13-1, JR06-11-1, JRB-6-1
8 (VIII)	4	JRB7-3-2, JRB07-4, JRB7-2, JR07-1-1

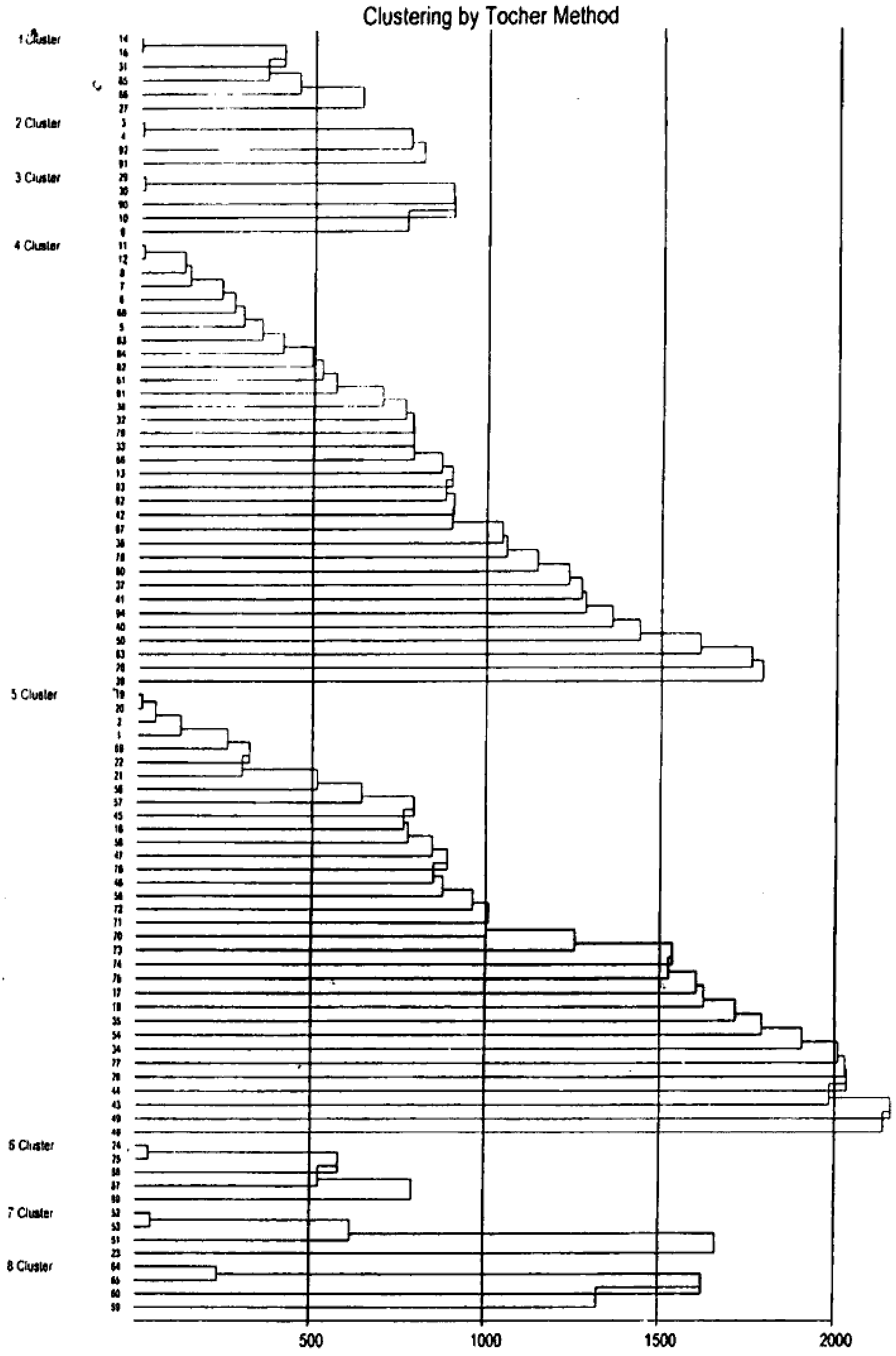


Fig 1 Composition of clusters of rice bean genotypes by Tocher's method

4.6.5 Intra and inter cluster divergence (D^2 values)

The estimation of intra and inter cluster D^2 values was done according to the method given by Singh and Choudhary (1977) are presented in Table 4.7.

All clusters showed intra cluster distance. Cluster VIII recorded maximum intra cluster value (17.35) followed by cluster V (15.72), where as the minimum intra cluster value was found in cluster I (5.86).

The inter cluster D^2 values was minimum (12.30) between the accessions of cluster I and II. Maximum inter cluster D^2 values (176.50) was found between cluster II and cluster VII.

Cluster I (5.86) had six accessions and was found nearest to cluster II (12.30) followed by cluster III (22.96), IV (30.74), VIII (56.55), VI (62.79), V (75.57), VII (130.27) exhibit distance value.

Cluster II (9.29) comprised of four accessions and was near to Cluster I (12.30) followed by cluster IV (36.74) cluster III (46.88), VIII (68.46), VI (101.79), V (111.10) and VII (176.50)

Cluster III (10.12) comprised of five accessions and was found nearest to cluster I (22.96) followed by cluster V (26.27) cluster IV (33.43), III (36.15), II (46.88), VIII (49.89) and cluster VII (70.49).

Cluster IV (11.44) consist of 33 accessions and was found nearer to cluster VIII (25.96), I (30.74), III (36.15), II (36.74), V (56.33), VI (95.88) and VII (97.45) had distant estimates.

Cluster V (15.72) comprised of thirty three accessions and was found nearer to cluster VI (32.73), III (33.43), V (44.48), IV (56.32), VII (58.30), I (75.57) and Cluster II (111.10) exhibited distant values.

Table 4.7 Inter and Intra cluster value in rice bean

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group 1.	**5.86**	12.30	22.96	30.74	75.57	62.79	130.27	56.55
Group 2.		**9.29**	46.88	36.74	111.10	101.79	176.50	68.46
Group 3.			**10.12**	36.15	33.43	26.27	70.49	49.89
Group 4.				**11.44**	56.33	95.88	97.45	25.96
Group 5.					**15.72**	44.48	32.73	58.30
Group 6						**7.82**	78.75	110.04
Group 7.							**14.22**	67.36
Group 8.								**17.35**

Cluster VI (7.82) comprised of five accessions and was found nearer to cluster III (26.27) followed by V (44.48), I (62.79), VII (78.75), IV (95.88), II (101.79) cluster VIII (110.04).

Cluster VII (14.22) comprised of four accessions and was found nearer to cluster V (32.73), VIII (67.36), III (70.49), VI (78.75), IV (97.45), I (130.27) and II (176.50).

Cluster VIII (17.35) consist of four accessions and was found nearer to cluster IV (25.96), III (49.89), I (56.55), V (58.30), VII (67.36), II (68.46) and VI (110.04).

4.6.6 Cluster mean values of different characters

The cluster means of all the eleven characters are summarized in Table 4.8.

The accessions grouped in to cluster II had the highest mean value (149.00) for days to 50% flowering followed by cluster VI (147.40), I (146.75), III (131.20), IV (101.20), VIII (93.50), V (93.35) and cluster VII (80.50).

The accession grouped in cluster I had the highest mean (130.58) for plant height (cm) followed by cluster III (128.53), VI (127.57), VII (116.91), II (112.35), V (106.36), IV (100.95) and VIII (95.96).

For number of branches per plant, cluster I had highest cluster mean value (4.42) followed by cluster V (3.86), IV (3.71), III (3.30), VIII (3.25), VII (3.00), VI (2.80) and cluster II (2.75).

The highest cluster mean value for number of leaves per plant was observed in cluster number II (28.63), followed by cluster IV (28.17), VIII (23.50), V (23.35), III (23.00), I (19.08), VI (18.10) and VII (17.88).

For leaf stem ratio, cluster IV had the highest cluster mean value (1.22), followed by cluster V (1.01), III (0.97), II and VII (0.96), VII (0.89), I (0.87) and VI (0.76).

Table 4.8 Cluster mean values of 11 traits studied in rice bean germplasm

Characters ⇨ Cluster number ⇩	Days to 50% flowering	Plant height (cm)	Branches/ plant	Leaves/ plant	Leaf stem ratio	Green fodder yield/ day	Dry fodder yield/ plant	Dry fodder yield/ day	Crude protein (%)	Crude fiber (%)	Green fodder yield / plant
I	146.75	130.58	4.42	19.08	0.87	1.69	22.66	0.18	14.57	33.88	106.63
II	149.00	112.33	2.75	28.63	0.96	0.98	17.97	0.20	14.44	34.94	88.54
III	131.20	128.53	3.30	23.00	0.97	1.06	28.53	0.23	14.89	34.62	133.21
IV	101.20	100.95	3.71	28.17	1.22	1.13	24.60	0.20	14.89	33.70	100.79
V	93.35	100.36	3.86	23.35	1.01	1.36	25.10	0.20	14.83	34.19	152.31
VI	147.40	127.57	2.80	18.10	0.76	0.99	26.10	0.26	14.46	35.87	164.57
VII	80.50	116.91	3.00	17.88	0.89	1.66	25.22	0.18	17.46	31.84	164.98
VIII	93.50	95.96	3.25	23.50	0.96	1.22	25.50	0.20	17.34	33.92	107.53

The highest cluster mean value for green fodder yield per day was observed in cluster number I (1.69), followed by VII (1.66), V (1.36), VIII (1.22), IV (1.13), III (1.06), VI (0.99) and II (0.98).

Cluster number III exhibited maximum cluster mean value (28.53) for dry fodder yield per plant followed by cluster VI (26.10), VIII (25.50), VII (25.22), V (25.10), IV (24.60), I (22.66) and II (17.97).

Cluster number VI had maximum cluster mean value (0.26) for dry fodder yield per day followed by cluster III (0.22), II , IV , V and VIII (0.20), I and VII (0.18).

The accession grouped in cluster VII had the highest mean (17.46) for crude protein percentage followed by cluster VIII (17.34), III and IV (14.89), V (14.83), I (14.57), VI (14.46) and cluster II (14.44).

The accession grouped in cluster VI showed the highest mean (35.87) for crude fiber percent followed by cluster II (34.94), III (34.62), V (34.19), VIII (33.92), I (33.88), IV (33.70) and cluster VII (31.84).

For green fodder per plant, cluster VII was showed highest cluster mean value (164.98), followed by VI (164.57), V (152.31), III (133.21), VIII (107.53), I (106.63), IV (100.79) and II (88.54).

4.7 Molecular analysis

Genetic diversity is the first and foremost step in any research programme. However, to have a reliable estimate of genetic relationship and genetic diversity generally a large number of polymorphic markers are required. DNA marker represent very effective tool for analyzing genetic diversity in any finger printing programme.

RAPD and ISSR have been extensively used in many crops as a genetic marker for assessment of genetic diversity and have proved highly efficient for assessment of genetic diversity and successful in characterizing individual accession, which are consistent with their pedigree. These markers are mostly dominant and detect variation in both coding as well as non coding regions of genome.

The objective of the present study was to find out genetic relationship among selected 38 accessions in rice bean (*Vigna umbellata*), using RAPDs and ISSRs markers.

Genomic DNA was isolated from selected 38 accessions of rice bean germplasm. After PCR amplification, products were resolved on agarose for the generation of fingerprints.

4.7.1 RAPD analysis

Initially, 15 randomly selected primers were screened for RAPD analysis and a total of 8 decamer primers showing amplification were selected for final RAPD PCR analysis (Table 3.8). The PCR reactions were carried out using a single decamer primer at a time.

Selected 8 decamer primer amplified 49 RAPD marker loci. The size of amplified markers ranged from 240-1600bp. Maximum of 10 bands were scored by two primers i.e. OPC-09 and OPC-11, while minimum 4 bands were produced by 4 primers i.e. OPC-10, OPC-13, OPC-16 and OPC-19. The sequences of these primers and their per cent GC content are presented in Table 4.9.

Table 4.9. Number of bands obtained using RAPD primers

Sl. №.	Primer	Total Bands	Monomorphic Bands	Polymorphic Bands	% polymorphism	PIC Values
1	OPC-09	10	0	10	100.00	0.709184
2	OPC-10	4	4	0	0.00	0.0
3	OPC-11	10	0	10	100.00	0.709184
4	OPC-13	4	4	0	0.0	0.0
5	OPC-15	5	0	5	100.00	0.709184
6	OPC-16	4	4	0	0.0	0.0
7	OPC-19	4	4	0	0.0	0.0
8	OPH-20	8	7	1	12.5	0.084184
Total		**49**	**23 (46.93)**	**26 (53.06)**	**312.5**	
Average		**6.125**		**3.25**	**39.06**	**0.27**

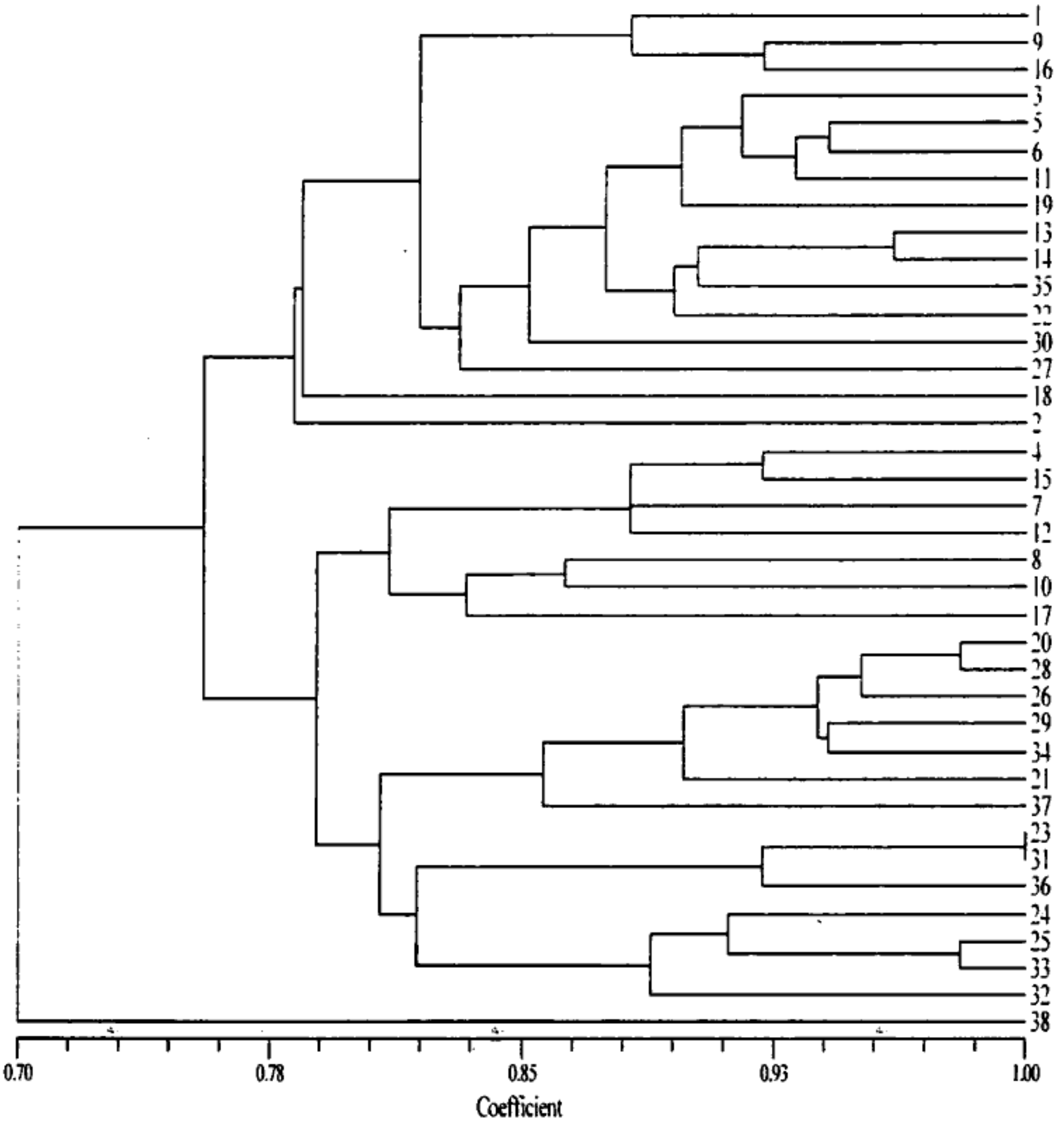


Fig 4.2 Dendogram generated showing relationship among rice bean germplasm using RAPD markers

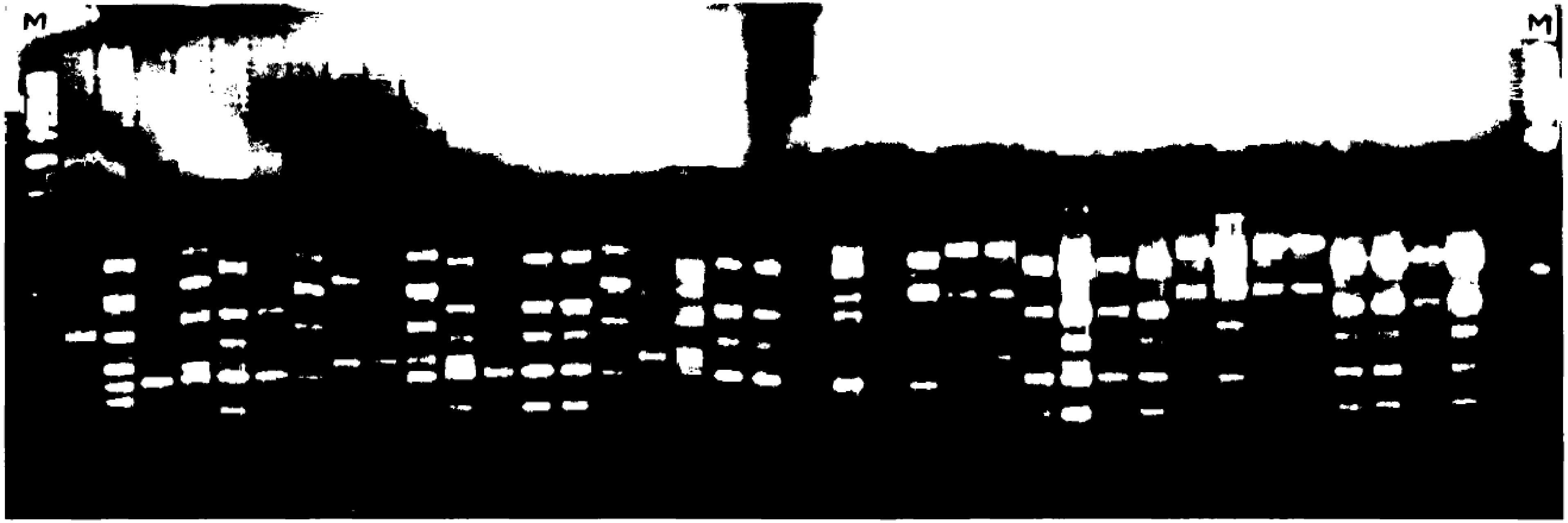
Table 4.10 Genetic distance among rice bean genotypes based on RAPD analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	1.00																																					
2	0.86	1.00																																				
3	0.84	0.75	1.00																																			
4	0.69	0.67	0.76	1.00																																		
5	0.86	0.76	0.90	0.78	1.00																																	
6	0.84	0.75	0.92	0.73	0.94	1.00																																
7	0.71	0.73	0.75	0.86	0.73	0.75	1.00																															
8	0.78	0.73	0.78	0.86	0.76	0.75	0.76	1.00																														
9	0.88	0.78	0.80	0.76	0.82	0.80	0.75	0.86	1.00																													
10	0.73	0.78	0.69	0.80	0.71	0.69	0.86	0.86	0.80	1.00																												
11	0.88	0.78	0.92	0.76	0.94	0.92	0.71	0.78	0.84	0.73	1.00																											
12	0.75	0.69	0.82	0.90	0.76	0.78	0.88	0.80	0.78	0.78	0.82	1.00																										
13	0.84	0.78	0.88	0.80	0.90	0.88	0.75	0.78	0.80	0.73	0.92	0.82	1.00																									
14	0.84	0.75	0.88	0.76	0.90	0.92	0.71	0.75	0.80	0.69	0.92	0.82	0.96	1.00																								
15	0.73	0.71	0.76	0.92	0.75	0.73	0.90	0.86	0.80	0.84	0.76	0.86	0.76	0.73	1.00																							
16	0.88	0.78	0.80	0.76	0.82	0.80	0.78	0.82	0.92	0.84	0.84	0.78	0.80	0.80	0.76	1.00																						
17	0.75	0.80	0.75	0.78	0.73	0.75	0.80	0.84	0.78	0.82	0.71	0.73	0.71	0.71	0.82	0.75	1.00																					
18	0.76	0.75	0.80	0.69	0.78	0.80	0.75	0.71	0.76	0.69	0.80	0.75	0.84	0.80	0.73	0.69	0.71	1.00																				
19	0.82	0.76	0.90	0.75	0.88	0.90	0.80	0.73	0.75	0.71	0.90	0.80	0.90	0.86	0.78	0.75	0.69	0.86	1.00																			
20	0.73	0.71	0.76	0.84	0.78	0.76	0.78	0.82	0.76	0.80	0.80	0.78	0.76	0.73	0.84	0.76	0.78	0.73	0.78	1.00																		
21	0.73	0.75	0.76	0.80	0.78	0.76	0.75	0.78	0.73	0.73	0.76	0.75	0.73	0.69	0.76	0.69	0.82	0.76	0.78	0.92	1.00																	
22	0.82	0.84	0.82	0.75	0.80	0.86	0.76	0.73	0.78	0.75	0.86	0.80	0.90	0.90	0.78	0.78	0.73	0.78	0.84	0.75	0.67	1.00																
23	0.75	0.76	0.78	0.75	0.80	0.82	0.84	0.76	0.75	0.82	0.78	0.76	0.78	0.75	0.82	0.71	0.76	0.78	0.88	0.82	0.78	0.80	1.00															
24	0.76	0.82	0.80	0.73	0.78	0.80	0.78	0.75	0.69	0.76	0.80	0.78	0.80	0.76	0.73	0.69	0.78	0.76	0.86	0.76	0.80	0.78	0.90	1.00														
25	0.76	0.78	0.80	0.76	0.78	0.80	0.82	0.75	0.69	0.76	0.80	0.82	0.80	0.76	0.73	0.73	0.78	0.76	0.86	0.84	0.88	0.75	0.82	0.92	1.00													
26	0.73	0.75	0.76	0.84	0.78	0.76	0.82	0.82	0.76	0.84	0.80	0.78	0.76	0.73	0.84	0.80	0.82	0.69	0.78	0.96	0.88	0.75	0.82	0.80	0.88	1.00												
27	0.82	0.76	0.82	0.82	0.88	0.82	0.73	0.80	0.82	0.75	0.86	0.76	0.86	0.86	0.78	0.82	0.80	0.75	0.76	0.78	0.78	0.76	0.73	0.78	0.78	0.82	1.00											
28	0.71	0.73	0.75	0.82	0.76	0.75	0.76	0.80	0.78	0.78	0.78	0.76	0.75	0.71	0.82	0.75	0.80	0.75	0.76	0.98	0.94	0.73	0.80	0.75	0.82	0.94	0.76	1.00										
29	0.69	0.67	0.73	0.84	0.78	0.76	0.75	0.78	0.73	0.76	0.76	0.75	0.76	0.76	0.80	0.73	0.78	0.69	0.75	0.96	0.88	0.75	0.78	0.73	0.80	0.92	0.78	0.94	1.00									
30	0.84	0.80	0.88	0.73	0.82	0.88	0.75	0.71	0.78	0.65	0.84	0.82	0.84	0.88	0.73	0.76	0.76	0.78	0.82	0.65	0.71	0.86	0.71	0.76	0.76	0.65	0.78	0.65	0.65	1.00								
31	0.75	0.76	0.78	0.75	0.80	0.82	0.84	0.76	0.75	0.82	0.78	0.76	0.78	0.75	0.82	0.71	0.76	0.78	0.88	0.82	0.78	0.80	1.00	0.90	0.82	0.82	0.73	0.80	0.78	0.71	1.00							
32	0.73	0.78	0.76	0.76	0.71	0.73	0.78	0.78	0.65	0.80	0.73	0.78	0.76	0.73	0.73	0.69	0.82	0.73	0.75	0.76	0.80	0.75	0.78	0.88	0.88	0.80	0.78	0.75	0.73	0.73	0.78	1.00						
33	0.75	0.76	0.82	0.78	0.76	0.78	0.80	0.76	0.67	0.75	0.78	0.80	0.78	0.75	0.75	0.71	0.80	0.75	0.84	0.86	0.90	0.73	0.80	0.90	0.98	0.90	0.80	0.84	0.82	0.75	0.80	0.90	1.00					
34	0.75	0.73	0.75	0.86	0.84	0.78	0.80	0.80	0.78	0.82	0.82	0.80	0.82	0.78	0.82	0.78	0.76	0.71	0.80	0.94	0.86	0.76	0.84	0.78	0.86	0.94	0.80	0.92	0.94	0.67	0.84	0.75	0.84	1.00				
35	0.86	0.80	0.82	0.82	0.92	0.86	0.76	0.76	0.86	0.75	0.90	0.80	0.90	0.90	0.82	0.86	0.73	0.75	0.80	0.78	0.71	0.88	0.76	0.71	0.71	0.78	0.88	0.76	0.78	0.82	0.76	0.67	0.69	0.84	1.00			
36	0.78	0.80	0.78	0.78	0.84	0.82	0.76	0.84	0.82	0.86	0.86	0.76	0.82	0.78	0.82	0.78	0.80	0.75	0.80	0.86	0.78	0.84	0.92	0.86	0.78	0.86	0.80	0.84	0.82	0.71	0.92	0.78	0.76	0.88	0.84	1.00		
37	0.65	0.67	0.65	0.80	0.75	0.73	0.75	0.75	0.73	0.76	0.73	0.75	0.73	0.73	0.76	0.69	0.78	0.73	0.71	0.84	0.80	0.71	0.78	0.73	0.76	0.84	0.71	0.86	0.88	0.63	0.78	0.69	0.75	0.90	0.75	0.82	1.00	
38	0.69	0.67	0.69	0.69	0.67	0.69	0.67	0.75	0.73	0.76	0.73	0.71	0.69	0.69	0.69	0.73	0.71	0.61	0.67	0.76	0.69	0.71	0.67	0.65	0.73	0.76	0.63	0.75	0.76	0.65	0.67	0.69	0.71	0.78	0.67	0.75	0.73	1.00

Plate-2 : Electrophoresis banding pattern of RAPD amplification products by primer OPC-09 resolved on 1.5% agarose gel

* M- Molecular weight marker
* Sample number as per the table no.- 3.2

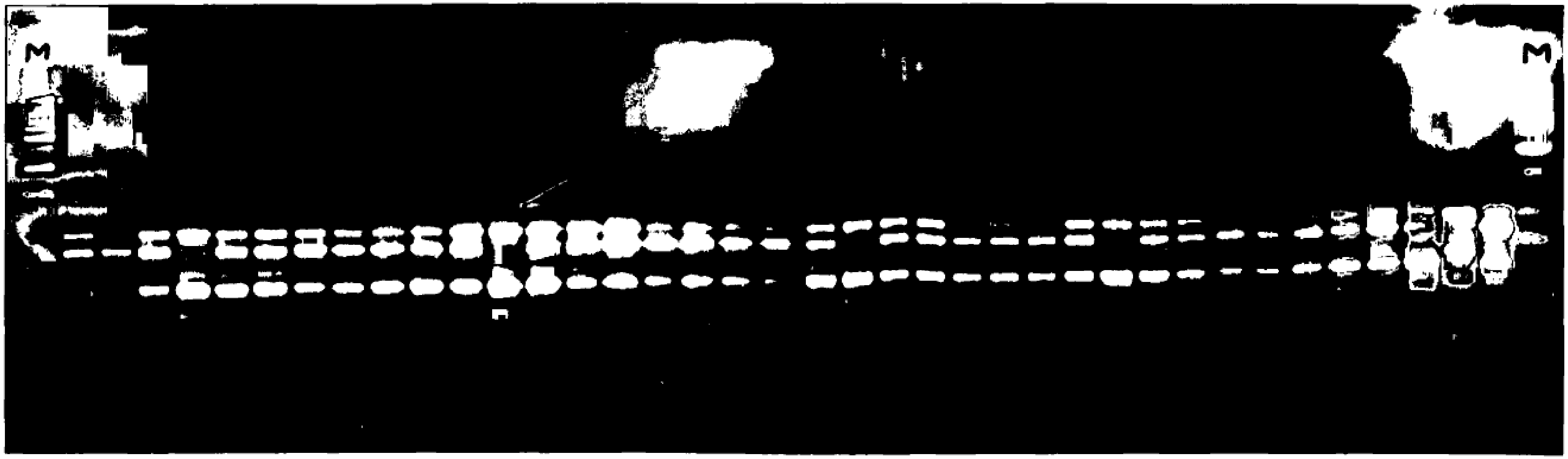
Plate-3 : Electrophoresis banding pattern of RAPD amplification products by primer OPC-11 resolved on 1.5% agarose gel



* M- Molecular weight marker

* Sample number as per the table no.- 3.2

Plate-4 : Electrophoresis banding pattern of RAPD amplification products by primer OPC-15 resolved on 1.5% agarose gel



* M- Molecular weight marker

* Sample number as per the table no.- 3.2

Out of these total 49 bands, 26 bands (53.06%) were polymorphic and remaining 23 bands (46.93%) were monomorphic. Average number of total bands per primer was 6.125, while average number of polymorphic bands per primer was 3.25. Among 8 RAPD primers, 4 produced polymorphic bands. Out of these four primers, OPC-09 and OPC-11 produce 10 electromorphs each showing (100.0%) polymorphism. Other 2 primers namely OPC-15 and OPH-20 generated polymorphic markers sharing 100.0 and 12.50 polymorphism respectively. Specific bands were also amplified by OPC-15 primer. Primer OPC-15 amplified a specific electromorph only in accession 37 of molecular weight about 245 bp.

Genetic diversity for a specific locus/marker can be evaluated by Polymorphic Information Content (PIC). The range of PIC scores of RAPDs markers ranged up to 0.709 with an average of 0.27.

4.7.2 Cluster analysis using RAPD markers

To group 38 accessions, a dendogram was generated by unweighted pair group method with "UPGMA" sub programme of "NTSYS-pc". As per cluster analysis, 38 accessions of rice bean germplasm positioned in to six major clusters (Fig. 2).

The first cluster contained three accessions 1, 9, and 16 The Second cluster occupied thirteen accessions 3, 5, 6, 11, 19, 13, 14, 35, 22, 30, 27, 18 and 2. The third cluster had seven accessions 4, 15, 7, 12, 8, 10 and 17. The fourth cluster contained seven accessions 20, 28, 26, 29, 34, 21 and 37. The fifth cluster comprised of three accessions 23, 31 and 36. The last sixth cluster contained five accessions 24, 25, 33, 32 and 38. Among these accessions samples 18 and 2 were closely related with each other but differed from all other accessions, while the sample # 38 (Accession JRB07-1-1) showed the highest diversity from all other accessions in dandogram analysis (Table 4.10).

4.7.3 ISSR analysis

A set of 20 primers was screened for 38 accessions of rice bean. Out of the 20 primers, 5 primers amplified successfully. Nucleotide sequences of ISSR primers are presented in Table 3.11.

Responding 5 ISSR primers amplified 31 ISSR marker loci. The size of the amplified markers ranged from 230-1100 bp. Maximum numbers of bands i.e. 7 were amplified by primer UBC-880 and UBC-821, while minimum numbers of bands i.e. 5 were obtained with primer UBC-851. Out of these 31 loci, 9 were polymorphic (1.80%) across all the rice bean accessions. Per cent polymorphism ranged from 0 to as high as 83.33. Average numbers of total bands per primer were 6.20, while average numbers of polymorphic bands per primer were 1.80 (Table 4.11).

Genetic diversity for a specific locus/marker can be evaluated by Polymorphic Information Content (PIC). The range of PIC scores of ISSR markers was found to be 0 (primer UBC-821, UBC-851, UBC-853) to 2.60 (primer UBC-885).

4.7.4 Cluster analysis using ISSR Markers

Based on ISSR markers, pair wise genetic similarity among 38 rice bean accessions was estimated and a dendogram was generated by UPGMA cluster analysis based on similarity coefficient (Fig 3).

The cluster analysis grouped rice bean accessions into five major clusters. The first cluster contained two accessions 1 and 6. The Second cluster occupied seven accessions 3, 24, 8, 9, 20, 21 and 23. The third cluster comprised six accessions 4, 5, 25, 26, 31 and 32. The fourth cluster contained eleven accessions 7, 10, 13, 14, 15, 16, 17, 18, 19, 11 and 12 and the last cluster had eleven accessions 22, 29, 30, 33, 35, 36, 27, 28, 34, 37 and 28. Accession 22 exhibited high molecular diversity as compared to other accessions as per UPGMA analysis (Table 4.12).

Table 4.11 Number of bands obtained using ISSR Primer

S. No.	Primer	Total Bands	Monomorphic Bands	Polymorphic bands	% polymorphism	PIC values
1	UBC-880	7	3	4	57.14	0.260
2	UBC-885	6	1	5	83.33	0.259
3	UBC-821	7	7	0	0.0	0.0
4	UBC-851	5	5	0	0.0	0.0
5	UBC-853	6	6	0	0.0	0.0
	Total **(Average)**	31 (6.20)	22	9 (1.80)	140.47 (28.09)	 (2.59)

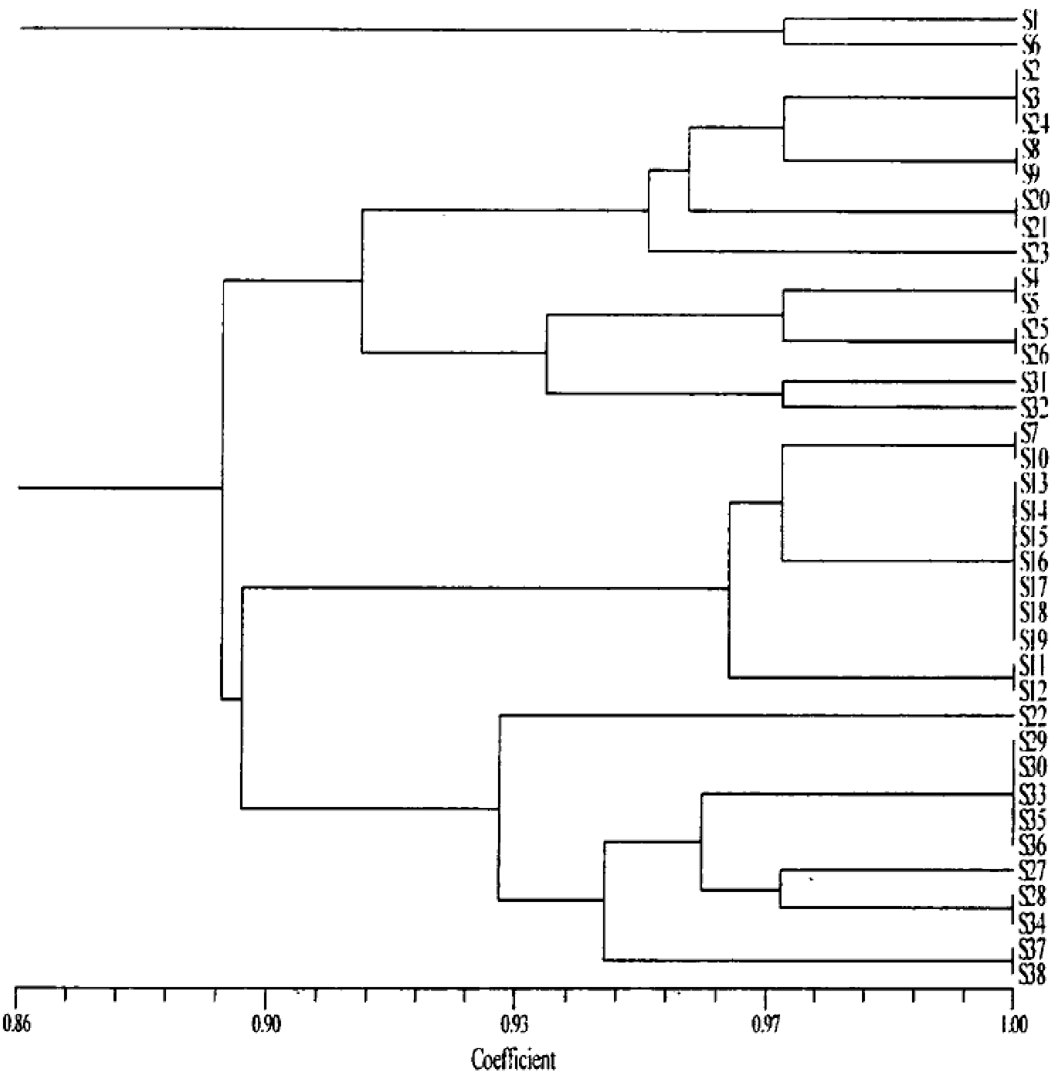
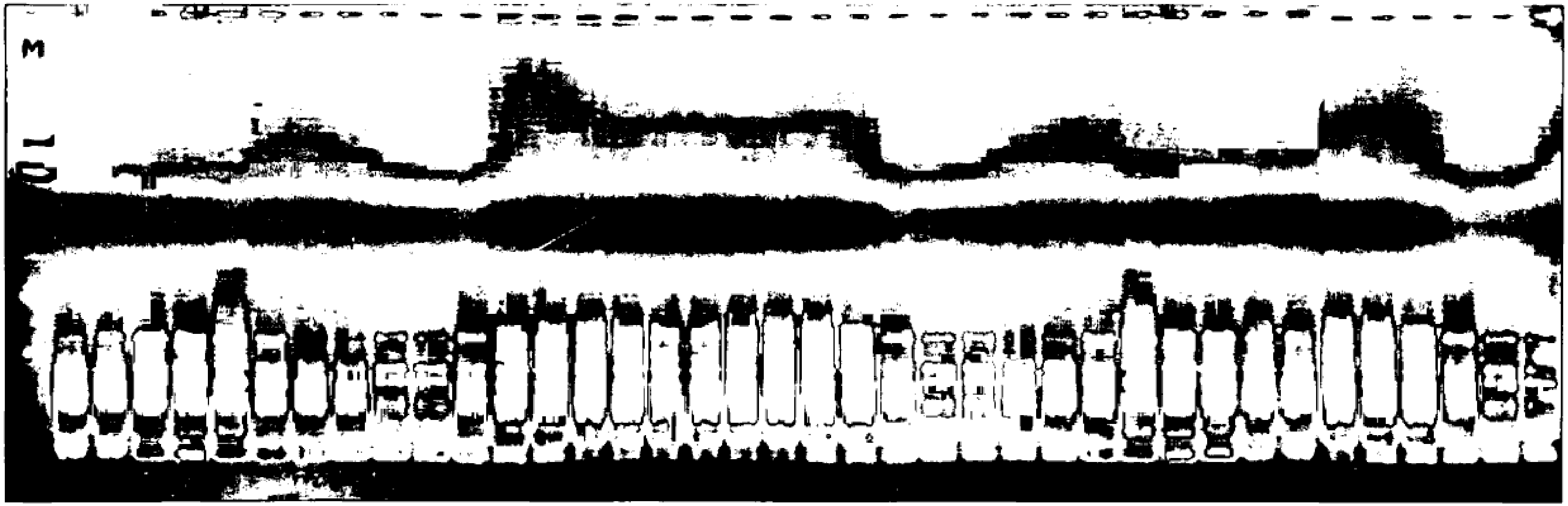


Fig 4.3 Dendrogram generated showing relationship among rice bean germplasm using ISSR markers

Table 4.12 Genetic distance among rice bean genotypes based on ISSR analysis

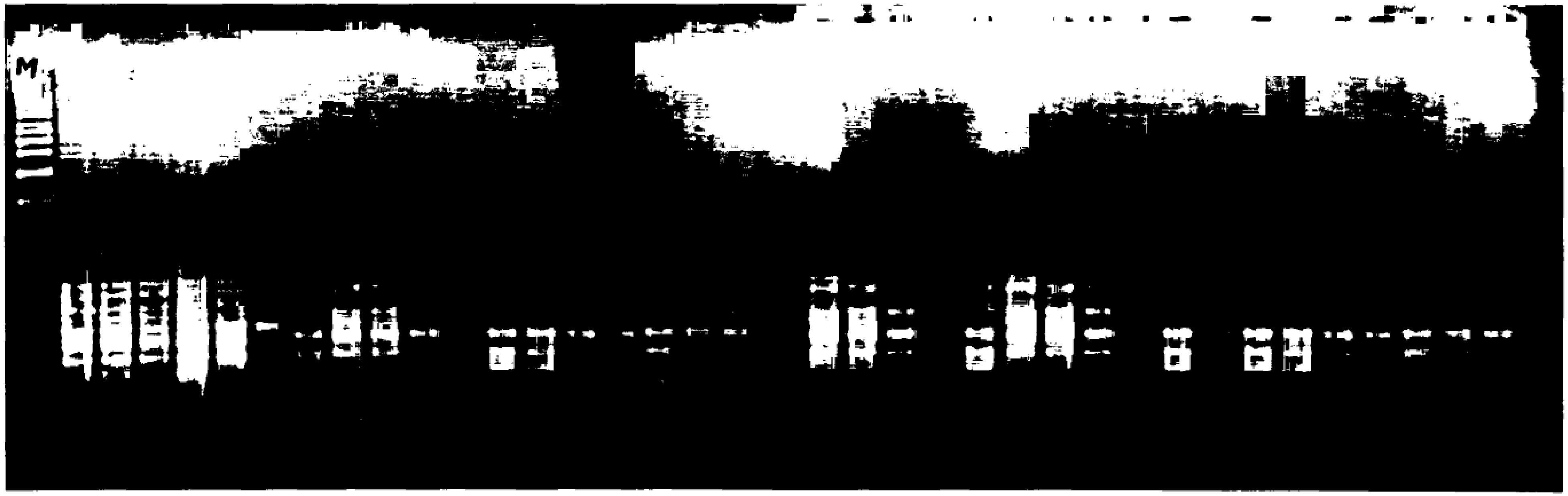
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38
S1	1.00																																					
S2	0.90	1.00																																				
S3	0.90	1.00	1.00																																			
S4	0.87	0.90	0.90	1.00																																		
S5	0.87	0.90	0.90	1.00	1.00																																	
S6	0.97	0.87	0.87	0.90	0.90	1.00																																
S7	0.87	0.90	0.90	0.87	0.87	0.84	1.00																															
S8	0.94	0.97	0.97	0.94	0.94	0.90	0.94	1.00																														
S9	0.94	0.97	0.97	0.94	0.94	0.90	0.94	1.00	1.00																													
S10	0.87	0.90	0.90	0.87	0.87	0.84	1.00	0.94	0.94	1.00																												
S11	0.81	0.84	0.84	0.94	0.94	0.84	0.94	0.87	0.87	0.94	1.00																											
S12	0.81	0.84	0.84	0.94	0.94	0.84	0.94	0.87	0.87	0.94	1.00	1.00																										
S13	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00																									
S14	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00																								
S15	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00	1.00																							
S16	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00	1.00	1.00																						
S17	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00	1.00	1.00	1.00																					
S18	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00	1.00	1.00	1.00	1.00																				
S19	0.84	0.87	0.87	0.90	0.90	0.87	0.97	0.90	0.90	0.97	0.97	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00																			
S20	0.87	0.97	0.97	0.94	0.94	0.90	0.87	0.94	0.94	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	1.00																		
S21	0.87	0.97	0.97	0.94	0.94	0.90	0.87	0.94	0.94	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	1.00	1.00																	
S22	0.87	0.90	0.90	0.81	0.81	0.84	0.87	0.87	0.87	0.87	0.81	0.81	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.87	0.87	1.00																
S23	0.87	0.97	0.97	0.87	0.87	0.84	0.87	0.94	0.94	0.87	0.81	0.81	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.94	0.94	0.87	1.00															
S24	0.90	1.00	1.00	0.90	0.90	0.87	0.90	0.97	0.97	0.90	0.84	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.97	0.97	0.90	0.97	1.00														
S25	0.90	0.94	0.94	0.97	0.97	0.94	0.90	0.97	0.97	0.90	0.90	0.90	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.97	0.97	0.84	0.90	0.94	1.00													
S26	0.90	0.94	0.94	0.97	0.97	0.94	0.90	0.97	0.97	0.90	0.90	0.90	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.97	0.97	0.84	0.90	0.94	1.00	1.00												
S27	0.87	0.90	0.90	0.87	0.87	0.84	0.94	0.94	0.94	0.94	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.87	0.87	0.94	0.87	0.90	0.90	0.90	1.00											
S28	0.84	0.87	0.87	0.90	0.90	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.90	0.90	0.90	0.84	0.87	0.94	0.94	0.97	1.00										
S29	0.81	0.90	0.90	0.87	0.87	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.87	0.90	0.90	0.90	0.94	0.97	1.00									
S30	0.81	0.90	0.90	0.87	0.87	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.87	0.90	0.90	0.90	0.94	0.97	1.00	1.00								
S31	0.81	0.84	0.84	0.94	0.94	0.84	0.87	0.87	0.87	0.87	0.94	0.94	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.87	0.87	0.87	0.81	0.84	0.90	0.90	0.94	0.97	0.94	0.94	1.00							
S32	0.84	0.87	0.87	0.97	0.97	0.87	0.84	0.90	0.90	0.84	0.90	0.90	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.84	0.84	0.87	0.94	0.94	0.90	0.94	0.90	0.90	0.97	1.00						
S33	0.81	0.90	0.90	0.87	0.87	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.87	0.90	0.90	0.90	0.94	0.97	1.00	1.00	0.94	0.90	1.00					
S34	0.84	0.87	0.87	0.90	0.90	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.90	0.90	0.90	0.84	0.87	0.94	0.94	0.97	1.00	0.97	0.97	0.97	0.94	0.97	1.00				
S35	0.81	0.90	0.90	0.87	0.87	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.87	0.90	0.90	0.90	0.94	0.97	1.00	1.00	0.94	0.90	1.00	0.97	1.00			
S36	0.81	0.90	0.90	0.87	0.87	0.84	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.94	0.94	0.94	0.87	0.90	0.90	0.90	0.94	0.97	1.00	1.00	0.94	0.90	1.00	0.97	1.00	1.00		
S37	0.87	0.84	0.84	0.87	0.87	0.90	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.87	0.87	0.94	0.81	0.84	0.90	0.90	0.94	0.97	0.94	0.94	0.94	0.90	0.94	0.97	0.94	0.94	1.00	
S38	0.87	0.84	0.84	0.87	0.87	0.90	0.87	0.87	0.87	0.87	0.87	0.87	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.87	0.87	0.94	0.81	0.84	0.90	0.90	0.94	0.97	0.94	0.94	0.94	0.90	0.94	0.97	0.94	0.94	1.00	1.00

Plate-5 : Electrophoresis banding pattern of ISSR amplification products by primer UBC-880 resolved on 1.5% agarose gel



* M- Molecular weight marker
* Sample number as per the table no.- 3.2

Plate-6 : Electrophoresis banding pattern of ISSR amplification products by primer UBC-885 resolved on 1.5% agarose gel



* M- Molecular weight marker
* Sample number as per the table no.- 3.2

4.7.5 Comparison of RAPD and ISSR markers for genetic diversity assessment

During the present investigation, two marker systems were used to evaluate molecular diversity in 38 rice bean accessions. Both marker systems generated polymorphism and could be distinguished among all the 38 rice bean accessions. RAPD markers showed higher level of polymorphism as compared to ISSR. A total of 49 loci were amplified by RAPD primers, out of them 23 were polymorphic. ISSR primers amplified 31 loci however; only 9 polymorphic bands were detected. Similarity patterns were observed for these marker systems. The correlation was calculated between these two molecular markers using the Mantel Statistic Test (Mantel, 1967). The Mantel's Z-test statistics revealed significant common pattern between the two dendograms when the matrices of cophentic value were compared.

4.8 Comparison between phenotypic and DNA polymorphism

On the basis of phenotypic analysis the genetic divergence of 94 rice bean accessions has been analyzed through the statistical distance (Mahalanobis's D^2 analysis). Clusters were formulated between the pairs of corresponding uncorrelated values of any two accessions considered together. During the present investigation 8 clusters were formed on the basis of intra and inter cluster divergence. For molecular polymorphism analysis, 38 accessions were selected on the basis of higher green fodder yield. These accessions with higher green fodder yield were positioned in Cluster V, VI and VII of genetic divergence analysis among 94 accessions. Subsequent RAPD analysis of 38 accessions resulted in the formation of 6 clusters similarly, the ISSR analysis resulted in the formation of 5 clusters.

DISCUSSION

DISCUSSION

A plant breeder has to face a tedious task to select genetically diverse plants for a fruitful hybridization programme, unless information provided to him viz, genetic variation and genetic divergence present in the available genetic material. In this chapter the present investigations have been interpreted and discussed in light of research work carried out in India and abroad. The discussion is confined to the relevant topics viz genetic variability, correlation coefficient analysis, path coefficient analysis, genetic divergence and molecular analysis.

5.1. Analysis of variance

The analysis of variance revealed that the accessions were significantly differing for all the eleven traits, indicating existence of genetic variability among the accessions for all the traits under study. Similar results were observed by Singh *et al.* (2005) for rice bean.

5.2 Parameters of genetic variability

The main objective of present investigation was to determine the nature and amount of genetic variability present in 94 accessions of rice bean included in this study. It is the most essential requirement for further successful crop improvement programme. Variability found in a broad sense is to achieve success in a plant breeding programme since it provides an opportunity for plant breeder to use his knowledge and skill in making useful selections. This information will be useful in deciding suitable selection criteria for genetic improvement of rice bean.

5.2.1 Coefficient of variation

All the traits under study show highly significant treatment sum of square values indicating the presence of genetic variability in the germplasm. Similar observations were reported by many other workers in forage cowpea for plant height, pod per plant and seed weight (Chauhan *et al.*, 2003); in mung bean and rice bean for seed weight, pods per plant and seeds per pod (Singh *et al.*, 2005); in rice bean for grain yield, pod number, branch per plant, plant height, node number per plant, seed weight and dry matter yield (Manohar *et al.*. 2005); and, in rice bean for plant height, branches per plant, flowering time, pod clusters per plant, pods per cluster, pods per plant, pod length, seeds per pod, seed weight and days to maturity (Sarkar *et al.*, 2007)

As we are aware that genetic features are predominately responsible for expression of their attributes. Similar findings on divergence during present investigation reveal that selection can be made effectively on the basis of phenotypic performance.

During the present investigations, phenotypic coefficients of variation (PCV) values were higher than the genotypic coefficient of variation (GCV) values for all the traits indicating the variable environmental influence on the expression of the traits. The high estimates of PCV and GCV were observed for the traits like green fodder yield per day, number of leaves, dry fodder yield per day, green fodder yield, leaf stem ratio, days to 50% flowering, dry fodder yield, number of branches and plant height. Results for plant height and yield per plant are in close harmony with the findings of rice bean cultivars (Singh *et al.*, 1997); leaf area per plant, plant height and number of branches (Thakur, 1990); for seed yield per plant, effective nodes per plant, pods per plant, days to 50% flowering, pod maturity, 100 seed weight and plant height in rice bean (Das *et al.*, 1997); for number of branches and leaves, leaf stem ratio, dry fodder yield and green fodder in fodder cowpea (Borah and Khan, 2000); for grain yield per plant, plant height and test weight in rice bean (Manohar *et al.*, 2005); for forage yield, dry matter yield, pod number and plant height in rice bean(Bhattacharya *et al.*, 2005); for protein yield per plant, green fodder per day, fiber yield per day, crude fiber percent and number of nodules per plant in rice bean (Jhariya, 2007). Low estimates of PCV and GCV were observed for the trait crude protein percent. Similar results were observed by Thakur (1990) for days to harvest and crude protein in forage soybean which indicates low influence of environment.

5.2.2 Heritability and genetic advance

Heritability denotes the proportion of genetically controlled variability expressed for a particular trait or a set of traits is a very important biometrical tool for guiding plant breeders for adoption of appropriate breeding procedures. High heritability in broad sense is helpful in identifying appropriate trait for selection and enables the breeder to select superior accessions on the basis of phenotypic expression of quantitative traits. Maximum selection response if the variance exhibited by the population is largely due to additive genetic effects.

During the present investigations, crude protein percent and leaf stem ratio showed high heritability along with high genetic advance. Thus these traits are under additive gene control with negligible environmental effect and will be useful from selection point of view. The results were in close a proximity to that of in cowpea for green forage yield, leaf area, number of nodules, leaf stem ratio (Thaware *et al.*, 1991); in cowpea for number of branches, number of leaves, leaf stem ratio, dry matter yield, green fodder yield, plant height (Borah and Khan, 2000). It revealed that the phenotypes were the true representative of their accessions and selection based on phenotypic performance would be reliable. High heritability estimates with low genetic advance was observed for crude fibre percent. It is indication of non additive gene action. The high heritability is being exhibited due to favorable influence of environment rather than accession and such type of selection may not be rewarding.

5.3 Correlation coefficient analysis

Variations in quantitative traits provide the basis for selection in plant breeding programme. The knowledge of association among the traits is useful to the breeder for improving the efficiency of selection. Correlation coefficient analysis measures the mutual relationship between plant traits and determines the component trait on which selection can be made for genetic improvement of yield. Investigation regarding the presence of component and nature of association among themselves is essential and pre-requisite for improvement in yield. Correlation coefficient provides a clear picture of the extent of association between a pair of traits and indicates whether simultaneous improvement of the correlated traits is possible or not.

Correlation is the relationship between the two attributes and the strength of relationship is measured in terms of correlation coefficient, a positive correlation between desirable traits is helpful to the plant breeder because it helps in simultaneous improvement of both the traits. While a negative correlation on the other hand shall hinder the simultaneous expression of both the traits with high values. In such situation some economic compromises has to be made.

During the present study, correlation coefficient value at phenotypic level was found greater than at genotypic level for trait pairs: plant height with branches per plant, days to flowering with leaf stem ratio, branches per plant with leaves per plant as well as leaf stem ratio, plant height with green fodder yield per day, leaf stem ratio with dry fodder yield per day, crude protein percent with dry fodder yield per day as well as crude fibre percent.

It was found that the apparent correlation of these traits is not only due to genes responsible for expression of traits but also due to favorable influences of environment. However, rest of the trait pairs exhibited genotypic correlation coefficient greater than the phenotypic correlation coefficient. It indicates that genetically strong correlation exists between the traits.

During the present investigation, significant positive correlation of green fodder yield per plant was found with traits crude protein percent and crude fibre percent. These findings are in agreement Kumar (1979) for green fodder yield per plant with number of branches and green fodder yield per day fodder cowpea

Other trait pairs showing significant positive correlation were days to flowering with plant height, plant height with dry fodder yield per plant and dry fodder yield per day, number of branches with dry fodder yield per plant, number of leaves with leaf stem ratio and green fodder yield per day, leaf stem ratio with green fodder yield per day, green fodder yield per day with dry fodder yield per plant, dry fodder yield per day and crude protein percent, dry fodder yield per plant with crude fibre percent, dry fodder yield per day with crude protein percent. These results indicate that simultaneous selection is possible for all these traits pairs. These results are in close agreement with the findings of Mehta *et al.* (2007) in fodder soybean for stem girth, green fodder yield per day, protein yield, dry fodder yield per plant, dry fodder yield per day with plant height. The results also show similarity with the findings of Singh *et al.* (1997) since pods per plant, seed weight and pod lengths were positively correlated with seed yield in rice bean.

Negative correlation between pairs of traits was exhibited by days to flowering with number of branches per plant; green fodder yield per day and dry fodder yield; plant height with number of branches per plant; leaf stem ratio and green fodder yield per day; number of branches per plant with green fodder yield per day as well as dry fodder yield per day; and, leaf stem ratio with dry fodder yield per plant as well as crude protein percent. It indicates that simultaneous improvement of some pair of traits might not be an easy task for example, delay in 50% flowering reduces the green fodder yield and ultimately affect the protein yield. This might be due to utilization of energy required for vegetative growth in the seed formation. Singh *et al.* (1997) for plant height and days to maturity were negatively correlated with seed yield in rice bean.

5.4 Path coefficient analysis

Path coefficient analysis is one of the widely used biometrical techniques in plant breeding that resolves the complex correlation matrix and provides a clear picture of direct and indirect effect of each variable on other dependent variable. Path coefficient analysis was carried out at genotypic and phenotypic level. In a biological system, however the relationship may exist in a very complex form. It is therefore, essential to study the relationship among variables in a comprehensive way. As a result, it helps in understanding the causal system in a better way, since it enables portioning the total correlation coefficient into direct and indirect effects of various traits.

During the present investigation, path analysis was carried out for traits under study using genotypic and phenotypic correlation coefficient and taking green fodder yield as dependable variable. Green fodder yield was set as a dependable variable in order to find out the causal factor and so as to identify the component responsible for producing green fodder yield per plant.

Salient findings indicated that the trait plant height had at par correlation coefficient value with its direct effect on green fodder yield. This indicates highly significant relationship with green fodder yield and direct selection for this trait will be beneficial for yield improvement. Thus, this trait can be ascertained as the most important component of green fodder yield.

The trait dry fodder yield showed negative direct effect on green fodder yield on the other hand exhibited significant positive correlation. This indicates that the indirect effect was the cause of correlation and the indirect causal factors are to be considered simultaneously for selection.

Trait days to 50% flowering revealed significant negative correlation and negative direct effect on green fodder yield per plant. Under such situation direct selection for these traits should be avoided and indirect selection should be preferred. All other traits influenced green fodder yield (indirect effect) mostly through days to 50% flowering, dry fodder yield per day, number of leaves and number of branches indicating presence of majority of the variation in fodder yield.

Although, the trait number of branches per plant did not exhibited direct effect on green fodder yield but expressed high indirect effect through green fodder yield per day. Hence, it can be concluded that green fodder yield per day, dry fodder yield per plant, dry fodder yield per day, number of leaves and number of branches are important traits. Similar results were observed by Kumar and Mishra (1981) for dry matter yield in fodder cowpea; Borah and Khan (1999) for dry matter yield, number of leaves and days to 50% flowering in fodder cowpea; Mehta *et al.* (*2007*) for dry fodder yield per plant, dry fodder yield per day, protein yield, number of branches and protein yield per day in forage soybean.

5.5 Genetic Divergence

The choice of parents is of paramount importance in any breeding programme. It is rather a difficult task for a plant breeder. Selection of parents on the basis of per se performance is good but there is a possibility of related lines being chosen resulting in limited or no advances under selection, and therefore, there is a need for emphasis on a wide genetic base. Selection of the parents on the basis of geographical diversity is another way of choosing parents, but this needs to be supplemented with the information on genetic diversity. In formulating successful breeding programme for evolving superior cultivars, knowledge of genetic divergence among parents is of utmost importance since a cross involving genetically diverse parents and is likely to produce high heterotic effect as well as a broad range of variability could be expected in the segregating generations.

For assessment of genetic divergence among the traits under study, the D^2 analysis has been a perfect test available among several multivariate analysis methods. The importance of this multivariate analysis had been greatly emphasized by assessment of genetic diversity in biological population (Mahalanobis, 1936).

Genetic divergence analysis based on Mahalanobis D^2 statistics, 94 accessions were found to be grouped into eight clusters. Out of these eight clusters, cluster IV and V had large population consisting of 33 accessions each. The pattern of distribution of accessions into different clusters was random across the geographical boundaries suggesting that the genetic diversity was more of a dominant factor towards total divergence among the accessions. Many cultivars collected from the same location were grouped into different clusters. This suggested that genetic drift, natural selection and diverse environmental conditions within a country cause more diversity in comparison to geographical isolation.

Contribution of different plant traits towards genetic divergence is important for the purpose of further selection and choice of parents. Hence selection for divergent accessions based on these traits might be useful for exploitation of hybrid vigour. Analysis revealed that days to 50% flowering, plant height, number of leaves per plant, green fodder yield per day, and dry fodder yield per day were the major contributors towards total divergence.

The intra-cluster distance was found to be highest in cluster VIII. The highest genetic diversity among the accessions in cluster VIII may be due to both natural and artificial selection forces. Whereas, cluster III, IV, V and VI showed low intra cluster distances as compared to cluster VIII. The intra cluster distance in cluster I was found to be low, which indicates that accessions belonging to this cluster were genetically close to each other.

The inter cluster distance was highest between cluster II and cluster VII, indicating that the accessions of these clusters might be used as parents in hybridization programme to get the highest heterotic effects or to select a good variant accession from the segregating population.

Cluster mean values for different traits revealed that the cluster mean values excelled in respect of different traits. It was found that cluster mean values for different traits viz. days to 50% flowering, plant height, number of leaves per plant, number of branches per plant, green fodder yield per plant and green fodder yield per day were greater for cluster I and II. This strongly indicates that high variability for the above traits was responsible for diversity among all accessions.

No work has been reported earlier on genetic divergence in rice bean germplasm. However, studies have been conducted in mung bean (Ramanujam *et al.*, 1974); in pigeonpea (Chattopadhyay *et al.*, 2005); in exotic and indigenous mung bean accessions (Prakash *et al.*, 2006); in chickpea (Sandhu *et al.*, 2006) and in horse gram (*Macrotyloma uniflorum*) by Sunil *et al.* (2008).

5.6 Molecular analysis

Although, significant interaction is present between accessions and environment, visible morphological traits are limited in number. The classification based on molecular analysis will be far more than the actual phenotypic variability present. Genetic information at DNA level could be more reliable to reflect the suitable genetic difference between the accessions as it is normally not subjected to environmental variation.

Genetic diversity analysis is possible using various molecular markers such as RAPD, ISSR, STMS and AFLP *etc.* It greatly depends upon type of markers used, their distribution in the genome, loci they amplify, level of polymorphism and reproducibility. PCR based molecular markers (RAPDs, ISSRs, STMSs *etc.*) are preferred over hybridization based markers like RFLPs for genetic diversity analysis, because they permit use of smaller amount of crudely prepared DNA from each individual accession and also reduce the time, labour and operational cost of DNA extraction (Sant *et al.*, 1999).

A prerequisite for taking advantage of these techniques is good quality genomic DNA. During the present investigation genomic DNA was extracted from the leaves samples of rice bean. The DNA was purified by phenol: choloroform: isoamyl alcohol (25:24:1). The PCR conditions were also standardized for RAPD and ISSR marker analysis.

During the present investigation out of 94 accessions of rice bean, 38 were selected on the basis of diversity analysis at phenotypic level exhibiting better fodder and crude protein yield. The genomic DNA of 38 accessions was explored using 12 ISSR and 15 RAPD markers to assess genetic variability within them.

Randomly selected 8 decamer primer amplified 49 RAPD marker loci. The size of amplified fragments ranged from 240-1600bp. Out of these 49 bands, 26 bands (53.06%) were polymorphic revealing presence of diversity among the accessions under study. (Muthusamy *et al.* 2009) also observed high degree of polymorphism in rice bean landraces. Other pulses exhibiting substantial diversity within germplasm are Italian common bean (Marotti *et al.*, 2005), domesticated cowpea and its wild progenitor (Ba *et al.*, 2004), common bean landraces (Maciel *et al.*, 2004), cowpea (Smith *et al.* 1997). Primer OPC-15 amplified a specific electromorph of 245 bp in accession JRB07-54-3 (Sample 37). This primer could be used to identify this specific accession with the highest green fodder yield, dry fodder yield per day and crude fiber percent among all accessions under study. It can be predicted that this marker may be associated with these traits. It should be investigated further to know its linkage with any specific trait / gene.

The cluster analysis for selected 38 accessions of rice bean using 8 RAPD markers was done using NTSYS-pc software programme. The cluster analysis grouped accessions into six clusters. The first cluster contained three accessions 1, 9, and 16. The Second cluster occupied thirteen accessions 3, 5, 6, 11, 19, 13, 14, 35, 22, 30, 27, 18 and 2. The third cluster consisted of seven accessions 4, 15, 7, 12, 8, 10 and 17. The fourth cluster had seven accessions 20, 28, 26, 29, 34, 21 and 37 The fifth cluster contained three accessions 23, 31 and 36 and the last cluster consisted of five accessions 24, 25, 33, 32 and 38. Among these accessions JRB06-9-1 (sample 18) and BFRB-3-1 (Sample 2) were closely related with each other but differed from all other accessions. Therefore, accession JRB07-1-1 (sample 38) showed the highest diversity as compared to all other accessions as per dendrogram analysis. This accession also produced highest number of leaves and can be used as a parent in a hybridization programme for developing fodder rice bean varieties.

The ISSR technique was more useful for the study of diversity in rice bean in terms of quality and quantity of data inputs as compared to RFLP and RAPD. The ISSR markers are one of the most efficient markers which permit detection of polymorphism in microsatellite loci without previous knowledge of the DNA sequence (Tautz, 1989).Significantly the efficiency of technique has been demonstrated in molecular characterization even at varietal level of a species (Hauntula *et al.*, 1996).

A set of five ISSR primers (UBC primers) were used to analyze genetic diversity among selected 38 accessions, these primers were consisted of 16-20 bases. These five ISSR primers amplified 31 ISSR marker loci. The size of the amplified fragments ranged from 230-1100 bp. Out of these 31 loci, 9 were found to be polymorphic (1.8%) across all the rice bean accessions under study. Ajibade *et al.* (2000) reported that ISSR primers generate 3 to 26 markers with average of 12.94 per accession in *Vigna*. Similar results have been reported also with ISSR markers by Marotti *et al.* (2005) among Italian common bean landraces and Muthusamy *et al.* (2009) in rice bean landraces.

Based on ISSR markers, genetic diversity among 38 accessions of rice bean was estimated and a dendrogram was generated by UPGMA cluster analysis based on similarity coefficient. The cluster analysis grouped 38 accessions of rice bean into five major clusters. The first cluster contained two accessions 1 and 6 .The Second cluster comprised seven accessions 3, 24, 8, 9, 20, 21 and 23. The third cluster occupied six 4, 5, 25, 26, 31 and 32. The fourth cluster contained eleven accessions 7, 10, 13, 14, 15, 16, 17, 18, 19, 11 and 12 and the last cluster eleven accessions 22, 29, 30, 33, 35, 36, 27, 28, 34, 37 and 28. Accession JRB06-10 (sample 22) exhibited highest diversity among 38 accessions in UPGMA analysis. Fang *et al.* (2006) studied in cowpea and Ajibade *et al.* (2004) in *Vigna* also found the similar results.

5.7 Comparison between phenotypic and molecular marker polymorphism analysis

The nature and amount of genetic variability present for forage yield and its components in rice bean can be estimated by the phenotypic diversity (D^2) analysis, which had been a perfect test available for several multivariate analyses. D^2 analysis based on Mahalanobis D^2 statistics grouped 94 accessions into eight clusters. Accessions in Clusters V, VI and VII were found with high green fodder yield, crude protein and dry fodder yield. Further, such 38 accessions representing Clusters V, VI and VII were selected to detect the genetic diversity at molecular level using RAPD and ISSR markers. Merely eight RAPD primers generated 49 DNA bands of molecular weight ranging from 240-1600bp, out of these, 26 bands (53.06%) were polymorphic. Cluster analysis grouped the accessions into six clusters. RAPD primer OPC-15 amplified a specific electromorph of 245 bp in Sample 37 (accession JRB07-54-3). This primer can be further used to identify this specific accession. While the accession JRB07-1-1 (sample 38) showed the highest diversity as compared to all other accessions.

Among the selected 38 accessions a set of only five ISSR primers amplified 31 ISSR markers loci. The size of the amplified fragments ranged from 230-1100 bp. Out of these 31 loci, 9 loci were found to be polymorphic (1.80%). The cluster analysis grouped rice bean accessions into five major clusters. Accession JRB06-10 (sample 22) demonstrated the highest diversity. Thus the most diverse accessions according to molecular analysis were JRB07-54-3 (sample 37), JRB06-10 (sample 22), BFRB-3-1 (sample 2), JRB06-9-1 (sample 18) and JRB07-1-1 (sample 38).

During the present investigation with rice bean germplasm, huge amount of diversity was observed as revealed by the dendrogram developed from both phenotypic and molecular analysis. Therefore, it can be concluded that phenotypic or molecular analysis in accessions may be insufficient for assessing the unambiguous diversity of the rice bean germplasm and selection of parents for further breeding programme. In addition, the excellent attributes of RAPD and ISSR markers could be of great importance for cultivar identification and estimating genetic variability among the selected rice bean accessions. As suggested by Lepse *et al.* (2005) in cucumber, phenotypic analysis simultaneously with molecular marker analysis will be more appropriate for detection of diversity among the genotypes.

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

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Summary

The present investigation entitled "Studies on phenotypic and molecular polymorphism in rice bean (*Vigna umbellata*)" was conducted at Seed Breeding Farm, National Seed Project Unit, Development of Plant Breeding and Genetics, JNKVV, Jabalpur (M.P.) during Kharif. This study was carried out with 94 rice bean germplasm including a check Bidhan-1 in Randomized Complete Block Design (RCBD) with two replications. The second part of the investigation i.e. molecular analysis was carried out at Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

The main objectives of this investigation were to measures the genetic variability, correlation coefficient, path coefficient, genetic divergence and polymorphism at molecular level for fodder yield and its components. At phenotypic level observations were recorded for 11 traits viz. days to 50% flowering, plant height, branches per plant, leaves per plant, leaf stem ratio, green fodder yield per plant (g), green fodder yield per day (g), dry fodder yield per plant (g), dry fodder yield per day (g), crude protein (%) and crude fibre (%). The mean data obtained were subjected to analysis of variance as per the design, which revealed that the mean square estimates were significant for all the traits indicating sufficient diversity among the accessions for the characters studied.

The high estimates of genotypic and phenotypic coefficient of variance were recorded for the traits i.e. leaves per plant (70.29) followed by green fodder yield per day (44.19), branches per plant (40.87), leaf stem ratio (26.92), dry fodder yield per day (25.88), green fodder yield per plant (22.44), plant height (21.98), days to 50% flowering (21.29) and dry fodder yield per plant (17.61).

High heritability supplemented with high genetic advance was exhibited by the traits crude fibre percent, crude protein percent and leaf stem ratio. High heritability with low genetic advance was exhibited by crude fibre percent hence there is a limited scope for selection of this trait and selection pressure may be applied to successive generation.

The study of correlation coefficient analysis among the fodder yield per plant and yield contributing traits at phenotypic and genotypic level revealed that the green fodder yield possess positive and significant correlation with crude protein percent (0.3530) and crude fibre percent (0.8610).

Path coefficient analysis revealed high positive direct effect on green fodder yield recorded by leaves per plant subsequently by plant height and crude fibre percent. While, leaf stem ratio showed highest negative direct effect on green fodder yield followed by crude protein percent, days to 50% flowering, dry fodder yield per plant, branches per plant, dry fodder yield per day and green fodder yield per day.

It was observed that the traits, plant height, leaves per plant, green fodder yield per day (g) and crude fibre percent are with the positive direct effect on yield. Therefore, selection pressure may be applied on these traits for genetic improvement of yield and quality attributes of rice bean.

On the basis of D^2 analysis, the 94 accessions were grouped into eight clusters, irrespective of the geographic divergence, indicating no parallelism between geographic and genetic diversity. The intra-cluster distance was highest in cluster VIII followed by cluster V, I and the minimum inter-cluster distance was observed between I and II, while the maximum inter-cluster distance was exhibited by II and cluster VII. It would therefore be logical to include accessions belonging to these different clusters in hybridization programme creating wider spectrum of variability and to exploit hybrid vigour.

The inter-cluster mean values for days to 50% flowering, plant height, leaves per plant, branches per plant, green fodder yield per plant and green fodder yield per day were distant for cluster I and II. This strongly indicated that diversity for the above traits is responsible for diversity of accessions.

Out of 94 accessions of rice bean, 38 were selected on the basis of diversity analysis at phenotypic level showing better fodder and crude protein yield. The genomic DNA of 38 accessions was subjected to amplification with RAPD and ISSR markers for diversity analysis.

Randomly selected 8 decamer primer amplified 49 RAPD marker loci. Out of these 49 bands, 26 bands (53.06%) were polymorphic. Average bands per primer were 6.125, while average polymorphic bands per primer were 3.25. The range of PIC scores of RAPD markers was 0 to 0.709 with an average of 0.27.

Five ISSR primers amplified 31 ISSR marker loci. Among these 31 loci, 9 were polymorphic (1.80%) across all the rice bean accessions. Percent polymorphism ranged up to 83.33. Average bands per primer were 6.20, while average polymorphic bands per primer were 1.80. The range of PIC scores of ISSR markers was 0 to 2.60.

The statistical distance (Mahalanobis's D^2) between the pairs of 94 accessions of rice bean was found to be 56.69% for green fodder yield per plant, 13.29% for crude protein percentage and 1.17% for dry fodder yield per plant, contributed maximum towards genetic divergence. The 38 accessions were selected on the basis of high green fodder and protein yield for molecular polymorphism analysis. Eight decamer primers amplified 49 RAPD marker loci, out of these 26 (53.06%) were polymorphic. Five ISSR primers amplified 31 marker loci. Out of these 31 loci, 9 were polymorphic (1.80%) across all the rice bean accessions. Phenotypic analysis together with RAPD and ISSR markers are appropriate for detection of diversity among the accessions.

Conclusions

Analysis of variance revealed highly significant variance for all the traits studied depicting greater variability in the existing material. The high estimates of genotypic and phenotypic coefficient of variation indicate greater diversity for green fodder yield per day, leaves per plant, dry fodder yield per day, green fodder yield, leaf stem ratio, days to 50% flowering, dry fodder yield per plant, branches per plant and plant height traits.

Traits crude protein percent and leaf stem ratio contained a good amount of heritable additive genetic component which lead to the conclusion that these two traits can be improved through direct selection as they are under additive gene control with negligible environmental effect.

The correlation of green fodder yield with other traits indicated the advantage of upgrading rice bean through simultaneous selection of leaves per plant, plant height and crude fibre percent since they had positive direct effects on green fodder yield.

Study of genetic divergence revealed a high inter-cluster distance between cluster II and VII suggesting accessions present within these two clusters may be considered for better divergent from each other. Traits days to 50% flowering, plant height, leaves per plant, branches per plant, green fodder yield and green fodder yield per day exhibited wider variability in cluster I and II indicating that variability for these traits was invariably responsible for the diversity of rice bean germplasm.

During study on RAPD markers with 38 rice bean accessions cluster analysis created six major clusters. Accession JRB07-1-1 showed the highest diversity from all other accessions and also produced highest number of leaves establishing itself as a parent in a hybridization programme for developing improved fodder rice bean varieties.

RAPD primer OPC-15 amplified a specific electromorph in accession JRB07-54-3 of 254bp. This primer could be used to identify accessions with the higher green fodder yield, dry fodder yield per day and crude fiber percent in rice bean.

Five ISSR primers amplified 31 marker loci and grouped rice bean accession into five major clusters. Accession JRB06-10 showed the highest diversity among 38 accessions. Comparative study of RAPD and ISSR markers with 38 rice bean accessions revealed JRB07-54-3, JRB06-10, BFRB-3-1, JRB06-9-1 and JRB07-1-1 as the most diverse accessions.

A comparative study between phenotypic and molecular diversity offers an opportunity for assessing reliable and effective genetic diversity among the accessions. It will provide a means to formulate a breeding strategy for the genetic enhancement of rice bean, a potential pulse fodder crop.

Suggestions for further work

1. Germplasm shall be explored for dual purpose type rice bean i.e. fodder cum seed type.

2. To judge the phenotypic stability, the yield performance along with component traits shall be assessed over seasons and locations.

3. The accessions JRB07-54-3, JRB06-13 and JRB-06 showed higher fodder yield potential JRB06-2-1, BFRB-8-1 and JRB07-11-1 having high dry fodder yield per plant and JRB-6-1 having high crude protein percent, these accessions can be involved in crossing programme.

4. Accessions BFRB-3-1, JRB06-9-1, JRB07-1-1, and JRB06-10 showing higher molecular diversity may be included in the hybridization programme for rice bean genetic improvement.

5. Accessions JRB07-54-3 found to be highest for green fodder yield, dry fodder yield per day and crude fiber percent among rice bean germplasm should be investigated further to know its linkage with any specific trait/gene.

6. Germplasm may be explored with trait specific primers for identification of QTL's using association mapping.

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APPENDIX

Appendix: 1 Weekly Metrological Data

Weeks	Temperature		Relative humidity		Rainfall	Rainy Days
	Min.	Max.	Min.	Max.	000.0	00
2 April -8 April	16.9	34.2	21	65	000.0	00
9 to 15	18.3	38.1	13	56	000.0	00
16 to 22	18.8	40.0	10	47	000.0	00
23 to 29	19.8	41.9	9	43	000.0	00
30 April to 6 May	24.6	41.7	12	29	000.0	00
7-13	23.8	39.5	17	40	000.6	00
14-20	26.5	41.1	17	38	009.8	02
21-27	24.2	40.1	22	55	000.0	00
28 to 03 June	24.8	41.5	19	45	011.4	01
4-10	24.7	38.8	46	74	057.6	03
11-17	24.6	35.8	68	86	256.4	05
18-24	22.3	29.2	17	91	171.1	05
25 June to 1 July	23.3	31.7	70	92	116.7	02
2-8	24.1	29.5	67	89	064.2	04
9-15	23.8	32.4	77	90	049.3	02
16-22	24.4	31.3	78	86	032.3	03
23-29	24.1	31.3	64	91	273.4	05
30 July to5 August	22.4	29.9	67	96	217.7	04
6-12	22.2	30.5	81	95	002.0	00
13-19	22.8	31.8	83	91	008.0	01
20-26	21.8	32.1	70	91	015.6	01
27 August to 2 Sep.	22.2	35.4	63	91	007.2	01
3-9	22.3	32.6	67	90	005.6	01
10-16	22.1	30.6	59	90	078.0	03
17-23	21.1	32.0	66	90	000.0	00
24-30	19.9	33.2	70	88	013.4	02
1 Sep to 7 October	20.6	34.1	54	86	000.0	00
8-14	18.2	32.3	56	83	000.0	00
15-21	14.1	31.0	44	88	000.0	00
22-28	11.1	32.2	32	89	000.0	00
29 Oct. to 4 Nov.	10.8	30.9	26	85	000.0	00
5-11	10.5	29.6	24	89	000.0	00
12-18	13.2	28.0	81	85	000.0	00
15-25	10.2	27.4	37	87	000.0	00
26 Nov.to 2 Dec.	7.7	27.7	35	90	000.0	00
3-9	7.4	29.1	32	91	000.0	00
10-16	9.7	27.5	31	92	000.0	00
17-23	9.1	25.8	37	90	000.0	00
24 Dec. to 31Dec.	6.9	25.9	40	90	000.0	03
1 Jun to 7 Jun	9.6	22.4	32	89	037.8	00
8-14	13.3	26.8	45	96	000.0	00
15-21	10.6	27.5	71	92	000.0	00
22-28	9.2	26.7	42	92	000.0	00
29 Jun to 4 Feb.	9.1	29.9	39	90	000.0	00
5-11	10.9	28.6	35	87	000.0	00
12-18	9.7	31.0	33	88	000.0	00
19-25	11.3	33.1	29	78	000.0	00
26 Feb. to 4 March	12.3	35.9	21	79	002.0	00
5-11 March	14.1	34.8	21	69	000.0	00

Appendix: 2 Mean performances of different genotypes of rice bean

Treatments	Days To 50% flowering	Plant height (cm)	No. of branches/ plant	No. of leaves/ plant	Leaf stem ratio	Green fodder yield/ day	Dry fodder yield/ plant	Dry fodder yield/ day	Crude protein (%)	Crude fiber (%)	Green fodder yield /plant
T_{1}	108.00	154.70	3.50	28.50	1.02	1.64	27.67	0.28	14.42	28.46	162.70
T_{2}	102.00	120.65	3.00	35.00	1.11	1.62	27.23	0.25	14.38	28.57	161.65
T_{3}	149.00	118.17	2.50	30.50	0.92	0.57	17.78	0.12	14.59	33.21	83.10
T_{4}	149.00	110.70	3.00	29.00	0.85	0.57	17.85	0.12	14.53	33.28	84.25
T_{5}	111.00	130.35	2.50	38.50	0.75	0.94	22.18	0.19	14.00	30.41	108.60
T_{6}	100.00	134.50	4.50	20.50	1.07	0.95	22.13	0.18	14.02	30.41	107.45
T_{7}	98.00	110.75	2.50	34.00	10.08	1.07	17.96	0.18	13.32	34.27	99.45
T_{8}	100.00	97.70	3.50	25.00	0.77	1.03	18.00	0.18	13.38	34.24	100.70
T_{9}	114.00	151.80	2.50	18.00	1.50	1.22	26.26	0.25	14.92	33.27	133.20
T_{10}	114.00	137.90	3.00	16.00	0.68	1.20	26.32	0.25	14.91	33.22	130.35
T_{11}	102.00	110.00	4.50	17.50	0.92	1.03	22.76	0.22	13.64	32.91	105.40
T_{12}	102.00	105.55	2.50	31.50	0.71	1.02	22.74	0.23	13.63	32.89	104.70
T_{13}	115.00	148.15	4.50	24.00	0.71	0.94	19.49	0.17	14.22	38.72	108.55
T_{14}	146.00	124.40	5.50	29.50	0.64	1.48	23.42	0.15	14.43	34.03	117.50
T_{15}	146.50	121.35	6.00	15.50	0.61	1.49	22.91	0.15	14.40	34.10	117.75
T_{16}	100.00	117.75	2.50	38.50	1.21	1.04	26.17	0.17	15.25	34.00	156.40
T_{17}	102.00	130.80	3.50	16.00	1.02	1.11	28.06	0.20	16.63	36.00	149.20
T_{18}	100.00	131.40	3.50	15.00	0.94	1.13	27.68	0.19	16.66	36.04	147.35
T_{19}	101.00	119.92	3.00	34.50	0.89	1.65	29.05	0.29	14.35	29.42	162.30
T_{20}	102.00	126.47	3.50	14.00	0.84	1.67	29.02	0.29	14.33	29.42	160.80
T_{21}	100.00	120.15	3.50	32.50	0.81	1.64	32.32	0.34	13.77	34.22	163.15
T_{22}	98.00	110.55	3.50	35.00	0.94	1.65	32.00	0.32	13.74	34.42	163.95
T_{23}	100.00	127.60	2.00	21.00	0.83	1.57	29.41	0.29	18.21	28.92	157.35
T_{24}	148.00	120.19	2.50	12.00	0.70	1.12	28.41	0.19	13.75	38.62	165.45
T_{25}	147.00	138.25	2.50	17.00	0.62	1.14	28.45	0.19	13.78	38.57	162.70
T_{26}	100.00	100.55	4.00	28.00	0.79	1.72	25.85	0.27	17.02	32.96	110.85
T_{27}	149.00	142.50	4.50	17.50	1.07	0.75	22.68	0.15	13.32	33.79	101.25
T_{28}	115.00	128.65	5.50	18.50	1.36	0.91	18.75	0.16	13.37	37.76	138.15
T_{29}	152.00	121.85	3.00	17.50	1.17	0.94	29.84	0.19	15.16	36.70	136.15
T_{30}	151.00	124.65	4.50	31.00	0.79	0.95	29.89	0.19	15.18	36.72	137.60
T_{31}	150.00	120.20	5.00	15.50	0.70	0.92	26.78	0.19	15.16	36.71	101.55
T_{32}	95.00	122.20	2.00	22.50	0.96	1.10	29.92	0.17	14.51	34.01	108.70
T_{33}	93.00	79.20	2.50	30.00	1.17	0.99	28.63	0.16	14.96	33.79	98.30
T_{34}	90.00	92.00	6.50	35.50	1.13	1.11	27.75	0.15	14.67	32.29	130.15
T_{35}	100.50	99.40	7.00	17.50	1.09	0.89	32.32	0.16	15.52	33.53	137.80
T_{36}	102.00	79.00	6.50	24.50	1.06	0.88	30.84	0.18	15.51	36.65	111.95
T_{37}	104.00	57.20	5.00	24.50	1.12	0.90	26.12	0.18	15.49	29.43	83.90
T_{38}	100.00	132.25	4.50	26.50	1.08	0.92	26.27	0.18	13.36	34.42	83.95
T_{39}	111.00	79.40	4.50	18.50	0.93	1.02	18.96	0.27	16.63	39.91	109.05
T_{40}	100.00	50.95	2.50	131.50	0.98	0.99	19.00	0.23	16.06	38.62	101.65
T_{41}	96.00	93.60	4.00	21.50	0.88	0.92	25.53	0.21	16.64	30.36	98.95
T_{42}	94.00	95.70	4.00	18.00	1.02	0.76	26.01	0.22	15.86	30.63	97.85
T_{43}	93.00	59.15	4.50	19.50	1.19	0.63	29.02	0.21	15.77	36.02	130.10
T_{44}	90.00	65.20	2.50	10.50	1.01	0.69	29.73	0.19	15.06	36.62	128.35
T_{45}	100.00	99.35	2.50	32.50	0.74	1.51	22.79	0.18	14.99	37.76	164.85
T_{46}	107.00	66.60	3.00	27.00	0.74	1.53	21.23	0.17	14.82	37.71	160.35
T_{47}	111.00	82.15	3.50	11.00	0.64	1.50	20.94	0.17	14.75	34.42	162.75
T_{48}	61.00	107.70	2.50	23.50	0.69	1.75	22.72	0.17	15.57	34.58	157.75
T_{49}	64.00	87.80	2.00	22.50	1.02	1.79	22.67	0.13	15.44	34.02	154.65
T_{50}	83.00	103.50	3.00	10.50	0.87	1.74	31.36	0.12	13.89	32.95	108.65
T_{51}	60.00	109.75	3.00	14.00	0.82	1.76	31.38	0.13	17.00	32.92	164.00
T_{52}	82.00	118.25	3.00	18.50	0.95	1.63	20.00	0.14	17.11	32.79	169.65
T_{53}	80.00	112.05	4.00	18.00	0.97	1.68	20.10	0.14	17.53	32.75	168.90
T_{54}	82.00	123.05	2.50	16.50	0.97	1.63	18.23	0.14	15.54	32.66	136.50
T_{55}	100.00	118.05	2.50	22.50	0.80	1.53	18.66	0.20	15.51	33.21	149.75
T_{56}	97.00	121.60	2.50	14.50	1.04	0.97	27.34	0.23	15.14	36.68	162.45
T_{57}	93.00	115.75	2.00	19.50	1.11	1.01	27.43	0.20	15.15	33.23	163.25
T_{58}	93.00	89.30	2.50	12.50	1.22	0.91	23.28	0.18	13.65	34.17	157.85
T_{59}	88.00	77.50	2.00	41.50	1.02	0.93	23.18	0.18	18.00	34.04	90.05
T_{60}	86.00	57.65	3.00	10.50	1.01	1.00	24.07	0.24	17.96	28.93	93.90
T_{61}	92.00	110.85	3.50	16.00	1.31	0.88	22.13	0.23	13.36	27.99	92.55
T_{62}	92.00	123.85	3.50	13.00	1.00	0.77	22.38	0.22	15.50	38.35	95.95
T_{63}	96.00	138.00	6.50	26.50	0.85	1.58	25.95	0.21	16.65	37.38	110.65

Treatments	Days To 50% flowering	Plant height (cm)	No. of branches/ plant	No. of leaves/ plant	Leaf stem ratio	Green fodder yield/ day	Dry fodder yield/ plant	Dry fodder yield/ day	Crude protein (%)	Crude fiber (%)	Green fodder yield /plant
T_{64}	98.00	110.60	5.50	24.50	0.88	1.54	25.91	0.19	16.69	39.47	120.75
T_{65}	102.00	138.10	2.50	17.50	0.92	1.41	28.83	0.19	16.73	33.24	125.40
T_{66}	110.00	101.85	3.00	15.00	1.03	1.11	30.27	0.16	15.16	31.33	99.10
T_{67}	107.00	93.90	2.50	34.50	1.03	1.00	32.29	0.17	15.48	32.30	100.65
T_{68}	107.00	100.00	6.50	29.50	0.86	1.61	22.76	0.16	13.99	36.64	98.75
T_{69}	97.00	148.40	7.50	27.50	0.78	1.65	26.73	0.28	14.79	36.56	158.50
T_{70}	81.00	68.50	7.50	27.50	1.07	1.58	19.87	0.27	14.68	37.23	160.70
T_{71}	84.00	55.00	7.00	17.50	0.97	1.52	23.57	0.18	14.62	37.86	162.75
T_{72}	79.00	123.50	5.00	22.00	1.23	1.33	18.81	0.20	14.01	33.34	157.75
T_{73}	77.00	123.00	5.50	18.00	1.02	1.34	19.96	0.16	14.07	30.39	148.25
T_{74}	76.00	113.00	5.00	28.00	1.51	1.29	20.24	0.14	14.07	32.65	143.25
T_{75}	82.00	116.00	4.50	12.50	1.33	1.55	30.44	0.15	14.78	34.27	141.20
T_{76}	97.00	87.50	2.00	17.00	0.80	1.42	17.72	0.15	14.42	34.49	162.15
T_{77}	98.00	87.00	2.50	35.00	1.06	1.73	29.05	0.29	15.51	36.27	129.35
T_{78}	98.00	91.50	3.50	28.00	1.11	1.72	29.11	0.29	15.53	34.21	108.25
T_{79}	101.00	92.50	4.00	23.50	0.92	1.57	28.42	0.28	15.05	34.16	101.75
T_{80}	100.00	75.50	3.50	19.50	0.81	1.55	28.36	0.23	15.53	35.10	83.85
T_{81}	98.00	87.50	3.50	33.50	0.83	0.87	28.31	0.22	14.40	33.16	89.25
T_{82}	110.00	100.50	3.50	31.50	0.76	0.89	22.20	0.21	14.42	34.01	88.30
T_{83}	108.00	97.50	3.00	34.50	1.01	0.78	22.80	0.19	14.62	32.18	97.55
T_{84}	105.00	86.00	3.50	14.50	1.03	0.96	23.04	0.19	14.29	30.32	91.95
T_{85}	149.00	137.55	2.50	10.00	1.11	0.96	19.97	0.18	15.04	32.36	100.05
T_{86}	140.00	137.45	3.00	26.50	1.09	0.93	20.24	0.24	15.05	32.27	101.70
T_{87}	154.00	134.95	2.50	24.00	0.91	0.86	26.65	0.30	15.24	31.02	161.75
T_{88}	150.00	148.35	3.50	11.50	0.84	0.93	26.77	0.31	15.13	37.00	162.70
T_{89}	138.00	96.10	3.00	26.00	0.73	0.92	20.22	0.32	14.44	34.18	170.25
T_{90}	125.00	106.45	3.50	32.50	0.73	0.98	30.32	0.29	14.29	33.21	128.75
T_{91}	151.00	114.40	3.00	29.50	0.87	1.42	17.46	0.28	14.35	34.95	97.45
T_{92}	147.00	106.05	2.50	25.50	1.21	1.39	18.81	0.27	14.28	38.35	89.35
T_{93}	100.00	99.00	2.50	31.50	1.07	1.54	19.96	0.19	15.52	34.18	108.40
T_{94}	109.50	102.40	3.00	31.50	0.89	1.51	20.05	0.13	15.95	33.28	110.50

VITA

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