Genetic Diversity and Gene-action in *Vigna mungo* Based on Morphological and Biochemical Markers

By

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In the name of Allah, the Compassionate, the merciful

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DECLARATION

This is to certify that this dissertation submitted by Abdul Ghafoor, is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the dissertation requirements for the degree of Ph. D. in Biological Sciences.

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Dedicated

to the researchers for their scientific achievements who work for prosperity and poverty alleviation

ABSTRACT

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Blackgram (*Vigna mungo* L.) germplasm consisting 484 accessions was evaluated for qualitative and quantitative characters alongwith investigations regarding harvest index. Out of this material, a representative sample consisting of 111 accessions was used for cluster and principal component analyses based on morphological and biochemical techniques (SDS-PAGE for seed proteins). Biochemical markers were also used for detection of QTLs in blackgram germplasm. Forty two accessions, selected on the basis of geographic origin were analyzed for cluster and PCA to investigate association between morphological/biochemical and geographic parameters. Forty pure-lines of *Vigna* spp. were analyzed for morphological and biochemical (seed protein & RAPD) characters to study cluster analysis and association between morphological and biochemical genetic diversity. Finally inheritance of quantitative and qualitative traits were investigated in F_1 and F_2 populations involving diverse parents.

In germplasm evaluation, high variation was observed for days to flowering, maturity, branches, pods, biological yield, grain yield and harvest index. For pod length, seeds per pod and seed weight, low variance was observed and hence improvement for these traits seemed to be difficult in the local germplasm used in present study. MYMV was associated with all the characters under study except with seeds/pod, seed weight and harvest index where it was insignificant. The decrease in branches was 12.20%, in pods 15.40%, in biomass 14.07% and grain yield 13.93%. From the germplasm analyzed, accessions with best performance for individual characters were identified which are suggested to be exploited for their genetic potential in future breeding programme. Highest selection scores were observed for harvest index ranging from 30.1 to 35.0% followed by 25.1 to 30.0%, and hence 25.1-35.0% harvest index would be one of the criteria for future selection. Keeping in view the results regarding HI and other statistics, 50 high yielding accessions/genotypes have been identified for further use.

In a representative sample consisting of 111 genotypes, genetic variance was observed for quantitative characters and seed protein. Out of 29 protein subunits, 20 were polymorphic and 9 were monomorphic. On the basis of banding pattern, gel was divided into four regions and first three regions were used for recording data. SDS-PAGE provided a powerful tool for germplasm discrimination based on genetic differences in

seed storage protein comparison in blackgram. SDS-PAGE was also observed important for preliminary detection of QTLs. Out of 240 combinations for detection of QTLs, 24 were observed significant in detection of QTLs and hence could be used for screening purposes. PCA and cluster analyses based on quantitative traits proved to be best method for grouping accessions for specific traits to select desirable parents for breeding programme. Data analysed for geographic distribution based on provinces, altitude and crop-ecological zones revealed that among all three geographic parameters, 16 accessions out of 42, which were about 40% of the total, grouped together on the basis of provincial distribution only. The separation on the basis of PC_1 and PC_2 for provincial distribution revealed 5 groups.

Forty selected pure-lines of *Vigna* spp. were analysed for RAPD, SDS-PAGE and quantitative traits which revealed that SDS-PAGE proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo,* whereas within *Vigna mungo* a low level of genetic diversity was observed and no clear differentiation was exhibited either for agronomic characteristics or origin as various clusters consisted of mixed genotypes from different origins. Out of 53 primers, 46 revealed amplification and out of these, 36 exhibited polymorphism and hence could be used for *Vigna* DNA fingerprinting. SDS-PAGE and RAPD exhibited high relationship which indicated that both these biochemical markers could be used for studying inter-specific genetic diversity among *Vigna* spp. Grouping of 40 genotypes on the basis of three parameters revealed that *Vigna radiata* was quite different in biochemical analyses (RAPD & SDS-PAGE), whereas one *V. radiata* (45727) was related to *V. mungo* based on quantitative traits. All 40 genotypes shared grouping for all three parameters (morphological, SDS-PAGE & RAPD) and it was observed that 8 genotypes out of 40 (20%) shared all three groups. Cluster analysis showed that many genotypes from same origins were grouped separately or vice versa.

Eleven parents (9010, 9024, 9025, 9102, 9104, 9105, 9106, MM 5-60, MM 33-40, Korea & Mash 1) were used to study inheritance of qualitative characters like, pubescence, seed coat colour, presence of spot on the seed and pod colour. All four qualitative characters revealed monogenic nature of inheritance segregating in Mendelian 3:1 ratio. The hairiness pattern was observed dominant over non-hairiness; brown seed

coat colour dominant over green seed coat colour. Presence of spots on seed coat was dominant to absence of spots and black pods were dominant over brown pods in blackgram. Seven hybrids (MM 33-40/9105, Mash 1/MM 5-60, 9106/MM 33-40, 9104/MM 33-40, MM 33-40/9104, Korea/MM 33-40 and Mash 1/MM 33-40) revealed strong linkage between spots on seed coat and pod colour in the present research material and suggested to be used for preliminary mapping in blackgram.

Results regarding inheritance of quantitative traits indicated the presence of both additive and non-additive genetic variation, but the magnitude varied. The major portion of GCA variance was observed for seed weight only, whereas for all other characters, SCA or Reciprocal variance contributed more towards genetic variance. Genotype "9020" produced the highest GCA effects for pods, pod length, seeds/pod, seed weight, biomass and grain yield; Mash 1 was best general combiner for plant height; Mash 3 best for branches and 9026 proved to be the best general combiner for pods/branch and HI. The genotype "9026" gave negative GCA effects for most of the characters (plant height, branches, pods, seed weight, biological yield and grain yield) but it exhibited best general combiner for pods/branch and HI. In the present investigation it can be concluded that hybrids involving genotypes 9020, Mash 1 and 9026 could be examined carefully for selection in the proceeding generations to select superior transgressive segregants.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS AND ABBREVIATIONS

AJK	Azad Jammu & Kashmir
AVRDC	Asian Vegetable Research & Development Centre, Taiwan
BB, Bb, bb	allelic notion for pod colour
CC, Cc, cc	allelic notion for seed coat colour
BR/PL	number of branches per plant
BY	biological yield per plant (g)
DF	days to flowering after planting
DM	days to maturity
electrophoresis	a method of separating and analyzing charged molecules of proteins and nucleic acids by their differential rates of movement under the influence of an electric field
enzyme	one group of proteins produced by living cells which acts as a catalyst in specific biochemical reaction
GCA	general combining ability
gel	an inert porous matrix used for electrophretic separation of proteins and DNA; the concentration of compounds comprising the gel can be altered within large limits
gene	the basic unit of inheritance, chemically a DNA sequence which codes for the production of a specific protein molecule
genome	a basic unit used to describe the chromosome makeup of an individual or the complete set of genes of an organism
genotype	the sum-total of the genes affecting the expression of a character; also employ for a variety or cultivar
GY	grain yield per plant (g)
HI	harvest index expressed in percent
HH, Hh, hh	allelic notion for plant hairiness
hybrid	first generation progeny (F_1) from a cross produced through controlling pollination between two parents
locus	any site on a chromosome which has defined genetically; a locus may be a gene, part of gene, or set of genes
masl	meter above sea level
monogenic	controlled qualitatively by one or few genes

multigenic	controlled by many genes
MYMV	Mungbean Yellow Mosaic Virus
NARC	National Agricultural Research Centre, Islamabad, Pakistan.
NIAB	Nuclear Institute for Agriculture & Biology, Faisalabad, Pakistan
NA	northern areas of Pakistan
NWFP	north western frontier province
PAGE	polyacrylamide gel electrophoresis; a type of electrophoresis employing gels made from the synthetic material, acrylamide
PCA	Principle Component Analysis
PCR	polymerase chain reaction
PGRI	Plant Genetic Resources Institute
pH	a term used to describe the acidity or alkalinity of a system
PH	plant height (cm)
phenotype	a sum-total of the expression of a character
P/BR	pods per branch
P/PL	pods per plant
PL	pod length (cm)
polymorphism	existing in many different forms
progeny	the generation obtained after crossing two or more parents
protein	a high molecular weight compound composed of a range of amino acids; they are the products of genes; seeds generally contain four kinds of proteins of different solubility properties; storage proteins (prolamins and globulins), enzyme proteins (albumins), and structural proteins (glutenins)
QTL	quantitative traits loci
RAPD	randomly amplified polymorphic DNA
RFLP	restricted fragment length polymorphism
SCA	specific combining ability
SD	standard deviation
SDS	sodium dodecyl sulphate, a powerful detergent
SE	standard error
SOV	source of variation
SS, Ss, ss	allelic notion for seed spots
SW	weight of 100 seeds in grams
t/ha	tonnes per hectare

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ABDUL GHAFOOR

INTRODUCTION

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The genus *Vigna* is a tropical plant and comprises about 150 species, most of which are found in Africa and Asia (Faris, 1965; Verdcourt, 1970). Seven species of this genus are cultivated as pulse crops mostly in Asia, Africa and some parts of Latin America (Anishetty & Moss, 1987). It is generally considered that two of these seven cultivated species are of African origin (subgenus *Vigna*) and five are Asiatic origin (subgenus *Ceratotropis*). The Asiatic group consists of mungbean/greengram (*Vigna radiata* L. Wilczek), blackgram/mash/urd (*Vigna mungo* L. Hepper), mothbean (*Vigna aconitifolia* Jack. Marechal), azuki bean (*Vigna angularis* Willd, Ohwi & Ohashi) and ricebean (*Vigna umbellata* Thunb, Ohwi & Ohashi). Mungbean and blackgram have been major pulses in Asia since from ancient times (Paroda & Thomas, 1987). At present, mungbean cultivation spreads worldwide because it is easily digested than blackgram (Smartt, 1990). Phylogenetically *V. radiata* and *V. mungo* are considered as closely related and thought to be the descendent of common ancestor. *Vigna sublobata* was thought to be the ancestral progenitor of both *V. mungo* and *V. radiata* (Smartt, 1980), however, it was shown subsequently from the evidence of morphological diversity of *V. sublobata*, that two distinct types exists. *Vigna radiata* var *sublobata* and *Vigna mungo* var. *silvestris* are wild forms of *V. radiata* and *V. mungo*, respectively (Arora *et al.* 1973; Miyazaki, 1982; Chandel, 1984). Subcontinent had been considered to be the region with greatest genetic diversity in mungbean and blackgram (Vavilov, 1926, 1951; Singh *et al.* 1974; Zeven & de Wet, 1982).

The subgenus *Ceratotropis* can be subdivided into two groups based on the seedling characters, i.e., 1) "mungbean group" showing an epigeal germination and 2) "azuki bean group" showing hypogeal germination. Marechal *et al.* (1978) published a monograph on *Phaseolus-Vigna* complex, and the taxonomic system they proposed was widely accepted (Tomooka *et al.* 1995). Recently, biochemical markers have been used to reveal the phylogenetic relationships of *Vigna* species (Yasui *et al.* 1985 for low molecular weight carbohydrates, Jaaska & Jaaska 1990 for isozymes, Rao *et al.* 1992 for seed proteins, Fatokun *et al.* 1993 for RFLP, Vaillancourt & Weeden, 1993 for chloroplast DNA, Zink *et al.* 1994 for RFLP of the phytohemagglutinin genes and Kaga, 1996 for RAPD).

Plant breeding, the induced evolution changed the phyto history in the recent past and the improvement in crop plants are mainly based on the presence of genetic variation either natural or induced through gene recombinant, mutation etc. Cereals are more researched as compared to legumes, but even among legumes, blackgram may be considered the most neglected although it is one of the important legumes of Asia. The scope of plant genetic improvement through the manipulation of available genetic variability is still equally believed by all the plant scientists. Sound breeding programme in any field crop depends mainly upon the availability of genetic variability either existing and/or created, i.e., mutation, gene recombination etc. (Anon., 1996, 1997).

Variances of relatively highly heritable, quantitative genetic markers provide one estimate of genetic diversity. Sokal, (1965) advocated calculating generalized variance-covariance matrix derived from morphological characters as indices of intra-population diversity. Various numerical taxonomic techniques (Nei, 1987; Weir, 1990; Brown & Weir, 1983) have been successfully used to classify and measure the pattern of phenotypic diversity in the relationship of germplasm collections in a variety of crops by many scientists as in blackgram (Shanmugam & Shreerangaswamy, 1982; Dasgupta & Das, 1984 & 1985), yellow yam (Akoroda, 1983), mungbean (Singh, 1988; Ramana & Singh, 1987), Indian mustard (Gupta *et al.* 1991), cole crops (Dias *et al.* 1993), corn (Revilla & Tracy, 1995), pea (Amurrio *et al.* 1991, 1993, 1995); soybean (Perry & McIntosh, 1991); ryegrass (Humphreys, 1991); foxtail millet (Li *et al.* 1995); alfalfa (Smith *et al.* 1991, 1995; Warburton & Smith, 1993), cotton (Brown, 1991; Goodman, 1968), and lentil (Ahmad, *et al.* 1997).

One of the approach for building gene-pool is to collect material from diverse geographical origins with a concentration of accessions from proposed centres of diversity in individual samples. Representative samples from the complete geographical range of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel, 1984; Frankel & Soule, 1981; Frankel, *et al.* 1995; Brown, 1978; Beuselinck & Steiner, 1992), because, genetically heterogeneous populations produce more and stable yield than genetically homogeneous lines (Simmonds, 1979).

More than a decade has passed since plant biotechnology started attracting special attention which is the witness of many successes and failure that ultimately made man

enable to see the true capability of plant biotechnology. Because of the expressions "engineering of genes" and "gene controlling everything", people tend to have an illusion that genes are alive. Those having such illusion outnumber those who do not by far among scientists, however, genes are not living things; they are merely macromolecular compounds. The understanding of the gene knowledge made genetic transformation possible which is the most promising technique because of its ability to introduce only the targeted genes. Successful examples can be observed in a variety of crops including legumes (Nakajima, 1994).

Among biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm. Seed storage proteins have been used as genetic markers in four major areas: 1) analyses of genetic diversity within and between accessions, 2) plant domestication in relation to genetic resources conservation and breeding, 3) genome relationship and 4) as a tool in crop improvement. SDS-PAGE is considered to be a practical reliable method because seed storage proteins are largely independent of environmental fluctuation (Gepts, 1989; Murphy *et al.* 1990).

The knowledge of genetic diversity is useful tool in gene-bank management and planning experiments because it facilitates efficient sampling and utilization of germplasm either by identifying and/or eliminating duplicates in the gene stock ultimately resulting in the development of core collection philosophy. Advancement in molecular biology has introduced DNA markers which is an attractive method for genotype identification (Ewens, 1981; Caetano-Anolles *et al.* 1991; Paterson *et al.* 1991; Cho *et al.* 1994; Samec, 1993; Samec & Nasinec, 1995, 1996; Margale *et al.* 1995). Several kinds of DNA markers have been recognized i.e., Restriction Fragment Length Polymorphism (Boststein *et al.* 1990), Polymerase Chain Reaction (PCR) based markers (Saiki *et al.* 1988), AFLP etc. Random Amplified Polymorphic DNA (RAPD) has been reported useful tool for evaluation of intraspecific variation (De Wet *et al.* 1983; Dhagat & Singh 1983; dos Santos *et al.* 1994; Thormann *et al.* 1994; Ribu & Hilu, 1996). RAPD markers are generated by PCR with a single short oligonuleotide of arbitrary sequence and provide genetic information at DNA level and a huge number of RAPD markers can be obtained in

a limited time (Engels, 1981; Demeke *et al.* 1992; Clark & Lanigan, 1993; Campos *et al.* 1994; He *et al.* 1995; Kaga *et al.* 1996). One of the practical application of the knowledge of genetic diversity is to design population for mapping experiments because it facilitates efficient sampling and utilization of germplasm resources. The researchers can use genetic similarity information to make decision regarding the choice for selecting superior genotypes for improvement or to be utilized as parents for the development of future cultivars through hybridization.

Polygenic morphological traits also often serve as genetic markers for various plant germplasm management and taxonomic applications (Stuessy, 1990) but their lower heritabilities and substantial genotype-environment interaction can dramatically increase the complexity and expense of assaying them, although computerised imaging systems may assist with this effort. Further, evaluation of available genetic stocks to assess the genetic variation for economically important characteristics is a pre-requisite for combining desirable genes in a single genotype. The magnitude and type of genetic variation in a population helps in selection of parents, which after hybridization are likely to produce the best recombinants for desirable traits such as high yield, resistant to diseases etc. Combining ability analysis is one of the techniques frequently employed to study the nature of genetic variation for particular plant traits and to select the potential parents which would produce the best recombinations (Murty, 1975). The technique has been effectively used for such purpose in pea (Singh *et al.* 1987b), mungbean (Malhotra, *et al.* 1979; Wilson *et al.* 1985) and blackgram (Singh & Singh, 1971; Singh, *et al.* 1987a; Ghafoor *et al.* 1993a). Since information in this respect is meagre in blackgram, therefore, this technique was also included in the present study to analyse selected blackgram genotypes for combining ability effects and gene-action.

Pulses, in general, give lower yield than cereals and the reason for this is not difficult to understand. Unlike cereals, pulses have been grown for centuries under marginal conditions of moisture and soil fertility. Blackgram or mash (*Vigna mungo* L. Hepper) is an important summer pulse crop of many South Asian countries including Pakistan, India, Bangladesh, Thailand and Korea. In Pakistan it is cultivated under a wide range of agro-ecological zones mainly of rainfed nature. At present it is cultivated over an area of 57,400 ha with

28,400 tonnes production (Anon., 1997). An average yield of 496 kg/ha is very low as compared with its potential, and yield obtained in many other countries. Among pulses, blackgram is the least researched crop, as no international centre admits this as a mandate crop. Keeping in view the importance of the crop, a wide range of local germplasm of blackgram collected from various parts of Pakistan in the last decade was evaluated under field condition for various qualitative and quantitative traits for further utilization by the breeders. The objective of this study was to determine the genetic diversity of blackgram germplasm on the basis of morphological characters, SDS-PAGE and RAPD analyses and to find the influence of molecular markers (protein and DNA) for the expression of QTL for practical exploitation of biochemical techniques in crop improvement.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 *Morphological Characters*

Estimates of genetic diversity and relationship between germplasm collections are very important for facilitating efficient germplasm collection, evaluation and utilization. Many tools are now available for identifying desirable variation in the germplasm including total seed protein, isozymes and various types of DNA markers. However, morphological characterization is the first step in the description and classification of crop germplasm (Smith & Smith, 1989; Singh & Tripathi, 1985). Broschat, (1979) considered PCA a useful data reduction technique which worked by removing intercorrelations among variables (components). By using PCA, not only the number of comparisons between treatment means reduced, but the meaningfulness of these comparisons is enhanced. Interactions among two or more variables may be pointed out by such analysis. In taxonomy, it can be used to express multidimensional inter-OTU (Operational Taxonomic Unit) distances in 3 or fewer dimensions which can be readily conceptualised. Additional applications of this technique will certainly be found as its use becomes more widespread in fields of biological sciences, where it has been used extensively for more than two decades.

Dasgupta & Das, (1984) conducted multivariate analysis in blackgram and considered it a method of choosing parents for hybridization using D^2 analysis. Data on 12 characters of forty strains of blackgram collected from India and Nepal were used. The genotypes were grouped into seventeen different clusters and no clear association was observed between clusters and geographical origin. Similarly genetic divergence was conducted in 38 genotypes of blackgram by Dasgupta & Das, (1985) using D^2 statistics. No relationship was observed between geographic distribution and genetic divergence of the varieties. Flowering time and seed size exhibited maximum contribution to the total divergence. Environmental conditions exerted considerable impact on the number and composition of clusters. Suggestion has been made for selecting suitable stable diverse parents so as to initiate a crossing programme for increased grain yield in blackgram.

Seventy two landraces of pea (*Pisum sativum*) evaluated for 19 morphological characters exhibited broad genetic diversity as reported by Amurrio *et al.* (1993). Significant positive correlation of flowering was also reported with shoot height, and

maturity and seven landraces were selected for special attention for having promising breeding value. Amurrio *et al.* (1995) reported a wide genetic diversity in 105 pea landraces at the intraspecific level based on 19 quantitative characters. Taxonomically useful results were provided and 6 groups were established but the grouping pattern of these landraces did not reflect any association with geographic origin. Smith *et al.* (1991) studied principal components and average cluster analysis in alfalfa and established six geographically distinct groups. Significant regional variation was observed within the germplasm evaluated but ecotypes from neighbouring countries were generally closely associated. All elite germplasm fell in one group and this revealed that only a small portion of genetic diversity has been used in formal breeding. Multivariate analysis have been used to successfully classify and order variation observed in both qualitative and quantitative traits in a collection of crop germplasm (Singh, 1988; Peeters & Martinelli, 1989; Caradus *et al.* 1989). Rumbaugh *et al.* (1988) used discriminant analysis of morphological and agronomical characters to place 146 accessions of alfalfa from Morocco into five geographical groups that were defined initially based on the area of collection.

Virmani *et al.* (1983) classified mungbean germplasm into various groups for different traits. Bakhsh *et al.* (1992) categorised lentil germplasm on the basis of quantitative traits and suggested the utilization of short statured lentil germplasm for crop improvement. The high yielding accessions selected from the local germplasm might prove their superiority in advance testing under various agro-ecological conditions (Ghafoor *et al.* 1989). They classified blackgram germplasm and selected eleven pure-lines for further exploitation. In an other study on mungbean, Ghafoor *et al.* (1992) selected twenty eight genotypes on the basis of high yield potential and resistance to diseases. Singh & Srivastava, (1985) categorised pea germplasm into various groups. The genetic diversity between *V. radiata* and *V. mungo* was reported by Chen *et al.* (1983) and Egawa, (1988) who observed irregular meiotic configuration with a high frequency of univalent formation in *V. radiata* and *V. mungo* hybrids with low pollen fertility (Miyazaki, 1982). Germplasm evaluation must be considered the first step in plant breeding programme and it is commonly based on a simultaneous examination of a large number of populations for several characters of both agronomic and physiological interest (Pezzotti

et al. (1994). Results reported by Falcinelli *et al.* (1988) and Veronesi & Falcinelli, (1988a, b) showed multivariate analysis to be a valid system to deal with germplasm collection. Nevertheless, the qualitative traits must be often used for separating varieties when a limited range of quantitative traits is found in certain groups (Sneedon, 1970). Smith *et al.* (1995) conducted average linkage cluster and PCA and reported the utility of these results in preservation and utilization of germplasm.

The landraces of tetraploid wheat from two provinces (Shewa and Tigray) of Ethiopia were found to be distinctly different (Pecetti *et al.* 1996). This divergence was attributed to the differences in environmental conditions between them. Wide differentiation among landraces within each province was also present. The proportion of total variance due to differences among agrotypes within landraces was by far the greatest found in this study. Various reasons were advocated for the occurrence of a great diversity in wheat, such as isolation from other wheat germplasm, primitive farming systems, heterogeneous environments, field mixture and natural cross fertilization due to field mixtures. Knowledge on the pattern of variation for important morpho-agronomic traits is needed for a proper improvement and better exploitation of gene pool (Jain *et al.* 1975).

Bekele, (1985) thoroughly discussed the importance of a hierarchical approach to quantitatively define the variance in the centre of genetic diversity over a range of micro environments. Subdividing the variance into its components may assist in genetic resources conservation and utilization by determining the relative contribution of different levels of variability to the total diversity available in any one area. This would enable planning of future germplasm sampling, establishment of *in-situ* gene conservation, or use of appropriate gene pools in crop improvement for specific plant attributes (Bekele, 1984; Pecetti *et al.*, 1992). Ahmad *et al.* (1997) reported that first two canonical components contributed 85% of the variation between lentil genotypes. It was observed that cluster analysis on the basis of quantitative characters were phenotypically more distinct and exhibited more breeding value. Though cluster analysis grouped together accessions with greater morphological similarity, the cluster did not necessarily include all the accessions/genotypes from the same or nearby sites. Ahmad *et al.* (1997) reported

phylogenetic relationship of 15 genotypes of the genus *Lens* and 7 of their interspecific hybrids were determined by morphological (quantitative and qualitative) characters. The first multivariate analysis was conducted on quantitative characters and second analysis was conducted on qualitative traits. Perry & McIntosh, (1991) characterised soybean germplasm from 78 countries for seventeen traits and determined variation within and among all regions for most of the characters. Canonical discriminant analysis and clustering of the canonical means delineated four regional clusters: i) India and Africa; ii) China, Europe, New World and Southeast Asia; iii) Korea and Japan; and iv) Southwest Central Asia. The clusters containing the Korean and Chinese accessions were the most diverse. Based on the diversity and number of accessions, Africa, India and Southeast Asia seemed underrepresented in the collection. One approach for building gene pool is to collect/acquire plant germplasm from diverse geographical origins with a concentration of accessions from proposed centre of diversity. This should capture inherent and unexploited diversity in the individual samples. Representative samples from the complete geographical range of the crop species can help to ensure that co-adapted gene complexes (or correlated adaptations) are conserved (Frankel, 1984). According to Brown, (1978), maximum genetic conservation would be achieved by sampling populations from as many distinct environments as possible. The breeding programme mainly depends upon magnitude of genetic variability as suggested by Shanmugam & Shreerangaswamy, (1982) in blackgram.

Singh *et al.* (1991) examined the organisation of diversity for morphological and agronomic characters in 306 landraces of cultivated common bean (*Phaseolus vulgaris* L.) by analysing data for multivariate statistical analysis and observed genetic variance within and between groups. Kumar & Arora, (1992) presented observation on 40 genotypes of chickpea collected from various geographical regions for 18 characters including seed yield. Multivariate analysis revealed 10 clusters. No definite relationship was observed between genetic diversity and geographical distribution. Based on inter-cluster distance, maximum hybrid vigour was observed among most diverse genotypes. Tawar *et al.* (1988) conducted genetic divergence using D^2 analysis in 34 diverse genotypes of mungbean and observed five clusters. Cluster I and cluster II had eight genotypes each

while cluster III had six genotypes. Similarly cluster IV and V had five and seven genotypes, respectively. Variability observed in the parents was related to genetic diversity of the parents selected under study. First canonical root contributed 88% of the total variation. Inclusion of such genotypes from distinct clusters and their implication in mungbean breeding programme was suggested.

Malhotra & Singh, (1971) while working on genetic divergence in blackgram reported narrow range of variability for 100-seed weight and pod length, whereas Shanmugam & Shreerangaswamy, (1982) while studying 45 genotypes of blackgram reported that yield per plant contributed maximum to the genetic diversity. Mishra & Rao, (1990) reported thirteen clusters in a comparative study of D^2 and meteroglyph analysis in 117 genotypes of chickpea. Cluster I had the maximum number of genotypes. Meteroglyph analysis did not show similar type of clustering as observed in D^2 analysis, but canonical analysis showed similar type of clustering. Gupta *et al.* (1991) and Dias *et al.* (1993) reported no association between morphological characters and geographical origin whereas Revilla & Tracy, (1995) observed a low level of morphological variability amongst widely used open-pollinated sweet corn cultivars.

Clements & Cowling, (1994) investigated the pattern of morphological diversity in relation to geographical origins of 157 accessions of wild *Lupinus angustifolius* using multivariate technique. Genetic diversity was extremely large for most of the morphological traits, with significant variation detected among localities in Greece, and within and between collection sites for same trait. Thirteen groups were identified by hierarchical clusters analysis. Accessions from northern Greece grouped together as later flowering, shorter, and smaller seed size, but some accessions from southern Greek Islands were grouped with the northern mainland types. Multivariate analysis provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level (Rouamba *et al.* 1996). Laghetti *et al.* (1998) suggested collecting expedition to the areas where genetic erosion takes place in cowpea alongwith areas where existing genetic diversity has not yet gathered (Padulosi, 1993).

Rabbani *et al.* (1998a) determined the extent of diversity and relationship among 52 accessions of *Brassica* germplasm from Pakistan for 35 morphological characters

using cluster and principal component analyses. The germplasm was categorised into six groups. Landrace group was primarily associated with morphological differences among the accessions and secondarily with the breeding objectives and horticultural uses. The germplasm collected from Pakistan showed a comparatively low level of phenotypic variation which revealed that the evaluated germplasm appears to have a narrow genetic base which undergoes a high level of genetic erosion. Though cluster analysis grouped together accessions with greater morphological similarity, the clusters did not necessarily include all the accessions from the same or nearby sites. Simply inherited characters are important for plant description (Kurlovich, 1998) and are mainly affected by the consumers preference, socio-economic scenario and natural selection. Nakayama *et al.* (1998) reported that foxtail millet landraces with low amylose allele were distributed only in Southeast Asian mainly because of preference followed by selection.

2.2 Biochemical Analysis

2.2.1 Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)

Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz, 1979; Murphy *et al.* 1990; Khan, 1992; Das & Mukarjee, 1995). It can also be used as a promising tool for distinguishing cultivars of particular crop species (Cooke, 1984; Ferguson & Grabe, 1986; Gardiner & Forde, 1988; Gadgil, *et al.* 1989; Koranyi, 1989; Moller & Spoor, 1993; Ahmad, *et al.* 1995; Jha & Ohri, 1996). However, few studies indicated that cultivar identification was not possible with the SDS-PAGE method, as electrophoretic patterns of proteins were similar among the cultivars (Ladizinsky & Adler, 1975; Raymond *et al.* 1991; Ahmad & Slinkard, 1992; de Vries, 1996). In case where the seed proteins fail to detect differences to identify the particular specie, 2-D electrophoresis is suggested.

Seed protein polymorphism may serve as genetic markers for plant germplasm management because they can be quite polymorphic, generally substantially more so than are isozymes (Gepts, 1990), and the variability is generally highly heritable (Smith &

Smith, 1986). Such proteins [e.g., zeins (*Zea* L.), gluten (*Triticum* L), phaseolins (*Phaseolus* L.)], often organ or tissue specific, get assayed from seeds where they often function in storage.

Although seed proteins can be fractionated by high-performance liquid chromatography (Smith & Smith, 1986) and other techniques, Polyacrylamide Gel Electrophoresis (PAGE), generally in Sodium Dodecyl Sulphate (SDS) gels is currently the favoured technique for rapid analysis (Cooke, 1984), whereas two-dimensional electrophoresis, often incorporating isoelectric focusing, may be required for certain more demanding applications (Celis & Bravo, 1984; Beckstrom-Sternberg, 1989). Protein fractionation by SDS-PAGE is relatively rapid and inexpensive compared to isozyme and DNA analyses. In contrast, two-dimensional (2-D) electrophoresis will often reveal an astounding number of different seed proteins simultaneously, but it is relatively slow and demands considerable technical skill and experience. Furthermore, sophisticated and expensive computer analytical software may be needed for reproducible analysis of the patterns formed by the hundreds of different polypeptides so revealed (Higginbotham *et al.* 1991). In order to determine the distribution of diversity in germplasm, phenotypic and genotypic variation within and between countries and regions have been examined for several important crop species including barley (Tolbert *et al.* 1979, Ruiz *et al.* 1997); durum wheat (Jain *et al.* 1975; Bogyo *et al.* 1980); safflower (Wu & Jain, 1977); rice (Holcomb *et al.* 1977); orchardgrass (Pezzotti *et al.* 1994); lentil (Erskine & Muehlbauer, 1991); Lupin (Clements & Cowling, 1994) and soybean (Perry & McIntosh, 1991). Variation partitioned in a hierarchical fashion by area indicated that greater levels of diversity were found in the larger geographic sub-divisions but characters differed in their contribution to this diversity. Van Hintum & Elings, (1991) considered rare alleles, occurring in one or two apparently random populations to be mutants, migration or the results of other coincidental events.

As with isozyme analysis, seed protein polymorphism may be interpreted according to a locus/allele model (with co-dominant alleles) following determination of their genetic control (Gepts, 1990). Conventional biometrical approaches have been used to estimate variance due to genotypes x environment inter-actions, and to estimate the number of

genes controlling individual quantitative character. The use of molecular markers to locate genes controlling quantitative traits has further facilitated the analysis of such traits (Stuber *et al.* 1982; Kahler & Wehrhahan, 1986; Edwards *et al.* 1987; Kjaer *et al.* 1991; Mansur *et al.* 1993; Tahir & Muehlbauer, 1995). Detection of QTL into individual genetic components by use of biochemical markers has been demonstrated in tomato (Tanksley *et al.* 1982), garden pea (Kneen *et al.* 1984) and lentil (Hoffman *et al.* 1986; Tahir & Muehlbauer, 1995).

Ladizinsky (1979) and Ladizinsky & Hymowitz (1979) considered seed protein an additional approach for species identification and a useful tool for tracing back the evolution of various groups of plants. They recommended this technique for resolving some specific taxonomic problems in crop plants. The highly uniform protein profiles of cultivated polyploid plants not only permits a relatively quick identification of their diploid progenitors but is also of practical value for plant breeders. Uniformity of protein profiles suggests that these polyploids evolve from a few diploid genotypes and consequently represent only a small segment of genetic variability.

Damania *et al.* (1983) used PAGE of storage proteins (*prolamines*) to screen 64 landraces of wheat and barley from Nepal and the Yemen Arab Republic and two cultivars for comparison. Altogether 3168 single seeds were examined and the advantages gained by using the vertical slab gel method were recognised. The extent of variation present within populations of landraces could be assessed easily and rapidly using SDS-PAGE. Differences in ploidy levels of wheat were also detected and investigated. Ferguson & Grabe, (1986) identified the genetically different perennial ryegrass by SDS-PAGE of seed proteins. They also observed that the banding patterns were not affected by year and location of production, class of certified seed, or variability and vigour of seed.

Thakare *et al.* (1987) reported major differences of *vicillin*, the major seed storage protein using SDS-PAGE in *Vigna mungo* and *V. radiata*. *V. mungo* and *V. radiata* showed species specific pattern with a considerable homology. They observed 4 major peptides in all *V. mungo* accessions except one (U-196) which was a radiated mutant. Low level of intra-specific variation was also reported for *V. mungo*. Kumamura *et al.* (1988) screened 3000 mutant lines of rice using SDS-PAGE and compared with that of original

variety. Determination of extracted protein in the starchy endosperm of mutants revealed changes in the contents of prolamin and glutelin but not globulin. Ahmad & Slinkard, (1992) reported phylogenetic relationship among *Cicer* species based on SDS-PAGE data and suggested *Cicer reticulatum* as the wild progenitor of cultivated chickpea. The basic criterion of phylogenetic relationship is gene homology, which in many cases can not be measured directly because of reproductive barriers between species. The fractionation of "non-essential" seed storage protein by polyacrylamide gel electrophoresis (PAGE) is used as an additional tool for assessing species relationship (Margoliash & Fitch, 1968; Sammour, 1989). Tomooka *et al.* (1992) analysed 581 accessions of mungbean by SDS-Polyacrylamide gel electrophoresis and reported eight protein types based on the combination of four albumin bands and three globulin bands. The frequency of each protein type strain showed a clear geographical cline. Rao *et al.* (1992), while conducting biochemical analysis on *Vigna* spp., observed that seed proteins were useful to detect inter-specific variation from mixed germplasm, and recommended SDS-PAGE as useful technique for gene bank management.

Moller & Spoor, (1993) used SDS-PAGE for discrimination and identification of *Lolium* spp. and reported differences in the resulting seed protein banding patterns for identification. Das & Mukarjee, (1995) while working on seed protein for species homology and genetic relationship among nine wild, two horticultural and one semi-cultivated species of *Ipomoea* reported three major groups on the basis of cluster analysis. Przybylska & Przybylska, (1995) reported a marked differences in smooth-seeded and rough-seeded species of *Lupinus* based on SDS-PAGE analysis. The rough-seeded species formed a rather homogenous group, well distinguishable from the smooth-seeded species.

de Vries, (1996) reported patterns of achene proteins of *Lactuca sativa* cultivars, mutually compared with *Lactuca saligna*, *L. serriola* and *L. virosa* on the basis of SDS-electrophoresis. *L. virosa* and *L. saligna* were easily identified and characterised by typical banding patterns. *L. sativa* and *L. serriola* shared the same banding patterns. They further reported that cultivar identification was not possible with the help of SDS-PAGE technique. Jha & Ohri, (1996) conducted experiment on seed protein in 9 accessions of cultivated *Cajanus cajan* and 10 wild *Cajanus* species using SDS-PAGE. They reported a

considerable variation among protein profiles of different accessions of *Cajanus cajan* while those of wild species were very specific and distinctly different from each other. Relative similarities between various taxa were estimated by Jaccard's similarity index and cluster analysis was performed to produce a UPGMA dendrogram. The clustering of 10 wild species and *C. cajan* more or less agree with their sectional classification and available data based on morphological characters, crossabilty, genome pairing in hybrids and nuclear RFLPs. Singh *et al.* (1996) reported little variation for protein bands in groundnut which indicated that most of the accessions were the members of same conservative species.

Tahir *et al.* (1996) detected a novel high molecular weight glutenin subunit in a hexaploid wheat landraces collected from Baluchistan, Pakistan using SDS-PAGE. Relationships between geographical parameters and morphological and biochemical characters were studied in landraces of barley by Ruiz *et al.* (1997). They reported high correlation between morphological and geographical parameters. Associations for some proteins and altitude were also detected. However, obvious geographical patterns were not found for characters such as growth habit, spike density and tillering capacity. The geographical parameters that had the most correlation with morphological traits was the longitude at the collection site.

Yoshida *et al.* (1997) used SDS-PAGE in blackgram for investigation of globulin properties in buffer (pH 3, 8 or 10) and one major 8S band was observed in all three environments. Globulin in *Vigna mungo* was observed as a group of heterogeneous proteins and separated into two fractions (α and β). SDS-PAGE of 8S globulin protein indicated three major bands with apparent molecular weights of 55, 45 and 33 kd, and several other minor bands.

2.2.2 Randomly Amplified Polymorphic DNA (RAPD)

Among several techniques available for assessing the genetic variability, relatedness and structure among crop germplasm collections, DNA-based markers provide powerful and reliable tools for discerning variations within crop germplasm and for studying evolutionary relationships (Gepts, 1993). Among molecular markers, random amplified polymorphic DNAs (RAPDs) have increasingly been employed in genetic

research owing to their speed and simplicity (Williams *et al.* 1990; Welsh & McClelland, 1990). RAPDs require no previous sequence information for the fingerprinting of cultivar genomes and have been used widely for estimating genetic variation at the population level and among closely related species (Rafalski, *et al.* 1991; Williams *et al.* 1993). The technique has been found successful to resolve various levels of inter- and intra-specific polymorphism, which facilitates assessment of genetic relationships, definition of regional grouping and identification of individual accessions (Vierling *et al.* 1994). Since the genetic differences are reflected as the presence or absence of RAPD fragment and a huge number of bands can be obtained in a limited time. RAPD analysis has been successfully utilised in many crops such as Brassica (Demeke *et al.* 1992) and *Vigna* (Kaga *et al.* 1996). The molecular markers, RAPDs have been widely used: 1) for determining the genetic relationships between different related species (Demeke *et al.* 1992; Thormann *et al.* 1994; Ren *et al.* 1995); 2) for the identification of cultivars and genotypes (Hu & Quiros, 1991; Samec, 1993; Samec & Nasinec, 1995); and (3) for estimating the genetic relationships and diversity among crop germplasm (Kresovich *et al.* 1992; Jain *et al.* 1994; Mailer *et al.* 1994; Farnham, 1996). Biochemical markers can be used for mapping purpose (Kiss *et al.* 1993; Timmerman *et al.* 1994).

RAPDs have been shown to provide a level of resolution equivalent to RFLPs for determining genetic relationships among *B. oleracea* genotypes (dos Santos *et al.* 1994) and among *B. napus* breeding lines (Hallden *et al.* 1994). The contribution of genetic markers to gene mapping or to plant breeding have been reviewed by Burr *et al.* (1983), Tanksley *et al.* (1982, 1989), Tanksley & Orton, (1983), Tanksley, (1983), Helentijaris *et al.* (1985), Beckmann & Soller, (1986), Soller & Beckmann, (1988), Edwards, (1992) and Dudley, (1993). Smith & Smith, (1992) have thoroughly reviewed the role of genetic markers in fingerprinting commercial germplasm.

Kresovich & McFerson (1992) have highlighted the important role of genetic diversity assessment in plant genetic resource management. One simple estimate of the genetic diversity in a given taxon, germplasm collection, or geographic region is the number of taxa included in the larger unit (e.g., the number of subspecies found in a species in a given region). Accordingly, diversity estimates derived from genetic marker data may be more valuable than

counts of taxa for most germplasm management applications, because such estimates can be on conserving genes rather than taxa.

Halward *et al.* (1991) used molecular markers in peanut and reported that initial studies did not reveal significant levels of detectable genetic polymorphism within *A. hypogaea*, even among diverse germplasm lines, despite the abundant morphological and physiological variability present within these genetic resources. Devos & Gale, (1992) reported the validity of RAPD as a genetic marker system in wheat and suggested many applications where RAPD will find great utility in crop genetics. The tagging of QTLs in individual populations is likely to be facilitated by the use of this technique. Joshi & Nguyen, (1993) used RAPD for investigation of genetic diversity in 15 varieties of wheat. Most of the spring and winter wheats were clustered together and the results were expected to be useful in the identification of suitable parents for the development of a mapping population for tagging agronomically important traits. Paran & Michelmore, (1993) converted RAPD markers to Sequence Characterized Amplified Regions (SCARs) and then designed pairs of PCR primers specific to the individual RAPD polymorphism, whereas, RAPD fragments were employed as RFLP probes by Schulz *et al.* (1994) and Wilkie *et al.* (1993).

Connolly *et al.* (1994) interpreted the RAPD data for fingerprinting sweet potato genotypes. They observed high level of polymorphism as from 170 bands from 36 decamer primers, 132 fragments (77.6%) were polymorphic. The pattern analysis enabled the grouping of cultivars and the identification of primers which gave the greatest discrimination among the cultivars. Yu & Nguyen, (1994) investigated genetic variation of nine upland and four low land rice cultivars using RAPD method via PCR involving 42 random primers and 260 bands. High level of polymorphism was observed as 40 primers showed polymorphism. They considered RAPD technology useful to detect genetic variation at the DNA level and suggested to use in future for determining the best choice of parents in order to generate mapping populations for tagging drought resistance traits.

RAPD markers were used to distinguish between six different *Lens* taxa representing cultivated lentil and its wild relatives (Sharma *et al.* 1995). Twenty four 10-mer primers generated 88 polymorphic bands in 54 accessions. The data showed that, of

the taxa examined, *L. orietalis* was the most similar to cultivated lentil. The level of variation detected within cultivated lentils suggested that RAPD markers may be an appropriate technology for the construction of genetic linkage maps between closely related *Lens* accessions. Abo-elwafa *et al.* (1995) conducted RAPD analysis in lentil involving 11 microsperma, 9 macrosperma and 16 wild relatives with forty 10.mer primers. Fifty bands were recorded, out of which 90% were polymorphic. The results revealed that variation in cultivated lentil was lower than that of wild relatives. Microsperma and macrosperma cultivars were indistinguishable by identified RAPD markers.

Link *et al.* (1995) examined three groups of faba bean inbred lines by means of RAPD. Out of 59 primers, 35 were informative and yielded 365 bands, 289 of which were polymorphic with a mean of 8.3 bands per primer. Cluster and PCA identified European small seeded lines and Mediterranean lines as distinct groups with European large seeded lines located in between. They concluded that RAPD is useful for classification of germplasm and identification of divergent heterotic groups in fababean. Mackill, (1995) classified rice germplasm using RAPD technology and reported that tropical and temperate *japonica* groups were genetically different but the boundary between them was not firm. Discriminant analysis showed that the distance between the two *japonica* groups was much lower than between *japonica* and *indica*, and that several cultivars were intermediate between these two. Multani & Lyon, (1995) reported the possibility to differentiate closely related cultivars of cotton using RAPD markers. Skroch & Nienhuis, (1995) analysed ten snap bean genotypes with the help of RAPD technique. RAPD-derived relationships among genotypes were reported for the ten genotypes included in the study. The data demonstrated that many informative, polymorphic RAPDs can be found among snap bean cultivars. These RAPDs may be useful for the unique identification of bean germplasm, and applications of molecular markers to bean breeding. Nienhuis *et al.* (1995) reported genetic relationship among landraces of lima bean as measures by RAPD markers. They thought that plant breeders can use RAPD markers to organize genetic resources into related groups to make more informed decisions regarding choice of parents.

Virk *et al.* (1995a) classified rice germplasm into two groups based on RAPD and all 31 of one group were classified as *indica*; however, eight of other group were designated as *japonica or javanica* while the others were *indica*. These findings have an immediate practical application by rice breeders as the RAPD technology provides a fast method for classifying relatively uncharacterised accessions as *japonica* or *indica*. Virk *et al.* (1995b) identified duplicate accessions within rice germplasm using RAPD analysis and they thought that it is not possible to prove that 2 accessions are genetically identical without sequencing their entire genome.

Ahmad *et al.* (1996) studied genetic relationship in *Lens* species using RAPD markers. One hundred and fifty eight reproducible bands were observed from 15 primers. Genetic distance between each of the accessions was calculated from simple matching coefficients. Jianhua *et al.* (1996) thought that RAPD banding patterns are useful in identifying genotypes of various crops. In particular, this technology has applications in plant breeding, seed production and seed testing programme. They observed that artificial and natural seed deterioration, contamination by pathogens, and different seed production environments did not generally affect the stability of RAPD markers in soybean seed. Further, the use of RAPD banding patterns appears applicable to a number of crops and can be successfully used to enhance and determine the genetic purity of individual seeds.

The genetic variation among 23 accessions of 5 species in the subgenus *Ceratotropis*, genus *Vigna*, were investigated by RAPD analysis by Kaga *et al.* (1996). A total of 404 fragments amplified with 24 primers were scored and analysed for cluster analysis. The accessions were separated into two main groups with an average of 70% differences. Within the main group, five subgroups were recognised. Wild forms were always grouped with their most closely related cultivated forms and they showed variation in each species.

The genomic DNA for 42 pea genotypes, representing four wild and cultivated subspecies were analysed by Samec & Nasinec, (1996) using RAPD reactions. Amplification of eight decamer primers generated 149 polymorphic bands. Genetic similarities of RAPD profiles were estimated and each genotype was clearly identified from the others which revealed that RAPD technology is a rapid, precise and sensitive

technique for identification of pea genotypes. Virk *et al.* (1996) evaluated diverse Asian rice germplasm to identify association between various quantitative traits and RAPD molecular markers using multiple regression analysis. This has allowed to predict for other samples of germplasm for quantitative traits. Such predictive capability is possible because of the availability of extensive diversity held in gene-banks, and can be used in future to facilitate the exploitation of that biodiversity. For mapping and isolation of QTLs, the method would provide information to guide the selection of parental material for hybridization and markers to show linkage to QTLs. The procedure can also be used as an initial screening method for the identification of QTLs. Ribu & Hilu, 1996 considered RAPD technique an effective descriptor in studying inter-specific variation in the crop because of high polymorphism.

Iqbal *et al.* (1997) used RAPD to evaluate the genetic diversity of elite commercial varieties of cotton. Twenty two varieties belonging to *Gossypium hirsutum* L. and one to *G. arboreum* L. were analysed for 50 random decamer primers using polmerase chain reaction (PCR). In total 349 bands were amplified, 89.1% of which were polymorphic. The diploid cotton was very much distinct from tetraploid *hirsutum* based on cluster analysis. Further, analysis revealed that the intervarietal genetic relationship of several varieties was related to their centre of origin and most of the varieties were observed with narrow genetic base. They suggested to use the results to select possible parents to generate a mapping population. Genetic diversity based on RAPD markers has been reported by Rabbani *et al.* (1998b) in brassica germplasm collected from Pakistan. A total of 198 polymorphic amplified products were obtained from 30 decamer primers. Based on pairwise comparison of RAPD amplified products, genetic similarity was estimated using UPGMA cluster analysis. Low level of genetic variability was reported to be attributed to the selection for similar traits and horticultural use. The study demonstrated that the RAPD is a simple and fast technique to compare the genetic relationship and pattern of variation among accessions of crop.

2.3 *Inheritance*

2.3.1 *Qualitative Traits*

Polymorphic, highly heritable morphological traits were some of the earliest genetic markers employed in scientific investigations (Mendel, 1866; De Vries, 1912), and they may still be optimal for certain plant germplasm management. Morphological assays generally require neither sophisticated equipment nor preparatory procedures, so monogenic or oligogenic morphological markers are generally simple, rapid and inexpensive to score. Until recently, scientific plant classification was based nearly exclusively on morphological traits (Stuessy, 1990), some of which may serve as genetic markers (Gottlieb, 1984); Hilu, 1984) suitable for plant germplasm management (Stanton *et al.* 1994).

Sen & Jana, (1963) investigated inheritance of pod colour and found that black pod colour is dominant over straw colour and this character is controlled by a single gene whereas, Ramaiah & Samolo, (1992) reported two complimentary genes for controlling pod colour in blackgram. Sen & Ghosh, (1959) reported 3:1 ratio in green gram, whereas Chaudhari & Thombre, (1975) observed single gene dominance for pod colour in pigeopea. Inheritance of prostrate growth habit, simple leaf type and thick stem was studied in chickpea by Singh & Singh, (1992) where all the characters were observed monofactorial recessive inheritance. The segregation in back crosses also confirmed the findings of F_2 generation. Monogenic recessive inheritance for simple leaf type was reported by Rao *et al.* (1980) and Dahiya *et al.* (1984). Pathak & Singh, (1983) observed 3:1 ratio for blackish to brown colour in green gram. The flower colour in peanut has been reported incomplete dominant that segregates in a ratio of 1 (red):2 (intermediate red) :1 (yellow) by Habib *et al.* (1980), whereas genetics of testa colour expression has been reviewed by Wynne & Coffelt, (1982) and Senapati & Roy, (1990) who reported seven loci to be involved in the expression of testa phenotypes. Dasgupta & Das, (1987) investigated inheritance of pod length and cluster number in blackgram and observed wide genetic variability for these characters in two crosses, hence, suggested selection of desirable segregants for improving cluster number and pod length. Rao *et al.* (1989) reported monogenic recessive inheritance of multifoliate leaf in blackgram. The linkage

studies for biochemical and morphological markers have been conducted by Kazan *et al.* (1993) in chickpea; Zamir & Tadmor, (1986) and Muehlbauer *et al.* (1989) in lentil; Weeden & Marx, (1984, 1987) in pea and Koenig & Gepts, (1989) in *Phaseolus.*. They observed distorted ratios i.e., deviation from normal assortment (9:3:3:1) and considered it might be due to linkage for some alleles.

2.3.2 Quantitative inheritance

Determining genetic control of morphological traits and, thereby, distinguishing homology from analogy may be more costly and complicated than with other genetic markers (Camussi *et al.* 1985). Indeed, Smith & Smith, (1992) argue strongly that lower heritabilities or uncertain homologies make most morphological markers essentially unsuited for certain germplasm management applications, especially for accurately determining genetic proximities among elite cultivars, lines or hybrids. Khan, (1973) and Frey, (1975) advocated population breeding using natural out crossing in pigeonpea where reproductive biology permits the option. Pedigree or bulk methods directed towards pure-lines have been used successfully by Gupta & Saxena, (1985).

Positive correlation among yield and its components has been reported by Rani & Rao, (1981 in blackgram and, in mungbean, Tomar *et al.* (1973) and Khalid *et al.* (1984) observed positive correlation of yield with yield components, whereas Malik *et al.* (1987) reported negative correlation of yield with maturity, pod length and seed weight. Malik *et al.* (1983) investigated maximum relative selection efficiency for branches per plant in mungbean. Malhorta *et al.* (1974) observed positive association of yield with days to maturity, plant height, pods and pod length, whereas negative with seed weight. Donald, (1962), Lal, (1967), Singh, (1977), Singh *et al.* (1980), Patel & Shah, (1982), Malik *et al.* (1981, 1986), Khan & Malik, (1989) and Ghafoor *et al.* (1993b) gave emphasis for the selection of legume genotypes on the basis of high harvest index.

On the basis of advanced generation performance, the crossing of selected F_1 plants in all possible combinations is suggested to be made by Jensen, (1970) which may result in breaking some undesirable linkages and release greater genetic diversity. Singh *et al.* (1975) reported significant GCA and SCA effects in a 6 parent diallel in lentil. They suggested selection of superior segregants based on number of branches during early stage

of growth and pods at the time of maturity. Singh *et al.* (1987b) conducted GCA and SCA in 10 parent half diallel of pea and reported additive type of gene action for days to flowering, plant height, branches, pods, seeds/pod, pod length, days to maturity, seed yield and harvest index. For combining seed yield and protein contents, they suggested multiple crosses followed by further inter matings among desirable sergeants. Plant height and seed size are known to be highly heritable in legumes (Munoz & Abrams, 1971; Khan & Rachie, 1972; Sharma *et al.* 1973; Seehaiah *et al.* 1993). Malhotra *et al.* (1979) conducted diallel analysis in mungbean and observed that from segregating generation of biparental populations, desirable plants were possible to select, and then selected plants were suggested to be used as one of the parents in other conventional breeding programmes. Wilson *et al.* (1985) reported both additive and non-additive genetic variance but predominance of non-additive yield contributing traits in mungbean.

Singh *et al.* (1987a) conducted combining ability analysis in 10 parent half diallel in blackgram and reported highly significant GCA and SCA effects for days to maturity, branches, clusters per plant, pods, grain yield, harvest index and protein contents. However, the estimates of SCA were observed greater than GCA, indicating the predominance of non-additive gene action, similarly Reddy *et al.* (1979a, b) also reported the predominance of non-additive gene-action in pigeonpea. Combing ability was investigated in pigeonpea by Mehetre *et al.* (1988) and Saxena *et al.* (1989) who reported both additive and non-additive gene effects for flowering, maturity, plant height, plant spread, branches, pods and grain yield. However, the additive gene effects were predominant. Most of the crosses involving best combiner as one of the parents exhibited high SCA effects. Thus the crosses involving diverse type of parents and showing high SCA effects for grain yield could successfully be utilised for exploitation in pigeonpea. From a diallel cross, GCA and SCA were studied by Saxena & Sharma, (1989) in mungbean and additive gene action was observed for branches, clusters/plant, pods/plant, pods/cluster, seeds/pod and yield. Parents with good GCA were suggested to be exploited in breeding programme and some hybrids were also identified as good specific combinations and thus recommended for yield improvement in mungbean. Similar results were also reported by Chowdhury, (1986) in mungbean.

Singh *et al.* (1992) examined combining ability for yield and its components in beans (*Phaseolus vulgaris* L.) involving 64 parents of diverse origin. Positive GCA effects were found for yield in 19 parents, seed weight (6 of the 19), seeds per square meter (20 parents), pods per square meter (19 parents) and days to maturity (29 parents). Negative GCA was observed for days to maturity in 25 parents. The observed intermediate growth habit alongwith earliness and determinate growth habit with lateness could very likely be recombinants and might therefore, facilitate simultaneous improvement.

Ghafoor *et al.* (1993a) conducted combing ability analysis in a 5-parent diallel in blackgram for grain yield and its components. Non-additive gene-actions contributed the major portion of genetic variance for all the characters studied. Biparental mating among selected F_2 segregants followed by selection in advance generations was suggested. One genotype (87/88) was observed as the best general combiner for pods and grain yield, thus recommended for further exploitation in blackgram breeding programme to develop high yielding varieties. Six genotypes and their fifteen F_1 hybrids were evaluated by Seehaiah *et al.* (1993) for combining ability analysis and indicated that non-additive gene-action was more important than additive gene-action for all the characters except plant height. The parents with high GCA were recommended to be used in the hybrid programme for blackgram improvement. Ram & Singh, (1993) reported high variance for seed yield, pods and branches in blackgram alongwith high estimates of heritability and genetic advance which were influenced by additive gene-action. High heritability and low genetic advance as observed for 100-seed weight and pod length may be due to non-additive gene-action. For plant height, both additive and non-additive gene-actions were observed. Shanmugasundaram & Rangasamy, (1994) observed highly significant GCA and SCA in F_1 and F_2 in 20 crosses of blackgram for yield and its components. Out of six parents, CO 4, CO 5 and T 9 were observed good general combiners and ten crosses were recorded with high SCA and thus suggested to exploit for blackgram improvement.

Sharma & Pandey, (1996) reported genetic variance and combining ability on 7 yield components in a one way 6-parents diallel. Both additive and non-additive genetic components were involved in the expression of these traits with a predominance of the former. Selection from segregating populations of crosses with high SCA values was

suggested to lead genetic improvement for seed yield in blackgram. Sharma & Sood, (1991), Singh & Singh, (1993) and Chauhan & Singh, (1993, 1997) reported non-additive genetic variance for yield and its components in lentil, whereas Dahiya & Brar, (1977) observed the importance of GCA effects in pigeonpea. Since the end product of breeding programme in self pollinated crops is usually a pure-line, there is no scope for exploiting high non-additive genetic variance in self pollinated crops. Kunta *et al.* (1997) conducted combining ability experiment in soybean and observed significant GCA effects for pods/plant, seeds/pod, seed weight, plant height and harvest index, whereas for yield, pods/plant, plant weight and harvest index, SCA effects were significant. They identified best combinations and suggested evaluation of particular signgle-scross for seed weight, where GCA effects weigh high.

MATERIALS AND METHODS

MATERIALS AND METHODS

The research project comprised of five experiments conducted under laboratory, green-house and field condition. A summary of experiments is presented as under;

Experiment	Number of accessions/genotypes	Experimental condition
Morphological characters	484	Field
SDS-PAGE	111	Field, Laboratory
Geographic distribution	88 42 accessions collected 30 advance breeding lines 16 donated from NRS	Field SDS-PAGE in Lab.
RAPD	40 pure-lines of *Vigna*	Laboratory, green-house, field
Inheritance		
Qualitative	13 populations	Green-house, field
Quantitative	6 parent diallel	Field

The materials and methods involved in each experiment vary accordingly and hence discussed separately for each experiment.

Experiment I

3.1 Genetic Diversity Based on Morphological Characters

3.1.1 Germplasm Collection

Legumes are very important crops of Pakistan and widely grown especially on the marginal lands for sustainable agriculture. The local germplasm/land-races are valuable source for agricultural prosperity due to high adaptability, good in quality and resistance for biotic and abiotic stresses. Collection of legume germplasm started during 1981 and the expeditions continued till 1996 to collect the germplasm which is under the threat of genetic erosion with the introduction of improved varieties. The germplasm collected represents a wide eco-geographic variation from dry mountainous region to irrigated plains and sandy arid region of Pakistan. These areas lie between 24 and 37° N latitude and 61 and 78° E longitude. The

altitude of collection sites ranged from less than 100 to more than 3000 meters above sea level (Fig. 3.1.1).

3.1.2 Experiment Material

Four hundred and eighty four blackgram/mash germplasm accessions/genotypes from local origin were evaluated for various agronomical traits in an augmented design under field conditions at National Agricultural Research Centre (NARC), Islamabad (33.40 ° N and 73.07° E). The experiment was planted on 25th July, 1995 for morphological characterization and agronomic evaluation, whereas the same set of germplasm was also planted on 28th July, 1997 for screening against Mungbean Yellow Mosaic Virus (MYMV). Out of 484 accessions, thirty two genotypes were advance breeding lines obtained from Pulses Programme, NARC, Islamabad, whereas other 452 accessions were obtained from gene-bank of Plant Genetic Resources Institute (PGRI), NARC, Islamabad. These accessions were collected by the PGRI scientists from various parts of the country during the past more than one decade. During 1995, one row of 4 meter length for each accession/genotype was planted with 75 cm and 10 cm inter and intra-row spacing, respectively. Three approved varieties, viz., Mash 1, Mash 2 and Mash 3 were repeated as check after every 20 rows. Recommended cultural practices were followed throughout the crop season. Pesticides were sprayed to save the crop from infestation of pests especially white fly, a vector for MYMV but during 1997, the crop was not sprayed by any pesticide and the white fly population was allowed to grow which ultimately increased the incidence of MYMV infection. For plant characters and agronomic traits, data were recorded following IPGRI descriptors for *Vigna* spp. (IBPGR, 1985). Whereas, for disease infection, the presence (1) or absence (0) was recorded on visual assessment basis depending upon the symptoms. The data for days to flowering and maturity were recorded on line basis. Days to flowering was recorded at 50% of flowering, whereas days to maturity was observed when about 90% pods turned brown/black after days of planting. Other quantitative data, i.e., branches, pods, grain yield (g) and biomass (g) were recorded on ten competitive plants selected randomly and then averaged to per plant basis. Pod length (cm) and seeds per pod were recorded on ten pods selected at random within each accession/genotype. Pods per branch were calculated and expressed as pods per unit branch, whereas seed density was calculated and expressed as seeds per unit area by using the formula "1-(pod length/seeds)".

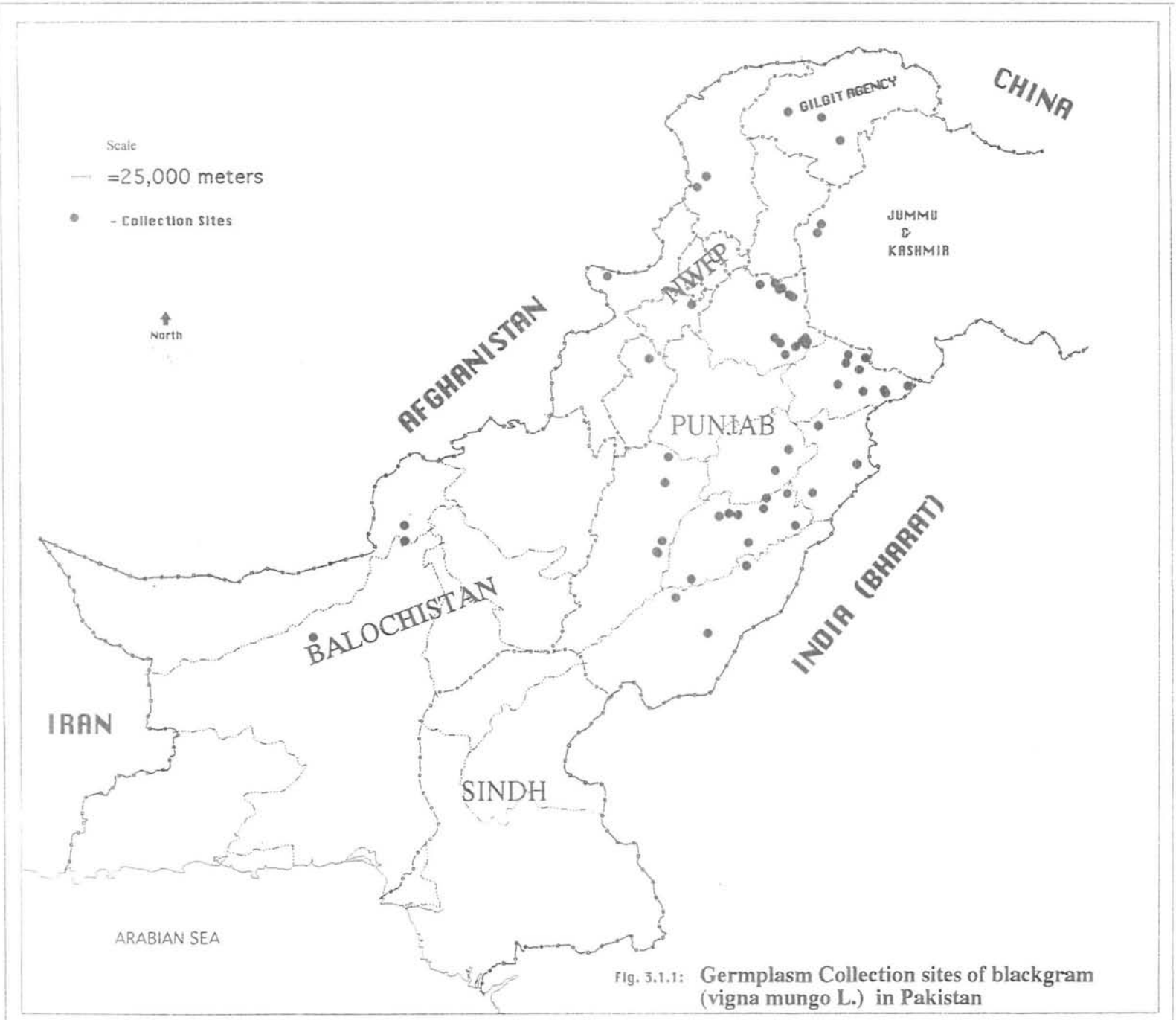


Fig. 3.1.1: **Germplasm Collection sites of blackgram (vigna mungo L.) in Pakistan**

Seed weight was recorded after counting 100 seeds by seed counter and weighed in grams. Harvest index was determined as economic yield expressed in percentage over total biomass.

3.1.3 Statistical Analysis

The data recorded were averaged and analyzed for simple statistics (mean, standard deviation, variance), frequency distribution and simple correlation coefficients using computer software "Microsoft EXCEL, Version 7.0" for windows 95 following the methods of Steel & Torrie, (1981). Twelve quantitative traits (days to flowering, days to maturity, branches/plant, pods/plant, pods/branch, pod length, seeds/pod, seed density, seed weight, biomass, grain yield and harvest index) were also analyzed by numerical taxonomic techniques using the procedure of Principal Component (PC) Analysis (Sneath & Sokal, 1973) using the computer software "STATISTICA" and "SPSS" for windows. To avoid the effect due to difference in scale, means of each character were standardized prior to analysis. The qualitative data for plant hairiness (*HR, Hr, hr*), seed coat colour (*BR, Br, br*) and spots on the seed coat (*SS, Ss, ss*) were used for mean comparison based on qualitative classes using "*t*" statistics with the help of computer software "SPSS" and "MS EXCEL". This analysis was conducted to detect the response of qualitative data on QTLs. The disease data were also analyzed for significance of MYMV with other Quantitative Traits Loci (QTLs) using "*t*" statistics.

Experiment II

3.2 Biochemical (SDS-PAGE) Basis of Genetic Diversity

3.2.1 Plant Material

From blackgram germplasm consisting 484 accessions and evaluated during 1995, one hundred and eleven genotypes were picked up as plant material for SDS-PAGE analysis. Out of 111 genotypes, 79 accessions were selected from the germplasm collections and others were advance breeding lines including three approved varieties (Mash 1, Mash 2, Mash 3) obtained from Pulses Programme, NARC, Islamabad. Only those accessions which were observed homozygous on the basis of protein patterns were included in this study.

3.2.2 *Protein extraction*

For the extraction of proteins, single seed was ground to fine powder with mortar and pestle. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in eppendorf tube with a small glass rod. The extraction buffer contained the following final concentrations: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol Blue (BPB) was also added to the sample buffer as tracking dye to watch the movement of protein in the gel. To purify extraction, the homogenate samples were mixed thoroughly by vortexing and centrifuged at 15,000 rpm for 5 minutes at RT. The extracted crude proteins were recovered as clear supernatant, transferred into new 1.5 ml eppendorf tubes and stored at -20 ^{o}C until electrophoresis.

3.2.3 *Electrophoresis*

Seed protein was analyzed through slab type SDS-PAGE using 11.25% Polyacrylamide gel. Electrophoresis was carried out at 100 V for half hour and then at 150 V until the Bromophenol blue marker reached the bottom of the gel (approximately two and half hour). In order to check the reproducibility of the method two separate gels were run under similar electrophoretic conditions. The molecular weights of the dissociated polypeptides were determined by using molecular weight protein standards "MW-SDS-70 kit" containing Albumin, Bovine Plasma (66 kd), Albumin, Egg Ovalbumin (45 kd), Pepsin Porcine Stomach Mucosa (34.7 kd), Trypsinogen, Bovine Pancreas, PMSF treated (24 kd), β-Lactoglobulin, Bovine Milk (18.4 kd) and Lysozyme, Egg White (14.3 kd) from Sigma Chemical Company, USA.

SDS-PAGE of total seed protein was carried out in Polyacrylamide slab gels in the discontinuous buffer system according to the method of Laemmli, (1970). Vertical gel slabs were prepared in a glass sandwich which was tightened by a set of plastic clips lined with a band of foamed silicon rubber. The separating gels contained 11.25% of Acrylamide and 0.135% by weight of N.N-methylene-bis-acrylamide in 1 M Tris-HCl buffer (pH 8.8) with 0.27% SDS. The gels were polymerised chemically by the addition of 20 µl by volume of tetramethylethylene-diamine (TEMED) and 10% ammonium persulfate

(APS). The stacking gels consisted of 30% Acrylamide and 0.8% N.N-methylene-bis-acrylamide in 0.25 M Tris-HCl buffer (pH 6.8) containing 0.2% SDS. The stacking gels were polymerised chemically in the same way as for the separation gel. The electrode buffer contained Tris-glycine (9.0 g Tris HCl and 43.2 g glycine per 3 litres buffer solution at a pH 8.9) with 3.0 g (0.1%) SDS. Six µl of protein supernatant were applied into the wells in stacking gel sample wells with a microsyringe.

3.2.4 Staining and destaining

After electrophoresis, the gels were stained with 0.2% (w/v) coomassie brilliant blue R250 dissolved in a solution containing 10% (v/v) acetic acid, 40% (v/v) methanol and water in the ratio of 10:40:60 (v/v) for one hour. Gels were then destained by washing with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and water in the ratio of 5:20:75 (v/v) until the colour of background disappeared and electrophoresis bands were clearly visible. After destaining, the gels were dried using Gel Drying Processor for about 100 minutes.

3.2.5 Data analysis

Depending upon the presence or absence of polypeptide bands, similarity index was calculated for all possible pairs of protein types. To avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of the bands was taken as indicative. The scores were "1" for the presence and "0" for the absence of a band. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, Jaccard's similarity index (S) was calculated for all possible pairs of protein types electrophoregrams by the following formula (Sneath & Sokal, 1973):

$$S = W/(A + B - W)$$

where "W" is the number of bands of common mobility, "A" the number of bands in protein type "A" and "B" is the number of bands in protein type "B".

The similarity matrix thus generated was converted to a dissimilarity matrix (Dissimilarity = 1- similarity) and used to construct dendrogram by the unweighed pair-group method with arithmetic means (Sneath & Sokal, 1973). All the analyses were

carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993) and "STATISTIA" for windows 95.

From the perspective of statistical genetic analysis, genetic-marker data fall into two broad categories; 1) quantitative traits (e.g. many agronomical features) with continuous variation governed by several to many genes; and 2) biochemical data e.g., genetically molecular markers with discrete phenotypes governed by one to several genes. Importantly, these two types of traits may simply be variants of a single genetic theme, distinguishable only by the magnitude of allelic substitution effects (Comstock, 1978; Robertson, 1989). The quantitative data and SDS-PAGE data were analyzed for simple statistics, cluster analysis and PCA by using the standard procedures with the help of computer software "STATISTICA" and "SPSS" for windows 95. Association of biochemical markers with genes affecting the variation of QTL were determined by dividing quantitative traits into 2 groups on the basis of presence or absence of protein peptide. The group means of quantitative characters were calculated, and 2-tailed *t-test* was applied to compute the probabilities that two group means were equal, using computer software "SPSS" and "MS EXCEL" that has been referred by Mansur *et al.* (1993); Muhelbauer *et al.* (1989); Tahir & Muehlbauer, (1995). In addition to this, correlation coefficients were also computed to determine association between protein pattern and QTLs.

Experiment III

3.3 Geographic Distribution Pattern in Relation With Morphological and Biochemical Traits

3.3.1 Experiment Material

Keeping in view the importance of variation expressed by the diversity in environment, blackgram germplasm selected on the basis of geographic origin was studied for morphological and protein patterns. Four hundred and eighty four blackgram accessions/genotypes were evaluated and characterized for various agronomical and morphological traits under field condition at NARC, Islamabad (33.40° N and 73.07° E) during 1995 (experiment I). Out of these, 32 accessions were selected on the basis of geographic distribution, 16 were donated by national research stations (NRS) and 30 advance lines

alongwith one check variety (Mash 1) were also included in the study. Ten plants of each accession/genotype were selected at random during 1995 and their progenies were planted during July, 1997. One row of 2 meter length for each plant progeny was planted with 75 cm and 10 cm inter and intra-row spacing, respectively. Recommended cultural practices were followed throughout the crop season to get healthy crop. Pesticides were sprayed twice to save the crop from the infestation of pests especially white fly, a vector for MYMV. Plant and agronomic characters were recorded following IPGRI descriptors for *Vigna* spp. Days to flowering were recorded when 50% plants started flowering and days to maturity were recorded at 90% maturity when pods turned brown/black. Other quantitative data i.e., branches, pods, grain yield (g) and biomass (g) were recorded on ten competitive plants selected randomly and then averaged to per plant basis. Pod length (cm) and seeds per pod were recorded on ten pods selected at random within each accession/genotype. Pods per branch were calculated and expressed as pods per unit branch, whereas seed density was calculated and expressed as seeds per unit area by using the formula "1-(pod length/seeds)". The seed weight was recorded after counting 100 seeds by seed counter and weighed in grams. Harvest index was determined as economic yield expressed in percentage over total biomass.

3.3.2 Statistical Analysis

The data recorded were averaged and analyzed for simple statistics (mean, standard deviation, variance) using computer software "Microsoft EXCEL" for windows 95 (Appendix II). The data were grouped according to provincial distribution, agro-ecological zones and altitude for comprehensive pattern of geographic distribution.

The crop agro-ecological zones were followed by FAO, (1976) considering the physiography, climate and soils in which the country has been divided into 17 crop ecological zones. A summary of these zones is given below:

	Zones	Areas and climate
1	Thatta zone	Indus delta with clayey and silty soil. The climate is marine sub-tropical.
2	Hyderabad zone	Frost free part of Indus floodplains. The soils are well drained clayey and loamy.

3	Sukkur zone	Arid part of the Indus plains, with less than 200 mm rainfal, very hot summer and mild but not frostless winter. Well drained and restricted drainage areas with clayey and loamy soils.
4	Faisalabad zone	Semi-arid belt (rainfall 200 to 400 mm) of the Indus plains, it has loamy and clayey, well drained soils. May and June are very hot but dry and July and August are hot with high humidity.
5	Gujranwala zone	Sub-humid part (rainfall 400 to 500) of the Indus plains, it has clayey soils with restricted drainage and loamy soils with good drainage. May and June are hot dry but July and August are hot humid. December, January and February are also rainy.
6	Peshawar zone	Covering the Peshawar valley with mild early summer and cold but rainy winter, it has clayey soils in the central part but loamy on the sloping sides. The central part has high incidence of frost, while the sloping sides are nearly frost free.
7	Thal zone	Sandy soils with arid to semi-arid sub-tropical (rainfall 150 to 350 mm)
8	D.I. Khan-Sibi zone	Long strip of piedmont plain on the west bank of Indus with arid area which depends on the storm water originating from Sulaiman mountain range. Clayey and loamy soils.
9	Gujrat zone	It covers the Himalayan piedmont plain with a gently, continuous slope and receive 500 to 1000 mm rainfall (70% in summer; 30% in winter).
10	Rawalpindi zone	Sub-humid part (rainfall 500 to 900 mm) of the Potohar with silty soils (wind-deposited silt).
11	Talagang zone	Semi-arid part of the Potohar Upland, with300 to 500 mm rainfall and mainly loamy soils.
12	Muree-Swat zone	Sub-humid to humid (precipitation 500 to 1400 mm) highlands-1000 to 5000 meters high with steep slopes. Summer is pleasant but winters are cold.
13	Chitral zone	It is arid and semi-arid area of high mountains (more than 3000

		masl) with precipitation of 150 to 500 mm.
14	Quetta-Loralai zone	Mountain area with large, nearly level valleys and 200 to 300 mm precipitation. The soil in the valleys is deep, highly calcareous loams.
15	Mekran-Jhalawan zone	It is a plateau, 500 to 1500 meter high and climate is arid sub-tropical with 50 to 300 mm rainfall. The soils are highly calcareous loams.
16	Tharparker zone	Sand ridges with 200 to 300 mm rainfall occurring mainly during July and August, soils are very sandy.
17	The Cholistan zone	Sandy ridges with arid climate.

Quantitative traits were also analyzed by numerical taxonomic techniques using the procedures of principal component (PC) and cluster analyses (Sneath & Sokal, 1973) with the help of computer software "STATISTICA" and "SPSS" for windows 95. To avoid the effect due to differences in scale, means of each character were standardized prior to analysis.

Experiment IV

3.4 Detection of Genetic Variation by Random Amplified Polymorphic DNA (RAPD)

3.4.1 Plant material

Forty pure-lines of *Vigna* were selected to study the extent of genetic variation based on RAPD analysis. DNA extraction and RAPD analysis were conducted at Legume Breeding Lab. NARC, Tsukuba, Japan during 1996. Seeds of each genotype were planted between papers in the petri plates. The seed was disinfected with 99.95% ethanol and washed with distilled water to avoid contamination and the plates were placed in the dark at RT. After the emergence of plumule, the plates were shifted in the incubation room at 30°C under normal light. After 5 days of transfer, the first 2 leaves were cut with disinfected scissors and dipped in liquid nitrogen (LN) for 10 minutes. After this, leaf samples were preserved in -80°C till the extraction of DNA.

3.4.2 *DNA extraction for RAPD analysis*

Approximately 0.3 g of primary leaves just after 5 days of emergence, collected and frozen at -80 ° C were ground in liquid nitrogen (LN) mixed vigorously with extraction buffer, 20% SDS and mercaptoethanol and incubated at 65 ° C for 10 minutes. Proteins of the solution were removed by the addition of phenol/chloroform/isoamylealcohol (25:24:1). The DNA was precipitated with isopropanol by incubation at -20 ° C for 30 minutes, and then centrifuged at 12,000 rpm for 15 minutes. The pellet was washed with 70% ethanol, dried and then suspended in 1 ml of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). DNA was digested with 1 µg RNase and incubated at 37 ° C to remove the RNA from the solution. DNA quantity was estimated with spectrophotometer for dilution for PCR. The DNA thus obtained was diluted to 100, 50, 10, 5 and 2.5 ng/µl to confirm the actual dilution for PCR. Using the mini-gel method (Sambrook *et al.* 1989), the appropriate DNA amount for PCR amplification was determined by visual comparison with lambda DNA of known concentration.

3.4.3 *RAPD analysis*

PCR was performed in the 0.5 ml reaction tubes with 10 µl of DNA solution containing 2.5 ng of genomic DNA, 1 µl of primer, 0.2 µl of 20 mM dNTP, 2 µl of 10X buffer, 1.6 µl $MgCl_2$. and 0.2 µl of Taq Polymerase. The volume in the tube was made 20 µl by adding 10 µl of H_2O. Twelve mer Operon oligonucleotide primers were used for PCR. The reaction mixture was overlaid with a drop of mineral oil to avoid evaporation. Amplification reaction was carried out at the Perkin Elmer thermocycler with 94° C for 1 minute, 30 ° C for 1 minute and 72 ° C for 2 minutes for 45 cycles. 72 ° C was applied for complete annealing prior to storage at 4 ° C. DNA extraction, RNase digestion and PCR amplification for RAPD analysis were carried out as described in the appendix III.

The amplified fragments were recorded after the electrophoresis on 13% Poly Acrylamide Gel (PAGE), with the composition of 33.06 ml H_2O, 9.6 ml Acrylamide (39:1), 4.8 ml of 10 X TBE, 0.48 ml of 10% fresh APS and 0.06 ml of TEMET. The solution was mixed thoroughly and poured into the glass plates to let the gels polymerise. Before applying the samples mixed with dye, gels were allowed for pre loading run at 150 constant voltage for 30 minutes. Eight µl of each sample was applied in the well and electrophoresis at 150

constant voltage was conducted for 70 minutes. The molecular marker X 174/*Hae* III digest was used as 5 µl . After the completion of electrophoresis, the gels were stained in ethidium bromide (200 ml TE and 10 µl ethidium bromide). Amplification was observed on the UV illuminator and Polaroid 667 was used for photographs.

3.4.4 Data analysis for RAPD

RAPD behaves as dominant markers (Clark & Lanigan, 1993), thus, they tend to be bistate (present - absent) type of scoring. Photographs from ethidium bromide stained polyacrylamide gels were used to score the data for RAPD analysis. Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored as present (1) or absent (0) for each of the primer-accession combinations. Since DNA samples consisted of a bulk sample of DNA extracted from individual plants, a low intensity for any particular fragment may be explained by the lesser representation of that specific sequence in the bulk sample of DNA. Therefore, the intensity of the bands was not taken into account and the fragments with the identical mobility were considered to be the identical fragments. Only major bands were scored and faint bands were not considered. The molecular size of the amplification products was calculated from a standard curve based on the known size of DNA fragments of a marker X 174/*Hae* III digest. The presence and absence of the bands was scored in a binary data matrix. Pair-wise comparisons of the genotypes based on the presence or absence of unique and shared amplification products were used to generate similarity coefficients. DNA band shared by all the genotypes were excluded from the data analysis since they are not informative (Clark & Lanigan, 1993).

The resulting similarity coefficients were used to evaluate the relationships among the accessions with a cluster analysis using an unweighed pair-group method with arithmetic averages (UPGMA) and then plotted in the form of a dendrogram using computer software "STATSTICA" for windows.

Experiment V

3.5 Inheritance

For inheritance of quantitative characters which are complex in nature, 6 parent diallel was conducted. Six genotypes/varieties (9020, 9025, 9012, Mash 1, Mash 2 and 9026) were crossed among each other in all possible combinations in a diallel under green-house conditions during spring seasons of 1994 and 1995. Thirteen other crosses involving 11 parents (9010, 9024, 9025, 9102, 9104, 9105, 9106, MM 5-60, MM 33-40, Korea and Mash 1) were also conducted under green-house conditions for the inheritance of qualitative or simply inherited characters.

3.5.1 Qualitative Characters

Seeds obtained from all F_1 crosses and parents were planted under green-house to have maximum plants in F_2 population during 1997. Plants were allowed to grow in an insect free green-house at 30-35°C temperature. Data for plant pubescence was recorded at the flowering stage for hybrids MM 33-40/9104, Mash 1/MM 33-40, Korea/MM 33-40, Mash 1/MM 5-60 and 9104/MM 33-40. Data on seed colour was recorded when the seed was fully matured in eight crosses (MM 33-40/9104, Mash 1/MM 33-40, Korea/MM 33-40, 9106/MM 33-40, 9104/MM 33-40, 9010/MM 33-40, 9025/MM 33-40 and MM 33-40/9105). The spots on seed coat were also recorded after harvesting individual plants at maturity for all the thirteen crosses because one of the parents in all crosses used was either green seeded or brown seeded without spots on seed coat. Pod colour was recorded for seven hybrids (Korea/MM 33-40, 9106/ MM 33-40, 9104/ MM 33-40, MM 33-40/9104, MM 33-40/9105, Mash 1/MM 5-60 & MM 33-40/9024) after harvesting individual plants. Data thus recorded were analysed with the help of chi-square (χ^2) most commonly used to test hypothesis concerning the frequency distribution of one or more populations. In agricultural research this test is useful for obtaining an objective approximation of goodness of fit for attribute data obtained from particular experimentation. There are three important applications of χ^2 in the analysis of attribute data.

i. Test for a fixed ratio hypothesis.
ii. Test for independence in a contingency table.
iii. Test for homogeneity of ratio.

In this study, we used χ^2 for a fixed ratio hypothesis using data from F_2 segregating population of each cross as described by Gomez & Gomez (1984). Nine hybrids were investigated for the analysis of linkage among genetic markers in F_2 generation using the computer programme "LINKAGE 1" of Suiter *et al.* (1983). Three hybrids (Mash 1/MM 33-40, 9025/MM 33-40, 9010/MM 33-40) were investigated for detection of QTLs from F_2 segregating generation using "*t*" statistics. Association of qualitative markers with genes affecting the variation of seed weight was determined by dividing quantitative traits into 2 groups on the basis of dominance or recessiveness of mono-genic locus. Group mean of quantitative characters were calculated, and 2-tailed *t-test* was applied to compute the probabilities that two group means were equal, using computer software "SPSS" and "Microsoft Excel, version 7.0".

3.5.2 Quantitative Characters

The seeds of 6 parent diallel (15 direct crosses and 15 indirect crosses) alongwith parents were obtained and planted under field condition during summer 1997. The experiment was planted in a randomised complete block design (RCBD) with three replicates at the experimental fields of NARC, Islamabad. One row of each cross/parent was dibbled by keeping 35 and 10 cm spacing between and within rows, receptively. Recommended cultural practices were followed throughout the crop season. Pesticides were sprayed to save the crop from the infestation of pests especially white fly , a vector for MYMV. For plant and agronomic characters, data were recorded following IPGRI descriptors for *Vigna* spp. The quantitative data i.e., plant height (cm), branches, pods, grain yield (g) and biomass (g) were recorded on ten competitive plants selected randomly and then averaged to per plant basis. Pod length (cm) and seeds/pod were recorded on ten pods selected at random within each accession/genotype. Pods/branch were calculated and expressed as pods per unit branch. Seed weight was recorded after counting 100 seeds by seed counter and weighed in grams, whereas harvest index was determined as economic yield expressed in percentage over total biomass.

3.5.3 *Statistical Analysis*

Analysis of variance was conducted for each character using the average values following the method of Steel & Torrie, (1981). Genotypic variance ($G\delta^2$), phenotypic variance ($P\delta^2$) and heritability in broad sense (h^2) were calculated according to the methods used by Singh & Choudary, (1979). Combining ability analysis was conducted with the help of a computer Programme written in "BASIC" using the design of combining ability analysis corresponding to the Method I, Model I of Griffing (1956).

RESULTS

RESULTS

4.1 Genetic Diversity Based on Morphological Characters

Basic statistics for measured quantitative traits, viz., days to flowering, days to maturity, branches per plant, pods per plant, pods per branch, pod length, seeds per pod, seed density, seed weight, biological yield, grain yield and harvest index is presented in Table 4.1.1. High variance (expressed as percent of means) was observed for days to flowering, days to maturity, branches, pods, biological yield, grain yield and harvest index. For other characters, viz., pod length, seeds per pod and seed weight, a low variance was observed and hence improvement for these traits seemed to be difficult in the local germplasm used in present study. Days to flowering ranged from 30 to 65 days after planting with a mean value of 47.2±0.46 days. Maturity period ranged from 60-125 days after planting, branches ranged from 2 to 60 BR/PL, pods from 3 to 195 P/PL. Pods per branch were calculated to find the best plant type/shape with maximum reproductive branches and it ranged from 0.2 to 11.3 P/BR. Pod length ranged between 3.1and 7.1 cm, seeds/pod from 2.8-8.2, seed density from -0.11 to 0.45, seed weight from 2.84-6.45 g, biological yield from 5.11-194.35 g, grain yield from 1.05-40.0 g and harvest index from 5.03-51.98%.

4.1.1 Qualitative Traits

Plant traits of qualitative nature with distinct classes like plant pubescence, growth habit, leaf shape, seed colour and seed spots were recorded on line basis following IPGRI descriptors for *Vigna mungo* and the tabulated results are presented in Table 4.1.2. In the present germplasm material, 381 accessions/lines were observed hairy which were 78.72% of the population, whereas, 103 accessions were glabrous which were 21.28%. Growth habit was recorded as erect, semi-erect and spreading or prostate types. Seventy two (14.88%) accessions were erect, three hundred eighty six (79.75%) were semi-erect, while only twenty six (5.37%) were spreading types. Leaf shape was recorded as delate, ovate, lanceolate, rhombic and obvate. Phenotypic distribution of different leaf shapes showed the predominance of delate leaf shape (70.66%), followed by ovate (23.34%), lanceolate (4.13%), obvate (1.24% and rhombic (0.62%). Seed colour was observed as brown or green. In blackgram, it was observed critically that the seed coat colour is either brown or

Table 4.1.1:- Basic statistics for 12 quantitative traits in 484 accessions of *Vigna mungo*

Traits	Mean±SE	δ^2	δ	δ^2 (%)	Range
Days to flowering	47.2±0.46	101.91	10.10	215.9	30-65
Days to maturity	85.4±0.60	175.38	13.24	205.4	60-125
Branches per plant	15.2±0.38	68.59	8.28	451.9	2-60
Pods per plant	43.8±1.21	704.12	25.54	1607.6	3-195
Pods per branch	3.06±0.07	2.26	1.50	73.9	0.2-11.3
Pod length (cm)	4.5±0.02	0.21	0.46	4.7	3.1-7.1
Seeds per pod	6.2±0.03	0.52	0.72	8.4	2.8-8.2
Seed density	0.26±0.004	0.0074	0.0860	2.8	-0.11-0.45
Seed weight (g)	4.77±0.03	0.31	0.55	6.5	2.84-6.45
Biological yield (g)	44.44±1.30	812.13	28.50	1827.5	5.11-194.35
Grain yield (g)	10.02±0.32	48.15	6.94	480.5	1.05-40.0
Harvest index (%)	23.28±0.41	79.99	8.94	343.6	5.03-51.98

δ^2 -Variance, δ- Standard deviation and δ^2 (%)- Variance expressed as percent of means.

Table 4.1.2:- Frequency distribution of qualitative traits in 484 accessions of *Vigna mungo*

Plant trait	Frequency	Percent
Hairiness		
Hairy	381	78.72
Glabrous	103	21.28
Growth Habit		
Erect	72	14.88
Semi-erect	386	79.75
Spreading	26	5.37
Leaf Shape		
Deltate	342	70.66
Ovate	113	23.34
Lanceolate	20	4.13
Rhombic	3	0.62
Obvate	6	1.24
Seed Colour		
Green	6	1.24
Brown	478	98.76
Seed Spots		
Present	477	98.55
Absent	7	1.45

green and it is the presence of black spots which makes its phenotypic appearance blackish. Four hundred and seventy eight accessions which were 98.76% of the population were with brown seed coat and only six accessions (1.24%) were with green seed coat colour. Similarly the spots on the seed coat were also observed and it was noted that four hundred and seventy seven (98.55%) accessions were having spots on the seed coat, while only seven (1.45%) accessions were without spots on the seed coat.

4.1.2 Mungbean Yellow Mosaic Virus (MYMV)

The same set of germplasm was planted under field condition during the year 1997 for screening against Mungbean Yellow Mosaic Virus (MYMV) which is a viral disease and has serious threat to blackgram and mungbean cultivation. MYMV is vectored by white fly (*Bemisia tabaci*) and the disease spread is favoured by hot dry climate. The crop was not sprayed by any pesticide so that white fly population could increase for disease spread. The disease data was recorded on visual basis for 2 categories under natural condition; tolerant (0) or susceptible (1) for preliminary investigation. The data thus recorded were summarized for frequency distribution and it was observed that 334 accessions which were 69.01% of the population, proved to be tolerant to MYMV, whereas remaining 150 were susceptible to the disease (Table 4.1.3). Data were also analyzed to see the relationship of disease with QTLs using *"t"* statistics and it was observed that MYMV is associated significantly with all the characters under study except with seeds/pod, seed weight and harvest index where it was insignificant. The decrease in branches was 12.20%, in pods 15.40%, in biological yield 14.07% and grain yield 13.93%.

4.1.3 Distribution of Quantitative Traits

The frequency distributions for various quantitative traits (days to flowering, days to maturity, branches, pods, pods/branch, pod length, seeds/pod, seed density, seed weight, biological yield, grain yield/plant and harvest index) are presented in the graphic form (Figs. 4.1.1 to 4.1.12). For days to flowering, maximum accessions (103) which were 21.28% of the population, flowered within the range of 51-55 days after planting. It was followed by eighty eight accessions which flowered in less than 35 days after planting (Fig. 4.1.1). Eleven accessions (45307, 45321, 45371, 45358, 45372, 45317, 45127, 45357, 9025, 45308, 45708) were selected on the basis of early flowering as presented in Table 4.1.4. Maximum accessions

Table 4.1.3:-Classification of MYMV and its significance for other QTLs in 484 accessions of *Vigna mungo*

Plant Traits	Mean values			
	Tolerant	Susceptible	Difference	Difference (%)
Days to flowering	48.05±0.56	45.39±0.79	2.67±0.97**	5.46
Days to maturity	86.74±0.77	82.55±0.86	4.19±1.15**	4.83
Branches per plant	15.82±0.46	13.90±0.64	1.93±0.79**	12.20
Pods per plant	46.01±1.48	38.93±2.01	7.08±2.50**	15.40
Pods per branch	3.14±.08	2.89±0.12	0.25±0.14*	7.96
Pod length (cm)	4.55±0.02	4.44±0.04	0.11±0.05*	2.42
Seeds per pod	6.28±0.03	5.97±0.07	0.31±0.079	4.94
Seed density	0.27±0.004	0.25±0.008	0.24±0.009**	7.41
Seed weight (g)	4.80±0.03	4.70±0.05	0.10±0.06	2.08
Biological yield (g)	46.14±1.59	40.65±2.21	6.49±2.72**	14.07
Grain yield (g)	10.48±0.39	9.02±0.54	1.46±0.66**	13.93
Harvest index (%)	23.42±0.46	22.98±10.11	0.44±0.95	1.88
Frequency	334 (69.01%)	150 (30.99%)		

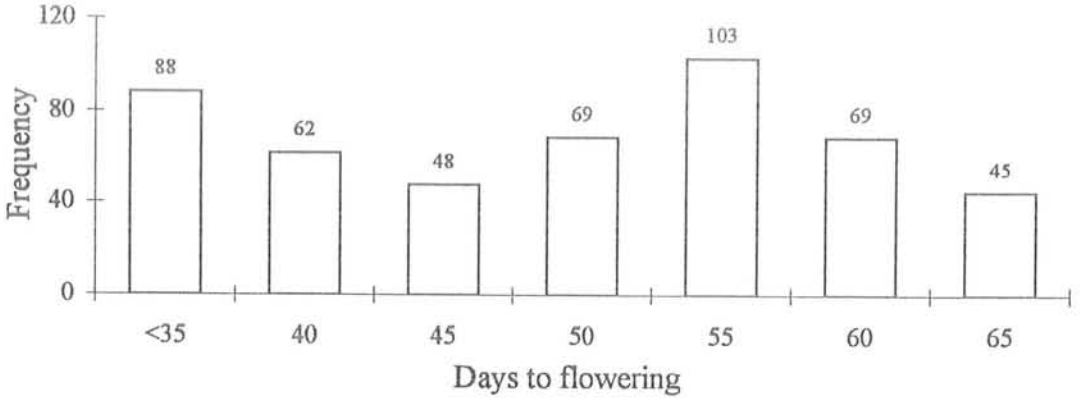


Fig. 4.1.1:- Frequency distribution for days to flowering in blackgram germplasm

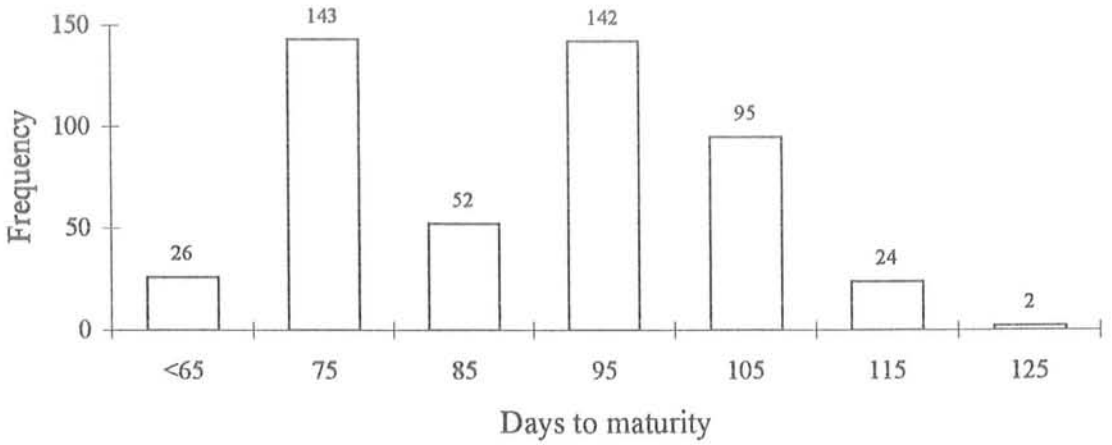


Fig. 4.1.2:- Frequency distribution for days to maturity in blackgram germplasm

(143) which were 29.55 percent of the total, matured between 66 and 75 days, followed by one hundred and forty two accessions with maturity range of 86-95 days . Twenty six accessions took less than 65 days to mature after planting (Fig. 4.1.2).

For branches/plant the results are depicted in Fig. 4.1.3. It was observed that maximum accessions (239) which were 49.38% of the total have 10 to 18 branches per plant, and it was followed by less than 9 branches per plant with the frequency value of 114 accessions (23.55%). Ten accessions were observed to be bushy and produced more than 36 branches per plant. Pods per plant ranged from 3 to 195 and on the basis of class interval, it was observed that one hundred and fifty seven accessions produced 41 to 60 pods per plant which was followed by the group from 21 to 40 (139 accessions) and these two groups can be considered medium pod bearing which were about 61% of the population (Fig. 4.1.4). Seventeen accessions produced more than 100 pods/plant. Three hundred and seventy eight accessions which were 78.10% of the population, produced up to 4 pods/branch. Six accessions (45127, 45336, 45348, 45060, 45072, 45052) produced more than 8 pods per unit branch (Fig. 4.1.5). Pod length ranged from 3.1-7.1 cm and the frequency distribution is presented in Fig. 4.1.6. Maximum accessions (391) which were 80.79% of the population exhibited 4.1 to 5.0 cm pod length, while only seven accessions (45223, 45101, 45429, 45334, 45332, 45226, 45353) produced long pods (>6 cm). Maximum accessions (222) produced 6.1 to 7.0 seeds/ pod and it was followed by 189 accessions (5.1-6.0 seeds/pod). Forty seven accessions (9.71%) were observed with more than 7 seeds/pod (Fig. 4.1.7). Seed density ranged from -0.11 to 0.45 in the present germplasm and frequency distribution presented in Fig. 4.1.8 revealed that maximum accessions (232) which were 47.93% of the population gave SD from 0.21 to 0.30. Seven accessions (45814, 45404, 45051, 45696, 45395, 45078, 45315) produces high SD and hence could be used to improve seed density (Table 4.1.4). The frequency distribution regarding seed weight as depicted in the Fig. 4.1.9 revealed that one hundred and seventy eight accessions were having 4.6 to 5.0 g seed weight, followed by 4.1-4.5g, where one hundred and fifteen accessions were observed. About 82% of the population produced 4.1 to 5.5 g seed weight and four accessions (45120, 45114, 45102, 45222) were having high seed weight (more than 6.0 g) and hence could be utilized for the manipulation of this trait in developing

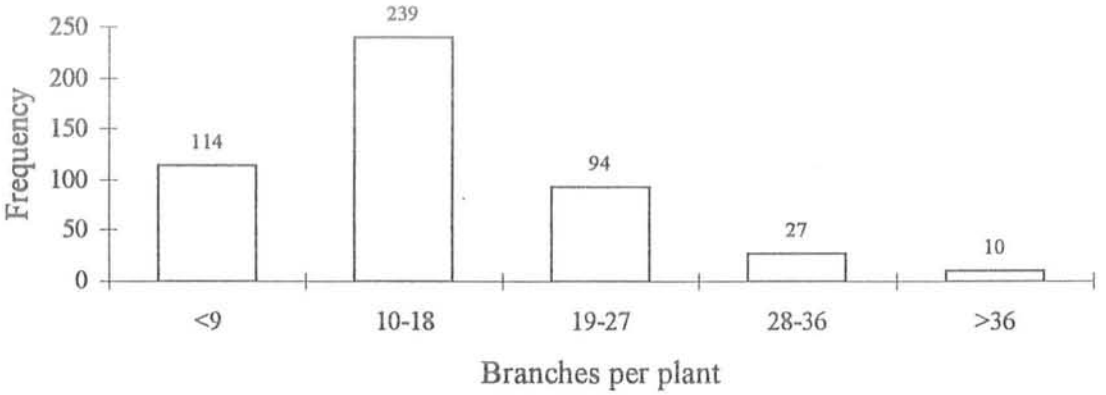


Fig. 4.1.3:- Frequency distribution for branches in blackgram germplasm

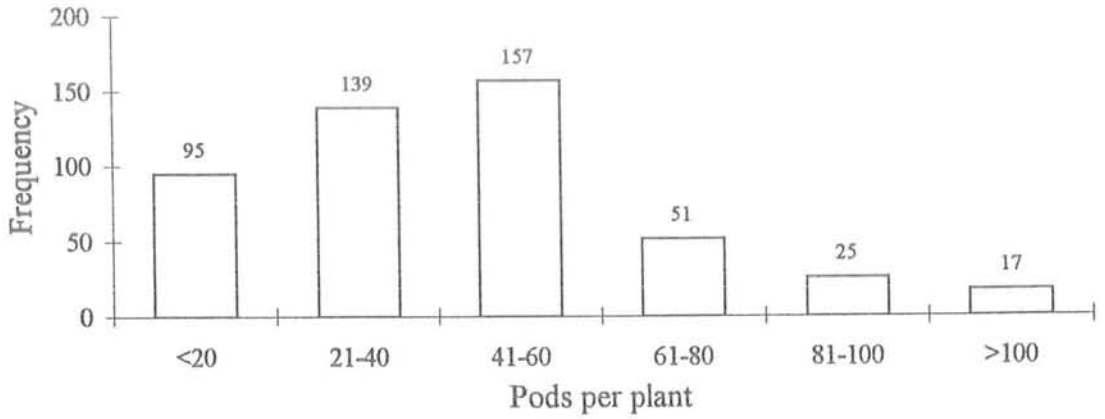


Fig. 4.1.4:- Frequency distribution for pods in blackgram germplasm

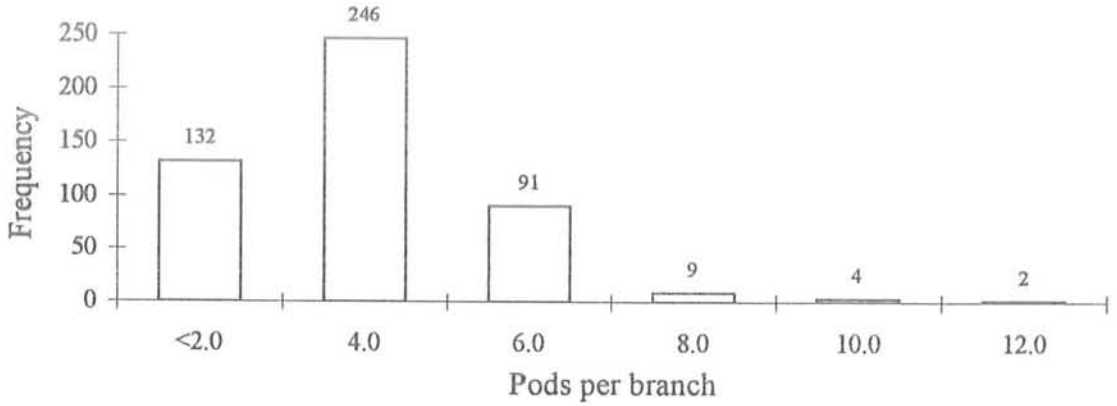


Fig. 4.1.5:- Frequency distribution for pods/branch in blackgram germplasm

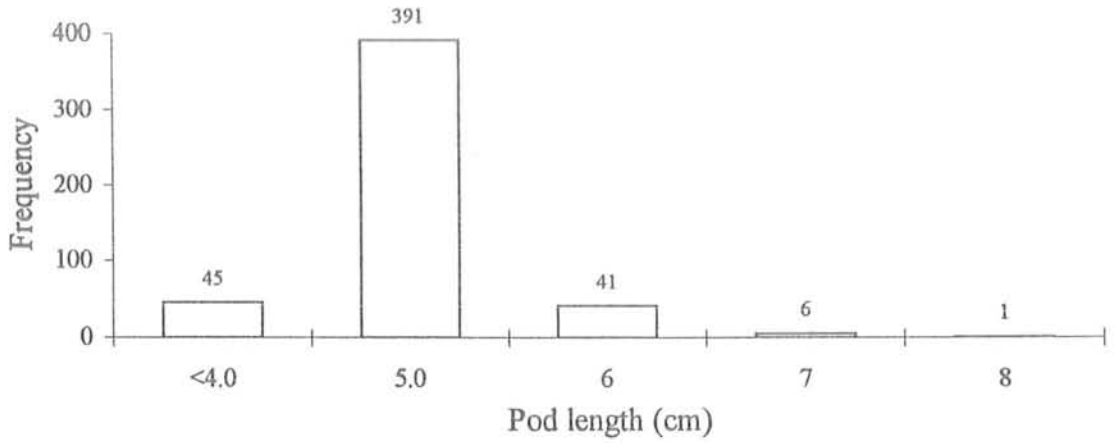


Fig. 4.1.6:- Frequency distribution for pod length (cm) in blackgram germplasm

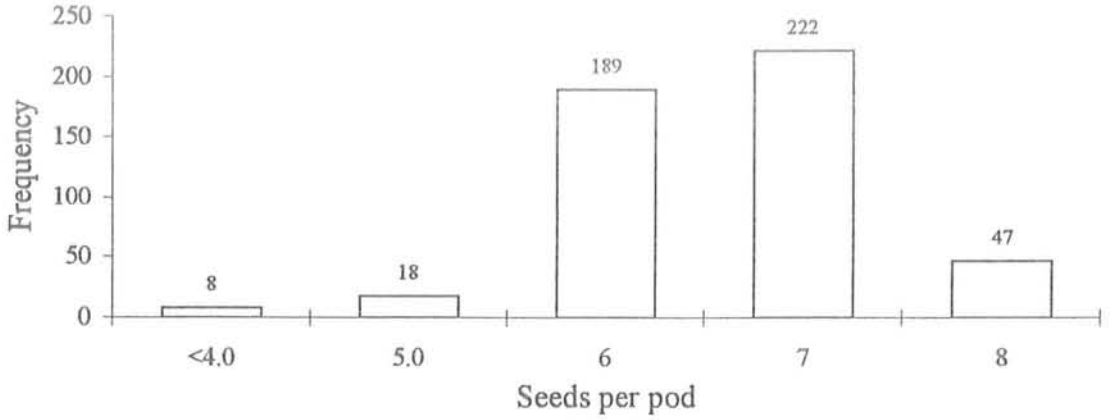


Fig. 4.1.7:- Frequency distribution for seeds/pod in blackgram germplasm

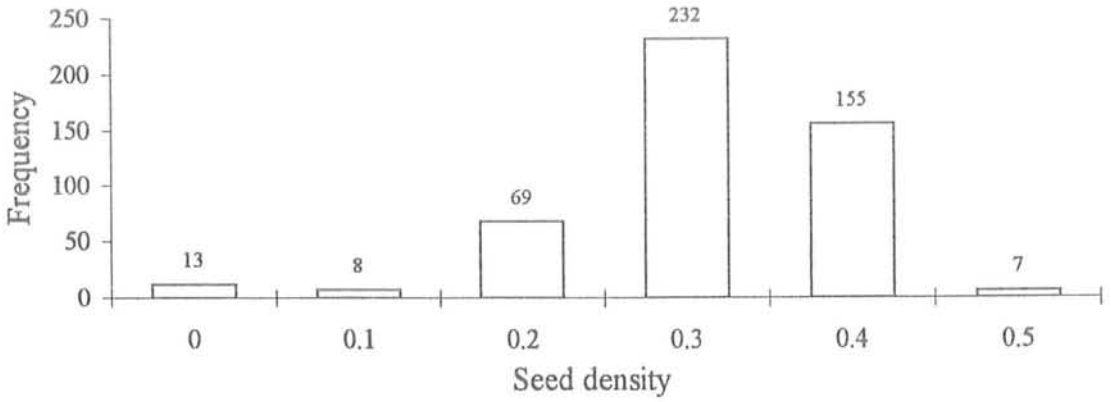


Fig. 4.1.8:- Frequency distribution for seed density in blackgram germplasm

bold seeded blackgram cultivars as high seed weight in any grain crop is preferred by the consumers.

The biological yield ranged from 5.11 to 194.35 g per plant. Frequency distribution revealed that maximum number of accessions (152) which were 31.40% of the population produced 20.1 to 40.0 g biological yield. It was followed by the range 40.1-60.0 g where 115 accessions were observed (Fig. 4.1.10). Twenty four accessions (4.96%) produced more than 100 g biological yield, whereas only three accessions (45311, 45037, 45230) produced more than 150 g biological yield per plant and hence were selected for high biological yield production. On the basis of grain yield per plant, the germplasm ranged from 1.05 to 40.0 g and the frequency distribution presented in Fig. 4.1.11, revealed that the maximum accessions (116) which were 23.97% of the population produced 4.1 to 8.0 g grain yield/plant which was followed by one hundred and ten accessions which produced less than 4.0 g. About 67% of the total germplasm under investigation produced the grain yield up to 12.0 g per plant which is considered the medium range of grain yield in blackgram. In the present material, twenty three accessions were observed as high yielding since they produced more than 24.0 g grain yield per plant. The accessions which produced more than 20 g grain yield per plant are listed in Table 4.1.4 and could be utilized for improving yield potential of blackgram. Frequency distribution regarding harvest index in blackgram as presented in the Fig. 4.1.12, revealed that the maximum accessions (115) which were 23.76% of the total, produced harvest index ranging from 20.1 to 25.0% followed by eighty eight accessions producing 25.1 to 30.0% harvest index. Forty one accessions gave more than 35% harvest index in the present study (Table 4.1.4). From the germplasm analyzed for frequency distribution and simple statistics, the accessions with the best performance for individual characters were selected and presented in Table 4.1.4 which can be exploited for their genetic potential in future breeding programme.

4.1.4 Significance of Qualitative Characters in Determining QTLs

Out of five qualitative characters studied, 3 (hairiness, seed coat colour, spots on seed coat) were observed into 2 distinct classes and hence were used for mean comparison for assessment of QTLs with qualitative markers. Hairiness was denoted by *HH* (hairy), *hh* (non-

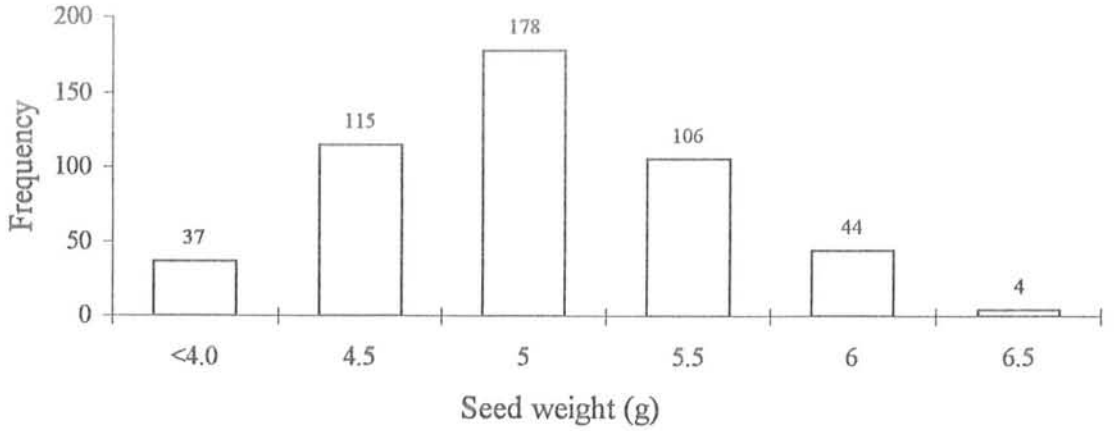


Fig. 4.1.9:- Frequency distribution for seed weight (g) in blackgram germplasm

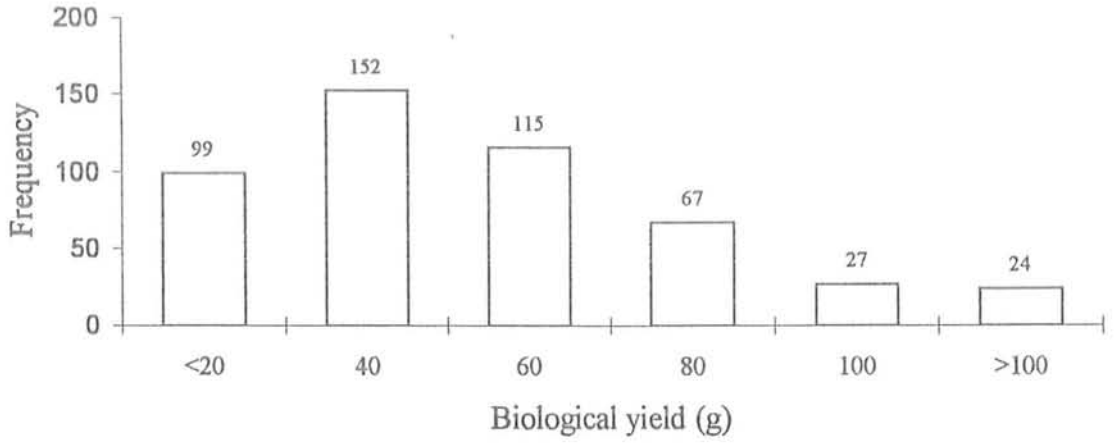


Fig. 4.1.10:- Frequency distribution for biological yield (g) in blackgram germplasm

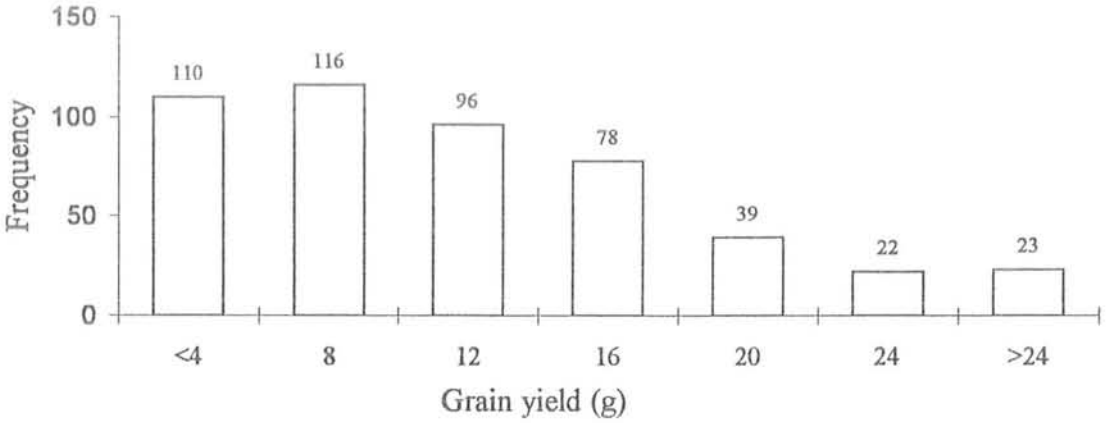


Fig. 4.1.11:- Frequency distribution for grain yield (g) in blackgram germplasm

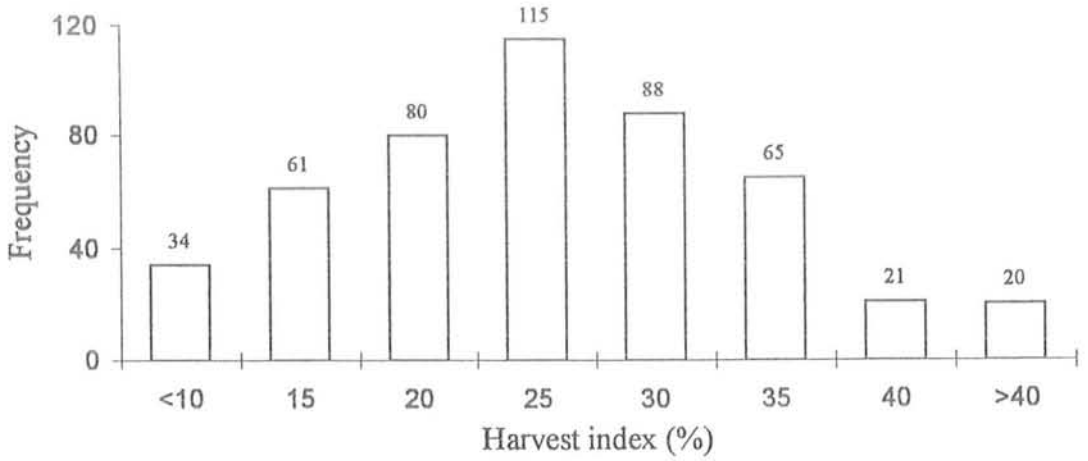


Fig. 4.1.12:- Frequency distribution for harvest index (%) in blackgram germplasm

Table 4.1.4:-The selected accessions on the basis of best performance for specific characters

Character	Range	Accessions selected
Days to flowering	<30 days	45307, 45321, 45371, 45358, 45372, 45317, 45127, 45357, 9025, 45308, 45708
Days to maturity	<65 days	92019, 92052, 92018, 9093, 9039, 45358, 45819, 45820, 9025, 9005, 9007, 9006, 92092, 92024, 9029, 45372, 45357, 45708, 45364, Mash 3, 45814, 45818, 45817, 93017, 9056
Branches/plant	>40	45152, 45178, 45225, 45315, 45096, 45714
Pods/plant	>100	45152, 45060, 45775, 45225, 45311, Mash 1, 45222, 45098, 45037, Mash 3, 45721, 45072, 45309, 45099, 45119, 45094, 45704
Pods/branch	>8	45127, 45336, 45348, 45060, 45072, 45052
Pod length	>6 cm	45223, 45101, 45429, 45334, 45332, 45226, 45353
Seeds/pod	>8 seeds	45429, 45304
Seed density	>0.40	45814, 45404, 45051, 45696, 45395, 45078, 45315
Seed weight	>6 g	45120, 45114, 45102, 45222
Biological yield	>150 g	45311, 45037, 45230
Grain yield	>20g	45775, 45152, 45060, Mash 1, 45221, 45138, 45222, 45063, 45124, 45037, 45717, 45098, 45206, 45721,45225, 45695, 45347, 45055, 45051, 45094, 45072, 9020, 45207, 45036, 45350, 45751, Mash 3, 45136, 45007, 45616, 45333, 45352, 45218, 45047, 45342, 45712, 45091, 9026, 45157, 45336, 45344, 45724, 45074, 45308, 45167
Harvest index	>35%	45339, 45431, 45306, 45609, 45166, 45649, 45727, 45724, 45101, 45387, 45100, 45190, 45207, 9026, Mash 1, 45024, 45326, 45797, Mash 3, 45168, 45349, 45795, 45019, 45013, 45050, 45163, 45072, 45040, 45714, 45169, 45228, 45152, 45171, 9010, 9012, 45080, 45094, 45167, 45318, 45347, 45341

hairy); seed coat colour by *CC* (brown), *cc* (green) and spots on seed coat by *SS* (spots present), *ss* (spots absent). The qualitative data with 2 distinct classes were analyzed to establish relationship between qualitative traits and QTLs under study (Table 4.1.5). Plant pubescence has significant relationship with all the characters except with pods/branch and seed density. It was observed that in general hairy (*HH*) plant genotypes facilitate early maturity, whereas glabrous (*hh*) plants were late in maturity. All other plant characters exhibited increasing effect with the presence of *hh* genes which revealed that this gene has association in increasing yield and yield components. Maximum difference in pods/plant (11.49±3.09), biological yield (9.17±3.26) and grain yield (2.86+0.81) was observed. Similarly for seed coat colour, the germplasm was categorized into brown seeded (*CC*) and green seeded (*cc*); and it was observed that *CC* genes have an increasing effect for most of the yield contributing characters. The difference was significant for flowering (9.56±1.00 days), maturity (22.67±1.77 days), branches (8.80±3.40), pods (16.22±7.29), pods/branch (1.89±0.18), biological yield (15.42±5.61 g), grain yield (4.55±1.78 g) and harvest index (6.00±2.67 %). The average value of accessions with *CC* gene for days to maturity is 85.16±0.60 which is significantly less than accessions with *cc* gene (107.83±1.66). Similar results were observed for spots on seed coat (*SS*). In the present study *HH, CC & SS* genes proved their significance in determining QTLs which might be exploited for selection to improve yield potential.

4.1.5 Principal Component Analysis (PCA)

Variance was further studied by PCA, and a principal components matrix for 12 quantitative characters is given in Table 4.1.6. The first five components with eigenvalues more than 1 contributed 79.5% of the variability amongst 484 accessions evaluated for twelve quantitative traits. Principal component 1 had 28.3% of the total variation, PC_2 17.2%, PC_3 12.9% and PC_4 had 11.0% of the total variation. Characters that contributed more positively to PC_1, were, branches (0.5994), pods (0.9071), biological yield (0.8495) and grain yield (0.9168), whereas days to flowering, maturity and pod length contributed least to first component. Days to flowering (0.8043) and maturity (0.8252) contributed maximum genetic variance to PC_2; seeds per pod (0.6347) and seed density (0.9343) were assessed significant for PC_3. Pod length contributed maximum (0.6841) for PC_4 and pods per branch contributed maximum (0.6435) for PC_5. All the characters under study

Table 4.1.5:- Mean comparison of QTLs on the basis of qualitative characters in 484 accessions of *Vigna mungo*

	Plant Pubescence			Seed Coat Colour			Spots on Seed Coat		
	Glabrous	Hairy	Difference	Green	Brown	Difference	Absence	Present	Difference
DF	49.03±0.95	46.74±0.52	2.30±1.08*	56.67±0.88	47.11±0.46	9.56±1.00**	56.67±0.88	47.11±0.46	9.56±1.0**
DM	88.20±1.15	84.69±0.69	3.51±1.34**	107.83±1.66	85.16±0.60	22.67±1.77**	107.83±1.66	85.16±0.60	22.67±1.77**
BR/PL	17.22±0.78	14.68±0.43	2.54±0.89	23.92±3.38	15.12±0.38	8.80±3.40*	23.92±3.38	15.12±0.38	8.80±3.40*
P/PL	52.86±2.80	41.37±1.31	11.49±3.09**	28.78±7.19	44.00±1.22	16.22±7.29*	28.78±719	44.00±1.22	16.22±7.29
P/BR	3.32±0.17	2.99±0.7	0.32±0.18	1.20±0.16	3.09±0.07	1.89±0.18**	1.20±0.16	3.09±0.07	1.89±0.18**
PL	4.63±0.06	4.49±0.02	0.14±0.06*	4.77±0.19	4.51±0.02	0.26±0.19	4.77±0.19	4.51±0.02	0.26±0.19
S/P	6.30±0.06	6.15±0.04	0.15±0.07*	6.03±0.20	6.18±0.03	0.15±0.20	6.03±0.20	6.18±0.03	0.15±0.20
SD	0.26±0.008	0.26±0.004	0	0.20±0.031	0.26±0.004	0.08±0.032	0.20±0.031	0.26±0.004	0.06±0.03
SW	4.96±0.05	4.72±0.03	0.23±0.06**	4.62±0.26	4.77±0.03	0.15±0.26	4.62±0.26	4.77±0.03	0.15±0.26
BY	51.66±0.92	42.48±1.43	9.17±3.26**	29.21±5.46	44.63±1.31	15.42±5.61*	29.20±5.46	44.63±1.31	15.42±5.61**
GY	12.28±0.73	9.42±0.34	2.86±0.81**	5.53±1.75	10.08±0.32	4.55±1.78*	5.53±1.75	10.08±0.32	4.55±1.78**
HI	24.86±0.82	22.86±0.46	2.00±0.94*	17.35±2.64	23.36±0.41	6.00±2.67	17.35±2.64	23.36±0.41	6.00±2.67*

*, ** - significant at 5 and 1% level of probability, respectively.

DF- days to flowering, DM- days to maturity, BR/PL- branches per plant, P/PL- pods per plant, P/BR- pods per branch, PL- pod length, S/P- seeds per pod, SD- seed density, SW- seed weight, BY- biological yield, GY- grain yield, HI- harvest index.

Table 4.1.6:- Principal components (PCs) for 12 quantitative characters in 484 accessions of *Vigna mungo*

		PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
Eigen value		3.3958	2.0689	1.5507	1.3147	1.2123	0.9511
Proportion of variation		28.3	17.2	12.9	11.0	10.1	7.09
Commutative variance		28.3	45.5	58.5	69.4	79.5	87.4
	Communality	Eigen Factors					
Days to flowering	0.7103	0.1640	0.8043	-0.0133	-0.1031	-0.1604	0.2166
Days to maturity	0.7745	0.1994	0.8252	-0.0933	-0.1788	-0.1145	0.0871
Branches per plant	0.8610	0.5994	-0.1398	-0.1358	0.3594	-0.5785	-0.2286
Pods per plant	0.8789	0.9071	-0.1985	-0.0517	-0.1167	0.0208	-0.1226
Pods per branch	0.9145	0.4443	-0.1609	0.0591	-0.5231	0.6435	0.1280
Pod length (cm)	0.9831	0.1779	0.2906	-0.3169	0.6841	0.5464	-0.1117
Seeds per pod	0.9885	0.3978	0.3023	0.6347	0.5070	0.2809	0.0123
Seed weight (g)	0.3653	0.2884	0.4013	-0.3361	-0.0438	-0.0788	0.1102
Seed density	0.9699	0.2402	0.0443	0.9343	-0.0646	-0.1822	0.3994
Biological yield (g)	0.7836	0.8495	0.0711	-0.0880	-0.2209	0.0155	-0.3150
Grain yield (g)	0.9030	0.9168	-0.2149	-0.1157	0.0025	-0.0535	0.1184
Harvest index (%)	0.4097	0.2379	-0.5159	-0.0834	0.2752	-0.0653	0.7180

contributed genetic variance positively towards PC_1. Seven characters (days to flowering, maturity, pod length, seeds per pod, seed weight, seed density and biological yield) exhibited positive effect on PC_2. In more detail, the first PC which explained 28.3% of the variance is positively associated with all the characters and four important characters (branches, pods, biological yield and grain yield) exhibited more positively, whereas days to flowering, maturity and pod length contributed least. This means that the populations with high PC_1 values are high yielding and formed by medium maturing plants characterized by low seed density and harvest index. Seven characters contributed positively for PC_2 where three characters (days to flowering, days to maturity and seed weight) were observed with highest values for PC_2. It is evident that nine important plant characters contributed more positively to first 3 principal components and hence these could be established important for the material under investigation. The second component is strongly associated with maturity and moderately with seed weight, contributing 17.2% of the total variance. The population with high PC_2 values are characterized by late flowering and maturity. The populations in this component are associated negatively with harvest index, grain yield, branches, pods and pods/branch which revealed that the accessions in the population failed in appropriate partitioning of economic yield which ultimately reduced harvest index.

4.1.6 Harvest Index, a Selection Criterion

Harvest index is considered as an important trait in improving grain yield in most of the crops. Green revolution in cereals is largely supported by the evidence that tremendous increase in harvest index was made which in turn enhanced the world wide cereal productivity. Similar emphasis is being given in legumes to select genotypes with appropriate harvest index. Harvest index in legumes is very tricky and sensitive to environmental fluctuations and it is imperative to find the optimum range of harvest index. In order to find the optimum harvest index alongwith other desirable traits, all the accessions were classified into various groups on the basis of harvest index classes (Table 4.1.7). These results gave interesting clue for the selection of high yielding blackgram cultivars from local germplasm. The accessions with harvest index less than 10.0%, produced the highest mean values for biological yield (53.00±6.97 g), followed by 50.38±0.59% in HI range of 15.1-20.0. The accessions falling in

Table 4.1.7:- Analysis on the basis of harvest index class intervals in 484 accessions of *Vigna mungo*

Characters	Harvest index range (%)						
	<10.0	10.1-15.0	15.1-20.0	20.1-25.0	25.1-30.0	30.1-35.0	>35.0
Days to flowering	48±1.94	50±1.23	52±1.01	47±0.97	45±1.03	44±1.10	44±1.53
Days to maturity	89±2.30	92±1.78	91±1.27	84±1.16	82±1.39	81±1.60	81±1.72
Branches per plant	14.1±1.43	13.3±0.90	14.6±0.76	15.9±0.88	15.6±0.73	15.6±0.90	17.0±1.92
Pods per plant	36.1±4.48	32.0±2.77	41.8±2.47	45.1±2.36	48.7±2.99	48.7±2.88	50.0±5.97
Pods per branch	2.68±0.28	2.54±0.17	2.99±0.15	3.15±0.14	3.30±0.19	3.26±0.15	3.23±0.25
Pod length (cm)	4.5±0.07	4.5±0.06	4.5±0.05	4.4±0.03	4.6±0.05	4.6±0.07	4.5±0.09
Seeds per pod	5.9±0.13	6.1±0.11	6.2±0.08	6.1±0.06	6.4±0.08	6.3±0.08	6.1±0.11
Seed density	0.23±0.02	0.25±0.01	0.26±0.01	0.27±0.01	0.27±0.01	0.27±0.01	0.25±0.01
Seed weight (g)	4.68±0.07	4.68±0.07	4.85±0.07	4.88±0.06	4.70±0.05	4.68±0.06	4.81±0.08
Biological yield (g)	53.00±6.97	40.30±3.46	50.38±0.59	46.04±2.55	44.44±2.99	39.48±2.53	35.26±3.53
Grain yield (g)	4.43±0.65	5.16±0.47	8.98±0.64	10.35±0.58	12.17±0.81	12.74±0.83	14.12±1.32
Frequency	34	61	80	115	88	65	41

Table 4.1.8:- Ranking of variables based on harvest index using communality index as multiplying factor

	I	II	III	Selection Score
Multiplying factor	3*C	2*C	1*C	
<10	BY	PL	-	3.73
10.1-15.0	-	PL	-	1.97
15.1-20.0	-	PL, SD,BY, SW	S/P	7.19
20.1-25.0	SD, SW	BR	DF, DM, PODS, PL, BY	9.86
25.1-30.0	P/BR, PL,S/P, SD	DF, DM, PODS	BR, GY	18.06
30.1-35.0	DF, DM, PL, SD	PODS, P/BR, S/P, GY	BR	18.54
>35	DF, DM, BR, PODS, GY	PL	P/BR, SD, SW	16.60

C- communality of individual character as observed in PCA.

DF- days to flowering, DM- days to maturity, BR- branches/plant, PODS- pods/plant, P/BR- pods/branch, PL- pods length, S/P- seeds/pod, SD- seed density, SW- seed weight, BY- biological yield, GY- grain yield, HI- harvest index.

the range of 20.1 to 25.0% gave the best values for seed density (0.27±0.01) and seed weight (4.88±0.06 g). Harvest index range of 25.1-30.0 gave the best values for pods/branch (3.30±0.19), pod length (4.6±0.05 cm), seeds/pod (6.4±0.08) and seed density (0.27±0.01). The accessions from 30.1 to 35.0 HI produced the best value for flowering (44±1.53 days), maturity (81±1.60 days) and pod length (4.6±0.07 cm), whereas the accessions with more than 35.0% HI gave the best values for flowering (44±1.53 days), maturity (81±1.72 days), branches (17.0±1.92), pods (50.0±5.97) and grain yield (14.12±1.32 g). It is obvious from results that generally the accessions having >35% HI gave the best values for days to flowering , maturity, branches, pods and grain yield. However, due to high SE for most of the values, results may not prove consistency, therefore, selection scores (SC) were established for various HI ranges (Table 4.1.8). For calculation of SC, the ranking of characters within HI range was sorted out, first top three values were picked and multiplied in reverse order, i.e., the first rank was multiplied with number 3, second with 2 and third with 1. To treat all the characters at same level, the product was multiplied with the communality values of individual characters as observed in Principal Component Analysis. These products were then aggregated within the HI range and termed as selection score and plotted in the graphic form as Fig. 4.1.13. Although the preliminary study for finding the best HI it was revealed that HI, more than 35% may prove the best for selection but after a comprehensive sorting and analysis, the highest SC (18.54%) was observed in the range of 30.1-35.0% followed by 25-30.0% HI range (18.06). On the basis of these results, best accessions for specific characters were identified and listed in Table 4.1.4. Keeping in view the results regarding HI and SC, fifty high yielding accessions/genotypes were identified and the data for these have been presented in Appendix I for further use.

4.1.7 Correlation Analysis

The correlation coefficients were computed among all the quantitative traits; days to flowering, days to maturity, branches, pods, pods/branch, pod length, seeds/pod, seed density, seed weight, biological yield, grain yield and harvest index. Correlation analysis was conducted for total germplasm and the lines selected on the basis of previous findings (listed in Table 4.1.4). The results regarding correlation revealed that days to flowering was positively correlated with days to maturity, seed weight and biological yield in both the populations,

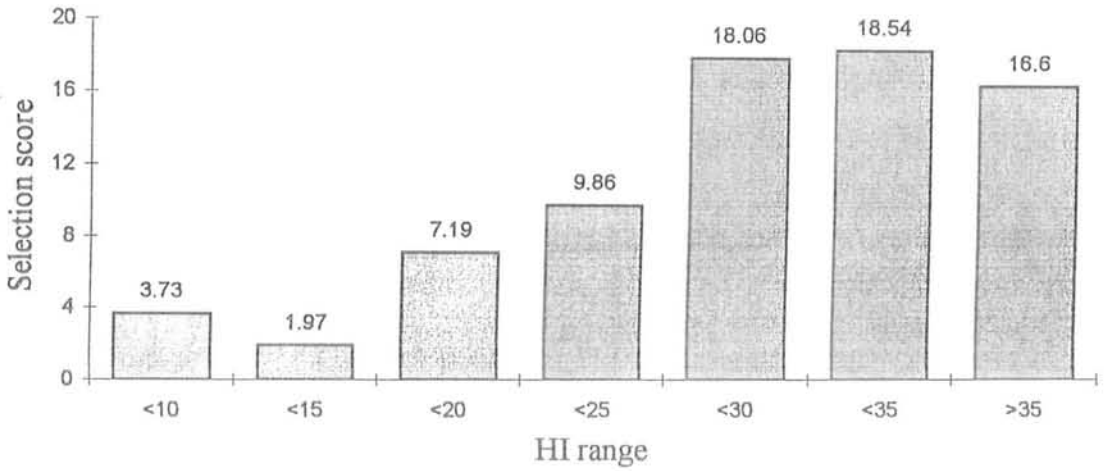


Fig. 4.1.13:- Selection score on the basis of HI and PCA in blackgram

Table 4.1.9:-Correlation coefficients among 12 quantitative characters for two populations in blackgram germplasm

		Days to flowering	Days to maturity	Branches per plant	Pods per plant	Pods per branch	Pod length	Seeds per pod	Seed density	Seed weight	Biological yield	Grain yield
Days to maturity	P	0.8404***										
	S	0.9141***										
Branches/plant	P	0.0069	0.0454									
	S	0.0622	0.1165									
Pods/plant	P	-0.0103	0.0314	0.5969***								
	S	0.1608	0.2439**	0.5582***								
Pod/branch	P	-0.0473	-0.0244	-0.2068**	0.5307***							
	S	0.1425	0.2022*	-0.2947**	0.4545**							
Pod length	P	0.0989*	0.1443**	0.1220*	0.1058*	0.0328						
	S	0.1503	0.0810	0.0337	0.0138	0.0039						
Seeds/pod	P	0.0999*	0.1495**	0.1742**	0.2395**	0.1226**	0.5138***					
	S	0.3006**	0.2707**	0.1322	0.2298**	0.1181	0.4060**					
Seed density	P	0.0007	0.0140	0.0847*	0.1593**	0.0929*	-0.2947**	0.6514***				
	S	0.1129	0.1451	0.0873	0.1844*	0.0915	-0.6008***	0.4796**				
Seed weight	P	0.2668**	0.2833**	0.1370**	0.1289**	0.0168	0.0662	0.0397	-0.0205			
	S	0.3642**	0.3985**	0.1270	0.1349	0.0769	0.0877	0.1083	-0.0021			
Biological yield	P	0.1464**	0.1829**	0.4920**	0.7502***	0.3623**	0.0902*	0.2235**	0.1520**	0.2575**		
	S	0.2979**	0.4137**	0.4544**	0.7249***	0.3072**	0.0011	0.1688	0.1491	0.2169*		
Grain yield	P	0.0171	0.0279	0.5283***	0.8088***	0.3928**	0.1190**	0.2548**	0.1624**	0.2401**	0.7952***	
	S	0.2572**	0.3527**	0.4913**	0.8095***	0.3331**	0.0122	0.2016*	0.1654	0.2750*	0.7758***	
Harvest index	P	-0.2162**	-0.2826**	0.1022*	0.1797**	0.1339**	0.0569	0.0891*	0.0581	-0.0017	-0.1256**	0.3939**
	S	-0.0757	-0.0970	-0.1529	-0.1520	-0.0425	-0.0412	-0.1000	-0.0463	0.0551	-0.5001**	0.0038

P- correlation in pooled germplasm, S- correlation in selected germplasm. *, ** and *** significant at P<0.05, 0.01 and 0.001

whereas in case of pods, pod length, seeds/pod and grain yield, the correlation coefficients differ and it seems that selection pressure changed the relationship among various characters (Table 4.1.9). Days to maturity had significantly positive correlation with seeds per pod, seed weight and biological yield in both the populations. Branches showed significantly positive correlation with pods, biological yield and grain yield for both the populations, whereas it has significantly negative correlation with pods/branch. In general, branches exhibited the correlation in the same direction in all the characters except with days to flowering and harvest index; and with harvest index it differed significantly. Pods had significant correlation with pods/branch, seeds/pod, seed density, biological yield and grain yield in both the populations. Pods/branch had positively significant correlation with biological yield and grain yield for both populations, whereas it had significantly positive correlation with harvest index in pooled population but negative in selected population. Pod length showed significantly positive association with seeds/pod and significantly negative with seed density in both cases. Seeds per pod had significantly positive association with seed density and biological yield. Seed weight showed significantly positive association with biological yield and grain yield for both populations. Biological yield, the important character in determining the yield in blackgram, showed significantly positive correlation with days to flowering, days to maturity, branches, pods, pods/branch, seed weight and grain yield, whereas significantly negative with harvest index for both the populations. Grain yield revealed positively significant correlation with branches, pods, pods/branch, seeds/pods, seed weight and biological yield in both cases whereas with harvest index it was positively significant for pooled germplasm and insignificant for selected genotypes.

4.2 Biochemical (SDS-PAGE) Basis of Genetic Diversity

All the accessions selected for the analysis of SDS-PAGE exhibited a considerable genetic variance for quantitative characters and thus represents the germplasm collections from which this material was derived (Table 4.2.1). High variance was observed for all the characters except pod length, seeds/pod, seed density and seed weight. Similar pattern of genetic variation was also observed in the analyses of total germplasm and hence the results obtained by this study could be of broader spectrum. SDS-PAGE was conducted in various combinations and it was revealed that 11.25% acrylamide gel concentration, 6 µl

Table 4.2.1:- Basic statistics for quantitative characters in 111 accessions of blackgram

Characters	Mean±SE	σ	σ^2	Range
Days to flowering	43.2±0.88	9.32	86.92	30-60
Days to maturity	80.8±1.38	14.57	212.17	60-110
Branches/plant	18.9±0.90	9.45	89.34	2.6-62.0
Pods/plant	53.9±3.05	32.21	1037.33	7.6-195
Pods/branch	3.2±0.16	1.72	2.97	0.3-10.6
Pod length (cm)	4.4±0.028	0.290	0.084	3.7-5.5
Seeds/pod	6.2±0.058	0.609	0.371	4.2-7.8
Seed density	0.279±0.007	0.069	0.005	-0.05-0.43
Seed weight (g)	4.62±0.056	0.589	0.347	3.24-6.01
Biological yield (g)	49.57±2.79	29.35	861.68	5.85-127.57
Grain yield (g)	12.55±0.83	8.78	77.04	1.35-39.21
Harvest index (%)	25.37±0.79	8.34	69.54	5.98-49.34

δ^2 -Variance, δ- Standard deviation

of sample gave the best resolution. The electrophoretic seed protein profiles for most of the accessions were similar, therefore, these were omitted and finally 46 accessions representing the research material were used to construct dendrogram based on SDS-PAGE and quantitative data. In total, 29 protein bands were recorded ranging from the Molecular Weight (MW) of 24 to 66 kd. Many protein subunits of lower MW were also observed but due to inconstancy in reproducibility they were not recorded. Occasionally, variation was also observed in the density or sharpness of a few bands but this variation was not taken in consideration. Out of 29 protein subunits, 20 were polymorphic and 9 were monomorphic (Table 4.2.2). Only polymorphic bands were included in PCA and constructing of dendrogram. On the basis of banding pattern, gel was divided into four regions (Fig. 4.2.1). Region I had 6 bands of more than 66 kd MW of which 5 were polymorphic. Region II ranged from 34 to 66 kd having ten protein peptides, out of which 5 were polymorphic. In this region, the protein bands were observed with high degree of variation in quantitative term and it may be specific to blackgram. Thirteen protein bands were observed in the region III ranging from 24 to 34 kd MW, among them, 10 were polymorphic (Table 4.2.2). Region IV consisted of many weakly stained bands of MW lower than 24 kd and hence were not recorded as the protein peptides with low MW may not be reproducible.

The results obtained after rapid SDS-PAGE electrophoresis showed that the method provided a powerful tool for reliable germplasm discrimination based on genetic differences in seed storage protein comparison in blackgram. Many accessions which were observed similar based on protein pattern were excluded from cluster and Principal Component Analyses.

4.2.1 Significance of Protein Subunits on QTLs

Based on the combination of various banding patterns, 29 protein bands were recognised among all the accessions/cultivars screened and gel was divided into 4 regions (Fig. 4.2.1). As already mentioned, out of 29 protein bands, 20 were polymorphic in nature and 13 bands exhibited significance in detecting one or the other QTLs as observed by comparison of means using "*t*" statistics (Table 4.2.3). Six bands were recorded in the region I and 5 were polymorphic. Protein band 1 was observed with significant mean

Table 4.2.2: Summary of protein peptides observed in blackgram

	MW	Band No.	Total	Polymorphic	Monomorphic	Significant for QTLs
Region I	>66.0kd	B1, B2, B3, B3a, B3b, B3c	6	5 (B1, B2, B3, B3a, B3b)	1	4
Region II	34-66kd	B4, B4a, B5, B5a, B6, B7, B8, B9, B9a, B9b	10	5 (B4a, B5, B5a, B9a, B9b)	5	2
Region III	24-34kd	B9c, B10, B10a, B10b, B10c, B10d, B11, B11a, B12, B12a, 12b, B13, B13a	13	10 (B10, B10a, B10b, B10c, B10d, B11a, B12a, B12b, B13, B13a)	3	7
Total			29	20	9	13

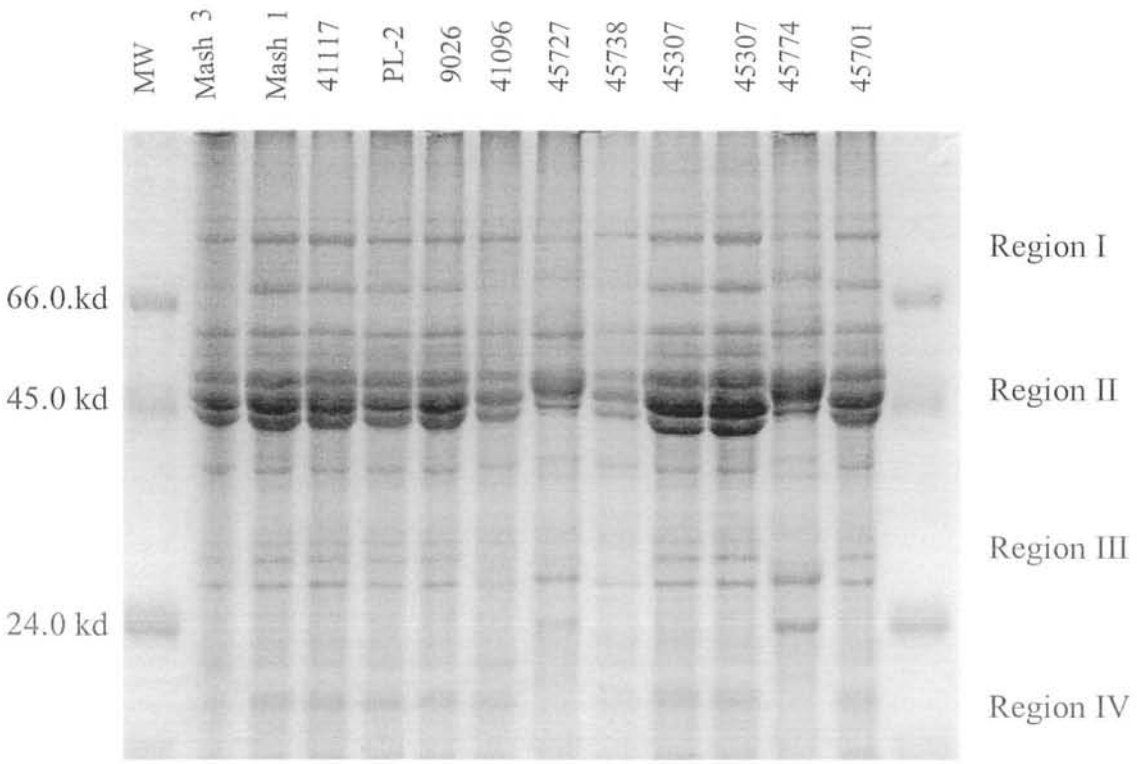


Fig. 4.2.1:- Seed protein profile of *Vigna* spp. The molecular marker used in this gel was SDS-70 KIT.

Table 4.2.3:- QTL detection based on protein peptides in blackgram germplasm

Trait	B1	B2	B3	B3a	B3b	B4a	B5	B5a
DF	0.05±1.88	2.38±2.57	0.64±2.57	3.04±2.51	2.81±5.77	2.23±3.81	3.46±8.05	10.34±1.98***
DM	2.29±2.97	0.26±3.78	1.62±3.65	7.60±2.96*	0.32±6.98	4.21±4.05	1.04±8.12	8.24±2.08***
BR/PL	0.77±1.82	0.02±2.12	1.65±2.47	4.79±2.06*	2.12±4.81	1.32±3.72	9.74±12.53	2.12±4.81
P/PL	2.96±6.61	6.67±10.06	3.51±10.45	10.87±6.30	19.35±9.77	1.78±11.28	1.98±41.12	20.96±8.67
P/BR	0.77±0.44*	0.15±0.49	0.06±0.52	0.14±0.41	0.47±0.38	0.19±0.56	0.12±2.78	0.59±0.47
PL	0.01±0.16	0.03±0.49	0.07±0.91	0.04±0.07	0.01±0.09	0.05±0.11	0.24±0.20	0.17±0.12
S/P	0.17±0.12	0.24±0.17	0.34±0.16*	0.26±0.16	0.20±0.71	0.16±0.24	0.21±0.40	0.75±0.19**
SD	0.02±0.02	0.03±0.02	0.03±0.02	0.04±0.01*	0.01±0.07	0.01±0.02	0.02±0.02	0.06±0.03
SW	0.01±0.12	0.17±0.16	0.16±0.16	0.25±0.16	0.40±0.48	0.22±0.23	0.34±0.44	0.08±0.33
BY	4.06±6.04	5.17±8.64	3.25±0.71	1.93±7.37	25.98±5.78*	9.28±10.44	5.51±3.60	27.01±5.56**
GY	0.42±1.79	1.17±2.42	0.36±2.46	0.66±2.05	10.11±1.09***	1.23±2.99	9.56±2.41	11.32±1.73**
HI	1.86±1.81	1.65±2.79	0.25±2.75	0.14±2.31	4.82±1.52*	1.88±2.79	14.70±2.62*	6.02±2.39
	1	0	1	3	3	0	1	5

-cont.-

Trait	B8	B9	B9a	B9b	B10	B10a	B10b
DF	0.52±2.21	0.59±2.10	0.47±1.98	0.34±2.52	2.08±2.88	2.87±2.99	3.16±3.28
DM	0.78±3.11	0.57±2.95	2.05±3.48	2.01±4.63	5.19±0.23	0.73±0.84	0.42±3.86
BR/PL	1.17±2.60	0.02±2.24	2.85±2.17	2.16±1.93	1.43±2.60	0.89±2.09	4.40±3.60
P/PL	4.53±8.01	5.31±7.45	6.09±6.90	7.60±7.77	3.49±11.29	8.81±8.21	16.58±12.39
P/BR	0.43±0.31	0.19±0.34	0.65±0.37*	0.12±0.54	0.14±067	0.37±0.32	0.18±0.34
PL	0.00±0.06	0.04±0.06	0.05±0.07	0.06±0.10	0.05±0.07	0.01±0.92	0.10±0.09
S/P	0.13±0.15	0.13±0.14	0.29±0.11**	0.31±0.13*	0.04±0.16	0.22±0.19	0.03±0.19
SD	0.01±0.01	0.01±0.01	0.04±0.01**	0.04±0.02**	0.01±0.02	0.02±0.01*	0.01±0.02
SW	0.07±0.11	0.06±0.11	0.25±0.13*	0.19±0.16	0.01±0.16	0.20±0.21	0.06±0.22
BY	0.55±6.60	6.18±6.44	8.91±6.32	12.65±7.25*	3.87±9.92	20.42±9.55*	24.51±10.12*
GY	0.05±2.20	0.89±2.07	1.78±1.84	1.40±2.34	1.33±2.86	4.60±2.76	2.72±3.16
HI	1.24±1.88	2.15±1.78	0.52±1.62	2.30±1.78	0.10±1.97	0.95±3.07	6.31±2.18*
	0	0	4	3	0	2	2

-cont.-

	B10c	B10d	B11	B12	B12a	B12b	B13
DF	0.64±3.18	2.36±4.23	0.95±4.06	2.51±5.48	6.23±5.41	3.46±8.05	2.51±5.48
DM	3.11±3.98	0.86±4.88	1.09±5.29	0.02±5.33	2.42±6.81	1.00±10.10	0.02±5.33
BR/PL	0.70±3.73	3.66±6.54	4.31±3.44	6.44±5.28	9.80±6.60	3.33±3.33	6.44±5.28
P/PL	1.45±12.14	7.50±20.42	3.72±12.19	5.49±15.67	9.02±18.64	8.46±12.15	5.49±15.67
P/BR	0.58±0.30*	0.47±0.37	0.71±0.43	1.08±0.54	1.24±0.86	0.38±0.21	1.08±0.54
PL	0.00±0.10	0.10±0.14	0.00±0.09	0.04±0.28	0.04±0.27	0.16±0.30	0.04±0.15
S/P	0.33±0.18*	0.29±0.15*	0.13±0.21	0.05±0.28	0.21±0.31	0.01±0.40	0.05±0.28
SD	0.04±0.03	0.05±0.02	0.01±0.02	0.00±0.02	0.01±0.01	0.02±0.01***	0.00±0.02
SW	0.16±0.23	0.02±0.35	0.06±0.27	0.28±0.25	0.43±0.27	0.68±0.09**	0.28±0.25
BY	14.79±9.97	15.83±15.77	10.50±13.98	13.38±11.98	18.68±10.57	26.13±12.28	13.38±11.98
GY	3.04±2.47	0.27±3.98	0.91±3.16	4.05±3.62	5.77±2.74	4.94±4.41	4.05±3.62
HI	10.58±2.41***	7.78±2.14**	3.99±3.05	0.30±3.46	2.33±5.05	2.62±2.28	0.30±3.46
	3	2	0	0	0	2	0

difference (0.77±0.44) for pods/branch. Band 2 did not indicate significant differences for any character. Band 3 gave significant difference (0.34±0.16) for seeds/pod. Protein band 3a gave significant mean differences for maturity (7.60±2.96 days), branches/plant (4.79±2.06) and seed density (0.04±0.01), whereas band 3b indicated significant differences for biological yield (25.98±5.78 g), grain yield (10.11±1.09 g) and harvest index (4.82±1.52 %). In region I, among six protein bands having MW more than 66 kd, four protein peptides were found with significant differences for QTL and 2 bands (3a and 3b) gave significant differences for six important yield contributing characters, hence could be used for screening blackgram germplasm. Region II (34 to 66 kd) consisted of ten protein peptides and out of these 5 were polymorphic. Out of 5 polymorphic protein peptides, 2 were observed significant for QTL detection, whereas others were insignificant. Band 5 revealed significant differences for harvest index (14.70±2.62 %); 5a gave significant differences for flowering (10.34±1.98 days), maturity (8.24±2.08 days), seeds/pod (0.75±0.19), biological yield (27.01±5.56 g) and grain yield (11.32±1.73 g). This protein peptide of region II proved very important for detection of important yield contributing characters. The region III consisted 13 bands and out of these 10 were polymorphic and 7 protein peptides were recorded important for QTL association (Table 4.2.2). Protein band 9a gave significant differences for pods/branch (0.65±0.37), seeds/pod (0.29±0.11), seed density (0.0.4±0.01) and seed weight (0.25±0.13 g), while band 9b exhibited significant differences for seeds/pod (0.31±0.13), seed density (0.04±0.02) and biological yield (12.65±7.25 g). These two protein peptides were observed important for seed characters. Band 10a indicated significance for seed density and biological yield with differences of 0.02±0.01 and 20.42±9.55 g, respectively. Band 10b exhibited significant differences for biological yield (24.51±10.12 g) and harvest index (6.31±2.18 %); 10c was observed significant for pods/branch (0.58±0.30), seeds/pod (0.33±0.18) and harvest index (10.58±2.41 %); 10d gave significant differences for seeds/pod (0.29±0.15) and harvest index (7.78±2.14 %). Protein peptides (10, 11, 12, 12a, 13) in region III did not prove any significance for QTL detection. Band 12b was observed important for seed characters, i.e., seed density (0.02±0.01) and seed weight (0.68±0.09 g).

To detect QTL association between SDS-PAGE and morphological characters, correlation among protein peptides and transformed data for quantitative characters were also conducted using computer software SPSS for Windows. Thirty two significant differences observed from a total of 240 mean comparisons using "*t*" statistics are presented alongwith the results of correlation coefficients in Table 4.2.4 and summary of these results are given in Table 4.2.5. The band 5a affected days to flowering as accessions with negative signal for this protein peptide have mean value of 43.7±0.89 for days to flowering, whereas its presence gave mean value of 33.3±1.76 indicating a difference of about 10 days in flowering alongwith significant correlation coefficient. This protein peptide was observed significant for both "*t*" and "*r*" statistics, hence it can be said more confidently that this protein peptide has association with days to flowering. The protein bands B3a and B5a exhibited significant differences for maturity with mean differences of 7.60±2.96 and 8.24±2.08 days, respectively. Band 3a revealed a significant difference of 4.79±2.06 for branches/plant. Three protein peptides (B1, B9a, B10c) were observed with significant differences for pods/branch with mean values of 0.77±0.44, 0.65±0.37 and 0.58±0.30, respectively. It was positively associated for B1 and B9a and hence these two peptides could be preferred for screening blackgram germplasm for pods/branch. Seeds/pod were affected by maximum bands; B5a revealed maximum mean difference of 0.75±0.19, followed by 0.34±0.16 observed for B3. Five peptides (B3, B5a, B9a, B9b, B10c) were observed significant for both *t* and *r* statistics in case of seeds/pod. Protein peptides B9a and B12b revealed significant differences for seed weight with mean differences of 0.25±0.13 and 0.68±0.09 g, respectively and it was also significantly associated as observed in correlation analysis (Table 4.2.4). Presence of band 12b gave high seed weight average (5.30±0.07 g) and hence blackgram germplasm could be screened for seed weight using this protein marker for practical improvement. For biological yield, 5 bands (B3b, B5a, B9b, B10a, B10b) were observed significant. Maximum difference (27.01±5.56) was observed in case of B5a and it was followed by B3b with a mean difference of 25.98±5.78 g and in both these cases, positive signal of bands increased biological yield. Four bands (B5a, B9b, B10a, B10b) were observed significant for both "*t*" and "*r*" statistics. Protein bands B3b and B5a gave significant

Table 4.2.4:- Protein subunits significant for detecting QTLs in blackgram

QTL	Band	Absence	Presence	*t* statistics	*r* statistics
DF	B5a	43.7±0.89	33.3±1.76	10.34±1.97**	0.1624*
DM	B3a	79.78±1.54	87.39±2.53	7.60±2.96**	0.1420
	B5a	81.2±1.41	73.0±1.53	8.24±2.08***	0.0136
BR/PL	B3a	19.71±0.99	14.92±1.80	4.79±2.06*	0.1887*
P/BR	B1	3.67±0.41	2.91±0.13	0.77±0.44*	0.2107*
	B9a	3.34±0.19	2.70±0.32	0.65±0.37*	0.1703*
	B10c	3.21±0.18	2.64±0.24	0.58±0.30*	0.1009
S/P	B3	5.92±0.14	6.26±0.06	0.34±0.16*	0.2008*
	B5a	6.2±0.06	6.9±0.18	0.75±0.19**	0.1951*
	B9a	6.29±0.07	6.00±0.08	0.29±0.11**	0.2135*
	B9b	6.26±0.06	5.95±0.11	0.31±0.13*	0.1839*
	B10c	6.24±0.06	5.91±0.17	0.33±0.18*	0.1694*
	B10d	6.22±0.06	5.93±0.13	0.29±0.15*	0.1159
SD	B3a	0.27±0.007	0.31±0.012	0.036±0.014**	0.1917*
	B9a	0.29±0.008	0.25±0.010	0.038±0.013***	0.2537***
	B9b	0.29±0.007	0.25±0.014	0.041±0.015**	0.2194*
	B10a	0.28±0.007	0.30±0.012	0.024±0.014*	0.1111
	B12b	0.28±0.007	0.26±0.00	0.019±0.007***	0.0382
SW	B9a	4.70±0.06	4.45±0.11	0.25±0.13*	0.1964*
	B12b	4.62±0.06	5.30±0.07	0.68±0.09***	0.1551*
BY	B3b	48.87±2.83	74.85±5.04	25.98±5.78**	0.1440
	B5a	48.84±2.83	75.85±4.79	27.01±5.56**	0.1496*
	B9b	51.62±3.04	38.97±6.58	12.65±7.25*	0.1595*
	B10a	47.36±2.86	67.78±9.11	20.42±9.55*	0.2172*
	B10b	47.14±2.82	71.65±9.72	24.51±10.12*	0.2505***
GY	B3b	12.28±0.84	22.38±0.70	10.11±1.09***	0.1874*
	B5a	12.25±0.84	23.56±1.51	11.32±1.73***	0.2097*
HI	B3b	25.24±0.81	30.06±1.28	4.82±1.52*	0.0949
	B5	39.81±2.51	25.10±0.78	14.70±2.62*	0.2354**
	B10b	25.99±0.81	19.68±2.69	6.31±2.81*	0.2271**
	B10c	26.42±0.78	15.84±2.82	10.58±2.41***	0.3801***
	B10d	25.79±0.81	18.01±1.98	7.78±2.14***	0.2117*

*, ** and *** significant at P<0.05, 0.01 and 0.001

DF- days to flowering, DM- days to maturity, BR/PL- branches/plant, P/PL- pods/plant, P/BR- pods/branch, PL- pod length, S/P- seeds/pod, SD- seed density, SW- seed weight, BY- biological yield, GY- grain yield, HI- harvest index.

Table 4.2.5:- Summary for QTL detection based on SDS-PAGE markers in blackgram

Bands	DF	DM	BR/PL	P/BR	S/P	SD	SW	BY	GY	HI	*	**	Total
B1				**							0	1	1
B3					**						0	1	1
B3a		*	**			**					1	2	3
B3b								*	**	*	2	1	3
B5										**	0	1	1
B5a	**	*			**			**	**		1	4	5
B9a				**	**	**	**				0	4	4
B9b					**	**		**			0	3	3
B10a						*		**			1	1	2
B10b								**		**	0	2	2
B10c				*	**					**	1	3	3
B10d					*					**	1	1	2
B12b						*	**				1	1	2
*	0	2	0	1	1	2	0	1	0	1	8	24	32
**	1	0	1	2	5	3	2	4	2	4	24		
Total	1	2	1	3	6	5	2	5	2	5	32		

differences of 10.11±1.09 and 11.32±1.73, respectively for grain yield and it was significant for both statistics. The positive signal in both protein bands increased grain yield and hence can be exploited for germplasm screening for yield potential. Harvest index was observed significant for five bands, i.e., B3b, B5, B10b, B10c and B10d. Except B3b, all others were also significantly associated and negative signal gave high harvest index.

Two protein peptides revealed significant differences for days to maturity; one for branches per plant; three for pods per branch, six for seeds per pod, five for seed density, two for seed weight, five for biological yield, two for grain yield and five for harvest index. In total, thirty two cases were recorded with significant mean differences which revealed that about 13.3% of the total polymorphic bands showed importance in detecting QTL on the basis of protein peptide in blackgram. The summary of these results as presented in Table 4.2.5 revealed that twenty four combinations out of 240 with two stars were observed significant for "*t*" and "*r*" statistics and other eight combinations with one star were observed significant for *t* statistics only. It is evident from Table 4.2.5 that thirteen protein peptides distributed in all three regions were observed significant for detecting QTLs in blackgram. B3a and B5a were observed significant for maturity characters; B3b and B5a were important for grain yield; B9a, B9b and B12b were important for seed characters and B5, B10b, B10c and B10d were significant for detection of harvest index.

4.2.2 PCA Based on Agronomic Characters

Variance was further studied by PCA using 12 quantitative characters. The first five components for which eigenvalues were greater than 1, contributed 82.8% of the variation amongst 111 accessions evaluated for twelve quantitative traits (Table 4.2.6). The contribution by first five PCs was slightly higher in this case as compared to PCs in total population where it was 79.5%. Principal component 1 had 33.5% of the total variation, PC_2 16.8%, PC_3 12.2% and PC_4 had 11.9% of the total variation. Characters which contributed more positively to PC_1, were pods (0.8515), seeds/pod (0.6288), seed weight (0.5244), biological yield (0.8456) and grain yield (0.9135). Days to flowering and maturity contributed maximum genetic variance to PC_2 , whereas branches/plant and

Table 4.2.6:- Principal components based on 12 quantitative characters in 111 selected genotypes of blackgram

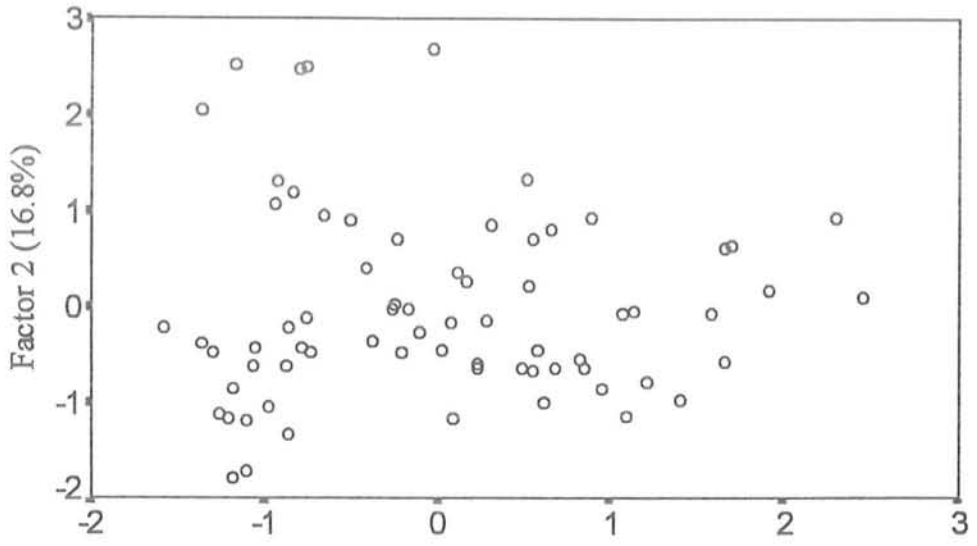
	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
Eigen values	4.02	2.02	1.46	1.43	1.01	0.75
Proportion of variance	33.5	16.8	12.2	11.9	8.4	6.3
Commutative variance	33.5	50.3	62.5	74.4	82.8	89.1
	Eigen Factors					
	1	2	3	4	5	6
Days to flowering	0.4044	0.5794	0.2490	0.3904	0.0975	0.3850
Days to maturity	0.3411	0.7130	0.2324	0.3043	0.0449	0.2217
Branches/plant	0.3629	0.2435	0.7124	0.1020	0.4492	0.0408
Pods/plant	0.8515	0.1749	0.1647	0.2311	0.1456	0.0277
Pods/branch	0.4760	0.4256	0.6653	0.1708	0.2039	0.0610
Pod length (cm)	0.3287	0.3720	0.3459	0.2876	0.6962	0.0045
Seeds/pod	0.6288	0.1734	0.3030	0.5462	0.3906	0.1482
Seed density	0.4105	0.4643	0.0882	0.7550	0.0411	0.1357
Seed weight (g)	0.5244	0.4585	0.0171	0.2028	0.1518	0.1848
Biological yield (g)	0.8456	0.0862	0.1658	0.1582	0.2240	0.3261
Grain yield (g)	0.9135	0.1557	0.0563	0.2125	0.1308	0.0640
Harvest index (%)	0.3962	0.5253	0.3422	0.1796	0.0417	0.6048

pods/branch gave the highest value for PC_3 with PCA values 0.7124 and 0.6653, respectively. Seed density and pod length were assessed significant for PC_4 and PC_5, with values 0.7550 and 0.6962, respectively. Harvest index contributed maximum to PC_6. The variation for pods/branch and harvest index was scattered unevenly among various components. Five important characters namely pods, seeds/pod, seed weight, biological yield and grain yield exhibited maximum contribution, whereas most of the other characters were not observed very important for the contribution in PC_1. It indicates that the populations with high PC_1 values are high yielding and characterised with low seed density and harvest index. Days to flowering, maturity and seed weight contributed maximum to PC_2. It is evident that seven important plant characters contributed more positively to first 2 principal components and if we consider PC_3, nine important characters contributed more positively and first 3 components shared 62.5% of the total variance in the present material and hence these could be established important for the material under investigation. The second component is strongly associated with maturity and moderately with seed weight, contributing 16.8% of the total variance.

First 3 components which contributed 62.5% of the total variance, were plotted graphically to observe the relationship between blackgram germplasm for these components. The separation on the basis of PC_1 and 2 revealed 2 major groups and other accessions were scattered throughout the plot (Fig. 4.2.2). Most of the genotypes were observed overlapping in the graph and protein pattern was also identical when observed critically, therefore, finally 46 genotypes were retained for further cluster analyses based on agronomic characters and protein bands.

4.2.3 Cluster analysis

A Euclidean dissimilarity coefficient matrix was calculated for 46 blackgram accessions from the morphological data and phenogram constructed is presented in the Fig. 4.2.3. The cluster diagram using Ward's method revealed 2 major groups and if it is observed critically, 7 clusters were observed. The group A consisted of 4 and group B consisted of 3 clusters. All the advance breeding lines were observed in the group A, whereas in group B, no advance breeding line was recorded. As this cluster analysis is based on agriculturally important characters, hence advance breeding lines were



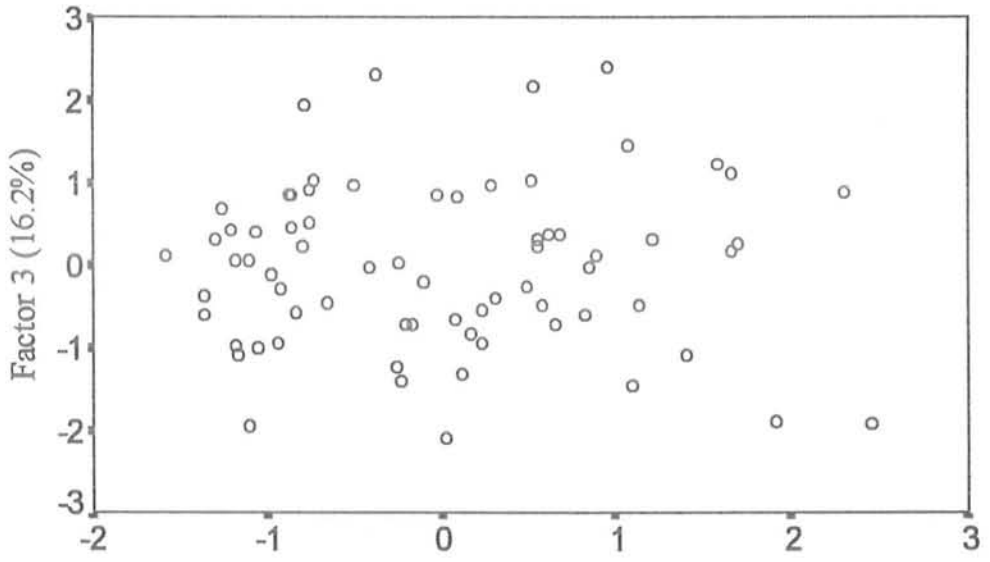


Fig. 4.2.2 :- Scattered diagram of first three PCs for 12 quantitative characters in blackgram

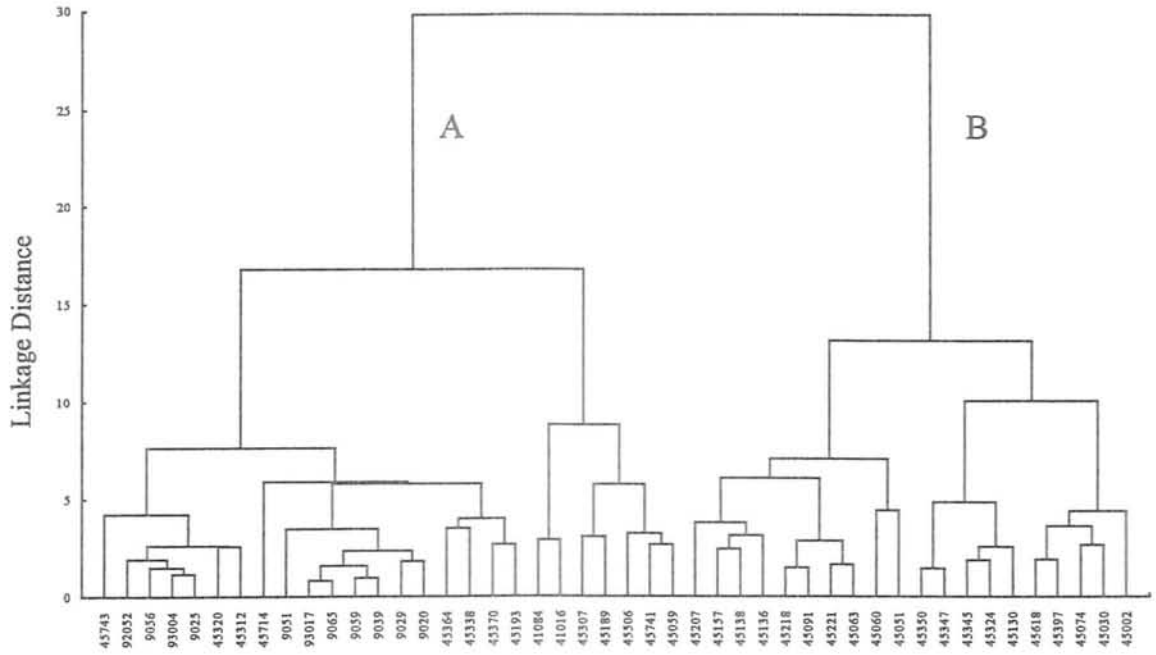


Fig. 4.2.3:- Phenogram based on 12 quantitative characters in blackgram

categorised in one group which may be because of selection pressure for high yield potential and other related characters.

It is evident from the Table 4.2.7 that seven accessions (45743, 92052, 9056, 93004, 9025, 45320, 45312) were observed in Cluster I, twelve (45714, 9051, 93017, 9065, 9059, 9039, 9029, 9020, 45364, 45338, 45370, 45193) in cluster II, two (41084, 41016) in cluster III, five (45307, 45189, 45506, 45741, 45059) in cluster IV of group A. Ten accessions (45207, 45157, 45138, 45136, 45218, 45091, 45221, 45063, 45060, 45051) were observed in cluster V, five accessions (45350, 45347, 45345, 45324, 45130) were observed in cluster VI and five (45618, 45397, 45074, 45030, 45002) in cluster VII of group B. All the advance breeding lines were present in clusters I and II. Both the green seeded genotypes (41084, 41016) were grouped together in cluster III of group A. The cluster I consisted 7 accessions, out of which one (45743) was collected from Northern Areas of Pakistan, 45320 and 45312 were from Punjab and others were advance breeding lines. In this cluster, the genotypes gave average of 34.29±4.07 days to flowering, 66.50±6.11 days to maturity and 4.26±1.77 g grain yield, therefore, were classified as early maturing with lower grain yield (Table 4.2.8). Cluster II consisted of 12 genotypes and all were originated from Punjab either as germplasm collections or as advance breeding lines of research institutes. This cluster is also categorised as early flowering (37.68±4.30 days), maturing (69.31±6.55 days) with medium grain yield (9.10±4.34 g). Cluster III consisted of two green seeded genotypes which were catagorized as late maturing (109.25±1.77 days to maturity) and low yielder (3.94±2.94 g). A mixture of genotypes from Punjab and Baluchistan comprising of cluster IV were categorised as medium maturing (83.80±11.65 days) and low yielder (5.50±2.03 g) with low harvest index (12.48±6.24 %). High SD in this cluster indicted that accessions of this cluster may be mixture of diverse origins. Cluster V consisted of 10 genotypes collected from Punjab and categorised as high yielding (25.84±4.16 g) and high harvest index (29.20±5.52 %), maturing late (92.30±10.59 days), but high variance for days to maturity indicated that there is a scope of selection for maturity. Cluster VI consisted of 5 genotypes, all from Punjab, falling in high yielding range (17.23±7.56 g) but earlier than cluster V with mean value of 71.20±1.79 for days to maturity. Cluster VII was also consisting of 5 genotypes

Table 4.2.7:- Clusters based on quantitative characters in blackgram

Group	Cluster	Frequency	Accessions
Group A	Cluster I	7	45743, 92052, 9056, 93004, 9025, 45320, 45312
	Cluster II	12	45714, 9051, 93017, 9065, 9059, 9039, 9029, 9020, 45364, 45338, 45370, 45193
	Cluster III	2	41084, 41016
	Cluster IV	5	45307, 45189, 45506, 45741, 45059
Group B	Cluster V	10	45207, 45157, 45138, 45136, 45218, 45091, 45221, 45063, 45060, 45051,
	Cluster VI	5	45350, 45347, 45345, 45324, 45130
	Cluster VII	5	45618, 45397, 45074, 45030, 45002

Table 4.2.8:- Mean and standard deviation for clusters based on quantitative characters in blackgram

Character	Group A				Group B		
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
DF	34.29±4.07	37.68±4.30	56.50±1.41	46.40±12.76	49.60±8.78	32.60±2.51	53.20±3.96
DM	66.50±6.11	69.31±6.55	109.25±1.77	83.80±11.65	92.30±10.59	71.20±1.79	94.40±5.27
BR/PL	9.50±4.49	21.28±8.57	17.83±0.00	12.58±4.25	22.24±4.78	14.90±3.78	15.15±4.67
P/PL	24.44±8.31	36.47±12.21	16.50±9.90	34.97±15.38	93.10±26.86	76.15±9.84	61.32±20.35
P/BR	2.86±1.21	1.95±0.72	0.99±0.66	2.98±1.68	4.46±1.93	5.37±1.54	4.06±0.44
PL	4.28±0.21	4.49±0.24	5.28±0.29	4.38±0.21	4.57±0.25	4.54±0.22	4.32±0.24
S/P	5.86±0.11	6.27±0.50	6.35±0.21	5.40±0.32	6.76±0.39	6.04±0.38	6.36±0.55
SD	0.27±0.03	0.28±0.03	0.17±0.02	0.19±0.07	0.32±0.06	0.25±0.02	0.32±0.03
SW	4.13±0.41	4.50±0.49	5.18±0.95	5.36±0.34	5.25±0.45	4.45±0.31	4.22±0.35
BY	20.51±12.52	34.71±13.36	19.74±10.19	50.92±23.88	91.13±20.78	62.94±16.32	72.85±26.53
GY	4.26±1.77	9.10±4.34	3.94±2.94	5.50±2.03	25.84±4.16	17.23±7.56	10.90±5.42
HI	22.40±5.63	26.83±7.27	20.57±2.60	12.48±6.24	29.19±5.52	26.51±5.93	16.26±7.65

and catagorized as late in maturity (94.40±5.27 days), medium yielding (10.90±5.42 g) with medium harvest index (16.26±7.65 %). It was noted that the genotypes from clusters III, IV and V were categorised as bold seeded with an average seed weight of 5.18±0.95, 5.36±0.34, and 5.25±0.45 g, respectively and hence are suggested to be utilized for improvement of seed weight in blackgram. The genotypes in the clusters V and VI of group B were observed high yielding, whereas genotypes of clusters I, II and VI were early maturing and hence selected genotypes from respective clusters are suggested to be used in crop improvement programme.

4.2.4 PCA Based on Protein Peptides

As already mentioned that genetically diverse 46 accessions were derived from 111, based on protein pattern and for these, variance was studied by PCA. The variance was rather more scattered on the basis of protein pattern as compared with quantitative characters. The first eight components contributed 75.6% of the variation amongst selected 46 accessions (Table 4.2.9). Principal component 1 had 19.7% of the total variation, PC_2 11.3%, PC_3 9.7%, PC_4 9.1% and PC_5 8.8% of the total variation, respectively. The protein bands which contributed more positively to PC_1, were B3, B10b, B12a, B12b and B13. Three bands (B10, B10c, B10d) contributed maximum genetic variance to PC_2 , whereas four bands (B3b, B5, B5a and B11a) exhibited the highest value for PC_3. The bands (B1, B2 and B9b) were assessed significant for PC_4 and B9a contributed more positively to PC_5. Other components were observed with varying degrees of variance. It was noted that out of fifteen bands, which contributed more positively for first 5 components, eleven were also found significant for detection of QTLs (Table 4.2.5). First three components which contributed about 40.8 % of the total variation were plotted as scatter diagram (Fig. 4.2.4). The separation on the basis of PC 1 and 2 revealed 1 major group and other accessions scattered which revealed the extent of variation that many accessions included in the present study were more similar on SDS-PAGE basis. The accessions 45136, 45157, 45059, 45060, 45347, 45002, 45741, 45714, 45312, 45370 and 45218 were observed separate from major group, hence more diversified on SDS-PAGE basis. One advance breeding line 9029 was also away from major group and morphologically, it is glabrous, early and low yielding genotype. Almost

Table 4.2.9:- Principal components based on SDS-PAGE in 111 selected genotypes of blackgram

	PC_1	PC_1	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9	PC_{10}
Eigen value	4.93	2.83	2.44	2.29	2.20	1.54	1.37	1.30	0.97	0.92
variance	19.7	11.3	9.7	9.1	8.8	6.2	5.5	5.2	3.9	3.7
	19.7	31	40.8	49.9	58.7	64.9	70.4	75.6	79.5	83.1
	Eigen Factors									
Protein bands	1	2	3	4	5	6	7	8	9	10
B1	0.2182	0.3841	0.0965	0.4529	0.2907	0.2191	0.1312	0.3245	0.1683	0.2132
B2	0.4146	0.4553	0.1071	0.5463	0.1073	0.3266	0.0972	0.2770	0.0545	0.1650
B3	0.5208	0.5154	0.0517	0.4475	0.1636	0.2868	0.0574	0.2600	0.0739	0.0655
B3a	0.0525	0.1018	0.1011	0.1357	0.2401	0.4630	0.2834	0.3009	0.0585	0.4949
B3b	0.2419	0.3832	0.5725	0.3663	0.0371	0.2236	0.0386	0.0079	0.0459	0.1212
B4a	0.1021	0.0576	0.3781	0.0799	0.1449	0.4326	0.0757	0.1106	0.2766	0.0014
B5	0.3651	0.3344	0.6387	0.0376	0.0939	0.2996	0.2123	0.1344	0.0448	0.0455
B5a	0.2457	0.4059	0.5574	0.3321	0.1449	0.1683	0.0337	0.0085	0.0762	0.1352
B9a	0.0854	0.2636	0.1410	0.5329	0.6177	0.0247	0.2140	0.1869	0.0506	0.0191
B9b	0.2109	0.0969	0.0489	0.5358	0.4971	0.2145	0.2109	0.0009	0.3693	0.1836
B10	0.2743	0.4315	0.0482	0.3039	0.1819	0.1874	0.1797	0.3694	0.2844	0.0576
B10a	0.3627	0.1670	0.1703	0.1241	0.2673	0.0726	0.3647	0.5398	0.2239	0.3215
B10b	0.5738	0.4030	0.3246	0.0353	0.0987	0.0959	0.4400	0.0884	0.1320	0.0457
B10c	0.4165	0.5693	0.1343	0.1841	0.0731	0.0110	0.0490	0.2629	0.3762	0.0375
B10d	0.5315	0.6787	0.1571	0.0686	0.1062	0.0067	0.1248	0.1704	0.2161	0.0009
B11a	0.4237	0.2443	0.6587	0.2864	0.0720	0.1641	0.1337	0.0134	0.1901	0.2231
B12a	0.8232	0.2366	0.3595	0.0983	0.0033	0.0195	0.1862	0.1724	0.1190	0.0486
B12b	0.7026	0.4619	0.0296	0.0836	0.0472	0.1403	0.1320	0.2197	0.2802	0.0992
B13	0.8967	0.0732	0.0459	0.2416	0.0771	0.0510	0.2064	0.0072	0.0928	0.1517
B13a	0.0320	0.0443	0.0196	0.1591	0.3176	0.1998	0.2459	0.4213	0.3778	0.4801

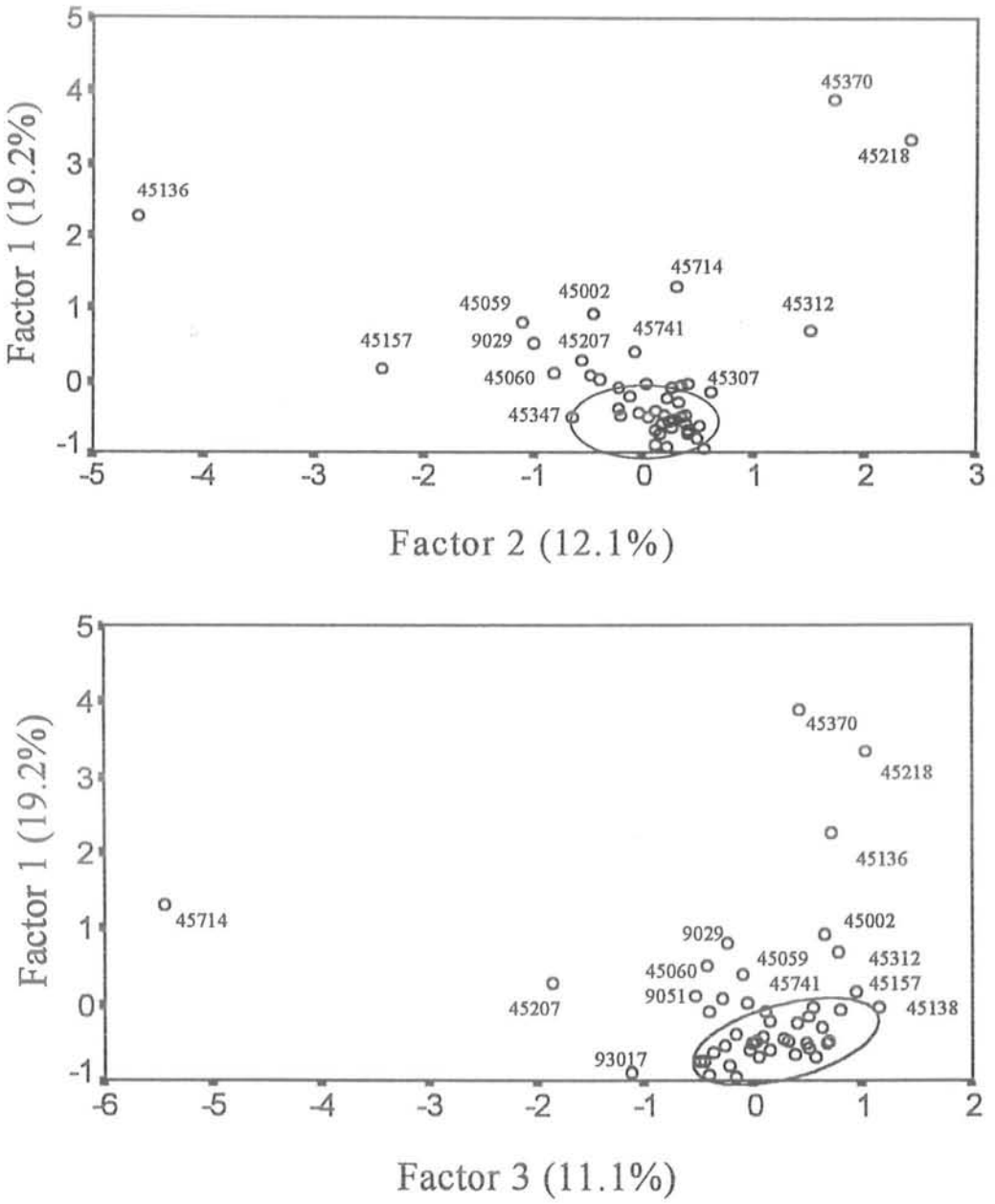


Fig. 4.2.4:- Scattered diagram of first three PCs based on 20 SDS-PAGE bands in blackgram

similar pattern was observed in diagram for PC_1 and PC_3 where one major group and others (same accessions as in PC_1 & PC_2) were scattered. The main cluster was observed near the origin (zero on XY scale) for both combinations.

4.2.5 Cluster analysis

The dendrogram of total seed proteins based on dissimilarity matrix using UPGMA showed a division into three major groups, A, B and C (Fig. 4.2.5). Group A comprised of 2 clusters, group B also of 2 clusters, whereas the group C consisted of one cluster only. It is evident from the results that 11 accessions were observed in the group A, twenty four in the group B and 11 in the group C (Table 4.2.10). The advance breeding lines were distributed in all three groups. Green seeded genotypes (41084, 41016) were present together in the cluster III of group B (Table 4.2.10). Group A consisted of 11 genotypes and out of these, two genotypes (9059, 9029) were advance breeding lines. Group B comprised of 24 genotypes of diverse origins. Similarly group C comprising of 11 genotypes also did not reveal any clear indication of geographic distribution. In general cluster analysis based on SDS-PAGE in blackgram germplasm did not reflect any clue either for agronomic preference and/or geographic distribution.

The analysis for each cluster was performed (Table 4.2.11) which revealed that the genotypes comprising of cluster I gave mean maturity of 84.75±15.91 days and grain yield of 13.22±12.01g. Cluster II was observed medium maturing (81.00±11.49 days), high yielding (16.42±7.86 g) with medium to high harvest index (25.65±7.53 %). Cluster III of group B was medium in maturity (78.06±21.56 days) and medium yielding (7.15±5.78 g). The accessions of cluster IV matured in 79.63±13.42 days and gave medium yield of 14.75±8.41 g and exhibited medium harvest index (24.64±5.91 %). Similarly, accessions grouping in cluster V matured in 79.93±13.81 days, produced 12.69±10.33 g yield per plant and 20.83±11.85 % of harvest index. In the present study, clusters based on SDS-PAGE did not give any indication for quantitative variation as 4 out of 5 clusters were categorised as high yielding and medium maturity, whereas cluster III was also medium in maturity and grain yield. For most of other characters, no clear observation was recorded which could facilitate selection on the basis of SDS-PAGE for improving agronomic traits in blackgram from the material under investigation. Further

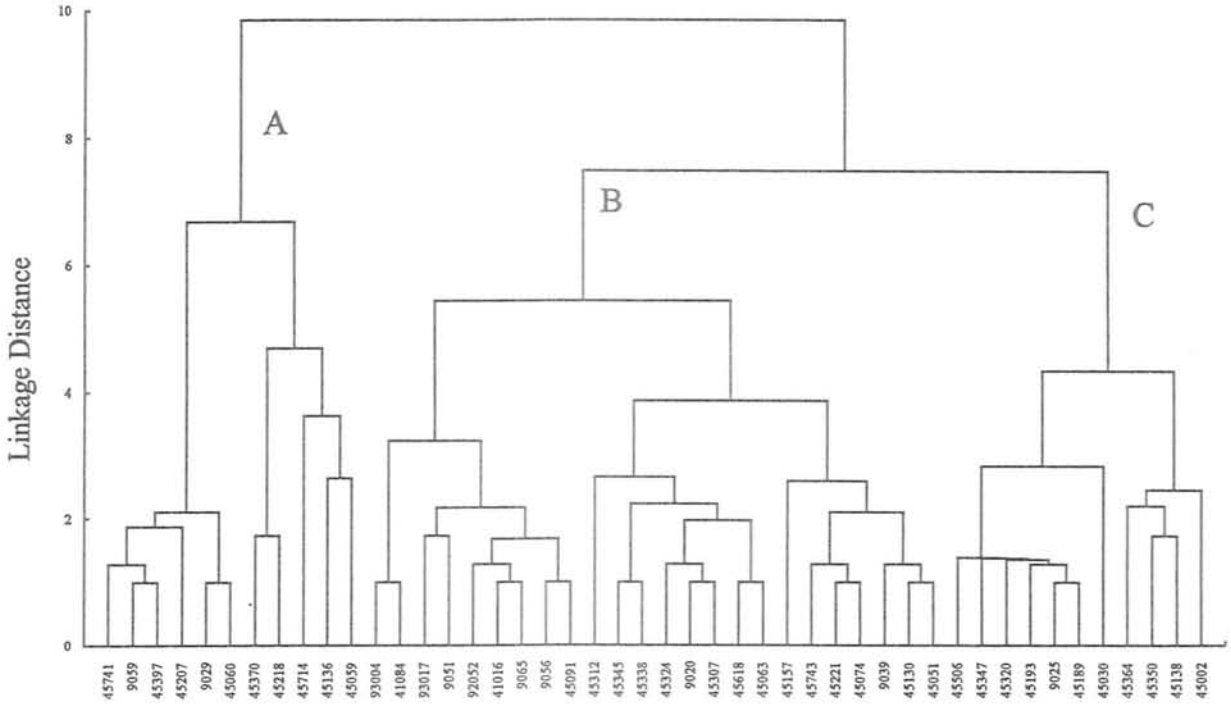


Fig. 4.2.5:- Dendrogram for 46 accessions of blackgram based on SDS-PAGE markers

Table 4.2.10:- Clusters based on protein peptides in blackgram

Group	Cluster	Frequency	Accessions
Group A	Cluster I	6	45741, 9059, 45397, 45207, 9029, 45060
	Cluster II	5	45370, 45218, 45714, 45136, 45059
Group B	Cluster III	9	93004, 41084, 93017, 9051, 92052, 41016, 9065, 9056, 45091
	Cluster IV	15	45312, 45345, 45338, 45324, 9020, 45307, 45618, 45063, 45157, 45743, 45221, 45074, 9039, 45130, 45051
Group C	Cluster V	11	45506, 45347, 45320, 45193, 9025, 45189, 45030, 45364, 45350, 45138, 45002

Table 4.2.11:- Mean and SD of quantitative characters for clusters based on SDS-PAGE in blackgram

	Group A		Group B		Group C
Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
DF	48.00±8.26	41.00±12.08	44.72±9.03	41.33±9.88	40.88±12.46
DM	84.75±15.91	81.00±11.49	78.06±21.56	79.63±13.42	79.93±13.81
BR/PL	15.86±2.80	25.08±11.64	18.74±7.68	15.75±6.02	16.60±3.46
P/PL	67.31±54.52	48.70±34.32	35.35±19.49	59.19±25.59	58.04±23.92
P/BR	4.11±2.79	2.12±1.14	1.94±0.76	4.10±1.69	3.49±1.21
PL	4.40±0.24	4.41±0.32	4.58±0.44	4.46±0.30	4.53±0.22
S/P	6.07±0.42	6.16±0.61	6.12±0.23	6.31±0.66	6.31±0.71
SD	0.27±0.04	0.28±0.05	0.25±0.05	0.29±0.08	0.28±0.06
SW	4.71±0.74	4.90±0.54	4.45±0.69	4.71±0.63	4.75±0.61
BY	55.52±36.70	62.35±26.01	28.77±19.77	61.74±32.79	61.18±27.59
GY	13.22±12.01	16.42±7.86	7.15±5.78	14.75±8.41	12.69±10.33
HI	24.62±12.46	25.65±7.53	23.75±3.89	24.64±5.91	20.83±11.85

high variance for most of the characters in almost all the clusters also revealed that the genotypes in various clusters may be from different origins but sharing similar protein peptides.

4.3 Geographic Distribution

The research material for this experiment comprised of 42 accessions which were selected from local germplasm on the basis of geographic distribution. Thirty advance lines obtained from national breeding programmes and 16 genotypes donated from national research stations were also included in the study (Table 4.3.1). The germplasm was evaluated for morphological traits and SDS-PAGE. The data regarding SDS-PAGE and morphological characters were analyzed on the basis of geographic parameters, i.e., provinces, agro-ecological zones and altitude. The agro-ecological zones were followed according to FAO, (1976). The results for all three parameters for morphological and biochemical factors are presented as under;

4.3.1 Provinces

The results regarding SDS-PAGE on the basis of provinces alongwith advance lines and donated germplasm are presented in Table 4.3.2 which revealed that in total 20 polymorphic bands were observed. The sign "+" indicates the presence of protein band, "-" indicates the absence and "+/-" indicates the heterozygosity of the protein bands in the germplasm. Band 1 exhibited variation for all the provinces followed by the protein peptide 2, 3, 8, 9 and 9b which gave polymorphism in all provinces except AJK (bands 2, 3 & 9b) and Punjab (bands 8 & 9). The protein bands 5a and 6 exhibited heterozygosity for the accessions from NWFP only. The accessions from northern areas exhibited variability for SDS-PAGE in nine protein peptides. The accessions from N.W.F.P revealed maximum variation for SDS-PAGE where variation was observed for all the twenty polymorphic protein peptides. It was followed by the accessions from Punjab where variation for 15 protein peptides, heterozygosity was observed. The lowest degree of polymorphism was observed in the accessions from AJK and Baluchistan where heterozygosity was observed for three and six protein sub-units, respectively. This may be because of low number of samples involved and thus it can be suggested to arrange expeditions from those areas to capture the maximum variability because both these locations (AJK and Baluchistan) are diverse in nature.

Table 4.3.1:- Summary of germplasm evaluated for biochemical, qualitative and quantitative traits

Province/location	Number of accessions collected	Accessions planted	Accessions evaluated
Punjab	249	14	14
Sindh	7	5 *	0
NWFP	35	15	15
Baluchistan	7	2	2
Azad Jammu & Kashmir	2	1	1
Northern Areas	10	10	10
Sub-total	310	47	42
Varieties/advance lines	-	30	30
Donated germplasm in the gene-bank	105	16	16
Total evaluated			88

*- The accessions collected from Sindh province could not mature at NARC, Islamabad and hence excluded from evaluation and analyses.

Table 4.3.2:- SDS-PAGE variation in accessions based on provincial distribution

Bands	Punjab	N.W.F.P.	Baluchistan	AJK	Northern Areas	Advance lines	Donated germplasm
	Number of samples						
	140	150	20	10	100	300	160
Band 1	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Band 2	+/-	+/-	+/-	-	+/-	+/-	+/-
Band 3	+/-	+/-	+/-	+	+/-	+/-	+/-
Band 3a	+/-	+/-	-	-	-	-	+/-
Band 3b	-	+/-	-	-	+/-	-	+/-
Band 4a	+/-	+/-	-	-	-	-	-
Band 5	+/-	+/-	+	+	+	+	+
Band 5a	-	+/-	-	-	-	-	-
Band 6	+	+/-	+	+	+	+	+
Band 8	+	+/-	+/-	+/-	+/-	+/-	+/-
Band 9	-	+/-	+/-	+/-	+/-	+/-	+/-
Band 9a	+/-	+/-	+	+	+/-	+/-	+/-
Band 9b	+/-	+/-	+/-	+	+/-	+/-	+/-
Band 10	+/-	+/-	+	+	+	+/-	+
Band 10a	+/-	+/-	-	+	-	-	-
Band 10b	+/-	+/-	-	+	+/-	-	+/-
Band 10d	+/-	+/-	-	-	-	-	-
Band 11	+/-	+/-	+	+	+	+/-	+/-
Band 11a	+/-	+/-	-	-	-	-	-
Band 13	+/-	+/-	+	+	+	+	+

+ indicates the presence of protein band, - indicates the absence and +/- indicates the heterozygosity of the protein bands.

The means alongwith SE and variance for morphological traits presented in the Table 4.3.3 revealed that advance lines were observed earlier in flowering (38.1±0.58 days) and maturity (65.9±0.67 days) whereas, the accessions from Baluchistan were late maturing (102.6±1.82 days). High variance were recorded for days to maturity in accessions from Punjab (278.1), N.W.F.P. (179.1) and in donated germplasm (175.7). In advance breeding lines, early maturity and low variance indicated the influence of selection pressure on blackgram germplasm as modern blackgram cultivars are being selected on the basis of earliness and high yield potential to be incorporated in various cropping patterns. The accessions from Baluchistan gave the maximum variance for branches per plant (174.8) followed by advance lines (65.5). High variance for maturity in accessions from Punjab (278.1), N.W.F.P (179.1), NA (171.6) and in donated germplasm (175.7) indicated that considerable genetic variability for maturity is present in these areas which could be collected and utilized for developing high yielding cultivars for various maturity groups suitable for different cropping patterns. Pods per plant exhibited high genetic variation in the accessions collected from almost all the areas including in the donated germplasm where the highest variance (638.8) was recorded and this may be because of diversity in collection/selection criteria. Low variance for pods was observed in the advance lines which indicated the impact of selection pressure as most of germplasm is selected for high yield potential by the breeders. The same pattern was also observed for pods/branch, whereas for characters like pod length, seeds/pod, seed density and seed weight, the variance was low. Low variance for these characters is supposed to be related to narrow genetic base as these characters are least affected by the environments. High variance was also observed for biological yield, grain yield and harvest index in all accessions collected from all the locations including donated germplasm. In the germplasm donated from other institute, high variance for DF, DM, BR/PL, P/PL, P/BR, PL, S/P, BY, GY and HI indicated the importance of this germplasm and further improvement for many agronomic characters through simple selection from this material. The graphic presentation of mean values and variance for maturity based on provinces revealed that advance lines has no further potential for selecting short duration cultivars, because these lines matured up to 66 days after planting and no genetic variation was observed. The accessions from NWFP and Punjab revealed a blaze for selection for maturity. On the other hand, material collected from AJK,

Table 4.3.3:- Quantitative variation in accessions based on provincial distribution

Traits	Punjab	N.W.F.P	Baluchistan	AJK	Northern Areas	Advance lines	Donated
	Number of samples						
	140	150	20	10	100	300	160
DF	46.8±0.71 70.9	43.9±0.73 79.9	52.4±1.19 28.15	48..0±0.26 0.67	49.77±1.08 104.9	38.1±0.58 10.2	47.3±0.61 64.2
DM	84.0±1.41 278.1	78.6±1.09 179.1	102.6±1.82 66.04	95.3±0.26 0.68	92.74±1.38 171.6	65.9±0.67 13.3	90.4±1.02 175.7
BR/PL	20.1±0.66 60.3	12.3±0.50 37.0	23.08±2.96 174.8	12.6±1.80 32.27	15.69±0.72 46.11	18.9±1.48 65.5	15.79±0.57 55.5
P/PL	37.3±1.33 246.4	25.8±1.64 404.4	43.18±4.76 452.3	27.4±4.49 202.04	35.94±1.90 323.7	37.0±1.88 106.5	33.1±1.94 638.8
P/BR	1.93±0.05 0.34	2.02±0.08 0.92	2.12±0.20 0.81	2.27±0.33 1.11	2.31±0.08 0.58	2.23±0.12 0.40	2.12±0.08 1.18
PL	4.53±0.03 0.13	4.28±0.02 0.08	4.61±0.06 0.08	4.92±0.11 0.13	4.67±0.03 0.07	4.38±0.03 0.03	4.53±0.03 0.18
S/P	6.20±0.04 0.17	5.90±0.05 0.32	6.65±0.09 0.15	6.26±0.17 0.28	6.32±0.06 0.35	6.01±0.04 0.06	6.14±0.05 0.50
SD	0.27±0.00 0.0	0.27±0.00 0.0	0.31±0.01 0.00	0.21±0.01 0.00	0.26±0.01 0.00	0.27±0.01 0.0	0.25±0.01 0.01
SW	4.41±0.06 0.46	3.86±0.07 0.78	4.45±0.08 0.12	5.11±0.16 0.25	4.5±0.07 0.39	4.23±0.06 0.12	4.44±0.04 0.26
BY	32.47±1.52 323.87	18.19±1.18 208.4	36.40±3.81 290.26	28.35±4.66 217.5	29.39±1.58 225.0	23.98±1.68 85.01	24.72±1.17 230.9
GY	6.14±0.30 12.89	4.52±0.42 26.85	10.05±1.05 21.99	5.95±1.18 13.95	7.48±0.43 16.86	6.57±0.49 7.32	6.71±0.47 37.38
HI	20.12±0.69 66.34	21.68±0.74 81.72	28.24±1.00 19.9	20.87±2.34 54.95	26.14±0.74 49.47	27.58±0.81 19.63	26.08±0.84 120.9

Upper row presents mean±SE and lower row is variance for specific character.

Baluchistan and NA was late maturing and exhibited low to medium variance (Fig. 4.3.1). Similarly for grain yield, germplasm collected from Baluchistan gave the highest mean yield but this may be misleading because of small sample size and hence further collection from this area is suggested to find the accurate indication for yield potential and genetic variation. Anyhow, this material need further testing under a wide range of ago-ecological climate to confirm adaptability. Although germplasm collected from NWFP, gave high variance but the average grain yield was minimum and hence the selection for further improvement may not increase yield potential (Fig. 4.3.2). The donated germplasm exhibited high values for mean yield alongwith high variance and hence simple selection can be practiced for yield potential improvement in blackgram. The germplasm collected from Baluchistan gave the highest average yield but matured very late, whereas advance lines and the accessions collected from N.W.F.P. produced medium to low grain yield and hence selected parents from these identified diverse origin are suggested to be used in breeding programme.

4.3.2 Altitude

The data for germplasm evaluated, was categorized with an interval of 200 meter above sea level of altitude and the results for 20 polymorphic protein peptides are presented in Table 4.3.4. Maximum variation was observed for protein band 1, 2, 3, 9a and 9b. The bands 2, 3, 9a & 9b gave heterozygosity for nine sets based on altitude intervals, followed by the bands 1 which exhibited variation in eight sets of collection sites. The band 6 exhibited variation for one set of altitude interval (1201-1400 masl). The bands 4a, 5, 5a and 10d gave heterozygosity in two sets of collection sites. On the basis of altitude, maximum variation was observed in the accessions collected from 601 to 800 and 1001 to 1200 masl, where 14 protein peptides out of 20 were polymorphic. It was followed by the accessions collected from 201 to 400 masl where 12 bands exhibited variation. The lowest level of variation was observed in the accessions collected from 2201 to 2400 masl (two loci), 1801 to 2000 masl (four loci), 401 to 600 (four loci) and 2001 to 2200 (five loci) and 0 to 200 masl (six loci). The accessions collected from high altitudes did not produce high degree of variability for most of the loci. A high level of polymorphism on the basis of SDS-PAGE, was recorded in the accessions collected from 600 to 1200 masl.

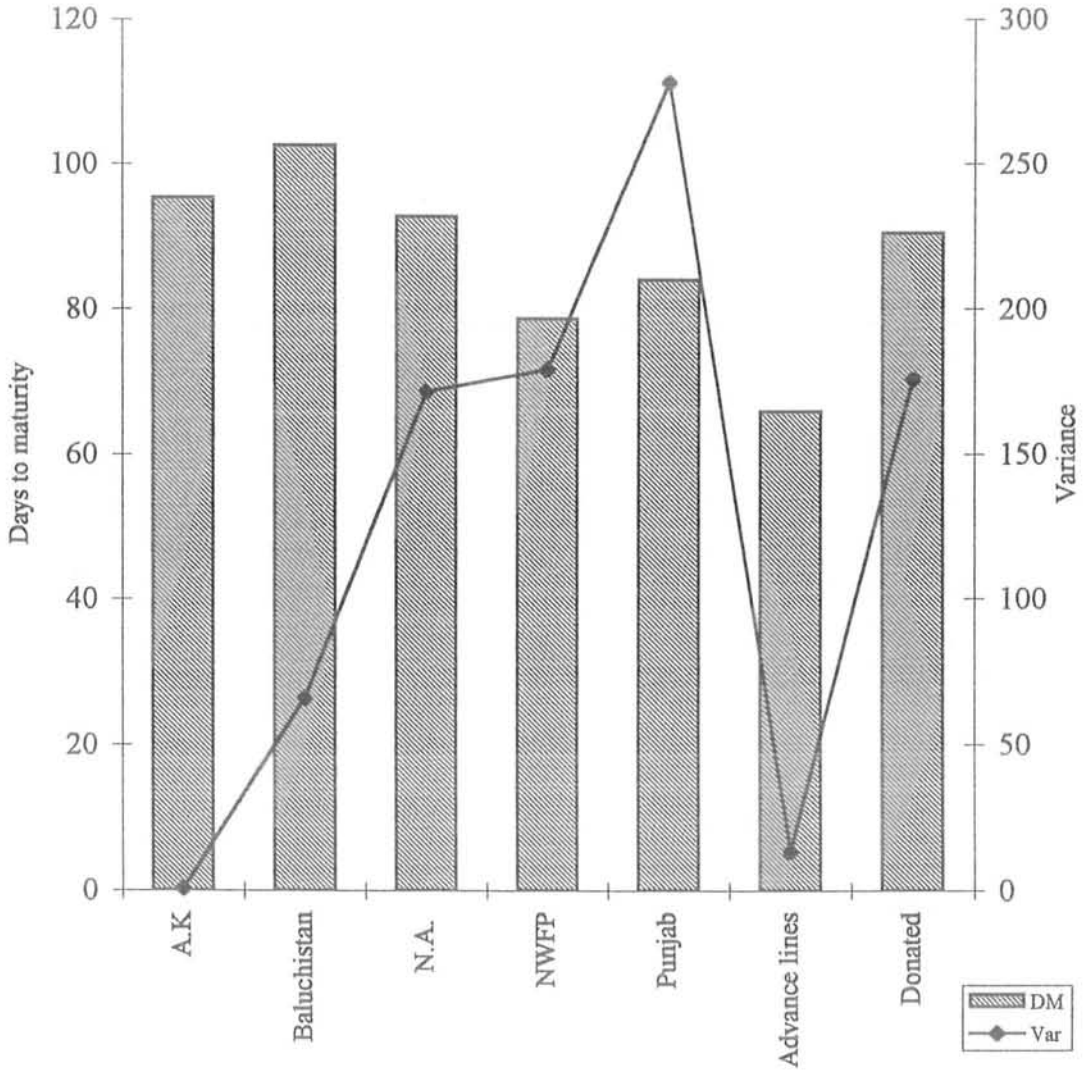


Fig. 4.3.1:- Days to maturity and variance based on provincial distribution

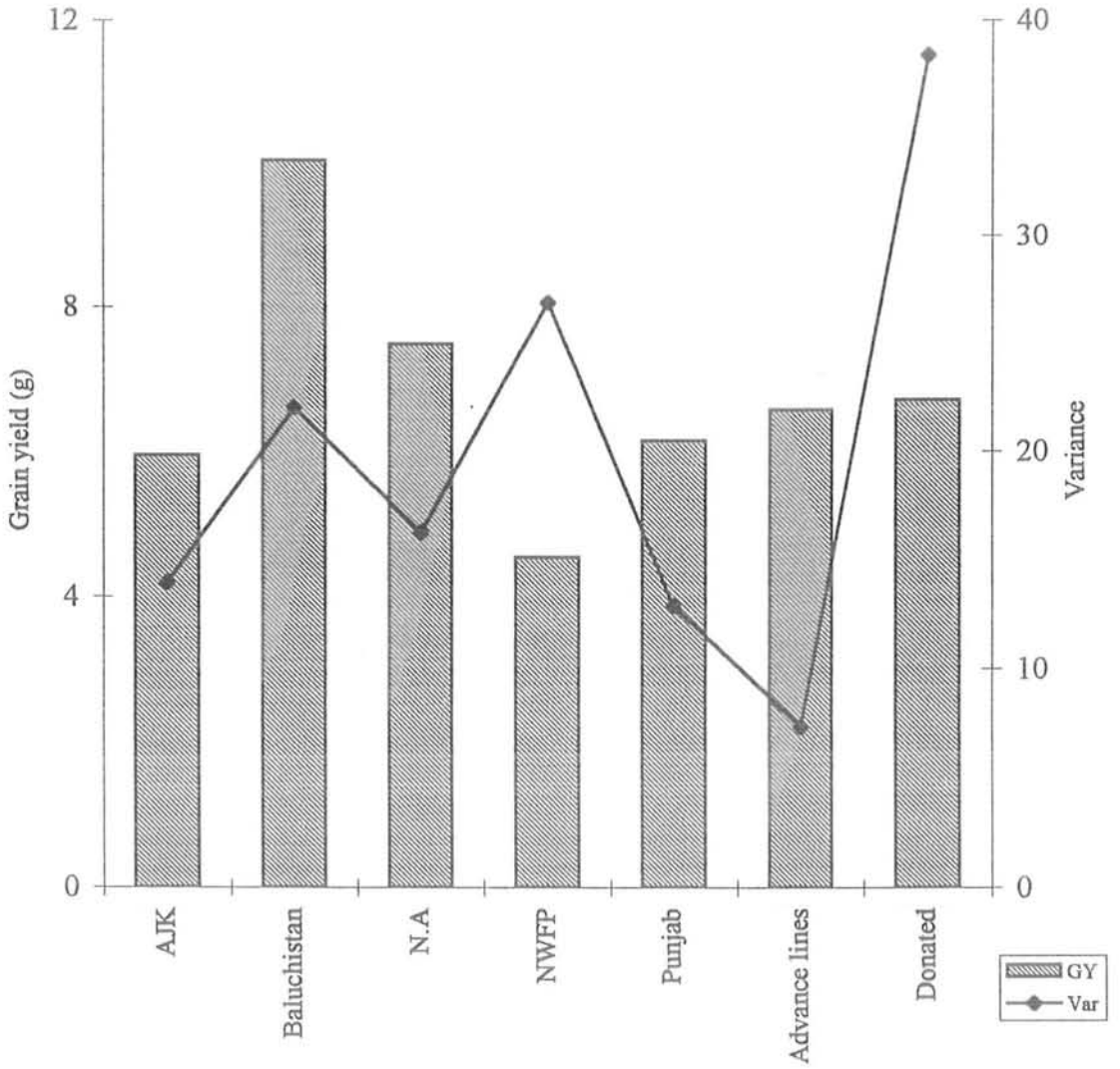


Fig. 4.3.2:- Grain yield and variance based on provincial distribution

Table 4.3.4:- SDS-PAGE variation in accessions based on altitude

	Altitude (masl)											
Bands	200	400	600	800	1000	1200	1400	1600	1800	2000	2200	2400
	Number of samples											
	40	60	20	30	20	50	20	60	70	20	20	10
1	+/-	+/-	+	+/-	+	+/-	+/-	+/-	+/-	+	+/-	+
2	+/-	+/-	+/-	+/-	-	+/-	+/-	+/-	+/-	-	+/-	-
3	+/-	+/-	+/-	+/-	+	+/-	+/-	+/-	+/-	+	+/-	+
3a	-	+/-	-	+/-	+	+/-	+/-	-	+/-	-	-	-
3b	-	-	-	-	+/-	+/-	-	-	+/-	-	-	-
4a	-	+/-	-	+/-	+	-	-	-	-	-	-	-
5	+	+/-	+	+	+/-	+	+	+	+	+	+	+
5a	-	-	-	-	+/-	-	-	+/-	-	-	-	-
6	+	+	+	+	+	+	+/-	+	+	+	+	+
8	+	+	+	+/-	+	+/-	+	+/-	+/-	+/-	+	+
9	-	-	-	+/-	-	+/-	-	+/-	+/-	+/-	-	-
9a	+/-	+/-	-	+/-	-	+/-	+/-	+/-	+/-	+	+/-	+/-
9b	+/-	+/-	-	+/-	-	+/-	+/-	+/-	+/-	+	+/-	-/+
10	+	+/-	+/-	+/-	+/-	+/-	-	+/-	+	+	+	+
10a	+/-	+/-	+/-	+/-	-	-	+	-	+/-	+/-	-	-
10b	-	+/-	-	-	-	-	+/-	-	+/-	+/-	-	+
10d	-	-	-	-	+/-	+/-	-	-	-	-	-	-
11	+	+	+	+/-	+/-	+/-	+	+	+	+	+	+
11a	-	+/-	-	+/-	+/-	+/-	+/-		-	-	-	-
13	+	+	+	+/-	+/-	+/-	+	+	+	+	+	+

+ indicates the presence of protein band, - indicates the absence and +/- indicates the heterozygosity of the protein bands.

The mean values and variance for agronomic traits presented in Table 4.3.5 revealed that the accessions collected from zero to 200 masl were late in maturity (97±2.22 days) and gave the highest variance (295.4). It was followed by the accessions collected from 1401 to 1600 masl with an average maturity of 82.4±2.00 days with high variance (240.5). The accessions collected from 401 to 1400 masl were early to medium in maturity coupled with low to medium variance. The accessions from 0 to 400 masl gave high variance for maturity, pods, biological yield, grain yield and harvest index. It was observed that the material under investigation gave high variation for maturity, branches, pods, biological yield, grain yield and harvest index for most of the collection sites based on altitude. But high yielding germplasm with other good economic characters was observed in the collections from 0 to 600 masl and then from high altitude (> 1600 masl). The germplasm from these sites had high grain yield alongwith high genetic variance which gave the clue that landraces may be present and hence more collection mission may be organized to collect maximum variability for practical utilization. The low variance was observed for pod length, seeds per pod, seed density and seed weight which might be related to narrow genetic base of blackgram in Pakistan. The material collected from 1801 to 2000 masl gave fairly high mean values for SW (5.14±0.13 g) and thus may be exploited for the development of high yielding cultivars alongwith bold seeds.

The graphic presentation of mean values and variance for maturity based on altitude revealed that the accessions collected from 401 to 1400 masl were in general early maturing but due to low variance except for 1001 to 1200 masl. further improvement for earliness is restricted. Accessions collected from 0 to 400 masl and 1401 to 1800 masl though were late in maturity but exhibited high genetic variance and thus indicated the scope for further selection (Fig. 4.3.3). Crossing among selected parents from these identified groups may produce desirable recombinants for further selection. Similarly for grain yield (Fig. 4.3.4), germplasm collected from 0 to 400 masl gave medium yield alongwith medium variance, whereas germplasm collected from 1601 to 2400 masl exhibited medium to high mean grain yield alongwith medium to high degree of genetic variation revealing the scope of further collection and selection to build a gene pool with desirable variability for practical exploitation in blackgram improvement programme.

Table 4.3.5:- Quantitative variation in accessions based on altitude

Traits	Altitude (masl)											
	200	400	600	800	1000	1200	1400	1600	1800	2000	2200	2400
	Number of samples											
	40	60	20	30	20	50	20	60	70	20	20	10
DF	51.7±1.23 60.60	47.7±1.18 84.1	40.4±0.11 0.25	38.9±0.35 3.8	40.6±0.11 0.26	44.9±1.47 108.6	39.2±0.22 0.96	44.9±1.47 129.2	51.2±0.90 56.9	54.6±1.98 78.5	43.0±1.44 41.7	51.2±0.51 2.6
DM	97.0±2.72 295.4	84.5±1.89 214.0	68.9±0.37 2.8	71.5±0.30 2.7	73.9±0.81 13.2	77.4±1.80 161.9	68.7±0.32 2.01	82.4±2.00 240.5	96.0±1.52 162.3	101.1±1.32 34.73	88.8±1.95 75.7	96.2±0.29 0.84
BR/PL	23.8±1.51 91.4	19.1±0.91 49.9	15.7±0.68 9.38	15.8±1.21 43.6	16.9±1.82 66.1	10.0±0.83 34.31	10.9±0.41 3.29	12.1±0.63 23.8	18.7±1.16 94.5	15.7±1.28 32.8	15.5±1.29 33.5	16.6±2.73 74.27
P/PL	40.6±3.15 396.8	36.8±1.94 226.2	30.7±1.54 47.4	32.5±2.49 186.7	26.6±3.38 227.9	17.0±1.85 170.42	18.6±1.27 32.5	25.7±2.49 373.0	41.1±2.33 378.6	39.5±4.67 436.4	35.3±3.65 266.4	51.6±9.29 862.9
P/BR	1.77±0.09 0.34	2.02±0.09 0.44	1.98±0.08 0.14	2.12±0.13 0.48	1.57±0.11 0.24	1.71±0.10 0.45	1.66±0.06 0.08	2.05±0.13 1.03	2.36±0.11 0.81	2.44±0.13 0.35	2.28±0.15 0.46	3.21±0.46 2.11
PL	4.64±0.08 0.27	4.55±0.04 0.10	4.43±0.02 0.01	4.41±0.03 0.02	4.23±0.03 0.02	4.15±0.04 0.07	4.16±0.04 0.04	4.39±0.05 0.12	4.69±0.03 0.07	4.71±0.04 0.04	4.66±0.07 0.09	4.58±0.08 0.06
S/P	6..32±0.07 0.18	6.22±0.06 0.20	6.20±0.06 0.08	5.90±0.05 0.08	5.91±0.06 0.07	5.86±0.08 0.34	5.73±0.12 0.27	5.85±0.09 0.54	6.44±0.06 0.23	6.50±0.11 0.26	6.26±0.11 0.23	6.20±0.19 0.35
SD	0.27±0.01 0.01	0.27±0.01 0.0	0.28±0.01 0.0	0.25±0.00 0.02	0.28±0.01 0.0	0.29±0.01 0.0	0.27±0.01 0.0	0.24±0.01 0.0	0.27±0.01 0.0	0.27±0.01 0.0	0.25±0.01 0.0	0.26±0.01 0.0
SW	4.47±0.15 0.93	4.54±0.07 0.29	4.14±0.05 0.0	4.10±0.13 0.48	3.86±0.14 0.39	3.54±0.12 0.70	3.56±0.09 0.18	4.10±0.12 0.85	4.57±0.05 0.19	5.14±0.13 0.32	4.31±0.07 0.11	4.82±0.14 0.19
BY	38.31±3.30 435.92	34.23±2.44 356.0	25.26±1.79 64.22	18.57±1.48 65.90	19.34±2.24 100.65	12.87±1.64 134.21	9.82±0.83 13.86	21.44±2.07 256.36	33.38±1.68 198.06	34.19±3.64 264.28	25.82±2.67 142.65	35.03±6.45 416.32
GY	6.49±0.73 21.15	6.60±0.45 12.40	5.79±0.51 5.11	4.20±0.41 4.94	3.41±0.45 4.00	2.76±0.42 8.83	4.68±0.13 0.35	4.81±0.68 27.63	8.78±0.51 18.45	9.30±1.31 34.12	6.95±0.65 8.37	11.98±2.35 55.42
HI	17.06±1.28 65.47	20.98±1.07 69.22	23.13±1.10 24.06	22.69±1.42 60.72	17.97±1.01 20.31	21.06±0.90 40.55	17.68±0.80 12.72	20.04±1.47 128.94	26.45±0.79 44.12	25.88±1.49 44.28	27.94±1.39 38.79	34.06±3.12 97.61

Upper row presents mean±SE and lower row is variance for specific character.

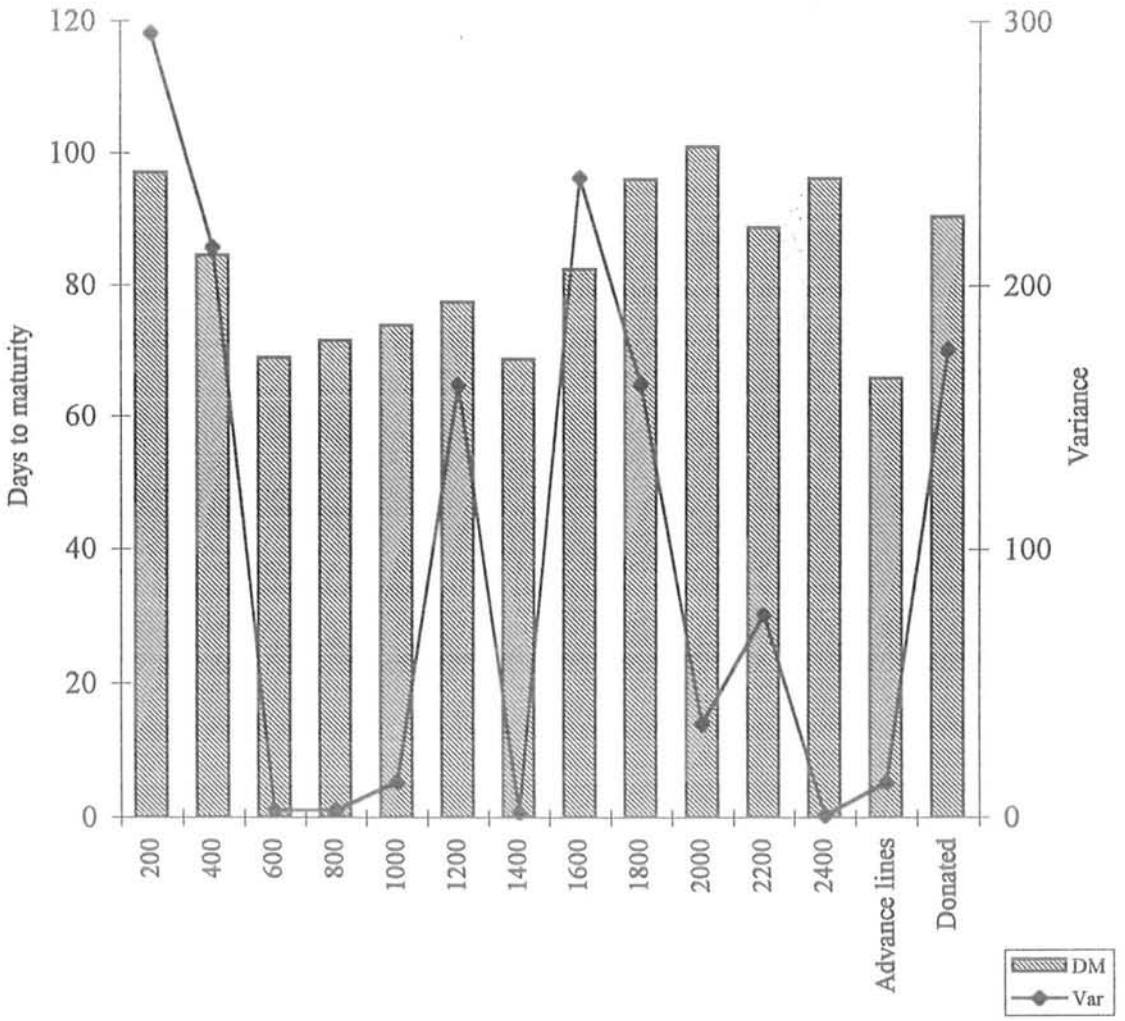


Fig. 4.3.3:- Days to maturity and variance based on altitude

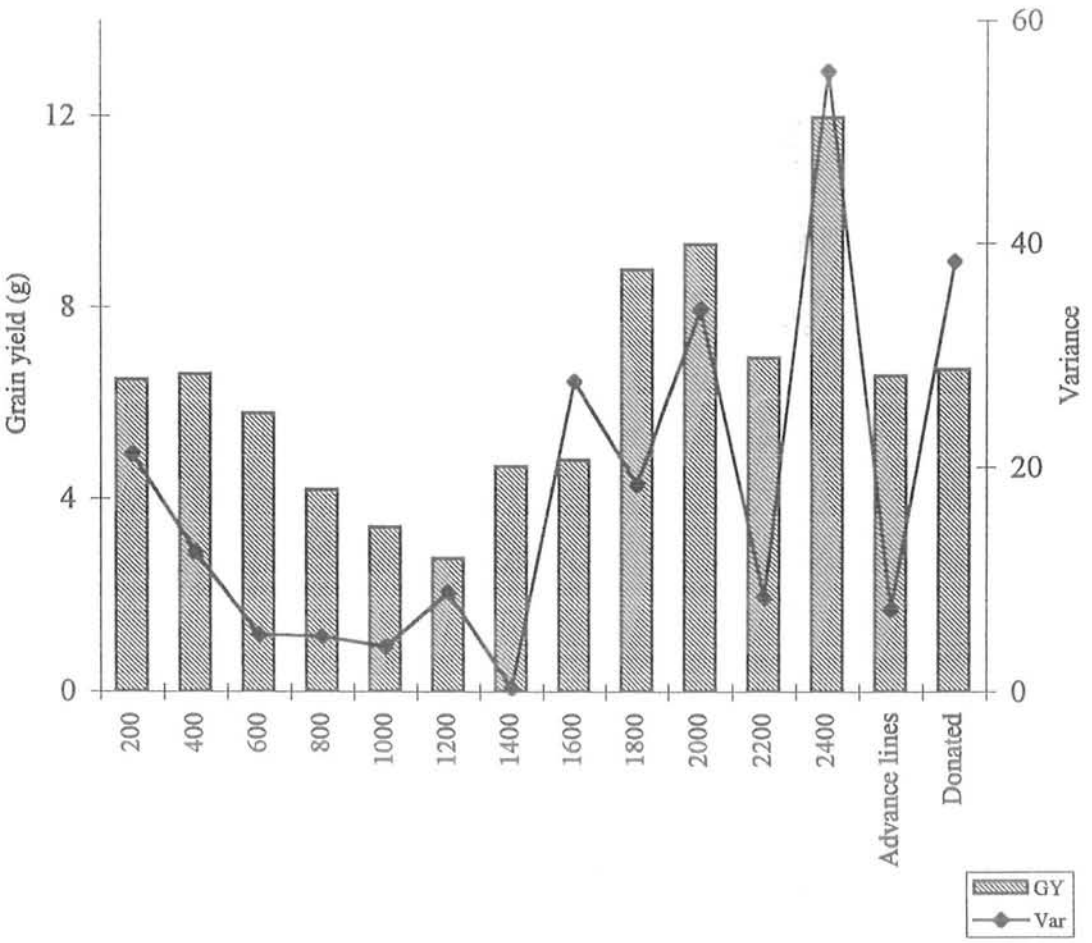


Fig. 4.3.4:- Grain yield and variance based on altitude

4.3.3 *Crop ecological Zones*

The germplasm analyzed for SDS-PAGE, was also categorized on the basis of crop ecological zones (Table 4.3.6). In total, 17 crop ecological zones have been identified on the basis of soil and climate in the country as presented in the Fig. 4.3.5. Blackgram germplasm has been collected from 10 zones and the remaining zones are not yet explored. The results regarding SDS-PAGE revealed that the protein peptides 10 and 10a gave variation for maximum zones (6 out of 10), followed by the bands 1, 2, 3 and 9b (5 out of 10). No variation in polymorphism was observed for the zones 3 and 5. High degree of heterozygous polymorphism was recorded in the zone 10 (13 loci), zone 12 (19 loci) and zone 13 (15 loci). The accessions from zone 12 which comprises sub-humid and humid areas of Murree, Swat, Dir, etc., gave the maximum variation, followed by the accessions from zone 13 which consists of high mountains of NWFP and NA. The lowest degree of polymorphism was observed in the accessions from zone 6 and 9 where the germplasm varied at 3 and 2 loci, respectively.

The morphological traits presented in Table 4.3.7 revealed that the accessions from zones 6 and 13 gave high degree of variation for days to flowering; accessions from zones 5, 6, 7 and 13 for days to maturity; accessions from zone 14 for branches. Pods per plant was observed with high variability scattered throughout the germplasm. High variance as recorded for days to maturity in accessions from zone 6 (245.1), zone 13 (221.47) and zone 7 (193.3). The accessions from zone 6 exhibited high variance for maturity, pods, biological yield, grain yield and harvest index. The accessions collected from zone 13 and 14 gave maximum variance for most of the agronomic characters and thus the germplasm from these areas should be investigated carefully for exploitation of genetic variation. The germplasm collected from zones 9 and 10 was early in maturity and low yielder with low variability for both the characters, whereas the germplasm collected from the zone 3, 4 and 14 was late in maturity but high yielder with medium to high levels of variability, therefore, the selected genotypes from these two groups of zones are suggested to be used in breeding programme to combine important plant characters in one genotype. The graphic presentation of mean values and variance for maturity indicated that accessions from zones 9 and 10 were short duration with the lowest variability (Fig. 4.3.6). The accessions from zones 5, 6, 7 and 13 were observed late in maturity

Table 4.3.6:- SDS-PAGE variation in accessions based on crop-ecological zones

	Crop Zones									
Bands	3	4	5	6	7	9	10	12	13	14
	Number of samples									
	10	10	20	20	30	40	40	60	170	20
Band 1	+	-	+	+	+/-	-	+/-	+/-	+/-	+/-
Band 2	-	+	-	-	+/-	+	+/-	+/-	+/-	+/-
Band 3	+	-	+	+	+/-	-	+/-	+/-	+/-	+/-
Band 3a	-	-	-	-	+/-	-	+/-	+/-	+/-	-
Band 3b	-	-	-	-	-	-	-	+/-	+/-	-
Band 4a	-	-	-	-	+/-	-	+/-	+/-	-	-
Band 5	+	+/-	+	+	+	+	+	+/-	+	+
Band 5a	-	-	-	-	-	-	-	+/-	+/-	-
Band 6	+	+	+	+	+	+	+	+	+/-	+
Band 8	+	+	+	+/-	+	+	+	+/-	+/-	+/-
Band 9	-	-	-	+/-	-	-	-	+/-	+/-	+/-
Band 9a	+	+/-	+	+	-	-	+/-	+/-	+/-	+
Band 9b	-	-	+	+/-	-	-	+/-	+/-	+/-	+/-
Band 10	+	+/-	+	+	+/-	+/-	+/-	+/-	+/-	+
Band 10a	-	+/-	-	-	+/-	+/-	+/-	+/-	+/-	-
Band 10b	-	+/-	-	-	-	-	-	+/-	+/-	-
Band 10d	-	-	-	-	-	-	+/-	+/-	-	-
Band 11	+	+	+	+	-	+	+/-	+/-	+	+
Band 11a	-	-	-	-	+/-	-	+/-	+/-	+/-	-
Band 13	+	+	+	+	+	+	+/-	+/-	+	+

+ indicates the presence of protein band, - indicates the absence and +/- indicates the heterozygosity of the protein bands.

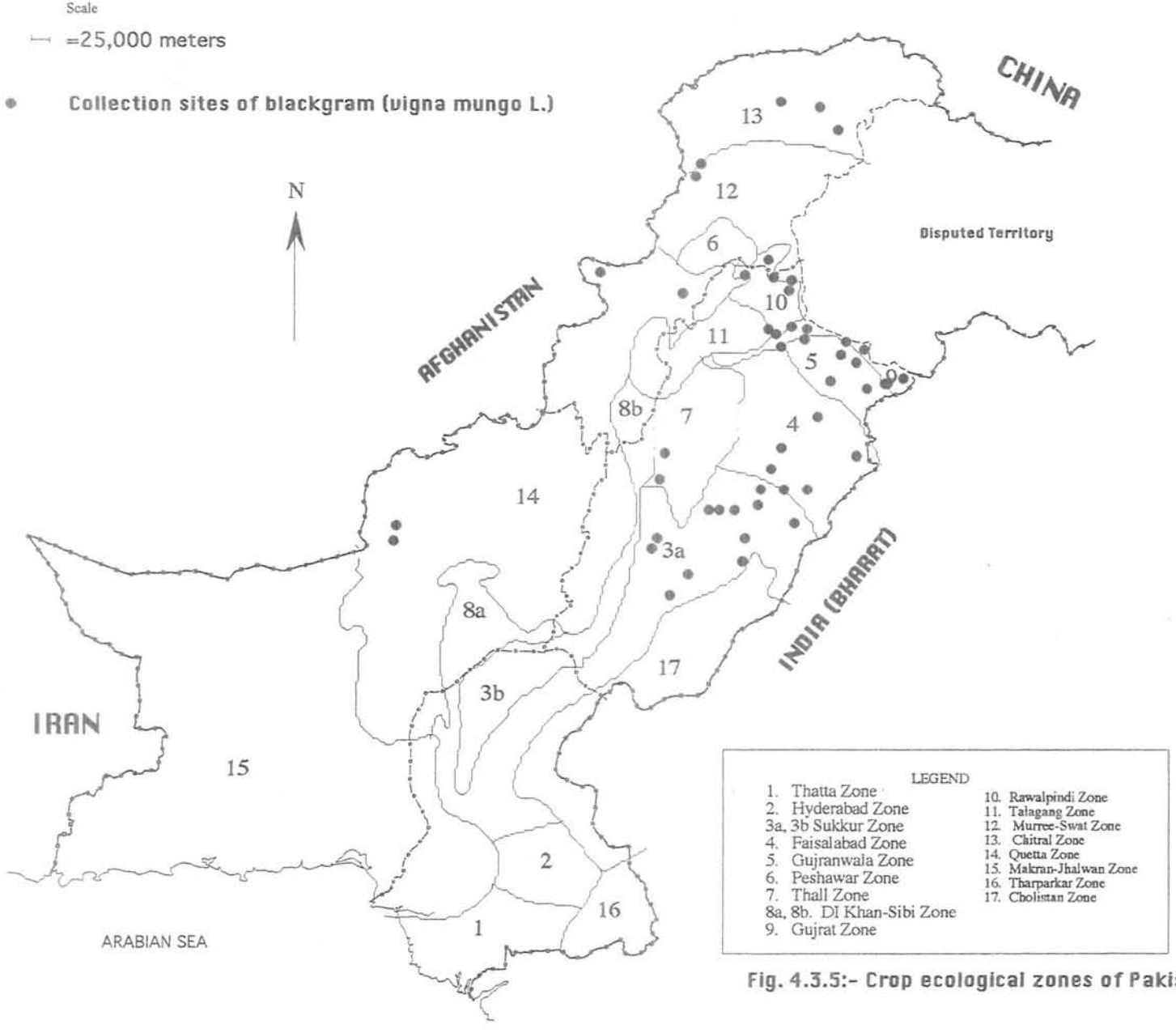


Fig. 4.3.5:- Crop ecological zones of Pakistan

Table 4.3.7:- Quantitative variation in accessions based on crop-ecological regions

Traits	Crop ecological zones									
	3	4	5	6	7	9	10	12	13	14
	Number of samples									
	10	10	20	20	30	40	40	60	170	20
DF	57.5±0.17 0.28	63.2±0.25 0.62	53.1±0.56 6.31	55.2±3.04 185.3	49.2±1.42 60.3	39.7±0.20 1.60	40.6±0.12 0.61	41.2±0.43 11.2	46.52±0.76 99.41	52.4±1.19 28.15
DM	110.5±0.17 0.28	107.4±0.16 0.27	98.7±2.72 148.5	95.2±3.50 245.1	88.3±2.54 193.3	70.5±0.65 16.97	70.7±0.29 3.45	76.2±1.17 82.8	86.1±1.14 221.47	102.6±1.82 66.04
BR/PL	35.7±0.78 6.05	24.8±1.46 21.41	19.9±1.26 31.74	12.5±1.34 36.09	21.6±1.38 57.5	14.93±0.85 29.1	16.58±1.03 42.56	9.2±0.61 22.1	15.3±0.49 40.61	23.1±2.96 174.83
P/PL	60.2±3.94 155.6	56.0±4.06 164.94	26.6±2.62 137.26	25.6±3.25 210.9	39.6±2.91 253.7	30.0±1.73 119.2	31.0±2.49 247.6	15.9±1.49 133.2	35.1±1.52 392.95	43.2±4.76 452.34
P/BR	1.67±0.07 0.05	2.27±0.11 0.13	1.39±0.14 0.41	2.03±0.14 0.40	1.91±0.09 0.27	2.10±0.10 0.41	1.85±0.11 0.47	1.74±0.10 0.61	2.29±0.07 0.82	2.12±0.20 0.81
PL	4.45±0.06 0.04	5.04±0.05 0.02	5.14±0.09 0.18	6.64±0.08 0.13	4.31±0.02 0.02	4.39±0.02 0.02	4.30±0.04 0.06	4.26±0.05 0.16	4.50±0.02 0.08	4.61±0.06 0.08
S/P	6.50±0.03 0.01	6.52±0.07 0.05	6.44±0.11 0.23	6.04±0.18 0.61	6.09±0.09 0.23	6.21±0.06 0.12	5.73±0.08 0.26	5.91±0.07 0.29	6.15±0.04 0.31	6.65±0.09 0.15
SD	0.31±0.01 0.0	0.23±0.01 0.0	0.20±0.01 0.0	0.23±0.01 0.0	0.29±0.01 0.0	0.29±0.01 0.0	0.25±0.01 0.0	0.28±0.01 0.0	0.26±0.00 0.0	0.31±0.01 0.0
SW	4.29±0.09 0.08	4.68±0.09 0.09	5.38±0.11 0.26	4.60±0.25 1.25	4.32±0.16 0.75	4.14±0.06 0.14	3.84±0.12 0.55	3.51±0.11 0.68	4.40±0.05 0.38	4.45±0.08 0.12
BY	48.02±3.86 149.21	53.0±1.85 34.34	30.72±2.01 80.79	27.56±5.05 509.45	46.01±4.46 596.5	19.01±1.10 48.58	22.88±1.44 82.57	12.58±1.44 24.14	25.50±1.12 211.82	36.40±3.81 290.26
GY	13.13±1.17 13.78	10.29±0.83 6.94	5.19±0.54 5.90	5.99±1.15 26.61	5.27±0.58 10.27	5.01±0.42 6.99	4.26±0.46 8.30	2.56±0.34 6.98	6.73±0.39 25.82	10.05±1.05 21.99
HI	27.15±0.26 0.68	19.19±1.15 13.12	16.64±1.64 53.85	23.99±1.67 56.09	11.53±0.80 19.17	25.82±0.82 33.6	17.57±1.55 96.29	20.30±0.80 38.88	24.60±0.65 72.29	28.24±1.00 19.90

Upper row presents mean±SE and lower row is variance for specific character.

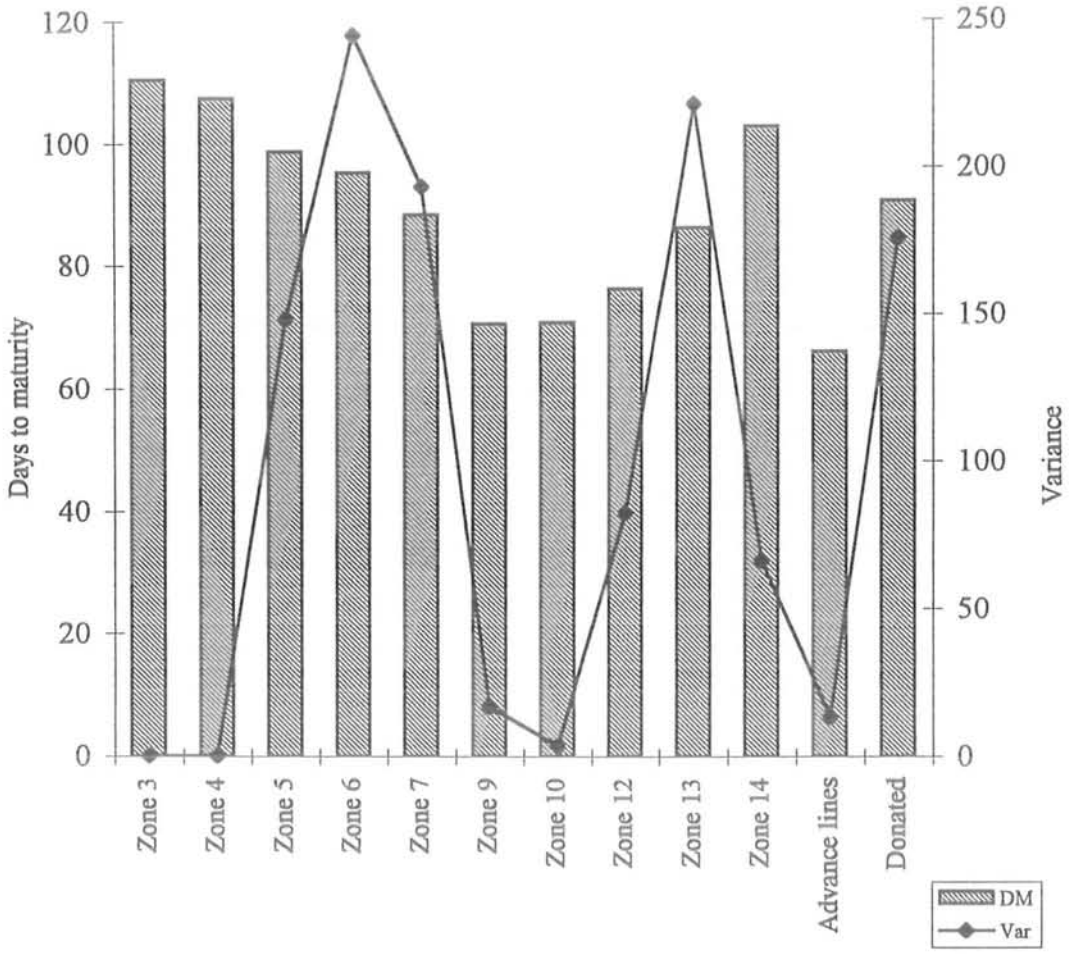


Fig. 4.3.6:- Days to maturity and variance based on crop-ecological zones

with maximum genetic variability indicating the presence of genetic diversity for maturity in these zones. The germplasm collected from zones 9 and 10 was early and exhibited low genetic variability similarly as in advance lines which had been selected by the breeders on the basis of best performance. In general, the accessions collected from zones 3, 4, 9 and 10 gave low genetic variability. The graphic presentation for grain yield in Fig. 4.3.7 revealed that the accessions collected from zone 3 gave the highest mean grain yield with medium variability and thus high yielding blackgram cultivars can be selected from this material. The germplasm collected from the zone 6, 13 and 14 as observed promising especially from zone 14 where high mean value alongwith high genetic variation revealed that careful selection should be practiced for improvement of yield potential in blackgram. The germplasm with high mean values alongwith low to medium genetic variance should be exploited through simple selection.

4.3.4 Cluster analysis

Forty two accessions, selected on the basis of geographic distribution, were included in the cluster analysis for morphological characters. The advance lines and donated germplasm was excluded from this analysis because of unknown origin. A Euclidean dissimilarity coefficient matrix was calculated and phenogram constructed is presented in Fig. 4.3.8. Cluster diagram using Ward's method revealed 2 major groups and if dendrogram is observed critically, 12 clusters were observed in total. The group A & B consisted of 6 clusters each. The geographic data for clusters alongwith accessions are presented in Table 4.3.8, which revealed that cluster I comprised of 4 accessions (45743, 45796, 45713, 45415) from NA and Punjab. Cluster II consisted of 3 accessions all sharing same geographic criteria as all were collected from Punjab. Cluster III, VI, VII, X and XI were made of accessions from different origins, whereas clusters II, IV, V, VIII and IX comprised of the accessions from same origin. The accession 41016 which was collected from Punjab did not share any cluster and observed independent of any cluster. It is observed that out of 42 accessions, 16 were grouped as influenced by the geographic basis and hence it can be said that about 40% of the germplasm under study has genetic diversity associated with geographic origin.

The means and SD for each cluster is given in the Table 4.3.9 which revealed that clusters III, IV and VI were early in maturity i.e. maturing in less than 70 days. Out of

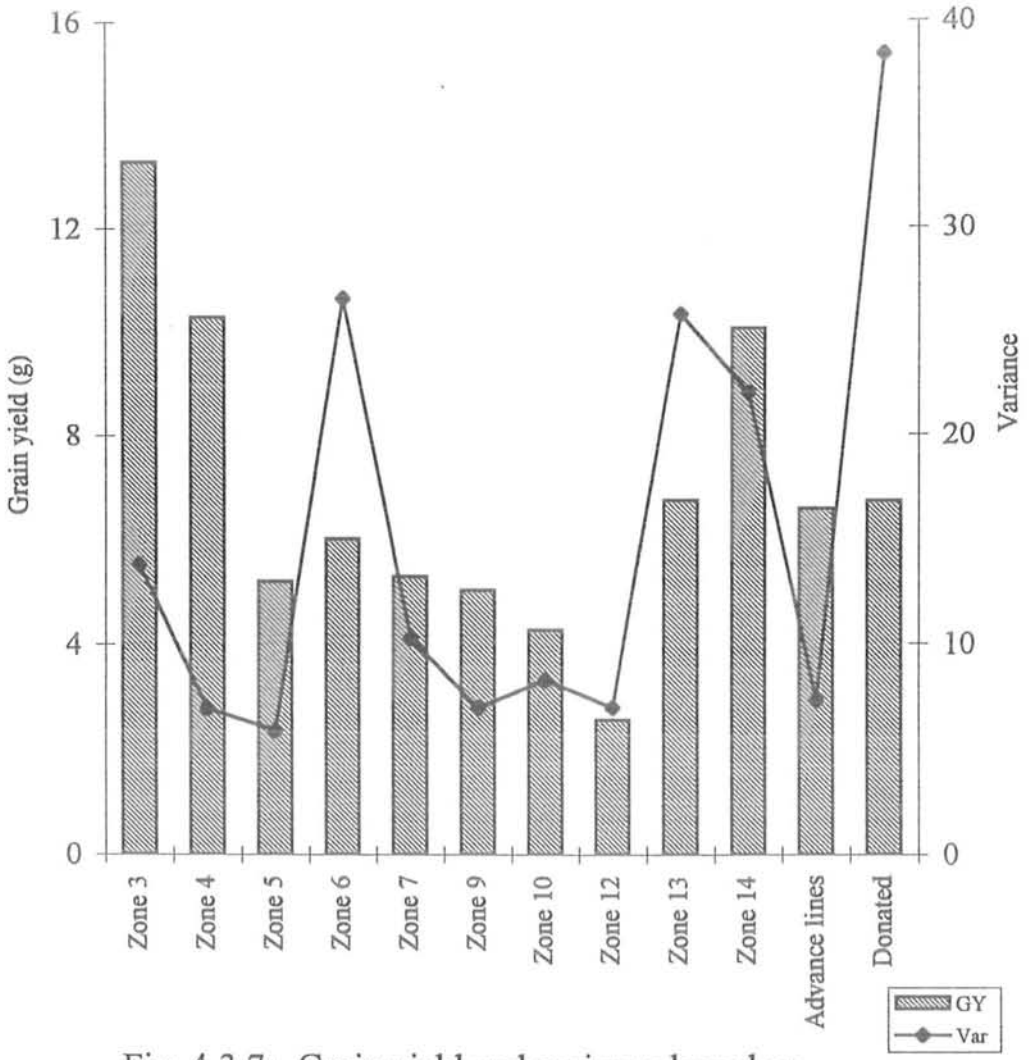


Fig. 4.3.7:- Grain yield and variance based on crop-ecological zones

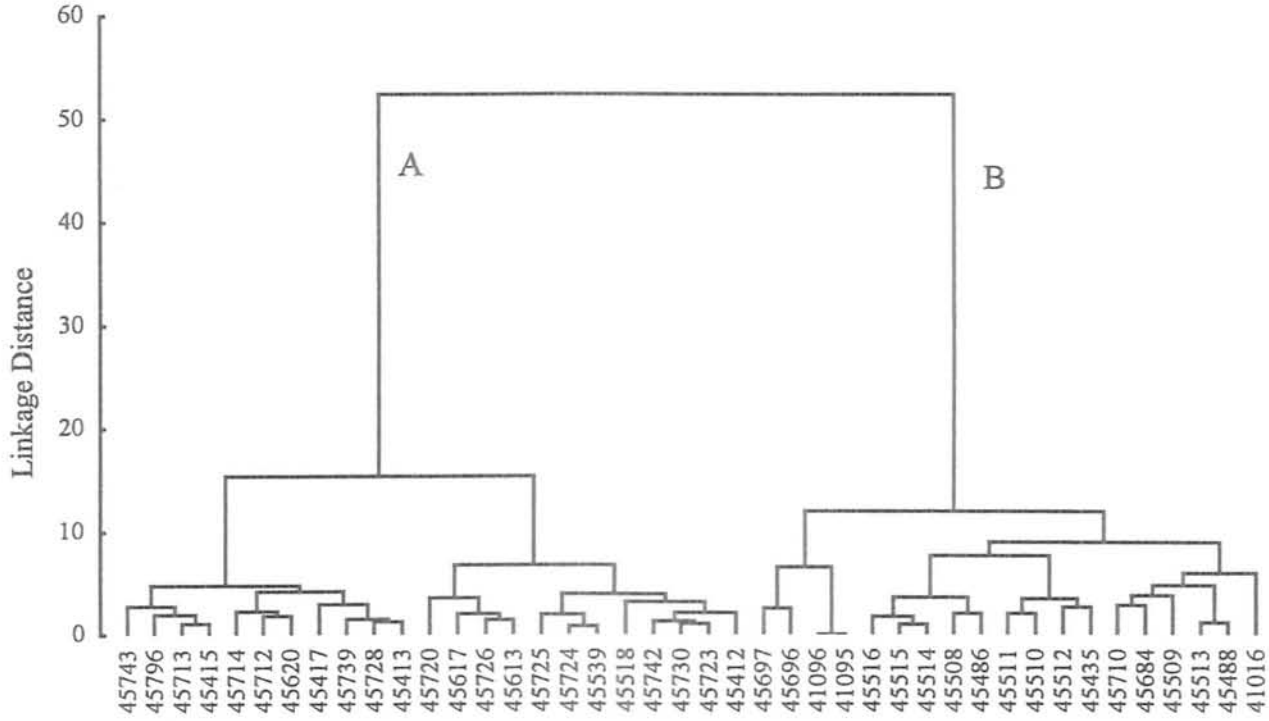


Fig. 4.3.8:- Phenogram based on quantitative characters for geographic distribution

Table 4.3.8:- Clusters based on quantitative characters in blackgram

Group	Cluster	Accessions	Altitude	Province	Zone
Group A	Cluster I	45743	1560	NA	13
		45796	2020	NA	13
		45713	330	P	9
		45415	650	P	10
	Cluster II	45714	400	P	9
		45712	300	P	9
		45620	450	P	9
	Cluster III	45417	830	P	10
		45739	1780	NA	13
		45728	800	N	13
		45413	540	P	10
	Cluster IV	45720	1470	N	10
		45617	1050	N	12
		45726	1100	N	12
		45613	1250	N	13
	Cluster V	45725	1200	N	12
		45724	1200	N	12
		45539	960	N	13
	Cluster VI	45518	1480	NA	13
		45742	800	N	13
		45730	1440	N	13
		45723	1270	N	13
		45412	170	P	7
Group B	Cluster VII	45697	150	P	7
		45696	220	P	7
		41096	1700	B	14
		41095	190	P	5
	Cluster VIII	45516	1650	NA	13
		45515	1650	NA	13
		45514	2160	NA	13
	Cluster IX	45508	2270	N	13
		45486	1480	NA	13
	Cluster X	45511	2170	NA	13
		45510	1820	N	13
		45512	1750	NA	13
		45435	250	P	4
	Cluster XI	45710	270	P	5
		45684	1610	AK	12
		45509	1190	N	13
		45513	1920	NA	13
		45488	1640	B	14
	Cluster XII	41016	200	P	5

Table 4.3.9:- Mean and standard deviation for clusters based on quantitative characters in blackgram

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI
DF	40.0±2.31	39.5±1.50	39.6±1.51	40.0±0.33	39.8±2.11	37.3±1.45	56.3±2.08	51.4±2.70	49.6±2.33	65.0±2.45	51.5±7.92
DM	75.1±6.3	71.7±4.95	70.4±0.74	69.5±0.39	74.3±3.27	69.4±1.45	106.3±7.33	96.8±1.66	95.4±1.13	108.7±1.81	94.8±5.46
BR	13.4±3.56	15.1±3.85	18.1±4.98	8.32±2.19	9.4±0.80	11.9±075	31.5±7.20	20.4±6.17	16.3±0.49	21.1±3.95	14.9±4.60
PODS	26.4±9.88	29.8±6.43	37.8±2.13	12.6±2.95	13.62±1.14	22.1±4.67	53.5±11.47	47.63±5.01	54.5±4.03	47.5±8.08	28.1±1.57
P/BR	1.95±0.19	2.10±0.51	2.22±0.47	1.57±0.36	1.54±0.20	1.89±0.31	1.71±0.07	2.47±0.44	3.44±0.32	2.29±0.32	2.02±0.48
PL	4.41±0.08	4.42±0.08	4.45±0.13	3.99±0.06	4.24±0.07	4.25±0.03	4.40±0.08	4.67±0.20	4.57±0.03	4.84±0.16	4.71±0.21
S/P	5.85±0.15	6.37±0.06	5.99±0.06	5.37±0.20	6.05±0.15	5.84±0.21	6.33±0.29	6.42±0.09	6.35±0.21	6.45±0.19	6.44±0.31
SD	0.25±0.01	0.31±0.01	0.26±0.01	0.25±0.03	0.30±0.02	0.27±0.03	0.30±0.02	0.27±0.03	0.28±0.03	0.25±0.03	0.26±0.04
SW	3.93±0.27	4.19±0.23	4.42±0.18	3.04±0.20	3.27±0.30	3.85±0.43	4.59±0.53	4.48±0.16	4.49±0.47	5.11±0.57	4.87±0.20
BY	14.41±5.24	19.99±4.12	28.15±3.23	9.20±3.21	10.14±2.54	13.89±2.04	51.92±6.76	41.29±13.69	33.82±1.71	45.51±6.73	28.09±3.88
GY	3.50±1.35	5.48±1.71	6.31±1.03	1.38±0.70	1.94±0.53	1.99±0.42	11.76±2.37	8.10±2.13	12.14±0.22	10.99±1.10	5.90±0.96
HI	25.51±2.08	26.53±2.80	22.48±3.67	17.04±8.05	19.14±0.44	14.91±3.63	23.40±6.50	22.72±9.42	35.77±2.41	24.41±4.59	21.57±6.75

these early maturing clusters, the accessions in clusters IV and V were collected from NWFP from an altitude from 960 to 1470 masl. The accessions collected from these areas can be used for the development of photo-insensitive cultivars. The variability for pod length, seeds per pod and seed density was limited and scattered throughout all clusters. The cluster X which consisted of 4 accessions from different origins gave the highest seed weight (5.11±0.57 g) but the variation for this character is limited in local germplasm. The clusters VII, VIII, IX and X were late maturing (> 95 days) but they produced high mean yield, i.e., 12.14±0.22 (cluster IX), 11.76±2.37 (cluster VII), 10.99±1.10 (cluster X) and 8.10±2.13 g (cluster VIII). Out of these 4 clusters with high grain yield, clusters VIII and IX were from the same origin. It was noted that the genotypes from clusters II, III, IV and VI were categorised as short duration with low to medium grain yield, whereas accessions from clusters VII, VIII, IX and X were late maturing but produced high grain yield and the accessions from these two identified sets may be inter-crossed in a selective diallel fashion to break undesirable linkage of these important characters in blackgram.

Cluster analysis on the basis of SDS-PAGE was conducted for 31 accessions because remaining 11 accessions did not vary for any locus based on SDS-PAGE and hence excluded from cluster analysis. Dendrogram based on UPGMA revealed 3 major groups (Fig. 4.3.9). Critical classification revealed that the group A consisted 2 clusters; B consisted 2, and C consisted 3 clusters. The geographic data for clusters alongwith accessions are presented in Table 4.3.10 which revealed that cluster I comprised of 4 accessions (45714, 45617, 45518, 45435) from NWFP, NA and Punjab. No clear mark for any of three geographic parameters was observed in this cluster. Cluster II consisted 2 accessions (45620, 45415) which shared same geographic criteria as both were originated from same province. Cluster III consisting of 4 accessions also sharing similar geographic origin as all were collected from high mountains with altitude ranging from 1610 to 2270 masl. Cluster IV comprised of 8 accessions from different origins. Cluster V consisting of 4 accessions (45743, 45725, 45724, 45539) also shared similar geographic parameters, whereas clusters VI and VII comprised of 4 and 5 accessions, respectively originated from various sources. The means and variance for each cluster is given in Table 4.3.11 which revealed that grouping of various accessions on the basis of SDS-PAGE did not

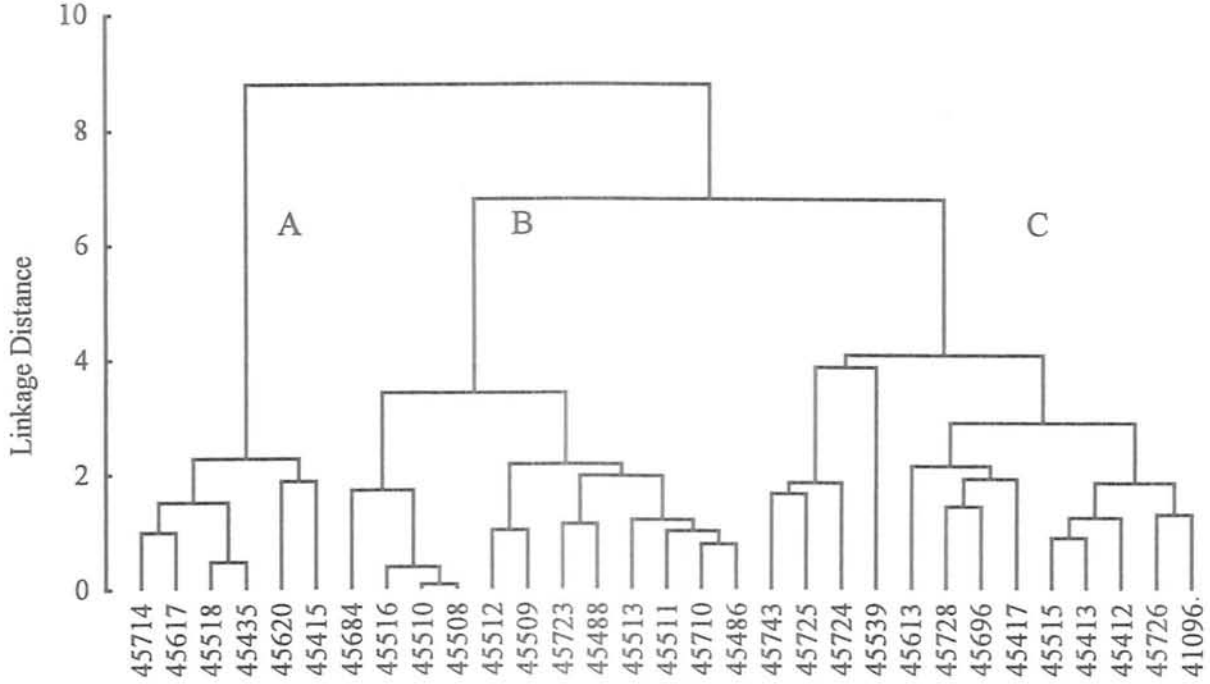


Fig. 4.3.9:- Dendrogram based on SDS-PAGE markers for geographic distribution

Table 4.3.10:- Clusters based on SDS-PAGE in blackgram

Group	Cluster	Accessions	Altitude	Province	Zone
Group A	Cluster I	45714	400	P	9
		45617	1050	N	12
		45518	1480	NA	13
		45435	250	P	4
	Cluster II	45620	450	P	9
		45415	650	P	10
Group B	Cluster III	45684	1610	AK	12
		45516	1650	NA	13
		45510	1820	N	13
		45508	2270	N	13
	Cluster IV	45512	1750	NA	13
		45509	1190	N	13
		45723	1270	N	13
		45488	1640	B	14
		45513	1920	NA	13
		45511	2120	NA	13
		45710	270	P	5
		45486	1480	N	13
Group C	Cluster V	45743	1560	NA	12
		45725	1200	N	12
		45724	1200	N	12
		45539	960	N	12
	Cluster VI	45613	1250	N	13
		45728	800	N	13
		45696	220	P	7
		45417	830	P	10
	Cluster VII	45515	1650	NA	13
		45413	540	P	10
		45412	170	P	7
		45726	1100	N	12
		41096	1700	B	14

Table 4.3.11:- Mean and standard deviation for clusters based on SDS-PAGE in blackgram

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
DF	44.8±12.50	40.8±0.64	53.3±6.75	53.6±11.03	40.4±2.01	43.0±7.39	45.3±8.29
DM	79.7±18.5	70.1±3.89	98.2±5.80	95.2±13.82	75.7±3.85	77.4±13.65	83.5±19.35
BR	12.9±8.38	16.0±0.75	15.68±2.55	16.42±4.69	9.1±0.85	19.1±6.83	18.9±10.22
PODS	27.1±20.56	30.9±8.32	42.9±11.43	34.6±11.46	13.7±0.93	32.9±13.10	38.3±17.75
P/BR	2.11±0.53	1.89±0.32	2.72±0.38	2.20±0.70	1.60±0.21	1.75±0.27	2.02±0.45
PL	4.40±0.46	4.43±0.05	4.70±0.15	4.63±0.22	4.29±0.11	4.29±0.19	4.36±0.32
S/P	5.98±0.51	6.15±0.35	6.26±0.04	6.45±0.28	5.98±0.19	5.82±0.31	6.07±0.45
SD	0.26±0.04	0.28±0.05	0.25±0.02	0.28±0.03	0.28±0.04	0.26±0.02	0.28±0.02
SW	4.13±0.74	4.02±0.06	5.04±0.39	4.69±0.53	3.34±0.28	4.37±0.84	3.83±0.60
BY	22.28±20.92	17.46±0.60	35.32±6.05	30.47±10.45	9.26±2.72	30.20±22.0	28.34±15.29
GY	4.47±4.23	4.00±0.15	9.68±3.16	7.38±3.46	1.87±0.45	5.40±3.26	6.94±4.93
HI	19.61±6.63	23.70±0.51	27.10±5.53	23.94±8.09	20.87±3.48	19.11±3.95	22.93±7.62

reveal a precise clustering for agronomic characters. The cluster II, comprising of 2 accessions originating from Punjab was observed early in maturity (70.1±3.89 days) and medium grain yield (4.00±0.15 g). The clusters III, IV and VII were late in maturity and medium yielding.

Among all three geographic parameters, it was observed that 16 accessions out of 42, which were about 40% of the total, grouped together on the basis of provincial distribution, whereas on the basis of other two geographic parameters, no geographic pattern was observed for association between SDS-PAGE or morphological characters and geographic distribution. Therefore, data related to provincial distribution were plotted in a scatter diagram on the basis of first two components which contributed 70% of total variation (Fig. 4.3.10). The separation on the basis of PC_1 and PC_2 for provincial distribution revealed 5 groups. The major group on the left, 10 accessions were from N.W.F.P., 2 from NA and 1 from Punjab, a group in the middle, 7 accessions from Punjab, 2 from NA and 1 from NWFP. Three groups were observed on the right side of the graph. Out of these, one group consisting of 4 accessions from NWFP and NA may be considered from similar geographic origin, whereas other 2 groups consisted a mixture of various origins.

4.4 *Random Amplified Polymorphic DNA (RAPD)*

Forty pure-lines of *Vigna* spp. selected for analysis of RAPD were also evaluated for morphological characters and SDS-PAGE. Out of 40 pure-lines, 3 (45727, 45761 & 45774) were blackgram like mungbean, whereas 6 (41117, 41065, 41084, 41085, 41096 & 41016) were green seeded mungbean like blackgram genotypes. On the basis of SDS-PAGE, out of 20 polymorphic bands, 14 prominent and reproducible bands were used for analysis and presentation. Genetic diversity among *Vigna* genotypes was estimated based on the number of different protein peptides between the 2 compared. Table 4.4.1 indicates the number of different peptides in all possible combinations of 40 *Vigna* genotypes. Data showed that 15 genotypes gave similar protein pattern and thus excluded from the cluster analysis, therefore, cluster analysis was conducted for 25 genotypes and the dendrogram is presented in Fig. 4.4.1. The dendrogram of total seed proteins based on dissimilarity matrix using UPGMA showed a division into 2 main groups. The group A

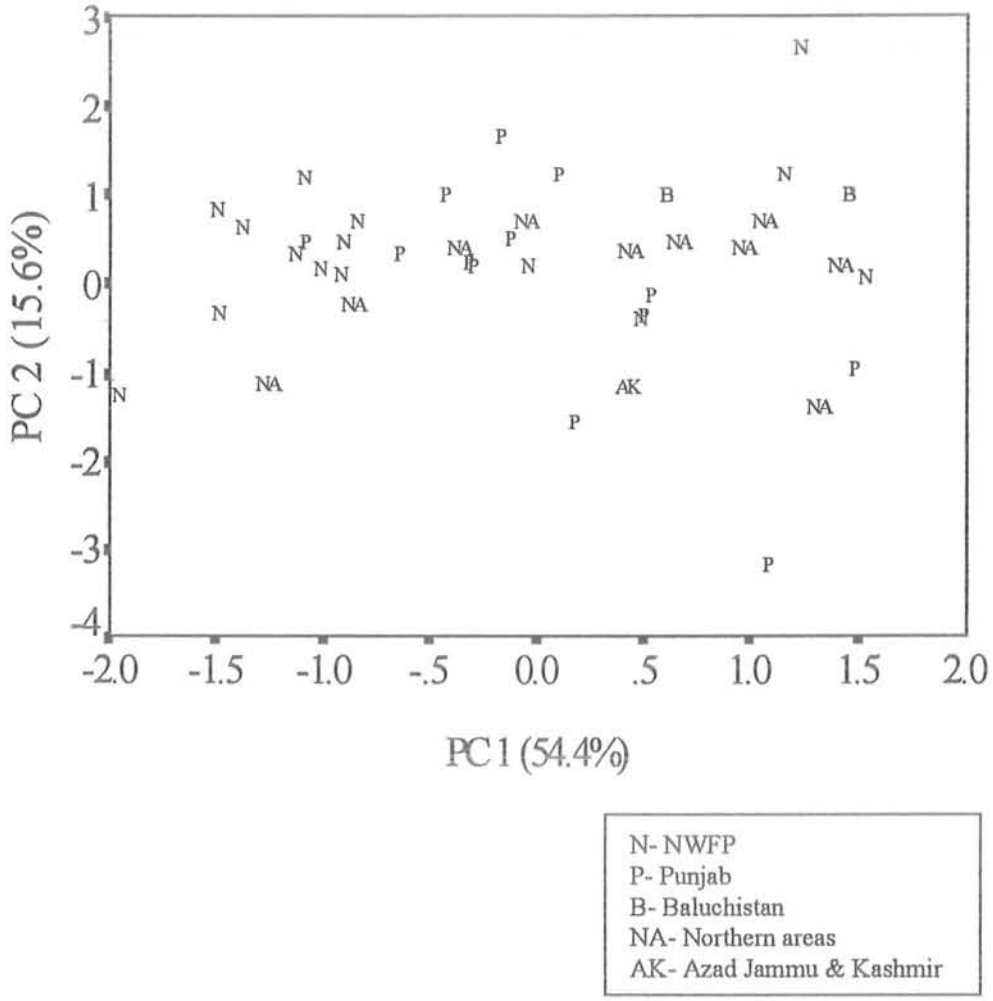


Fig. 4.3.10:- Scattered diagram of first two PCs on the basis of provinces

Table 4.4.1: The number of different protein peptides detected between 3 *Vigna radiata* and 37 *V. mungo* genotypes.

	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	39	40
M3	1	2	2	2	1	1	3	1	1	1	2	0	3	2	6	3	0	0	0	1	10	3	5	2	1	3	2	1	2	4	4	7	2	2	2	5	2	3	2
9006		1	1	3	2	2	4	2	2	0	3	1	2	3	7	4	1	1	1	2	11	4	6	3	2	4	3	2	3	5	5	8	3	3	3	6	3	4	3
9039			0	4	1	1	3	1	1	1	4	2	3	4	8	5	2	2	2	3	10	5	5	2	3	5	4	3	4	6	6	7	4	4	4	7	4	5	4
9047				4	1	1	3	1	1	1	4	2	3	4	8	5	2	2	2	3	10	5	5	2	3	5	4	3	4	6	6	7	4	4	4	7	4	5	4
9067					3	3	5	3	3	3	4	2	5	4	8	5	2	2	2	3	12	5	7	4	1	3	0	1	2	2	2	9	4	4	4	3	4	5	4
9010						0	2	0	0	2	3	1	4	3	7	4	1	1	1	2	9	4	4	1	2	4	3	2	3	5	5	6	3	3	3	6	3	4	3
M1							2	0	0	2	3	1	4	3	7	4	1	1	1	2	9	4	4	1	2	4	3	2	3	5	5	6	3	3	3	6	3	4	3
9093								2	2	4	1	3	2	1	5	2	3	3	3	2	7	4	2	1	4	6	5	4	3	7	3	8	5	5	5	6	5	4	5
41117									0	2	3	1	4	3	7	4	1	1	1	2	9	4	4	1	2	4	3	2	3	5	5	6	3	3	3	6	3	4	3
PL-2										2	3	1	4	3	7	4	1	1	1	2	9	4	4	1	2	4	3	2	3	5	5	6	3	3	3	6	3	4	3
9026											3	1	2	3	7	4	1	1	1	2	11	4	6	3	2	4	3	2	3	5	5	8	3	3	3	6	3	4	3
41096												2	1	0	4	1	2	2	2	1	8	3	3	2	3	5	4	3	2	6	2	9	4	4	4	5	4	3	4
M2													3	2	6	3	0	0	0	1	10	3	5	2	1	3	2	1	2	4	4	7	2	2	2	5	2	3	2
9086														1	5	2	3	3	3	2	9	4	4	3	4	6	5	4	3	7	3	10	5	5	5	6	5	4	5
9080															4	1	2	2	2	1	8	3	3	2	3	5	4	3	2	6	2	9	4	4	4	5	4	3	4
45727																3	6	6	6	5	4	7	7	6	7	9	8	7	6	10	6	7	8	8	8	9	8	7	8
45738																	3	3	3	2	7	4	4	3	4	6	5	4	3	7	3	8	5	5	5	6	5	4	5
45703																		0	0	1	10	3	5	2	1	3	2	1	2	4	4	7	2	2	2	5	2	3	2
41065																			0	1	10	3	5	2	1	3	2	1	2	4	4	7	2	2	2	5	2	3	2
45426																				1	10	3	5	2	1	3	2	1	2	4	4	7	2	2	2	5	2	3	2
9035																					9	2	4	1	2	4	3	2	1	5	3	8	3	3	3	4	3	4	3
45761																						9	7	8	11	11	12	11	10	12	10	3	10	10	10	11	10	9	10
41084																							2	3	4	2	5	4	3	3	5	8	1	1	1	2	1	2	1
45740																								3	6	4	7	6	5	5	5	8	3	3	3	4	3	2	3
9092																									3	5	4	3	2	6	4	7	4	4	4	5	4	5	4
9005																										4	1	2	3	3	3	8	3	3	3	4	3	4	3
41085																											3	2	3	1	5	8	1	1	1	2	1	2	1
9025																												1	2	2	2	9	4	4	4	3	4	5	4
9056																													1	3	3	8	3	3	3	4	3	4	3
45737																														4	2	9	4	4	4	3	4	5	4
45304																															4	9	2	2	2	1	2	3	2
45736																																11	6	6	6	3	6	5	6
45774																																	7	7	7	10	7	8	7
9065																																		0	0	3	0	1	0
45701																																			0	3	0	1	0
9020																																				3	0	1	0
9014																																					3	4	3
45729																																						1	0
9029																																							1

Any two genotypes with 0 value were similar for protein banding pattern.

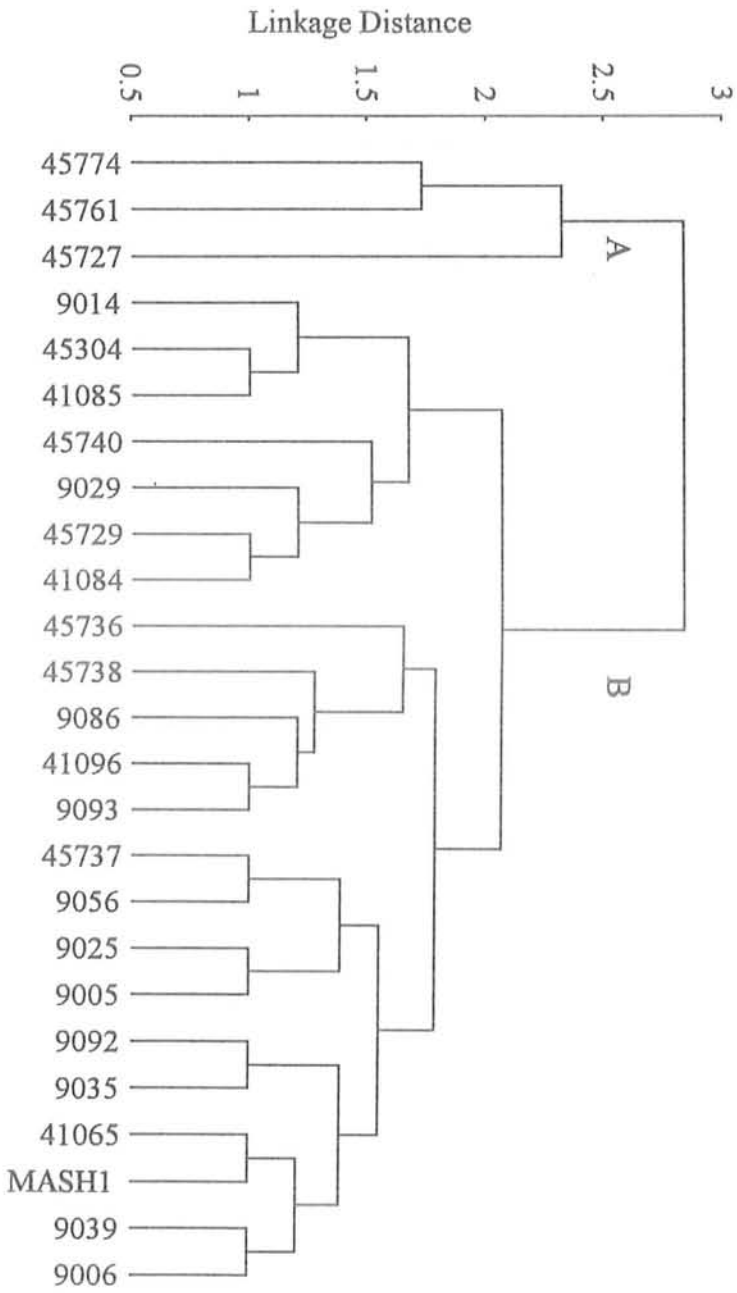


Fig. 4.4.1:-Dendrogram based on SDS-PAGE markers in 25 genotypes of *Vigna* spp.

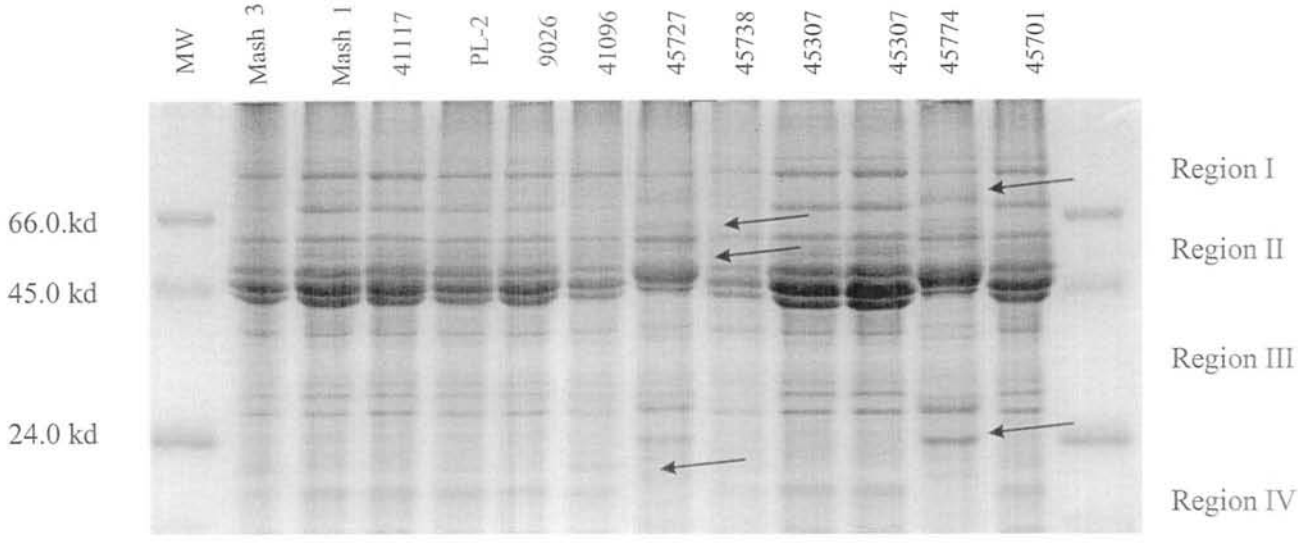


Fig. 4.4.2:- Variation in Seed proteins of *Vigna* spp. The molecular marker used in this gel was SDS-70 KIT from SIGMA chemical company. The arrows indicate variation in various regions.

consisted 2 clusters, whereas group B comprised of 3 clusters. The cluster I comprised of 2 genotypes (45774, 45761) and cluster II one genotype (45727). The group A, consisted 3 genotypes and all of these were *Vigna radiata,* hence clearly differed from other clusters. One band closer to MW of 66.0 kd was observed in all the blackgram genotypes, whereas in mungbean, a band of higher MW was observed (Fig. 4.4.2). This band could be used for identification of these two species. A very thick band of 45.0 Kd MW was specific for *V. radiata*. Two bands of approx. 40.0 and 34.7 Kd MW, respectively were also specific to genotypes 45727, 45761 and 45774, hence these specific bands alongwith others mentioned could be used for identifying *Vigna radiata* from mixed germplasm. Cluster III consisted of 7 genotypes, i.e., 9014, 45304, 41085, 45740, 9029, 45729, 41084 (Table 4.4.2). Out of these, 41084 & 41085 were green seeded blackgram, 9029 was advance breeding line and others were pure-lines selected from local germplasm in the gene-bank, PGRI. Cluster IV consisted 5 genotypes (45736, 45738, 9086, 41096, 9093) and cluster V consisted 10 genotypes, viz., 45737, 9056, 9025, 9005, 9092, 9035, 41065, Mash 1, 9039 and 9006. Out of these 45737 and 41065 were obtained from Gene-bank, PGRI and others were advance lines. Some bands were observed more frequently, while others were specific to some genotypes. In general, cluster analysis based on SDS-PAGE proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo,* whereas within *Vigna mungo* a low level of genetic diversity was observed and no clear differentiation was observed either for agronomic characteristics or origin as various clusters consisted of mixed genotypes from different origins.

Analysis for each cluster was performed and means alongwith standard deviation are presented in Table 4.4.3. The results revealed that genotypes comprising of cluster I were *Vigna radiata* and gave mean maturity of 67.5±9.19 days, grain yield of 4.41±0.55 g and harvest index of 13.57±0.41%. The genotype 45727 exhibited mean performance of 80.2±10.35 days for maturity, 1.04±0.52 g for grain yield and 5.42±0.95% for harvest index. Cluster III gave average values of 84.4±17.70 days for maturity, 6.10±5.49 g for yield and 20.25±8.80% for harvest index. The genotypes of cluster IV were medium in maturity (80.8±16.88 days) and high in grain yield (7.35±5.12 g) with medium harvest index (22.31±12.99%). In the present study, clusters I and V could be categorised as early

Table 4.4.2:- Clusters based on SDS-PAGE markers in 25 genotypes of *Vigna* spp.

Cluster	Frequency	Genotypes
Cluster I	2	45774, 45761
Cluster II	1	45727
Cluster III	7	9014, 45304, 41085, 45740, 9029, 45729, 41084
Cluster IV	5	45736, 45738, 9086, 41096, 9093
Cluster V	10	45737, 9056, 9025, 9005, 9092, 9035, 41065, Mash 1, 9039, 9006
Cluster VI	15 (homozygous for SDS-PAGE)	41016, PL-2, 9020, 45701, 45703, 45426, 9065, 41117, 9047, 9010, 9080, 9067, 9026, Mash 2, Mash1 3

Table 4.4.3:- Mean and standard deviation for clusters based on SDS-PAGE in *Vigna* spp.

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI (Homozygous for SDS-PAGE)
DF	39.0±1.41	34.0±1.21	45.0±8.70	41.5±9.42	38.2±7.09	45.3±8.54
DM	67.5±9.19	80.2±10.35	84.4±17.70	80.8±16.88	70.2±14.55	79.8±17.08
PH	50.5±2.12	48.3±3.21	40.4±12.23	41.6±17.24	30.8±14.28	36.8±9.42
BR/PL	6.5±0.71	3.1±0.99	21.6±8.68	19.3±14.39	15.9±7.57	16.1±10.15
P/PL	20.0±1.41	5.0±1.51	36.9±19.30	37.4±19.80	31.4±14.60	38.2±31.0
P/BR	3.08±0.12	1.67±0.09	2.09±1.59	2.30±0.68	2.10±0.93	2.41±0.82
PL	7.65±0.21	4.45±0.31	4.61±0.61	4.43±0.55	4.38±0.16	4.52±0.50
S/P	11.7±0.71	6.2±0.51	6.16±0.98	6.12±0.43	5.83±0.36	5.9±0.79
SD	0.35±0.02	0.19±0.01	0.24±0.07	0.28±0.06	0.25±0.04	0.23±0.09
SW	3.12±0.06	3.79±0.03	4.59±0.66	4.51±0.43	4.29±0.21	4.43±0.61
BY	32.57±5.05	19.19±3.45	31.02±16.79	30.68±11.10	21.86±5.11	24.17±15.41
GY	4.41±0.55	1.04±0.52	6.10±5.49	7.35±5.12	5.16±2.33	7.33±6.13
HI	13.57±0.41	5.42±0.95	20.25±8.80	22.31±12.99	22.71±7.76	29.51±9.14

maturing with medium grain yield, whereas other clusters were late in maturity and gave medium to high grain yield. The genotypes comprising the cluster V were early in maturity (70.2±14.55 days), medium yielder (5.16±2.33 g) and medium harvest index (22.71±7.76%). Fifteen homozygous lines (41016, PL-2, 9020, 45701, 45703, 45426, 9065, 41117, 9047, 9010, 9080, 9067, 9026, Mash 2 & Mash1 3) on the basis of SDS-PAGE were kept in cluster VI which gave average performance of 79.8±17.05 days to maturity, 7.33±6.13 g for grain yield and 29.51±9.14% for harvest index. Most of these genotypes were advance breeding lines including 2 improved varieties (Mash 2 & Mash 3) and hence the selection pressure for agronomic characters might have influenced the protein banding pattern. The variance for most of the characters in almost all the clusters gave a clue that the genotypes in various clusters may be from different origins but sharing similar protein peptides. The results further indicated that grouping of genotypes did not reflect morphological status and hence this technique could more confidently be used for inter-specific genetic diversity rather than intra-specific for *Vigna* spp.

4.4.1 DNA amplification

For RAPD analysis, fifty three 12 mer primers (Operon) were studied to find the initial results for amplification. Figure 4.4.3 shows the amplification profiles generated by primer "OPD 20" (5′ -AAGACCCTACGA- 3′) for *Vigna* spp. Fifty three primers were tested using 4 genotypes (9010, PL-2, 41065, 45737) for DNA amplification initially. Among them, 46 revealed amplification for the present material, whereas 7 primers failed to amplify DNA. Out of amplified primers, 36 exhibited polymorphism and hence could be used for *Vigna* DNA fingerprinting (Table 4.4.4). Finally, out of 36 polymorphic primers, 11 were used for RAPD analysis of all 40 genotypes.

4.4.2 Primers data

Each of the 11 primers varied greatly for the ability to resolve variability among 40 genotypes. Some of the primers generated several markers and were able to show a high level of genetic diversity, while others produced few markers and detected little variability. Eleven primers generated a total of 131 reproducible and score able amplification products across all the genotypes, out of which 107 (80.2 %) fragments were

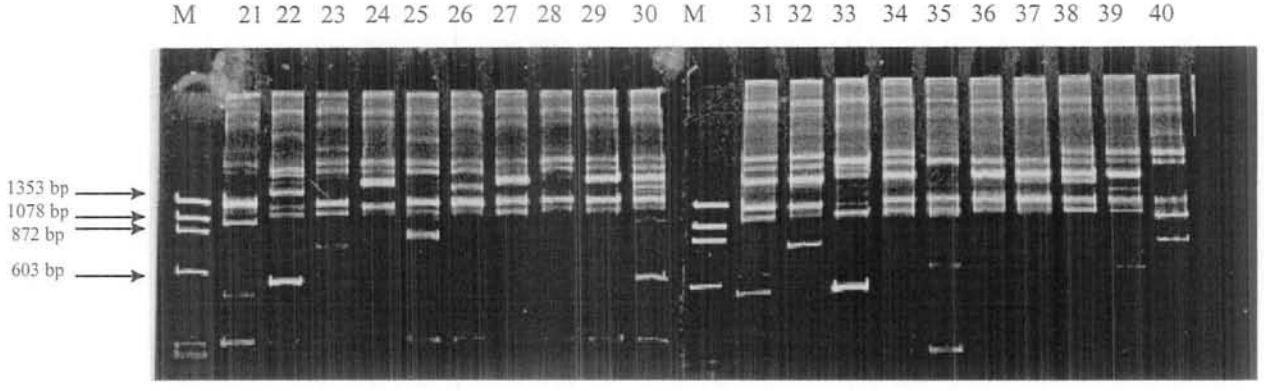


Fig. 4.4.3:- Segregation of RAPD markers with primer "OPD 20" among 20 genotypes of *Vigna* spp. The markers are indicated by arrows with molecular size. Molecular marker X 174/*Hae* III digest was used. The genotypes (22 & 33) were *Vigna radiata* while all others were *V. mungo*.

Table 4.4.4:- Primer used for preliminary DNA amplification in *Vigna mungo*

Sr.No.	Primer	Sequence (5′ to 3′)		Sr.No.	Primer	Sequence (5′ to 3′)	
1-	D 20	AAGACCCTACGA	+/-	28-	C 54	ATGCAGAAGTCT	+
2-	D 10	AGGGCCATGATA	+/-	29-	C 95	GTCCACGTGAAG	+
3-	D 40	TCCGAGTATCTG	+/-	30-	C 17	GTTATGCAAGGG	+
4-	F 30	GGAGAGGAAATG	+/-	31-	C 61	AAGAGGGTTGAC	+
5-	F 21	AGCAACAATCCG	+/-	32-	C 75	GATGGTGACGAA	+/-
6-	F 16	ACTAACCTGGAC	+/-	33-	C 85	ACTTTGACAGCG	+/-
7-	D 69	CGCTCCAAATCA	+/-	34-	C 27	GAGGACGTTAAA	+/-
8-	D 91	CTGAAGTATCCC	-	35-	C 67	CCAAGATCCATT	+/-
9-	D 76	CACTTCAACCAG	+/-	36-	C 71	TTCAACATCGAC	+/-
10-	D 65	TACAGCCACTTG	+/-	37-	D 25	GCCATCCGTACA	+/-
11-	D 20	AAGACCCTACDA	+/-	38-	D 45	AGGGTCGTTGCA	+/-
12-	C 20	ACTCACAAATTG	+/-	39-	C 32	TTGCCTCGAGCT	-
13-	C 19	CAGGATGACCTA	+	40-	C 39	CCTAAGGGGTCT	+/-
14-	C 24	AACGAGCAGAAC	+/-	41-	C 78	CTCGAAACGCGT	-
15-	C 26	CACGTTATCGCA	+/-	42-	C 91	CACCTGGCATGG	+/-
16-	C 41	CAGACAGGGTAT	+/-	43-	Z 10	CCCGTGCACGGA	-
17-	C 42	AAGGTCGAACGT	+	44-	C 63	ACTGTTATAACG	-
18-	C 44	GGCAACATAGTA	+/-	45-	F 04	TTGAGTAGTTGC	-
19-	C 92	ATCGACGGAGAA	+/-	46-	D 86	ATGGGCCAGAAG	+/-
20-	C 93	GTCACTCGGATA	+	47-	D 87	GACGCCTATGTC	+/-
21-	C 96	ACCAAGAAAGGG	+/-	48-	D 89	CAGTCTAAGCGG	+/-
22-	C 97	AAGACGGTGGTA	+/-	49-	F 48	GTCCTGCTAACC	-
23-	C 98	ACCAACGTGTAC	+/-	50-	A 73	AGCACTAAATCT	+/-
24-	F 22	AAGATCAAAGAC	+/-	51-	B 32	ATCATCGTACGT	+/-
25-	C 48	ATGAGTGGACAC	+	52-	B 28	GGACAAGTAATG	+/-
26-	C 72	AGATTGCAAGAA	+	53-	D 67	AACACCTCGATC	+/-
27-	C 50	CTTCCTTGGTCA	+				

+ represents amplification of primer, - no amplification and +/- represents polymorphism obtained by the primer

polymorphic in one or other of the 40 genotypes. The rest of the 24 (19.8%) bands were monomorphic for the present material (Table 4.4.5). The levels of polymorphism varied with different primers among various genotypes. The number and size of amplified fragments also varied with different primers. A maximum of 18 fragments out of which 14 were polymorphic were generated by primer OPD 69, followed by OPF 21 producing 17 fragments, out of which 13 were polymorphic (Table 4.4.5). Each of primers OPF 22, OPD 67 and OPC 96 produced 9 polymorphic fragments. Many primers generated genotype-specific amplification products. The primer OPB 32 produced 1 specific band of high MW (>1353 bp) in 2 genotypes (45761, 45774) which were mungbean whereas, same band was absent in 45727 which was also mungbean. Two bands of high MW were absent in 45761 and 45774, whereas these were present in all other genotypes. Primer OPF 21 produced specific band of 1078 bp in all the genotypes except 45761 where it was absent and hence it could be specific for this genotype. Four bands in this primer were observed specific for 45761 and 45774, whereas 45727 did not share any band with these two genotypes although 45727 was also *Vigna radiata* but genetically diverse from 45774 and 45761 on the basis of this primer. The primer OPC 92 produced 7 polymorphic bands and one band of > 1353 bp was observed in all three mungbean genotypes, therefore, this primer may be specific for mungbean genotypes included in present study. Primer OPA 73 produced 6 out of 11 bands specific for 45761 and 45774. Five specific bands for 45761 and 45774 were observed in the primer OPD 69 where 14 polymorphic bands were observed. Primer OPF 22 exhibited 2 specific bands for 41016 which was a green seeded genotype. This band may not be related to the appearance of seed coat colour because in present research material six genotypes (41117, 41096, 41065, 41084, 41086, 41016) were green seeded and no such polymorphism was observed which could be associated with seed coat colour. One band in middle of 60-310 bp was observed specific for 45761 and 45774 in the primer OPF 22. The primer OPC 67 gave 5 polymorphic bands, one at 1078 bp was specific to 45761 and 45774. Similarly, OPC 96 and OPC 26 also produced specific bands for 45761 and 45774. One band in the middle of 1078-1353 bp for the primer OPC 26 was observed specific for all three mungbean genotypes (45727, 45761, 45774) and hence could be exploited for identification of mungbean cultivars.

Table 4.4.5:- Primes selected for polymorphism in 40 genotypes of *Vigna* spp.

Primer	Sequence	Total amplified bands					Polymorphic Bands	Monomorphic bands	Percent polymorphism
		Molecular weight (bp)							
	Sequence (5′ to 3′)	1353 & >	1078-1353	872-1078	603-872	<603			
OPF 22	AAGATCAAAGAC	4	2	0	2	1	9	0	100.0
OPD 69	CGCTCCAAATCA	3	2	2	4	3	14	4	77.8
OPF 21	AGCAACAATCCG	2	2	2	4	3	13	4	76.5
OPA 73	AGCACTAAATCT	5	2	1	1	2	11	1	91.7
OPC 92	ATCGACGGAGAA	4	1	0	1	1	7	4	63.6
OPD 20	AAGACCCTACGA	5	3	2	3	0	13	1	92.9
OPD 67	AACACCTCGATC	5	1	1	2	0	9	2	81.8
OPC 26	CACGTTATCGCA	3	3	0	0	0	6	1	85.7
OPC 96	ACCAAGAAAGGG	3	2	2	2	0	9	0	100.0
OPC 67	CCAAGATCCATT	2	1	1	1	0	5	2	71.4
OPB 32	ATCATCGTACGT	7	2	1	1	0	11	5	68.8
Total		43	21	12	21	10	107	24	

4.4.3 *Cluster analysis*

Genetic diversity in *Vigna* was carried out on the basis of number of different RAPD fragments between the 2 genotypes compared and Table 4.4.6 shows the number of different fragments in all possible combinations of 40 *Vigna* genotypes. Based on similarity coefficients using RAPD data, cluster analysis was performed by UPGMA in order to determine the relationships among different genotypes. The cluster analysis suggested the existence of two major groups (Fig. 4.4.4) for 40 genotypes based on RAPD. One group consisting of 2 genotypes (45774, 45761) differed from others. As these 2 genotypes were distinctly diverse from rest of the germplasm (either mungbean or blackgram), therefore, this cluster was excluded from the analysis in second set for precise clustering (Fig. 4.4.5). Cluster I consisted of two genotypes which were blackgram like mungbean. Cluster II, III, IV, V, and XI consisted single genotype as, 41016, 45737, 45727, PL-2 and 9010, respectively (Table 4.4.7). The genotype 41016 was a green seeded blackgram, PL-2 was an exotic genotype obtained from AVRDC and 45727 was a *Vigna radiata* genotype but genetically different from other two genotypes (45774, 45761). Other genotypes 45737 and 9010 were of local origin but did not share any group on the basis of eleven primers under investigation. Cluster VI consisted of 5 genotypes, out of which 4 (9014, 9020, 9005, 9092) were advance breeding lines. Cluster VII consisted of 2 genotypes (45729, 9025), cluster VIII consisted of 6 genotypes, out of which 5 (45703, 45736, 45740, 45426, 45738) were obtained from gene-bank and 9056 was advance breeding line. Cluster IX consisted of 5 genotypes, four of these (41085, 41084, 41065, 41117) were green seeded. Two genotypes (41096, 9047) comprised cluster X, whereas cluster XII consisted of 13 genotypes. Cluster XII included advance breeding lines and approved varieties of blackgram except 45304 which was obtained from gene-bank.

The analysis for each cluster was performed and mean alongwith standard deviation for each cluster is presented in Table 4.4.8. The results revealed that the genotypes comprising of cluster I gave mean maturity days of 67.5±9.19, grain yield of 4.41±0.55 g and harvest index of 13.57±0.41%. These genotypes, as already mentioned, were mungbean and similarly these gave high values for pod length (7.65±0.21 cm) and

Table 4.4.6:- The number of different RAPD fragments detected between 3 *Vigna radiata* and 37 *V. mungo* genotypes

	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	39	40
Mash 3	7	5	13	13	11	6	8	15	18	10	16	11	11	16	18	20	23	20	17	9	72	17	16	15	12	23	17	19	24	10	16	64	15	11	16	16	17	12	25
9006		8	12	8	12	7	11	12	21	7	13	6	12	15	17	19	24	15	16	8	73	12	17	14	13	18	16	22	23	13	17	63	14	14	19	17	14	11	24
9039			10	14	14	5	7	10	17	11	17	10	10	15	19	19	22	17	18	12	71	16	17	18	17	18	18	18	19	11	17	61	14	14	19	15	16	11	22
9047				18	24	13	13	16	25	17	13	12	14	19	19	21	26	19	20	18	71	18	21	22	21	18	26	24	27	15	21	59	16	20	23	21	22	19	26
9067					20	13	17	14	21	9	19	10	16	13	23	17	22	17	16	14	75	14	17	14	13	20	16	24	25	19	17	63	14	14	19	19	18	17	30
9010						15	15	18	23	15	21	14	18	17	25	27	26	19	24	14	71	22	23	18	17	26	18	24	23	17	25	65	22	14	21	21	20	13	24
Mash 1							8	15	16	8	18	9	7	12	16	18	25	18	15	11	72	15	16	17	16	19	17	19	22	10	14	60	15	13	18	14	13	8	21
9093								15	20	12	16	11	13	16	18	22	23	16	19	13	70	15	16	17	16	17	19	19	20	12	16	60	17	13	18	16	17	14	21
41117									17	13	19	12	12	15	23	15	16	9	14	16	73	12	13	16	13	10	14	14	17	13	17	59	8	12	17	15	14	17	20
PL-2										18	22	21	15	20	26	18	19	24	17	25	70	21	16	19	18	21	17	21	20	18	18	62	17	17	18	16	19	16	27
9026											16	9	9	12	20	22	25	16	19	13	74	15	20	13	12	19	17	23	26	16	20	62	15	13	18	18	15	14	25
41096												17	19	22	24	24	25	20	21	17	74	19	20	19	16	21	23	25	26	16	18	66	19	19	20	20	23	20	27
Mash 2													12	9	15	19	22	13	18	12	77	14	15	14	15	16	18	20	21	13	15	61	14	16	21	19	16	11	26
9086														11	17	17	22	17	14	14	73	18	15	16	13	16	18	16	19	11	17	57	12	12	13	13	14	15	20
9080															18	16	21	14	13	19	74	17	14	15	14	17	15	19	20	14	16	60	13	11	16	16	15	12	25
45727																20	23	20	17	21	70	19	16	23	20	21	25	19	22	16	16	60	19	23	24	22	15	20	29
45738																	7	14	5	19	76	13	6	17	14	13	11	11	16	14	8	62	9	17	14	14	11	16	19
45703																		15	12	24	75	18	9	20	17	16	16	14	17	17	13	61	12	18	17	21	18	23	24
41065																			11	15	74	7	16	15	12	9	13	13	18	14	16	60	11	15	18	14	15	14	19
45426																				16	73	10	7	16	11	14	12	12	19	13	9	63	8	14	11	9	12	13	22
9035																					77	14	19	12	11	18	16	18	23	11	17	67	18	16	17	15	16	13	22
45761																						75	76	77	76	71	73	75	68	76	78	26	73	69	74	72	69	74	75
41084																							15	14	13	10	10	16	25	17	13	63	14	16	19	13	12	13	20
45740																								19	14	15	13	11	16	12	6	62	9	13	14	16	13	18	21
9092																									7	16	12	20	23	17	17	65	16	12	13	17	16	15	22
9005																										15	11	17	22	12	12	62	11	9	8	12	13	16	21
41085																											14	14	19	13	15	59	14	16	15	13	14	19	14
9025																												14	21	19	15	63	16	12	15	15	10	13	20
9056																													15	13	13	67	12	18	19	17	16	19	22
45737																														18	18	60	19	21	20	18	17	20	23
45304																															12	62	11	13	14	14	15	14	19
45736																																62	13	17	14	14	15	14	21
45774																																	61	57	62	64	57	64	65
9065																																		12	15	17	14	19	22
45701																																			9	17	16	17	20
9020																																				8	19	18	19
9014																																					15	10	21
45729																																						13	20
9029																																							23

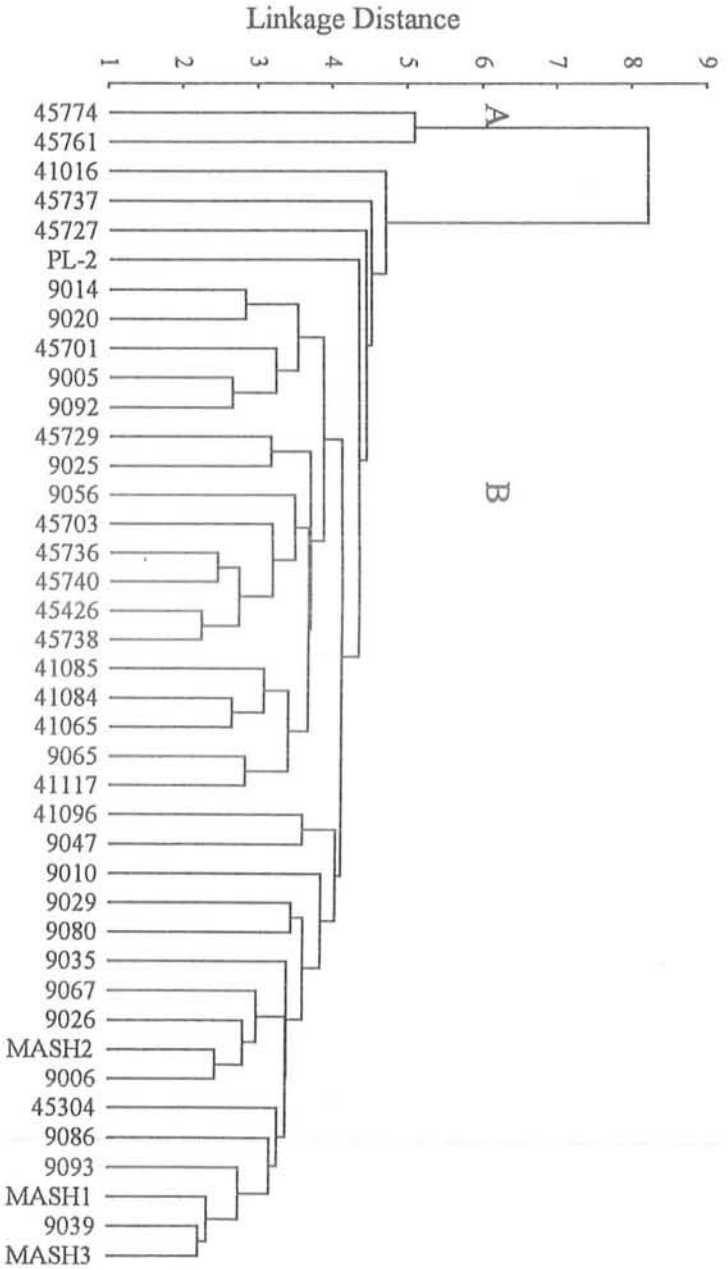


Fig. 4.4.4:-Dendrogram based on RAPD markers in 40 genotypes of *Vigna* spp.

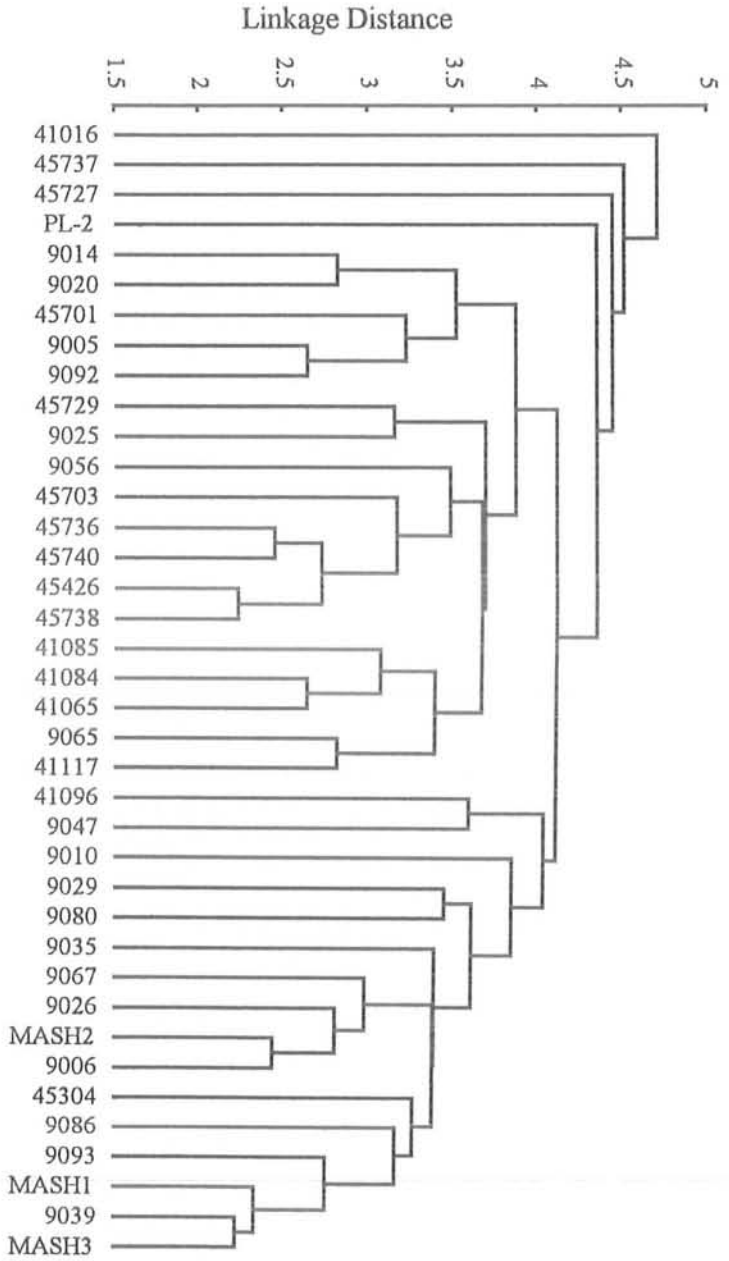


Fig. 4.4.5:-Dendrogram based on RAPD markers in 38 genotyeps of *Vigna* spp.

Table 4.4.7:- Clusters based on RAPD markers in 40 genotypes of *Vigna* spp.

Cluster	Frequency	Genotypes
Cluster I	2	45774, 45761
Cluster II	1	41016
Cluster III	1	45737
Cluster IV	1	45727
Cluster V	1	PL-2
Cluster VI	5	9014, 9020, 45701, 9005, 9092
Cluster VII	2	45729, 9025
Cluster VIII	6	9056, 45703, 45736, 45740, 45426, 45738
Cluster IX	5	41085, 41084, 41065, 9065, 41117
Cluster X	2	41096, 9047
Cluster XI	1	9010
Cluster XII	13	9029, 9080, 9035, 9067, 9026, Mash 2, 9006, 45304, 9086, 9093, Mash 1, 9039, Mash 3

Table 4.4.8:- Mean and standard deviation for clusters based on RAPDs in *Vigna* spp.

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII
DF	39.0±1.41	56±0.99	32±2.58	34.0±1.21	47±1.53	41.8±11.28	35.8±3.18	44.5±8.51	50.0±8.38	50.0±10.61	38±6.01	38.5±4.16
DM	67.5±9.19	111±3.15	75±4.09	80.2±10.35	76±3.45	74.6±17.41	69.8±7.42	79.0±13.73	94.6±21.78	90.0±28.99	67±12.02	69.1±5.29
PH	50.5±2.12	40±1.01	40±1.24	48.3±3.21	26±3.12	28.8±10.89	25.0±7.07	55.0±5.48	42.1±8.18	34.5±3.54	32±2.12	30.7±10.48
BR/PL	6.5±0.71	17.8±0.21	7.0±2.04	3.1±0.99	9.0±0.56	24.9±8.83	12.9±0.82	11.63±10.68	16.37±5.58	25.08±14.97	7.7±1.21	18.9±10.45
P/PL	20.0±1.41	23.5±0.68	8.4±1.01	5.0±1.51	23±2.45	48.2±10.88	35.0±9.43	20.97±10.67	23.0±11.39	47.3±18.27	21.7±9.51	46.8±30.80
P/BR	3.08±0.12	1.47±0.09	1.20±0.10	1.67±0.09	2.56±0.08	2.24±0.82	2.64±1.05	2.27±0.70	1.61±0.84	2.03±0.51	2.87±0.21	2.64±1.24
PL	7.65±0.21	5.5±2.14	4.0±1.24	4.45±0.31	4.3±1.11	4.51±0.28	4.37±0.07	4.05±0.54	4.78±0.45	4.39±0.09	4.4±0.82	4.56±0.38
S/P	11.7±0.71	6.5±0.12	5.2±0.80	6.2±0.51	5.6±0.92	6.32±0.61	6.20±0.57	5.23±0.87	5.80±0.43	6.20±0.42	5.9±0.99	6.14±0.64
SD	0.35±0.02	0.16±0.00	0.23±0.01	0.19±0.01	0.23±0.01	0.28±0.03	0.29±0.08	0.21±0.14	0.20±0.07	0.29±0.03	0.25±0.01	0.26±0.03
SW	3.12±0.06	5.86±0.01	4.23±0.02	3.79±0.03	3.80±0.09	4.26±0.25	4.17±0.28	4.86±0.66	4.30±0.50	4.55±0.37	4.96±0.02	4.35±0.35
BY	32.57±5.05	26.94±1.21	17.35±2.14	19.19±3.45	14.90±2.04	29.35±6.73	29.13±9.67	23.04±11.40	18.66±7.61	33.89±19.98	7.03±2.65	28.43±17.76
GY	4.41±0.55	6.03±0.16	1.35±0.09	1.04±0.52	4.95±1.04	8.09±1.96	5.02±0.40	2.69±1.29	3.77±2.18	9.71±4.83	2.61±1.15	9.23±6.63
HI	13.57±0.41	22.41±2.14	7.78±1.14	5.42±0.95	33.22±3.68	28.13±4.04	17.59±4.03	12.26±4.95	21.84±10.73	29.49±3.31	38.59±5.12	32.32±5.11

seeds per pod (11.7±0.71). Cluster II, III, IV, V and XI consisted of single genotype in each case. Out of these, 41016 was late in maturity, while others (45737, 45727, PL-2, 9010) were early to medium in maturity. Cluster VI consisted 5 genotypes and produced an average of 74.6±17.41 days to maturity, 8.09±1.96 g yield and 28.13±4.04% harvest index. The genotypes comprising cluster VII were early in maturity (69.8±7.42 days), medium yielding (5.02±0.40 g) and medium harvest index (17.59±4.03%). Cluster VIII exhibited an average of 79.1±13.73 days for maturity, 2.69±1.29 g of grain yield and 12.26±4.95% of harvest index. Cluster IX was categorized as late maturing (94.6±21.78 days) and low yielding (3.77±2.18 g) alongwith medium harvest index (21.84±10.73%), whereas cluster X was categorized as late maturing (90.0±28.99 days), high yielding (9.71±4.83 g) and high harvest index (29.49±3.31%). Thirteen genotypes comprising cluster XII gave average performance of 69.1±5.29 for days to maturity, 46.8±30.80 for pods/plant, 9.23±6.63 g for grain yield and 32.32±5.11% for harvest index. As already mentioned that most of the genotypes in this cluster were advance breeding lines which have been selected for high yield potential and other economic characters and thus were grouped together. In the present study, clusters VI, and XII could be categorised as high yielding and early maturity, whereas cluster VII could be considered early maturing and medium yielding. The cluster X was observed high yielding but late maturing. High variation for most of characters in some of the clusters revealed the scope of selection for improvement.

4.4.4 Cluster based on quantitative characters

Euclidean dissimilarity coefficient matrix was calculated for 40 *Vigna* genotypes from the agronomic data and phenogram constructed is presented in the Fig. 4.4.6. The cluster diagram using Ward's method revealed 10 clusters as presented in the Table 4.4.9. Cluster I consisted three genotypes (45740, 45701, 41096), of these 41096 was a green seeded genotype. Cluster II consisted single genotype (45426), which was a pure-line selected from local germplasm. The cluster III consisted 5 genotypes (41084, 41085, 41065, 41016, 41117) and all of these were green seeded blackgram lines, categorised as late maturing and low yielding. Two blackgram like mungbean genotypes (45774, 45761) constituted cluster IV and cluster V consisted of 5 genotypes (45736, 45737, 9056,

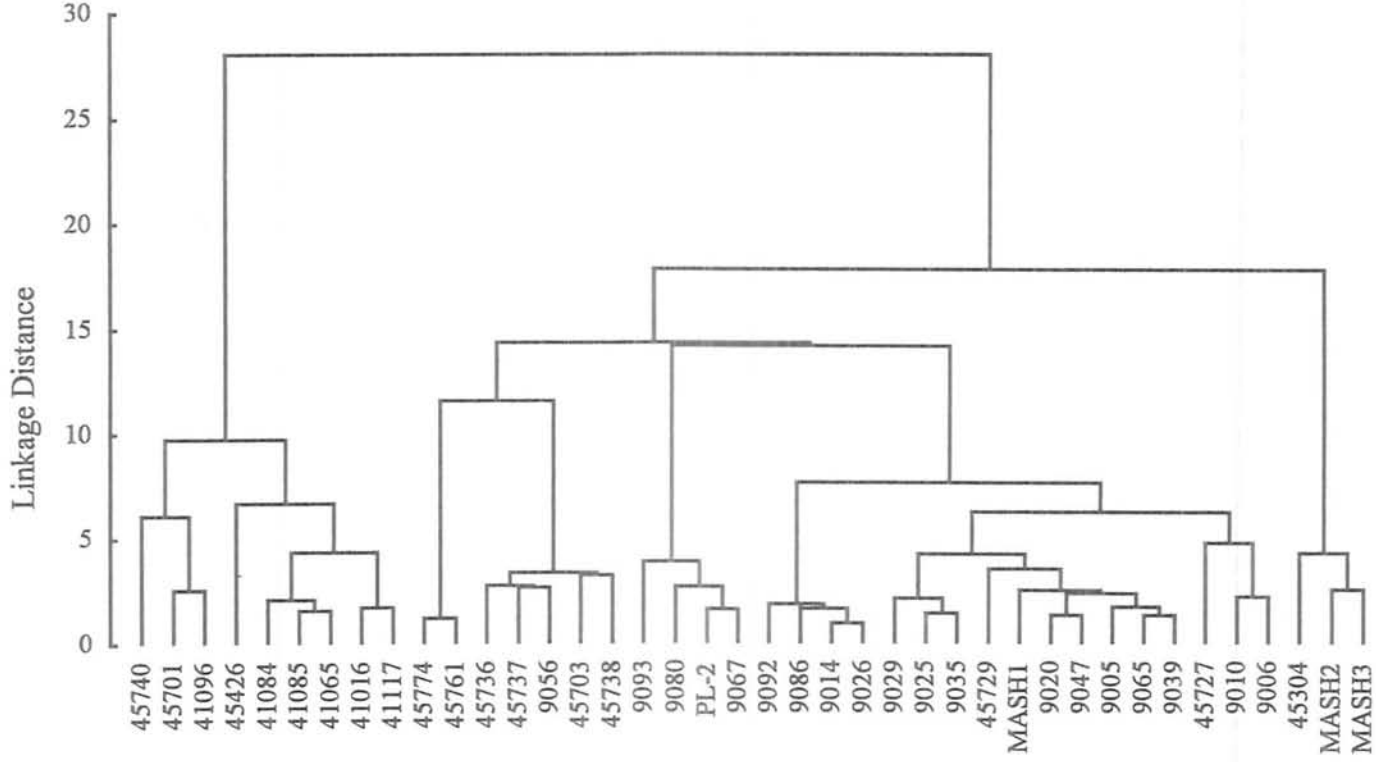


Fig. 4.4.6:-Phenogram based on quantitative characters in 40 genotypes of *Vigna* spp.

Table 4.4.9 :- Clusters based on quantitative characters in 40 genotypes of *Vigna* spp.

Cluster	Frequency	Genotypes
Cluster I	3	45740, 45701, 41096
Cluster II	1	45426
Cluster III	5	41084, 41085, 41065, 41016, 41117
Cluster IV	2	45774, 45761
Cluster V	5	45736, 45737, 9056, 45703, 45738
Cluster VI	4	9093, 9080, PL-2, 9067
Cluster VII	4	9092, 9086, 9014, 9026
Cluster VIII	10	9029, 9025, 9035, 45729, Mash 1, 9020, 9047, 9005, 9065, 9039
Cluster IX	3	45727, 9010, 9006
Cluster X	3	45304, Mash 2, Mash 3

45703, 45738). Clusters VI & VII consisted of 4 genotypes each, of these all were advance breeding lines except PL-2 which was an exotic lines obtained from AVRDC. Cluster VIII included 10 genotypes which were also advance breeding lines and improved variety (Mash 1) except 45729, a pure-line screened from the local germplasm. Each of clusters IX and X consisted of 3 genotypes, out of which 2 were advance breeding lines (9010, 9006), two were approved varieties (Mash 2, Mash 3) and one (45304) was pure-line screened from germplasm, while 45727 was *V. radiata*. As this cluster analysis is based on agriculturally important characters, hence advance breeding lines were categorised mostly in clusters VI, VII, VIII, IX and X. It may be because of selection pressure for high yield potential and other related traits. Further, it is important to note that one *V. radiata* genotype (45727) was grouped with *V. mungo* for clustering based on quantitative characters. This clustering on quantitative data should be related with qualitative and biochemical (protein & DNA) data for precise classification and genetic diversity.

Analysis for each cluster was performed and mean alongwith standard deviation for each cluster is presented in Table 4.4.10. The results revealed that the genotypes comprising of cluster I gave mean days of 101.8±10.61 for maturity, 9.67±4.23 g for grain yield and 21.73±8.87% for harvest index, respectively. These genotypes were catagorized as late maturing and high yielding. Cluster III consisted of 3 genotypes and gave the mean performance of 109.0±1.41 for days to maturity, 3.81±2.05 g for grain yield and 17.51±5.71 % for harvest index, hence these genotypes were catagorized as late maturing and low yielding. The cluster IV consisted of 2 *Vigna radiata* genotypes (45774, 45761) which gave the mean values for maturity (67.5±9.19 days), for grain yield (4.41±0.55 g) and harvest index (13.57±0.41%), respectively. These genotypes were also confirmed as *Vigna radiata* by SDS-PAGE and RAPD analyses. Cluster V consisted of 5 genotypes which were early in maturity (73.8±12.05 days) but low yielding (2.12±0.84 g) alongwith low harvest index (11.16±5.76%). The genotypes comprising cluster VI were medium maturing (75.8±4.19 days) and medium yielding (4.64±3.54 g) but high harvest index (35.80±2.20%) which might be due to low biomass (13.34±10.64 g) exhibited by the genotypes of this cluster. The genotypes of clusters VII, VIII and IX were also early in

Table 4.4.10:- Mean and standard deviation for clusters based on quantitative characters in of *Vigna* spp.

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
DF	56.8±4.54	52.3±3.54	55.3±1.79	39.0±1.41	38.9±7.64	43.0±5.83	38.6±3.71	38.6±3.37	35.3±1.89	34.7±1.15
DM	101.8±10.61	89.9±6.24	109.0±1.41	67.5±9.19	73.8±12.05	75.8±4.19	67.8±4.35	67.7±4.13	67.7±3.88	67.7±3.79
PH	44.3±14.29	50.2±5.24	45.6±4.83	50.5±2.12	52.0±8.37	26.0±6.68	24.5±6.45	27.3±7.99	43.3±9.87	41.0±1.73
BR/PL	29.7±7.72	3.0±1.21	17.8±3.97	6.5±0.71	8.25±2.50	8.5±3.70	33.4±1.70	16.3±2.55	6.6±3.22	26.1±12.04
P/PL	55.7±14.04	10.1±5.21	19.6±7.54	20.0±1.41	16.8±6.32	21.7±15.99	45.7±3.74	37.4±11.38	19.8±13.93	92.01±20.46
P/BR	2.03±1.03	3.33±1.25	1.19±0.40	3.08±0.12	2.05±0.53	2.28±0.87	1.51±0.23	2.36±0.68	2.77±1.05	4.10±1.03
PL	4.33±0.66	4.05±0.57	5.08±0.43	7.65±0.21	4.14±0.55	4.28±0.29	4.49±0.17	4.41±0.14	4.09±0.58	4.99±0.50
S/P	6.2±1.32	3.8±2.41	5.82±0.54	11.7±0.71	5.6±0.47	5.8±0.40	6.1±0.10	6.1±0.28	5.37±1.01	6.72±1.19
SD	0.30±0.05	-0.05±0.01	0.16±0.03	0.35±0.02	0.26±0.06	0.26±0.03	0.26±0.03	0.27±0.04	0.23±0.04	0.25±0.06
SW	4.73±0.92	5.21±1.21	4.80±0.70	3.12±0.06	4.47±0.45	4.05±0.26	4.15±0.13	4.39±0.29	3.82±0.18	4.68±0.47
BY	44.10±3.54	13.87±2.15	21.62±6.19	32.57±5.05	19.73±5.86	13.34±10.64	28.36±2.07	24.32±6.42	17.63±11.11	55.05±7.66
GY	9.67±4.23	1.94±0.98	3.81±2.05	4.41±0.55	2.12±0.84	4.64±3.54	7.84±1.92	6.41±1.78	4.27±6.02	19.57±3.67
HI	21.73±8.87	14.07±6.15	17.15±5.77	13.57±0.41	11.16±5.76	35.80±2.70	27.80±4.22	27.08±5.98	24.16±8.73	35.90±5.07

maturity and medium to high yielding. The genotypes comprising cluster X gave an average of 67.7±3.79 days for maturity, 19.57±3.67 g for grain yield and 35.90±5.07% for harvest index, hence could be considered early maturing and high yielding which might be due to approved varieties as 2 of three members of this cluster were approved high yielding varieties.

The comparison of genetic diversity based on morphological, SDS-PAGE and RAPD were plotted in a scatter diagram (Fig. 4.4.7). It was observed that genetic diversity based on SDS-PAGE and RAPD exhibited high relationship (r=0.7224) which indicated that both these biochemical markers could be used for studying inter-specific genetic diversity among *Vigna* spp. Although, the grouping on the basis of quantitative characters revealed low intra-cluster variation, but in comparison with both biochemical markers, insignificant association between dissimilarities was observed. Further, one *V. radiata* genotypes (45727) was closely related to *V. mungo* on the basis of quantitative characters with high intra-cluster variance. The grouping of 40 genotypes on the basis of three parameters revealed that *Vigna radiata* was quite different in biochemical analyses (RAPD & SDS-PAGE). In general, all 40 genotypes shared grouping for all three parameters (morphological, SDS-PAGE & RAPD) and it was observed that 8 out of 40 (20%) genotypes shared all three groups. Cluster analysis showed that many genotypes from same origins were grouped separately or vice versa, which may be because of frequent exchange of germplasm by the breeders or transport of grain to various markets from where the seed of various origins is disseminated throughout the country.

4.5 Inheritance

4.5.1 Inheritance of Qualitative Characters

Eleven parents, viz., 9010, 9024, 9025, 9102, 9104, 9105, 9106, MM 5-60, MM 33-40, Korea and Mash 1 were used to study the inheritance of qualitative characters like, pubescence, seed coat colour, presence of spot on the seed and pod colour. The genotypes used for this study included advance breeding lines (9010, 9024, 9025, 9102, 9104, 9105 & 9106); approved variety (Mash 1); pure-lines obtained from NIAB (MM 33-40 & MM 5-60) and exotic (Korea). The genotypes; 9010, 9024, 9025, 9102, MM 5-60 & MM33-40 were true breeding hairiness, whereas 9104, Korea and Mash 1 were

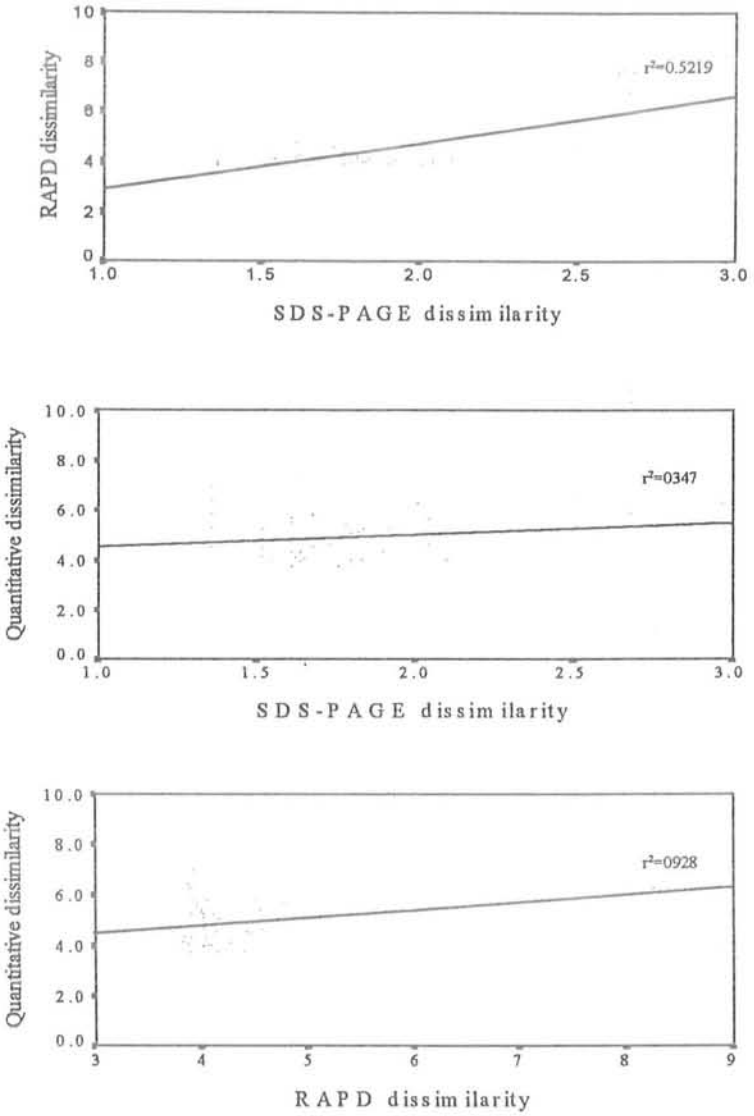


Fig. 4.4.7:- Association among genetic distances based on quantitative, SDS-PAGE and RAPD dissimilarities

glabrous. All the genotypes were brown seeded except MM 33-40 which had green seed coat colour. All were with black pods and black spots on the seeds except MM 5-60 and MM 33-40 which produced brown pods without spots on seeds. The true breeding plants were crossed to study specific qualitative characters and discussed as under:

4.5.2 Pubescence

Five hybrids were used to investigate the inheritance of pubescence in blackgram. Out of parents, 9104, Korea and Mash 1 were non-hairy or glabrous, whereas others were having hairs on plants or pubescent. The allelic notion for this character was assigned as *HH* (dominant homozygous hairy); *Hh* (heterozygous hairy) and *hh* (homozygous recessive non-hairy). The F_1 plants were all having hairs for each hybrid either female parent was kept pubescent or glabrous, suggesting *hh* alleles recessive to *HH, Hh* types. The F_2 segregation for all the crosses showed 3:1 ratio which fit for goodness by χ^2 method (Table 4.5.1). This 3:1 ratio indicated the monogenic nature of this character.

4.5.3 Seed coat colour

It was observed in blackgram that the seed coat colour was either brown or green in the material included in this study and black appearance of seed was mainly due to the presence of black spots on seed coat. Hence seed coat colour was catagorized as brown or green and analysed for inheritance (Table 4.5.2). The observation on seed coat colour was taken at maturity because recording in immature seeds may mislead and data will be biased. One parent produced green seed coat colour (MM 33-40) and hence it was used for hybridization with other parents. The allelic notion was given as CC (homozygous brown); Cc (heterozygous brown) and cc (recessive green). Eight crosses were studied for inheritance of this character. The F_1 plants were all having brown seed coat colour with slightly diffused black spots for each hybrid, either female parent was kept brown or green, suggesting the dominance of brown seed coat in nature, whereas green being recessive. The F_2 population segregated in a 3:1 ratio for all the crosses except 9025/MM 33-40, in which χ^2 did not fit well for 3:1 ratio (Table 4.5.2). This 3:1 ratio indicated the monogenic nature of seed coat colour in blackgram.

Table 4.5.1:- Segregation for pubescence in the F_2 population in blackgram

Cross	Observed		Expected ratio	χ^2	P at 5%
	Pubescence (*HH, Hh*)	Glabrous (*hh*)			
MM33-40/9104	62	20	3:1	0.016	0.90
Mash 1/MM 33-40	45	16	3:1	0.049	0.82
Korea/MM 33-40	274	93	3:1	0.022	0.88
Mash 1/MM 5-60	169	57	3:1	0.006	0.94
9104/MM 33-40	81	26	3:1	0.028	0.87

Table 4.5.2:- Segregation for seed coat colour in the F_2 population in blackgram

Cross	Observed		Expected ratio	χ^2	P at 5%
	Brown (*CC, Cc*)	Green (*cc*)			
MM33-40/9104	60	22	3:1	0.146	0.70
Mash 1/MM 33-40	45	16	3:1	0.051	0.82
Korea/MM 33-40	285	82	3:1	1.381	0.24
9106/MM 33-40	105	32	3:1	0.197	0.66
9104/MM 33-40	86	21	3:1	1.648	0.20
9010/MM 33-40	107	34	3:1	0.060	0.81
9025/MM 33-40	90	42	3:1	3.27	0.07
MM 33-40/9105	200	67	3:1	0.005	0.91

4.5.4 *Spots on Seed coat*

It is the presence of black spots on seed coat which makes the phenotypic expression of most of *Vigna mungo* genotypes blackish. These spots can be seen in fully matured seeds, whereas in immature seeds these spots are not developed and difficult to observe. The presence or absence of these spots on seed coat colour were observed and analysed for inheritance (Table 4.5.3). Observations on spots were taken at maturity to avoid any confusion or bias. Allelic forms were assigned as *SS* (homozygous spots present); *Ss* (heterozygous spots present) and *ss* (recessive spots absent). Two parents (MM 5-60 & MM 33-40) used in hybridization programme were without spots on seed coat, whereas all others were having spots and hence the phenotypic expression was black. The parent "MM 5-60" was brown seeded without spots and "MM 33-40" green seeded without spots. Thirteen hybrids involving 11 parents were studied for inheritance of this character. All the F_1 plants were observed with spots for each hybrid either female parent was kept spotted or un-spotted which indicated the dominant nature of this character, whereas the un-spotted nature was recessive. The F_2 population segregated in a ratio of 3:1 for all the crosses which fit for goodness by χ^2 method with slight variation in probability (Table 4.5.3). This 3:1 ratio revealed the presence of monogenic gene action for phenotypic expression on seed coat of blackgram.

4.5.5 *Pod colour*

Two types of pod colour have been observed in the local blackgram germplasm, i.e., black and brown and these were assigned allelic notion *BB,* (homozygous black); *Bb* (heterozygous black) and *bb* (recessive brown). Pod colour was recorded in F_1 and F_2 on the basis of black or brown colour and the data were analysed for inheritance (Table 4.5.4). Among parents used in the study, MM 5-60 and MM 33-40 were brown podded, whereas all others had black pod colour. Seven crosses involving 8 parents were used to study inheritance of this character. All the F_1 plants were observed with black pod colour for each hybrid which revealed the presence of dominance for black pod colour, whereas brown pods being recessive in blackgram. The F_2 population segregated in a 3:1 ratio for

Table 4.5.3:- Segregation of seed spots in the F_2 population of blackgram

Cross	Observed		Expected ratio	χ^2	P at 5%
	present (*SS*)	Absent (*ss*)			
Mash 1/MM 33-40	49	12	3:1	0.923	0.34
Korea/MM 33-40	284	83	3:1	1.113	0.29
9106/MM 33-40	99	38	3:1	0.547	0.46
9104/MM 33-40	78	29	3:1	0.252	0.62
MM 33-40/9104	62	20	3:1	0.016	0.90
9010/MM 33-40	107	34	3:1	0.59	0.80
9025/MM 33-40	101	31	3:1	0.161	0.69
MM 33-40/9105	201	67	3:1	0.00	1.00
Mash 1/MM 5-60	175	51	3:1	0.714	0.40
9105/MM 5-60	130	44	3:1	0.008	0.93
9102/MM 5-60	106	41	3:1	0.655	0.42
9025/MM 5-60	16	7	3:1	0.362	0.55
MM 33-40/9024	218	74	3:1	0.02	0.89

Table 4.5.4:- Segregation of pod colour in the F_2 population of blackgram

Cross	Observed		Expected ratio	χ^2	P at 5%
	Black (*BB*)	Brown (*bb*)			
Korea/MM 33-40	280	87	3:1	0.508	0.49
9106/MM 33-40	107	30	3:1	0.703	0.40
9104/MM 33-40	85	22	3:1	1.125	0.29
MM 33-40/9104	62	20	3:1	0.016	0.90
MM 33-40/9105	205	63	3:1	0.318	0.57
Mash 1/MM 5-60	180	46	3:1	2.602	0.11
MM 33-40/9024	207	85	3:1	2.630	0.10

all the crosses which fit for goodness by χ^2 method with slight variation in probability (Table 4.5.4). This 3:1 ratio indicated the monogenic nature of this character.

In the present investigation, all four qualitative characters revealed monogenic nature of inheritance segregating in Mandelian 3:1 ratio. The hairiness pattern was observed dominant over non-hairiness; brown seed coat colour dominant over green seed coat colour. The presence of spots on seed coat was dominant to absence of spots and black pods were dominant over brown pods in blackgram.

4.5.6 Linkage analysis

Inheritance of qualitative characters revealed single gene dominance involved for pubescence (*HH, Hh*), seed colour (*CC, Cc*), spots (*SS, Ss*) and pod colour (*BB, Bb*). Further, analysis of linkage among these characters was carried out and the results regarding linked loci are presented in the Table 4.5.5. Nine hybrids were investigated for the analysis of linkage among genetic markers in F_2 generation using the computer programme "LINKAGE 1" of Suiter *et al.*, (1983). The joint segregation of independent assortment revealed that out of nine hybrids, 7 exhibited linkage for various character pairs. The summary of linked loci presented in the Table 4.5.6 revealed that hairiness was weakly linked with seed colour in one hybrid (Korea/MM 33-40) whereas, in other hybrids, no linkage was observed for this character pair. In this hybrid, out of 367 observed plants, 119 were recombinants and 248 were parental types which slightly deviate from the normal 9:3:3:1 ratio of independent assortment. Weak linkage between hairiness and pod colour was observed in the hybrid Mash 1/MM 5-60. The deviation from 9:3:3:1 ratio in this hybrid was observed in low magnitude where 91 recombinants out of 226 plants were observed. The hybrid 9104/MM 33-40 exhibited medium linkage between seed colour and pod colour. In this hybrid, out of 107 plants observed in F_2, twenty three plants were recombinants and 84 were parental types. All the seven hybrids (MM 33-40/9105, Mash 1/MM 5-60, 9106/MM 33-40, 9104/MM 33-40, MM 33-40/9104, Korea/MM 33-40 and Mash 1/MM 33-40) revealed strong linkage between spots and pod colour in the present research material. In the hybrid "MM 33-40/9104" six recombinants out of 82 were observed which indicated strong linkage for this character pair. Eleven recombinants were produced out of 107 plants in the hybrid "9104/MM 33-

Table 4.5.5:- Joint segregation for 4 morphological marker loci in F_2 population of blackgram

Hybrid	Loci	Number of plants/observations -/-	-/+	+/-	+/+	χ^2	p	r
MM 33-40/9104	*HH:CC*	4	16	18	44	0.6284	0.43	0.43±0.09
	HH:SS	5	15	15	47	0.0053	0.94	0.49±0.08
	HH:BB	4	16	16	46	0.2765	0.60	0.45±0.09
	CC:SS	7	15	13	47	0.8996	0.34	0.43±0.09
	CC:BB	8	14	12	48	2.3374	0.13	0.39±0.09
	SS:BB	17	3	3	59	52.6918	0.00	0.08±0.11
9104/MM 33-40	*HH:CC*	4	22	17	64	0.3917	0.53	0.45±0.08
	HH:SS	6	20	23	58	0.2817	0.60	0.46±0.08
	HH:BB	4	22	18	63	0.5634	0.45	0.44±0.08
	CC:SS	9	12	20	66	3.2823	0.07	0.38±0.08
	CC:BB	10	11	12	74	11.7120	0.00	0.27±0.09
	SS:BB	20	9	2	76	57.0676	0.00	0.11±0.09
9106/MM 33-40	*HH:CC*	12	33	20	72	0.4099	0.52	0.46±0.66
	HH:SS	12	33	26	66	0.0383	0.84	0.50±0.06
	HH:BB	9	36	21	71	0.1411	0.71	0.47±0.06
	CC:SS	11	21	27	78	0.9178	0.34	0.44±0.07
	CC:BB	7	25	23	82	0.00	0.99	0.50±0.06
	SS:BB	23	15	7	92	45.8797	0.00	0.18±0.08
Korea/MM 33-40	*HH:CC*	28	65	54	220	4.3277	0.04	0.42±0.04
	HH:SS	18	75	65	209	0.7569	0.38	0.46±0.04
	HH:BB	15	78	52	222	0.3777	0.53	0.47±0.04
	CC:SS	24	58	59	226	2.6701	0.10	0.43±0.04
	CC:BB	19	63	48	237	1.7090	0.19	0.44±0.04
	SS:BB	53	30	14	270	149.44	0.00	0.14±0.05
Mash 1/MM 33-40	*HH:CC*	3	12	13	33	0.3989	0.53	0.44±0.10
	HH:SS	4	11	11	35	0.0463	0.83	0.47±0.10
	HH:BB	3	12	12	34	0.2260	0.63	0.45±0.11
	CC:SS	5	11	10	35	0.5188	0.47	0.44±0.10
	CC:BB	6	10	9	36	1.9493	0.16	0.38±0.11
	SS:BB	13	2	2	44	41.3359	0.00	0.06±0.13
Mash 1/MM 5-60	*HH:SS*	10	47	41	128	1.1003	0.29	0.44±0.05
	HH:BB	6	51	40	129	4.5413	0.03	0.37±0.06
	SS:BB	30	21	16	159	60.1259	0.00	0.19±0.06
MM 33-40/9105	*CC:SS*	10	38	57	163	0.5414	0.46	0.46±0.05
	CC:BB	12	36	51	169	0.0724	0.79	0.48±0.05
	SS:BB	52	15	11	190	145.4304	0.00	0.10±0.06
9010/MM 33-40	*CC:SS*	12	22	21	86	3.5332	0.06	0.39±0.07
9025/MM 33-40	*CC:SS*	11	31	20	70	0.2509	0.62	0.47±0.07

-/- and +/+ are homozygous recessive and homozygous dominant, whereas -/+ and +/- are heterozygous dominant. *HH*- denotes hairiness; *CC*- seed colour brown; *SS*- the spots present on seed coat and *BB*- black pod colour.

Table 4.5.6:- Linkage groups observed in various hybrids of blackgram

Traits	*Seed coat colour*	*Pod colour*
Hairiness	Korea/MM 33-40	Mash 1/MM 5-60
Seed coat colour		Korea/MM 33-40 9104/MM 33-40
Seed spots		MM 33-40/9105 Mash 1/MM 5-60 9106/MM 33-40 9104/MM 33-40 MM 33-40/9104 Korea/MM 33-40 Mash /MM 33-40

40" whereas, 22 recombinants out of 137 genotypes were observed in the cross "9106/MM 33-40. Forty four recombinants out of 367 were produced in the hybrid "Korea/MM 33-40". Similarly in the hybrid "Mash 1/MM 33-40" out of 61 plants 57 were parental types and only 4 plants were observed as recombinants indicating a strong linkage for this character pair. The linkage between alleles *SS:BB* were observed in all the hybrids under study.

The association of qualitative characters with seed weight was studied for 3 crosses (Mash 1/MM 33-40, 9025/MM 33-40 & 9010/MM 33-40) and results are presented in the Table 4.5.7. The hybrid Mash 1/MM 33-40 exhibited significant relationship between seed colour and seed weight. It is evident from the data that green seed coat colour produced higher seed weight (4.63g) in the segregating population of this cross and the difference was observed significant statistically using "*t*" test. Similarly the hybrid 9025/MM 33-40 also revealed association between seed colour and seed weight indicating significant difference, whereas for the cross 9010/MM 33-40, no association was observed between seed colour and seed weight. In hybrids, Mash 1/MM 33-40 and 9025/MM 33-40, female parents (Mash 1 & 9025) were having low to medium seed weight, whereas in third hybrid, parent 9010 was a bold seeded having higher seed weight at par with MM 33-40. The genes for seed weight might be segregating qualitatively but the number may be high. Pooled data also exhibited significant differences for seed weight on the basis of seed coat colour. Green seeded plants gave an average seed weight of 5.25 g per 100 seeds and hence the allele *"cc"* might have some influence in the expression of seed weight in blackgram.

4.5.7 Quantitative Characters

The analysis of variance for 6 parent diallel revealed significant differences among parents and hybrids for all the characters under study (Appendix IV). Analysis of variance regarding combining ability as presented in Table 4.5.8, revealed significant differences for GCA among parents and hybrids for all the characters except pods/branch. SCA was significant for all the characters except branches/plant and pods/branch, whereas reciprocal effects were significant for all the characters except harvest index. The present results indicated the presence of both additive and non-additive genetic variation. It is

Table 4.5.7:- QTL detection for seed weight based on monogenic characters in F_2 generation of blackgram

Plant Trait	*Loci*	Frequency	Mean±SD	Difference±SE
		Mash 1/MM-33-40		
Hairiness	*HH, Hh*	39	4.42±0.54	0.01±0.17ns
	hh	16	4.43±0.59	
Seed colour	*CC, Cc*	48	4.39±0.56	0.24±0.12*
	cc	7	4.63±0.46	
Spots	*SS, Ss*	44	4.39±0.54	0.18±0.19ns
	ss	11	4.57±0.57	
		9025/MM-33-40		
Seed colour	*CC, Cc*	166	5.35±0.66	0.20±0.10*
	cc	52	5.55±0.61	
Spots	*SS*	172	5.40±0.66	0.04±0.11ns
	ss	46	5.37±0.64	
		9010/MM-33-40		
Seed colour	*CC, Cc*	106	5.05±0.62	0.08±0.11ns
	cc	34	4.97±0.52	
Spots	*SS*	107	5.03±0.57	0.00±0.13ns
	ss	33	5.03±0.67	
		Combined		
Seed colour	*CC, Cc*	320	5.01±0.77	0.24±0.0.08**
	cc	93	5.25±0.66	
Spots	*SS*	323	5.06±0.77	0.00±0.08
	ss	90	5.06±0.72	

Table 4.5.8:- Mean squares for general combining ability in a 6 parent diallel of blackgram

SOV	DF	PH	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
GCA	5	193.82**	199.82**	754.61**	0.52ns	0.2679**	0.8080**	1.10**	905.04**	86.99**	22.19**
SCA	15	46.12**	50.00ns	579.81**	0.45ns	0.0617**	0.1756**	0.11**	241.76**	30.66**	17.77**
Reciprocal	15	50.83**	102.72**	1135.48**	0.61**	0.0223**	0.0677**	0.13**	408.12**	56.90**	9.18ns
Error	105	6.20	29.75	188.09	0.28	0.0086	0.0321	0.02	95.37	9.08	6.73

** is significant at 1% level. SOV- source of variation, GCA- general combining ability effects and SCA- specific combing ability effects.

Table 4.5.9:- Variance and percentage (lower row) due to GCA, SCA, Reciprocal and Error Effects in a 6 parent diallel of Blackgram

Source of Variation	Plant Height	Branches	Pods per plant	Pod per branch	Pod length	Seeds per pod	Seed weight	Biological yield	Grain yield	Harvest index
GCA	12.42	12.53	15.62	0.0062	0.0174	0.0531	0.083	55.67	4.75	0.40
	19.37	13.84	1.77	1.13	27.44	28.51	38.91	14.19	9.45	2.71
SCA	23.19	11.76	227.45	0.0945	0.0310	0.0832	0.0544	85.00	12.53	6.41
	36.16	12.99	25.70	17.14	48.90	44.66	25.50	21.66	24.93	43.40
Reciprocal	22.32	36.48	473.69	0.1606	0.0067	0.0179	0.0577	156.38	23.91	1.23
	34.80	40.30	53.53	29.14	10.57	9.61	27.05	39.85	47.56	8.32
Error	6.20	29.75	168.10	0.2898	0.0083	0.0321	0.0182	95.37	9.08	6.73
	9.67	32.87	18.99	52.59	13.09	17.23	8.53	24.30	18.06	45.56

believed by plant scientists that both additive and non-additive gene-actions in most of the cases are mixed with each other and it is not possible to detect the real situation regarding gene-action for quantitative characters. The variance observed due to combining ability effects were partitioned and expressed in term of percentage to get precise information (Table 4.5.9). It is quite evident from the results that GCA effects were higher in case of seed weight (38.91%), whereas in all other cases, either SCA or Reciprocal effects were high indicating predominance of non-additive (dominance, epistasis or mixed) gene-action for these characters. Highly significant reciprocal effects alongwith high variance for branches, pods/plant, biomass and grain yield indicated the importance of maternal contribution of the parents used in hybridization. Further, high contribution of reciprocal effects also restricted the bulking of hybrids sharing same parentage but reciprocally mated and hence, these should be handled separately for selection, in later segregating generations. High SCA variance for plant height (36.16%), pod length (48.90%) and seeds/pod (44.66%) revealed the importance of non-additive gene-action for these characters and hence selection for improvement of these characters is suggested by delayed selection in later generations when the genes are fixed and expressed fully. High variance due to error was observed for pods/branch (52.59%) and harvest index (45.56%), both of these characters were calculated and expressed as a product of two variables and hence more influenced by the environmental fluctuation. As both these character also expressed non-additive variance involved and hence, the selection for these should also be delayed and done more precisely as these are important yield components. In the present study it is observed that both additive and non-additive genetic variation is present but the magnitude varied. The major portion of GCA variance is observed for seed weight only, whereas for all other characters, SCA or Reciprocal variance contributed more towards genetic variance.

4.5.8 General Combining Ability (GCA) Effects

Means, SD and GCA effects presented in Table 4.5.10 revealed that variety "Mash 3" was the tallest among the parents used in the study having 56.8 cm plant height, followed by "Mash 1" which produced 53.5 cm plant height. The variance was high for "Mash 3" and "9025" which revealed that bulk selection from these cultivars could be

Table 4.5.10:- Means, standard deviation and General Combining Ability (GCA) Effects in 6 Parents of Blackgram

Parents	Plant height	Branches	Pods	Pods per branch	Pod length	Seeds per pod	Seed weight	Biological yield	Grain yield	Harvest index
9020	50.5±3.88	33.3±10.84	91.8±29.96	2.78±0.57	5.30±0.52	6.9±0.53	5.83±0.51	73.46±22.01	24.22±7.00	33.10±3.06
	3.90	3.51	9.72	-0.09	0.29	0.50	0.47	13.53	3.99	-1.46
9025	47.0±6.57	25.8±17.74	78.0±41.60	3.30±1.44	4.80±0.18	6.3±0.54	4.25±0.26	48.60±20.36	14.76±9.34	31.27±5.09
	-4.78	-3.23	-7.74	-0.08	-0.11	-0.14	-0.33	-7.72	-3.07	-0.71
9012	41.7±2.37	27.3±15.40	99.3±36.38	4.15±1.16	4.50±0.23	5.7±0.52	4.23±0.32	46.21±14.45	16.87±5.33	36.56±3.56
	0.53	1.35	-1.72	0.08	-0.05	-0.13	0.01	-0.34	-0.68	-0.82
Mash 1	53.5±2.64	30.5±12.25	113.0±28.44	3.78±0.44	4.62±0.07	6. 5±0.60	5.55±0.19	75.33±19.83	30.97±5.39	41.39±3.27
	4.41	0.81	1.2	-0.11	-0.09	-0.03	0.23	2.68	1.64	1.46
Mash 3	56.8±6.59	37.5±13.91	108.0±25.51	3.05±1.09	4.98±0.21	6.5±0.44	4.64±0.35	95.64±18.09	23.75±5.73	40.61±6.61
	0.69	4.02	8.06	-0.19	-0.07	-0.23	-0.20	2.78	0.79	-0.38
9026	46.5±3.74	31.0±7.73	98.7±22.47	3.25±0.54	5.28±0.21	7.2±0.35	4.33±0.25	50.58±10.72	21.20±4.07	41.88±2.52
	-4.75	-6.47	-9.53	0.39	0.04	0.03	-0.18	-10.93	-2.67	1.91
$\delta^2 (G_i-G_i)$-	1.03±1.02	4.96±2.23	31.35±5.60	0.05±0.22	0.001±0.03	0.005±0.073	0.003±0.055	15.89±3.99	1.51±1.23	1.12±1.06

Upper row is mean±SD and lower row is GCA effect for specific character.

practised to improve the uniformity for plant height. The GCA effects were positive for the genotypes 9020, 9012, Mash 1 and Mash 3. The best GCA was recorded in the parent Mash 1 (4.41). It was followed by 9020 (3.90) whereas, 9025 and 9026 produced significantly negative GCA effects with values of -4.78 and -4.75, respectively. As plant height in blackgram is highly influenced by the fluctuation in environments and hence short statured genotypes are preferred to get the maximum grain yield, therefore, the genotypes with minimum plant height and GCA with negative magnitude could be utilized in breeding programme for the development of high yielding blackgram cultivars. Mash 3 produced the highest values of mean performance (37.5 branches/plant) and GCA (4.02) for branches which was followed by "9020" for mean performance of 33.3 branches/plant and GCA value of 3.51. The genotypes, 9020, 9012, Mash 1 and Mash 3 produced GCA effects of positive magnitude, whereas 9025 and 9026 were observed with negative GCA effects.

The parent "Mash 1" produced maximum pods (113.0) but it could not give high GCA effect although it was positive. The genotype "9020" produced 91.8 pods/plant and the highest GCA effect (9.72). It followed by Mash 3 which produced 108.0 pods per plant and 8.06 GCA effect. Three cultivars (9025, 9012, 9026) produced GCA effects with negative magnitude, 9026 and 9012 being the lowest in GCA with values of -9.53 and -7.74, respectively. When pods were calculated on unit branch basis, the genotype "9012" produces 4.15 pods/branch and 0.08 GCA, whereas the best general combiner for this character was "9026" which gave GCA value of 0.39. Improvement of blackgram on the basis of productive branches is one of the best criteria and the genotypes with high mean performance and GCA for this important character should be utilized in breeding programme for yield potential. Therefore, the hybrids between parents like Mash 1 and 9012 should be observed carefully to select the desirable recombinants with high number of productive branches.

There is a little variation in local blackgram germplasm for pod length, seeds/pod and seed weight, but the parents included in the present investigation were selected on the basis of maximum diversification available in the local genetic stock. The genotype "9020" was selected on the basis of high pod length (5.3cm) and high seed weight (5.83 g). The

same genotype proved to be the best general combiner for pod length, seeds/pod and seed weight with GAC values of 0.29, 0.50 and 0.47, respectively which were positive and significant. Mash 3 gave the highest mean performance for biomass and the genotype 9020 gave maximum GCA (13.53) for biomass. Three cultivars (9020, Mash 1, Mash 3) produced positive GCA effects for biomass, whereas 9025, 9012 and 9026 gave GCA with negative effect. Mash 1 produced the highest grain yield (30.97 g), followed by 9020 (24.22 g). The genotype "9020" gave the highest GCA effect (3.99) for grain yield and it was followed by Mash 1 (1.64). Three genotypes (9025, 9012, 9026) produced negative GCA for this character, 9025 being the lowest in general combining with GCA value of -3.07. Harvest index is very much influenced by the environmental fluctuation and very difficult to predict in most of the legumes. In the present breeding material, the genotype "9026" gave the highest HI (41.88%), followed by Mash 1 (41.39%), while the lowest HI (31.27) was observed in 9025. The harvest index in blackgram has a wide range but the parents were selected on the basis of agronomic characters and hence, the range for HI was not very wide. The best general combiner for HI was "9026" which produced GCA value of 1.91 and it was followed by Mash 1 for mean performance (41.39 %) and GCA (1.46) for HI. Four cultivars (9020, 9025, 9012, Mash 3) produced negative GCA effects, 9020 being the lowest. In the present study, it is revealed that the genotype "9020" produced the highest GCA effects for pods, pod length, seeds/pod, seed weight, biomass and grain yield; Mash 1 best general combiner for plant height; Mash 3 best for branches and 9026 proved to be the best general combiner for pods/branch and HI. Two cultivars (9025 & 9012) did not produce any worth as for as general combing is concerned, although later produced high pods/branch but failed to prove its superiority in GCA effects. The genotype "9026" gave negative GCA effects for most of the characters (plant height, branches, pods, seed weight, biomass and grain yield) but it exhibited best general combiner for pods/branch and HI which are derived parameters and more influenced by the environmental fluctuation. In the present investigation it can be concluded that the hybrids involving genotypes 9020, Mash 1 and 9026 could be examined carefully for selection in the proceeding generations.

4.5.9 Specific Combing Ability (SCA) Effects

The estimates of SCA effects are presented in Table 4.5.11 which revealed that the highest SCA effects (4.78) were observed in the hybrid 9020/9012 and the same hybrid gave the maximum mean performance for plant height (58.3 cm). The hybrid 9025/Mash 3 gave the highest SCA effects (5.61) for branches/plant, followed by the hybrid 9012/Mash 1 with SCA value of 4.91. The average performance was observed the best for the cross 9020/Mash 1 which produced 48.7 branches/plant. Pods/plant is highly variable characters and the hybrid 9020/9026 produced the maximum SCA effect (29.32) which gave an average performance of 136.0 pods/plant alongwith high variance indicating the expectation of transgressive segregants in later generations. Highest SCA effects (0.67) for pods/branch were observed in the hybrid 9025/Mash 1, whereas the cross 9012/Mash 3 gave the highest mean performance. In case of pod length, the cross 9025/9012 was the best for SCA effects (0.14), followed by 9012/Mash 3 with 0.09 SCA value. As regards seeds/pod, the cross "9020/9026" gave the highest SCA effects (0.25) and mean performance (7.25 seeds), whereas it was followed by the cross "9020/9012" which gave the SCA value of 0.17 and average seeds of 6.9. The best mean performance was observed by the hybrid 9020/9026 which was followed by 9020/9012 and 9020/Mash 1 with mean value of 6.9 seeds in both cases. It is observed in the results that the hybrids involving 9020 as one of the parents gave higher mean performance and SCA effects for seeds/pod. The parent "9090" is a bold seeded and the hybrids involving this as one of the parents like 9020/9012 and 9020/Mash 1 gave the best average performance but because of additive nature of gene action for seed weight, the cross "9020/9012" produced negative SCA effects, whereas the hybrid "9020/Mash 1" produced positive SCA effects because both of these parents were bold seeded. The hybrid "9012/Mash 3" revealed the highest SCA effects (15.36), followed by the hybrid 9020/Mash 1(13.57) which also gave the best average biomass of 110.75 g per plant. For grain yield, the hybrid "9020/9026" gave the best SCA effects of 6.45 and it was followed by the hybrid "9012/Mash 3 (5.32) which produced the average grain yield of 30.92 g. The best average grain yield of 37.71 g was produced by the hybrid. 9020/Mash 1, but this hybrid gave low GCA of 1.49. The cross "9025/Mash 1" was observed with the best SCA effects of 3.78 and followed by the

Table 4.5.11:- Means, standard deviation and Specific Combining Ability (SCA) Effects in 6 parents Diallel of Blackgram

Hybrid	Plant Height	Branches	Pods	Pods per branch	Pod length	Seeds per pod	Seed weight	Biological yield	Grin yield	Harvest index
9020/9025	30.3±11.90	10.0±10.42	32.5±31.36	3.35±1.49	4.73±0.30	6.8±0.39	4.92±0.39	20.86±25.12	8.04±8.88	39.85±5.35
	-2.83	-4.09	-8.85	0.10	-0.06	0.15	0.01	-7.21	-1.20	3.19
9020/9012	58.3±4.50	45.3±30.65	140.0±41.82	4.72±2.96	5.11±0.09	6.9±0.19	5.11±0.22	86.6±20.08	29.17±5.83	33.92±2.02
	4.78	4.13	7.13	0.24	0.03	0.17	-0.24	-0.07	0.21	0.01
9020/Mash 1	57.3±1.25	48.7±6.24	152.0±22.91	3.14±0.43	5.00±0.09	6.9±0.09	5.11±0.30	110.75±15.46	37.71±4.81	34.13±1.58
	-0.10	1.46	-1.28	-0.14	0.05	-0.07	0.18	13.57	1.49	-5.63
9020/Mash 3	56.4±4.54	44.5±18.92	142.0±61.98	3.13±0.36	4.63±0.16	6.4±0.40	4.39±0.34	77.12±44.40	26.78±12.77	38.35±7.61
	-1.61	1.04	4.86	-0.11	-0.15	-0.01	-0.12	-3.31	-0.59	1.83
9020/9026	56.0±9.87	29.3±8.3	136.0±39.07	4.72±1.14	4.94±0.14	7.25±0.49	4.72±0.44	70.92±21.58	28.24±8.07	40.74±5.79
	4.01	3.66	29.32	0.39	0.08	0.25	-0.05	12.51	6.45	1.98
9025/9012	47.0±2.76	23.3±6.73	55.0±14.50	2.47±0.48	4.49±0.13	5.8±0.53	4.31±0.28	32.24±8.05	10.98±2.44	35.33±5.81
	-3.83	-1.80	-19.91	-0.81	0.14	0.15	0.18	-8.90	-2.03	1.58
9025/Mash 1	46.0±4.03	31.5±10.20	130.0±37.27	4.18±1.25	4.47±0.12	6.4±0.28	4.20±0.27	58.74±17.72	23.71±7.32	41.37±7.14
	2.17	-0.39	19.92	0.67	-0.05	0.15	-0.09	3.00	3.21	3.78
9025/Mash 3	36.7±1.25	37.7±1.70	134.3±7.36	3.60±0.21	4.23±0.02	5.6±0.16	3.87±0.05	70.58±1.84	25.59±1.37	36.23±1.00
	-5.19	5.61	16.19	-0.06	-0.23	-0.43	-0.24	14.23	3.86	-2.42
9025/9026	37.3±3.98	21.75±6.07	84.8±30.13	3.90±1.16	4.53±0.15	6.3±0.22	4.08±0.13	38.92±16.35	14.16±5.99	36.78±3.59
	0.07	0.89	2.65	0.08	-0.10	-0.21	-0.11	-3.27	-2.14	-1.30
9012/Mash 1	56.0±2.64	47.8±12.25	105.7±28.44	2.33±0.67	4.68±0.07	6.45±0.59	4.48±0.19	78.4±19.83	22.57±5.39	29.25±3.27
	3.41	4.91	-5.4	-0.82	0.02	0.09	0.05	1.84	-1.26	-2.64
9012/Mash 3	51.8±5.10	34.0±12.09	135.3±44.88	4.9±1.87	4.72±0.17	6.4±0.50	4.73±0.47	86.25±34.88	30.92±10.95	39.10±6.20
	3.83	1.45	15.55	0.46	0.09	0.16	0.21	15.36	5.32	0.99
9012/9026	33.5±2.96	34.5±10.61	107.8±33.92	3.28±0.81	4.56±0.20	6.05±0.19	4.71±0.19	73.64±25.51	27.07±8.79	37.06±4.07
	-1.99	-0.81	3.51	0.38	-0.16	-0.07	0.26	6.78	2.16	-0.63
Mash 1/Mash 3	57.8±5.10	41.8±12.09	91.5±44.88	2.25±1.87	4.61±0.17	6.1±0.50	4.40±0.47	47.81±34.88	17.70±10.95	38.16±6.20
	1.2	3.86	2.26	-0.33	0.05	0.04	-0.35	-9.20	-3.14	-0.07
Mash 1/9026	45.8±2.96	27.8±10.61	92.3±33.92	3.42±0.81	4.67±0.20	6.2±0.19	4.80±0.19	48.90±25.51	22.15±8.79	45.19±4.08
	-6.65	-6.27	-22.53	0.07	-0.15	-0.34	-0.22	-17.29	-5.38	3.60
Mash 3/9026	39.0±4.12	25.5±10.99	88.0±30.06	3.63±0.52	4.59±0.08	5.75±0.17	4.66±0.13	52.41±17.64	19.57±7.56	36.93±7.46
	-4.75	-8.98	-27.26	0.04	-0.16	-0.32	0.11	-9.27	-5.02	-4.20
$\delta^2(S_{ii}\text{-}S_{ij})$	8.27±2.88	39.67±8.29	250.80±15.84	0.39±0.62	0.01±0.11	0.043±0.21	0.0243±0.16	127.16±11.28	12.10±3.48	8.97±2.99

Upper row is mean±SD and lower row is SCA effect for specific hybrid.

cross Mash 1/9026" with SCA value of 3.60 and the same hybrid was the best for average harvest index production.

4.5.10 Reciprocal Effects

The reciprocal effects were investigated and the results are presented in Table 4.5.12. The results revealed that the hybrid "/Mash 3/9020" was the best for RCA effects (6.44) in case of plant height and it followed by 9026/9020 with RCA value of 6.13. In case of branches/plant, the hybrid "Mash 1/9020" was the best for RCA effects (10.46) and the same cross the best for pods/plant with RCA value of 38.88, whereas these were followed by the cross "Mash 1/9012" with RCA value of 8.25 (for branches) and "9012/9020" in case of pods/plant with RCA value of 21.38. The mean performance was the best by the hybrid "9025/9020" for both these characters, i.e., 47.3 for branches and 160.0 for pods/plant, but RCA effects were negative in both cases. The cross "/Mash 3/9020" was the best in RCA effects (1.11) for pods/branch. The hybrid "9026/9020" produced the longest pods (5.31 cm) but the RCA value was negative (-0.18), whereas the highest RCA effects (0.15) were observed in the hybrid "9026/Mash 1" which gave medium pod length of 4.36 cm in the present study. The highest RCA value (0.22) for seeds/pod was observed in the hybrid "9026/9025" which was followed by a cross "Mash 3/9012" with RCA effects of 0.20 and 6.0 seeds/pod. Most of the hybrids with good performance were those involving 9020 as one of the parents but could not prove their superiority for RCA effects. Similar results were observed for seed weight where the hybrids (Mash 1/9020, Mash 3/9020) with high seed weight failed to exhibit high RCA effects. The hybrid "9026/Mash 1" was the best for RCA effects (0.31) whereas it gave poor average seed weight of 4.19 g per 100 seeds. Mash 1/9020 proved to be the best for biomass with RCA value of 19.08 followed by the hybrid "9026/9012" with RCA value of 16.24 and they gave the average biomass of 72.58 and 41.17 g, respectively. For grain yield, the hybrid "9026/Mash 1" which was low in average yield production (10.27 g), but it gave the best RCA effects (5.94), whereas the best hybrid (9025/9020) for grain yield (36.63 g) gave negative RCA value of -14.29. This might be due to complexity in gene-action, but because of best performance, these crosses are to be looked into carefully as some superior transgressive segregants might be expected in the succeeding generations

Table 4.5.12:- Means, standard deviation and Reciprocal effects in 6 parents Diallel of Blackgram

Hybrid	Plant Height	Branches	Pods	Pods per branch	Pod length	Seeds per pod	Seed weight	Biological yield	Grin yield	Harvest index
9025/9020	56.3±7.74	47.3±18.38	160.0±49.61	3.40±1.72	4.93±0.17	7.0±0.28	4.72±0.29	100.13±38.82	36.63±13.72	37.19±3.83
	-13.00	-18.62	-64.12	-0.02	-0.01	-0.10	0.10	-39.63	-14.29	1.33
9012/9020	54.0±5.26	37.5±13.58	97.3±35.49	2.63±0.45	4.86±0.12	7.0±0.27	4.74±0.32	63.42±26.15	23.10±8.92	36.55±4.04
	2.17	3.92	21.38	1.05	0.12	-0.07	0.18	11.59	3.03	-1.31
Mash 1/9020	53.0±5.26	27.8±13.58	74.3±35.49	3.08±0.45	4.92±0.12	6.7±0.27	6.01±0.32	72.58±26.15	21.76±8.93	29.65±4.04
	2.17	10.46	38.88	0.02	0.04	0.14	-0.45	19.08	3.97	2.23
Mash 3/9020	43.5±1.50	37.5±0.50	110.5±11.50	2.97±0.35	4.92±0.08	6.9±0.10	5.26±0.09	72.65±10.90	26.82±6.80	36.33±3.91
	6.44	3.50	15.63	0.08	-0.14	-0.25	-0.43	2.23	-0.02	0.86
9026/9020	44.0±2.00	37.0±2.00	130.0±5.00	3.52±0.05	5.31±0.01	7.1±0.10	5.11±0.03	83.09±2.05	32.52±1.40	39.11±0.72
	6.13	-3.87	3.00	0.60	-0.18	0.08	-0.20	-6.09	-2.14	0.81
9012/9025	43.5±5.01	34.3±10.90	93.3±26.77	2.80±0.56	4.89±0.32	6.8±0.60	4.76±0.31	57.62±18.02	22.70±6.45	39.78±3.74
	-4.62	-5.50	-19.12	-0.16	-0.20	-0.47	-0.23	-12.69	-5.86	-2.22
Mash 1/9025	51.5±5.01	27.8±10.90	103.8±26.77	3.67±0.56	4.47±0.32	6.4±0.60	4.76±0.31	60.95±18.02	25.09±6.45	42.75±3.74
	-2.75	1.88	13.13	0.25	0.01	0.02	-0.28	-1.11	-0.69	-0.67
Mash 3/9025	38.7.±1.25	40.0±1.63	105.7±7.36	2.64±0.15	4.36±0.05	5.6±0.16	3.93±0.06	71.78±3.58	22.79±1.10	31.76±0.43
	-1.00	-1.17	14.32	0.49	-0.07	0.01	-0.03	-0.59	1.40	2.23
9026/9025	37.8±5.15	25.5±9.94	93.0±32.78	3.75±0.79	4.56±0.14	5.9±0.21	4.02±0.21	41.12±15.55	15.30±5.77	38.04±4.57
	-0.25	-1.87	-4.12	0.08	-0.02	0.22	0.03	-1.15	-0.97	-0.63
Mash 1/9012	54.8±2.37	31.3±15.40	89.3±36.28	2.85±1.16	4.53±0.23	6.2±0.52	5.43±0.31	53.75±14.45	22.06±5.33	41.77±3.56
	0.63	8.25	8.25	-0.26	0.08	0.13	-0.48	12.32	0.26	-6.26
Mash 3/9012	52.3±11.90	45.5±13.80	115.0±27.52	2.67±0.52	4.66±0.18	6.0±0.47	4.65±0.42	73.14±29.88	25.15±8.25	35.50±17.25
	-0.25	-5.25	9.88	1.11	0.03	0.20	0.04	6.56	2.89	1.80
9026/9012	48.0±6.07	18.5±9.69	83.8±21.81	5.30±2.06	4.54±0.08	6.4±0.22	4.31±0.28	41.17±11.96	15.78±4.64	38.88±5.30
	-7.25	8.00	12.00	-1.01	0.01	-0.17	-0.05	16.24	5.64	-0.91
Mash 3/Mash 1	48.8±6.53	40.5±16.38	138.5±60.93	3.39±0.41	4.59±0.09	6.3±0.50	4.27±0.27	68.48±31.97	26.11±11.06	38.89±2.86
	4.5	0.63	-23.50	-0.56	0.01	-0.07	0.06	-10.33	-4.20	-0.36
9026/Mash 1	38.5±7.00	13.3±8.30	53.0±26.88	4.15±1.43	4.36±0.17	5.9±0.44	4.19±0.21	23.80±17.57	10.27±5.90	43.76±14.54
	3.61	7.25	19.63	-0.36	0.15	0.15	0.31	12.55	5.94	0.71
9026/Mash 3	33.5±1.50	16.5±0.50	61.5±5.50	3.72±0.22	4.45±0.03	6.0±0.00	4.14±0.06	36.54±7.97	11.86±1.72	33.00±2.49
	2.75	4.50	13.25	-0.03	0.07	-0.12	0.26	7.94	3.85	2.10
$\delta^2 (R_6\text{-}R_{41})$	6.20±2.49	29.75±5.45	188.09±13.71	0.29±0.54	0.009±0.092	0.032±0.18	0.018±0.134	95.37±9.77	9.08±3.01	6.73±2.59

Upper row is mean±SD and lower row is Reciprocal effect for specific hybrid.

when genes are fixed. The hybrids "Mash 1/9020" and "Mash 3/9025" were the best for harvest index in case of RCA effects (2.23 in both cases) although they gave medium harvest index of 29.65 and 31.76%, respectively in the present study. Maximum harvest index of 43.76 % was produced by the hybrid "9026/Mash 1" and it was followed by Mash 1/9025 with 42.75 % of harvest index.

4.5.11 Correlation Analysis

The correlation coefficients were computed for parents, F_1 and F_2 separately between grain yield and all the quantitative traits (Table 4.5.13). The sample size for calculating the correlation was kept 10 plants for parents and F_1, whereas 30 plants for F_2 population. In total, correlation coefficients were calculated for 6 parents and 18 F_1 & F_2 populations. The association of grain yield with plant height was positive in all the parents, and all the F_1 populations except for 9020/Mash 1 and Mash 3/9025, whereas it was positive for all crosses in F_2 except 9020/Mash 1, Mash 1/mash 3 and Mash 3/9025. The selection criteria for individual hybrids might differ according to gene-action involved and nature of correlation, hence should be handled carefully according to the investigation for specific character. Grain yield exhibited positive association with branches for all parents, F_1 and F_2 except Mash 3/9025 where it was negative for F_1 generation. The results regarding association with pods gave similar pattern as with branches/plant. The correlation between grain yield and pods/branch revealed that it was negative for all the parents except Mash 1 and 9026 but it was insignificant in all cases. Pod length exhibited positive association with grain yield for all the parents except mash 1 and six hybrids exhibited negative association with grain yield. Out of parents, only Mash 3 exhibited significant positive association of pod length with grain yield. Seed weight exhibited positive association with grain yield for all the parents, but it was significant in Mash 3 only. The correlation of grain yield with biomass was significantly positive for all the parents, F_1 and F_2 populations. Harvest index was significantly positive with grain in two parents (9025, 9012), whereas it was insignificant negative for Mash 1, Mash 3 and 9026. Out of 18 combinations, the correlation between this character pair was significantly positive for four crosses in F_1, whereas significantly negative in 2 crosses. In F_2, correlation between this character pair was significantly positive in nine hybrids out of eighteen. The correlation of grain yield with plant height, branches, pods and biomass revealed the validity of these correlation

Table 4.5.13:- Correlation coefficients between grain yield and 10 quantitative traits in various hybrids for F_1 and F_2 generations of blackgram

		PH	BR/PL	P/PL	P/BR	PL	S/P	SD	SW	BY	HI
9020	Parent	0.8275**	0.9081**	0.8641**	-0.2708	0.3323	0.6830**	0.2907	0.4341	0.9620**	0.0142
9025	Parent	0.7590**	0.8511**	0.9857**	-0.3508	0.4095	0.2563	0.1181	0.1562	0.9480**	0.7010**
9012	Parent	0.5181*	0.8822**	0.9611**	-0.1116	0.1832	-0.1468	-0.3462	0.2840	0.9703**	0.5487*
Mash 1	Parent	0.6164*	0.9269**	0.9972**	0.1490	-0.0718	0.8621**	0.7307**	-0.3027	0.9845**	-0.1644
Mash 3	Parent	0.8774**	0.5973**	0.9097**	-0.2114	0.8270**	0.7040**	0.4125	0.8325**	0.9287**	-0.4290
9026	Parent	0.1845	0.6706**	0.9340**	0.1829	0.1557	0.6326**	0.6621**	-0.3639	0.9851**	-0.4854
9020/9025	F_1	0.9488**	0.9202**	0.9275**	0.0830	0.8996**	-0.2653	-0.6478**	0.4903	0.9746**	-0.3803
9020/9025	F_2	0.4028*	0.6627**	0.8998**	0.0816	0.4580*	0.0697	-0.2269	0.2509	0.8461**	0.0383
9020/9012	F_1	0.6035*	0.9260**	0.9948**	-0.5521*	-0.8563**	0.5286*	0.6692**	-0.5944*	0.9841**	-0.5468*
9020/9012	F_2	0.4496*	0.5454**	0.8835**	0.6123**	0.2813	0.3351	0.1879	0.3022	0.7008**	0.3513
9020/M1	F_1	-0.7516**	0.5455*	0.9972**	0.6121*	0.3211	0.9261**	0.5339*	-0.8512**	0.9429**	0.0009
9020/M1	F_2	-0.1762	0.2898	0.9734**	0.4691**	-0.3365	0.0654	0.6987**	-0.1449	0.2317	0.7008**
9020/M3	F_1	0.0410	0.9436**	0.9831**	0.5400**	0.1371	0.0084	-0.0859	0.5677*	0.9864**	-0.4792
9020/M3	F_2	0.1975	0.5738**	0.8420**	0.6125**	0.2116	0.4680**	0.3287	0.3298	0.8757**	0.5799**
9025/9020	F_1	0.6180*	0.8207**	0.9409**	-0.4917	0.6882**	0.2936	-0.3811	0.6017*	0.9885**	-0.0096
9025/9020	F_2	0.1339	0.7088**	0.8543**	-0.0403	0.6105**	0.7584**	0.6094**	0.5368**	0.8052**	0.5584**
9025/M3	F_1	0.5827*	0.1504	0.9879**	0.8136**	-0.7211**	0.4187	0.6070*	-0.9979**	0.9915**	0.9901**
9025/M3	F_2	0.8223**	0.8527**	0.9392**	-0.3303	0.5824**	0.4731**	0.2260	-0.1127	0.9567**	-0.0009
9025/9026	F_1	0.4895	0.7900**	0.9721**	-0.0143	0.4269	0.4889	0.1088	0.5484*	0.9773**	-0.4766
9025/9026	F_2	0.5780**	0.6746**	0.8792**	0.4278*	0.6027**	0.2279	-0.2769	0.7311**	0.9151**	0.5527**
9012/9020	F_1	0.7525**	0.8825**	0.9574**	0.3099	0.2098	-0.0116	-0.2634	0.1864	0.9830**	0.4804
9012/9020	F_2	0.1016	0.8157**	0.8398**	0.2516	0.3428	0.4921**	0.1402	0.0980	0.8086**	0.2492
9012/9025	F_1	0.4395	0.8359**	0.9816**	0.2092	0.2238	0.2697	0.2725	-0.5910*	0.9718**	-0.5397*
9012/9025	F_2	0.1697	0.6851**	0.7334**	0.0119	0.0630	-0.1018	-0.1253	0.3259	0.6771**	0.4465*
9012/M1	F_1	0.7892**	0.9163**	0.7332**	-0.0966	-0.1007	0.4993	0.5054	0.4358	0.9253**	0.1984
9012/M1	F_2	0.1983	0.8312**	0.8121**	-0.1122	0.4560*	0.5984**	0.4862**	0.3404	0.9374**	0.2399
9012/M3	F_1	0.1274	0.7449**	0.9846**	0.4723	0.6416**	0.1423	-0.1029	0.3229	0.9635**	-0.1299
9012/M3	F_2	0.2614	0.8516**	0.8872**	0.1088	0.2589	-0.0144	-0.1970	0.2048	0.9510**	0.1971
9012/9026	F_1	0.0554	0.6537**	0.9367**	0.6957**	0.0883	0.0941	0.0029	0.3429	0.9779**	-0.4766
9012/9026	F_2	0.5709**	0.8172**	0.9082**	0.3270	0.5290**	0.4990**	0.2733	0.2338	0.9335**	0.3362

-cont.-

		PH	BR/PL	P/PL	P/BR	PL	S/P	SD	SW	BY	HI
M1/9020	F_1	0.3600	0.4679	0.7756**	0.1402	0.7254**	0.6542**	0.4869	-0.1243	0.8495**	0.6831**
M1/9020	F_2	0.5121**	0.8263**	0.8555**	0.6395**	0.1186	0.4517*	0.5356**	0.2710	0.6782**	0.6003**
M1/9012	F_1	0.2743	0.7288**	0.3516	-0.7233**	0.5983*	-0.1440	-0.5022	0.3673	0.9346**	0.1652
M1/9012	F_2	0.5801**	0.7120**	0.9434**	0.5542**	0.6072**	0.0923	-0.3590	0.4185*	0.8847**	0.2053
M1/M3	F_1	0.5608*	0.8476**	0.9431	-0.4197	0.4822	-0.0361	-0.4528	0.6772	0.9489	-0.1959
M1/M3	F_2	-0.3890	0.3750	0.8817**	0.5463**	0.0393	0.0359	-0.0015	0.0575	0.7177**	0.7228**
M1/9026	F_1	0.6476**	0.5326*	0.9700**	0.4553	0.4490	0.0059	-0.2783	-0.2895	0.7928**	0.8434**
M1/9026	F_2	0.3369	0.6901**	0.9517**	0.7056**	0.4487*	0.4989**	0.3431	0.6830**	0.8106**	0.4575*
M3/9025	F_1	-0.4580	-0.9899**	-0.6668**	-0.0757	-0.9453**	0.3721	0.5597*	-0.6008*	0.9623**	0.0408
M3/9025	F_2	-0.0948	0.8276**	0.8878**	0.7150	0.1258	0.1004	0.0319	0.5666**	0.9740**	0.2296
M3/9026	F_1	0.1292	0.9818**	0.9810**	-0.6783**	-0.2208	0.2442	0.2408	0.1180	0.8460**	0.6508**
M3/9026	F_2	0.5840**	0.7196**	0.8565**	0.1305	0.4444*	0.1741	-0.0943	0.3977	0.9743**	0.7164**
Combined	F_1	0.4539**	0.7771**	0.8844**	-0.0639	0.3572**	0.3354**	0.0925*	0.3476**	0.9418**	0.0180
Combined	F_2	0.2205**	0.6955**	0.8391**	0.2465**	0.3488**	0.2760**	0.0895	0.2718**	0.8129**	0.3735**

coefficients in a wider mixed populations either in different pure-lines, whereas the differences in association among populations of same cross for characters like, pods/branch, pod length, seeds/pod, seed density and seed weight revealed that selection for these characters should be carefully conducted to improve blackgram for specific character. The correlation of grain yield with other characters for pooled populations of F_1 and F_2 revealed the importance of plant height, branches, pods, pod length, seeds/pod, seed weight and biomass in improving yield potential of blackgram.

DISCUSSION

DISCUSSION

5.1 Genetic Diversity Based on Morphological Characters

In order to maintain, evaluate and utilise germplasm efficiently and effectively, it is important to investigate the extent of genetic diversity it contains. Smith & Smith (1989) considered morphological characherization as first step in description and classification of crop germplasm. The breeding programme mainly depends upon magnitude of genetic variability (Shanmugan & Shreerangaswamy, 1982; Smith *et al.* 1991). In present investigation, blackgram germplasm was evaluated for qualitative and quantitative characters alongwith MYMV reaction. For qualitative characters, a considerable level of variability was observed for plant pubescence, plant type and leaf shape, whereas seed coat colour and spots on the seed coat exhibited a low level of variation as most of the accessions were brown seeded with black spots. Only six accessions were green seeded and seven accessions were without seed spots. Both these traits were present in six accessions together but in one accession, the seed coat colour was brown and spots were absent. Qualitative characters are important for plant description (Kurlovich, 1998) and are mainly influenced by the consumers preference, socio-economic scenario and natural selection. Nakayama *et al.* (1998) reported that foxtail millet landraces with low amylose allele were distributed only in Southeast Asia mainly because of preference followed by selection. Some of these traits are reflected to some biotic/abiotic stresses. Prostate plant type is preferred for planting under rainfed conditions as it facilitates in moisture conservation. The plants with lanceolate leaf shape (narrow leaves) in most cases are drought tolerant and hence, the plants with these characters may be utilized for breeding blackgram for rainfed conditions. Hairiness crop is more tolerant against some insects, whereas glabrous cultivars facilitate in harvest and trample.

The important yield traits; days to flowering, maturity, branches, pods, pods/branch, biomass, grain yield and harvest index exhibited high range alongwith high variation which, in general revealed that the selection for these economic traits is effective in developing high yielding varieties of blackgram. Subdividing the variance into its components assists genetic resources conservation, utilization and it enables planning for use of appropriate gene pools in

crop improvement for specific plant attributes (Bekele, 1984, 1985; Pecetti *et al.* 1992, 1996). For pod length, seeds per pod, seed density and 100-seed weight, low genetic variability seemed to restrict the scope of selection for these traits in the present germplasm collection. Hence, the genes for these important economic traits should be investigated or exploited from other sources, i.e., inter-specific hybridization, mutation etc. Large scale testing of broad base germplasm needs to be built up by making extensive local collection and obtaining germplasm from abroad to develop a sound breeding programme (Jain *et al.* 1975; Ghafoor *et al.* 1992). Brown (1978) and Laghetti, *et al.*(1998) advocated that maximum genetic conservation would be achieved by sampling population from as many environments as possible. The classification of germplasm gave rise to some elite lines for specific characters and the accessions for days to flowering (11), days to maturity (25), branches (6), pods (17), pod length (7), seeds/pod (2), seed weight (4), biomass (3), grain yield (45) and harvest index (41) have been selected and suggested for exploitation in breeding programme. It was observed that some of these accessions possessed desirable genes for more than one character and hence these could be utilized directly or included in hybrid programme for varietal development. Selection on the basis of best performance has already been suggested by many researchers like Donald, (1962), Lal, (1967), Singh, (1977), Singh *et al.* (1880) and Khan & Malik, (1989). As observed in the present study and referred by the earlier researchers (Malik *et al.* 1881; Malik *et al.* 1986; Ghafoor *et al.* 1993b) that biomass is significantly associated with pods and branches/plant, therefore, this trait should be considered while selecting genotypes for high number of pods and ultimately grain yield.

Analysis regarding HI range revealed that the accessions having more than 35% HI exhibited the best values for days to flowering, days to maturity, branches, pods and grain yield. But most of the values showed high SE which restricted the prediction and reproducibility of the results. Hence precise investigation regarding Selection Score (SC) revealed that the highest SC values were observed for HI range of 30-35% (18.54) and 25-30% (18.06) which gave a clue for the future selection for blackgram improvement. The association of qualitative traits with QTLs revealed that the accessions with *"HR"* genes were early, whereas the accessions with *"hr"* genes had increasing effects for pods, seed weight, biomass and grain yield. Seed coat colour and seed spots also exhibited significant differences

for days to flowering, maturity, branches, pods, biomass and grain yield. The genes *"HR", BR"* and *"SS"* being a good indicator for better agronomic characters, has been identified as markers and are suggested to be used for screening blackgram germplasm.

Correlation is a measure of the degree to which variables vary together or a measure of intensity of association. Generally, a high magnitude of correlation with positive signs were observed between different traits for pooled (P) and selected (S) population. Out of 66 combinations of correlation coefficients, 55 values (83.3%) were observed with same signs and in 38 cases (57.6%), the correlations were significantly similar for both the populations and thus the results could be implied in a wider perspectives. Only 6 values in the present studies were observed significantly different in two populations (Table 5.1.1). It is observed that correlation of harvest index with other characters mainly differed in 2 populations which might be due to selection based on economic characters. Significant positive correlation of grain yield with other yield contributing characters has also been reported by Rani & Rao, (1981) in blackgram. In mungbean, Tomar *et al.*(1973) and Khalid *et al.*(1984) also observed positive correlation of yield with yield components, whereas, Malik *et al.* (1987) reported negative correlation of yield with maturity, pod length and seed weight. Malik *et al.* (1983) investigated maximum relative selection efficiency for branches per plant in mungbean, and Malhorta *et al.* (1974) observed positive association of yield with days to maturity, plant height, pod number and length, whereas negative with seed weight. Grain yield, the ultimate objective in blackgram breeding programme exhibited positive association with all the characters under study with varying degrees of significance. High correlation of grain yield with branches, pods/plant, pods/branch, seeds/pod, seed weight and biological yield for both the populations revealed that these characters are really yield contributing characters in either case. For selected population, grain yield showed significantly positive correlation with days to flowering and maturity also, indicating the importance of selection pressure for these traits. Malik *et al.* (1987) and Ghafoor *et al.* (1993b) reported positive association of grain yield with biological yield. Negative association of biological yield with harvest index showed physiological inefficiency for appropriate partitioning of total dry matter towards economic yield. Consequently the varieties with low grain yield attained low harvest index.

Table 5.1.1:- Summary of correlation analysis for pooled (P) and selected (S) populations.

		DF	DM	BR	P/PL	P/BR	PL	S/P	SD	SW	BY	GY
DM	P	+***										
	S	+***										
BR	P	+	+									
	S	+	+									
P/PL	P	-	+	+***								
	S	+	+*	+***								
P/BR	P	-	-	-**	+***							
	S	+	+*	-**	+**							
PL	P	+	+**	+*	+*	+						
	S	+	+	+*	+	+						
S/P	P	+*	+**	+*	+**	+**	+***					
	S	+**	+**	+	+**	+	+***					
SD	P	+	+	+*	+**	+	-**	+***				
	S	+	+	+	+*	+	-***	+**				
SW	P	+**	+**	+**	+*	+*	+	+	-			
	S	+**	+**	+	+	+*	+	+	-			
BY	P	+**	+**	+**	+***	+**	+*	+**	+**	+**		
	S	+**	+**	+***	+***	+**	+	+	+	+*		
GY	P	+	+	+***	+***	+**	+**	+**	+**	+**	+***	
	S	+**	+**	+**	+***	+**	+	+*	+	+**	+***	
HI	P	-**	-**	+*	+**	+**	+	+*	+	-	-**	+**
	S	-	+	-	-	-	-	-	-	+	-**	+

*, ** and *** represents significant at P>0.05, P>0.01 and P>0.001, respectively.

Total correlation coefficients studied	66	
Correlation with same signs	55	83.3 %
Significantly with same signs	38	57.6 %
Correlation with different signs	11	16.7 %
Significantly with different signs	6	9.1 %

Construction of SC on the basis of PCA and HI revealed that the most economic range of HI was 25-35% and thus future blackgram cultivars may be selected on the basis of this range. In previous studies conducted by Patel & Shah, (1982) and Ghafoor *et al.* (1993b), high selection indices were obtained in blackgram where harvest index ranged from 26 to 36%. The results obtained in this study supported the approach that the germplasm with high harvest index (25 to 35 %) might be future potential source for blackgram breeding. On the basis of these results, high yielding accessions combined with other good agronomic characters were identified from the groups constructed on the basis of harvest index and these selected accessions are suggested for further testing under wide range of agro-ecological conditions to utilize for selection/breeding of high yielding blackgram cultivars. Virmani *et al.* (1983) evaluated mungbean germplasm and classified into various groups based on different traits and identified accessions with high yield potential for future utilization. Bakhsh *et al.* (1992) categorised lentil germplasm on the basis of quantitative traits and observed that short statured lentil genotypes were high yielding with other good agronomic characters. They suggested exploitation of selected genotypes for lentil improvement in future. According to Ghafoor *et al.*, (1989), high yielding accessions selected from the local germplasm might prove their superiority in advance testing under various agro-ecological conditions. They classified blackgram local germplasm for various agronomic characters and selected eleven high yielding pure-lines for further exploitation. From these initially selected pure-lines, two varieties have been developed and approved for general cultivation. In a study on mungbean Ghafoor *et al.*, (1992) selected twenty eight genotypes on the basis of high yield potential and resistance to diseases. Singh & Srivastava, (1985) categorised pea germplasm into various groups. Germplasm evaluation must be considered the first step in any plant breeding programme and it is commonly based on a simultaneous examination of a large number of populations for several characters of both agronomic and physiological interest (Pezzotti *et al.* (1994).

In the present germplasm evaluation, all the characters contributed genetic variance positively for PC_1 and seven (DF, DM, PL, S/P, SW, SD & BY) exhibited positive effect on PC_2. The first PC explained 28.3% of the variance and was positively associated with all the characters. The populations with high PC_1 values were

characterized as high yielding with low seed density and harvest index. It was observed that nine important plant characters contributed more positively in first 3 principal components and hence these could be established important for the material under investigation. The second component was associated with maturity and high seed weight, contributing 17.2% of the total variance. Results reported by various researches (Holcomb *et al.* 1977; Camussi *et al.* 1985; Falcinelli *et al.* 1988 and Veronesi & Falcinelli, 1988a, 1988b) showed multivariate analysis to be a valid system to deal with germplasm collections.

The grouping of accessions by multivariate methods in this study is of practical value to the breeders of blackgram. Representative accessions may be chosen from particular groups for hybrid programme with other approved varieties. Several potentially important agronomic types have been identified and these may be exploited for genetic potential to transfer the desirable genes and this, alongwith biochemical analysis will also facilitate in assembling a core collection of accessions from the large genetic resources collection (Tolbert *et al.* 1979; Frankel, 1984; Singh, 1988; Clements & Cowling, 1994 and Vierling, *et al.* 1994). Tawar *et al.* (1988) conducted genetic divergence in 34 diverse genotypes of mungbean and grouped into five clusters. They observed that variability in the parents was related to genetic diversity. Inclusion of such genotypes from distinct clusters and their implication in mubgbean breeding programme was suggested.

The association of QTL with easily identifiable gene markers could permit a rapid and precise transfer of QTL into superior crop cultivars (Tanksley *et al.* 1989; Tahir & Muehlbauer, 1995). In the present study, 3 qualitative characters (hairiness, seed coat colour, spots on seed) were detected significant for various QTLs. Variation at qualitative loci in the vicinity of QTL in blackgram germplasm may be an indication of genetic variation potentially available to breeding programmes which can be used potentially for crop improvement.

From the present investigation, it was determined that blackgram germplasm from Pakistan displayed a wide range of diversity for most of the traits studied alongwith some accessions with unique characters. This could enable us to identify, select and combine some potential landraces to induce evolution for important traits in one genotype with broad based genetic background. Quite often, the quantitative traits are economically

important (Amurrio *et al.* 1995). Moreover, if one of the goals is to bring together varieties with genetically similar characteristics, quantitative characters may be useful for grouping. Nevertheless, the qualitative traits must be often used for separating varieties when a limited range of quantitative traits if found in certain groups (Sneedon, 1970). Malhotra & Singh, (1971) reported a narrow range of variability for 100-seed weight and pod length in blackgram, whereas Shanmugam & Shreerangaswamy, (1982) reported that yield per plant contributed maximum to the genetic diversity. Mishra & Rao, (1990) reported thirteen clusters in a comparative study of D^2 and meteroglyph analyses in 117 genotypes of chickpea.

5.2 SDS-PAGE and its Significance in Determining QTLs

Seed proteins have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz, 1979; Khan, 1992; Das & Mukarjee, 1995). It is a promising tool for distinguishing cultivars of a particular crop species (Cooke, 1984; Ferguson & Grabe, 1986; Gardiner & Forde, 1988; Gadgil, *et al.* 1989; Koranyi, 1989; Jha & Ohri, 1996). However, a few studies indicated that cultivar identification was not possible with the SDS-PAGE (Ladizinsky & Adler, 1975; Raymond *et al.* 1991; Ahmad & Slinkard, 1992; de Vries, 1996).

The variance for SDS-PAGE was low for the samples analysed and similar results have also been reported by Thakare, *et al.* (1987) in blackgram who reported limited intraspecific genetic diversity in blackgram. However, Damania *et al.* (1983), Kumamura *et al.* (1988), Fergouson & Grabe, (1986) and Jha & Ohri, (1996) have reported a considerable range of variation among cereals, rice, ryegrass and pigeonpea genotypes, respectively on the basis of seed proteins. In present investigation, all three regions exhibited variation with major differences in the region III, where among 13 bands 10 were recorded as polymorphic in nature and out of these polymorphic bands, 7 were found significant in detecting QTL with mean comparison for various characters. Moller & Spoor, (1993) suggested 5 regions in *Lolium* spp. and observed major differences in the regions B, C and D. The present study supported the previous results of Murphy *et al.* (1990) who used different crops but indicated potential power of electrophoresis techniques for determining the extent of genetic variation in crop germplasm. In the

present studies, some specific protein bands were observed for some genotypes and hence these peptides may serve as markers for specific genotypes (Gepts, 1990; Smith & Smith, 1986). Przybylska & Przybylska, (1995) reported markers for smooth-seeded and rough-seeded species of *Lupinus* based on SDS-PAGE analysis. Rao *et al.* (1992) considered that seed proteins of *Vigna* are useful to distinguish the species, and some of black seeded mungbean accessions and green seeded blackgram accessions mixed in the germplasm were also clearly identified and separated for gene bank management. It was also possible to identify *Vigna mungo* and *Vigna radiata* in the present investigations on the basis of SDS-PAGE as it has already been reported by Thakare *et al.* (1987) and Rao *et al.* (1992). Yoshida *et al.* (1997) observed two fragments of globulin in blackgram using SDS-PAGE. Similarly, phylogenetic relationships have also been reported by Margoliash & Fitch, (1968), Sammour, (1989), Tomooka *et al.* (1992) in legumes. In present studies, intra-specific variation was limited among blackgram accessions. Similar results had already been reported by Thakare *et al.* (1987), who observed differences between *Vigna mungo* and *V. radiata* but intra-specific variation within *V. mungo* was limited.

Intraspecific variation for identification of various accessions/genotypes is possible by the use of SDS-PAGE in blackgram by critical observation of regions II and III, especially in region III where many lower molecule weight bands occur. The accessions with similar banding patterns may be duplicated in the germplasm, but these are suggested to be confirmed by the use of 2-D electrophoresis focusing as suggested by earlier researchers (Celis & Bravo, 1984; Beckstrom-Sternberg, 1989 and Higginbotham *et al.* 1991). Tahir *et al.* (1996) detected HMW glutenin subunit in hexaploid wheat using SDS-PAGE which was specific for some accessions collected from Baluchistan, Pakistan. In the present studies intraspecific variation was limited and it was observed that SDS-PAGE alone did not exhibit high level of intraspecific variation, therefore, diverse accessions based on SDS-PAGE are suggested to be acquired from various sources, preferably from centre of diversity to build a broad based gene-pool with maximum variability. Further, for better management of genebank, a precise comprehensive knowledge of agricultural and biochemical data (protein and DNA) is essential to eliminate duplicates which will ultimately help in making core collection of blakgram germplasm.

Besides genetic variation, screening analysis for marker bands to detect QTLs were carried out and some significance of protein peptides were observed in determining QTLs in blackgram. In total, 20 polymorphic bands were analysed in comparison with 12 quantitative characters and out of 240 combinations, 24 exhibited significant differences for means using both "*t*" and "*r*" statistics. It is also worth mentioning that both these tests were in good agreement (r= 0.7508) and hence can be used independently but for better understanding, both of these statistics are recommended to use for detecting QTLs in germplasm management (Fig. 5.2.1). The factors or loci affecting variation in quantitative traits may occur as individual genes or gene clusters scattered throughout the genome, therefore, same quantitative traits may be expressed differently at several loci (Tahir & Muehlbauer, 1995). The use of molecular markers to locate genes controlling quantitative traits has been considered important in the analysis of such traits (Stuber *et al.* 1982; Stuber, 1992; Kahler & Wehrhahan, 1986; Edwards *et al.* 1987; Kjaer *et al.* 1991; Edwards, 1992; Mansur *et al.* 1993; Tahir & Muehlbauer, 1995). Detection of QTL into individual genetic components by use of biochemical markers has been demonstrated in tomato (Tanksley *et al.* 1982), garden pea (Kneen *et al.* 1984) and lentil (Hoffman *et al.* 1986; Tahir & Muehlbauer, 1995). Variation in 10 quantitative traits out of 12 was significantly associated with 13 protein peptides, however, the actual number of QTL might be fewer because several of these traits were correlated. The association of QTL with easily identifiable markers could permit the rapid and precise identification and transfer of QTL into superior crop cultivars (Tanksley, 1983). The amount of information provided by this marker based approach will depend on the type and number of markers, and their linkage relationship (Singh *et al.* 1991). Expansion of genetic base for blackgram breeding might be accomplished by systematic use of germplasm that differ from common banding pattern and known to be associated with variation in quantitative traits.

The link of protein pattern has already been reported by Murphy *et al.* (1990), whereas Moller & Spoor, (1993) could not detect any link for days to maturity, winter hardiness and disease. The frequency of these markers based of protein peptides for QTL are not very commonly observed since these protein subunits would tend to be simply inherited, whereas agriculturally important traits are usually polygenic in nature. The

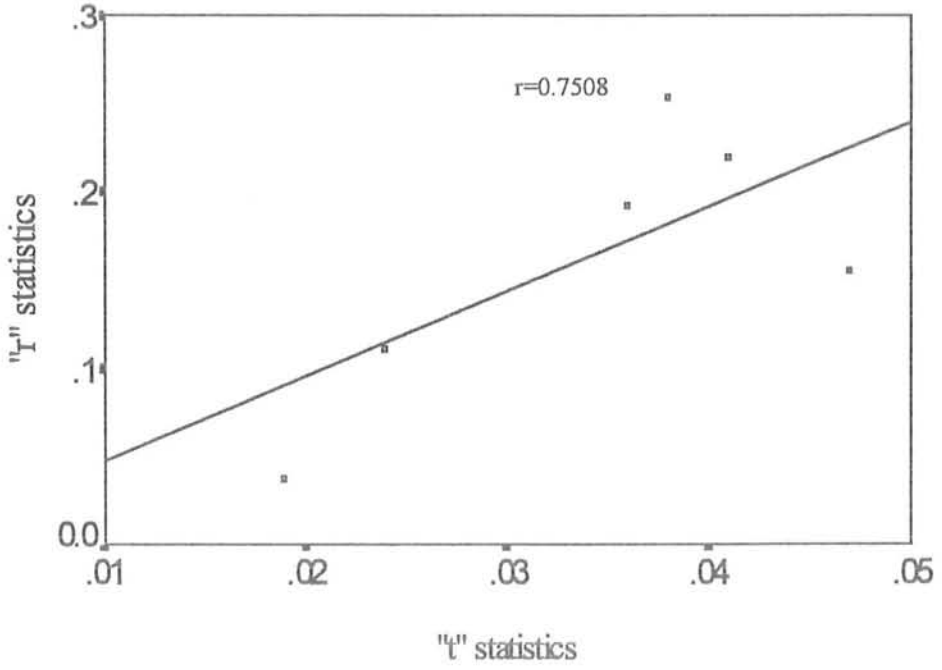


Fig. 5.2.1:- Association between *r* and *t* statistics for detecting QTL based on SDS-PAGE in blackgram

initial results are encouraging for locating factors that influence the expression of quantitative traits. However, the conclusions are specific to the sample investigated, and the environment in which the measurable traits were recorded.

In order to ensure the efficient and effective use of crop germplasm, its characterisation is imperative. In the present investigation, considerable variation for quantitative characters was observed in almost all the characters except pod length, seeds/pod and seed density. The greater part of genetic variation was accounted for by days to maturity, branches/plant, pods/plant, biomass and grain yield in PCA for quantitative characters. Multivariate analysis provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level (Rouamba *et al.* (1996). Broschat, (1979) considered PCA a powerful tool for data reduction which removes intercorrelations among components. Additional applications of this technique will certainly be found as its use becomes more widespread in fields of biological sciences, where it has been used extensively for more than two decades. Dasgupta & Das, (1984) considered multivariate analysis best for choosing parents for hybridization. They investigated 12 characters on forty strains of blackgram collected from India and Nepal, and grouped into seventeen different clusters. Genetic divergence conducted in 38 genotypes of blackgram by Dasgupta & Das, (1985) using D^2 statistics revealed no relationship between geographic distribution and genetic divergence. Flowering time and seed size exhibited maximum contribution to the total divergence. They further observed that environmental conditions exerted considerable impact on the number and composition of clusters. Suggestion has been made for selecting suitable stable diverse parents so as to initiate a crossing programme for increased grain yield in blackgram. Such studies would allow more efficient enhancement and use of genetic resources with a view to introduce desirable characteristics from landsraces into improved cultivars. Kresovich & McFerson, (1992) considered genetic diversity important in assessment of PGR management. Ahmad *et al.* (1997) reported that first two canonical components contributed 85% of the variation between lentil genotypes. It was observed that cluster analysis on the basis of quantitative characters were phenotypically more distinct and exhibited more breeding value. Though cluster analysis grouped together accessions with greater morphological similarity, the

cluster did not necessarily include all the accessions/genotypes from the same or nearby sites. Kumar & Arora, (1992) observed in chickpea that the varieties with narrow genetic base were affected more by seasonal variation than those with broader genetic base, particularly under rainfed condition. Under such circumstances, availability of genetically diverse genotypes for hybridization programme becomes imperative. Gupta *et al.* (1991), Dias *et al.* (1993), Amurrio *et al.* (1993, 1995) and Rabbani *et al.* (1998a) also reported no association between morphological characters and geographic origin. Revilla & Tracy, (1995) observed low level of morphological variability amongst widely used open-pollinated sweet corn cultivars. The grouping of some of the accessions based on irrigated areas and flood plains exhibited the association between morphological characters and geographical origin. This was due to easy exchange of germplasm between the neighbouring regions and perhaps same ancestors. Cluster analysis based on morphological characters was observed more reliable than on the basis of protein peptides which indicated that cluster analysis on the basis of quantitative characters have more breeding value in blackgram, but simultaneous study for both agronomical and biochemical analysis (protein and DNA) is suggested. Multivariate analysis have been used for classifying in both qualitative and quantitative traits in collection of crop germplasm (Peeters & Martinelli, 1989; Caradus *et al.* 1989; Rumbaugh *et al.* 1988).

From the present studies it was concluded that blackgram distributed throughout Pakistan over a wide agro-ecological range exhibited significant variation for all the quantitative characters except pod length, seeds/pod and seed density. Although variation was observed for total seed protein but the level was low, hence SDS-PAGE was not very effective for studying intra-specific genetic diversity in blackgram. The association of biochemical variation with QTL was also observed and can be used for germplasm screening and further exploitation for blackgram improvement. PCA and cluster analyses proved their validity to establish genetic diversity, and these statistics on the basis of quantitative characters revealed more reliability than SDS-PAGE. No geographic relationship was found in any case. The management of both qualitative and quantitative matrices are suggested to workout independently at the beginning and then the mixed one has advantages as;

- One gets a synthetic description of the most important characters of each cluster.
- The results have a useful biological significance because some of the traits chosen are directly related to adaptability to agronomic conditions, such as flowering, maturity, yield and yield contributing characters.
- Clusters analysis gives a general, morphological and physiological description of the main characteristics and the possible use of each one of the groups.
- The use of morphological characters gives a better resolution which has more significance for varietal description.

5.3 Geographic Distribution

In order to determine the distribution of diversity in germplasm, phenotypic and genotypic variation within and between countries and regions for various crops have been examined by many researchers as; Tolbert *et al.* (1979), Ruiz *et al.* (1997), Jain *et al.* (1975), Bogyo *et al.* (1980), Wu & Jain, (1977), Holcomb *et al.* (1977), Pezzotti *et al.* (1994), Erskine & Muehlbauer, (1991), Clements & Cowling, (1994) and Perry & McIntosh, (1991) in a variety of crop species. SDS-PAGE on the basis of provinces revealed that out of 20 polymorphic bands, Band 1 exhibited polymorphism for all the provinces followed by protein peptide Bands 2, 3, 8 and 9 which gave polymorphism in all provinces except AJK (bands 2 & 3) and except Punjab (bands 8 & 9). The accessions from NWFP revealed maximum polymorphism, followed by the accessions from Punjab. The lowest degree of polymorphism was observed in the accessions from AJK and Baluchistan. These areas are quite diverse in geographical features, however, a low level of variability might be due to lesser representation of material from these areas. Therefore, it is suggested that more expeditions may be arranged from those areas to capture the maximum variability because both these locations (AJK and Baluchistan) are diverse in nature. The differences according to geographical regions shown by the analysis of SDS-PAGE and morphological traits is useful in substantiating the postulated regions of diversity or gene centres. Genetic diversity on the basis of SDS-PAGE and agronomic characters in the present study indicated the worth in examining the centre of genetic diversity in blackgram using SDS-PAGE and other biochemical markers. According to Perry & McIntosh, (1991), differentiation according to geographical regions of origin is useful in substantiating the postulated regions of diversity or gene centres. The rare

alleles, each only occurring in one or two apparently random populations can be considered to be mutants, migration or the results of other coincidental events (Van Hintum & Elings, 1991). Alleles common in the restricted areas occur mostly in high mountainous areas. This could indicate that genetic material is introduced from foot hills of NWFP to high mountains of NWFP and NA. Migration of landraces into new regions, followed by some degree of contamination by mixture or out crossing with other landraces can be expected in the country like Pakistan, where germplasm movement is not restricted from one area to other. The areas with a high level of stress will present interesting tolerance to environmental stresses, but homogeneous mixtures, and hence, need less extensive sampling for genetic resources conservation purposes. Laghetti *et al.* (1998) considered oppressive prolonged drought a serious threat to the conservation of gene pool of *Vigna savi* in natural habitat and thus recommended germplasm collection mission for conservation of maximum genetic diversity from the areas under environmental stresses.

The grouping on the basis of altitude and crop ecological zones could not prove its worth for the present blackgram germplasm used, whereas clustering based on provincial distribution, 16 accessions out of 42 (40%) grouped together. Further, cluster analysis showed that many accessions from same origins were grouped separately which may be because of frequent exchange of germplasm by the breeders or transport of grain to different markets from where the seed of various origins is disseminated through out the country. According to Smith *et al.* (1995), linkage cluster and PCA are useful for preservation and utilization of germplasm. Though accessions grouped together with greater morphological similarity, the cluster did not necessarily include all the accessions/genotypes from the same or nearby sites. The grouping pattern of landraces did not reflect geographic origin for altitude and crop ecological zones which is in agreement with those presented by Singh & Tripathi, (1985) and Amurrio *et al.* (1995). Further Gupta *et al.* (1991), Dias *et al.* (1993), and Rabbani *et al.*, (1998a) also reported no association between morphological characters and geographic origin.

On the whole, multivariate approach has proved to be very useful tool in that it produced five clusters on the basis of provincial distribution much more differentiated if compared to the initial subdivision according to latitude and crop ecological zones of the

collecting sites only. The study confirmed the existence of a wealth of phenotypic divergence in the local blackgram germplasm. The variation within the country appears largely attributable to different provinces. Further collecting missions to main blackgram growing areas with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields close to motorable roads within individual province as already has been suggested by Pecetti *et al.* (1996) for tetraploid wheat. Laghetti *et al.* (1998) suggested collecting expedition to the areas of where genetic erosion takes place in cowpea alongwith the areas where existing genetic diversity has not been yet gathered (Padulosi, 1993).

5.4 Random Amplified Polymorphic DNA (RAPD)

Electrophoresis alongwith field evaluation adds information to taxonomy and should not be disassociated from morphological, anatomical and cytological observation (de Vries, 1996). Among several techniques available for assessing genetic variability, relatedness and structure among crop germplasm collections, DNA-based markers provide powerful and reliable tools for discerning variations within crop germplasm and for studying evolutionary relationships (Gepts, 1993). For better management of genebank, a precise comprehensive knowledge of agricultural and biochemical data (protein and DNA) is essential. Forty pure-lines of *Vigna* spp. selected for RAPD analysis were also evaluated for morphological characters and SDS-PAGE. Out of 40 pure-lines, 3 (45727, 45761 & 45774) were blackgram like mungbean, whereas 6 (41117, 41065, 41084, 41085, 41096 & 41016) were green seeded mungbean like blackgram which were confirmed by the application of SDS-PAGE and RAPD analyses. On the basis of SDS-PAGE, 15 genotypes gave similar protein pattern and thus excluded from the cluster analysis because genotypes with similar banding pattern give no taxonomic information (Williams *et al.* 1993). Singh *et al.* (1996) reported little variation for protein bands in groundnut which indicated that most of the accessions were the members of same conservative species. Cluster analysis based on seed protein revealed 2 main groups. The group A, in total consisted 3 genotypes (45774, 45761 & 45727) and all these three genotypes were *Vigna radiata,* hence clearly differed from other clusters. Band 4 at 45 kd MW was observed in

all the blackgram genotypes but absent in mungbean and hence this band could also be used for identification of these two species. Two bands (9c, & 14) were specific to genotypes 45727, 45761 and 45774. As these all three genotypes were *Vigna radiata* and hence these specific bands alongwith band 4 could be used for isolating *Vigna radiata* from mixed germplasm. The cluster pattern of blackgram genotypes indicated that the genetic variation within *Vigna mungo* is very narrow, which may necessitate the use of novel techniques/procedures for the creation of genetic variability in *Vigna mungo*. Similar findings were also reported by Ahmad *et al.* (1996) who observed a low level of genetic variation in lentil. Ladizinsky, (1979) and Ahmad *et al.* (1995) used morphological and seed protein comparisons but found no biological basis for separating closely related small and large seeded lentils. In general cluster analysis based on SDS-PAGE proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo*, whereas within *Vigna mungo* a low level of genetic diversity was observed and no clear differentiation was recorded either for agronomic characteristics or origin as various clusters consisted mixed genotypes from different origins.

Among molecular markers, RAPD is employed in genetic research owing to their speed and simplicity (Williams *et al.* 1990; Welsh & McClelland, 1990). RAPDs require no previous sequence information, therefore, have been used widely for estimating genetic variation at the population level and among closely related species (Rafalski, *et al.* 1991; Williams *et al.* 1993). RAPDs have been shown to provide a level of resolution equivalent to RFLPs for determining genetic relationships among *B. oleracea* genotypes (dos Santos *et al.* 1994) and among *B. napus* breeding lines (Hallden *et al.* 1994).

Fifty three 12 mer primers were tested on 4 genotypes (9010, PL-2, 41065, 45737) for preliminary DNA amplification. Out of 53 primers, 46 revealed amplification for present material, whereas seven primers failed to amplify. Out of amplified primers, 36 exhibited polymorphism and hence are suggested to be used for *Vigna* for fingerprinting and other DNA work. Some of the primers revealed characteristic fragments for some genotypes which were not produced in others. Finally out of 36 polymorphic primers, 11 were used for RAPD analysis of 40 genotypes. Some of the primers generated several markers and were able to show high genetic diversity, while others produced few markers

and detected little variability. Eleven primers generated 131 reproducible and score able amplification products across all the genotypes, out of which 107 (80.2 %) fragments were polymorphic in one or other of the 40 genotypes. The levels of polymorphism varied with different primers among various genotypes. RAPD has been found important to resolve various levels of inter-and intra-specific polymorphism, which facilitates assessment of genetic relationships, definition of regional grouping and identification of individual accessions (Connolly *et al.* 1994; Vierling *et al.* 1994; Link *et al.* 1995; Multani & Lyon, 1995; Nienhuis *et al.* 1995; Skroch & Nienhuis, 1995; Virk *et al.* 1995a, 1995a & 1996). The molecular markers, RAPDs have been used for determining the genetic relationships between different related species (Demeke *et al.* 1992; Devos & Gale, 1992; Thormann *et al.* 1994; Yu & Nguyen, 1994; Abo-elwafa *et al.* 1995; Mackill, 1995; Ren *et al.* 1995; Sharma *et al.* 1995; Ahmad *et al.* 1996; Iqbal *et al.* 1997; Ahmad *et al.* 1997), for the identification of cultivars and genotypes (Hu & Quiros, 1991; Rabbani *et al.* 1998b), for estimating the genetic relationships and diversity among crop germplasm (Kresovich *et al.* 1992; Jain *et al.* 1994; Mailer *et al.* 1994; Farnham, 1996), for plant breeding and seed testing programme (Jianhua *et al.* 1996) and for tagging agronomical characters (Joshi & Nguyen, 1993). Halward, *et al.* (1991) used molecular markers in peanut and did not observe significant level of polymorphism. This might be due to the material and primers used in experiment as selection of primer in this study is very important. Smith & Smith, (1992) have thoroughly reviewed the role of genetic markers in fingerprinting commercial germplasm. Many primers used in this study generated polymorphic fragments in two species of subgenus *Ceratotropis*, indicating the usefulness of RAPD analysis to disclose DNA polymorphism in this taxon (Kaga *et al.* 1996). Williams *et al.* (1993) pointed out the possibility that similar sized fragments with no sequence homology may bias the data obtained. Thormann *et al.* (1994) showed such biased data obtained from RAPD analysis compared with RFLP analysis. In our study, the proportion of common bands were low (19.8%). Based on unweighed-pair-group method, two main groups were distinguished in the present material. Numerical taxonomy based on various ecological, morphological, biochemical and physiological characteristics and RFLP analysis provided clear difference in subgenus *Ceratotropis*. In the present study, two

species from one group of subgenus *Ceratotropis* were included and among these, two groups were observed on the basis of SDS-PAGE and RAPD which differentiated genotypes. Genetic diversity between *V. radiata* and *V. mungo* was reported by Chen *et al.* (1983) and Egawa, (1988) who observed irregular meiotic configuration with a high frequency of univalent formation in *V. radiata* and *V. mungo* hybrids with low pollen fertility (Miyazaki, 1982). Many primers generated genotype-specific amplification products and thus preliminary mapping in blackgram is suggested. The contribution of genetic markers to gene mapping or to plant breeding have been reviewed by Burr *et al.* (1983), Tanksley, (1983), Tanksley & Orton, (1983), Helentijaris *et al.* (1985), Beckmann & Soller, (1986), Soller & Beckmann, (1988), Tanksley *et al.* (1989), Edwards, (1992), Stuber, (1992) and Dudley, (1993).

RAPD is known to be highly sensitive to the reaction conditions (Samec, 1993). In routine variety identification, the problems can arise with the RAPD pattern reproducibility and the evaluation of fingerprints when the assays are performed in various labs. and by different scientists (Samec & Nasinec, 1995, 1996). Therefore, it is always necessary to perform control reactions with standard genomic DNA. Evaluation of electrophoretic gels should be performed with an automatic computer system, which would circumvent doubts with the detection of very faint bands. However, in the recent time two approaches have been proposed, which could reduce an influence of conditions in individual labs.

Paran & Michelmore (1993) converted RAPD markers to Sequence Characterized Amplified Regions (SCARs) and then designed pairs of PCR primers specific to the individual RAPD polymorphism. SCARs are advantageous over RAPD markers as they detect only single locus, their amplification is less sensitive to reactions and they can potentially be converted into codominant markers. Second approach employs the RAPD fragments as RFLP probes. This approach was successfully used to detect polymorphism in species with either smaller genome (*Daucus carota*, Schulz *et al.* 1994) or larger genome (*Allium* spp., Wilkie *et al.* 1993). Probably, there may be variety-specific RAPD/RFPLs in *Vigna radiata* and *Vigna mungo* useful for fingerprinting/tagging purposes.

The validity of aforementioned procedures would be very reliable but unfortunately, obtaining of both SCARs primers and RFLP probes is time and labour consuming. Our study has shown that the RAPD is very efficient in the production of DNA polymorphism in *Vigna* spp. The methodology is relatively simple to perform, rapid and amenable to automation. The protocol is ready to use in breeding, for registration and control of the distribution of commercial cultivars, the control of seed purity and for the cataloguing of accessions in germplasm collection as well. In future prospects, the results of this study can lead to a creation of group/variety-specific probes, using PCR primers (Paran & Michelmore, 1993) or RFLP (Wilkie *et al.* 1993; Schulz *et al.* 1994). The results can also be used for mapping purpose (Kiss *et al.* 1993) as well as the rapid generation of markers for important agronomic traits (Timmerman *et al.* 1994).

Cluster analysis based on agriculturally important characters revealed that advance breeding lines were categorised mostly in clusters VI, VII, VIII, IX and X. It may be because of selection pressure for high yield potential and other related characters. This revealed that only a portion of genetic diversity has been exploited, and it is suggested to broaden the genetic base of cultivated blackgram involving diverse parents in breeding programme. High polymorphism among individual genotypes revealed that the RAPD technique is an effective descriptor in studying inter and intra-specific variation in the subgenus *Ceratotropis* of genus *Vigna*. The technique can resolve genetic variation and identify genotypes in the breeding programme (Ribu & Hilu, 1996). Grouping grmplasm into geographical entries and elucidating affinities among these groups can define gene pools and determine gene flow among populations.

Comparison of genetic diversity based on agronomical, SDS-PAGE and RAPD analyses revealed high relationship for genetic dissimilarities based on SDS-PAGE and RAPD with high r^2 value (0.5219). This indicated that there was no significant difference between the results from SDS-PAGE and RAPD analyses in determining inter-specific genetic diversity between *V. radiata* and *V. mungo* (Fig. 5.4.1). Hence, these could equally be used for studying inter-relationships and genetic diversity in *Vigna* spp. Kaga, (1996) reported comparison between RAPD and RFLP for estimating genetic relationships among *Ceratotropis* species and observed strong correlation between genetic dissimilarities. The grouping based on

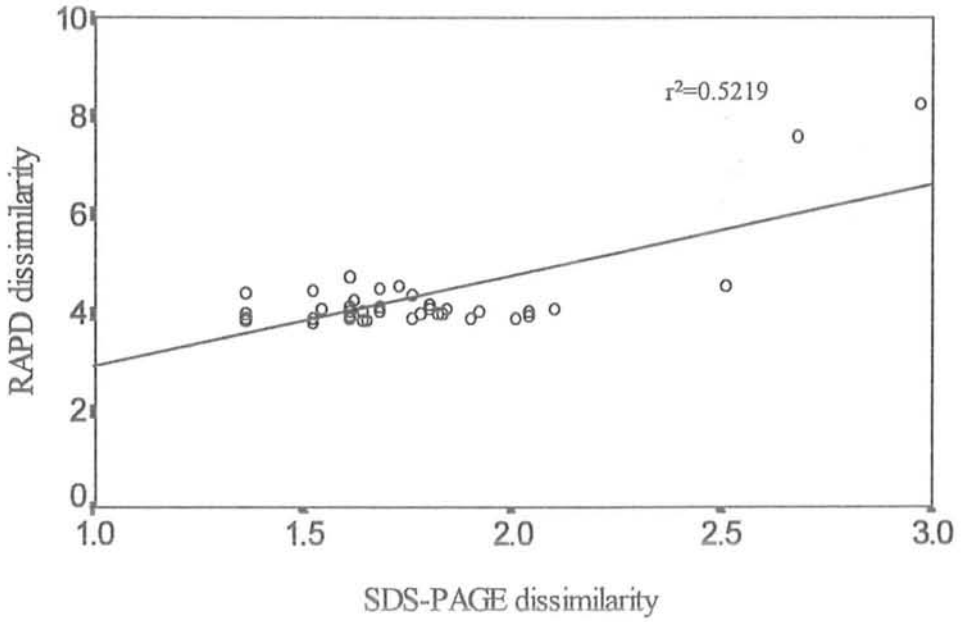


Fig. 5.4.1:- Association between genetic distances based on SDS-PAGE and RAPD dissimilarities

quantitative characters appeared with low intra-cluster variance for most of the characters, but in comparison with SDS-PAGE and RAPD analysis, insignificant association was observed. The grouping of 40 genotypes on the basis of SDS-PAGE and RAPD revealed that *Vigna radiata* was quite different as observed in banding pattern and cluster analysis. In general, all 40 genotypes shared grouping for three parameters (morphological, SDS-PAGE & RAPD) and it was observed that 8 out of 40 genotypes, which were 20% of the total, shared all three groups. Furthermore, cluster analysis shows that many genotypes from same origins were grouped separately which may be because of frequent exchange of germpalsm by the breeders or transport of grain to various markets from where the seed of various origins is disseminated throughout the country. Though cluster analysis grouped together accessions with greater morphological similarity, but the cluster did not necessarily include all the accessions/genotypes from the same or nearby sites.

5.5 Inheritance

5.5.1 Qualitative Inheritance

Polymorphic, highly heritable morphological traits were some of the earliest genetic markers employed in scientific investigations (Mendel, 1866; De Vries, 1912), and they may still be important as they require neither sophisticated equipment nor preparatory procedures. Monogenic or oligogenic morphological traits are generally simple, rapid and inexpensive to score. Until recently, scientific plant classification was based nearly exclusively on morphological traits (Stuessy, 1990), some of which may serve as genetic markers (Gottlieb 1984; Rao *et al.* 1989; Hilu, 1984) suitable for plant germplasm management (Stanton *et al.* 1994). In the present study, all the qualitative characters revealed monogenic segregation in 3:1 ratio for most of the hybrids. Hairiness (*HH, Hh*) was dominant over non-hairiness or glabrous (*hh*); brown seed coat colour (*CC, Cc*) was dominant to green (*cc*); presence of spots on seed (*SS, Ss*) was observed dominant over recessive absences of spots (*ss*) and black pod colour (*BB, Bb*) dominant to brown pod colour (*bb*). Monogenic inheritance for testa colour in groundnut has been reported by Senapati & Roy, (1990). Monogenic markers are useful in estimating the rate of out crossing in predominantly self pollinated crops. They also help in identification of F_1 hybrids in the breeding programme. Rao *et al.* (1989) reported monogenic recessive

inheritance of multifoliate foliate leaf in blackgram. Heterozygous are not possible to detect in case of complete dominance gene factors for morphological markers, therefore, the segregating ratios fit well in 3:1 chi-square fitness. Sen & Jana, (1963) studied inheritance of pod colour and found black colour dominant over straw colour in blackgram, whereas Ramaiah & Samolo, (1992) reported complementary gene action for pod colour. Similarly, Sen & Ghosh, (1959) observed complementary gene interaction in mungbean. Pathak & Singh, (1983) observed 3:1 ratio for blackish to brown colour in green gram. Chaudhari & Thombre, (1975) reported single gene dominance for pod colour in pigeonpea. Monogenic inheritance for simple leaf type in chickpea was reported by Rao *et al.* (1980) and Dahiya *et al.* (1984). Our results are in agreement with Sen & Jana, (1963), Chaudhari & Thombre (1975) and Pathak & Singh, (1983), but contradictory with Ramaiah & Samolo, (1992). This contradiction in results might be due to different genes in various parents involved in the hybridization. The inheritance of prostrate growth habit, simple leaf type and stem thickness was studied in chickpea by Singh & Singh, (1992) where all the characters were observed monofactorial recessive inheritance. The flower colour in peanut has been reported incomplete dominant that segregate in a ratio of 1:2:1 by Habib *et al.* (1980), whereas genetics of testa colour expression has been reviewed by Wynne & Coffelt, (1982) and Senapati & Roy, (1990) who reported seven loci to be involved in the expression of testa phenotypes.

Joint segregation of character pairs revealed normal distribution of independent assortment (9:3:3:1) for most of the character pairs but some distorted segregation for some crosses and character pair was also observed. All the character pairs segregated in a normal independent assortment except for *SS* vs *BB* in all the hybrids which was strongly linked in all cases. The character pair *HH* vs *CC* was linked in the hybrid Korea/MM 33-40 only; *HH* vs *BB* linked in the hybrid Mash 1/MM 5-60 and the allelic pair CC vs *BB* was linked in the hybrids Korea/MM 33-40 and 9104/MM 33-40 but this linkage was of weak to medium intensity. This deviation from normal assortment might be due to linkage for some alleles, and this type of distorted ratios have been observed by Kazan *et al.* (1993) in chickpea; Zamir & Tadmor, (1986); Muehlbauer *et al.* (1989) in lentil; Weeden & Marx 1984,1987 in pea and Koenig & Gepts (1989) in *Phaseolus* for morphological

markers inheritance. Hairiness was weakly linked with seed colour in one hybrid (Korea/MM 33-40), whereas in other hybrids, no linkage was observed for this character pair, which might be due to different genes involved for one of the loci. Hairiness and/or seed colour might be at different locus in various parents used for hybridization because in the hybrid "Korea/MM 33-40", glabrous parent "Korea" was exotic. Low level of linkage between hairiness and pod colour was observed in the hybrid "Mash 1/MM 5-60" which might also be due to different gene for seed colour and/or hairiness. Two hybrids "Korea/MM 33-40 and 9104/MM 33-40 exhibited weak linkage between seed colour and pod colour. All the seven hybrids revealed strong linkage between spots and pod colour in the present research material. As this linkage was observed in all the hybrids involved, therefore, it may be concluded that the genes for these characters might be from same origin. The linkage of blackgram proposed in this study contains 4 morphological loci. Morphological markers are in limited quantity in plants especially, blackgram because not much genetic work has been conducted so far on this crop. Five morphological loci have been reported by Kazan *et al.* (1993) in chickpea. The identified linkage of *SS* VS *BB* might be used for initial mapping of blackgram genome. The arrangements proposed were based on linkage observed between genetically diverse cultivated blackgram in the present study. The usefulness of the mapped marker loci should be realised when loci affecting QTLs including diseases and other economically important genes are added to the linkage group. The use of closely linked markers should facilitate breeding by giving a unique identity by tagging the genes of economic importance and by providing a mean of selection in the absence of nurseries and screening procedures that can be costly and time consuming.

The association of qualitative characters with seed weight was studied for 3 crosses (Mash 1/MM 33-40, 9025/MM 33-40, 9010/MM 33-40). It was evident from the data that the green seeded produced slightly higher seed weight (4.63g) in the segregating populations of the cross "Mash 1/MM 33-40". Mean difference was observed significant statistically using "*t*" test. The hybrid 9025/MM 33-40 also revealed association between seed colour and seed weight indicating significant difference. In this hybrid, green seeded plants produced bold seeds (5.55 g) with a statistically significant difference. The cross

"9010/MM 33-40" did not exhibit any influence of seed colour in the expression of seed weight. The association of seed colour with seed weight in the hybrids, Mash 1/MM 33-40 and 9025/MM 33-40 revealed that transgressive segregants for high seed weight to select bold seeded blackgram cultivars from these hybrids is possible. It is suggested to utilize diverse parents for both qualitative and quantitative characters for planning experiments for inheritance and mapping. Analysis of pooled data for QTL detection confirmed the association of seed coat colour and seed weight. In the cross "9025/MM 33-40" the cultivars with early maturity and high seed weight might be selected because, 9025 is a short duration cultivar with low seed weight. The hybrid "9010/MM 33-40" exhibited no linkage for seed colour Vs seed weight, which might be due to different genes involved for seed weight in different genotypes as it is concluded in many legumes that seed weight is controlled qualitatively but the genes vary in number and the segregation for this character will differ accordingly with the involvement of different parents used. Tahir & Muehlbauer, (1995) in a study on lentils reported linkage of 3 isozyme markers out of 10 for seed weight and used this marker for mapping. In present study, the initial results are encouraging for locating factors that influence the expression of seed weight. However, the conclusions are specific to the populations examined, and the environments under which the measurements were recorded. Further research is needed to establish the constancy of detected QTLs, and to locate their exact position in the blackgram genome.

5.5.2 Quantitative Characters

The analysis of combining ability in a 6 parent diallel indicated that both additive and non-additive gene effects were important for seed yield and its components in blackgram, however, non-additive gene effects are predominant. As in the present study, SCA and Reciprocal components of variance were more important for all the characters except seed weight indicating the importance of non-additive genetic variation for phenotypic expression of these characters. These results are similar to those of Reddy *et. al.*, (1979a, b), Chowdhury, (1986), Sharma & Sood, (1991), Ghafoor *et al.* (1993a) and Chauhan & Singh (1993, 1997) who reported predominance of SCA variance for yield and its components in legumes. Singh & Singh, (1993) and Chauhan & Singh, (1997) reported additive genetic variation involved for seed weight and non-additive for seed yield in lentil

and expected transgressive segregants in the proceeding generations for both characters in some of the crosses. However the researchers like Sharma *et al.* (1973) and Dahiya & Brar (1977) reported the importance of GCA, whereas Saxena *et al.* (1989) and Shanmugasundaram & Rangasamy, (1994) reported the presence of both GCA and SCA effects. Dasgupta & Das, (1987) investigated inheritance of pod length and cluster number in blackgram and observed wide genetic variability for these characters in two crosses, hence, suggested selection of desirable segregants for improving cluster number and pod length. Singh *et al.* (1975) and Singh *et al.*, (1992) reported significant GCA and SCA effects thus suggested selection of superior segregants based on number of branches during the early stage of growth and pods at the time of maturity. Although the reason for this difference in the results is not well understood but the differences in the results are mainly because of parents used and environments under which the experiment is conducted because polygenic characters are more influenced by the environmental fluctuations. Plant height and seed size are known to be highly heritable in legumes (Munoz & Abrams, 1971; Khan & Rachie, 1972; Sharma *et al.* 1973; Seehaiah *et al.* 1993). Wilson *et al.* (1985) observed the presence of both additive and non-additive genetic variance but predominance of non-additive for plant height, pods, pod length, seeds/pod, seed weight and grain yield in mungbean. Similarly, Sharma & Pandey, (1996) also observed both additive and non-additive gene action for yield and its components in blackgram. Ram & Singh, (1993) reported high variance for seed yield, pods and branches in blackgram which were influenced by additive gene-action. For plant height, both additive and non-additive gene-actions were observed. In present study, grain yield and its components were also observed having non-additive genetic variation. Estimates of the form of genetic variation have quite a fundamental influence on the identification of breeding strategies and methods. Presence of non-additive gene-action suggests that hybrids may provide a desirable alternative to pure lines or higher potential yields, assuming that epistasis is not additive x additive in nature (Mehetre, *et al.* 1988; Saxena & Sharma, 1989; Kunta *et al.* 1997).

On the basis of estimates of gene-action, Sharma *et al.* (1973) suggested the development of composite varieties. Dahiya & Brar, (1977) preferred the use of bulk

population improvement over pedigree methods, and Reddy *et al.* (1979b) suggested the exploitation of non-additive gene action through hybrids. Khan, (1973) and Frey, (1975) advocated population breeding using natural out crossing in pigeonpea crops where reproductive biology permits the option. Pedigree or bulk methods directed towards pure-lines have been used successfully by Gupta & Saxena, (1985). Thus the manipulation of these characters by simple selection or conventional breeding procedures should lead to only a partial exploitation of the total genetic variability existing or created in the population. The parents with best mean performance and GCA produced best recombinants in most of the cases (Table 5.5.1). Close relationship between performance and combining ability effects may be due to the presence of both additive and non-additive genetic variation for yield and its components.

Breeding by pedigree method would result in partial exploitation of additive and additive x additive types of gene-action. Under such situation any suitable methods of recurrent selections should be adopted (Singh *et al.* 1987a & b). It is also proposed to utilize diallel selective mating system to capitalize on additive genetic variance and enhance genetic recombinants, as pointed by Jensen, (1970) for the improvement of self pollinated crops. Therefore, for improvement of seed yield in blackgram, other breeding methods including biparental mating among selected F_2 segregants from crosses involving the parents 9020, Mash 1 and 9026 need special considerations. Single seed descendent (SSD) method for genetic improvement can also be used without loosing genes of economic importance. Malhotra *et al.* (1979) suggested that from further segregating generation of biparental populations, desirable plants can be selected and used as in other conventional breeding programme. Simultaneously, the hybrids involving the parents 9020, Mash 1 and 9026 may be exploited through modified diallel selective mating system (Frey, 1975). By this technique, improvement in the population can effectively be made and at the same time superior segregants are being expected for further improvement in blackgram. Since the end product of breeding programme is usually pure-lines, therefore, there is no scope for exploiting high non-additive genetic variation in a strongly self pollinated crop (Chauhan & Singh, 1997). GCA and SCA information shows the kind of progeny which must be evaluated for the relevant traits. If SCA effects are significant,

Table 5.5.1:- Summary of hybrids on the basis of mean performance, GCA and SCA in blackgram

Character	Best hybrid					Best for
	SCA		Mean		GCA	mean
	Direct Cross	Reciprocal	Direct Cross	Reciprocal		
Plant height	9020/9012	Mash 3/9020	9020/9012	9025/9020	Mash 1	Mash 3
Branches/plant	9025/Mash 3	Mash 1/9020	9020/Mash 1	9025/9020	Mash 3	Mash 3
Pods/plant	9020/9026	Mash 1/9020	9020/Mash 1	9025/9020	9020	Mash 1
Pods/branch	9025/Mash 1	Mash 3/9012	9020/9012 9020/9026	9026/Mash 1	9026	9012
Pod length	9025/9012	9026/Mash 1	9020/9012	9026/9020	9020	9020
Seeds/pod	9020/9026	9026/9025	9020/9026	9025/9020 9012/9020	9020	9026
Seed weight	9012/9026	9026/Mash 1	9020/9012 9020/Mash 1	9020/Mash 1	9020	9020
Biological yield	9012/Mash 3	Mash 1/9020	9020/Mash 1	9025/9020	9020	Mash 3
Grain yield	9020/9026	Mash 1/9020 Mash 3/9025	9012/Mash 3	9025/9020	9020	Mash 1
Harvest index	9025/Mash 1	Mash 3/9025	9025/Mash 1	9026/Mash 1	9026	9026

specific hybrid combinations must be evaluated. Alternatively, if GCA is significant and SCA not, then the performance of a single cross progeny can be adequately predicted on the basis of GCA. In the present experiment, GCA mean square was high for seed weight, and thus the performance of a particular single-cross for this character could be adequately predicted (Kunta *et al.* 1997).

CONCLUSION

CONCLUSION

⇒ In a preliminary study, blackgram germplasm consisting 484 accessions was evaluated for qualitative and quantitative characters alongwith MYMV reaction. For qualitative characters, a considerable variability was observed for plant pubescence, plant type and leaf shape, whereas seed coat colour and spots on the seed coat exhibited a low level of variation. Most of the accessions were brown seeded with black spots and only six accessions were green seeded, whereas seven accessions were without seed spots.

⇒ Correlation analysis for pooled germplasm and selected germplasm revealed that, out of 66 combinations, 55 correlation values were observed with same signs and in 38 cases the values were significantly similar for both the populations and thus the results could be implied in a wider perspectives. Only 6 values were observed significantly different in two populations.

⇒ Construction of selection score for harvest index on the basis of PCA and other yield contributing characters revealed that the most economic range of HI was 25-35%. On the basis of these results, high yielding accessions combined with other good agronomic characters were identified which are suggested for further testing under wide range of agro-ecological conditions to utilize for selection/breeding of high yielding blackgram cultivars.

⇒ Grouping of accessions by multivariate methods in the study proved its significance for practical use for the breeders working on blackgram. Representative accessions are suggested to be chosen from particular groups for hybrid programme with other approved varieties.

⇒ Three qualitative characters (hairiness, seed coat colour, spots on seed) were observed important for detection of various QTLs. Variation at qualitative loci in the vicinity of QTL in blackgram germplasm may be an indication of genetic variation potentially available to breeding programmes which can be used potentially for crop improvement.

⇒ In the present studies, intraspecific variation was limited and it was observed that SDS-PAGE alone did not exhibit high level of intraspecific variation, therefore,

diverse accessions based on SDS-PAGE are suggested to be acquired from various sources, preferably from centre of diversity to build a broad based gene-pool with maximum variability.

⇒ Twenty polymorphic bands protein peptides were analysed for comparison with 12 quantitative characters and out of 240 combinations, 24 exhibited significant differences for means. The factors or loci affecting variation in quantitative traits may occur as individual genes or gene clusters scattered throughout the genome, therefore, same quantitative traits may be expressed differently at several loci. Expansion of genetic base for blackgram breeding might be accomplished by systematic use of germplasm that differ from common banding pattern and known to be associated with variation in quantitative traits.

⇒ Multivariate analysis provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level. To establish high yielding pure-lines of blackgram, selected parents from diverse clusters are needed to be combined to have maximum useful transgressive segregants.

⇒ Cluster analysis based on morphological characters was observed more reliable than SDS-PAGE which indicated that quantitative characters might have more breeding values in blackgram, but simultaneous study for both agronomical and biochemical analyses (protein and DNA) is suggested.

⇒ The grouping on the basis of altitude and crop ecological zones could not prove its worth for the present blackgram germplasm used, whereas clustering based on provincial distribution, 16 accessions out of 42 (40%) grouped together. Further, cluster analysis showed that many accessions from same origins were grouped separately which may be because of frequent exchange of germplasm by the breeders or transport of grain to different markets from where the seed of various origins is disseminated through out the country.

⇒ Forty pure-lines of *Vigna* spp. selected for RAPD analysis were also evaluated for morphological characters and SDS-PAGE. On the basis of SDS-PAGE, out of 20 polymorphic bands, 14 prominent bands were used for analyses and presentation. SDS-PAGE was conducted for 40 genotypes, but 15 genotypes gave similar protein

pattern and thus excluded from cluster analysis, genotypes with similar banding pattern give no taxonomic information.

⇒ SDS-PAGE revealed specific bands for *Vigna radiata.*, hence these could be used for identification of these two species. Two bands (9c, & 14) were specific to genotypes *Vigna radiata* and hence these specific bands could be used for isolating *Vigna radiata* from mixed germplasm. SDS-PAGE proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo,* whereas within *Vigna mungo* a low level of genetic diversity was observed and no clear differentiation was recorded either for agronomic characteristics or origin as various clusters consisted mixed genotypes from different origins.

⇒ Out of 36 polymorphic primers, 11 were used for RAPD analysis of 40 genotypes. Each of the 11 primers varied greatly for the ability to resolve variability among 40 genotypes. Some of the primers generated several markers and were able to show high genetic diversity, while others produced few markers and detected little variability. Eleven primers generated 131 reproducible and score able amplification products across all the genotypes, out of which 107 (80.2 %) fragments were polymorphic in one or other of the 40 genotypes. The rest of the 24 (19.8%) bands were monomorphic for the present material.

⇒ Comparison of genetic diversity based on agronomical, SDS-PAGE and RAPD analyses revealed high relationship for genetic dissimilarities based on SDS-PAGE and RAPD, hence these could equally be used for studying inter-relationships and genetic diversity in *Vigna* spp.

⇒ Grouping of 40 genotypes on the basis of SDS-PAGE and RAPD revealed that *Vigna radiata* was quite different as observed in banding pattern and cluster analysis. In general, all 40 genotypes shared grouping for three parameters (morphological, SDS-PAGE & RAPD) and it was observed that 8 out of 40 genotypes, which were 20% of the total were grouped together for all three parameters.

⇒ Monogenic or oligogenic morphological traits are generally simple, rapid and inexpensive to score and manipulate in a biological system. In the present study, inheritance of qualitative characters revealed single gene dominance involved for

pubescence (*HH, Hh, hh*), seed colour (*CC, Cc, cc*), spots (*SS, Ss, ss*) and pod colour (*BB, Bb, bb*). Joint segregation of character pairs revealed normal distribution of independent assortment (9:3:3:1) for most of the character pairs but some distorted segregation for some crosses and character pair was also observed. All the character pairs segregated in a normal independent assortment except for *SS* vs *BB* in all the hybrids which was strongly linked in all cases.

⇒ Estimates of the form of genetic variation have quite fundamental influence of the identification of breeding strategies and methods for crop improvement. The presence of non-additive gene-action suggests that hybrids may provide a desirable alternative to pure lines or higher potential yields, assuming epistasis is not additive x additive in nature.

⇒ If SCA effects are significant, specific hybrids combinations must be evaluated. Alternatively, if GCA is significant and SCA not, then the performance of a single cross progeny can be adequately predicted on the basis of GCA. In the present experiment, GCA mean square was high for seed weight, and thus the performance of a particular single-cross for this character could be adequately predicted on the basis of GCA basis.

⇒ However, the conclusions are specific to the populations/material examined, and the environments under which the measurements were recorded, especially for quantitative characters. Further research is needed to establish the constancy of detected QTLs, based on morphological and biochemical (proteins and DNA) analyses and to locate their exact position in the blackgram genome for future use in crop development.

RECOMMENDATIONS

RECOMMENDATIONS

⇒ Qualitative characters are important for varietal description and screening for biotic and a-biotic stresses. Plants with lanceolate leaf shape are drought tolerant, and spreading type plants are preferred for planting under rainfed condition. The parents with unique characters may be utilized in blackgram breeding for a specific problem. As these characters are controlled monogenically, hence these could also serve as markers in hybridization.

⇒ For pod length, seeds per pod, seed density and 100-seed weight, low genetic variability seemed to restrict the scope of selection for these traits in the present germplasm collection. Hence, the genes for these important economic traits should be investigated or exploited from other sources, i.e., inter-specific hybridization, mutation, etc. Large scale testing of broad base germplasm needs to be built up by making extensive local collection and obtaining germplasm from abroad to develop a sound breeding programme.

⇒ Monogenic loci, being a good indicator for better agronomic characters are suggested to be used for screening and breeding blackgram germplasm.

⇒ As the best range for harvest index was observed from 25 to 35%, therefore this range should be kept as one of the selection criteria for future selection in blackgram improvement.

⇒ Several potentially important agronomic types have been identified and these are recommended to be exploited for genetic potential to transfer the desirable genes and this, alongwith biochemical analysis will facilitate in assembling a core collection of accessions from the large genetic resources collection.

⇒ Intraspecific variation to identify various accessions/genotypes is possible by the use of SDS-PAGE in blackgram but accessions with similar banding patterns may not be duplicates in the germplasm, hence these are suggested to be confirmed by the use of 2-D electrophoresis focusing and DNA analyses. For better management of genebank, a precise comprehensive knowledge of agricultural and biochemical data (protein and DNA) is essential to eliminate duplicates which will ultimately help in making core collection of blackgram germplasm.

⇒ The association of QTL with easily identifiable markers could permit rapid and precise identification and transfer of QTL into superior crop cultivars but amount of information provided by this marker based approach will depend on the type and number of markers, and their linkage relationship. Further, the actual number of markers might be fewer, hence there is a need to expand the level of markers based on RAPD and RFLP which will ultimately help in marker assisted breeding.

⇒ Genetic diversity for blackgram within country appears largely attributable to different provinces rather than altitude or crop-ecological zones. Further collecting missions to main blackgram growing areas with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields closer to motorable roads. Collection missions are proposed to be arranged within individual province to collect germplasm from the areas of genetic erosion alongwith the areas where existing genetic diversity has not been yet gathered.

⇒ Cluster pattern of blackgram indicated that genetic variation within *Vigna mungo* is very narrow, which may necessitate the use of novel techniques/procedures for the creation of use able genetic variability in *Vigna mungo*.

⇒ Based on unweighed-pair-group method, two main groups were distinguished in the present material. Numerical taxonomy based on various ecological, morphological, biochemical and physiological characteristics and RFLP analysis provided clear difference in subgenus *Ceratotropis*. In the present study, two species of subgenus *Ceratotropis* were included and among these, two groups were observed on the basis of SDS-PAGE and RAPD which differentiated genotypes. To have a better understanding for phylogenetic and evolutionary process of *Ceratotropis*, other species of this subgenus are suggested to be incorporated for future research.

⇒ For RAPD analysis, some of the primers revealed characteristic fragments for some genotypes which were not produced in others, hence genotype-specific amplification products are suggested to be used in preliminary mapping of blackgram. Further, evaluation of electrophoretic gels should be performed with an automatic computer system, which would circumvent doubts with the detection of very faint bands.

However, in the recent time two approaches have been proposed, which could reduce an influence of conditions in individual labs., i.e., Conversion of RAPD markers to Sequence Characterised Amplified Regions (SCARs) and then to design pairs of PCR primers specific to the individual RAPD polymorphism. SCARs are advantageous over RAPD markers as they detect only single locus, their amplification is less sensitive to reactions and they can potentially be converted into co-dominant markers. The second approach employs the RAPD fragments as RFLP probes. Probably, there may be some variety-specific RAPD/RFPLs in *Vigna radiata* and *Vigna mungo* useful for fingerprinting purposes.

⇒ Cluster analysis based on agriculturally important characters revealed that advance breeding lines included in research material were categorised mostly in specific clusters. This might be because of selection pressure for high yield potential and other related characters by which advance lines were developed. This revealed that only a portion of genetic diversity has been exploited for varietal development, hence genetically diverse parents are suggested to be used in breeding programme to broaden the genetic base. High polymorphism among individual genotypes revealed that the RAPD technique is an effective descriptor in studying inter-specific variation in the crop.

⇒ Identified linkage of *SS* vs *BB* might be used for initial mapping of blackgram genome. The arrangements proposed were based on linkage observed between genetically diverse cultivated blackgram for these loci in the present study. The usefulness of the mapped marker loci should be realized when loci affecting QTLs including diseases and other economically important genes are added to the linkage group. The use of closely linked markers should facilitate breeding by giving a unique identity by tagging the genes of economic importance and by providing a mean of selection in the absence of nurseries and screening procedures that can be costly and time consuming.

⇒ Close relationship between performance and combining ability effects may be due to the presence of both additive and non-additive genetic variation for yield and its components. Breeding by pedigree method would result in partial exploitation of additive and additive x additive types of gene-action. Under such situation any

suitable methods of recurrent selections should be adopted. It is also proposed to utilize selective diallel mating system to capitalize on additive genetic variance and enhance genetic recombinants. Therefore, for the improvement of seed yield in blackgram, breeding methods including biparental mating among selected F_2 segregants from crosses involving the parents 9020, Mash 1 and 9026 need special considerations. Single seed descendent (SSD) method for genetic improvement can also be used without loosing genes of economic importance.

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APPENDICES

Appendix I

Average performance of selected genotypes/accesstions of blackgram during 1995

Acc.	HR	YMV	DF	DM	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
9020	1	0	41	67	33.2	91.8	2.8	5.34	6.9	5.83	73.46	24.22	33.21
9026	1	0	39	66	31.0	98.7	3.3	5.28	7.2	4.33	50.58	21.20	41.88
45007	1	0	60	90	15.5	71.8	4.6	4.08	6.2	5.70	107.40	23.09	21.50
45036	1	1	62	98	27.0	87.0	3.2	5.32	6.2	4.27	123.54	23.88	19.33
45037	1	0	64	105	14.0	110.0	7.9	4.94	7.0	4.99	182.25	28.99	15.91
45047	1	1	63	100	16.0	55.0	3.4	4.42	6.0	4.92	80.71	21.66	26.84
45051	0	0	62	105	15.0	100.0	6.7	4.02	7.0	5.47	101.44	25.50	25.14
45055	0	0	66	108	20.0	83.3	4.2	4.42	6.6	4.88	109.35	25.71	23.51
45060	0	0	65	100	18.7	163.7	8.8	4.68	6.4	4.95	119.27	32.22	27.01
45063	1	0	61	98	25.0	80.0	3.2	4.46	6.8	5.59	113.87	29.10	25.55
45072	1	0	59	90	12.6	106.0	8.4	4.64	6.6	5.05	64.78	24.58	37.95
45074	0	0	59	89	12.0	50.0	4.2	4.48	6.8	4.50	82.41	20.46	24.82
45091	0	1	59	100	23.3	75.0	3.2	4.70	6.2	5.29	77.23	21.28	27.56
45094	0	1	60	98	37.0	102.0	2.8	4.30	7.2	4.41	67.80	24.71	36.45
45098	1	0	32	74	25.0	110.0	4.4	4.16	6.0	4.21	81.76	27.63	33.79
45124	1	0	50	90	17.0	73.0	4.3	4.00	6.0	5.18	122.15	29.02	23.76
45136	1	0	33	74	30.3	89.7	3.0	4.42	7.0	4.13	84.80	23.39	27.58
45138	1	0	50	92	25.0	90.0	3.6	4.70	7.4	5.40	87.48	30.46	34.82
45152	1	0	36	75	62.0	195.0	3.1	4.46	6.6	4.70	105.63	39.21	37.12
45157	1	1	34	75	19.0	70.0	3.7	4.56	7.2	5.18	68.44	21.04	30.75
45166	1	0	59	100	13.0	38.0	2.9	4.88	7.0	5.18	38.25	18.58	48.58
45167	1	0	56	95	16.5	40.0	2.4	4.70	5.8	5.08	55.80	20.19	36.18
45206	0	0	56	95	19.2	74.0	3.9	4.42	6.4	4.48	96.25	27.33	28.40
45207	1	0	49	88	16.0	93.0	5.8	4.41	6.4	5.40	57.20	24.20	42.31
45218	1	0	51	92	25.4	74.0	2.9	4.90	6.6	5.37	87.18	21.73	24.93
45221	0	0	59	99	25.0	95.0	3.8	4.84	6.6	5.71	114.40	30.49	26.65
45222	0	0	60	100	35.0	111.7	3.2	4.70	7.2	6.01	121.99	29.93	24.53
45225	0	0	47	88	52.5	155.0	3.0	5.02	5.8	4.87	127.57	26.53	20.80
45308	1	0	30	69	26.7	88.7	3.3	4.40	5.2	4.42	64.95	20.43	31.46
45311	1	0	32	74	23.0	126.0	5.5	3.86	5.6	4.12	194.35	19.01	9.78
45326	1	0	35	74	11.6	53.4	4.6	4.50	6.0	5.11	45.77	18.87	41.23
45333	1	0	55	95	14.0	58.3	4.2	4.26	5.0	5.75	71.31	22.72	31.86
45336	1	1	32	71	8.3	85.0	10.2	5.08	6.8	4.91	73.76	21.00	28.47
45342	1	1	37	72	22.0	85.0	3.9	4.46	6.4	4.53	65.94	21.57	32.71
45344	1	1	46	90	26.0	80.0	3.1	4.48	7.0	4.58	73.48	20.60	28.03
45347	0	0	35	70	14.0	63.2	4.5	4.82	6.6	4.33	74.31	26.26	35.34
45350	1	0	32	70	20.0	82.5	4.1	4.68	6.2	4.28	80.17	23.83	29.72
45352	1	0	48	90	15.3	61.7	4.0	3.94	5.6	4.47	70.28	21.92	31.19
45358	1	0	30	64	12.0	53.0	4.4	3.96	5.0	4.58	83.77	18.86	22.51
45616	1	0	37	72	12.0	71.0	5.9	4.68	6.2	4.64	95.82	22.95	23.95
45695	1	1	46	90	25.0	62.0	2.5	4.88	7.0	5.15	77.91	26.29	33.75
45712	1	0	49	89	16.3	81.3	5.0	4.86	7.8	4.41	84.79	21.47	25.33
45714	1	1	35	72	41.0	97.0	2.3	4.02	5.6	4.53	52.76	19.68	37.30
45717	1	1	32	70	19.3	81.3	4.2	4.22	5.8	4.66	107.03	28.91	27.01
45721	1	0	48	89	30.0	107.5	3.6	4.26	6.2	4.01	139.81	26.94	19.27
45724	1	1	35	74	15.0	30.0	2.0	4.68	5.4	3.80	42.85	20.46	47.75
45751	1	0	62	110	16.7	75.0	4.5	4.26	6.8	4.55	71.37	23.79	33.34
45775	0	0	37	75	37.5	162.5	4.3	4.82	6.6	4.40	135.17	40.25	29.77
Mash 1	0	0	41	75	30.5	113.0	3.8	4.62	6.5	5.55	75.33	30.97	41.39
Mash 3	1	1	34	65	37.5	108.0	3.1	4.98	6.5	4.64	59.64	23.75	40.61

Appendix II

Mean and standard deviation of pure-lines evaluated for geographic distribution during 1997

Sr. No.	Variety	DF	DM	BR	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
1.	9005	33.5±0.53	64.5±0.53	15.0±3.51	40.30±1.41	2.8±0.56	4.2±0.11	6.0±0.42	4.24±0.03	24.20±3.84	7.58±0.79	31.6±1.74
2.	9006	34.5±0.52	64.5±0.52	9.1±1.58	32.60±5.62	3.7±1.26	4.4±0.23	6.0±0.21	4.01±0.28	16.67±0.57	5.13±0.41	30.77±1.41
3.	9007	34.5±0.52	64.5±0.52	15.8±2.64	31.10±9.66	1.9±0.29	4.3±0.35	6.0±0.64	3.95±0.49	24.25±11.44	5.86±3.53	22.49±3.93
4.	9010	37.5±0.56	66.5±0.58	7.6±0.70	21.60±2.46	2.8±0.58	4.4±0.17	5.9±0.11	3.65±0.23	7.02±1.93	2.6±0.31	38.59±6.14
5.	9013	40.5±0.52	74.5±0.53	28.0±10.70	102.83±21.25	3.4±0.33	4.5±0.14	6.3±0.41	5.65±0.25	70.12±13.51	28.20±4.97	39.91±6.48
6.	9014	40.5±0.52	73.5±0.53	33.5±19.50	47.66±18.62	1.62±0.39	4.38±0.08	6.0±0.21	4.01±0.07	26.23±5.11	6.08±1.17	24.82±9.30
7.	9018	38.5±0.52	67.5±0.52	26.83±6.85	45.50±5.09	1.84±0.66	4.4±0.10	6.5±0.31	4.54±0.32	32.99±0.34	10.04±0.47	30.46±1.77
8.	9020	40.7±1.15	66.7±1.16	32.1±9.68	81.26±26.94	2.68±0.66	5.26±0.56	6.88±0.64	5.66±0.47	67.85±20.37	20.14±6.94	33.24±3.07
9.	9025	33.5±0.45	64.5±0.52	23.5±12.20	75.33±39.32	2.89±1.41	4.72±0.18	5.8±0.42	4.36±0.04	32.29±7.03	9.73±2.04	33.43±4.74
10.	9026	38.5±0.52	65.5±0.52	31.0±9.48	93.50±20.68	2.99±0.33	5.46±0.28	6.8±2.21	4.24±0.17	50.87±10.61	21.16±4.25	40.35±4.95
11.	9029	38.5±0.52	64.5±0.52	17.16±6.50	21.00±1.05	1.38±0.46	4.47±0.03	5.7±0.10	4.28±0.01	12.48±3.53	3.64±0.03	31.4±8.60
12.	9031	40.5±0.52	67.5±0.51	25.83±18.44	50.00±7.02	3.25±2.05	4.4±0.02	5.8±0.21	4.89±0.73	35.01±8.23	10.23±2.23	29.34±0.50
13.	9035	41.5±0.52	67.5±0.52	15.16±3.68	29.66±10.89	1.9±0.25	4.39±0.11	5.9±0.10	4.63±0.07	20.11±8.37	4.63±2.14	22.56±1.26
14.	9039	35.5±0.52	63.5±0.52	17.83±0.87	30.16±7.55	1.71±0.50	4.61±0.11	6.3±0.10	4.26±0.06	26.67±3.45	7.51±1.35	29.00±1.43
15.	9047	42.5±0.52	69.5±0.52	14.5±0.63	34.33±4.21	2.39±0.14	4.32±0.21	5.9±0.31	4.81±0.10	19.76±0.54	6.3.00±0.54	31.82±1.88
16.	9051	41.5±0.52	66.5±0.52	36.6±3.51	52.00±12.64	1.4±0.21	4.36±0.04	6.1±0.31	3.78±0.07	35.89±2.90	7.90±1.89	21.74±3.52
17.	9053	40.5±0.52	71.5±0.52	18.16±3.51	45.83±12.64	2.51±0.21	4.43±0.04	6.5±0.31	4.95±0.07	53.77±2.90	14.87±1.89	27.53±3.52
18.	9056	41.5±0.52	66±14.57	12.5±7.90	24.33±14.75	1.99±0.07	4.54±0.08	6.0±0.21	4.17±0.15	14.89±2.99	3.41±1.93	21.37±8.67
19.	9059	38.5±0.52	66±1.05	18.0±3.86	47.50±7.90	2.66±0.13	4.59±0.03	6.3±0.10	4.23±0.04	29.83±5.65	7.27±1.32	24.4±0.16
20.	9065	41.5±0.52	66.5±0.52	19.16±2.63	42.00±8.43	2.28±0.75	4.32±0.10	6.3±0.31	4.02±0.03	23.74±2.85	7.03±2.03	29.06±5.06
21.	9086	42±1.05	68.5±0.52	34.0±16.86	49.83±8.95	1.73±0.59	4.73±0.01	6.1±0.31	4.05±0.07	30.99±5.82	10.40±1.99	35.55±0.13
22.	9093	33.5±0.52	63.5±0.52	35.0±8.78	41.66±8.78	1.20±0.05	4.37±0.05	6.0±0.21	4.27±0.13	27.33±5.35	6.69±1.40	24.44±0.34
23.	92018	33.5±0.53	62.5±0.53	18.33±0.00	30.00±1.76	1.63±0.09	4.07±0.03	5.9±0.11	3.98±0.04	17.08±1.05	4.34±1.04	25.17±4.52
24.	92019	35.5±0.53	60.5±0.52	11.83±2.99	32.33±4.92	2.79±0.29	4.15±0.14	5.9±0.11	3.77±0.30	16.39±0.73	4.99±0.51	30.37±1.75
25.	92024	38.5±0.52	64.5±0.52	13.35±1.05	29.66±4.92	2.21±0.19	4.20±0.13	6.1±0.11	4.08±0.11	17.4±2.58	4.79±1.05	27.27±2.02
26.	92030	39.5±0.52	67.5±0.52	9.33±1.05	27.33±3.51	3.00±0.72	4.05±0.14	5.3±0.11	3.75±0.26	12.45±0.35	3.23±0.51	26.09±4.83
27.	92052	31.5±0.52	61.5±0.52	14.66±2.45	32.66±5.97	2.22±0.03	4.17±0.07	5.9±0.10	3.82±0.03	22.67±4.44	5.46±0.52	25.35±7.26
28.	92092	35.0±0.05	64.5±1.05	18.5±0.52	33.66±10.36	2.41±3.16	4.52±1.18	6.0±0.23	4.22±0.09	17.41±1.60	5.47±0.32	31.55±1.02
29.	93004	38.5±0.52	68.5±0.52	10.33±1.40	21.16±7.20	1.99±0.42	4.2±0.27	5.7±0.31	4.24±0.36	14.53±4.04	2.78±0.69	19.33±0.61
30.	93017	40.0±1.05	65.0±0.00	16.66±1.75	38.00±5.62	2.27±0.09	4.4±0.06	6.2±0.21	4.30±0.24	30.46±4.62	8.57±1.28	28.15±0.04

Mean and standard deviation of donated germplasm from NRS evaluated for geographic distribution during 1997

Sr. No.	Variety	DF	DM	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
1.	45028	65.1±2.02	97.2±2.15	10.4±3.85	50.5±14.15	5.01±1.25	4.7±0.85	7.01±0.56	4.80±0.14	72.99±14.51	15.02±7.53	20.57±6.98
2.	45126	39.8±0.79	78.8±0.79	16.6±8.29	53.0±26.14	3.27±1.12	4.5±0.21	6.36±0.36	4.97±0.27	29.85±12.50	11.74±6.10	37.94±7.29
3.	45131	40.0±0.67	78.6±1.07	28.0±4.27	100.2±11.42	3.60±0.20	4.4±0.09	6.36±0.25	4.52±0.36	58.23±8.71	22.90±3.06	39.43±2.02
4.	45134	49.4±1.35	96.4±0.52	13.1±6.92	25.8±12.54	2.05±0.82	4.8±0.27	6.70±0.30	4.31±0.36	25.65±13.35	4.85±2.53	20.33±8.41
5.	45138	47.0±0.82	95.5±0.53	21.2±3.01	85.2±28.08	3.25±0.38	4.6±0.14	6.98±0.44	5.24±0.34	86.86±12.24	22.04±9.91	38.66±6.10
6.	45139	45.7±5.58	89.510.02	13.8±7.35	33.9±29.50	2.47±1.79	4.3±0.29	6.28±0.75	4.72±0.16	30.96±21.44	7.12±7.75	18.35±11.47
7.	45141	66.1±1.45	108.2±1.14	9.7±3.74	16.4±7.49	1.87±1.05	4.6±0.30	5.64±0.82	4.47±0.41	23.32±4.35	3.10±1.69	12.86±5.85
8.	45145	45.6±1.07	79.8±1.23	23.2±11.38	58.2±31.93	2.44±0.25	4.3±0.23	6.68±0.51	3.90±0.31	39.19±25.06	13.04±8.07	33.72±2.48
9.	45154	44.1±1.51	88.2±1.56	18.8±6.54	79.5±26.85	4.24±1.99	4.7±0.85	5.60±0.66	5.95±1.21	82.36±30.12	17.04±9.15	20.69±3.99
10.	45156	45.5±0.53	80.0±1.65	16.0±1.05	21.0±3.16	1.33±0.29	4.3±0.11	5.80±0.21	4.16±0.04	19.23±4.34	5.73±0.28	31.60±8.89
11.	45158	45.4±0.97	81.6±0.84	14.2±2.82	35.8±3.54	2.50±0.77	4.4±0.25	6.42±0.60	4.48±0.38	23.58±12.28	7.81±4.54	33.06±4.96
12.	45159	44.7±0.67	79.3±0.67	10.6±3.20	24.0±6.58	2.32±0.49	4.0±.023	5.84±0.80	4.03±0.27	10.91±1.81	3.89±1.21	35.32±8.16
13.	45162	38.3±0.82	80.2±0.63	11.1±6.74	24.9±22.32	2.07±1.41	4.4±0.40	5.56±0.72	4.41±0.46	18.07±14.83	4.42±3.85	25.83±9.45
14.	45163	34.3±0.82	74.9±0.74	12.1±4.77	29.6±8.96	2.57±0.60	4.1±0.28	6.48±0.85	4.25±0.22	18.34±6.87	5.24±1.51	30.09±5.04
15.	45164	40.7±0.95	79.6±0.84	13.0±5.21	35.3±12.83	2.86±0.72	4.0±0.20	5.90±0.41	4.11±0.28	22.15±7.65	6.22±2.42	27.85±4.81
16.	45419	34.6±0.70	69.9±0.74	20.7±7.45	49.0±18.70	2.43±0.90	4.3±0.09	5.88±0.17	4.96±0.07	26.90±10.77	8.37±2.57	34.86±12.15

Mean and standard deviation of accessions evaluated for geographic distribution during 1997

Sr.No.	Accession	DF	DM	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
1.	45412	38.5±0.53	69.0±1.05	12.8±2.64	27.0±5.27	2.11±0.02	4.20±0.15	5.9±0.53	3.22±0.03	16.48±2.00	1.66±0.40	9.71±1.20
2.	45413	40.5±0.53	70.5±0.53	16.0±2.81	36.4±4.92	2.29±0.09	4.47±0.03	6.0±0.21	4.21±0.12	32.63±0.27	7.47±1.96	22.92±6.18
3.	45415	41.2±0.42	72.9±2.13	16.9±5.84	36.8±18.11	2.11±0.59	4.46±0.11	5.9±0.30	3.98±0.68	17.04±7.02	3.90±1.84	24.07±11.39
4.	45417	40.7±0.48	70.4±0.52	24.4±3.01	40.9±3.94	1.70±0.25	4.26±0.13	5.9±0.36	4.41±0.30	28.34±4.79	4.96±1.63	17.17±3.60
5.	45435	63.2±0.79	107.4±0.52	24.8±4.63	56.0±12.84	2.27±0.36	5.04±0.15	6.9±0.21	4.68±0.30	53.00±5.86	10.29±2.64	19.19±3.62
6.	45518	35.2±0.63	71.3±0.67	11.0±3.07	14.4±4.20	1.39±0.50	4.25±0.17	5.5±0.49	4.34±0.29	11.76±2.71	1.45±0.32	12.71±3.28
7.	45539	40.4±0.52	77.4±0.52	9.3±1.95	12.4±3.34	1.44±0.64	4.20±0.15	5.9±0.10	3.32±0.26	10.35±3.10	1.87±0.68	18.77±5.34
8.	45613	40.1±0.57	69.9±0.88	9.3±1.10	13.5±2.71	1.43±0.20	4.06±0.16	5.4±0.50	3.22±0.34	6.55±1.94	1.21±0.38	18.56±2.74
9.	45617	40.4±0.84	69.4±1.07	5.0±1.98	10.6±4.59	2.11±0.64	3.96±0.13	5.6±0.32	3.04±0.20	6.99±3.06	1.22±0.53	17.91±4.56
10.	45620	40.3±0.48	67.4±0.52	15.5±3.43	25.1±2.41	1.67±0.25	4.39±0.12	6.4±0.16	4.06±0.27	17.89±3.84	4.11±0.85	23.34±3.54
11.	45696	53.9±0.88	97.8±0.42	23.2±5.11	40.3±6.03	1.79±0.30	4.31±0.07	6.0±0.33	5.21±0.35	59.73±11.19	9.02±1.57	15.90±4.93
12.	45697	55.3±0.48	98.1±0.88	28.8±2.33	51.6±20.40	1.83±0.84	4.41±0.07	6.4±0.44	4.53±0.20	61.81±18.36	5.13±0.50	8.78±1.88
13.	45710	50.7±0.48	86.8±1.03	22.1±6.12	29.7±15.45	1.32±0.63	4.80±0.30	6.4±0.68	4.90±0.18	34.49±11.48	4.35±3.27	10.86±6.29
14.	45712	37.8±0.92	87.1±0.74	18.7±8.20	81.1±1972	4.96±1.58	4.81±0.07	7.4±0.22	4.05±0.36	84.74±21.06	21.40±14.43	27.61±6.87
15.	45713	40.2±0.42	67.1±0.74	14.6±2.45	30.6±5.07	2.11±0.19	4.29±0.18	5.7±0.25	3.98±0.32	16.08±4.68	3.58±0.56	23.7±7.06
16.	45714	36.0±0.53	70.5±0.53	41.0±12.81	97.2±21.93	2.67±0.86	4.05±0.03	5.3±0.11	4.46±0.39	52.35±21.03	18.93±6.30	36.92±3.43
17.	45720	40.0±1.05	69.0±1.05	9.3±2.46	10.0±5.97	1.30±0.98	4.00±0.23	5.1±0.53	2.76±0.05	13.52±3.38	0.72±0.27	6.14±3.51
18.	45723	38.5±0.53	67.5±0.53	12.5±0.53	23.7±1.73	1.89±0.06	4.26±0.19	6.1±0.11	3.90±0.05	13.09±1.31	2.15±0.33	16.80±4.20
19.	45724	37.5±0.53	74.7±0.67	15.2±9.74	30.0±12.33	2.11±0.97	4.62±0.83	5.5±0.29	3.74±0.45	42.57±12.60	20.40±10.88	39.01±6.08
20.	45725	41.6±0.52	70.9±0.74	8.6±2.22	14.5±3.04	1.77±0.54	4.20±0.14	6.2±0.33	2.95±0.27	7.50±1.36	1.45±0.34	19.64±4.58
21.	45726	39.6±0.70	69.7±0.67	9.5±7.22	16.4±19.01	1.45±0.82	3.92±0.30	5.4±0.84	3.13±0.55	9.73±13.78	2.38±3.28	25.57±7.09
22.	45728	37.4±1.51	71.3±0.67	19.3±8.18	37.0±8.11	2.09±0.60	4.52±0.12	6.0±0.30	4.66±0.64	26.17±6.18	6.41±1.63	24.80±5.33
23.	45730	36.5±0.71	69.2±0.63	11.5±3.32	21.5±7.21	1.87±0.35	4.27±0.22	5.9±0.55	4.13±0.44	15.61±5.67	2.43±0.94	15.93±4.50
24.	45739	39.7±0.95	69.5±0.53	12.7±2.71	36.7±11.88	2.82±0.38	4.54±0.10	6.0±0.32	4.42±0.26	25.45±6.50	6.42±2.12	25.04±4.72
25.	45742	38±0.67	70.4±0.52	11.6±2.44	23.6±8.77	2.17±0.92	4.26±0.05	5.8±0.23	3.67±0.35	12.51±4.17	2.29±0.56	19.20±4.11
26.	45743	41.9±0.88	79.9±0.57	8.3±3.77	13.7±7.05	1.80±0.83	4.43±0.08	5.7±0.21	3.56±0.29	6.63±2.30	1.66±0.59	26.06±7.09
27.	45486	47.9±0.88	94.6±0.52	15.9±5.15	57.3±18.76	3.66±0.75	4.55±0.19	6.5±0.33	4.15±0.18	32.61±10.98	12.29±4.60	37.47±4.28
28.	45488	47.3±1.16	94.7±0.67	10.5±3.34	26.2±12.59	2.58±1.09	4.76±0.28	6.8±0.50	4.62±0.35	24.79±12.79	6.96±3.39	29.33±6.22
29.	45508	51.2±1.62	96.2±0.92	16.6±8.62	51.6±29.38	3.21±1.45	4.58±0.25	6.2±0.59	4.82±0.43	35.03±20.40	11.98±7.44	34.06±9.88
30.	45509	65.3±0.67	102.2±1.87	16.7±5.66	29.8±14.89	1.79±0.64	4.35±0.19	6.1±0.59	5.03±0.16	27.57±13.29	6.26±3.87	23.19±6.80

-cont.-

Sr.No.	Accession	DF	DM	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
31.	45510	63.2±0.79	106.8±0.79	18.6±6.08	51.4±22.05	2.72±0.52	4.68±0.19	6.3±0.40	5.56±0.32	43.11±16.99	12.61±6.31	28.17±4.36
32.	45511	68.4±0.52	110.4±0.52	16.8±4.64	37.4±9.23	2.25±0.23	4.85±0.40	6.3±1.04	5.64±0.41	84.50±9.82	10.32±3.76	21.91±7.66
33.	45512	65.0±0.67	110.0±0.67	24.0±9.09	45±14.33	1.93±0.30	4.77±0.14	6.7±0.29	4.55±0.27	37.44±9.03	10.75±3.34	28.38±1.97
34.	45513	46.0±0.82	95.4±0.70	12.8±3.68	27.5±10.86	2.15±0.54	4.73±0.22	6.7±0.53	4.72±0.43	25.27±9.61	5.99±2.79	23.59±7.93
35.	45514	49.2±0.63	97.2±0.79	16.9±4.51	46.5±13.60	2.80±0.58	4.88±0.24	6.5±0.50	4.42±0.30	33.73±10.37	9.02±2.15	27.67±4.19
36.	45515	50.20±0.63	97.6±0.70	20.8±5.20	51.4±23.15	2.56±1.25	4.77±0.16	6.0.5±0.64	4.31±0.21	34.82±11.23	10.08±3.81	29.09±7.43
37.	45516	50.8±0.79	94.4±1.26	14.9±5.57	41±22.12	2.69±0.94	4.62±0.21	6.3±0.44	4.68±0.57	34.79±17.24	8.19±5.07	25.32±10.13
38.	45684	48.0±0.82	95.3±0.82	12.6±5.68	27.4±14.21	2.27±1.05	4.92±0.36	6.2±0.53	5.11±0.50	28.35±14.75	5.59±3.74	20.87±7.41
39.	45796	36.7±0.95	80.3±0.67	14.0±6.77	24.±19.94	1.77±0.23	4.44±0.17	6.0±0.36	4.20±0.33	17.91±7.38	4.88±1.87	28.20±8.01
40.	41016	55.5±0.52	110.5±0.52	17.8±4.39	23.5±5.44	1.46±0.66	5.48±0.16	6.5±0.10	5.85±0.01	26.94±2.65	6.02±0.48	22.40±0.40
41.	41095	57.5±0.53	110.5±0.53	35.7±2.46	60.2±12.47	1.67±0.23	4.45±0.20	6.5±0.11	4.29±0.28	48.01±12.22	13.13±3.71	27.15±0.82
42.	41096	57.5±0.53	110.5±0.53	35.66±2.46	60.16±12.47	1.67±0.23	4.45±0.20	8.5±0.11	4.28±0.28	48.01±12.22	13.12±3.71	27.15±0.82

Appendix III

LEGUMES DNA MINIPREP

Leaf Tissues Fresh (in ice box) or frozen at -80° C

Step 1-Weigh 1.0 g of plant leaves, preferably young leaves in autoclaved mortar and pastel.

Step 2-Add Liquid Nitrogen (LN); Grind gently, put some more LN and make fine powder.

Step 3-Take 15 ml Extraction Buffer and 1 ml of 20% SDS solution for one sample and mix in a separate tube.

Extraction Buffer	500 ml Extraction Buffer	
100 mM Tris-HCL pH 8	1 M Tris-HCL pH 8	50.0ml
50 mM EDTA pH 8	500 mM EDTA pH 8	50.0ml
500 mM NaCl	NaCl	14.61g
10 mM mercaptoethanol	12 µl per 15 ml EB	

Add mercaptoethanol at the time of use in only solution to be used for DNA extraction.

Add 16 ml of EB + 20% SDS in two steps; first add 8 ml of solution in grounded sample when it melts to avoid freezing.

Step 4-Transfer this mixture in a new 50 ml tube, the remaining 8 ml solution may be used for washing mortar and pastel, and transfer this also in the same tube.

Step 5-Mix thoroughly and incubate at 65° C for 10 minutes.

Step 6-Add 5 ml of 5 M potassium acetate, shake vigorously and incubate at 0° C (in ice) for 20 minutes.

5 M potassium acetate	
5 M potassium acetate =	120 ml
Glacial acetic acid =	23 ml
d H_2O =	57 ml
Total =	200 ml

Step 7-Spin tubes at 20,000* g for 20 minutes. [20,000* g can be attained by centrifuge at 12,000 RPM.

Step 8-Pour supernatant through a miracloth filter (Calbiochem) into a new clean 50 ml tube.

Step 9-Add 10 ml of Phenol/Chloroform/isoamylealcohal (25:24:1). Mix gently by several inverting. Spin at 12,000* g (10,000 RPM) for 20 minutes. Transfer 15 ml of supernatant in new 50 ml clean tube.

Step 10-Add 10 ml isopropanol, mix gently by several inverting and incubate at -20° C for 30 minutes.

Step 11-Spin at 12,000 RPM for 15 minutes. Pour off supernatant carefully. Add 70% Et OH and after 5-10 minutes wash DNA pellet by throwing off 70% Et OH carefully. Lightly dry DNA pellet by inverting the tube for 10-15 minutes.

Step 12-Add 1 ml of TE buffer. Transfer the dissolved DNA into 1.5 ml appendorf tube and spin at 15,000 RPM for 10 minutes and store in freezer (-20.0° C) till further use.

Purification of DNA

Step 1- Take 100 µl of original DNA in 1.5 ml appendorf tube.

Step 2- Add 1 µl of RNase; incubate at 30° C for 30 minutes.

Step 3- Add 100 µl of Phenol/Chloroform/Isoamylealcohal, mix gently, centrifuge at 15,000 rpm for 5 minutes.

Step 4- Take upper fraction (supernatant) to a new tube. Add 200 µl of 99.5% Ethanol (EtOH) and mix gently by several inverting. Incubate at -20° C for 15 minutes.

Step 5- Centrifuge at 15,000 rpm for 15 minutes. Remove ethanol carefully.

Step 6- Add 300 µl of 70% ethanol for washing DNA pallet. Remove ethanol.

Step 7- Repeat step 6.

Step 8- Dry the DNA pallet at room temperature (RT) for 10-15 minutes and redissolve the DNA pallet in TE. Measure the DNA concentration (99µl of dH_2O + 1 µl of DNA) by spectrophotometer.

Reagent	Quantity	Step
Template DNA (20-50 ng)	5.0 µl	7
20 mM dNTP	0.2 µl	1
10X Buffer	2.0 µl	2
25 mM $MgCl_2$	1.6 µl	3
Primer	1.0 µl	6
Taq DNA Polymerase (1 unit)	0.2 µl	5
dH_2O	10.0 µl	4

Multiple samples can be mixed as premix.

Take a 1.5 ml tube, add all above listed reagents except template DNA stepwise as listed. Put 15 µl of this premix in the 0.5 ml tubes to be used for PCR machine. Then add 5 µl of DNA template in these tubes and cover the mixture with mineral oil. Before putting the tubes in the PCR machine, check the following programme;

94° C	1 minute	
94° C	1 minute	
30° C	1 minute	45 cycles
72° C	2 minutes	
72° C	5 minutes	
04° C	storage	

Take the samples from the machine when PCR action finished. Add 5 µl of dye for DNA electrophoresis into new tubes. Take the PCR product from the tubes carefully, not to carry oil in the new tubes, mix well and can be stored at 4° C till electrophoresis.

PAGE for DNA analysis

The PAGE (Polyacrylamide Gel Electrophoresis) for DNA is slightly different than that of protein as mentioned under;

dH_2O	33.06 ml
Acrylamide solution	9.60 ml (39:1) (Acrylamide:Bis-acrylamide)
10X TBE	4.80 ml
Ammonium Persulphate (APS, 10%)	0.48 ml or 480 µl
TEMED	0.06 ml or 60 µl

The APS should be used as fresh preparation, take 0.1 g of APS and add 1 ml of dH_2O, mix well to prepare 10% APS solution.

10X TBE

Tris	108.00 g
Boric acid	55.00 g
EDTA-2Na	7.44 g

Fill up to 1 liter with dH_2O

Run the gel at 150 Voltage constant for 30 minutes pre loading. Load the samples @ 8 µl and run the electrophoresis for one hour in 1X TBE buffer then take it out and stain in TE containing Ethidium Bromide (200 ml TE + 10 µl Ethidium Bromide) for 30-60 minutes and take a photograph with UV light illuminator.

Take the PCR products after the reaction and transfer into the new tubes in which 5 µl of dye is already added and from this DNA solution 8 µl is applied in each well carefully. The wells should be washed for good separation.

Appendix IV

Means, standard deviation, analysis of variance and genetic parameters in 6 parent diallel of blackgram, 1997

	Hybrid	PH	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
1.	9020/9020	50.5±3.88	33.3±10.84	91.8±29.96	2.78±0.57	5.30±0.52	6.9±0.53	5.83±0.51	73.46±22.01	24.22±7.00	33.10±3.06
2.	9020/9025	30.3±11.90	10.0±10.42	32.5±31.36	3.35±1.49	4.73±0.30	6.8±0.39	4.92±0.39	20.86±25.12	8.04±8.88	39.85±5.35
3.	9020/9012	58.3±4.50	45.3±30.65	140.0±41.82	4.72±2.96	5.11±0.09	6.9±0.19	5.11±0.22	86.6±20.08	29.17±5.83	33.92±2.02
4.	9020/Mash 1	57.3±1.25	48.7±6.24	152.0±22.91	3.14±0.43	5.00±0.09	6.9±0.09	5.11±0.30	110.75±15.46	37.71±4.81	34.13±1.58
5.	9020/Mash 3	56.4±4.54	44.5±18.92	142.0±61.98	3.13±0.36	4.63±0.16	6.4±0.40	4.39±0.34	77.12±44.40	26.78±12.77	38.35±7.61
6.	9020/9026	56.0±9.87	29.3±8.3	136.0±39.07	4.72±1.14	4.94±0.14	7.3±0.49	4.72±0.44	70.92±21.58	28.24±8.07	40.74±5.79
7.	9025/9020	56.3±7.74	47.3±18.38	160.0±49.61	3.40±1.72	4.93±0.17	7.0±0.28	4.72±0.29	100.13±38.82	36.63±13.72	37.19±3.83
8.	9025/9025	47.0±6.57	25.8±17.74	78.0±41.60	3.30±1.44	4.80±0.18	6.3±0.54	4.25±0.26	48.60±20.36	14.76±9.34	31.27±5.09
9.	9025/9012	47.0±2.76	23.3±6.73	55.0±14.50	2.47±0.48	4.49±0.13	5.8±0.53	4.31±0.28	32.24±8.05	10.98±2.44	35.33±5.81
10.	9025/Mash 1	46.0±4.03	31.5±10.20	130.0±37.27	4.18±1.25	4.47±0.12	6.4±0.28	4.20±0.27	58.74±17.72	23.71±7.32	41.37±7.14
11.	9025/Mash 3	36.7±1.25	37.7±1.70	134.3±7.36	3.60±0.21	4.23±0.02	5.6±0.16	3.87±0.05	70.58±1.84	25.59±1.37	36.23±1.00
12.	9025/9026	37.3±3.98	21.75±6.07	84.8±30.13	3.90±1.16	4.53±0.15	6.3±0.22	4.08±0.13	38.92±16.35	14.16±5.99	36.78±3.59
13.	9012/9020	54.0±5.26	37.5±13.58	97.3±35.49	2.63±0.45	4.86±0.12	7.0±0.27	4.74±0.32	63.42±26.15	23.10±8.92	36.55±4.04
14.	9012/9025	43.5±5.01	34.3±10.90	93.3±26.77	2.80±0.56	4.89±0.32	6.8±0.60	4.76±0.31	57.62±18.02	22.70±6.45	39.78±3.74
15.	9012/9012	41.7±2.37	27.3±15.40	99.3±36.38	4.15±1.16	4.50±0.23	5.7±0.52	4.23±0.32	46.21±14.45	16.87±5.33	36.56±3.56
16.	9012/Mash 1	56.0±2.64	47.8±12.25	105.7±28.44	2.33±0.67	4.68±0.07	6.5±0.59	4.48±0.19	78.4±19.83	22.57±5.39	29.25±3.27
17.	9012/Mash 3	51.8±5.10	34.0±12.09	135.3±44.88	4.9±1.87	4.72±0.17	6.4±0.50	4.73±0.47	86.25±34.88	30.92±10.95	39.10±6.20
18.	9012/9026	33.5±2.96	34.5±10.61	107.8±33.92	3.28±0.81	4.56±0.20	6.1±0.19	4.71±0.19	73.64±25.51	27.07±8.79	37.06±4.07
19.	Mash 1/9020	53.0±5.26	27.8±13.58	74.3±35.49	3.08±0.45	4.92±0.12	6.7±0.27	6.01±0.32	72.58±26.15	21.76±8.93	29.65±4.04
20.	Mash 1/9025	51.5±5.01	27.8±10.90	103.8±26.77	3.67±0.56	4.47±0.32	6.4±0.60	4.76±0.31	60.95±18.02	25.09±6.45	42.75±3.74
21.	Mash 1/9012	54.8±2.37	31.3±15.40	89.3±36.28	2.85±1.16	4.53±0.23	6.2±0.52	5.43±0.31	53.75±14.45	22.06±5.33	41.77±3.56
22.	Mash 1/Mash 1	53.5±2.64	30.5±12.25	113.0±28.44	3.78±0.44	4.62±0.07	6. 5±0.60	5.55±0.19	75.33±19.83	30.97±5.39	41.39±3.27
23.	Mash 1/Mash 3	57.8±5.10	41.8±12.09	91.5±44.88	2.25±1.87	4.61±0.17	6.1±0.50	4.40±0.47	47.81±34.88	17.70±10.95	38.16±6.20
24.	Mash 1/9026	45.8±2.96	27.8±10.61	92.3±33.92	3.42±0.81	4.67±0.20	6.2±0.19	4.80±0.19	48.90±25.51	22.15±8.79	45.19±4.08
25.	Mash 3/9020	43.5±1.50	37.5±0.50	110.5±11.50	2.97±0.35	4.92±0.08	6.9±0.10	5.26±0.09	72.65±10.90	26.82±6.80	36.33±3.91
26.	Mash 3/9025	38.7±1.25	40.0±1.63	105.7±7.36	2.64±0.15	4.36±0.05	5.6±0.16	3.93±0.06	71.78±3.58	22.79±1.10	31.76±0.43
27.	Mash 3/9012	52.3±11.90	45.5±13.80	115.0±27.52	2.67±0.52	4.66±0.18	6.0±0.47	4.65±0.42	73.14±29.88	25.15±8.25	35.50±17.25
28.	Mash 3/Mash 1	48.8±6.53	40.5±16.38	138.5±60.93	3.39±0.41	4.59±0.09	6.3±0.50	4.27±0.27	68.48±31.97	26.11±11.06	38.89±2.86
29.	Mash 3/Mash 3	56.8±6.59	37.5±13.91	108.0±25.51	3.05±1.09	4.98±0.21	6.5±0.44	4.64±0.35	95.64±18.09	23.75±5.73	40.61±6.61
30.	Mash 3/9026	39.0±4.12	25.5±10.99	88.0±30.06	3.63±0.52	4.59±0.08	5.7±0.17	4.66±0.13	52.41±17.64	19.57±7.56	36.93±7.46

-cont.-

	Hybrid	PH	BR/PL	P/PL	P/BR	PL	S/P	SW	BY	GY	HI
31.	9026/9020	44.0±2.00	37.0±2.00	130.0±5.00	3.52±0.05	5.31±0.01	7.1±0.10	5.11±0.03	83.09±2.05	32.52±1.40	39.11±0.72
32.	9026/9025	37.8±5.15	25.5±9.94	93.0±32.78	3.75±0.79	4.56±0.14	5.9±0.21	4.02±0.21	41.12±15.55	15.30±5.77	38.04±4.57
33.	9026/9012	48.0±6.07	18.5±9.69	83.8±21.81	5.30±2.06	4.54±0.08	6.4±0.22	4.31±0.28	41.17±11.96	15.78±4.64	38.88±5.30
34.	9026/Mash 1	38.5±7.00	13.3±8.30	53.0±26.88	4.15±1.43	4.36±0.17	5.9±0.44	4.19±0.21	23.80±17.57	10.27±5.90	43.76±14.54
35.	9026/Mash 3	33.5±1.50	16.5±0.50	61.5±5.50	3.72±0.22	4.45±0.03	6.0±0.00	4.14±0.06	36.54±7.97	11.86±1.72	33.00±2.49
36.	9026/9026	46.5±3.74	31.0±7.73	98.7±22.47	3.25±0.54	5.28±0.21	7.2±0.35	4.33±0.25	50.58±10.72	21.20±4.07	41.88±2.52
MS (genotypes)		277.02	375.97	3371.70	2.12	0.29	0.88	0.004	1.05	1631.25	199.82
MS (replicates)		3.03	166.97	519.18	.522	0.03	0.06	0.002	0.09	951.53	57.68
MS (error)		24.80	119.01	752.39	1.159	0.03	0.13	.001	0.07	381.48	36.31
F.R (genotypes)		11.16 **	3.15 **	4.481 **	1.83 **	8.878 **	6.847 **	2.77 **	14.45 **	4.27 **	5.50 **
F.R (replicates)		.123 ns	1.40 ns	0.69 ns	0.451 ns	0.56 ns	0.448 ns	1.15 ns	1.20 ns	2.49 ns	1.59 ns
SE		2.49	5.45	13.71	0.53	0.09	0.179	0.18	0.13	9.76	3.01
CD1		6.97	15.27	38.40	1.507	0.26	0.502	0.051	0.38	27.35	8.43
CD2		9.22	20.18	50.75	1.99	0.34	0.663	0.068	0.49	36.14	11.15
G σ^2		63.05	64.24	654.82	0.24	0.06	0.18	0.001	0.25	312.44	40.87
P σ^2		87.85	183.25	1407.21	1.40	0.09	0.31	0.002	0.32	693.92	77.19
Heritability (BS)		0.71	0.35	0.46	0.17	0.66	0.59	0.307	0.77	0.45	0.53