ASSESSMENT OF GENETIC DIVERSITY IN WORLD CHICKPEA GERMPLASM BASED ON MORPHOLOGICAL AND BIOCHEMICAL GENE MARKERS.

By

Falak Noor Gulbaz

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Dedicated To My Loving Mother (Late)

CERTIFICATE

This thesis by Falak Noor Gulbaz is accepted in this present form by the Department of Biological Sciences, Quaid-I-Azam University, Islamabad, as satisfying the thesis requirements for the Degree of Doctor of Philosophy in Biology (Genetics).

Supervisor (Dr. M. Ashraf) .....

External Examiner Setsuko Komatsu .....

External Examiner .....

Chairman .....

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(FALAK NOOR GULBAZ)

GLOSSARY OF TERMS AND ABBREVIATIONS

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
Gel	an inert porous matrix used for electrophoretic 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
Hybrid	first generation progeny (F_1) from a cross produces 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
Multigenic	controlled by many genes
PAGE	polyacrylamide gel electrophoresis; a type of electrophoresis employing gels made from the synthetic material, acrylamide
PGRI	Plant Genetic Resources Institute
PH	a term used to describe the acidity or alkalinity of a system
Phenotype	a sum-total of the expression of a character
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)
SDS	sodium dodecyl sulphate, a powerful detergent
t/ha	tonnes per hectare

ABSTRACT

Abstract

World core collection of Chickpea (*Cicer arietinum* L.) along with local accessions collected from all over the country was evaluated for six qualitative and four quantitative traits including disease (*Ascochyta rabiei*) reaction at National Agricultural Research Centre (NARC), Islamabad under rainfed conditions. Out of total 423 accessions, 360 accessions were exotic, sixty were local and three were improved varieties. The accessions differed significantly for plant traits of qualitative nature with distinct classes like growth habit, iron deficiency, flower colour, plant pubescence, plant pigmentation and pod size. All the exotic germplasm was badly infected with Ascochyta blight, hence sixty local accessions were selected for further investigation. These were evaluated on single plant progeny basis during winter 1997 for genetic diversity based on morphological, quantitative traits and biochemical markers. For quantitative traits, CV and genetic variance revealed that the results could be of broader spectrum. The negative association of days to flowering with days to maturity in the total germplasm, may be due to disease infection at the time of pod formation, caused forced maturity and little seed was obtained.

The total germplasm (423 accessions) was grouped into 10 clusters based on average linkage distance. Cluster I consisted of 60 accessions, cluster II-56; cluster III-73; cluster IV-53, cluster V-62, cluster VI-26, cluster VII-35, cluster VIII-20, cluster IX-23 and cluster X-15 accessions. All the exotic accessions were grouped in the clusters I, II, III, IV, VIII and X, whereas other clusters consisted of mixed accessions of local and exotic origin. Out of 63 local accessions (including 3 varieties), eleven were in the cluster V, sixteen in cluster VI, seventeen in cluster VII and nineteen in the cluster IX. As the number of accessions from various sources were grouped in a systematic way, therefore, relationship may be established between origin and clustering pattern. The selected accessions from various clusters are suggested to be used in crop improvement in future.

Variance studied by PCA revealed that cluster analyses grouped together accessions with greater genetic similarity, but clusters did not necessarily include all the accessions from same origin. Several potentially important agronomic types from particular groups have been

identified which may be exploited for genetic potential to transfer the desirable genes and this, along with biochemical analyses, will facilitate in assembling a core collection from the large genetic resources.

The accessions collected from Layyah district were better in evaluation as compared to other four major chickpea growing districts of Pakistan. Therefore, the selected accessions from this area could be tested under a wide range of environments or used in hybridization programme. As local material is better adapted, indicating the worth for improving seed weight in chickpea, hence it could be utilized by the breeders of chickpea by involving local and exotic chickpea parents in the breeding programme. In the present study, multivariate approach has proved to be more useful tool, it produced five clusters on the basis of provincial distribution much more differentiated when compared to the initial subdivision according to geographic sites of chickpea. The study confirmed the existence of a wealth of phenotypic divergence in the local chickpea germplasm. Further, collecting missions to main chickpea 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.

Correlation and path coefficient analyses conducted in a replicated trial revealed that pods per plant and 100-seed weight had the maximum contribution in determining grain yield, the ultimate product in chickpea under rainfed conditions. Further, it was observed that high indirect contribution was exhibited via secondary branches and harvest index by most of the yield components, hence these two traits along with pods per plant and 100-seed weight are suggested to be given emphasis while selecting high yielding chickpea cultivars for rainfed conditions. Correlation and path coefficient analyses indicated that pods per plant and 100-seed weight were potent contributors to grain yield through direct effects. Although, biological yield had significant association but exhibited negative direct effects. On the basis of performance, seven accessions produced higher grain yield than both the checks hence were selected for further evaluation under a wide range of environmental conditions.

The accessions evaluated for agronomic traits were also used for the analyses of SDS-PAGE through slab type gel electrophoresis using 10 samples for each accession. Although, all of these were not homozygous and polymorphism did exist for one or the other locus within various samples of the accessions. SDS-PAGE revealed that 11.25% acrylamide gel concentration, 6 µl of sample gave the best resolution. Out of sixty two accessions, 41 were homozygous on the basis of SDS-PAGE whereas others were heterozygous hence single seed descents could be isolated from these heterogeneous lines to establish pure-lines for future breeding programme. In total, 14 protein bands were recorded ranging from the Molecular Weight (MW) of 24 to 66 KDa. 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 gene bank, 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 chickpea germplasm.

In order to ensure the efficient and effective use of crop germplasm, its characterisation is imperative. In the present investigation, cluster analyses based on SDS-PAGE in local chickpea germplasm did not reflect any clue either for agronomic preference and/or geographic distribution. For most accessions and protein subunits, no clear observation was recorded which could facilitate selection on the basis of SDS-PAGE for improving agronomic traits in chickpea from the material under investigation. Further, 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.

Cluster analyses based on morphological characters was observed more reliable than on the basis of protein peptides which indicated that cluster analyses on the basis of quantitative characters have more breeding value in chickpea, but simultaneous study for both agronomical and biochemical analyses (protein and DNA) is suggested. From the

present studies, it was concluded that local chickpea germplasm collected from main chickpea growing areas exhibited significant variation for all the quantitative characters except seeds/pod and 100 seed weight. Although, variation was observed for total seed protein but the level was low. SDS-PAGE was not very effective for studying intra-specific genetic diversity in cultivated chickpea alone rather wild chickpea spp. could be included. Further, biochemical markers are suggested to increase by adding DNA techniques (RAPD, RFLP, ALP) for studying diversity related to germplasm collections. 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. Little geographic relationship was observed that could be enhanced by involving more diverse accessions in research material. Grouping of advance breeding lines in one cluster revealed that only a portion of genetic variance has been exploited for chickpea improvement in the past. If one of the goals is to bring together cultivars 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 is found.

From the present investigation, it was concluded that chickpea germplasm displayed a wide range of diversity for most of the traits studied and that there were only a few accessions with unique characters. The study revealed that the exotic accessions are not well adapted to our local environment and are reported to be badly infected by *Ascochyta rabiei*. Among local chickpea growing areas, district Layyah is reported to be the best region for obtaining higher grain yield. Multivariate approach proved to be very useful tool in that it produced different clusters on the basis of geographic distribution. Correlation and path coefficient analyses revealed that pods per plant and 100-seed weight had the maximum contribution in determining grain yield, the ultimate product in chickpea under rainfed conditions. On the basis of performance, seven accessions (Pak-52984, Pak-52983, Pak-52981, Pak-52979, Pak-52978, Pak-52975, and Pak-52974) produced higher grain yield than the check varieties. These high yielding accessions can be of great importance to the future chickpea breeders in producing superior cultivars during hybridization programme.

INTRODUCTION

INTRODUCTION

Chickpea (*Cicer arietinum* L.,) is a self pollinated, diploid annual grain legume with 2n=16 chromosomes. It belongs to the family Leguminosae/Febaceae and was one of the first grain legumes to be domesticated in the world. It is an important crop of the Indian-Pak subcontinent, West Asia, North Africa, South Europe, North and Central America. Chickpea most probably originated in an area of present day south-eastern Turkey and adjoining Syria. Vavilov (1926) indicated Hindustan and Mediterranean region as the centre of origin along with Ethiopia as a secondary centre of diversity. According to Ladizinsky (1975), the centre of origin is south-eastern Turkey.

The legume family has such distinctive characteristics that its members can frequently be recognised with only little experience. It is one of the three largest families with 600-genera and 12000-species. All major growth forms are represented: herbs, both annuals and perennials, shrubs, vines and trees. It is of worldwide distribution. While, less heterogeneous than the rose family, considerable variation is found among its members.

The genus Cicer includes both annuals and perennials. Plants are shrubby, generally grow less than one meter high, and are pubescent with glandular or aglandular hairs. Roots are robust, long and rich in starch. Stems are branched. The stipule is generally toothed and its shape is useful in chickpea taxonomy. Axillary racemes show 1-5 flowers, which are 5-50 mm long. Flowers are papilionaceous and 4 to 30 mm long. The general floral formula is K5/C5/A(9)+1/G1. Pods are acuminate, pubescent, characteristically inflated, and up to 3 cm long. They contain 1 to 10 seeds (1-3 in *C. arietinum*). Seeds vary from globular to bilobular, showing a characteristic beak. The maximum length of seeds of wild species is 4-6 mm but in cultivated species it can reach up to 15 mm. Seed colour is generally black, excepting in *C. arietinum*, which shows a wide variation. There are thick cotyledons without endosperm in the mature seed. The hilum is small. Germination is quick and hypogeal. The plants of annual species can complete their life cycle in from 90 to 180 days, depending on the climatic conditions. Chickpeas are often divided into two major groupings (van der Maesen 1972; Cubero 1975; Auckland and van der Maesen 1980) that correspond to difference in size, shape

and seed colour. The types that produce large round seeds (600mg) with ram head shape and are white or pale cream/beige coloured are referred to as kabuli types. Flowers of kabuli types are non-pigmented. The types that produce smaller seeds (20mg), that have an angular appearance with sharp edges, and are variously pigmented, are referred as desi types. These generally have pigmented flowers, stems and some times leaves.

Legumes have played a crucial role in agricultural production throughout history. This is obviously due to their capacity to fix nitrogen in association with rhizobia. Cost escalation of fossil fuel required for the manufacture of nitrogenous fertilizer has helped to increase awareness of the importance of the rhizobium-legume symbiosis. Chickpea is one of the most important legume crops of the world and cultivated under a wide range of agro-ecological conditions mainly of rainfed nature. Drought tolerance is a desirable characteristic for a crop such as chickpea which grows mainly on conserved soil moisture (Saxena, N.P. 1984). It is economical in production and gives good yield of grain with excellent source of protein. Its great popularity as a human food is due to its mild, acceptable flavour and to the unique ability of its principal proteins to form a good combination with cereals. A large proportion of its total nitrogen requirement can be met through symbiotic fixation (Islam and Saxena, 1981). Chickpea is considered to be an important low-input crop in the cropping systems of the semi-arid tropics. It is commonly believed to be a hardy crop which can be grown on marginal soils which are not suitable for other cereal crops such as wheat (Moolani and Chandra, 1970).

It has generally been found to be an ideal crop for rotation in the Indian-Pak subcontinent, as any crop succeeding chickpea grows well (Argikar, 1970). The nutritive value of a corn diet was significantly improved when it was supplemented with chickpeas (Usha et al., 1972). Chickpeas have a higher protein digestibility, which tends to place them on a par with other widely used pulses (Sumathi and Pattabiraman, 1976). It has relatively few insect pests and generally suffers little pest-caused loss when compared with most other semi-tropical leguminous crops. There appears to be two major reasons why insect pests are of relatively little importance on chickpea. The first being the plants are covered with glandular hairs which exude acid-droplets containing high concentrations of malic acid. The second is the early sowing of crop or just after the winter in almost all areas of the world in which it is of importance, so that it grows during

the period when insect activity and populations are at a minimum. Chickpea has staged a comeback in rainfed or partially irrigated areas because of its low cost of production, good price and relatively low infestation by insects (Singh, 1983).

Environmental conditions greatly affect the growth of crops. Among these, biotic stresses (disease) and abiotic stresses (mainly moisture) have the most severe effect on its productivity. Among biotic stresses (mainly diseases) that affect chickpea, *Ascochyta* blight is the most devastating worldwide, causing up to 100% yield losses in severely affected fields (Nene, 1984). Resistance breeding has relied on the use of screening germplasm or nurseries where disease epidemics are created artificially by inoculation with the pathogen and frequent sprinkler irrigation. With this approach, *Ascochyta* blight resistance sources have been identified and many resistant cultivars have been developed (Reddy and Singh, 1984; Nene and Reddy, 1987; Malik, 1991).

Further research is needed to reduce the risk of crop failure by understanding genetic diversity for disease and agronomic traits, to develop high yielding disease resistant cultivar. According to estimates conducted by United Nations and others, it is the general conclusion that world population will increase from 5 to 8 billion in the next two or three decades which means that we have to produce more food. If this task is to be accomplished, increased production must come not only from land already available but also from new lands brought under cultivation and by increasing cropping intensity. Genetics of disease and other characters have been reported by Malik (1991), however, the locations of the genes conferring resistance are not known. Since multiple genes appear to be responsible for most of the disease and agronomic traits, hence, knowledge of their genomic locations and linkage to molecular markers would facilitate gene transfer and pyramiding of the genes into acceptable genetic backgrounds through marker-assisted selection.

Molecular markers have been used to establish linkage maps for many crop species and they have been utilized to determine gene number for particular traits and for gene tagged in various crops (Welsh and McClelland, 1990). RAPD markers (Williams *et al.*, 1990; Welsh and McClelland, 1990) are simple and fast and have been employed widely for mapping genomes and for tagging resistance genes. Plant breeding has helped

to promote agricultural development and has caused significant yield increases and quality improvement in different crops. For instance, during 1970 to 1980 wheat, rice and corn yield increased by 22.6%, 18.7% and 31.6%, respectively in the world whereas soybean and broadbean yield increased by 13.5% and 17.3%, respectively (Stoskopf, 1985).

During the last few years, although chickpea never failed totally due to natural calamities but a wide fluctuation in the production is in evidence (Table 1.1). Crop production on dry lands fluctuates widely from year to year due to vagaries of weather. There is an immense need of improvement by best utilization of our existing genetic resources. Data on agronomic, morphological and physiological plant traits are generally used to characterize the varieties, however, such data may not provide an accurate identification of genetic diversity because environmental influence upon the expression of observed traits are difficult in scoring due to the presence of multiple allele or genes. Moreover, field-testing and evaluation of plant materials is often laborious and time consuming. Considering these difficulties, bio-chemical markers received more attention in recent years from the crop geneticists for the assessment of genetic variability. Further, the data reflect truly the genetic variability as bio-chemical markers are products of genes and their expression in not influenced by the environment.

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 use of seed protein electrophoresis have been able to detect qualitative and quantitative differences among cultivars for cultivar analysis in various crop species (Righetti and Bosisio, 1981;Cooke, 1984, Gupta and Robbelen, 1986; Gardiner and Forde, 1988; Gilliland, 1989).

Table 1.1: Area, production and yield of chickpea in Pakistan, India and World.

Year	Area (000 ha.)	Production (000 t)	Yield (kg/ha)
PAKISTAN			
1980-81	842.9	336.9	400
1981-82	901.6	293.7	326
1982-83	892.9	491.0	550
1983-84	919.6	521.9	568
1984-85	1013.7	523.7	517
Average	**914.1**	**433.4**	**474**
1985-86	1033.3	586.2	567
1986-87	1082.1	583.3	539
1987-88	820.6	371.5	453
1988-89	979.4	456.0	466
1989-90	1035.4	561.9	543
Average	**990.2**	**511.9**	**517**
1990-91	1091.5	531.0	486
1991-92	996.9	512.8	514
1992-93	1007.6	347.3	344
1993-94	1045.0	410.7	393
1994-95	1064.5	558.5	524
Average	**1041.1**	**472.1**	**453**
1995-96	1118	679.6	607
1996-97	1100.2	594.4	540
1997-98	1102.3	767.1	696
1998-99	1076.9	697.9	648
1999-00	971.8	564.5	581
Average	**1074**	**660.7**	**615**
INDIA			
1989-99	**7300**	**5754**	**788**
WORLD			
1989-99	**11194**	**9587**	**767**

Source: Agricultural Statistics of Pakistan; FAO year book of Asia statistics, 1999.

In this technique, researcher can identify variation in the physical and chemical properties of proteins. The size (or length) of a protein, RNA or DNA molecule is one of the most frequent measurements in molecular biology. Molecules of RNA, DNA and protein can be separated according to their size by electrophoresis. This technique depend upon the fact that dissolved molecules in an electric field move at a speed determined by the ratio of their charge to their mass. Molecules of proteins and nucleic acids are subjected to electrophoresis in a semi solid material made of agarose (a plant polysaccharide) or of a synthetic polymer such as polyacrylamide. The size of the such gels limits the rate at which molecules can move through them. Nucleic acids have identical charge to mass ratios that separate according to length, with the longer ones moving more slowly through the pores of the gel. Even very long nucleic acids(chain containing from 10,000 to 20,000 residues) that differ in length by a few percentage points can be separated from one another, and each individual chain can be isolated in mixtures containing chains of 500 nucleotides or less. Proteins chains can also be separated according to length. Both before and during electrophoresis, the proteins are continuously exposed to detergent SDS (Sodium Dodecyl Sulphate), a common cleaning agent found in toothpaste; its chemical formula is $CH_3(CH_2)_{11}SO_4^-Na^+$. Approximately, one molecule of detergent binds to each amino acid i.e., 1.4g of SDS binds to each gram of protein. At neutral pH, the detergent is negatively charged. SDS molecules repel one another, which forces the proteins with bound detergent in to rod like shapes endowed with similar mass to charge ratios. Proteins in this case are said to be denatured. As with nucleic acids, chain length (which is equivalent to mass) is the determinant for the separation of proteins during electrophoresis through polyacrylamide gels containing SDS. Again, even chains that differ in molecular weight by less than one percent can be separated. Forms of the same protein that are separated in this way are called allozymes. Because allozymes are specified by different alleles, allozymic variation is a direct reflection of underlying genetic variation.

Band colour on the gel mark the locations of very specific enzymes. The number of bands reveal the number of enzymes capable of reacting with the substrate. When members of a species are analyzed in this way, many of the bands are identical, revealing a high degree of genetic similarity, but there still may be considerable variation. The

degree of genetic relationship between different species, genera, families and even divisions can be estimated in the same way. The closer the banding pattern compare, the closer the genetic similarity is presumed to be.

Polyacrylamide gel electrophoresis (PAGE) of cereal storage proteins is a valuable tool for gauging variation in populations of landraces and cultivars. It appears to be as good marker systems as allozymes for this purpose. There are some limitations to the method. Basically, it underestimates the amount of genetic variation. First all types of enzymes are equally variable. If a researcher picks a "conservative" group of enzymes, he will now show as much variation as if he had picked another group. Second, not all changes in amino acid sequence of an enzyme will result in change in the electrical charge or shape of the molecule. This means that two slightly different molecules will still migrate to the same point on the gel, and in those cases, they will be scored by the experimenter as identical, even though they actually are different. Despite these drawbacks, gel electrophoresis allows taxonomists to estimate the degree of genetic similarity between organisms quickly and conveniently. Such an estimate is much more accurate than one based on morphological similarities (like leaf shape and flower structure). Most morphological traits are controlled by many genes, not single genes, and can be strongly affected by environment. But with many amino acids, we are dealing with single genes, and environmental influences are eliminated.

Over the last few years, there has been continuous increase in the number of systems applied for molecular genetic markers available. But, RAPD (random amplified polymorphic DNA) is the most widely used efficient method for identification of polymorphism at DNA level (Dos Santos *et al.*, 1994 and Thormann *et al.*, 1994). The RAPD markers are based on the amplification of DNA by polymerase chain reaction (PCR) using primers homologues to random target site in the genome. The advantage of this technique is in technical simplicity than RFLPs: the protocol requires less DNA and it produces results in a short time without radioactivity. While, its disadvantage lies in the fact that the random markers are dominant and the method needs sticker standardization for reproducibility (Williams *et al.*, 1990, Paran *et al.*, 1991 and Welsh *et al.*, 1991). The advantages make this method appropriate in genotype identification and in genetic

diversity studies (Veirling *et al.*, 1994, Dweikat *et al.*, 1993, Tinker *et al.*, 1993 and Yu & Nguyen 1994).

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 recombination, mutation etc. Cereals are more researched as compared to legumes, although among legumes, chickpea is the most researched crop because of two international centers, i.e., ICARDA and ICRISAT which have world mandate for this important legume. 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. (Ghafoor *et al.*, 2001).

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), kale crops (Das *et al.*, 1989), 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 approaches 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.

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).

Landraces are a useful source of genetic variation, and the greater the variation, the greater the chances of a landrace possessing gene combinations of interest to plant breeders. Evaluations of germplasm collections may take over three years. With the rapid genetic erosion occurring in many parts of the world, precious germplasm may be lost before additional collections can be made. Since the formation of the International Board for Plant Genetic Resources (IBPGR) in 1974, germplasm collecting activities have been intensified in the centres of diversity of major crops as well as in peripheral areas. Although, most of these collections have been lodged safely in various gene banks around the world for long-term storage, little evaluation has been undertaken to determine their worth as future sources of valuable genes.

Chickpea has abundant genetic variation for qualitative and quantitative traits. Some of the variations were described by Ayyar and Balasubramanian (1936, 37) when they reported the inheritance of flower colour. Singh et al., (1982) also reported variations in qualitative and many quantitatively inherited traits. 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. 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.

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. Keeping in view the importance of the crop, a wide range of local germplasm of chickpea 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 the present study is to multiply and select better land races to assess and evaluate the genetic variability based on biochemical (storage proteins) gene markers in local chickpea germplasm collection. The study also aims to relate the storage protein polymorphism with the genetic variation in morphological/physiological plant traits as means to enhance the breeder's efficiency for manipulating the desirable traits and to introgress economically important traits from the wild species to cultivated chickpea.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

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 also 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 conceptualized. 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.

The selection criteria vary from crop to crop depending upon the yield components and their contribution to grain yield. Some of these components have direct effect on the yield while others have indirect influence. Adhikari and Pandey (1982a) reported that seed yield per plant in chickpea and significant and positive correlation with pods per plant and primary and secondary branches per plant. Katiyar et al., (1981) also indicated that pods per plant had the highest direct effect on yield of chickpea but overall positive correlation between pods per plant and seed yields was reduced by a high negative indirect effect of pods per plant on seed yield via seeds per pod. Similar associations were reported by Khan et al., (1983). Salih (1982) found very little associations among seed size, the number of pods per plant, seeds per plant, and plant height in chickpea. According to Pandya and Pandey (1980), seeds per plant had a positive and high associations with number of pod per plant, number of branches and days to flowing and very

little association with 100-seed weight while plant height showed a negative correlation with seed yield. Such studies were carried out mainly with pure lines and similar information for segregating populations in limited.

Ram *et al.*, (1980) studied the segregating populations in chickpea and reported that pods and seeds per plant consistently showed the highest positive direct effect on seed yield in F2 and F3 generations in all the crosses studied. Knott (1972) carried out the F3 yield test in wheat and found that testing on a plot basis was more effective than on an individual plant basis, and expressing the yield of F3 lines as percentage of adjacent checks, following the moving average of check method, increased the efficiency of these tests. But Knott and Kumar (1975) found early generation yield testing of very little use in wheat. They concluded that reliable yield testing in wheat could be done only when a reasonable degree of homozygosity is reached.

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).

Stable grain yield is the most important trait the plant breeder wants to improve. It is the final product of several contributory factors and their interactions. It is naturally a complex character of many other traits, which again have inter relations among themselves. These inter-relations can be positive or negative. It is therefore important to determine such inter-dependence among these contributory characters which may facilitate the interpretation of results already obtained and provide the basis for planning more efficient breeding programs for the future. Correlation coefficients show patterns of association among yield components and growth attributes, indicating what complexities determine yield. Most of the studies on associations between yield and yield components have been carried out on homozygous populations, but it is realized that these fixed genotypes have some limitations in extrapolating data to genotypes in segregating populations. Such studies are therefore to be conducted on both homozygous genotypes and heterozygous and heterogeneous populations

to determine the important and stable character or characters on which selection is to be based.

Information is available for chickpea which shows the relationships between yield and its components and also among components in pure line cultivars. The relationships studied among eight different characters in nine chickpea lines showed that high positive correlation exist between plant height and inter node length, between number of days to flowering and number of nodes up to the first flower, between height at flower initiation and seed yield, between number of pods per plant and seed yield and between seed size and seed yield (Baluch and Soomro, 1968).

Sharma *et al.*, (1969) also carried out studies on correlation between yield and other characters in chickpea and found out that yield was positively correlated with eight morphological characters in the 44 lines studied .It was highly correlated genotypically, phenotypically and environmentally with number of flowers, number of pods, number of branches, number of seeds per pod and 100-seed weight. Plant height and pod length were also found to exhibit high significant genotypic correlations with yield, whereas pod width revealed a positive but non-significant correlation with yield. Important traits registered by Gill and Brar (1980) include plant height, protein and ascorbic acid content of the seed. These characters should be considered while making selection for yield and protein improvement. Yield and six components of yield were studied by Sandhu and Singh (1970) on sixty lines from thirteen countries and the results obtained revealed that the expected genetic advance for 100-seed weight and pod number per plant was high. The seed yield was found to be positively correlated with the number of primary branches, secondary branches and pods per plant. The importance of these three characters was further confirmed by the results obtained by Rang *et al.*, (1980), Khoragade *et al.*, (1985), Setty *et al.*, (1977) and Singh *et al.*, (1978). The correlation and path analysis carried out by Singh et al., (1978) on six yield components of 75 chickpea lines showed that a selection index based on high pod and primary branch number and a low secondary branch number should improve yield. The analysis of yield components by Adhikari and Pandey (1982a) in chickpea also emphasized the importance of number of pods per plant and both

primary and secondary branches which were positively associated with seed yield. Partial correlation and regression studies of Khorgade *et al.*, (1985) revealed that 100-seed weight and number of branches per plant were the most important yield determiners.

The selection indices studied by them indicated that the use of single character indices exhibited no higher efficiency than straight selection for yield alone except 100- seed weight. Setty *et al.*, (1977), Tyagi *et al.*, (1982), Shahi *et al.*, (1984) and Chowdhry and Khan (1974) observed a positive association of yield with 100-seed weight. Hundred seed weight was also found to be positively correlated with number of seeds per ten pods and secondary branches per plant (Chowdhry and Khan, 1974). On the other hand, Dobholkar (1973) and Raju *et al.*, (1978) obtained results which exhibited a negative correlation between seed yield and 100-seed weight, but a positive correlation between yield and number of pods per plant and seeds per pod. The results obtained by Dahiya *et al.*, (1983a) were not in favour of using 100-seed weight as a selection criterion since the varieties used were unstable for this character.

According to Setty *et al.*, (1977), days to flowering and days to maturity showed a negative correlation with seed yield. This was further supported by the report of Salih (1982) which revealed the significant negative correlation between seed yield and days to 50% flowering and maturity and the positive correlation between yield and plant population at harvest indicating the importance of earliness and good plant stand for high seed yields. The work of Setty *et al.*, (1977) showed that seed yield had a positive correlation with of branches, pods per plant, seeds per pod, pod yield and seed volume. The analysis of data collected on thirteen traits in 132 lines of chickpea showed that pods per plant and seed per pod were among the important components (Rang *et al.*, 1980). Tyagi *et al.*, (1982) and Shahi *et al.*, (1984) stressed the importance of pods per plant since it was significantly and positively correlated with seed yield per plant. They also noted that pods per plant had a positive association with number of primary and secondary branches, while seed protein exhibited a significant negative correlation with seed yield per plant, seed weight and plant height. Dobholkar (1973),

observed that the number of pods per plant was positively correlated with number of seeds per pod.

In chickpea, Dahiya *et al.*, (1983b) found positive and significant correlation's between F2 and F3, F2 and F4 and F3 and F5 generations. Dahiya *et al.*, (1984) also reported that the F3 yield trial selection method resulted in significant yield increases over both random and visual selection. These results have also shown that visual selection that visual selection and random selection were equally ineffective in the identification of high yielding lines. On the other hand, Mckenzie and Lambert (1961) concluded that F3 yield tests were of little value in predicting F6 yields in barley. Among the components studied by Adhikari and Pandey (1982a), plant height and node number between first and last pod exhibited a high negative genotypic correlation with the seed yield though the phenotypic correlations were non-significant and negative correlation with seeds per pod while it had a highly significant and positive correlation with plant height. This report also showed the highly significant and negative correlation (-0.95) between plant height and pods per plant can not be improved simultaneously. Significant negative correlation of plant height with number of pods per plant was observed in soybean while the association of plant height with seed yield per plant was positive (Sharma *et al.*, 1983). Islam *et al.*, (1982) found the number of pods per plant and the seed weight to be important components of yield. They also obtained a negative relationship between seed yield and plant height as Adhikari and Pandey (1982a). Singh *et al.*, (1980) proposed to increase the number of pods per plant, the seed size, the number of seeds per pod and the number of plants per unit area in tall plant types of chickpea.

Dahiya *et al.*, (1976) conducted an experiment to identify physiologically efficient genotypes in chickpea and found no correlation between total plant weight and effective and number. The results further indicated that in large-seeded types, the 100-seed weight contributed to an improved harvest index, whereas in small-seeded types the number of seeds per pod was important. The major yield contributing characters were, according to Govil *et al.*, (1980) vigorous growth, erect habit, early flowering but late maturing, numbers pods per secondary branch and per plant, numerous seeds per pod, resistance to *Fusarium oxyporium* f. sp.

Ciceri and small and less wrinkled seeds. The number of pods per plant, flower color and seed color, which were positively correlated with seed yield, were negatively correlated with leaf characters, height, days to flowering, pod size, seed size and degree of seed wrinkling (Govil, 1980).

Khan *et al.*, (1983) studied the variability, interrelationships and path coefficients for some characters in chickpea and found out the highest heritability values of 96% for number of pods per plant and 93% for number of primary branches. Other characters such as plant height, 100-seed weight and seed yield per plant exhibited 77%, 57% and 53%, respectively. The findings of Khorgade *et al.*, (1985) and Mohanty and Sahoo (1974) were similar to those of Khan *et al.*, (1983) indicating that several characters are not much affected by the environment. According to these results yield was positively and significantly associated with number of branches and number of pods per plant and thus these two characters are ideal for effective selection for seed yield.

The general, similar associations were reported for other pulse crops such as lentil, per, soybean, green gram and black gram. For instance, a positive as association between number of pods per plant and seed yield was observed in lentils (Tikka *et al.*, 1973) and Narsinghani *et al.*, 1978), soybeans (Sharma *et al.*, 1983, and Malik and Singh, 1982), pigeonpea (Shoran, 1982), mungbean (Gupta *et al.*, 1983), black gram (Rani and Rao, 1981), green gram (Malik *et al.*, 1983). Analysis of the components of yield done by Singh and Srivastava, (1985) for peas showed that days to 50% flowering, days to maturity, plant height and number of primary branches per plant were positively associated with grain yield as well as with each other indicating their efficiency for evolving high yielding varieties.

Number of primary and secondary branches was highly associated with seed yield in lentils (Dixit, 1974 and Tikka *et al.*, 1973). Hundred seed weight was also found to have a significant positive correlation with seed yield in green gram (Malik *et al.*, 1983), pigeonpea (Shoran, 1982), soybean (Malik and Singh, 1982) and black gram (Rani and Rao, 1981). But Narsinghani *et al.*, (1978) obtained significant negative genotypic and phenotypic correlation of seed weight with seed yield, days to flowering and pods per plant. Sandhu *et al.*, (1980) stressed the

importance of varieties with longer flowering duration and grain-filling period, i.e., flowering earlier and mature late will result in more productive varieties.

Chandra's report (1968) has shown that plant characters of chickpea are affected by environment, particular plant height and number of secondary branches. High genetic gains accompanied by the high heritability were observed for pods per plant, pod setting percentage, flowering duration and primary branches per plant while selection progress were expected to be greatest for seed weight and foliage colour. The association between various parameters suggested that selection for number of pods per plant and grain yield should lead to higher yields in favourable environments (Ramanujam and Gupta, 1973). These authors also suggested that an increase in number of pods per plant should be brought about by more pods per branches rather than by more branches per plant. The results obtained by Benjamini, (1981) and Gupta and Ramanujam, (1974) indicated that the number of pods per branches and the percentage of pods carrying two seeds instead of one, cause an increase in seed yield.

In summary most of the results reported on correlation between yield and yield components have shown that yield is positively associated with the number of primary and secondary branches and pods per plant. Selection based on the number of branches, number of pods, number of seeds, volume and weight of seeds was suggested to be very important and reliable in improving the yield (Setty *et al.*, 1977).

The review made by Smithson, (1985) showed that fruit number per plant has been significantly correlated with seed yield per plant in all of more than sixty cases reported, with correlation values ranging from 0.28 to 0.96. Also the number of seeds per plant was significantly and positively correlated with seed yield and fruit number per plant. Both primary and secondary branches play important roles since they are positively correlated with fruit number and yield per plant. For yield improvement in chickpea, Jain *et al.*, (1981) recommended to consider 100-seed weight, pods per plant, flowering period and harvest index in that order. The stability of yield was correlated with the stability of pod number and seeds per 100 grams. The partial regression analysis carried out by Sandhu and Singh, (1970) confirmed the importance of pod number per plant which had the strongest

influence on yield and indicated that the selection index based on this character accounted for 28% of variation in seed yield. Similar analysis done by Gupta *et al.*, (1972) exhibited that yield is mainly determined by the numbers of secondary branches, of pods per plant and of seeds per pod and a selection index based on these three characters was found to account for 80% of the total variation in yield.

Pod number determined yield per plant in pigeonpea (Singh *et al.*, 1982), soybean (Marwan, 1983), and lentil (Tikka *et al.*, 1973). This was mainly because it contains two primary components (Singh *et al.*, 1982), the number of seeds per pod and size of seed. According to Singh *et al.*, (1982) pods per plant had maximum efficiency followed by height at maturity when selection was based on single characters in pigeonpea. Selection based on a combination of these two characters lead to higher efficiency (110%) and was superior to selection for yield alone. Similarly, Shahi *et al.*, (1984) found pods per plant and 100-seed weight to be the most important characters in chickpea. Yield alone was good indicator for expected genetic improvement and the expected gain from index selection was considered not worth since it involves intensive labour and efforts of data recording.

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.*, (1990) examined the organization of diversity for morphological and agronomic characters in 306 landraces of cultivated common bean (*Phaseolus vulgaris* L.) by analyzing 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 Das *et al.*, (1989) 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 along with areas where existing genetic diversity has not yet gathered (Padulosi, 1993).

Rabbani *et al.*, (1998) 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. Bakhsh *et al.*, (1998) carried out experiments to estimate genetic variability and level of association of grain yield with its various components, separately in 18 parental lines, 28 F-1 and 19 F-2 generations. Highly significant genotypic differences were noted in these populations for characters like plant height, number of primary and secondary branches, pads per plant, 100-seed weight, biological yield, harvest index and grain yield. A comparison between F1, F2 and parental lines revealed that the range of inter-genotypic variation for the above mentioned characters in F-1 and F-2 was wider than that of parental lines. Generally, the genetic correlation coefficients were greater than those of phenotypic correlations in all the populations. Positive and highly significant genetic correlation of yield with plant height, number of primary and secondary branches, number of pods per plant, 100 seed weight and biological yield was observed in parental lines.

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.*, 1983; Koranyi, 1989; Moller & Spoor, 1993; Ahmad, *et al.*, 1992; 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); 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

recognized. 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.

MATERIALS AND METHODS

MATERIALS AND METHODS

The research project comprised of experiments conducted under laboratory, green-house and field condition.

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 wider adaptability, good in quality and resistance to biotic and abiotic stresses. Collection of chickpea 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

3.1.2 Experiment Material

Four hundred and twenty three chickpea germplasm accessions 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). Out of these, 360 represented a core collection received from USDA, 60 accessions were collected from five districts of Punjab and other three were approved varieties used as checks. The origin and characterization of all the accessions are given in the appendix 1. The experiment was planted on 25th October 1995 for morphological characterization and agronomic evaluation. One row 4 meter long for each accession was planted with 50 and 10 cm inter and intra-row spacing, respectively. Three approved varieties, viz., Punjab 91, Noor 91 and Paidar 91 were repeated as check after every 20 rows. Recommended cultural practices

were followed throughout the crop season. For plant characters and agronomic traits, data was recorded following IPGRI descriptors for chickpea (IBPGR, 1985).

3.1.3 Statistical Analysis

The data recorded was 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). Quantitative traits 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.

3.2 Evaluation of local sixty accessions

3.2.1 Experiment Material

During 1995, all the exotic germplasm was badly affected by *Ascochyta* blight, hence 60 local accessions were selected for further evaluation. These were selected on the basis of geographic origin and studied for morphological and protein patterns. Four hundred and twenty three accessions/genotypes were evaluated and characterized for various agronomical and morphological traits under field condition at NARC, Islamabad during 1995. Out of these, 60 accessions resistant to *Aschocyta* blight were selected on the basis of geographic distribution and two check varieties (Punjab 91 and Paidar 91) were also included in the study. Ten plants of each accession/genotype were sampled at random during 1995 and their progenies were planted during November 1996 and harvested during April-May, 1997. Two rows 2 meter long for each plant progeny were 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 and fungicides were sprayed to save the crop from the infestation of pests and diseases. Plant and agronomic characters were recorded following IPGRI descriptors for chickpea. 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. 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 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.

3.2.2 Statistical Analysis

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

3.3 Genetic and path analyses in selected pure-lines

3.4.1 Plant material

Thirty two genotypes of chickpea including two checks (Punjab 91 and Paidar 91) were evaluated for agronomic traits under field conditions at National Agricultural Research Centre (NARC), Islamabad, Pakistan. The experiment was planted during winter season of 1999 in randomised complete block design (RCBD) with four replications. Two rows 4 meter long for each genotype were planted in each replicate with 10 cm intra-row spacing, whereas inter-row distance was kept 50 cm. Pesticides and fungicides were sprayed to save the crop from infestation of pests and *Ascochyta rabiei*. For evaluation, data were recorded following descriptors for chickpea (IBPGR 1985). The data for days to flowering and maturity were recorded on line basis at 50% of flowering and 90% pod maturity and each genotype was represented by a single value. Other quantitative data, i.e., plant height, primary and secondary branches, pods, grain yield (g) and biological yield (g) were recorded on ten plants sampled randomly. Seed weight was recorded after counting 100 seeds in grams and harvest index was determined as economic yield expressed in percentage over total biomass.

3.3.2 *Data analysis*

The data recorded were analyzed for simple statistics, i.e., mean, standard deviation, variance, broad sense heritability and genetic advance. Broad sense heritability was estimated as a ratio between genotypic and phenotypic variance (Singh and Chaudhry, 1985). The averaged data were analyzed by numerical taxonomic techniques using the procedure of cluster and principal component analyses (Sneath and Sokal 1973) with the help of computer software "Statistica" and "SPSS" for Windows.

3.4 Biochemical (SDS-PAGE) Basis of Genetic Diversity

3.4.1 *Plant Material*

From chickpea germplasm consisting of 423 accessions and evaluated during 1995, sixty accessions were used for SDS-PAGE analysis. Only those accessions which were observed homozygous on the basis of protein patterns were included in this study.

3.4.2 *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 room temperature. 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.

3.4.3 *Electrophoresis*

Seed protein was analyzed through slab type SDS-PAGE using 11.25% Polyacrylamide gel. Electrophoresis was carried out at 100 V for half an 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 KDa), Albumin, Egg Ovalbumin (45 KDa), Pepsin Porcine Stomach Mucosa (34.7 KDa), Trypsinogen, Bovine Pancreas, PMSF treated (24 KDa), β-Lactoglobulin, Bovine Milk (18.4 KDa) and Lysozyme, Egg White (14.3 KDa) 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.

3.4.4 *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.

3.4.5 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 "STATISTICA" 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., molecular gene 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.

RESULTS

RESULTS

4.1 Evaluation of World Collection Germplasm.

4.1.1. Distribution of qualitative traits

A world core collection of chickpea germplasm comprising of 423 accessions was sown at National Agricultural Research Centre (NARC) in an Augmented Design during winter 1995. Plant traits of qualitative nature with distinct classes like growth habit, iron deficiency, flower colour, plant pubescence, plant pigmentation and pod size were recorded on line basis following IPGRI descriptors for chickpea and the tabulated results are presented in Table 4.1.1. Growth habit was recorded as erect, semi-erect and spreading or prostate types. Among the germplasm characterized, 201 accessions were erect types which were 47.5% of the total population, whereas, 184 accessions (43.5%) were semi-erect and thirty eight were spreading. Some of the chickpea genotypes show response to iron deficiency due to some physiological disorder, therefore, this trait was recorded as iron deficient or non-deficient types. Out of 423 accessions, 312 did not show symptoms of iron deficiency and these were 73.8% of the population, whereas rest of 26.2% exhibited iron deficiency.

Flower colour was recorded as dark pink, pink, light pink and white. Three hundred forty six accessions were pink flowered and only 77 (18.2%) gave white flowers, and these were all white seeded or Kabuli types. Twelve accessions were glabrous and all others (97.2 %) were pubescent. Out of these, 235 accessions were with less hairs while others were with dense hairs. Out of the total germplasm evaluated for plant pigmentation, 173 accessions which were 40.9% of the population, had no anthocyanin pigmentation, 248 (58.6%) had weak pigmentation and only two had strong anthocyanin pigmentation. Pod size was observed as small (< 15 mm), medium (15-20mm) and large (> 20mm) pods. One hundred and eighteen accessions which were 27.9% of the total population beard small pods, two hundred and fifteen (50.8%) were medium and 90 accessions (21.3%) gave large pods. The accessions with larger pods are suggested to be utilized for future selection and improvement for high seed weight as large pods contain bold seeds.

Table 4.1.1:- Classification of 423 accessions of chickpea for 5 qualitative traits and Ascochyta blight

Growth habit	Frequency	Percentage
Erect	201	47.5
Semi-erect	184	43.5
Spreading	38	9.0
Iron deficiency		
Non deficient	312	73.8
Iron deficient	111	26.2
Flower colour		
Dark pink	3	0.7
Pink	242	57.2
Light pink	101	23.9
White	77	18.2
Plant Pubescence		
Non-hairy	12	2.8
Less hairy	235	55.6
Dense hairy	176	41.6
Plant pigmentation		
No anthocyanin	173	40.9
Weak anthocyanin	248	58.6
Strong anthocyanin	2	0.5
Pod size		
Small < 15mm	118	27.89
Medium 15-20mm	215	50.83
Large > 20mm	90	21.28
Ascochyta		
Disease present	372	87.9
Disease absent	51	12.1

Ascochyta rabiei infection was observed under natural conditions and three hundred and seventy two accessions were infected with disease and only fifty one accessions were free of disease. Out of the total germplasm, 360 accessions were exotic, sixty were local and three were improved varieties. All the exotic germplasm was badly infected with disease and destroyed and no seed was harvested from exotic accession, hence for future study, sixty accessions were selected for further investigation.

4.1.2. Distribution of Quantitative Traits

Frequency distributions for four quantitative traits (days to flowering, days to maturity, branches and plant height) are presented in the graphic form (Figs. 4.1.1 to 4.1.4). For days to flowering, maximum accessions (152) which were 35.93% of the total population, flowered in less than 130 days after planting. It was followed by one hundred and one accessions which flowered from 131 to 135 days after planting (Fig. 4.1.1). Five accessions along with one check (PAK-52925, PAK-53098, PAK-53299, PAK-53300, PAIDAR-91, PAK-53099) matured late, i.e., > 150 days after planting. Maximum accessions (210) which were 49.65 percent of the total, matured between 211 and 215 days, followed by one hundred and forty five accessions with maturity range of 216-225 days. Nine accessions along with three checks (PAK-53250, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-53251, PAK-53252, PAK-52984, PAK-53253, PAIDAR-91, NOOR-91 and PUNJAB-91) took more than 220 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 one hundred and sixty four accessions which were 38.77% of the total have 6 to 10 branches per plant, and it was followed by 125 accessions which had 11 to 15 branches per plant. Eighteen accessions and three checks (PAK-52964, PAK-52965, PAK-52966, PAK-52967, PAK-52968, PAK-52969, PAK-52970, PAK-52971, PAK-52972, PAK-52973, PAK-52974, PAK-52975, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984, PAIDAR-91, NOOR-91 and PUNJAB-91) were observed bushy and produced more than 25 branches per plant. Frequency distribution for plant height presented in the Fig. 4.1.4 revealed that 99 accessions which were 23.40 percent of the total population were short statured and gave less than 40cm plant height. Maximum accessions (172) which were 40.66% of the population were in the range of 41-50cm and these were followed by 102

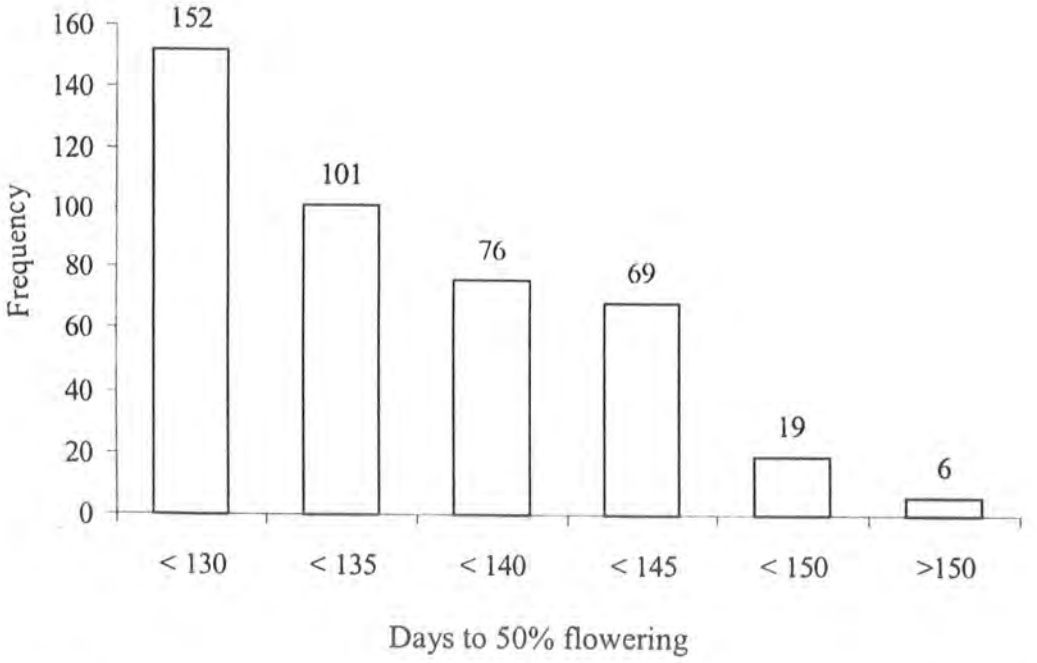

Fig. 4.1.1:- Frequency distribution for days to flowering in 423 accessions of chickpea

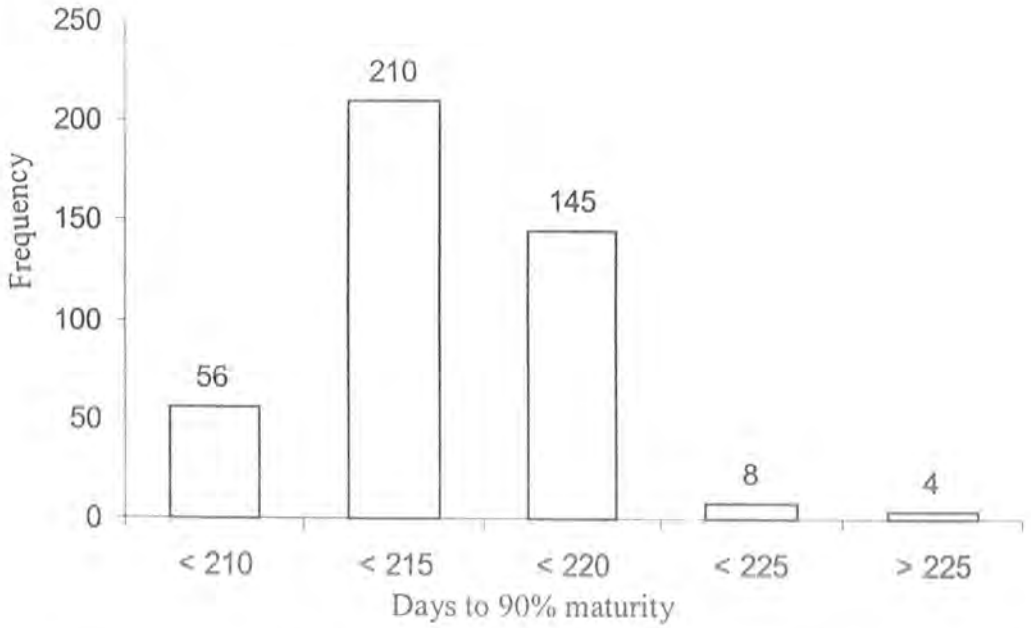

Fig. 4.1.2:- Frequency distribution for days to maturity in 423 accessions of chickpea

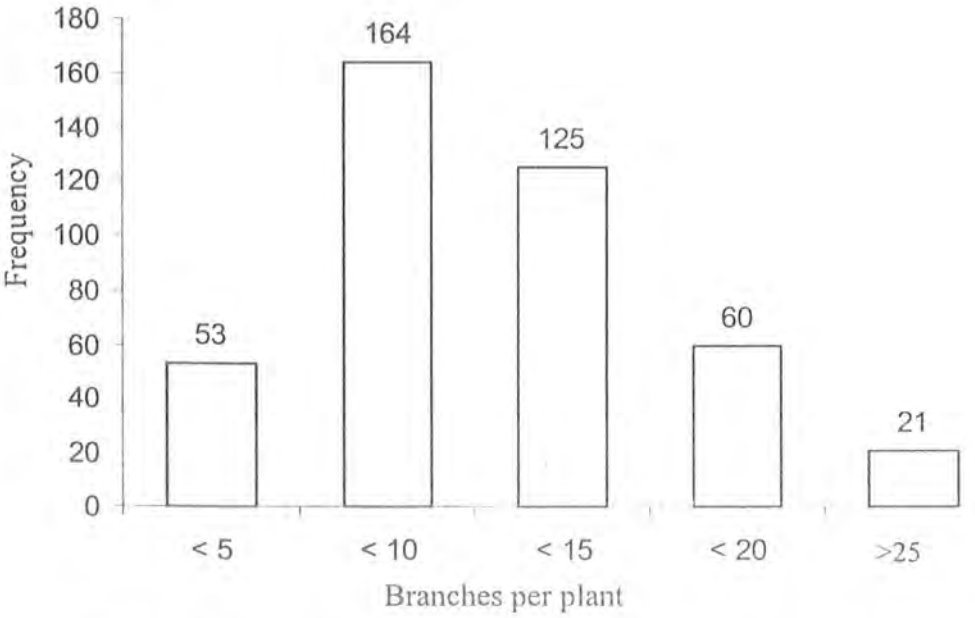

Fig. 4.1.3:- Frequency distribution for branches per plant in 423 accessions of chickpea

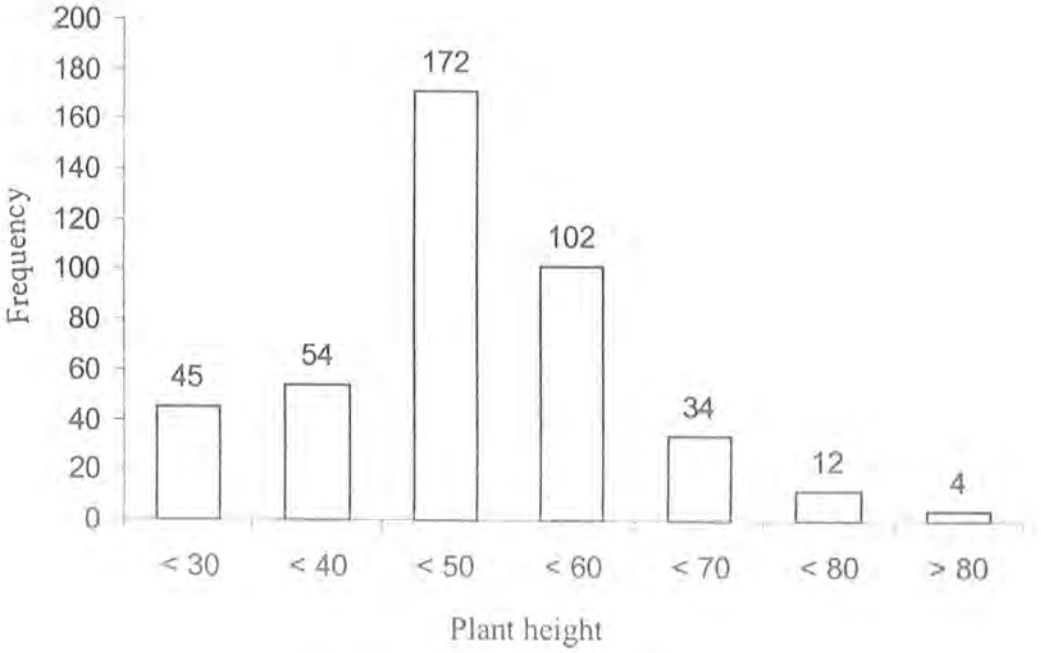

Fig. 4.1.4:- Frequency distribution for plant height in 423 accessions of chickpea

accessions which were in the range of 51-60cm of plant height. Sixteen accessions (PAK-53359, PAK-53360, PAK-53361, PAK-53362, PAK-53363, PAK-53364, PAK-53365, PAK-53366, PAK-52918, PAK-52919, PAK-52921, PAK-52922, PAK-52924, PAK-52925, PAK-52926, PAK-52927) were tall with plant height > 70cm.

4.1.3. Correlation Analysis

Simple correlation coefficients were computed among all the four quantitative traits; days to flowering, days to maturity, branches, and plant height. Due to disease infection, other data could not be recorded and only four quantitative traits were analyzed for correlation coefficients. Correlation analysis was conducted for total chickpea germplasm and the accessions selected on the basis of disease tolerance under went further evaluation because only sixty three accessions along with 3 checks could survive. The results regarding correlation revealed that days to flowering was negatively correlated with days to maturity in the total germplasm, whereas it was positively significant in the accessions selected. The negative association of days to flowering with days to maturity in the total germplasm was may be due to disease infection at the time of pod formation that caused forced maturity and little seed was obtained. Days to flowering and plant height had similar correlation, i.e., significantly positive in both the cases (Table 4.1.2). This association was similar due to late infection because maximum plant height had been attained before infection or pod formation. Days to maturity was positively correlated with branches per plant in total population and negatively in selected accessions. Similarly, branches per plant were also positively associated with plant height in total germplasm and negatively associated in selected accessions. Days to maturity exhibited positive correlation with plant height in both the population although it was slightly higher in selected accessions but 8% of total germplasm was of course higher than 10% of the selected accessions. The results in general revealed that maturity and branches per plant were more affected by environmental factors. The simple correlation coefficients presented in the Table 4.1.2 revealed negative correlation of days to flowering with maturity plant height, whereas other correlations were positive and maximum association (0.2145) was observed between plant height and number of branches per plant. The results indicated that the genotypes selected for early flowering may not necessarily mature early because maturity time is more influenced by

Table 4.1.2:- Phenotypic correlation coefficient among four quantitative traits in total germplasm and selected accessions of chickpea during 1995 and 1997

Quantiative traits	Germplasm/ Accessions	Days to flowering	Days to maturity	Branches per plant
Days to maturity	Total germplasm	-0.3376		
	Selected accessions during 1995	0.1425		
	Selected accessions during 1997	0.6429		
Branches per plant	Total germplasm	0.0124	0.1234	
	Selected accessions during 1995	0.2452	0.0984	
	Selected accessions during 1997	0.1411	-0.0065	
Plant height	Total germplasm	-0.2007	0.0870	0.2145
	Selected accessions during 1995	-0.1885	0.1245	0.1845
	Selected accessions during 1997	-0.2504	0.1027	-0.1965

environmental conditions at the time of harvesting during the months of April and May.

4.1.4. Cluster Analysis

The analysis of variance revealed significant differences for all the three quantitative traits (Table 4.1.3). The germplasm was grouped into 10 clusters based on average linkage (Table 4.1.4). Cluster I consisted of 60 accessions, cluster II of 56; cluster III of 73; cluster IV of 53, cluster V of 62, cluster VI of 26, cluster VII of 35, cluster VIII of 20, cluster IX of 23 and cluster X of 15 accessions. Approved cultivar, Paidar 91 was in the cluster VI, whereas as other two varieties (Noor 91, Punjab-91) were included in cluster VIII.

Greater number of exotic accessions were grouped in the clusters I, II, III, IV, VIII and X whereas other clusters consisted of mixed accessions of local and exotic origin. Out of 63 local accessions (including 3 varieties), eleven were in the cluster V, sixteen in cluster VI, seventeen in cluster VII and nineteen in the cluster IX. As the number of accessions from various sources were grouped in a systematic way, therefore, relationship may be established between origin and clustering pattern. Out of this total germplasm, sixty accessions which were of local origin were further evaluated to investigate the genetic diversity and its relationship with geographic origin based on quantitative traits and SDS-PAGE analysis. Exotic germplasm was totally destroyed due to the disease, *Ascochyta rabiei*.

The germplasm was divided in to ten clusters and the results regarding genetic distance among different cluster is presented in the Table 4.1.5. The genetic distance based on average linkage ranged from 0.5952 for the clusters II and IV to 1.5786 for the clusters I and IX. The genetic distance of cluster I was higher with most of the other clusters ranging from 1.0945 (with cluster X) to 1.5584 (with cluster VIII). Both of these cluster consisted of exotic germplasm and it is also important to note that cluster I consisted of all the 60 accessions of exotic origin. Similarly cluster II exhibited high genetic distance with cluster V, VIII and X. Out of these, cluster V consisted of both the exotic (51 accessions) and local germplasm (11 accessions). Cluster III exhibited genetic distance from 0.6524 (cluster III vs IV) to 1.3976 (with cluster I) and the cluster IV gave high range of

Table 4.1.3:- Analysis of variance for 423 chickpea accessions/genotypes evaluated during winter season, 1995.

SOV	df	Growth habit	Iron deficiency	Days to flowering	Flower colour	Days to maturity	Plant height	Plant pubescence	Ascochyta blight
Between	9	30.50	0.30	30.07	35.91	45.68	36.18	0.55	2.93
Within	413	0.36	0.19	0.37	0.24	0.03	0.23	0.24	0.07
F. ratio		85.44	1.58	82.05	150.07	1739.84	155.01	2.25	44.40
Significance P		0.00	0.12	0.00	0.00	0.00	0.00	0.02	0.00

Table 4.1.4:- Chickpea germplasm classified in to 10 clusters

Cluster	Number of accessions	Exotic	Local	Status
Cluster 1	60	All	0	Destroyed due to disease
Cluster 2	56	All	0	Destroyed due to disease
Cluster 3	73	All	0	Destroyed due to disease
Cluster 4	53	All	0	Destroyed due to disease
Cluster 5	62	51	11	Further evaluated
Cluster 6	26	10	16	Further evaluated
Cluster 7	35	18	17	Further evaluated
Cluster 8	20	All	0	Destroyed due to disease
Cluster 9	23	4	19	Further evaluated
Cluster 10	15	All	0	Destroyed due to disease

Table 4.1.5:- Genetic distance among 10 clusters of 423 chickpea accessions/genotypes based on 5 qualitative and 3 quantitative traits evaluated during winter season, 1995

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10
Cluster 2	1.3260									
Cluster 3	1.3392	0.7083								
Cluster 4	1.3976	0.5952	0.6524							
Cluster 5	1.4159	1.1019	0.7480	0.8215						
Cluster 6	1.2646	0.8454	1.0489	0.8667	0.7926					
Cluster 7	1.4498	0.9808	0.7767	1.0730	0.6647	0.7936				
Cluster 8	1.5584	1.2280	0.7804	0.7343	0.7849	1.3017	1.2946			
Cluster 9	1.5786	0.8614	1.1139	0.8998	0.9134	0.5173	0.8022	1.4222		
Cluster 10	1.0945	1.2230	0.9869	1.1971	0.8826	0.9530	0.9827	1.1857	1.3745	

genetic distance with all other clusters. Clusters V, VI and VII exhibited low to medium genetic diversity among one another, whereas medium to high genetic diversity with the clusters consisting of exotic germplasm. Cluster VIII also consisted of all the germplasm of exotic origin and had medium to high genetic distance with other clusters, whereas cluster IX consisted of both exotic (4 accessions) and local (19 accessions), exhibited the lowest genetic distance (0.5173) with cluster VI and the highest (1.5786) with cluster I. Therefore, this cluster might consist of diverse accessions on the basis of the qualitative traits. Table 4.1.6 represents the accessions grouped into ten clusters based on an average linkage distance.

4.1.5. Principal Component Analysis

Variance was further studied by PCA, and a principal components matrix for five qualitative traits (plant pubescence, growth habit, iron deficiency, flower colour, plant pigmentation), three quantitative characters (days to flowering, days to maturity, plant height) and disease reaction under natural infection is given in Table 4.1.7. The first four components with eigenvalues more than 1 contributed 70.6% of the variability amongst 423 accessions (both exotic and local) evaluated for nine traits. Principal component 1 had 23.64% of the total variation, PC_2 18.99%, PC_3 14.38% and PC_4 had 13.61% of the total variation. Characters that contributed more positively to PC_1 were days to maturity (0.867) and plant height (0.546), whereas days to flowering, qualitative traits and disease reaction contributed least to first component. Iron deficiency, flower colour, plant pigmentation and disease reaction gave negative contribution towards this component. Plant pigmentation and disease reaction contributed maximum genetic variance to PC_2; plant pubescence, growth habit and flower colour were assessed significant for PC_3, although the variation for growth habit was also contributed by PC_2 was 0.521, that was slightly lower than variation by PC_3 (0.570). Iron deficiency and days to flowering contributed maximum for PC_4 with values of 0.763 and 0.365, respectively. The first PC which explained 23.64% of the variance is positively associated with five characters and out of these, two were quantitatively inherited. The populations with high PC_1 values are late in maturity and tall staured.

Table 4.1.6.- Classification of chickpea germplasm into ten clusters based on average genetic distance

Cluster	f	Accessions
Cluster 1	60	53007, 53008, 53019, 53020, 53021, 53022, 53023, 53024, 53025, 53026, 53027, 53028, 53029, 53030, 53031, 53032, 53033, 53034, 53035, 53036, 53037, 53038, 53039, 53040, 53041, 53042, 53043, 53044, 53045, 53046, 53047, 53048, 53049, 53050, 53051, 53052, 53053, 53054, 53055, 53056, 53057, 53058, 53254, 53255, 53256, 53257, 53258, 53259, 53260, 53261, 53262, 53263, 53264, 53265, 53266, 53267, 53268, 53269, 53270, 53271
Cluster2	56	53009, 53010, 53011, 53012, 53013, 53014, 53016, 53017, 53095, 53098, 53112, 53115, 53116, 53127, 53129, 53133, 53135, 53136, 53155, 53157, 53158, 53160, 53161, 53164, 53165, 53166, 53169, 53170, 53172, 53173, 53193, 53194, 53195, 53197, 53199, 53203, 53205, 53209, 53210, 53211, 53212, 53218, 53219, 53220, 53221, 53223, 53224, 53225, 53227, 53236, 53237, 53238, 53243, 53245, 53246, 53252
Cluster 3	73	53015, 53018, 53059, 53060, 53061, 53062, 53063, 53064, 53065, 53066, 53067, 53069, 53070, 53071, 53074, 53075, 53078, 53079, 53080, 53081, 53082, 53083, 53084, 53086, 53087, 53088, 53089, 53090, 53091, 53092, 53093, 53101, 53102, 53103, 53107, 53109, 53137, 53138, 53139, 53140, 53141, 53142, 53143, 53145, 53146, 53147, 53148, 53149, 53151, 53152, 53153, 53154, 53156, 53174, 53175, 53176, 53177, 53178, 53180, 53181, 53182, 53184, 53185, 53189, 53190, 53200, 53201, 53202, 53206, 53232, 53233, 53234, 53253
Cluster 4	53	53077, 53094, 53096, 53097, 53099, 53110, 53111, 53113, 53114, 53121, 53122, 53123, 53124, 53125, 53126, 53130, 53131, 53132, 53134, 53159, 53162, 53163, 53167, 53168, 53171, 53191, 53192, 53196, 53198, 53204, 53207, 53208, 53213, 53214, 53215, 53216, 53217, 53226, 53228, 53229, 53230, 53231, 53235, 53239, 53240, 53241, 53242, 53244, 53247, 53248, 53249, 53250, 53251
Cluster 5	62	53272, 53274, 53275, 53277, 53278, 53279, 53283, 53285, 53286, 53287, 53288, 53291, 53292, 53293, 53294, 53295, 53296, 53297, 53301, 53302, 53303, 53305, 53306, 53307, 53308, 53309, 53311, 53312, 53313, 53314, 53315, 53340, 53341, 53344, 53346, 53347, 53348, 53350, 53351, 53352, 53353, 53355, 53356, 53357, 53358, 53360, 53361, 53363, 53364, 53365, 53366, 52938, 52942, 52944, 52956, 52957, 52961, 52962, 52965, 52973, 52974, 52975
Cluster 6	26	53318, 53319, 53320, 53321, 53322, 53323, 53324, 53325, 53326, 53327, 52926, 52927, 52928, 52929, 59230, 52931, 52932, 52933, 52934, 52935, 52963, 52964, 52966, 52967, 52968, PAIDAR-91
Cluster 7	35	53273, 53276, 53280, 53281, 53282, 53284, 53289, 53290, 53304, 53310, 53316, 53317, 53339, 53345, 53349, 53354, 53359, 53362, 52918, 52937, 52939, 52941, 52943, 52945, 52946, 52947, 52948, 52949, 52950, 52951, 52952, 52958, 52959, NOOR-91, Punjab-91
Cluster 8	20	53068, 53072, 53073, 53076, 53085, 53100, 53104, 53105, 53106, 53108, 53128, 53144, 53150, 53179, 53183, 53186, 53187, 53188, 53222, 53298
Cluster 9	23	53299, 53300, 53342, 53343, 52919, 52921, 52922, 52924, 52925, 52953, 52954, 52955, 52960, 52969, 52970, 52971, 52972, 52978, 52979, 52980, 52981, 52983, 52984
Cluster 10	15	53117, 53118, 53119, 53120, 53328, 53329, 53330, 53331, 53332, 53333, 53334, 53335, 53336, 53337, 53338

Table 4.1.7:- Principal Components (PCs) for 9 characters in 423 accessions of chickpea

	PC_1	PC_2	PC_3	PC_4
Eigen value	1.89	1.52	1.15	1.09
Proportion of σ^2	23.64	18.99	14.38	13.61
Commulative σ^2	23.64	42.63	57.01	70.62
	Eigen factor			
Plant pubescence	0.362	-0.665	0.414	-0.115
Growth habit	0.150	0.521	0.570	0.065
Iron deficiency	-0.065	0.259	0.076	0.763
Flower colour	-0.822	-0.380	0.079	0.039
Plant pigmentation	-0.117	0.218	-0.378	-0.576
Days to flowering	0.149	-0.105	-0.699	0.365
Days to maturity	0.867	0.296	-0.098	-0.085
Plant height	0.546	0.214	0.142	-0.099
Ascochyta rabiei	-0.521	0.670	00.040	-0.133

4.2. Evaluation of Sixty Local Accessions

4.2.1. Genetic Variation

Basic statistics for measured quantitative traits, viz., days to flowering, days to maturity, plant height, branches per plant, pods per plant, pods per branch, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index is presented in Table 4.2.1. High variance (expressed as percent of means) was observed for all the traits under study except seeds per pod where, a low variance was observed and hence improvement for this trait seemed to be difficult in the local germplasm used in present study. Days to flowering ranged from 124 to 155 days after planting with a mean value of 140±0.61 days. Maturity period ranged from 163-185 days after planting with a mean value of 170±0.58 days. Although, maturity range was not very high and a difference of only 3 weeks was observed between early and late maturing accessions, but at the time of harvest the earliness up to 3 weeks is considered sufficient for the preparation of land for next sowing. Plant height ranged from 37.6 to 66.9 and average value for this traits was 51±0.74 cm. High variance and range was observed for plant height. Branches ranged from 5.8 to 22.6 branches per plant, pods from 7.5 to 122.7. Pods per branch were calculated to find the best plant type/shape with maximum reproductive branches and it ranged from 0.7 to 13.1 pods per branch. High variance for pod characters (branches and pod number) was observed in the material used in the present study that could be exploited for crop improvement. Seeds per pod ranged between 0.5 and 1.8 seeds/pod with a mean value of 1.1±0.03 seeds along with low variance. Seed weight ranged from 10.88 to 27.49 g, biological yield from 14.14 to 50.65 g, grain yield from 1.74 to 18.35 g and harvest index from 7.15 to 56.62%. High variance for yield contributing traits was observed in the present material that could be utilized for improving yield potential of chickpea in future breeding programme.

4.2.2 Qualitative Traits

The germplasm comprising 62 accessions of chickpea along with 2 checks were also characterized for plant traits of qualitative nature with distinct classes like growth habit, iron deficiency, flower colour, plant pubescence, plant pigmentation and pod size following IPGRI descriptors for chickpea and the tabulated results are presented in Table 4.2.2. Growth habit was recorded as erect,

Table 4.2.1:-Range, means, SE and variance for 11 quantitative traits in 62 chickpea accessions along with two checks evaluated during 1997.

Quantitative traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140.03±0.61	4.80	23.05	124.00	155.00
Days to maturity	170.48±0.58	4.60	21.20	163.00	185.00
Plant height (cm)	51.00±0.74	5.83	33.98	37.60	66.90
Number of branches	12.31±0.46	3.60	12.99	5.80	22.60
Pods per plant	39.66±3.04	23.93	572.44	7.50	122.70
Pods per branch	3.70±0.30	2.37	5.62	0.68	13.10
Seeds per pod	1.13±0.03	0.22	0.05	0.47	1.81
100-seed weight (g)	15.78±0.60	4.72	22.31	10.88	27.49
Biological yield (g)	29.18±1.06	8.34	69.48	14.14	50.65
Grain yield (g)	7.19±0.44	3.47	12.05	1.74	18.35
Harvest index (%)	25.21±1.27	10.03	100.68	7.15	56.52

Table 4.2.2:- Classification of 62 accessions of chickpea for qualitative traits

Growth habit	Frequency	Percentage
Erect	45	72.6
Semi-erect	16	25.8
Spreading	1	1.61
Iron deficiency		
Non deficient	17	27.4
Iron deficient	45	72.6
Flower colour		
Dark pink	1	1.6
Pink	39	62.9
Light pink	22	35.5
White	0	0
Plant pigmentation		
No anthocyanin	32	51.6
Anthocyanin present	30	48.4
Pod size		
Small < 15mm	35	56.5
Medium 15-20mm	23	37.1
Large > 20mm	4	6.4

semi-erect and spreading or prostate types. Among the germplasm characterized, 45 accessions were erect types which were 72.6% of the total population, whereas, 16 accessions (25.8%) were semi-erect and one accession (1.6%) was spreading. Both the checks were erect type and it was noted that about one third of the local germplasm collected from five districts of Punjab was erect in nature. Some of the chickpea genotypes show response to iron deficiency due to some physiological disorder, therefore this trait was recorded as iron deficient or non-deficient types. Seventeen accessions, which were 27.4% of the material, did not show symptoms of iron deficiency, whereas rest of the forty five accessions (72.6%) exhibited iron deficiency. One check variety (Punjab-91) exhibited response to iron deficiency although under major chickpea growing areas (Thall desert), it does not respond to iron chlorosis. Flower colour was recorded as dark pink, pink, light pink and white. All the accessions involved in this study were having pink flowers and none gave white flowers, and hence all these accessions were of desi types. Further, all the accessions were pubescent and tolerant to disease. Out of the total germplasm evaluated for plant pigmentation, 32 accessions, which were 51.6% of the population, had no anthocyanin pigmentation, whereas rest of the material (30 accessions) had anthocyanin pigmentation. Pod size was observed as small (< 15 mm), medium (15-20mm) and large (> 20mm) pods. Thirty five accessions which were 56.5% of the total population beard small pods, twenty three (37.1%) were medium and other 4 accessions which were 6.5% gave large pods. The accessions with larger pods are suggested to be utilized for future selection and improvement for high seed weight as large pods contain bold seeds.

4.2.3. Distribution of Quantitative Traits

The frequency distributions for various quantitative traits (days to flowering, days to maturity, plant height, branches, pods/plant, pods/branch, seeds/pod, 100-seed weight, biological yield, grain yield/plant and harvest index) are presented in the graphic form (Figs. 4.2.1 to 4.2.11). For days to flowering, maximum number of accessions (30) which were 48.4% of the population, flowered within the range of 136-140 days after planting and it was followed by twenty three accessions which flowered from 141 to 145 days after planting (Fig. 4.2.1). Five accessions (Punjab-91, Paidar-91, PAK-52983, PAK-52932 and PAK-52922) were selected on the basis of early flowering as presented in Table 4.2.3. Maximum accessions (30) which were

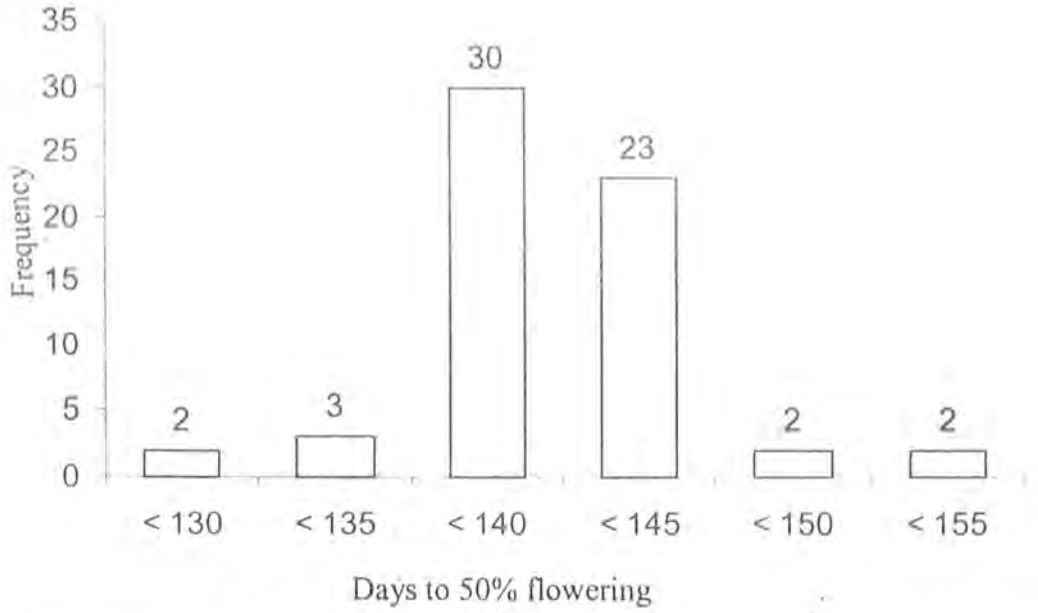

Fig. 4.2.1:- Frequency distribution for days to flowering in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

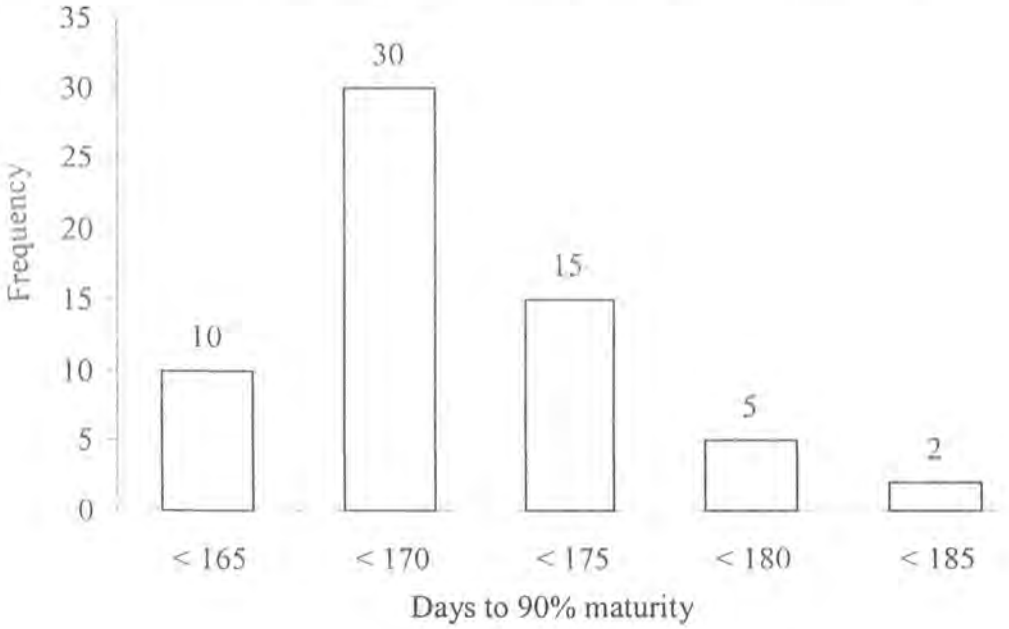

Fig. 4.2.2:- Frequency distribution for days to maturity in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

48.4 percent of the total, matured between 166 and 170 days, followed by fifteen accessions with maturity range of 171-175 days. Ten accessions (PAK-52983, PAK-52932, PAK-52922, PAK-52975, PAK-52931, PAK-52935, PAK-52953, PAK-52966, PAK-52971, PAK-52978) took less than 165 days to mature after planting and hence were considered short duration (Fig. 4.2.2). Seven accessions were late in maturity and took > 180 days to harvest after planting. For plant height, maximum accessions (22) which were 35.5% of the population, gave from 51 to 55 cm plant height and these were followed by nineteen accessions (30.7%) which were from 46 to 50 cm tall (Fig. 4.2.3). Eleven accessions (PAK-52983, PAK-52955, PAK-52979, PAK-52922, PAK-52933, PAK-52921, PAK-52984, PAK-52965, PAK-52937, PAK-52961, PAK-52942) were selected on the basis of short stature and these were less than 45 cm tall, whereas three genotypes (PAK-52968, Punjab-91, Paidar-91) including one accession and two varieties were tall stature.

For branches/plant the results depicted in Fig. 4.2.4 revealed that twenty seven accessions which were 43.6% of the total have 10 to 12 branches per plant, and it was followed by the range 13-15 with the frequency value of 14 accessions (22.6%). Twelve accessions (PAK-52971, PAK-52970, PAK-52973, PAK-52972, PAK-52975, PAK-52974, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984) were observed to be bushy and produced more than 15 branches per plant, hence these could be incorporated in the breeding programme to improve branches in chickpea. Pods per plant ranged from 7.5 to 122.75 and on the basis of class interval, it was observed that twenty nine accessions produced 21 to 40 pods per plant which was followed by the group from 41 to 60 pods with a frequency of fourteen accessions and these two groups constituted about 69% of the population (Fig. 4.2.5). Eight accessions (PAK-52974, PAK-52975, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984) produced more than 60 pods/plant, hence these are suggested to be tested under a wide range of agro-ecological conditions to develop superior cultivars.

Fifty two accessions which were 83.9% of the population, produced up to 6 pods/branch. Ten accessions (PAK-52961, PAK-52949, PAK-52966, PAK-52962, PAK-52964, PAK-52973, PAK-52960, PAK-52970, PAK-52984, PAK-52968) produced more than 6 pods per unit branch (Fig. 4.2.6). For seeds per pod, thirty five accessions which were 56.5% of the total produced 1.1 to 1.25 seeds per pod

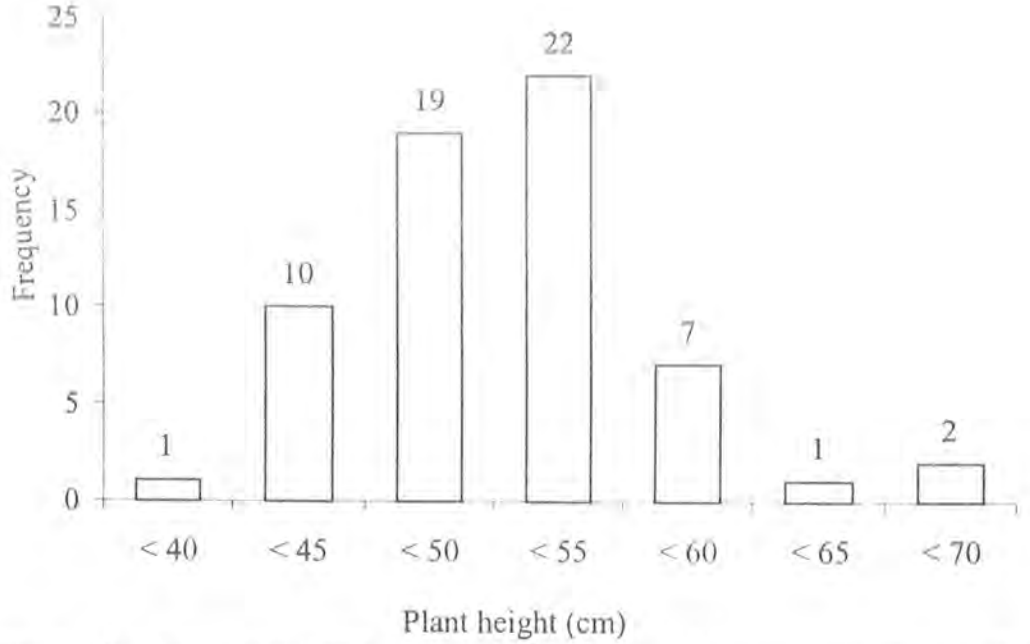

Fig. 4.2.3:- Frequency distribution for plant height in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

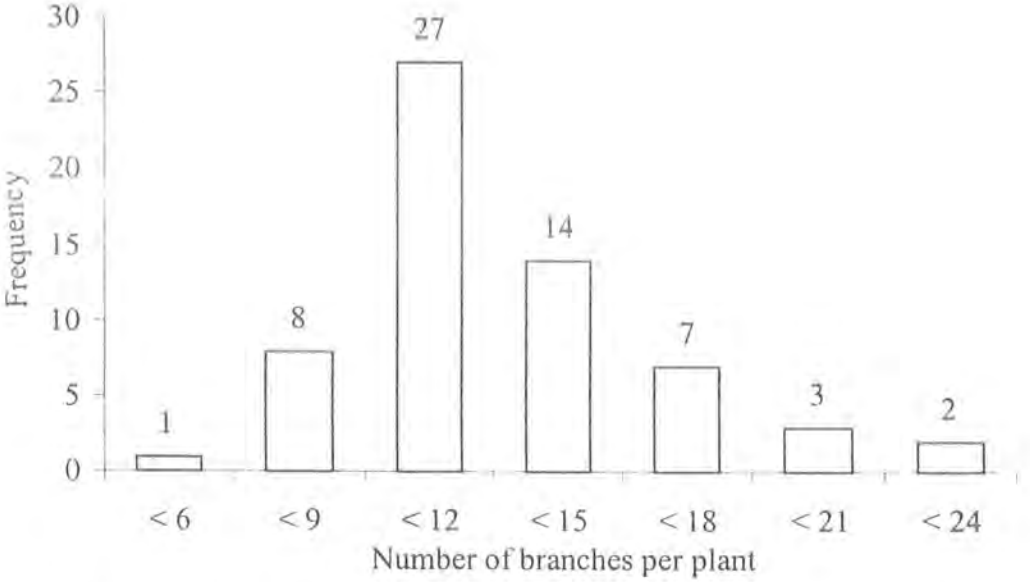

Fig. 4.2.4:- Frequency distribution for branches per plant in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

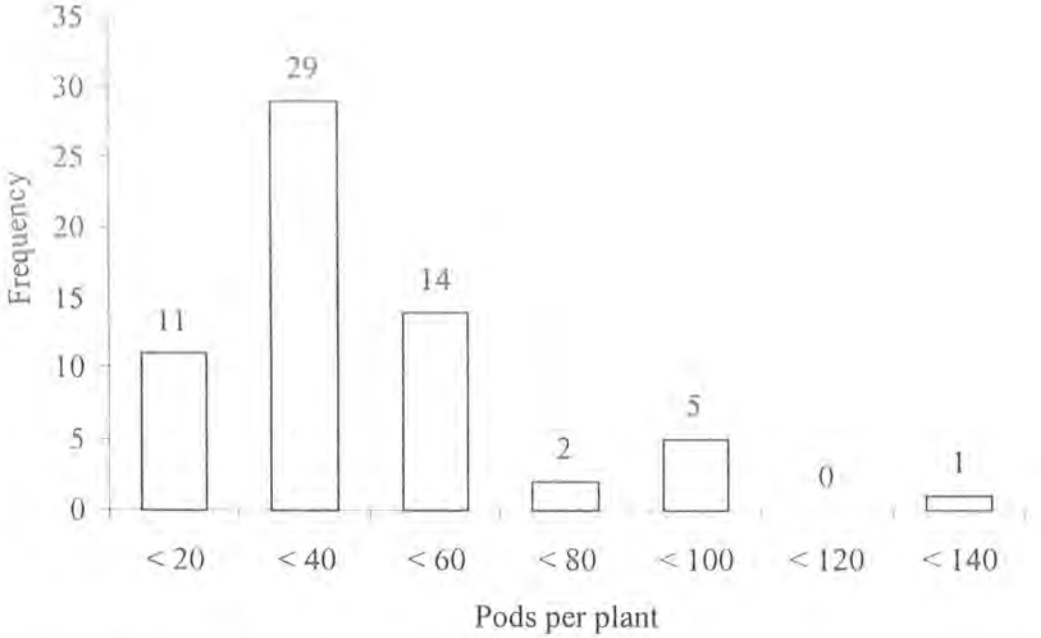

Fig. 4.2.5:- Frequency distribution for pods per plant in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

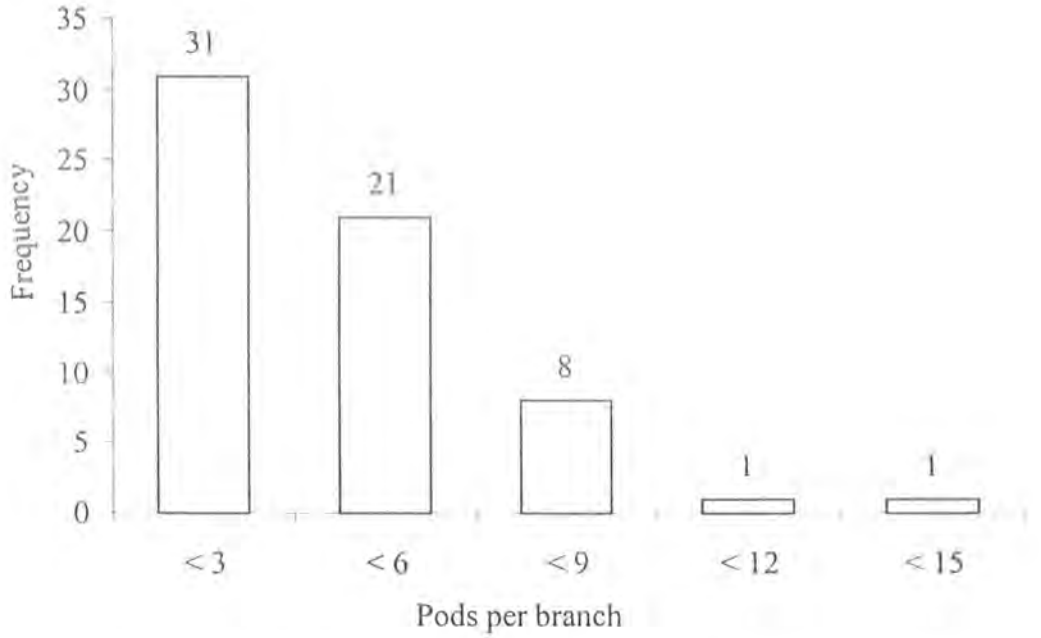

Fig. 4.2.6:- Frequency distribution for pods per branch in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

and it was followed by 13 accessions in the range up to one seed per pod (Fig. 4.2.7). Two checks (Paidar-91 and Punjab-91) and one accession (PAK-52984) produced high number of seeds per pod, hence were selected (Table 4.2.3). The frequency distribution regarding seed weight as depicted in the Fig. 4.2.8 revealed that twenty six accessions which were 41.9% of the population were having 12.1 to 16.0 g 100-seed weight, followed by the range up to < 12 g where fourteen accessions were observed. Eleven accessions (PAK-52972, PAK-52973, PAK-52974, PAK-52975, PUNJAB-91, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984) produced high seed weight (more than 20.0 g) and hence could be utilized for the manipulation of this trait in developing bold seeded chickpea cultivars as high seed weight in any grain crop is preferred by the consumers and high seed weight in desi types is not very frequently available in chickpea germplasm.

The biological yield ranged from 14.14 to 50.65 g per plant. Frequency distribution revealed that maximum number of accessions (29) which were 46.8% of the population produced 20.1 to 30.0 g biological yield and it was followed by the range 30.1-40.0 g where 18 accessions were observed (Fig. 4.2.9). Seven accessions (PAK-52975, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984) which were 11.3% of the total, produced more than 40 g biological yield whereas only one accession (PAK-52984) produced more than 50 g biological yield per plant and hence could be utilized for breeding chickpea for high biological yield production. On the basis of grain yield per plant, the germplasm ranged from 1.74 to 18.35 g and the frequency distribution presented in Fig. 4.2.10, revealed that the thirty three accessions which were 53.2% of the population produced 4.1 to 8.0 g grain yield/plant which was followed by fourteen accessions (22.6%) which produced 8.1 to 12.0 4.0 g. About 90% of the total germplasm under investigation, produced the grain yield up to 12.0 g per plant which is considered to be the medium range of grain yield in chickpea. In the present material, five accessions (PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984) and one check (Punjab-91) were observed as high yielding since they produced more than 24 g grain yield per plant. The accessions which produced more than 12 g grain yield per plant are listed in Table 4.2.3 and could be utilized for improving yield potential of chickpea.

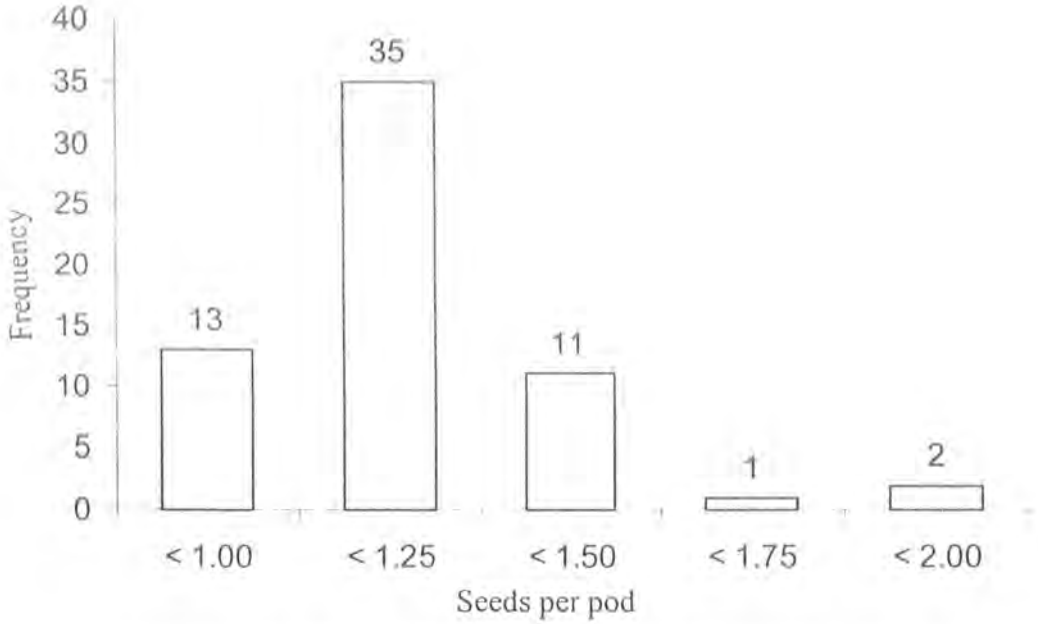

Fig. 4.2.7:- Frequency distribution for seeds per pod in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

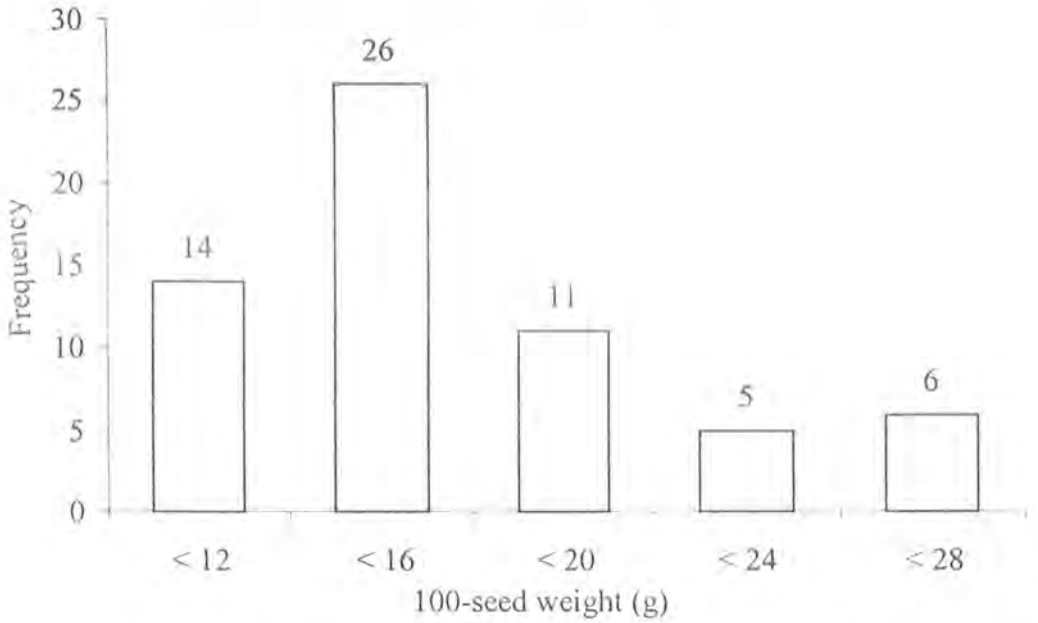

Fig. 4.2.8:- Frequency distribution for 100-seed weight in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

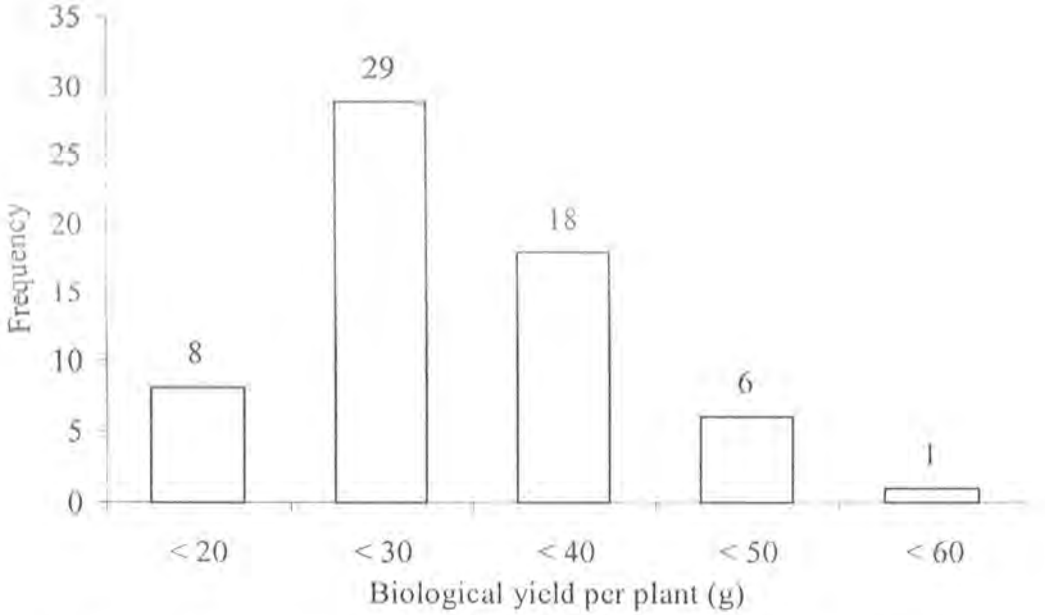

Fig. 4.2.9:- Frequency distribution for biological yield per plant in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

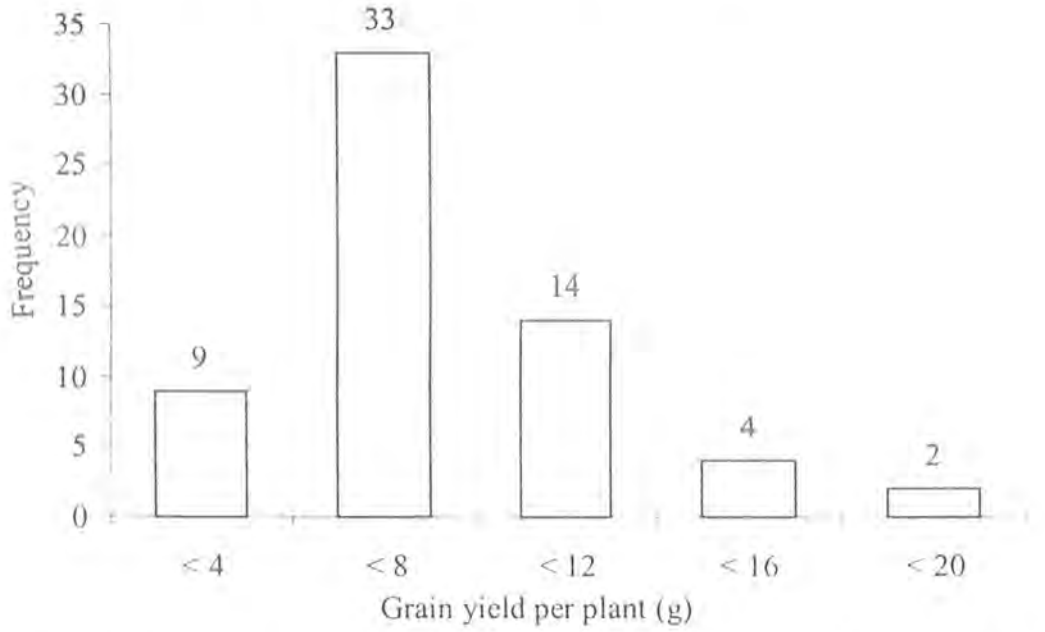

Fig. 4.2.10:- Frequency distribution for grain yield in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

Table 4.2.3:- Selected accessions for various traits

Character	Selection range	Accessions
Days to flowering	< 135 days	Punjab-91, Paidar-91, , 52983, 52932, 52922
Days to maturity	< 165 days	52983, 52932, 52922, 52975, 52931, 52935, 52953, 52966, 52971, 52978
Plant height	< 45 cm	52983, 52955, 52979, 52922, 52933, 52921, 52984, 52965, 52937, 52961, 52942
Branches per plant	> 15 branches	52971, 52970, 52973, 52972, 52975, 52974, 52978, 52979, 52980, 52981, 52983, 52984
Pods per plant	> 60 pods	52974, 52975, 52978, 52979, 52980, 52981, 52983, 52984
Pods per branch	> 6 pods	52961, 52949, 52966, 52962, 52964, 52973, 52960, 52970, 52984, 52968,
Seeds per pod	> 1.5 seeds	Paidar-91, 52984, Punjab-91
100-seed weight	> 20 g	52972, 52973, 52974, 52975, C 44, 52978, 52979, 52980, 52981, 52983, 52984
Biological yield per plant	> 40 g	52975, 52978, 52979, 52980, 52981, 52983, 52984
Grain yield per plant	> 12 g	52979, 52980, 52981, 52983, 52984, Punjab-91
Harvest index	> 30	52968, 52969, 52970, 52971, 52972, 52973, 52974, 52975, 52978, 52979, 52980, 52981, 52983, 52984, Punjab-91, Paidar-91

Frequency distribution regarding harvest index in chickpea as presented in the Fig. 4.2.11, revealed that the maximum accessions (29) which were 46.8% of the total, produced harvest index ranging from 20.1 to 30.0% and these were followed by thirteen accessions producing 10.1 to 20.0% harvest index. Sixteen accessions (PAK--52968, PAK-52969, PAK-52970, PAK-52971, PAK-52972, PAK-52973, PAK-52974, PAK-52975, PAK-52978, PAK-52979, PAK-52980, PAK-52981, PAK-52983, PAK-52984, Punjab-91, Paidar-91) gave more than 30% harvest index in the present study (Table 4.2.3). Both the checks also produced high harvest index and the accessions with higher harvest index than both the checks are suggested to be incorporated in the breeding programme. 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 4.2.3 which can be exploited for their genetic potential in future breeding programme.

4.2.4. Correlation Analysis

The correlation coefficients were computed among all the quantitative traits; days to flowering, days to maturity, plant height, branches per plant, pods per plant, pods/branch, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index. The results regarding correlation analysis are presented in the Table 4.2.4. The results regarding correlation revealed that days to flowering was significantly positive with days to maturity, whereas it was negative with plant height, seeds per pod, grain yield and harvest. Branches per plant exhibited significantly positive association with all the traits except days to flowering, days to maturity and plant height. Similarly, pods per plant had significantly positive correlation with all the traits except with days to flowering, days to maturity and plant height. 100-seed weight exhibited positive association with branches per plant, pods per plant, pods per branch, seeds per pod, grain yield, biological yield and harvest index. The positive association of 100-seed weight with seeds per pod was only possible because of the presence of large pod size accessions in the present material. Grain yield, biological and harvest index showed positively significant association with all the traits except with days to flowering, days to maturity and plant height. In the present study, out of eleven characters, eight were observed to be yield contributing for chickpea improvement.

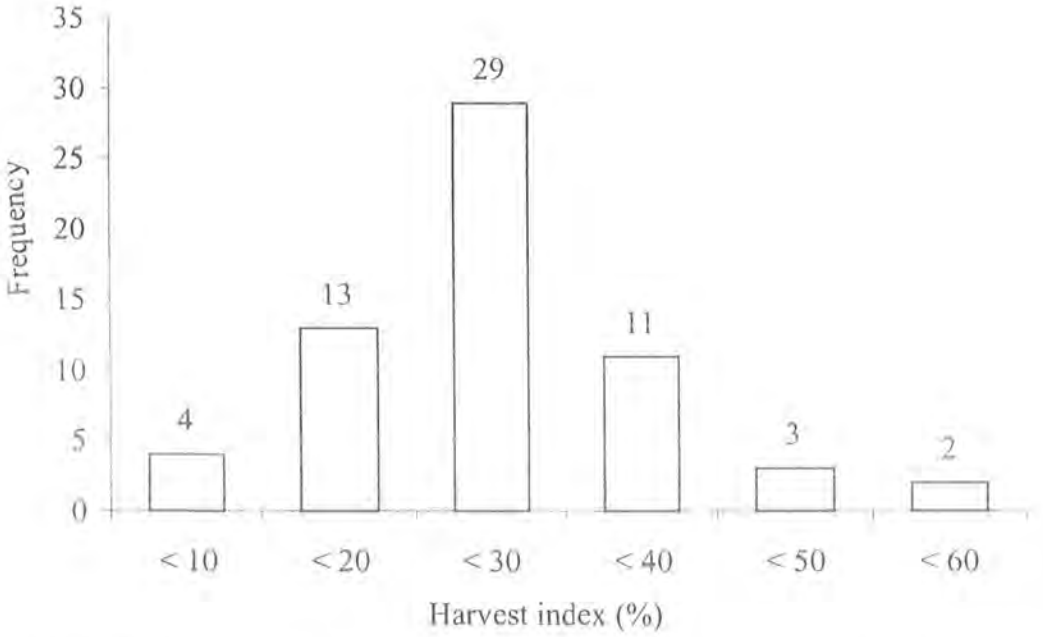

Fig. 4.2.11:- Frequency distribution for harvest index in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

Table 4.2.4:- Simple correlation coefficient among eleven quantitative traits in 62 accessions of chickpea evaluated during 1997

Quantitative Traits	Days to flowering	Days to maturity	Plant height (cm)	Number of branches	Pods per plant	Pods per branch	Seeds per pod	100-seed weight (g)	Biological yield (g)	Grain yield (g)
Days to maturity	0.6429**									
Plant height (cm)	-0.2504**	0.1027								
Number of branches	0.1411	-0.0085	-0.1965							
Pods per plant	0.0745	0.0459	-0.1449	0.9683**						
Pods per branch	0.0803	0.1054	0.1345	0.5721**	0.5946					
Seeds per pod	-0.3093**	0.0324	0.1449	0.7343**	0.7887	0.5496**				
100-seed weight (g)	-0.0461	0.0779	-0.0162	0.9160**	0.9580	0.5539**	0.8321**			
Biological yield (g)	0.0704	0.0180	-0.0754	0.9636**	0.9537	0.6076**	0.8106**	0.9400**		
Grain yield (g)	-0.2043**	0.1137	0.1259	0.8019**	0.8698	0.5428**	0.9498**	0.9379**	0.8753**	
Harvest index (%)	-0.3207**	0.0970	0.2354*	0.6999**	0.7755	0.5162**	0.9568**	0.8475**	0.7871**	0.9531**

4.2.5. *Principal Component Analysis (PCA)*

Variance due to quantitative traits was further studied by PCA, and a principal components matrix for eleven quantitative characters is given in Table 4.2.5. The first three principal components with eigenvalues more than 1 contributed 88.58% of the variability amongst 62 genotypes evaluated for eleven quantitative traits. Other components (PC_4 to PC_{11}) were less than unity hence could not prove their importance. Principal component 1 had 60.39% of the total variation, PC_2 16.45% and PC_3 11.75% of the total variation. Only PC_1 exhibited more than half of variability, hence considered cumulative of other components. Characters that contributed more positively to PC_1, were, branches (0.917), pods per plant (0.953), pods per branch (0.657), seeds per pod (0.919), 100-seed weight (0.968), biological yield (0.957), grain yield (0.963) and harvest index (0.909), whereas days to flowering contributed least to first component. Days to flowering (0.964) and maturity (0.689) contributed maximum genetic variance to PC_2 and plant height was assessed significant for PC_3. Days to maturity were contributed by all the factors but high effects were observed for PC_2.

All the characters under study contributed genetic variance positively towards PC_1 except days to flowering where it was negative. Eight characters (branches, pods per plant, pods per branch, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index) exhibited maximum effect on PC_1 and seven characters were positive for PC_2, out of which days to flowering and days to maturity contributed maximum. In more detail, the first PC which explained 60.39% of the variance is positively associated with all the characters except one and eight important yield contributing characters exhibited more positively, whereas days to flowering contributed least. This means that the populations with high PC_1 values are high yielding and formed by medium maturing plants characterized by high seed weight and harvest index. Seven characters contributed positively for PC_2 where days to flowering and days to maturity were observed with highest values for PC_2. It is evident that ten important plant characters contributed more positively to first 2 principal components and hence these could be established important for the material under investigation. The component 3 contributed maximum for plant height, although it had good share for days to maturity.

Table 4.2.5:- Principal Components (PCs) for 10 quantitative characters in 62 genotypes of chickpea

		PC_1	PC_2	PC_3
Eigen value		6.64	1.81	1.29
Proportion of σ^2		60.39	16.45	11.75
Commulative σ^2		60.39	76.83	88.58
	Communality		Eigen factor	
Days to flowering	0.944	-0.082	0.964	0.087
Days to maturity	0.872	0.667	0.689	0.627
Plant height	0.836	0.316	-0.415	0.814
Branches per plant	0.952	0.917	0.209	-0.261
Pods per plant	0.966	0.953	0.162	-0.179
Pods per branches	0.479	0.657	0.120	0.179
Seeds per pod	0.913	0.919	-0.234	0.120
100-seed weight (g)	0.943	0.968	0.048	-0.057
Biological yield per plant (g)	0.951	0.957	0.128	-0.139
Grain yield per plant (g)	0.954	0.963	-0.116	0.117
Harvest index (%)	0.934	0.909	-0.245	0.217

The first component is strongly associated with high yield potential and yield contribution traits, thus more related to reproductive phase, whereas second component is associated with days to flowering and days to maturity contributing 17.2% of the total variance, hence the populations in this component are more likely related to vegetative traits. The population with high PC_2 values are characterized by late flowering and maturity. The populations in this component are associated negatively with plant height, seeds per pod, grain yield and harvest index which revealed that the accessions in the population failed in appropriate partitioning of economic yield which ultimately reduced harvest index.

First 3 components which contributed 88.6% of the total variance, were plotted graphically to observe the relationship between 62 accessions of chickpea for these components. The factor 1 contributing 60.4% of the variability was kept as x-axis in both the cases, whereas factor 2 and 3 were plotted against y-axis simultaneously. The separation on the basis of both graphs gave similar results. As the accessions were plotted on the basis of geographic origin and source of seed collection, hence these were investigated to see whether the genetic diversity was related to geographic origin or not. The PC_1 and 2 revealed one group in the left upper half, one in the right upper half, one in between of these two groups and one consisting checks was observed in the lower half of the graph (Fig. 4.2.12). Similar results were observed in the Fig. 4.2.13 where factor 3 was plotted against y-axis instead of factor 2. Three clusters consisting of accessions were in the middle of the graph with similar pattern as in the graph plotted for factor 1 and 2. The only difference was that the varieties were shifted in the upper half of the graph.

4.2.6. Cluster analysis

A Euclidean dissimilarity coefficient matrix was calculated for 62 chickpea accessions from the morphological data and phenogram constructed is presented in the Fig. 4.2.14 and the accessions in each cluster are presented in the Table 4.2.6. The cluster diagram using Ward's method revealed 2 major groups and if it is observed critically, six clusters were observed. The group A consisted of 4 and group B consisted of 2 clusters. Both the checks were grouped together in cluster II. Cluster I consisted of five accessions, cluster II consisted of 2 checks (Punjab-91 and Paidar 91). Cluster III comprised of 12 accessions, cluster IV of seven accessions, cluster V of 22 accessions and cluster VI consisted of 13 accessions.

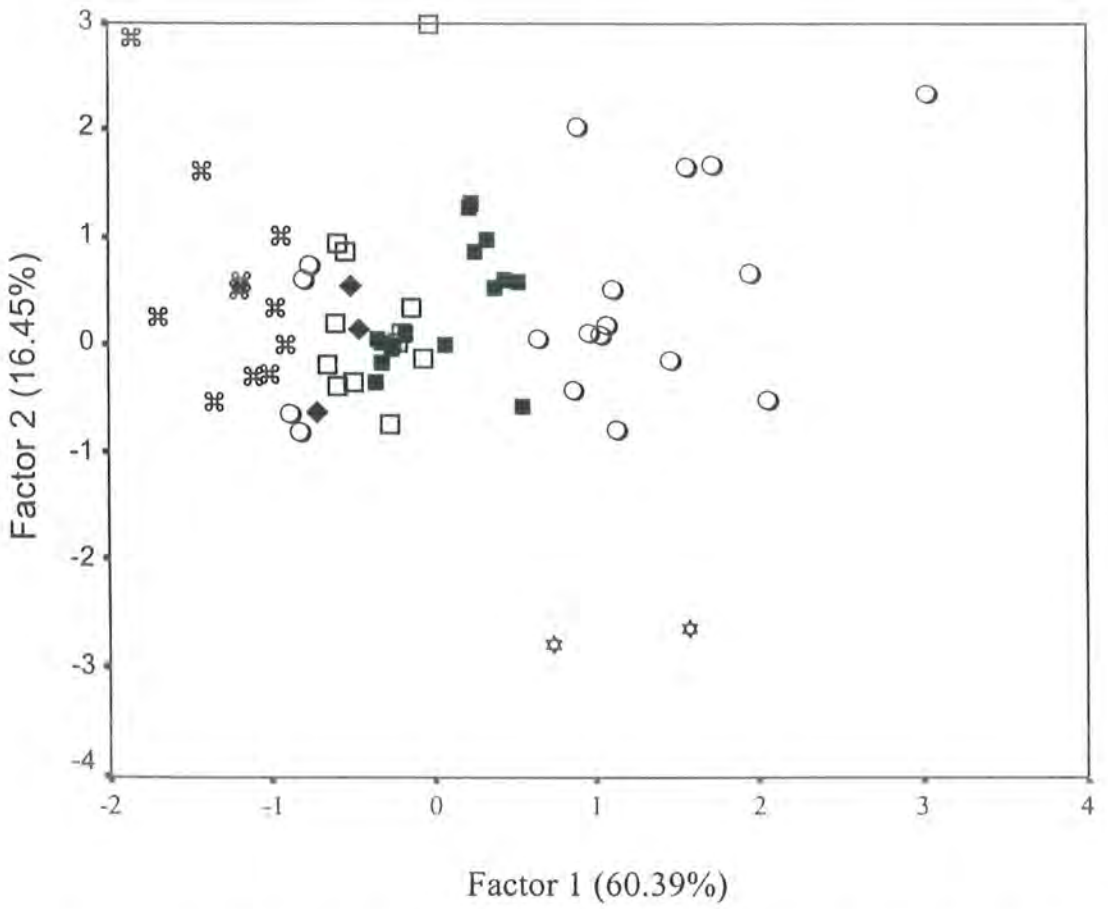

Fig.4.2.12:- Scattered diagram based on quantitative traits for first and second factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, ◆- District Mianwali, ⌘- Bahawalnagar and ✡- approved varieties

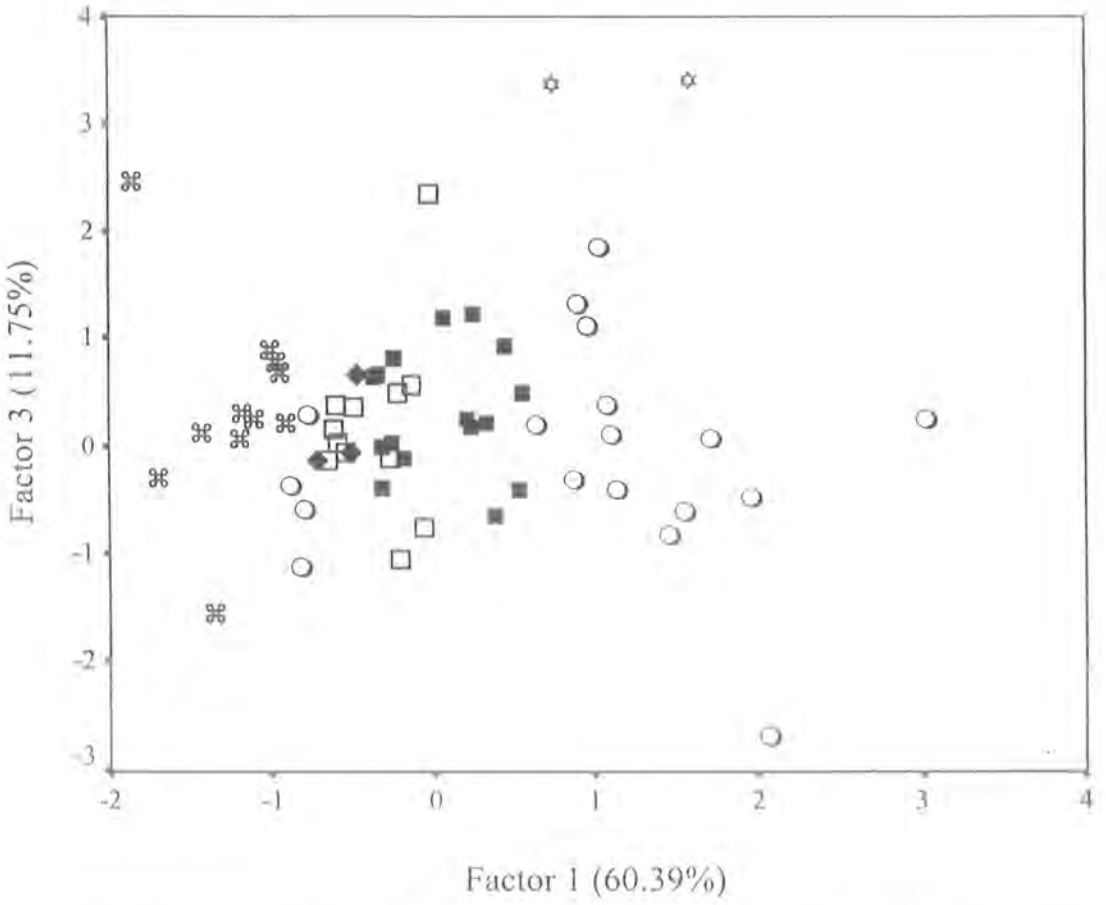

Fig.4.2.13:- Scattered diagram based on quantitative traits for first and third factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, ◆- District Mianwali, ⌘- Bahawalnagar and ✡- approved varieties

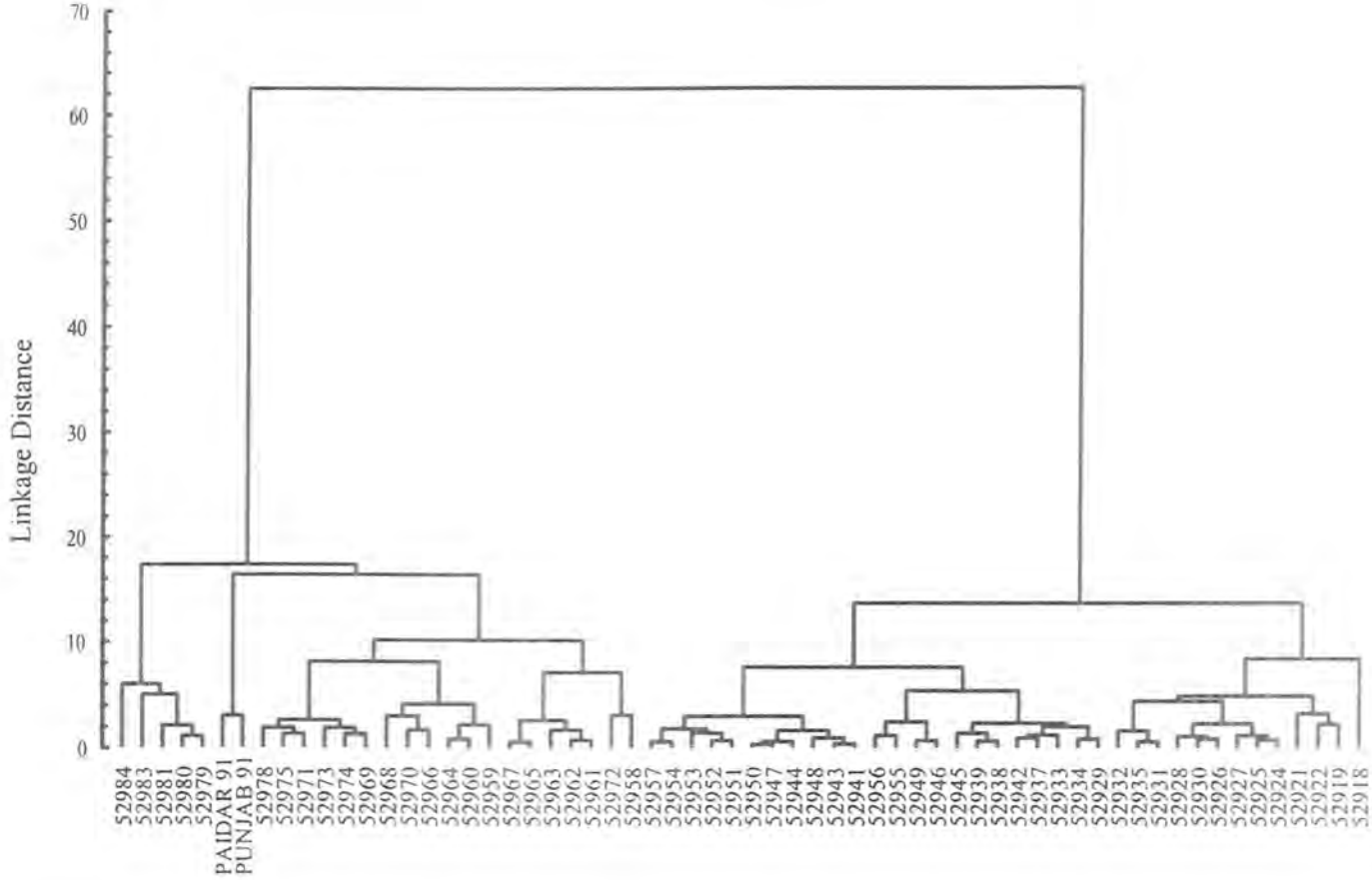

Fig. 4.2.14:- Cluster diagram of 62 chickpea accessions along with 2 checks based on quantitative traits

Table 4.2.6:- Clusters based on quantitative characters in chickpea

Group	Cluster	Frequency	Accessions
Group A	Cluster I	5	52984, 52983, 52981, 52980, 52979
	Cluster II	2	Paidar 91, Punjab-91
	Cluster III	12	52978, 52975, 52971, 52973, 52974, 52969, 52968, 52970, 52966, 52964, 52960, 52959
	Cluster IV	7	52967, 52965, 52963, 52962, 52961, 52972, 52958
Group B	Cluster V	23	52957, 52954, 52953, 52952, 52951, 52950, 52947, 52944, 52948, 52943, 52941, 52956, 52955, 52949, 52946, 52945, 52939, 52938, 52942, 52937, 52933, 52934, 52929
	Cluster VI	13	52932, 52935, 52931, 52928, 52930, 52926, 52927, 52925, 52924, 52921, 52922, 52919, 52918

As this cluster analysis is based on agriculturally important characters, hence both the checks were categorized in one group that may be because of selection pressure for high yield potential and other related characters.

Five accessions (PAK-52984, PAK-52983, PAK-52981, PAK-52980, PAK-52979) were observed in Cluster I, two approved varieties were in cluster II, twelve accessions (PAK-52978, PAK-52975, PAK-52971, PAK-52973, PAK-52974, PAK-52969, PAK-52968, PAK-52970, PAK-52966, PAK-52964, PAK-52960, PAK-52959) were in cluster III, seven accessions (PAK-52967, PAK-52965, PAK-52963, PAK-52962, PAK-52961, PAK-52972, PAK-52958) were in cluster IV. Group B comprised of two clusters, i.e., cluster V and VI which consisted of twenty three and thirteen accessions, respectively. The average performance and range along with genetic variance for six clusters are presented in the Tables 4.2.7 to 4.2.12. The members of cluster I were late in flowering (142±2.74 days), having high number of pods (98.5±6.16) and grain yield (13.68±0.95 g). The members of this cluster might be tested under a wide range of environments to select the best cultivars. The cluster II consisted of two approved varieties and his cluster gave high average values for most of the characters and exhibited the highest grain yield (15.15±3.20 g). But high variance for grain yield revealed that one of the varieties could not perform consistently thus gave high variance that could be lowered by simple selection of superior plants and further multiplication for general cultivation.

Cluster III consisted of 12 genotypes which gave average of 140±0.57 days to flowering, 169±0.92 days to maturity and 9.43±0.46 g grain yield, therefore, were classified as early to medium maturing with low to medium grain yield (Table 4.2.9). High genetic variance for yield and its contributing characters was observed that could be exploited through simple selection. Cluster IV consisted of 7 genotypes and gave the average grain yield of 8.10±0.11 g with low genetic variance, therefore, selected accessions from this cluster might be utilized in hybrid programme for crop improvement. This cluster was also categorized as medium maturing (175±2.06 days).

Cluster V consisted of maximum number (23) of genotypes that were categorized as early maturing (170±0.51 days) and low yielder (5.79±0.17 g). The accessions of this cluster gave 13.19±0.20 g 100-seed weight along with low to

Table 4.2.7:- Range, means, SE and variance for 10 quantitative traits for cluster 1 in chickpea

Quantitative traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	142±2.74	6.12	37.50	132	148
Days to maturity	172±2.69	6.02	36.30	163	178
Plant height (cm)	43.8±1.91	4.27	18.27	37.6	48.0
Number of branches	20.7±0.78	1.74	3.03	18.5	22.6
Pods per plant	98.5±6.16	13.78	189.79	89.6	122.7
Pods per branch	6.1±1.32	2.95	8.73	3.9	11.2
Seeds per pod	1.5±0.08	0.18	0.03	1.4	1.8
100-seed weight (g)	26.50±0.42	0.94	0.89	25.40	27.49
Biological yield (g)	45.27±1.44	3.22	10.40	42.32	50.65
Grain yield (g)	13.68±0.95	2.12	4.49	12.02	17.36
Harvest index (%)	40.41±1.76	3.93	15.46	35.71	45.14

Table 4.2.8:- Range, means, SE and variance for 10 quantitative traits for cluster 2 in chickpea. This cluster consisted of 2 approved varieties, i.e., Punjab 91 and Paidar 91

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	124±0.00	0.00	0.00	124	124
Days to maturity	175±1.00	1.41	2.00	174	176
Plant height (cm)	66.7±0.20	0.28	0.08	66.5	66.9
Number of branches	9.0±0.78	1.10	1.20	8.3	9.8
Pods per plant	78.3±0.55	0.78	0.61	37.7	38.8
Pods per branch	4.3±0.31	0.43	0.19	4.0	4.6
Seeds per pod	1.7±0.12	0.16	0.03	1.6	1.8
100-seed weight (g)	20.25±3.27	4.62	21.39	16.98	23.52
Biological yield (g)	27.57±6.40	9.05	81.92	21.17	33.97
Grain yield (g)	15.15±3.20	4.53	20.48	11.95	18.35
Harvest index (%)	55.44±1.08	1.53	2.33	54.36	56.52

Table 4.2.9:- Range, means, SE and variance for 10 quantitative traits for cluster 3 in chickpea

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140±0.57	1.96	3.84	136	143
Days to maturity	169±0.92	3.17	10.06	164	174
Plant height (cm)	55.4±1.12	3.88	15.02	49.7	62.7
Number of branches	15.1±0.46	1.59	2.54	12.9	17.5
Pods per plant	57.1±3.67	12.71	161.53	39.3	86.7
Pods per branch	6.0±0.82	2.84	8.05	2.8	13.1
Seeds per pod	1.2±0.02	0.07	0.00	1.13	1.33
100-seed weight (g)	19.52±0.82	2.83	8.02	15.37	24.35
Biological yield (g)	36.88±1.03	3.56	12.64	30.47	41.93
Grain yield (g)	9.43±0.46	1.59	2.54	7.33	11.73
Harvest index (%)	31.01±0.85	2.94	8.64	26.42	34.68

Table 4.2.10:- Range, means, SE and variance for 10 quantitative traits for cluster 4 in chickpea

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	145±2.02	5.34	28.48	140	155
Days to maturity	175±2.06	5.44	29.62	170	185
Plant height (cm)	48.0±1.54	4.07	16.58	44.1	54.4
Number of branches	13.8±0.44	1.17	1.36	12.9	16.4
Pods per plant	47.5±2.86	7.56	57.10	36.1	58.6
Pods per branch	4.3±0.58	1.54	2.37	2.6	6.7
Seeds per pod	1.2±0.02	0.06	0.00	1.1	1.3
100-seed weight (g)	17.09±0.78	2.05	4.21	14.99	21.19
Biological yield (g)	33.87±1.13	2.98	8.87	30.11	38.72
Grain yield (g)	8.10±0.44	1.15	1.33	7.01	10.56
Harvest index (%)	28.27±0.91	2.39	5.74	25.85	32.93

Table 4.2.11:- Range, means, SE and variance for 10 quantitative traits for cluster 5 in chickpea

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140±0.35	1.66	2.75	137	143
Days to maturity	170±0.51	2.44	5.96	165	175
Plant height (cm)	50.1±0.91	4.35	18.94	41.8	57.2
Number of branches	10.9±0.15	0.71	0.51	9.5	12.3
Pods per plant	28.8±1.02	4.91	24.07	18.8	35.2
Pods per branch	2.6±0.27	1.31	1.72	1.0	6.3
Seeds per pod	1.1±0.01	0.04	0.00	1.0	1.1
100-seed weight (g)	13.19±0.20	0.94	0.88	11.63	14.81
Biological yield (g)	26.07±0.54	2.59	6.71	20.83	29.90
Grain yield (g)	5.79±0.17	0.80	0.65	4.05	6.90
Harvest index (%)	22.29±0.58	2.80	7.82	15.48	25.57

Table 4.2.12:- Range, means, SE and variance for 10 quantitative traits for cluster 6 in chickpea

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	139.9±1.35	4.86	23.58	134	153
Days to maturity	169.5±1.66	5.98	35.77	163	185
Plant height (cm)	50.5±1.28	4.62	21.36	42.8	58.2
Number of branches	8.6±0.37	1.35	1.82	5.8	10.1
Pods per plant	16.2±1.21	4.36	19.02	7.5	21.1
Pods per branch	2.1±0.27	0.97	0.95	0.7	3.9
Seeds per pod	0.9±0.05	0.17	0.03	0.5	1.0
100-seed weight (g)	11.37±0.12	0.45	0.20	10.88	12.09
Biological yield (g)	19.11±0.66	2.39	5.72	14.14	23.15
Grain yield (g)	3.39±0.26	0.93	0.87	1.74	4.88
Harvest index (%)	12.87±0.97	3.49	12.20	7.15	18.33

high genetic variance for various characters. Thirteen accessions grouped together in cluster VI that were categorized early to medium maturing (169±1.66 days), low grain weight (11.37±0.12 g 100-seed weight) and low grain yield (3.39±0.26 g). The accessions of this cluster grouped together with greater similarity in poor performance. The genotypes in the clusters I and II were observed high yielding and medium maturing along with high seed weight, whereas genotypes of cluster III were early and medium yielding. Selected genotypes from clusters I and III are suggested to be used in crop improvement programme.

4.2.7. Harvest Index, an Important Selection Parameter

Harvest index is an important trait in determining yield potential of grain crops. Green revolution in cereals is largely considered due to tremendous increase in harvest index that enhanced the worldwide cereal productivity. Similar emphasis is being given in legumes to select genotypes with appropriate harvest index. Harvest index in legumes is very much sensitive to environmental fluctuations and it is imperative to find the optimum range of harvest index. In order to find the optimum harvest index along with other desirable traits, all the accessions were classified into various groups on the basis of harvest index classes (Table 4.2.13). Out of 62 genotypes, eight gave harvest index < 15 percent, nine have produced < 20 percent harvest index (Fig. 4.2.15). Maximum genotypes (17) exhibited harvest index from 20.1 to 25.0 percent and these were followed by 12 genotypes which produced from 25.1 to 30.0 percent harvest index. Nine genotypes were observed in the range of 30.1-35.0 percent harvest index, whereas seven genotypes gave more than 35 percent harvest index and these were suggested to be utilized in future breeding programme,

The accessions with harvest index less than 10.0%, produced 16.59±0.92 g biological yield and low grain yield of 2.35±0.21 g. Similarly, the accessions producing less than 20 percent harvest index did not show any worth and could be discarded at this stage from further evaluation. The accessions which gave harvest index of more than 25 percent were observed better for most of the characters. It was observed from the present study that the accessions with high harvest index should be evaluated for further crop improvement and the accessions with high harvest index might be used as one of the parents in hybridization programme.

Table 4.2.13:- Analysis on the basis of harvest index in 62 accessions

Characters	< 10 harvest index				< 15 harvest index				< 20 harvest index			
	Mean± SE	SD	Min.	Max.	Mean± SE	SD	Min.	Max.	Mean± SE	SD	Min.	Max.
Days to flowering	143± 3.92	7.83	135	153	140± 0.75	1.50	138	141	139± 0.99	2.96	134	143
Days to maturity	173± 4.73	9.46	163	185	170± 0.85	1.71	168	172	169± 1.42	4.27	163	175
Plant height	47.8± 3.07	6.15	42.8	56.3	51.6± 1.25	2.50	49.1	53.8	49.5± 1.56	4.69	43.1	58.2
Branches/plant	6.9± 0.45	0.90	5.8	7.9	8.9± 0.09	0.19	8.6	9.0	9.8± 0.11	0.33	9.2	10.2
Pods/plant	11.0± 1.86	3.72	7.5	15.7	16.6± 0.17	0.34	16.2	17.0	20.3± 0.44	1.31	17.6	21.7
Pods/Branch	1.1± 0.18	0.37	0.7	1.5	2.7± 0.58	1.16	1.3	3.9	2.6± 0.28	0.84	1.7	4.6
Seeds/pod	0.7± 0.09	0.18	0.5	0.8	0.9± 0.02	0.04	0.9	0.9	1.0± 0.01	0.04	0.9	1.1
100-SW	10.92± 0.01	0.03	10.88	10.95	11.25± 0.14	0.29	10.95	11.50	11.88± 0.07	0.20	11.57	12.10
Biological yield	16.59± 0.92	1.83	14.14	18.21	18.73± 0.26	0.53	18.23	19.33	21.95± 0.41	1.24	20.26	23.79
Grain yield	2.35± 0.21	0.43	1.74	2.67	3.23± 0.17	0.34	2.87	3.63	4.45± 0.13	0.38	3.85	4.94
Harvest index.	8.61± 0.56	1.11	7.15	9.55	12.89± 0.73	1.46	11.04	14.26	16.69± 0.45	1.36	15.01	18.97

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Characters	< 25 harvest index				< 30 harvest index				< 35 harvest index			
	Mean± SE	SD	Min.	Max.	Mean± SE	SD	Min.	Max.	Mean± SE	SD	Min.	Max.
Days to flowering	139± 0.33	1.35	137	142	142± 1.27	4.40	138	155	140± 1.21	3.63	136	149
Days to maturity	169± 0.45	1.84	165	173	172± 1.48	5.12	165	185	169± 1.59	4.76	164	180
Plant height	51.0± 1.03	4.27	41.8	57.2	50.8± 1.62	5.60	44.1	59.9	54.5± 1.36	4.09	49.7	62.7
Branches/plant	11.0± 0.11	0.45	10.3	11.6	13.2± 0.15	0.52	12.2	13.9	16.1± 0.32	0.97	14.7	17.5
Pods/plant	30.0± 0.76	3.13	21.8	34.7	43.9± 1.99	6.88	35.1	53.1	62.4± 3.44	10.32	54.4	86.7
Pods/Branch	2.6± 0.33	1.38	1.0	6.3	4.8± 0.52	1.80	1.6	7.1	5.6± 1.13	3.39	2.6	13.1
Seeds/pod	1.1± 0.01	0.03	1.1	1.1	1.2± 0.01	0.04	1.1	1.2	1.3± 0.01	0.03	1.2	1.3
100-SW	13.30± 0.17	0.69	12.31	14.27	16.09± 0.31	1.06	14.77	17.87	21.15± 0.56	1.67	19.33	24.35
Biological yield	26.45± 0.48	1.99	23.93	29.40	32.37± 0.65	2.25	29.81	36.47	38.99± 0.45	1.36	37.62	41.93
Grain yield	5.95± 0.14	0.57	5.11	6.72	7.59± 0.14	0.50	6.85	8.23	10.29± 0.39	1.18	8.49	11.73
Harvest index.	23.10± 0.34	1.41	20.69	24.98	27.12± 0.38	1.33	25.45	29.81	32.84± 0.40	1.19	30.82	34.68

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Cahracters	< 40 harvest index					< 45 harvest index					> 45 harvest index				
	Mean	±SE	SD	Min.	Max.	Mean	±SE	SD	Min.	Max.	Mean	±SE	SD	Min.	Max.
Days to flowering	145	±0.50	0.71	144	145	137	±4.50	6.36	132	141	132	±8.00	13.86	124	148
Days to maturity	176	±0.50	0.71	175	176	167	±3.50	4.95	163	170	176	±1.15	2.00	174	178
Plant height	44.9	±2.70	3.82	42.2	47.6	42.8	±5.20	7.35	37.6	48.0	59.1	±7.63	13.22	44	67
Branches/plant	19.1	±0.60	0.85	18.5	19.7	21.4	±0.95	1.34	20.4	22.3	13.6	±4.55	7.88	8	23
Pods/plant	90.1	±0.45	0.64	89.6	90.5	94.9	±1.35	1.91	93.5	96.2	66.4	±28.15	48.76	38	123
Pods/Branch	4.7	±0.33	0.46	4.4	5.0	4.9	±0.91	1.29	3.9	5.8	6.6	±2.32	4.02	4	11
Seeds/pod	1.4	±0.01	0.01	1.4	1.4	1.4	±0.04	0.05	1.4	1.5	1.7	±0.07	0.13	2	2
100-SW	25.56	±0.17	0.24	25.40	25.73	26.94	±0.44	0.62	26.50	27.37	22.66	±3.06	5.31	16.98	27.49
Biological yield	42.91	±0.59	0.83	42.32	43.50	44.96	±0.56	0.79	44.40	45.51	35.26	±8.53	14.78	21.17	50.65
Grain yield	12.29	±0.26	0.37	12.02	12.55	13.24	±0.05	0.08	13.19	13.30	15.89	±1.99	3.45	11.95	18.35
Harvest index.	36.36	0.64	0.91	35.71	37.00	42.10	0.14	0.20	41.95	42.24	52.01	3.49	6.04	45.14	56.52

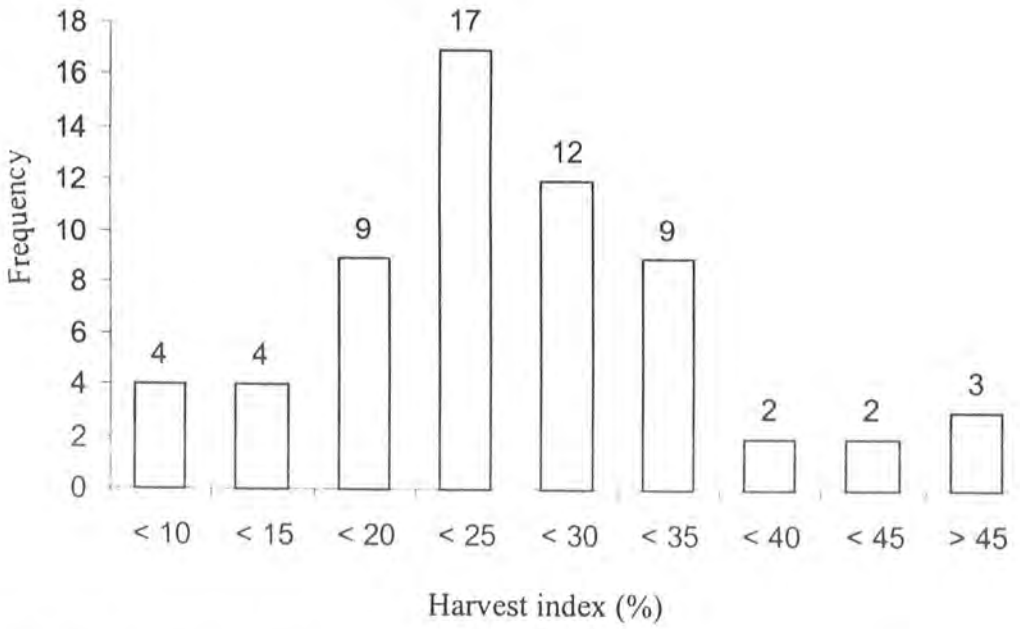


Fig. 4.2.15:- Frequency distribution for harvest index in 62 accessions along with 2 checks of chickpea collected from five districts of Punjab

On the basis of performance and results based on harvest index, eleven best accessions were identified and listed in Table 4.2.14. It was important to note that all these accessions were collected from the district Layyah from field areas. In total, the germplasm was collected from five chickpea growing districts of Punjab and seed was collected from field or market, but only the accessions collected from field areas of District Layyah could prove their superiority. Although, all the selected accessions were lower in grain yield than one of the check (Punjab-91), but superior single plants could be selected from these accessions for further testing. The progenies of selected single plants could be tested under adaptive yield trial to find better adaptive cultivars producing higher grain yield.

4.2.8. Correlation Analysis

The correlation coefficients were computed among all the quantitative traits; days to flowering, days to maturity, plant height, branches, pods, pods/branch, seeds/pod, 100-seed weight, biological yield, grain yield and harvest index. Correlation analysis was conducted for all the six clusters observed in the analysis (Table 4.2.15). Cluster II consisted of 2 approved varieties, therefore correlation coefficients for this cluster was not calculated. Further, both of these were approved varieties and selection from pure-lines could not be practiced. Days to flowering revealed positively significant correlation with days to maturity in all the clusters. Seeds per pod vs pods per plant, seed weight vs branches per plant and biological yield per plant vs pods per plant also exhibited significantly positive correlation coefficients among each other in all the clusters. Grain yield per plant gave significantly positive association with pods per plant, seeds per pod and biological yield per plant in all the clusters under study. Harvest index exhibited significantly positive correlation with branches per plant, 100-seed weight and biological yield per plant in all the clusters. Similar correlation coefficients for all the cluster could be exploited for chickpea improvement through simple selection from the germplasm.

Cluster I consisted of 5 accessions, and if this cluster was not taken in consideration, the character pairs, i.e., pods per plant vs branches per plant and seeds per pod vs branches per pod gave significantly positive association. The 100-seed weight exhibited significantly positive correlation with pods per plant and seeds per pod. Biological yield and grain yield gave positively significant association with branches and 100-seed weight in both the cases. Harvest index showed significantly

Accession No.	Origin	Source	D.F.	D.M.	P.Ht.	Br.No.	Pds/pt.	Pds/Br.	Sds/pd.	100S.W.	B.Y.	G.Y.	H.I.
52971	Layyah	Field	138	165	52.3	15.6	56.8	4.4	1.3	19.97	38.17	10.52	32.88
52972	Layyah	Field	149	180	53	16.4	58.6	2.6	1.3	21.19	38.72	10.56	32.93
52973	Layyah	Field	141	170	50.2	16.4	59.6	6.9	1.3	21.46	38.97	10.74	33.29
52974	Layyah	Field	140	170	54.9	16.9	65.3	4.4	1.3	21.57	39.43	10.81	33.40
52975	Layyah	Field	136	164	54.8	16.9	69	2.9	1.3	22.92	40.09	11.71	33.95
52978	Layyah	Field	138	165	49.7	17.5	86.7	5.2	1.3	24.35	41.93	11.73	34.68
52979	Layyah	Field	144	175	42.2	18.5	89.6	4.4	1.4	25.40	42.32	12.02	35.71
52980	Layyah	Field	145	176	47.6	19.7	90.5	5.0	1.4	25.73	43.50	12.55	37.00
52981	Layyah	Field	141	170	48	20.4	93.5	5.8	1.4	26.50	44.40	13.19	41.95
52983	Layyah	Field	132	163	37.6	22.3	96.2	3.9	1.5	27.37	45.51	13.30	42.24
52984	Layyah	Field	148	178	43.8	22.6	122.7	11.2	1.8	27.49	50.65	17.36	45.14
Punjab-91	Variety		124	176	66.5	9.8	38.8	4.0	1.8	23.52	33.97	18.35	54.36
Paidar-91	Variety		124	174	66.9	8.25	37.7	4.6	1.6	16.98	21.17	11.95	56.52

Table 4.2.15: - Phenotypic correlation coefficient among eleven quantitative traits in 62 genotypes of chickpea

Cluster I		DF	DM	PH.	Br/Pl	Pods/ Pl	Pods/ Br	Seeds/ P	SW	BY	GY
Days to maturity	Cluster 1	0.99**									
	Cluster 3	0.97**									
	Cluster 4	0.98**									
	Cluster 5	0.95**									
	Cluster 6	0.99**									
Plant height	Cluster 1	0.63	0.56								
	Cluster 3	0.11	0.22								
	Cluster 4	0.94**	0.91**								
	Cluster 5	-0.20	-0.19								
	Cluster 6	0.33	0.32								
Branches/plant	Cluster 1	-0.27	-0.31	-0.39							
	Cluster 3	-0.57	-0.50	-0.53							
	Cluster 4	0.12	0.14	0.36							
	Cluster 5	-0.52	-0.52	0.19							
	Cluster 6	-0.71**	-0.71**	0.13							
Pods/plant	Cluster 1	0.39	0.35	-0.10	0.74						
	Cluster 3	-0.65*	-0.62*	-0.52	0.90**						
	Cluster 4	-0.37	-0.40	-0.09	0.82**						
	Cluster 5	-0.54**	-0.55**	0.25	0.95**						
	Cluster 6	-0.72**	-0.73**	0.04	0.99**						
Pods/branch	Cluster 1	0.63	0.58	0.21	0.54	0.95**					
	Cluster 3	0.32	0.38	0.51	-0.21	-0.19					
	Cluster 4	-0.48	-0.41	-0.55	-0.45	-0.37					
	Cluster 5	0.06	-0.06	-0.13	-0.04	-0.01					
	Cluster 6	-0.27	-0.29	0.06	0.67*	0.66*					
Seeds/pod	Cluster 1	0.29	0.25	-0.18	0.81	0.99**	0.91				
	Cluster 3	-0.68*	-0.63*	-0.46	0.95**	0.89**	-0.13				
	Cluster 4	-0.17	-0.22	0.07	0.86**	0.92**	-0.44				
	Cluster 5	-0.58	-0.57	0.09	0.94**	0.90**	-0.06				
	Cluster 6	-0.71**	-0.71**	0.00	0.96**	0.96**	0.65				
100-seed weight	Cluster 1	-0.32	-0.37	-0.41	0.99**	0.73	0.53	0.80			
	Cluster 3	-0.64*	-0.58*	-0.50	0.98**	0.95**	-0.18	0.96**			
	Cluster 4	-0.04	-0.06	0.23	0.97**	0.92**	-0.47	0.94**			
	Cluster 5	-0.51*	-0.51*	0.19	0.98**	0.94**	-0.03	0.92**			
	Cluster 6	-0.55*	-0.58*	0.29	0.85**	0.84**	0.46	0.78**			
Biological yield	Cluster 1	0.25	0.20	-0.13	0.85	0.98**	0.90*	0.99**	0.84		
	Cluster 3	-0.65**	-0.58*	-0.43	0.94**	0.91**	-0.08	0.99**	0.97**		
	Cluster 4	-0.24	-0.28	0.02	0.87**	0.98**	-0.43	0.97**	0.96**		
	Cluster 5	-0.54**	-0.55**	0.17	0.98**	0.93**	-0.05	0.96**	0.98**		
	Cluster 6	-0.73**	-0.74**	0.03	0.95**	0.95**	0.57*	0.93**	0.90**		
Grain yield	Cluster 1	0.38	0.33	-0.04	0.77	1.00**	0.95*	0.99**	0.75	0.99**	
	Cluster 3	-0.66*	-0.62*	-0.61*	0.97**	0.89**	-0.32	0.92**	0.95**	0.90**	
	Cluster 4	0.03	0.04	0.27	0.99**	0.86**	-0.44	0.90**	0.98**	0.90**	
	Cluster 5	-0.55**	-0.56**	0.21	0.98**	0.96**	-0.04	0.96**	0.98**	0.99**	
	Cluster 6	-0.67**	-0.69**	0.17	0.94**	0.93**	0.57*	0.89**	0.96**	0.98**	
Harvest index	Cluster 1	-0.12	-0.20	-0.15	0.92**	0.79	0.68	0.84	0.95**	0.88**	0.83
	Cluster 3	-0.65*	-0.59*	-0.48	0.97**	0.87**	-0.19	0.99**	0.97**	0.98**	0.94**
	Cluster 4	-0.05	-0.07	0.21	0.96**	0.91**	-0.43	0.96**	0.99**	0.96**	0.97**
	Cluster 5	-0.61**	-0.60**	0.25	0.94**	0.97**	0.00	0.94**	0.92**	0.93**	0.96**
	Cluster 6	-0.62**	-0.62**	0.27	0.95**	0.93**	0.60*	0.90**	0.95**	0.96**	0.98**

positive correlation with pods per plant, seeds per pod and grain yield. The clusters III, IV, V and VI represent 88.7 percent of the germpalsm evaluated, hence the correlation coefficients observed for these clusters could also be considered in broader spectrum, hence used to improve chickpea through simple selection from the local germpalsm. In cluster IV, plant height exhibited positive correlation with days to flowering and days to maturity, whereas in other clusters insignificant association for these characters was observed.

In clusters V and VI, branches per plant had significantly negative association with days to flowering and days to maturity, whereas in other clusters it was insignificant. Similarly, pods per plant exhibited significantly negative correlation with days to flowering and maturity in clusters III, V and VI. Pods per plant, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index had significantly negative correlation with days to flowering and maturity in clusters III, V and VI.

These clusters consisted of forty eight accessions, hence from these accessions these results are needed to be given consideration while selecting superior cultivars of chickpea. In cluster III, grain yield also exhibited significantly negative correlation with plant height, whereas it was insignificant in other clusters. In cluster I and VI, pods per branch exhibited significantly positive association with pods per plant, whereas this character pair was insignificant and negative in other clusters. Similarly, in clusters I and VI, biological yield and grain yield exhibited positive correlation with pods per branch and this character pair was insignificantly negative in other clusters. The correlation coefficients observed with similar magnitude in all the clusters or clusters III, IV, V and VI, could be exploited for chickpea improvement. In the clusters where specific correlation pattern was observed, could be studied more precisely for better utilization of these linkages and to break undesirables linkages observed in some clusters, bi-parental selective mating system could be followed.

The graphic presentation of mean values and variance for grain yield based on six clusters revealed that cluster II which consisted of check varieties were high yielding and gave high standard error and variance (Fig. 4.2.16). High standard error observed in check varieties revealed that these might be influenced more by environmental fluctuations and yield under stress might be poor as compared to local

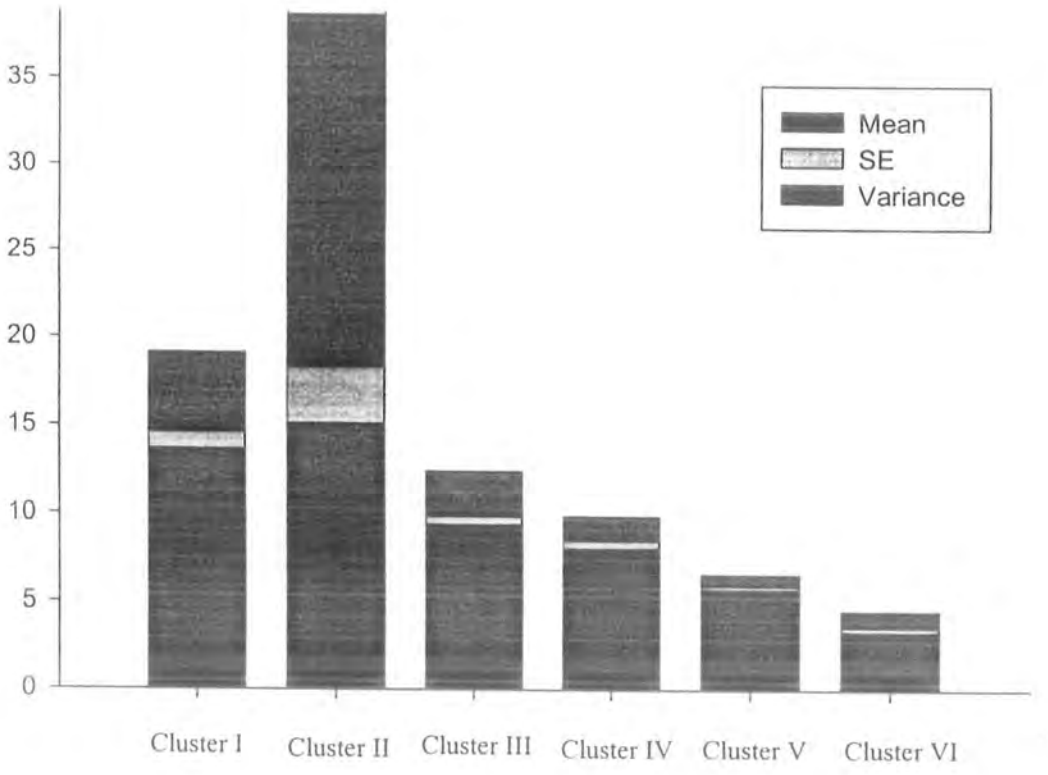


Fig. 4.2.16:- Mean, standard error and variance for grain yield in six clusters constructed on the basis of quantitative traits in chickpea

landraces. The cluster I consisted of five accessions (PAK-52984, PAK-52983, PAK-52981, PAK-52980, PAK-52979) gave high average grain yield and medium variance that gave an indication that selected accession from this cluster might be exploited for chickpea improvement.

The accessions of cluster VI gave low grain yield along with low variance. Similarly, graph for 100-seed weight was also plotted for average, standard error and variance in the Fig. 4.2.17. Similar trend was also observed in this trait. The accessions of cluster I gave high average value for 100-seed weight along with low standard error and low variance. These accessions could be tested under a wide range of environmental conditions of the country to identify the best high yielding bold seeded cultivars for future use. The clusters V and VI consisted of thirty six accessions and these gave low average 100-seed weight. Most of the germplasm collected locally was low yielding but better adapted and exhibited low deviation that indicated the worth that could be utilized by the breeders of chickpea by involving local and exotic chickpea parents in the breeding programme.

4.2.9. Selection of Superior Genotypes

On the basis of agronomic performance and statistical analyses, thirty one genotypes were identified and presented in the Table 4.2.3. Three accessions were selected for early flowering, 10 for maturity, 11 for short stature, 12 for branches per plant, 8 for pods per plant, 10 for pods per branch, one for seeds per pod, 10 for 100-seed weight, 7 for biological yield, 5 for grain yield and 14 were identified for high harvest index. Some of these accessions were better for more than one agronomic traits. The accessions PAK-52975 and PAK-52978 were better for days to maturity, branches per plant, pods per plant, 100-seed weight, biological yield and harvest index. The accessions PAK-52979, PAK-52980 and PAK-52981 were selected for the best performance for branches per plant, pods per plant, 100-seed weight, biological yield, grain yield and harvest index.

The accession PAK-52983 was observed better for nine important agronomic characters, i.e., days to flowering, days to maturity, plant height, branches per plant, pods per plant, 100-seed weight, biological yield, grain yield and harvest index. Similarly, accession PAK-52984 was identified superior on the basis of plant height, branches per plant, pods per plant, pods per branch, seeds per pod, 100-seed weight,

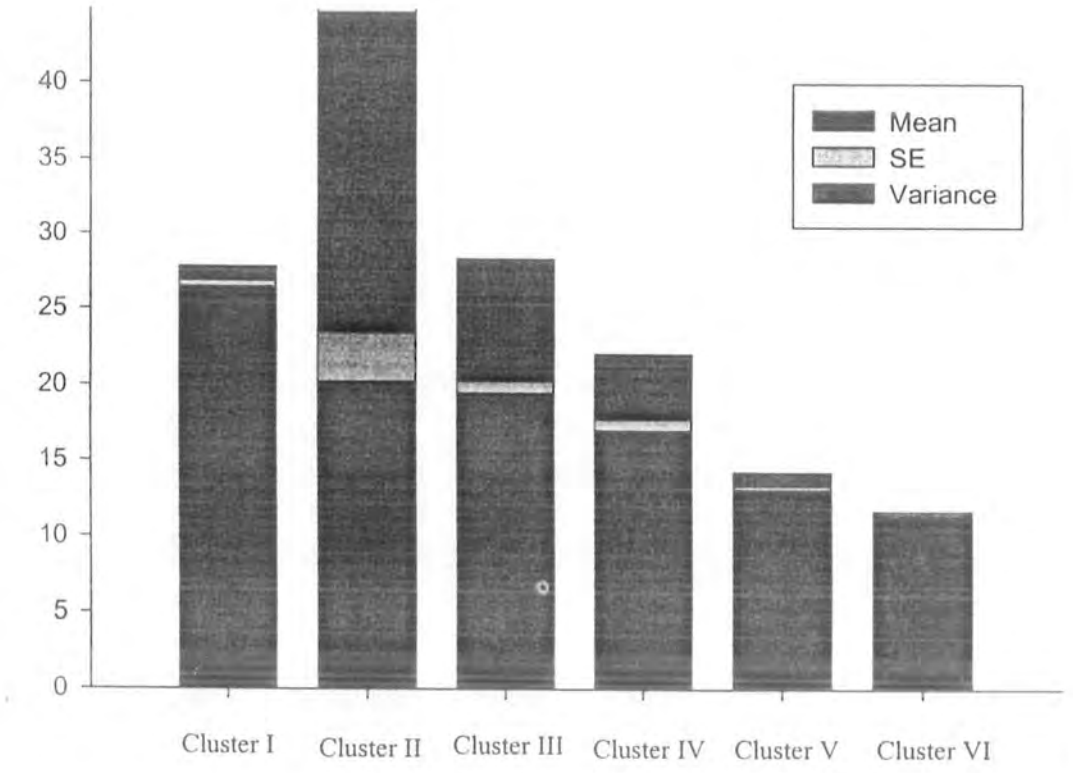


Fig. 4.2.17:- Mean, standard error and variance for 100-seed weight in six clusters constructed on the basis of quantitative traits in chickpea

biological yield, grain yield and harvest index. Other accessions were observed better for individual character and some associated with more. The check, Punjab 91 was better for days to flowering, seeds per pod and harvest index, whereas other check, Paidar 91 was better for days to flowering, seeds per pod, 100-seed weight, grain yield and harvest index. The accessions with better performance than both the checks could be exploited for future chickpea breeding.

4.3 Classification Based on Geographic Distribution

4.3.1. Genetic Diversity

All the sixty accessions included in the experiment were collected from five major chickpea growing districts of the Punjab, i.e., Bahawalnagar, Bhakkar, Khushab, Layyah and Mianwali (Table 4.3.1). These accessions along with 2 checks were studied for genetic variation attributed to various collection sites. Further, these accessions were either collected from the field areas or obtained from markets. The variance was also investigated according to the source of seed. Out of sixty accessions, 11 were collected from Bahawalnagar and out of these, 4 accessions were collected from the field areas and 7 were obtained from markets of the area. From the District Bhakkar, 14 accessions were collected from the field areas and 3 from markets.

Twelve accessions represent the District Khushab, and out of these, 9 were collected from farmers' fields and other as market samples. From the District Layyah, 14 accessions were collected from the fields and 4 were collected as market samples, where 2 accessions were obtained from market of Mianwali. These five districts of Punjab represents the major chickpea growing areas of the country because more than 80 percent of desi types are being cultivated in these five districts. The data was analyzed on the basis of collecting sites and sources, i.e., field or market.

4.3.2. Collecting Sites

The agronomic characters were analyzed on the basis of collecting sites and the results are presented in the Tables 4.3.2 to 4.3.6. Eleven accessions collected from Bahawalnagar ranged from 135 to 153 days to flowering with an average of 141±1.41 days after planting (Table 4.3.2). The accessions collected from this area were homogeneous in nature and low to medium variation was observed for most of the

Table 4.3.1:- The origin and source of chickpea germplasm evaluated during 1997

District	Collected from field	Market samples	Total
Bahawalnagar	4	7	11
Bhakkar	14	3	17
Khushab	9	3	12
Layyah	14	4	18
Mianwali	-	2	2
	41	19	60

characters. Average grain yield were low (3.13±0.23 g) along with low harvest index (12.00±0.92) for the accessions collected from Bahawalnagar. Low to medium variance and low average performance for most of the characters indicated that improvement from these accessions for further selection was limited. These accessions could better be utilized in the hybridization programme to create genetic variation.

The results of 17 accessions collected from the District Bhakhar are presented in the Table 4.3.3. The accessions collected from Bhakkar ranged from 165 to 175 days for maturity with an average of 170±0.73 days. The variance for pods per plant was high in the group of accessions that could be exploited through simple selection. The average pod number in the accessions collected from this district was 38.8±2.35 pods per plant with a range of 21.1 to 53.1 pods. The mean grain yield of the accessions collected from Bhakkar was 6.97±0.24 g per plant along with medium range of harvest index, i.e., 18.33-29.81%. This group consisted of 17 accessions and out of these, 14 were collected from farmers' fields and 4 were collected as market samples. This district is a major chickpea growing area and the germplasm collected from this area can be used in hybridization programme involving improved germplasm from either sources. The variation for seeds per pod was not enough for the germplasm evaluated in the present study.

Twelve accessions collected from the district Khushab were early in flowering and maturity with the average values of 140±1.03 and 170±1.2 days for flowering and maturity, respectively (Table 4.3.4). Low to medium variation was observed for most of the characters in the accessions collected from this area. Average grain yield was low (6.00±0.25 g) with a range of 4.94 to 7.01 g per plant. The accessions with maximum limits for grain yield and other components could be selected to test under a wide range of environments. Low to medium harvest index range (18.97-25.85%) was observed for the accessions collected from Khushab. Low to medium variance and low average performance for most of the characters indicated that improvement from these accessions for further selection was limited, therefore this material could be utilized in the hybridization programme to create genetic variation.

Basic statistics presented in the Table 4.3.5 revealed that the accessions collected from Layyah ranged from 132 to 149 for days to flowering and from 163 to

Table 4.3.2:- Range, means, SE and variance for 11 quantitative traits in 11 chickpea accessions collected from District Bahawalnagar

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	141±1.41	4.69	22.02	135	153
Days to maturity	172±1.71	5.66	32.07	163	185
Plant height (cm)	50.7±1.44	4.77	22.77	42.8	58.2
Number of branches	8.3±0.38	1.27	1.62	5.8	9.6
Pods per plant	15.2±1.21	4.01	16.11	7.5	19.9
Pods per branch	2.0±0.31	1.02	1.04	0.7	3.9
Seeds per pod	0.8±0.05	0.16	0.03	0.5	1.0
100-seed weight (g)	11.23±0.10	0.34	0.12	10.88	11.73
Biological yield (g)	18.49±0.61	2.02	4.08	14.14	21.06
Grain yield (g)	3.13±0.23	0.76	0.58	1.74	4.19
Harvest index (%)	12.00±0.92	3.05	9.30	7.15	15.54

Table 4.3.3:- Range, means, SE and variance for 11 quantitative traits in 17 chickpea accessions collected from District Bhakkar

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140±0.48	1.99	3.97	137	144
Days to maturity	170±0.73	3.02	9.13	165	175
Plant height (cm)	51.3±1.21	4.97	24.74	44.1	59.9
Number of branches	12.3±0.30	1.25	1.56	10.1	13.9
Pods per plant	38.8±2.35	9.71	94.19	21.1	53.1
Pods per branch	4.4±0.46	1.90	3.62	1.1	7.1
Seeds per pod	1.1±0.01	0.06	0.00	1.0	1.2
100-seed weight (g)	15.07±0.42	1.73	3.01	12.09	17.87
Biological yield (g)	30.15±0.92	3.78	14.25	23.15	36.47
Grain yield (g)	6.97±0.24	1.00	1.01	4.88	8.23
Harvest index (%)	25.44±0.69	2.84	8.07	18.33	29.81

Table 4.3.4:- Range, means, SE and variance for 11 quantitative traits in 12 chickpea accessions collected from District Khushab

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140.6±1.40	4.85	23.54	137	155
Days to maturity	170.5±1.51	5.23	27.36	165	185
Plant height (cm)	50.0±1.36	4.70	22.08	41.8	54.6
Number of branches	11.2±0.27	0.93	0.86	10.2	12.9
Pods per plant	29.9±1.51	5.23	27.34	21.7	36.1
Pods per branch	2.5±0.29	1.00	1.00	1.0	4.6
Seeds per pod	1.1±0.01	0.04	0.00	1.1	1.1
100-seed weight (g)	13.44±0.33	1.13	1.28	12.10	14.99
Biological yield (g)	26.83±0.84	2.91	8.45	23.79	30.11
Grain yield (g)	6.00±0.25	0.86	0.74	4.94	7.01
Harvest index (%)	23.14±0.69	2.38	5.65	18.97	25.85

Table 4.3.5:- Range, means, SE and variance for 11 quantitative traits in 18 chickpea accessions collected from District Layyah

Quantitative traits	Mean ±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140±1.03	4.37	19.06	132	149
Days to maturity	170±1.22	5.17	26.68	163	180
Plant height (cm)	49.9±1.46	6.18	38.16	37.6	62.7
Number of branches	16.0±0.95	4.04	16.34	9.8	22.6
Pods per plant	63.2±7.01	29.73	884.17	20.4	122.7
Pods per branch	5.0±0.74	3.12	9.76	1.8	13.1
Seeds per pod	1.3±0.05	0.19	0.04	1.0	1.8
100-seed weight (g)	20.59±1.28	5.43	29.49	11.91	27.49
Biological yield (g)	36.99±2.08	8.82	77.85	21.07	50.65
Grain yield (g)	9.96±0.84	3.57	12.75	4.41	17.36
Harvest index (%)	31.36±2.11	8.95	80.09	15.84	45.14

180 for days to maturity with an average of 140±1.03 and 170±1.22 days for flowering and maturity, respectively. The variance for pods per plant was high with a mean value of 62.3±7.01 pods per plant in the group of accessions that could be exploited through simple selection. The range for pod per plant in the accessions collected from this district was from 20.4 to 122.7 pods per plant and the accessions with high number of pods along with other yield components are suggested to be evaluated for further selection. The 100-seed weight in this group of accessions was also high with a mean value of 20.59±1.28 g per 100-seeds with a range of 11.91-27.49 g. The mean grain yield of the accessions collected from the district Layyah was high with a mean value of 9.96+0.84 g per plant with a range from 4.41 to 17.36 g per plant. The harvest index in these accessions ranged from 15.84 to 45.14 percent with a mean value of 31.36±2.11 percent.

This group consisted of maximum number of accessions included in the material, and out of 18 accessions these 14 were collected from farmers' fields and 4 were collected as market samples. This district is included in the major chickpea growing area and the germplasm collected from this area was better in evaluation as compared to the germplasm collected from other districts, therefore, the selected accessions from this group can be tested under a wide range of environments or used in hybridization programme involving improved germplasm from other sources.

Two accessions were collected from the District Mianwali and both the accessions were obtained from market. This might be due to the off season or less area under chickpea cultivation during the expedition year. Both of these accessions were of low to medium importance for most of the characters under study. The average days to flowering were 141±0.02 with a range from 141 to 143 days to flowering (Table 4.3.6). Days to maturity ranged from 170 to 172 with a mean value of 171±1.00. The variation for most of the characters was low along with low mean values, therefore, these accessions could be rejected at this stage from further evaluation. The graphic presentation of mean values and variance for grain yield based on collecting sites along with checks revealed that the varieties gave the highest mean yield along with high standard error and variance (Fig. 4.3.1). This was followed by the accessions collected from the district Layyah which gave high mean value and variance. High standard error and variance observed in check varieties indicated the influence of environmental fluctuations. The performance

Table 4.3.6:- Range, means, SE and variance for 11 quantitative traits in 2 chickpea accessions collected from District Mianwali

Quantitative traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	141±0.00	0.00	0.00	141	141
Days to maturity	171±1.00	1.41	2.00	170	172
Plant height (cm)	50.7±3.75	5.30	28.13	46.9	54.4
Number of branches	10.9±0.15	0.21	0.04	10.7	11.0
Pods per plant	29.9±0.35	0.49	0.24	29.5	30.2
Pods per branch	2.0±0.96	1.35	1.83	1.0	2.9
Seeds per pod	1.1±0.01	0.01	0.00	1.1	1.1
100-seed weight (g)	12.91±0.11	0.16	0.02	12.80	13.02
Biological yield (g)	25.30±0.12	0.16	0.03	25.19	25.42
Grain yield (g)	5.74±0.05	0.07	0.00	5.69	5.79
Harvest index (%)	22.61±0.07	0.09	0.01	22.54	22.67

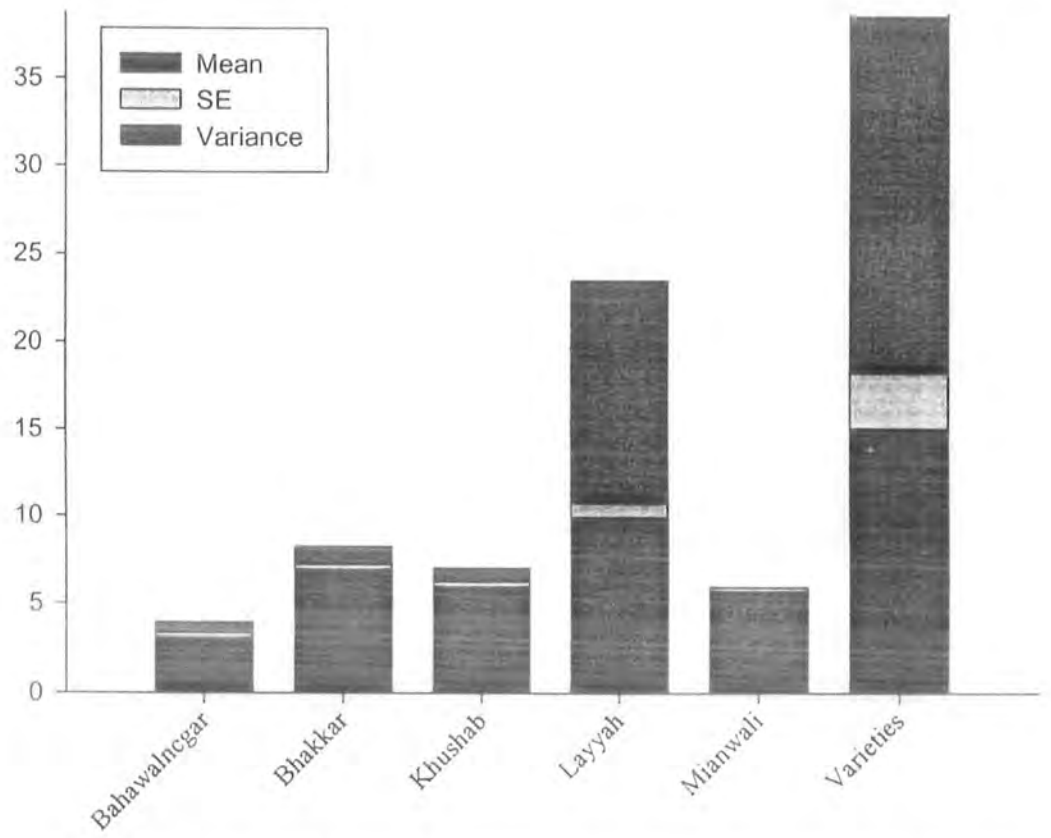


Fig. 4.3.1:- Mean, standard error and variance for grain yield in chickpea based on collecting sites of Punjab

under favourable environmental conditions might be better as compared to stress conditions.

The local germplasm is adaptive to various environmental stresses that could be utilized by involving these in the hybridization programme. The accessions collected from the districts of Bahawalnagar and Mianwali were of poor performance, hence could be excluded at this stage. The accessions originated from Khushab were also of poor performance but due to high number of accessions, these are preferred to be tested further for their performance. For 100-seed weight, the average values, standard error and variance were plotted in the Fig. 4.3.2. The accessions collected from Layyah exhibited better 100-seed weight along with high variation. This variation could be exploited through simple selection for improving 100-seed weight in chickpea. Although, varieties gave high 100-seed weight along with high variance but due to high standard error these may not be exploited further. The accessions collected from Bahawalnagar and Mianwali also gave poor performance for 100-seed weight as they gave low seed weight and lowest variance that limited any further improvement through selection from these accessions. Most of the germplasm collected locally gave low seed weight except the accessions collected from Layyah. As local material is better adapted, that indicated the worth for improving seed weight in chickpea, hence that could be utilized by the breeders of chickpea by involving local and exotic chickpea parents in the breeding programme. The germplasm was collected either from field areas or from markets and the data was analysed for basic statistics on the basis of collection sources and results are presented in the Tables 4.3.7 and 4.3.8. High average performance was observed by the material collected from farmers' fields that revealed scope of improvement through selection from this germplasm.

4.3.3. Cluster analysis

Sixty accessions, collected from five chickpea growing districts of the Punjab, were studied for cluster analysis based on agronomic characters. As already mentioned, both the checks were grouped together in the cluster II. Cluster I consisted of five accessions, i.e., PAK-54984, PAK-52983, PAK-52981, PAK-52980 and PAK-52979, all of these were collected from the District Layyah. Cluster III comprised 12 accessions and out of these eight accessions (PAK-52978, PAK-52975, PAK-52971, PAK-52973, PAK-52974, PAK-52969, PAK-52968,

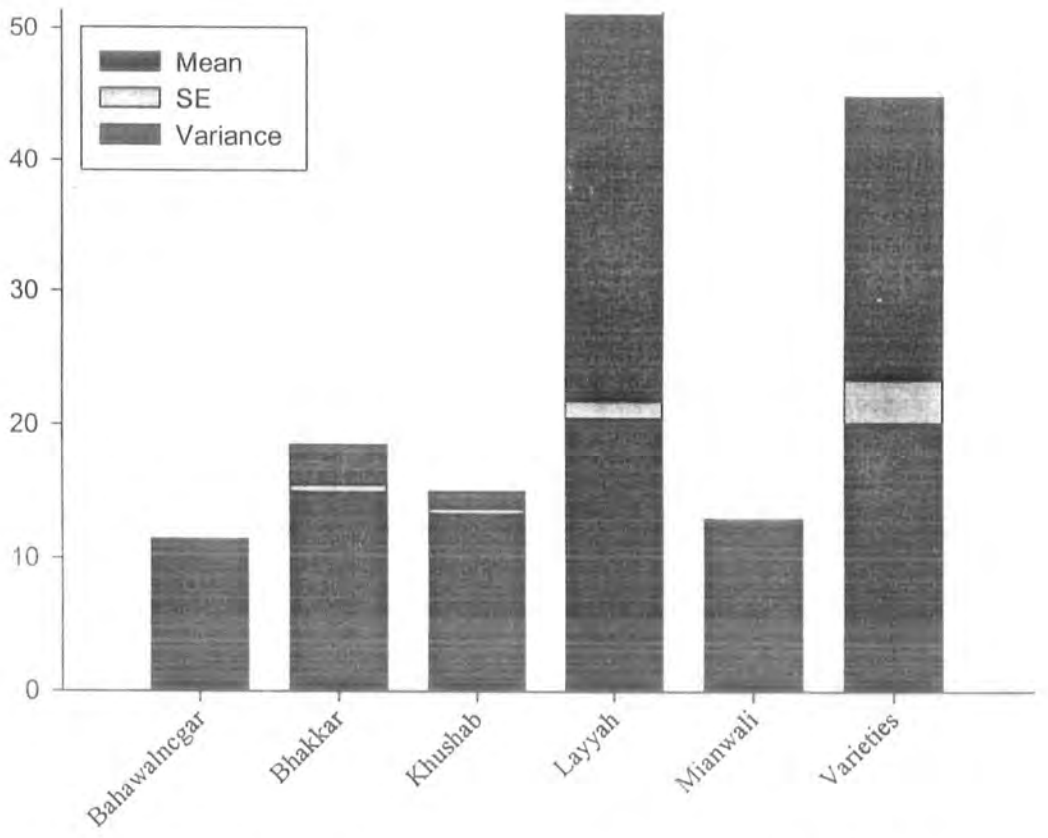


Fig. 4.3.2:- Mean, standard error and variance for 100-seed weight in chickpea based on collecting sites of Punjab

Table 4.3.7:- Range, means, SE and variance for 11 quantitative traits in 41 chickpea accessions collected from field areas of 5 Districts of Punjab

Quantitative traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	140±0.59	3.76	14.15	132	155
Days to maturity	170±0.68	4.38	19.19	163	185
Plant height (cm)	51.1±0.84	5.36	28.76	37.6	62.7
Number of branches	13.6±0.55	3.55	12.57	9.0	22.6
Pods per plant	46.9±3.95	25.30	640.20	17.0	122.7
Pods per branch	4.2±0.40	2.54	6.46	1.0	13.1
Seeds per pod	1.2±0.03	0.16	0.03	0.9	1.8
100-seed weight (g)	17.08±0.77	4.91	24.07	11.50	27.49
Biological yield (g)	32.25±1.22	7.80	60.77	19.33	50.65
Grain yield (g)	8.00±0.48	3.05	9.32	3.63	17.36
Harvest index (%)	27.34±1.15	7.38	54.45	14.26	45.14

Table 4.3.8:- Range, means, SE and variance for 11 quantitative traits in 19 chickpea accessions collected from markets of 5 Districts of Punjab

Quantitative traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	141±0.95	4.13	17.06	134	153
Days to maturity	171±1.18	5.13	26.36	163	185
Plant height (cm)	49.1±1.03	4.49	20.17	42.8	56.3
Number of branches	9.9±0.49	2.15	4.62	5.8	13.5
Pods per plant	24.1±2.74	11.93	142.31	7.5	50.2
Pods per branch	2.5±0.37	1.60	2.55	0.7	6.7
Seeds per pod	1.0±0.04	0.19	0.04	0.5	1.2
100-seed weight (g)	12.48±0.42	1.83	3.37	10.88	16.83
Biological yield (g)	22.74±1.26	5.51	30.40	14.14	33.81
Grain yield (g)	4.61±0.42	1.85	3.43	1.74	8.01
Harvest index (%)	17.43±1.53	6.66	44.34	7.15	27.50

PAK-52970) were collected from Layyah, where other four accessions (PAK-52966, PAK-52964, PAK-52960, PAK-52959) originated from the District Bhakkar. Both of these districts are adjoining and well known due to chickpea cultivation. Cluster IV consisted of seven accessions, out of which five accessions (PAK-52967, PAK-52965, PAK-52963, PAK-52962, PAK-52961) were collected from Bhakkar, one (PK-52972) from Layyah and one (PK-52958) originated from Khushab. Twenty three accessions were grouped together in cluster V and out of these eleven (PAK-52957, PAK-52954, PAK-52953, PAK-52943, PAK-52941, PAK-52956, PAK-52955, PAK-52939, PAK-52938, PAK-52942, PAK-52937) were collected from the District Khushab and it is important to note that in total twelve accessions were collected from Khushab and out of these 11 were grouped together in this cluster.

Seven accessions, viz., PAK-52952, PAK-52951, PAK-52950, PAK-52947, PAK-52948, PAK-52949 and PAK-52946 were collected from Khushab were grouped in this cluster. Both the accessions originated from the district Mianwali were also observed in this cluster. Two accessions (PAK-52933 and PAK-52934) of this cluster were collected from Layyah and one (PAK-52929) originated from Bahawalnagar. This cluster consisted of a mix population of accessions collected from various sites. Cluster VI consisted of thirteen accessions and out of these two (PAK-52932 and PAK-52931) were collected from Layyah, one (PAK-52935) from Bhakkar and all others (PAK-52928, PAK-52930, PAK-52926, PAK-52927, PAK-52925, PAK-52924, PAK-52921, PAK-52922, PAK-52919, PAK-52918) were from Bahawalnagar.

It is important to note that in cluster I, all the five accessions were from the District Layyah and all of these were collected as market samples. In cluster III, four accessions originated from Bhakkar and out of these 3 were market samples, whereas one was collected from farmer's field. Other eight accessions in this cluster originated from Layyah and all of these were collected from farmers' fields. Cluster IV comprised seven accessions and all of these were collected from fields' areas, five from Bhakkar, and one from Khushab and Layyah in each case. Cluster V consisted of 23 accessions from various sources and origin. Seven were collected from fields' areas of Bhakkar, two from Layyah and Mianwali as market samples in each case. Eleven accessions were from Khushab and out of these eight

were collected from field areas and three from markets, whereas one accession was collected from Bahawalnagar area. Out of thirteen accessions of cluster VI, ten originated from Bahawalnagar and out of these three were collected from farmers' field and seven from markets. One accession originated from field area of Bhakkar, whereas two accessions were collected from markets of district Layyah.

4.3.4. Principal Component Analysis (PCA)

Variance for principal components revealed that first three principal components with eigenvalues > 1 contributed 88.58% of the variability amongst 62 genotypes evaluated for eleven quantitative traits. All the characters under study contributed genetic variance positively towards PC_1 except days to flowering where it was negative. The first PC which explained 60.39% of the variance is positively associated with all the characters except one and eight important yield contributing characters exhibited more positively, whereas days to flowering contributed least. Ten important plant characters contributed more positively to first 2 principal components and hence these could be established important for the material under investigation. The component 3 contributed maximum for plant height, although it had good share for days to maturity. First 3 components which contributed 88.6% of the total variance, were plotted graphically to observe the relationship between 62 accessions of chickpea for these components. The factor 1 contributing 60.4% of the variability was kept as x-axis in both the cases, whereas factor 2 and 3 were plotted against y-axis simultaneously. The separation on the basis of both graphs gave similar results. As the accessions were plotted on the basis of geographic origin and source of seed collection, hence these were investigated to know whether genetic diversity was related to geographic origin or not. The PC_1 and 2 revealed one group in the left upper half, one in the right upper half, one in between of these two groups and one consisting checks was observed in the lower half of the graph. The accessions collected from Bahawalnagar were grouped together in the left side of the graph. The accessions from the district Bhakkar and Khushab were grouped in the middle of the accessions from Layyah and Bahawalnagar. Four accessions collected from the district Layyah were grouped closer to the accessions originated from Bahawalnagar. Fourteen accessions out of 18 originated from Layyah were clearly separated from other accessions. This cluster was on the right half of the graph. Two accessions

collected from Mianwali were closer to the accessions originated from Bahawalnagar and Khushab.

The checks were separated from all the germplasm and were observed in the lower half of the graph. Similar results were observed where factor 3 was plotted against y-axis instead of factor 2. Three clusters consisting of accessions from Layyah (three), Khushab, Mianwali and Bhakkar were in the middle of the graph with similar pattern as in the graph plotted for factor 1 and 2. The accessions collected from Bahawalnagar were grouped together in the left half, whereas fifteen accessions collected from the district Layyah were observed in the right half of the figure. The approved varieties were shifted to the upper side, i.e., above 3.0 level against y-axis.

The first 3 components were also plotted to observe the relationship between 60 accessions along with 2 checks on the basis of source. The germplasm was collected from five districts either from the field areas (41 accessions) or markets (19 samples) and graphic presentation gave a separation on the basis of source. Factor 1 was plotted against x-axis in both the cases, whereas factor 2 and 3 were kept against y-axis simultaneously. The accessions were plotted on the basis of seed source collection, hence these were investigated to see whether the genetic diversity was related to source or not. The PC_1 and 2 revealed that six accessions collected as market samples were separated from others, whereas others were mixed with in the left half of the graph (Fig. 4.3.3). In general, all the market samples except three were grouped in the left side of the graph. Twenty two accessions out of 41 collected from farmers' fields were separated in the right half of the graph, whereas others were on the right half and out of these eleven accessions were grouped together closer to the origin and eight accessions were mixed with the market samples. Both the checks included in the experiment were clearly separated in the lower half of the graph. Similar findings were observed in the Fig. 4.3.4 where factor 3 was plotted against y-axis instead of factor 2. The PC_1 and 3 revealed that ten accessions instead of six in case of PC_1 and 2 collected from markets were separated from others, whereas other nine samples were mixed with others. Three samples collected from markets were grouped in the right half of the graph in this case also and all others, either separated or mixed were in the left half. Twenty two accessions out of 41 collected from farmers' fields were

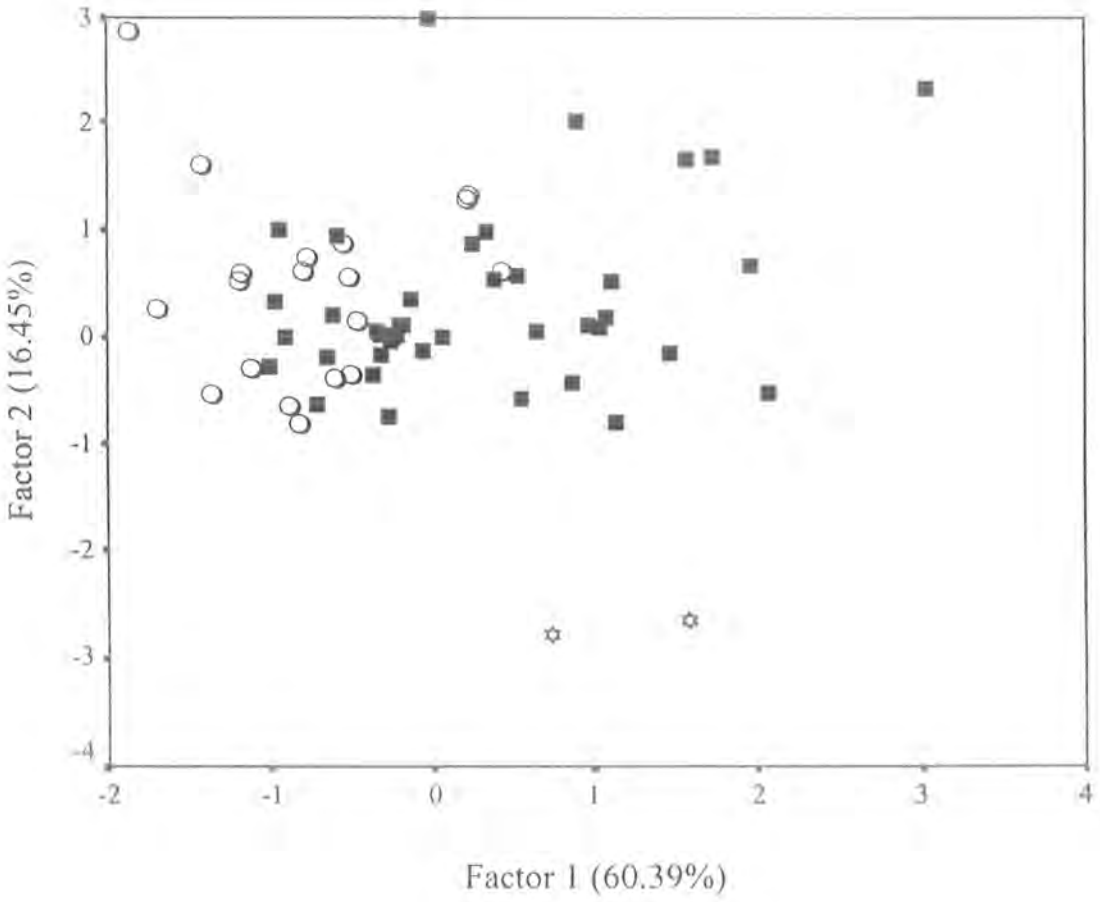


Fig.4.3.3:- Scattered diagram based on quantitative traits on the basis of collection criterion for first and second factors in chickpea. The marks represent as ○- market, ■- field and ✡- approved varieties

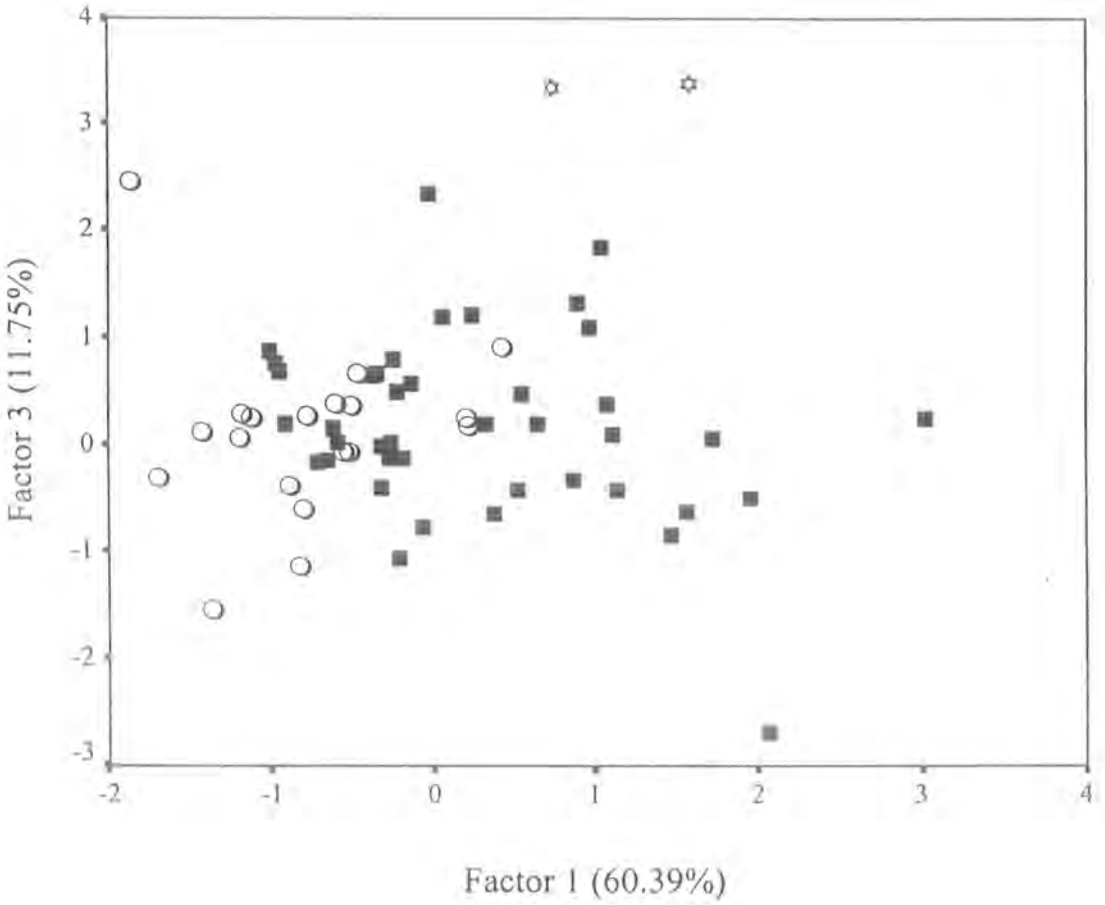


Fig. 4.3.4:- Scattered diagram based on quantitative traits on the basis of collection criterion for first and third factors in chickpea. The marks represent as ○- market, ■- field and ✡- approved varieties

separated in the right half of the graph, whereas others were on the left half. Nine accessions collected from field areas were mixed with market samples in the left half, whereas others could be observed clearly. Both the check varieties, Punjab 91 and Paidar 91 were clearly separated in the upper half of the graph.

4.4. Genetic and Path Analysis in Selected Pure-lines

4.4.1. Genetic Variance

Genotypes included in the study differed significantly for all the traits under study (Table 4.4.1). Medium to high genetic variance was observed for days to flowering, maturity, secondary branches and 100-seed weight, whereas for other characters, low to medium habitability (broad sense) along with low to medium genetic advance was observed (Table 4.4.2). Improvement of these traits through simple selection might be limited from germplasm used in the present study.

4.4.2. Multivariate Analyses

The first three components, with eigenvalues >1 contributed 83.38% of the variability amongst genotypes evaluated for 10 quantitative traits (Table 4.4.3). Other PCs (4 to 10) had eigenvalues less than 1. The first PC was more related to days to maturity, plant height, primary branches, 100-seed weight, grain yield and harvest index, whereas the second PC contrasts variables that relate solely to vegetative growth (flowering, maturity) with those that are associated with reproductive development, i.e., pods and biological yield. The variation for plant height and biological yield was distributed among all the components. Seven characters contributed positively to PC_1, thus this component is a weighted average of the characters. The characters with the greatest weight on this component suggested that this component reflects the yield potential of each accession. The characters with the greatest positive weight on PC_2 were pods and biological yield, whereas days to flowering and maturity had a substantial negative weight. These results suggest that this component reflects the tendency of each accession to emphasize vegetative, as opposed to reproductive growth. Although, PC_3 exhibited positive effects for all the characters but the magnitude was low except secondary branches, pods and biological yield. This suggests that the genotypes that emphasize vegetative growth tend to have low yield, whereas those that emphasize

Table 4.4.1: - Average performance of Chickpea accessions evaluated during 1999.

Genotypes	DF	DM	PH	PB	SB	Pods	SW	BY	GY	HI
52984	123	171	73.3	2.9	8.1	35.5	22.17	29.13	16.35	56.35
52983	123	174	62.5	3.3	8.4	26.2	20.84	27.26	14.15	50.64
52981	125	177	66.9	3.2	11.2	37.0	22.40	35.66	19.15	53.77
52980	122	174	49.5	3.1	7.9	40.1	21.22	32.66	17.75	54.34
52979	123	174	65.3	3.3	10.4	36.0	23.98	35.30	20.05	57.00
52978	124	174	64.6	2.7	8.2	38.6	21.11	37.52	18.75	52.08
52975	122	178	59.6	3.9	11.5	33.9	22.57	27.49	14.95	54.31
52974	121	174	66.5	2.9	8.2	39.1	23.52	28.68	15.50	53.48
52973	118	175	73.6	3.1	8.8	32.2	23.80	27.97	15.90	56.99
52972	117	174	72.2	2.4	9.6	36.6	20.90	27.82	16.20	58.35
52971	124	178	58.2	3.5	10.8	32.4	20.25	24.41	13.90	57.05
52970	123	179	51.5	2.7	8.9	30.7	21.50	18.08	10.85	59.31
52969	126	175	39.2	3.8	9.4	19.3	21.75	15.36	8.40	55.94
52968	124	178	64.3	3.8	9.1	28.5	22.52	27.17	15.30	56.55
52967	122	177	60.8	3.3	10.5	40.5	22.71	29.72	17.10	56.80
52966	124	179	65.0	3.9	11.6	41.4	21.62	29.97	15.45	51.59
52965	123	171	65.2	3.4	8.1	39.2	22.17	32.02	17.25	53.97
52964	122	171	63.2	2.6	10.4	28.9	21.23	20.90	11.65	55.97
52935	137	165	52.3	2.1	10.1	52.6	12.09	24.06	5.11	18.33
52937	142	173	44.5	2.7	10.2	64.6	12.10	24.11	5.16	18.97
52938	138	168	49.4	2.8	10.3	62.0	12.31	24.14	5.34	20.69
52939	140	170	50.0	2.3	10.3	72.4	12.36	24.32	5.50	20.77
52941	138	168	54.6	2.1	10.5	81.9	12.44	25.19	5.69	21.18
52942	142	173	44.6	2.3	10.5	2.69	12.48	25.42	5.79	22.06
52963	123	170	69.5	3.2	9.4	29.6	20.66	37.25	14.10	47.60
52962	123	173	57.2	2.8	8.8	28.3	20.85	22.15	12.50	57.35
52961	124	173	59.8	2.8	8.8	30.6	23.71	24.48	13.60	55.49
52960	122	171	66.5	2.9	8.7	36.0	22.78	26.90	14.90	55.67
Punjab-91	124	176	66.5	3.4	9.8	38.8	23.52	33.97	18.35	54.36
Paidar-91	124	174	66.9	3.1	8.25	37.7	16.98	21.17	11.95	56.52
MS(V)	15.30	27.63	242.45	0.69	7.07	149.47	9.14	186.98	41.52	47.77
MS(R)	15.44	1.47	125.00	1.23	18.83	899.01	.006	298.68	69.27	67.14
MS(E)	1.67	2.56	63.66	.31	1.17	71.18	0 001	61.46	10.26	11.19
F.RATIO(V)	9.13**	10.78**	3.80**	2.19**	6.04**	2.09*	242.1**	3.09**	4.05**	4.27**
F.RATIO(R)	9.21**	.57ns	1.96ns	3.88*	16.09**	12.63**	149.5**	6.75**	6.75**	6.00**
ST.ERROR	.64	.8	3.98	.28	.89	5.03	.003	5.27	2.25	2.79
CD1	1.81	2.24	11.17	.78	2.49	14.08	.009	14.78	6.30	7.81
CD2	2.39	2.96	14.76	1.04	3.29	18.61	.011	19.53	8.33	10.33

DF- Days to 50% flowering, DM- days to 90% maturity, PH-Plant height (cm), PB-primary branches per plant, SB- Secondary branches per plant, Pods- number of pods per plant, SW- 100-seed weight, BY- Biological yield per plant (g), GY- Grain yield per plant (g), HI- Harvest index (%).

Table 4.4.2:- Basic statistics for ten quantitative traits in 30 genotypes of chickpea

Quantitative Traits	Mean±SE	σ	σ^2	Minimum	Maximum
Days to flowering	126.10±1.29	7.09	50.30	117	142
Days to maturity	173.57±0.63	3.43	11.77	165	179
Plant height (cm)	60.11±1.65	9.03	81.52	39.2	73.6
Primary branches per plant	3.01±0.09	0.50	0.25	2.1	3.9
Secondary branches per plant	9.56±0.20	1.10	1.21	7.9	11.6
Pods per plant	38.44±2.82	15.43	238.20	2.7	81.9
100-seed weight (g)	19.95±0.75	4.12	16.97	12.09	23.98
Biological yield per plant (g)	27.34±0.99	5.41	29.26	15.36	37.52
Grain yield per plant (g)	13.22±0.86	4.69	21.96	5.11	20.05
Harvest index (%)	48.12±2.62	14.33	205.47	18.33	59.31

reproductive growth tend to have lower vegetative growth. The first 3 principal components contributed more than 80 percent of the variability, hence these were plotted to observe relationships between the clusters (Fig. 4.4.1 and 4.4.2). In both the figures, PC_1 that contributed 54.97% of the variation was kept as in the X-axis, whereas PC_2 and PC_3 were plotted simultaneously against Y-axis. Both of these figures gave similar results, although PC_1 vs. PC_3 gave clear separation of two groups. All the five accessions collected from the District Khushab were grouped in the left half and one accession from Bhakkar was mixed with these. Group B in cluster analysis was separated and clearly visible in the right half of the graph. This cluster consisted of accessions collected from the Districts of Bhakkar and Layyah along with two checks included in the material.

Euclidean dissimilarity coefficients of 30 genotypes ranged between 1.83 and 7.41 (Table 4.4.4). At the 25 percent linkage distance three clusters were observed, whereas clusters II and III unite to form one group (B) and cluster I constituted group A. Six accessions were grouped together in the group A (cluster I) and out of these 5 accessions were collected from the areas of District Khushab, whereas one (PAK-52935) was collected from District Bhakkar (Fig. 4.4.3). The group B consisted of twenty four accessions including two checks. These accessions were collected from the Districts of Layyah and Bhakkar. Cluster II consisted of seven and cluster III fifteen accessions, whereas one check (Punjab-91) was in cluster II and other (Paidar 91) was in cluster III. Mean values along with standard deviation for each cluster presented in Table 4.4.5 revealed that accessions collected from the District Khushab (cluster I) were late in flowering but early in maturity, short statured and low yielder. The average performance of the accessions grouped in cluster II and III (group B) were similar, as both of these were late in maturity and high yielding along with high seed weight and harvest index.

Genetic variance, phenotypic variance, heritability and genetic advance presented in the Table 4.4.6 revealed high proportion of genetic variation for days to flowering, days to maturity, secondary branches and 100-seed weight. The range for days to flowering, days to maturity and number of primary branches was low, but due to the adaptation of chickpea to Thall desert, the crop duration does not matter because of sole crop culture. For other characters, considerable range of the

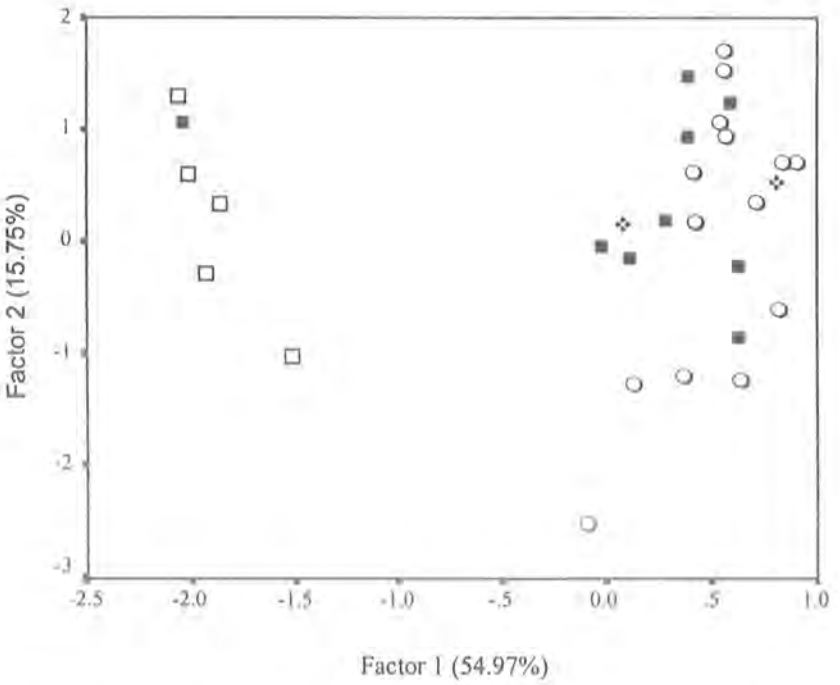


Fig. 4.4.1:- Scattered diagram based on quantitative traits for first and second factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, and ❖- approved varieties

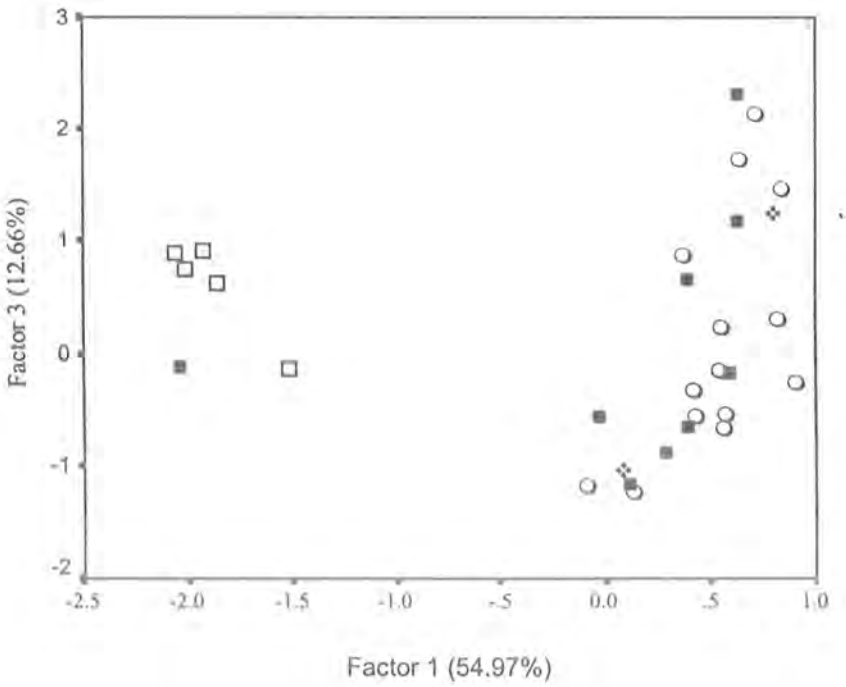


Fig. 4.4.2:- Scattered diagram based on quantitative traits for first and third factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, and ❖- approved varieties

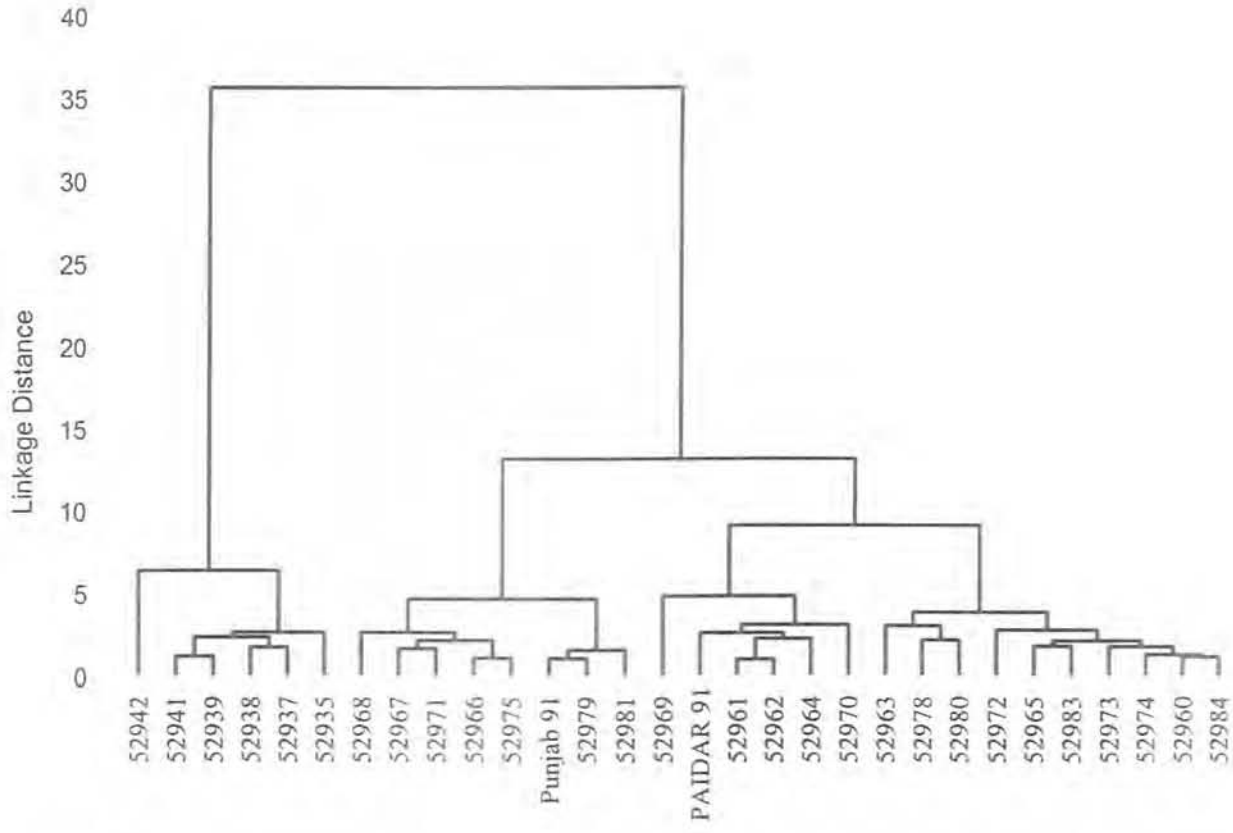


Fig. 4.4.3:- Cluster diagram of 30 chickpea genotypes selected on the basis of better performance from a broad based germplasm

Table 4.4.3:- Principal Components (PCs) for 10 quantitative characters in 30 genotypes of Chickpea

		PC_1	PC_2	PC_3
Eigen value		5.49	1.58	1.27
Proportion of σ^2		54.97	15.75	12.66
Commulative σ^2		54.97	70.72	83.38
	Communality	Eigen factors		
Days to flowering	0.915	-0.938	-0.065	0.178
Days to maturity	0.818	0.647	-0.584	0.243
Plant height (cm)	0.779	0.688	0.542	0.105
Primary branches	0.737	0.667	-0.446	0.305
Secondary branches	0.817	-0.291	-0.389	0.762
Pods/plant	0.608	-0.577	0.392	0.348
100-seed weight (g)	0.924	0.959	-0.029	-0.059
Biological yield/plant (g)	0.830	0.429	0.596	0.539
Grain yield/plant (g)	0.958	0.927	0.253	0.185
Harvest index (%)	0.952	0.948	-0.114	-0.201

Table 4.4.4.- Genetic distances among various pure-lines selected on the basis of the best performance.

	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
1	1.97	3.72	2.93	2.95	2.18	4.50	1.29	1.64	2.13	3.97	4.20	5.55	3.09	3.25	4.55	1.48	3.09	6.25	6.66	6.13	6.45	6.57	6.72	2.27	2.57	2.09	1.08	2.69	2.47
2		3.44	2.22	2.90	2.60	3.37	1.50	1.84	2.68	2.68	3.01	3.93	1.80	2.51	3.67	1.75	2.81	6.06	5.91	5.61	6.10	6.51	5.70	2.52	1.67	1.51	1.57	2.39	1.88
3			3.77	1.30	3.09	2.46	3.35	3.07	3.12	2.74	4.77	5.88	2.96	1.61	2.08	3.45	3.97	7.10	6.62	6.52	6.74	6.98	6.64	2.97	4.09	3.65	3.56	1.45	4.40
4				3.07	2.11	4.17	2.21	3.13	3.49	3.65	3.69	4.64	3.04	2.93	4.42	2.07	4.02	6.41	6.05	5.91	6.23	6.61	6.34	3.20	2.74	2.51	2.59	2.78	3.32
5					2.51	2.76	2.70	2.53	2.86	2.99	4.72	5.63	2.72	1.64	2.70	2.49	3.70	7.06	6.87	6.56	6.90	7.10	6.89	2.33	3.65	3.10	2.75	0.99	4.09
6						4.56	1.97	2.63	2.72	4.15	4.58	6.11	3.41	3.05	4.45	1.99	4.14	6.51	6.50	6.28	6.43	6.62	6.58	2.30	3.40	2.91	2.46	2.29	3.61
7							3.89	3.50	4.00	1.37	4.02	4.22	2.31	1.71	1.04	4.02	3.76	7.27	6.42	6.36	6.82	7.25	6.54	4.00	3.82	3.68	3.90	2.55	4.13
8								1.28	1.98	3.32	3.32	4.93	2.47	2.54	4.02	1.58	2.97	6.33	6.33	6.03	6.27	6.50	6.54	2.59	2.10	1.50	1.10	2.23	2.33
9									1.78	3.11	3.65	5.19	2.18	2.40	3.59	2.13	3.01	7.03	7.05	6.71	7.02	7.22	7.00	2.70	2.60	2.10	1.64	2.08	2.60
10										3.38	3.68	5.67	3.39	2.67	4.05	2.96	2.41	6.31	6.63	6.25	6.37	6.44	6.57	2.95	2.60	2.33	1.91	2.77	2.83
11											2.84	3.51	1.98	1.59	1.89	3.72	2.94	6.53	5.70	5.71	6.10	6.60	5.73	3.89	2.78	2.85	3.29	2.63	3.10
12												3.08	3.28	3.45	4.53	4.38	3.07	6.43	5.74	5.95	6.08	6.65	5.60	5.08	2.06	2.46	3.48	4.24	2.80
13													4.00	4.56	5.04	5.16	4.14	6.70	5.91	5.91	6.57	7.30	5.59	5.80	3.38	3.83	4.70	5.31	4.13
14														1.97	2.54	2.67	3.66	7.26	6.55	6.49	6.94	7.41	6.52	3.38	2.85	2.62	2.80	1.99	2.81
15															1.84	2.89	3.13	6.74	6.14	6.05	6.33	6.66	6.40	3.11	2.94	2.61	2.71	1.33	3.37
16																4.12	4.20	7.34	6.38	6.38	6.74	7.12	6.79	3.96	4.30	4.08	4.13	2.38	4.32
17																	3.62	6.38	6.46	5.97	6.43	6.65	6.77	1.99	2.82	2.36	1.60	2.20	2.85
18																		5.15	5.64	5.09	5.46	5.68	5.31	3.55	1.78	1.96	2.22	3.71	2.69
19																			2.95	1.80	2.06	2.17	4.19	5.93	5.47	5.73	5.78	7.00	5.61
20																				1.68	1.46	2.55	4.11	6.33	5.53	5.79	6.13	6.56	5.49
21																					1.36	2.00	4.31	5.70	5.22	5.48	5.59	6.38	5.18
22																						1.13	4.66	6.21	5.60	5.80	5.96	6.69	5.54
23																							5.49	6.35	6.00	6.15	6.14	6.96	5.90
24																								6.15	5.28	5.62	6.21	6.76	5.81
25																									3.45	3.05	2.33	2.39	3.62
26																										0.93	1.73	3.37	1.78
27																											1.21	2.82	2.17
28																												2.55	2.16
29																													3.56

1-52984, 2-52983, 3-52981, 4-52980, 5-52979, 6-52978, 7-52975, 8-52974, 9-52973, 10-52972, 11-52971, 12-52970, 13-52969, 14-52968, 15-52967, 16-52966, 17-52965, 18-52964, 19-52935, 20-52937, 21-52938, 22-52939, 23-52941, 24-52942, 25-52963, 26-52962, 27-52961, 28-52960, 29-Punjab 91, 30-Paidar-91

Table 4.4.5:- Analysis on the basis of clusters for 30 genotypes of chickpea

Characters	Cluster 1		Cluster 2		Cluster 3	
	Mean ± SE	σ	Mean ± SE	σ	Mean ± SE	σ
Days to flowering	140 ± 0.89	2.17	124 ± 0.38	1.07	122 ± 0.55	2.22
Days to maturity	170 ± 1.28	3.15	177 ± 0.55	1.55	173 ± 0.55	2.21
Plant height (cm)	49.2 ± 1.66	4.07	63.3 ± 1.17	3.32	62.6 ± 2.34	9.36
Primary branches per plant	2.4 ± 0.12	0.30	3.5 ± 0.10	0.29	3.0 ± 0.09	0.34
Secondary branches per plant	10.3 ± 0.07	0.16	10.6 ± 0.30	0.86	8.7 ± 0.17	0.68
Pods per plant	56.0 ± 11.41	27.94	36.1 ± 1.53	4.34	33.0 ± 1.45	5.80
100-seed weight (g)	12.30 ± 0.07	0.17	22.45 ± 0.40	1.14	21.57 ± 0.41	1.62
Biological yield per plant (g)	24.54 ± 0.25	0.60	30.46 ± 1.46	4.14	26.83 ± 1.58	6.31
Grain yield per plant (g)	5.43 ± 0.11	0.28	16.78 ± 0.78	2.22	14.36 ± 0.69	2.78
Harvest index (%)	20.33 ± 0.57	1.41	55.18 ± 0.70	1.99	55.00 ± 0.74	2.97

Table 4.4.6:- Analysis of variance and basic statistics for 30 genotypes of chickpea

Quantitaive traits	Mean squares			SE	CD_1	CD_2	$G\sigma^2$	$P\sigma^2$	h^2	GA
	Genotypes	Replicates	Error							
Days to flowering	15.30**	15.44**	1.67	0.64	1.81	2.39	3.41	5.08	0.67	2.62
Days to maturity	27.63**	1.47	2.56	0.80	2.24	2.96	6.27	8.83	0.71	3.65
Plant height (cm)	242.45**	125.00	63.66	3.98	11.17	14.76	44.70	108.36	0.41	7.43
Primary branches	0.69**	1.23*	0.31	0.28	0.78	1.04	0.10	0.41	0.23	0.26
Secondary branches	7.07**	18.83**	1.17	0.89	2.49	3.29	1.48	2.65	0.56	1.57
Pods per plant	149.47*	899.01**	71.18	5.03	14.08	18.61	19.57	90.75	0.22	3.55
100-seed weight (g)	9.14**	0.01**	0.20	0.00	0.01	0.01	2.24	2.44	0.92	2.48
Biological yield (g)	186.98**	298.68**	61.46	5.27	14.78	19.53	31.38	92.84	0.34	5.63
Grain yield (g)	41.52**	69.27**	10.26	2.25	6.30	8.33	7.82	18.08	0.43	3.18
Harvest index (%)	47.77**	67.14**	11.19	2.79	7.81	10.33	9.15	20.34	0.45	3.51

means was observed that indicated the scope of selection from these genotypes for crop improvement. Medium to high genetic variance was observed for days to flowering, maturity, secondary branches and 100-seed weight, whereas for other characters, low to medium heritability (broad sense) was observed. Improvement of these traits through simple selection might be limited from germplasm used in the present study. For the characters like, days to flowering, days to maturity and 100-seed weight, high heritability coupled with high genetic advance revealed the presence of additive gene effects, hence crop improvement through these important traits could be possible through simple selection. Genetic advance along with heritability estimates gives an indication for gene-action and the characters with high heritability coupled with high genetic advance are supposed to be controlled additively, hence could be exploited through simple selection. Chickpea breeders should consider heritability estimates along with genetic advance because h^2 alone is not a good indicator of the amount of usable genetic variability.

4.4.3 Correlation Coefficient Analysis

The results regarding genotypic, phenotypic and environmental correlation coefficients given in the Table 4.4.7 revealed that the genotypic correlation's were slightly higher than phenotypic ones for most of the characters, exhibiting high degrees of genetic association among traits under consideration. The environmental correlation coefficients were not much important in most of the cases except five combinations, i.e., primary branches Vs secondary branches, pods Vs biological yield, pods Vs grain yield, biological yield Vs grain yield where it was positive and biological yield Vs harvest index where it was negative. The significant environmental correlation indicated environmental influence which is quite expected in a crop like chickpea. The experiment was conducted under rainfed condition and hence environments played important role to determine correlation among characters, therefore, these results could only be valid for selection under rainfed conditions. Days to flowering exhibited significantly positive correlation with primary branches (0.5687), whereas negative with plant height (-0.5505), pods per plant (-0.7241) and harvest index (-0.8992). Short duration cultivars could be selected to improve the yield potential from present material.

Table 4.4.7:- Genotypic, phenotypic and environmental correlation among ten quantitative traits in chickpea

		DF	DM	PH	PBR	SBR	P/P	SW	BY	GY
DM	r_G	0.1218								
	r_P	0.0368								
	r_E	-0.1527								
PH	r_G	-0.5505**	-0.3419							
	r_P	-0.2426	-0.1950							
	r_E	0.1064	-0.0242							
PBR	r_G	0.5687**	0.7097**	-0.6098**						
	r_P	0.2870	0.2277	0.0223						
	r_E	0.1261	-0.1254	0.3127						
SBR	r_G	0.2240	0.9120**	-0.0800	0.4986*					
	r_P	0.0134	0.2888	0.0218	0.4315*					
	r_E	-0.0985	0.0233	0.0564	0.4218*					
P/P	r_G	-0.7241**	0.1988	0.9325**	-0.5589**	-0.2000				
	r_P	-0.0507	0.0068	0.3371	0.0468	0.0884				
	r_E	0.1291	-0.0532	0.2835	0.1196	0.1134				
SW	r_G	-0.1770	0.0902	0.1254	0.1406	0.2336	0.2179			
	r_P	-0.1449	0.0764	0.0809	0.0677	0.0844	0.0455			
	r_E	-0.0095	0.1996	0.1232	0.0640	0.0466	0.0605			
BY	r_G	-0.0732	-0.2198	0.9411**	-0.3561	-0.2473	0.8112**	0.6710**		
	r_P	-0.0807	-0.1247	0.3090	0.1924	0.1416	0.5290**	0.1565		
	r_E	-0.1195	-0.1557	0.1755	0.2721	0.1789	0.5020*	0.0931		
GY	r_G	-0.3280	0.0719	0.7880**	-0.3029	-0.1422	0.8345**	0.7044**	0.9121**	
	r_P	-0.1030	-0.0282	0.3679	0.1563	0.1620	0.6616**	0.2464	0.8690**	
	r_E	-0.0172	-0.0978	0.2662	0.2519	0.2058	0.6376**	0.1191	0.8603**	
HI	r_G	-0.8992**	0.5097*	-0.6249**	-0.5512**	0.0878	-0.9881**	0.177	-0.8058**	-0.8511**
	r_P	0.0069	0.1928	-0.0425	-0.1937	-0.0323	0.0440	0.0296	-0.4851*	-0.0699
	r_E	0.2779	0.2211	0.0319	-0.1679	-0.0398	0.0862	-0.0187	-0.4600*	-0.0211

DF- days to flowering, DM- days to maturity, PH- plant height (cm), PBR- primary branches per plant, SBR- secondary branches per plant, P/P- pods per plant, SW- 100-seed weight (g), BY-biological yield per plant (g), GY- grain yield per plant (g).

Days to maturity gave positive correlation with primary branches (0.7097) and harvest index (0.5097), whereas plant height had significant positive correlation with biological yield and negative with primary branches. 100-seed weight showed significant association with biological yield and grain yield. Grain yield was positively correlated with all the characters, except for harvest index where it was negative. It was negatively insignificant with days to flowering and secondary branches. Genetic improvement in chickpea is mainly focused on grain yield by the breeders of the country. Grain yield is a complex character which is the final product of many (some known and others unknown) independent variables. In the present study, grain yield was positively associated with biological yield and 100-seed weight but negatively with harvest index. To improve grain yield emphasis should be given on development of chickpea cultivars with higher seed weight and biological yield. The genotypes with low grain yield and high biological yield consequently produced low harvest index and this important combination, high biological yield and harvest index could be attained using bi-parental mating to break unwanted linkage for further improvement of the crop.

4.4.4 Path Coefficient Analysis

The genotypic correlation coefficients were partitioned into direct and indirect effects by various yield contributing characters (Table 4.4.8). The path coefficient analysis was carried out in this study to utilize a complete represent of the causal factors involved in determining the end product i.e., grain yield. The direct effects exhibited by secondary branches, pods and 100-seed weight were positive, whereas all the other characters gave negative direct effects. The highest direct effect of 1.9242 was exhibited by pods per plant and it was followed by secondary branches (1.2356) and 100-seed weight (1.2177). 100-seed weight and pods per plant also exhibited significant positive association with grain yield, hence could more confidently be exploited for crop improvement.

In the present study conducted under rainfed conditions, it is indicated that pods per plant and 100-seed weight had the maximum contribution in determining grain yield, the ultimate product in chickpea under rainfed conditions. Further, it was observed that high indirect contribution was exhibited via secondary branches and harvest index by most of the yield components, hence these two traits along with pods per plant and 100-seed weight are suggested to be given emphasis while

Table 4.4.8:- Direct (parenthesis) and indirect effect of independent variables with dependent variable (grain yield). The last column shows genotypic correlations of independent variables with grain yield.

Variables	Days to flowering	Days to maturity	Plant height	Primary branches	Secondary branches	Pods per plant	100-seed weight	Biological yield per plant	Harvest index	RG with Grain yield per plant
Days to flowering	(-0.4438)	-0.1477	0.8294	-0.4247	0.2768	-1.3934	-0.2155	0.0788	1.1121	-0.3280
Days to maturity	-0.0541	(-1.2127)	0.5150	-0.5300	1.1269	0.3825	0.1098	0.2366	-0.5022	0.0719
Plant height	0.2444	0.4146	(-1.5065)	0.4554	-0.0988	1.7942	0.1527	-1.2835	0.6157	0.7880
Primary branches	-0.2524	-0.8607	0.9187	(-0.7467)	0.616	-1.0755	0.1712	0.3833	0.5431	-0.3029
Secondary branches	-0.0994	-1.1059	0.1205	-0.3723	(1.2356)	-0.3848	0.2845	0.2662	-0.0865	-0.1422
Pods per plant	0.3214	-0.2411	-1.4048	0.4174	-0.2471	(1.9242)	0.2654	-1.1458	1.1742	1.0638
100-seed weight	0.0785	-0.1093	-0.1889	-0.1050	0.2887	0.4193	(1.2177)	-0.7222	-0.1744	0.7044
Biological yield per plant	0.0325	0.2666	-1.7964	0.2659	-0.3056	2.0482	0.8170	(-1.0764)	0.7939	1.0458
Harvest index	0.5010	-0.6181	0.9414	0.4116	0.1085	-2.2933	0.2155	0.8674	(-0.9852)	-0.8511

selecting high yielding chickpea cultivars for rainfed conditions. Correlation and path coefficient analyses indicated that pods per plant and 100-seed weight were potent contributors to grain yield through direct effects. On the basis of performance, seven accessions produced higher grain yield than both the checks, hence were selected and presented in the Table 4.4.9 for further evaluation under a wide range of environmental conditions.

4.5. Biochemical (SDS-PAGE) Basis of Genetic Diversity

All the accessions evaluated for agronomic traits were also used for the analysis of SDS-PAGE through slab type gel electrophoresis using 10 samples for each accession. Although, all of these were not homozygous and polymorphism did exist for one or another locus within various samples of the accessions. SDS-PAGE was conducted in various combinations and it was revealed that 11.25% acrylamide gel concentration, 6 µl of sample gave the best resolution. The electrophoretic seed protein profiles for most of the accessions were similar. Out of these sixty two accessions, 41 were homozygous on the basis of SDS-PAGE whereas others were heterozygous hence single seed descents could be isolated from these heterogeneous lines to establish pure-lines for future breeding programmme. In total, 14 protein bands were recorded ranging from the Molecular Weight (MW) of 24 to 66 KDa. Many protein subunits of lower MW were also observed but due to inconsistency 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 into consideration.

Out of 14 protein subunits, 8 were polymorphic and 6 were monomorphic. Only polymorph bands were included in PCA and constructing of dendrogram. On the basis of banding pattern, gel was divided into three regions (Fig. 4.5.1a & b). Region I had 4 bands of more than 66 KDa MW of which 3 were polymorphic. Region II ranged from 24 to 66 KDa 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. The quantitative intensity of bands were not recorded at present, although, these may provide some information specific to chickpea. Weak protein bands were observed in the region III of lower molecular

Table 4.4.9:- Performance of chickpea genotypes selected on the basis of evaluation during 1999

Genotypes	DF	DM	PH	Pods	SW	GY	HI
52984	123	171	73.3	39.5	22.17	18.35	56.35
52983	123	174	62.5	42.2	20.84	19.15	50.64
52981	125	177	66.9	37.0	22.40	19.15	53.77
52979	123	174	65.3	46.0	23.98	20.05	57.00
52978	124	174	64.6	38.6	21.11	18.75	52.08
52975	122	178	59.6	39.9	22.57	19.95	54.31
52974	121	174	66.5	39.1	23.52	19.50	53.48
Punjab-91	124	176	66.5	38.8	23.52	18.35	54.36
Paidar-91	124	174	66.9	37.7	16.98	11.95	56.52
F.ratio (V)	9.13**	10.78**	3.80**	2.09*	242.1**	4.05**	4.27**
F.ratio (R)	9.21**	.57ns	1.96ns	12.63**	149.5**	6.75**	6.00**
CD1	1.81	2.24	11.17	14.08	.009	6.30	7.81
CD2	2.39	2.96	14.76	18.61	.011	8.33	10.33

DF- Days to 50% flowering, DM- days to 90% maturity, PH-Plant height (cm), Pods- number of pods per plant,
SW- 100-seed weight, GY- Grain yield per plant (g), HI- Harvest index (%).

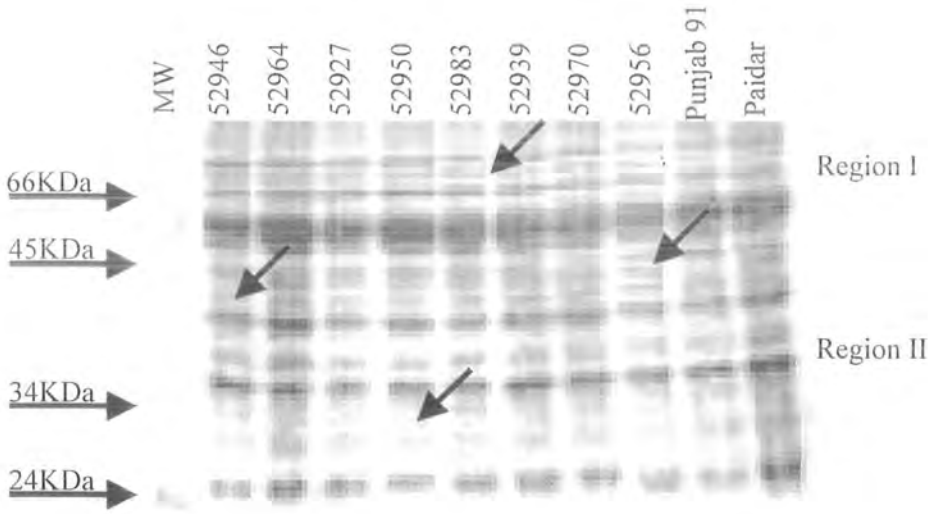


Fig.4.5.1a:- Total seed protein pattern in chickpea accessions obtained by slab type gel electrophoresis using SDS-PAGE. The marker SDS 70 from Sigma Chemicals was used as standard. The arrows indicate variation among various accessions involved in the evaluation.

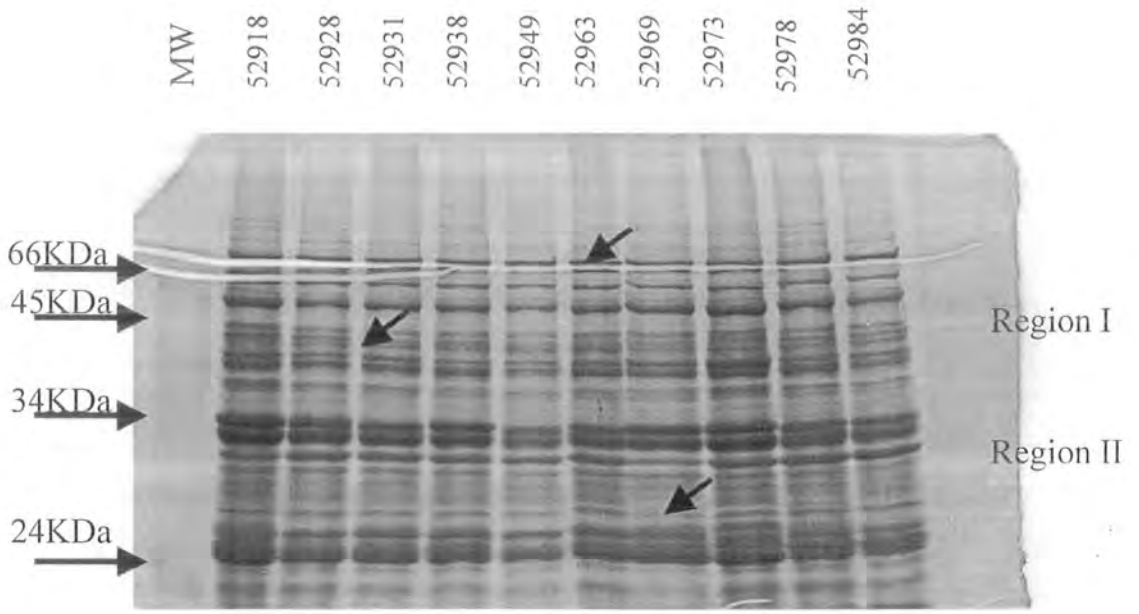


Fig.4.5.1b:- Total seed protein pattern in chickpea accessions obtained by slab type gel electrophoresis using SDS-PAGE. The marker SDS 70 from Sigma Chemicals was used as standard. The arrows indicate variation among various accessions involved in the evaluation.

weight, hence not recorded due to inconsistency in presence. Weakly stained bands 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 chickpea. Many accessions which were observed similar based on protein pattern were excluded from cluster and Principal Component Analyses.

4.5.1 PCA Based on Protein Peptides

As already mentioned that out of 62 accessions/genotypes, 41 accessions were homozygous and others were heterozygous. The analysis was performed for total material and homozygous lines separately. The variance was rather more scattered on the basis of protein pattern as compared with quantitative characters. The first three components contributed 56.41% of the variation amongst 62 accessions (Table 4.5.1). Principal component 1 had 25.30% of the total variation, PC_2 16.85% and PC_3 had 14.25% of the total variation, respectively. Other components exhibited eigenvalues less than unity, hence did not contribute for variance significantly. The protein bands which contributed more positively to PC_1, were B2, B4 and B13. Four bands (B5, B7, B12, B14) contributed maximum genetic variance to PC_2 , whereas one protein subunit (B11) exhibited the highest value for PC_3.

First three components which contributed 56.41% of the total variation were plotted as scatter diagram. The scatter diagram on the basis of PC 1 and 2 where Factor 1 was used as x-axis and factor 2 as y-axis is presented in the Fig. 4.5.2. The accessions were plotted on the basis of their origin. Many accessions overlap each other due to similarity on the basis of SDS-PAGE markers. Two approved varieties were separated in the left upper part of the graph whereas few accessions were marked separately in other cases. Two accessions from District Khushab and four from District Layyah were clearly separated whereas others were mixed together or overlapped to each other. Similar pattern was observed for PC 1 and 3 where Factor 1 was kept as x-axis and factor 3 as y-axis (Fig. 4.5.3). Approved varieties were clearly separated in the left half of the graph. From others, five accessions originated from the District Layyah were grouped into 2 groups,

Table 4.5.1:- Principal Components (PCs) for 8 SDS-PAGE markers in 62 accessions of chickpea

		PC_1	PC_2	PC_3
Eigen value		2.02	1.35	1.14
Proportion of σ^2		25.30	16.85	14.25
Commulative σ^2		25.30	42.15	56.41
	Communality		Eigen factors	
Band 2	0.700	0.830	-0.075	-0.078
Band 4	0.754	0.859	-0.102	0.075
Band 5	0.557	0.257	0.701	0.016
Band 7	0.539	-0.047	0.459	-0.571
Band 11	0.550	0.274	-0.151	0.672
Band 12	0.447	-0.169	0.556	0.331
Band 13	0.489	0.475	-0.209	-0.469
Band 14	0.475	0.447	0.505	0.143

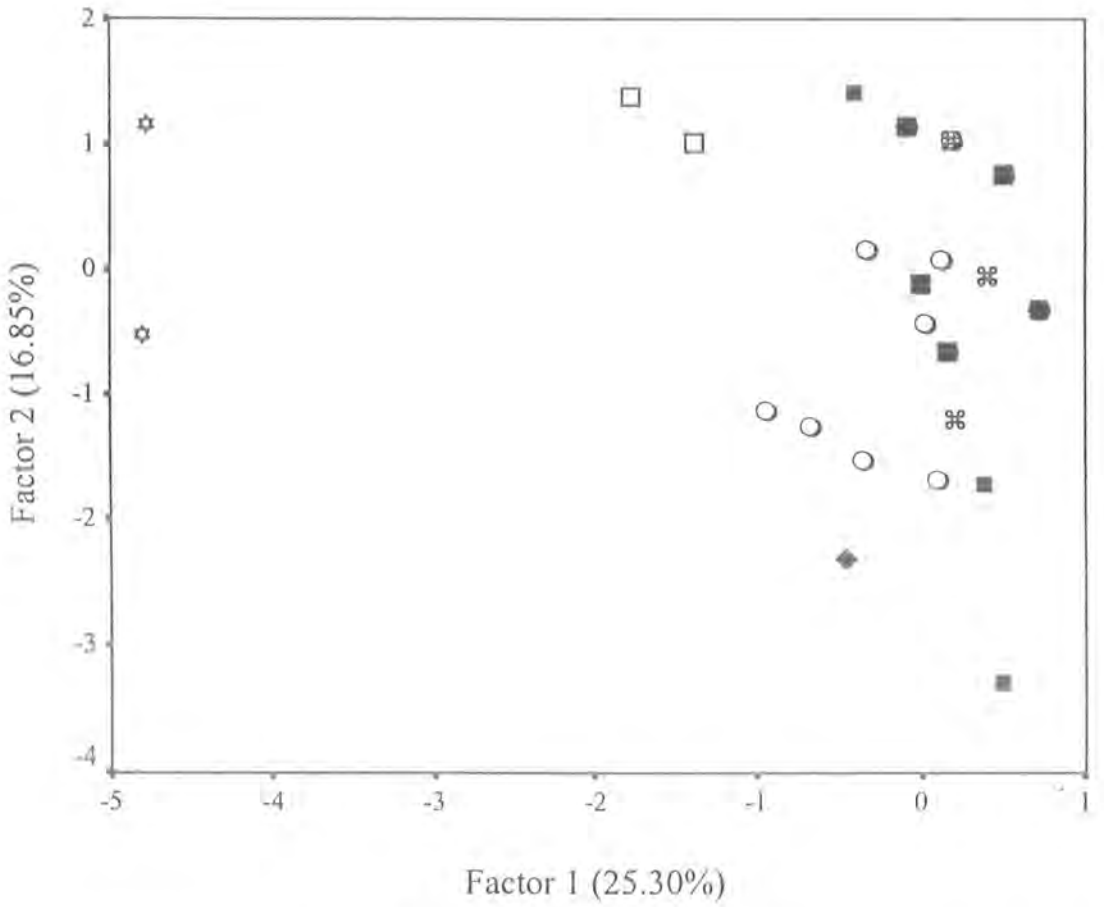


Fig.4.5.2:- Scattered diagram based on SDS-PAGE markers for first and second factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, ◆- District Mianwali, ⌘- Bahawalnagar and ✡- approved varieties

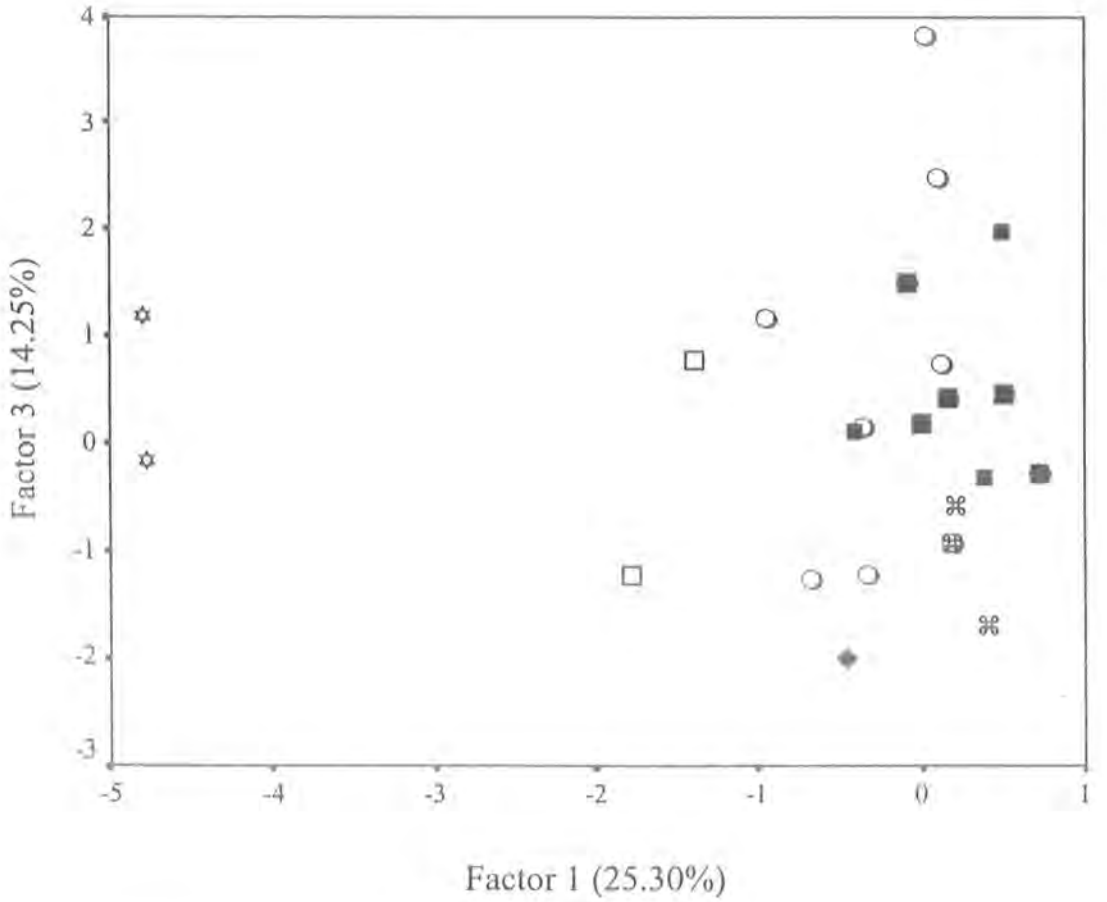


Fig.4.5.3:- Scattered diagram based on SDS-PAGE markers for first and third factors in chickpea. The marks represent as ○- District Layyah, ■- District Bhakkar, □- Districh Khushab, ◆- District Mianwali, ⌘- Bahawalnagar and ✡- approved varieties

one consisting of three accessions was in upper right part and one comprising of 2 accessions was observed in the lower right part of the graph. Two accessions originated from Khushab and Bahawalnagar in each case were also clearly separated from others, whereas others were mixed together or overlapped to each other. Although, both the graphs revealed similar pattern and did not indicate any clear differences on the basis of origin when analysed for SDS-PAGE, hence the separation on the basis of PC 1 and 2 could be considered better than on the basis of PC 1 and 3, because of high magnitude of variability contributed by factor 2 than 3.

4.5.2 Cluster analysis

The dendrogram of total seed proteins based for all the 62 accessions (including 2 checks) on dissimilarity matrix using UPGMA showed a division into five clusters (Fig. 4.5.4). Clusters I consisted of two approved varieties, cluster II consisted of one accession (PAK-52946), cluster III comprised of four accessions, viz., PAK-52984, PAK-52935, PAK-52934, PAK-52931, whereas cluster IV consisted of PAK-52932 accession. All the other accessions were grouped together to constitute one cluster at 1.5 linkage distance. Out of these 62 accessions, 41 were homozygous and others were heterozygous when analysed 10 samples in each accession. Homozygous accessions were used to construct dendrogram on the basis of SDS-PAGE separately as presented in the Fig. 4.5.5. The heterozygous accessions gave no information when combined with pure-lines, hence heterozygous accessions were suggested to evaluate under field conditions for isolation of superior lines for future breeding programme.

The dendrogram based on biochemical markers for 41 accessions exhibited seven clusters when cut at 1.2 linkage distance. Cluster I, II and III consisted of one accession in each case as PAK-52984 in cluster I, PAK-52935 in II and Paidar 91 in cluster III. Cluster IV consisted of four accessions (PAK-52972, PAK-52930, PAK-52952, PAK-52928), whereas cluster V comprised eight accessions (PAK-52978, PAK-52965, PAK-52964, PAK-52979, PAK-52966, PAK-52957, PAK-52951, PAK-52945). Twenty four accessions including one check were grouped together in cluster VI and remaining four accessions (PAK-52980, PAK-52981, PAK-52950, PAK-52924) were observed in cluster VII. In general, cluster analysis

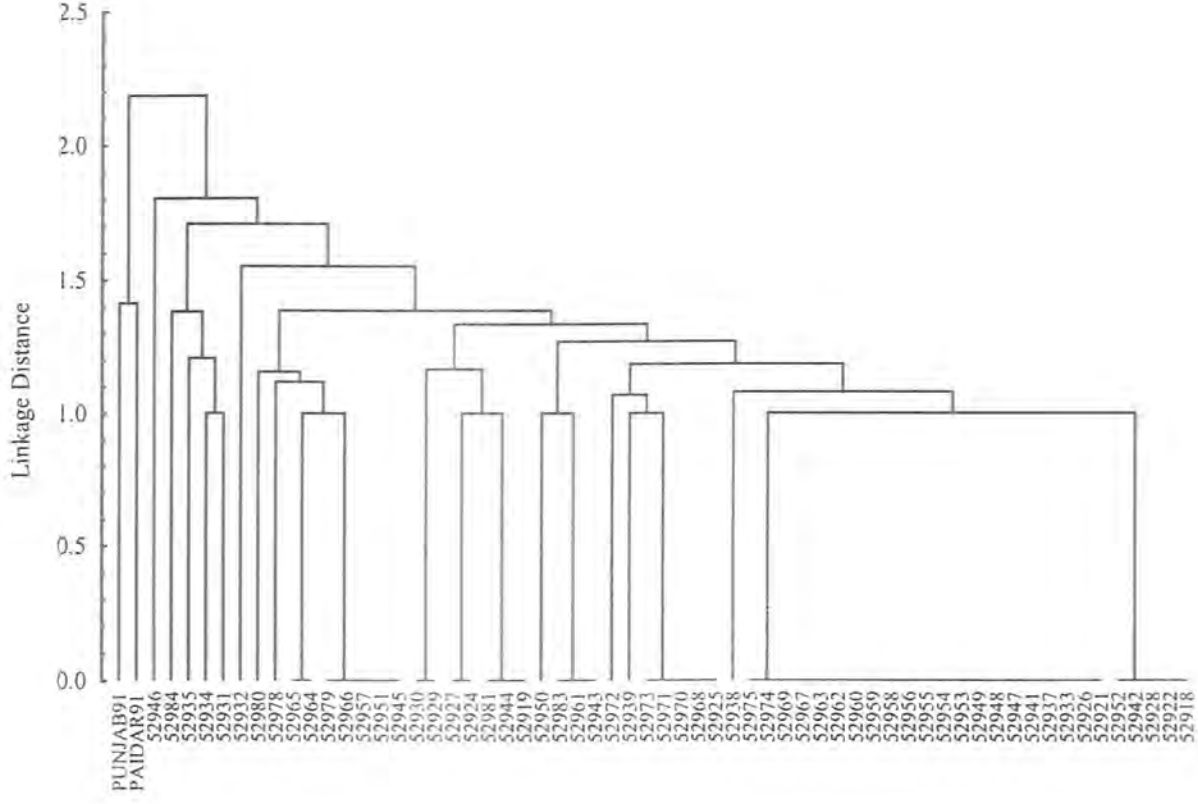


Fig. 4.5.4:- Cluster diagram of 62 chickpea accessions along with 2 checks based on SDS-PAGE markers

Fig. 4.5.5:- Cluster diagram of 41 homozygous chickpea accessions and 2 checks based on SDS-PAGE markers

based on SDS-PAGE in local chickpea germplasm did not reflect any clue either for agronomic preference and/or geographic distribution.

The genetic dissimilarities for various protein subunits presented in the Table 4.5.2) revealed that genetic distance varied from 1.00 (Band 2 vs Band 7) to 4.12 (Band 11 vs Band 13). Four protein types were observed at 3.0 linkage distance when compared among all the polymorphic bands. The protein subunits 11 and 13 were obviously different from other protein subunits, i.e., bands 2, 4, 5, 7, 12 and 14. For most accessions and protein subunits, no clear observation was recorded which could facilitate selection on the basis of SDS-PAGE for improving agronomic traits in chickpea from the material under investigation. Further, high variance for most of the characters in almost all the clusters also revealed that the genotypes in various clusters were may be from different origins but sharing similar protein peptides.

Table 4.5.2:- Genetic dissimilarities among various protein subunit types in chi ckpea

	B4	B5	B7	B11	B12	B13	B14
B2	1.73	2.24	1.00	3.32	2.45	3.16	2.45
B4		2.45	2.00	3.16	3.00	3.61	3.00
B5			2.45	3.74	3.00	3.61	2.65
B7				3.46	2.65	3.00	2.65
B11					3.61	4.12	3.61
B12						3.74	2.83
B13							3.74

DISCUSSION

DISCUSSION

5.1. Germplasm Evaluation

Identification of the superior genotypes of a plant species has always been important for crop improvement, genetic research and breeding programme (Ghafoor et al., 2001). Genetic improvement in any quantitative trait depends on effective selection among individuals that differ in phenotypic value, and effective selection is possible only when genetic variability is present. In order to utilize germplasm efficiently and effectively, it is important to investigate the extent of genetic diversity they contain. 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 and Smith, 1989; Singh and Tripathi, 1985). Oftentimes, evaluation may be regarded as an end in itself, but the best evaluation is one that relates to the plant breeder's needs.

In this study, world collection of chickpea germplasm along with local accessions collected from all over the country was evaluated for six qualitative and four quantitative traits including disease (*Ascochyta rabiei*) reaction at National Agricultural Research Centre (NARC), Islamabad under rainfed conditions. World core collection of chickpea comprising 423 accessions differed significantly for plant traits of qualitative nature with distinct classes like growth habit, iron deficiency, flower colour, plant pubescence, plant pigmentation and pod size. Two hundred and one accessions were erect types, 184 accessions were semi-erect and thirty eight were spreading.

Three hundred and twelve accessions did not show symptoms of iron deficiency. These symptoms of iron deficiency are associated with colder climate, because the accessions showing iron deficiency under colder region seldom show similar reaction under warm regions (Anonymous, 2001). Three hundred forty six accessions were pink flowered and only 77 gave white flowers, and these were all white seeded or Kabuli types. White coloured flowers are associated with Kabuli types but these two genes are independent of each other although these may be

closely linked. Twelve accessions were glabrous and all others were pubescent. Out of these, 235 accessions were with less hairs while others were with dense hairs. The accessions with larger pods are suggested to be utilized for future selection and improvement for high seed weight as large pods contain bold seeds. 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 large pod size could be used to improve pod and seed size in chickpea, because these two traits are interlinked to each other. Hairiness in crop gives more tolerance against some insects, whereas glabrous cultivars facilitate in harvesting and threshing (Ghafoor *et al.*, 2001).

The range of CV and genetic variance (σ^2) revealed that the results could be of broader spectrum. All the exotic germplasm lines badly infected by disease and sixty accessions could produce good quality seed and hence selected for further evaluation and study. Superior genotypes selected from preliminary evaluation of a broad based germplasm facilitate improvement of yield potential (Patel & Shah, 1982 and Ghafoor *et al.* 1993b). In the total germplasm, days to flowering was negatively correlated with days to maturity, whereas it was positively significant in selected accessions. The negative association of days to flowering with days to maturity in the total germplasm was may be due to disease infection at the time of pod formation that caused forced maturity and little seed was obtained.

Days to maturity was positively correlated with branches per plant in total population and negatively in selected accessions. Similarly, branches per plant were also positively correlated with plant height in total germplasm and negatively associated in selected accessions. Days to maturity exhibited positive correlation with plant height in both the population although it was slightly higher in selected accessions but 8% of total germplasm was of course higher than 10% of the selected accessions. The results in general revealed that maturity and branches per plant were more affected by environmental factors.

On the basis of performance and tolerance to *Ascochyta rabiei*, sixty accessions were selected for further evaluation and suggested for further testing under wide range of agro-ecological conditions to utilize for selection/breeding of high yielding chickpea cultivars. Selection on the basis of best performance has already been suggested by many researchers like Donald, (1962), Lal, (1967), Singh et al, (1977), Singh *et al.* (1980) and Khan & Malik, (1989). Virmani *et al.* (1983) evaluated mungbean germplasm and classified it 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, three 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 is 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). Dotlacil *et al.* (2000) reported that landraces and obsolete cultivars were better able to compensate for adverse environment, but at a lower level of yield potential than modern cultivars, hence local accessions selected from the present material need careful evaluation for utilizing its potential in crop improvement. It is suggested that cultivars with this broad environmental adaptation could give better results in organic or extensive farming systems. Whereas Krajewski and Drzazga (1999) showed no straight relationship between the characteristics of the lines and their origin, with a consistent tendency to produce stable, high yielding genotypes.

The effect of plant breeding on yield and its physiological determinants has been widely studied in chickpea. However, it is poorly understood, how, and to what extent, yield stability has been modified. There was a clear decrease in yield stability assessed in absolute terms as a consequence of chickpea breeding. Brancourt and Slinkard (1998) felt complications in breeding for improved varieties studying genotype environment interactions. A thorough understanding of the environments can improve the efficiency of breeding methods.

Subdividing the variance into its components assists the genetic resource conservation and their utilization. It enables planning for use of appropriate gene pools in crop improvement for specific plant attributes (Pecetti *et al.* 1996). Large scale testing of broad-based germplasm needs to be built up by making extensive local collection and introductions to develop a sound breeding programme (Ghafoor *et al.* 1992). Laghetti, *et al.* (1998) advocated that maximum genetic conservation would be achieved by sampling populations from as many environments as possible. Correlation coefficients revealed negative correlation of days to flowering with maturity and plant height, whereas other correlations were positive and maximum association was observed between plant height and number of branches per plant. The results indicated that the genotypes selected for early flowering may not necessarily mature early because maturity time is more influenced by environmental conditions at the time of harvesting during the months of April and May.

The germplasm was grouped into 10 clusters based on average linkage distance. Cluster I consisted of 60 accessions, cluster II of 56; cluster III of 73; cluster IV of 53, cluster V of 62, cluster VI of 26, cluster VII of 35, cluster VIII of 20, cluster IX of 23 and cluster X of 15 accessions. Approved cultivar, Paidar 91 was in the cluster VI, whereas as other two varieties (Noor 91, Punjab-91) were in cluster VIII. All the exotic accessions were grouped in the clusters I, II, III, IV, VIII and X whereas other clusters consisted of mixed accessions of local and exotic origin. Out of 63 local accessions (including 3 varieties), eleven were in the cluster V, sixteen in cluster VI, seventeen in cluster VII and nineteen in the cluster IX. As the number of accessions from various sources were grouped in a systematic way, therefore relationship may be established between origin and clustering pattern. Out of this total germplasm, sixty accessions which were of

local origin were further evaluated to investigate the genetic diversity and its relationship with geographic origin based on quantitative traits and SDS-PAGE analysis.

Genetic distance based on average linkage ranged from 0.5952 for the clusters II and IV to 1.5786 for the clusters I and IX. The genetic distance of cluster I was higher with most of the other clusters ranging from 1.0945 (with cluster X) to 1.5584 (with cluster VIII). Both of these clusters consisted of exotic germplasm and it is also important to note that cluster I consisted of all the 60 accessions of exotic origin. Similarly, cluster II exhibited high genetic distance with cluster V, VIII and X. Out of these, cluster V consisted of both the exotic (51 accessions) and local germplasm (11 accessions). Cluster III exhibited genetic distance from 0.6524 (cluster III vs IV) to 1.3976 (with cluster I) and the cluster IV gave high range of genetic distance with all other clusters. Cluster V, VI and VII exhibited low to medium genetic diversity among one another, whereas medium to high genetic diversity with the cluster consisting of exotic germplasm. Cluster VIII also consisted of all the germplasm of exotic origin and had medium to high genetic distance with other clusters, whereas cluster IX consisted of both the exotic (4 accessions) and local (19 accessions) exhibited the lowest genetic distance (0.5173) with cluster VI and the highest (1.5786) with cluster I. Therefore, this cluster might consist of diverse accessions on the basis of the qualitative traits. The selected accessions from various clusters are suggested to be used in crop improvement in future (Singh *et al.*, 1997a).

Variance studied by PCA revealed that first four components with eigenvalues more than 1 contributed 70.6% of the variability amongst 423 accessions evaluated for nine traits. Principal component 1 had 23.64% of the total variation, PC_2 18.99%, PC_3 14.38% and PC_4 had 13.61% of the total variation. Characters that contributed more positively to PC_1 were days to maturity and plant height, whereas days to flowering, qualitative traits and disease reaction contributed least to first component. Iron deficiency, flower colour, plant pigmentation and disease reaction gave negative contribution towards this component.

Plant pigmentation and disease reaction contributed maximum genetic variance to PC_2, whereas plant pubescence, growth habit and flower colour were

assessed significant for PC_3. Although, the variation for growth habit was also contributed by PC_2 (0.521), it was slightly lower than variation by PC_3 (0.570). Iron deficiency and days to flowering contributed maximum for PC_4 with values of 0.763 and 0.365, respectively. The first PC which explained 23.64% of the variance is positively associated with five characters and out of these two were quantitatively inherited. The populations with high PC_1 values are late in maturity and tall staured.

Though, cluster analysis grouped together accessions with greater genetic similarity, the clusters did not necessarily include all the accessions from same origin. Gupta *et al.* (1991), Das *et al.* (1989), Amurrio *et al.* (1995), Rabbani *et al.* (1998) and Ghafoor *et al.*, (2001) also reported lack of association between agronomic traits and origin.

5.2. Evaluation of Sixty Local Accessions

Green revolution was introduced by a tremendous increase in harvest index that enhanced cereal productivity, but due to low yielding ability of legumes, this group of crops could not be benefited through green revolution. To increase harvest index, high grain yield coupled with optimum biomass along with other yield components are suggested to combine together. High variance for measured traits was observed except for seeds per pod where, a low variance was observed and hence improvement for this trait seemed to be difficult in the local germplasm used in present study. High genetic variability observed for yield components could be exploited through simple selection for isolation of the best cultivars. Although, maturity range was not very high and a difference of only 3 weeks was observed between early and late maturing accessions, but at the time of harvest the earliness up to 3 weeks is considered sufficient for the preparation of land for next sowing. High variance for yield contributing traits was observed in the present material that could be utilized for improving yield potential of chickpea in future breeding programme.

The germplasm characterized for plant traits of qualitative nature with distinct classes revealed genetic variation for most of the characters. Some of the chickpea genotypes show response to iron deficiency due to some physiological disorder and it was observed that few lines exhibited response to iron deficiency

although under major chickpea growing areas (Thall desert), these does not respond to iron chlorosis (Anonymous, 2002). 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 the present investigation, local chickpea germplasm was evaluated for qualitative and quantitative characters along with chickpea blight reaction.

For qualitative characters, a considerable level of variability was observed for most of the traits. 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. Ghafoor *et al.*, (2001) considered that these traits reflect some biotic/abiotic stresses association. Prostate plant type is preferred for planting under rainfed conditions as it facilitates moisture conservation. The plants with narrow leaves in most cases are drought tolerant and hence, the plants with these characters may be utilized for breeding chickpea for drought areas.

Correlation is a measure of the degree to which variables vary together or a measure of intensity of association. The results regarding correlation revealed that days to flowering was significantly positive with days to maturity, whereas it was negative with plant height, seeds per pod, grain yield and harvest index. Branches per plant exhibited significantly positive association with all the traits except days to flowering, days to maturity and plant height. Similarly pods per plant had significantly positive correlation with all the traits except with days to flowering, days to maturity and plant height. 100-seed weight exhibited positive association with branches per plant, pods per plant, pods per branch, seeds per pod, grain yield, biological yield and harvest index. The positive association of 100-seed weight with seeds per pod was only possible because of the presence of large pod size accessions in the present material. Grain yield, biological yield and harvest index showed positively significant association with all the traits except

with days to flowering, days to maturity and plant height. In the present study, out of eleven characters, eight were observed to be yield contributing for chickpea improvement.

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 chickpea breeding programme exhibited positive association with all the characters under study with varying degrees of significance. High correlation of grain yield with yield contributing characters revealed the importance of these characters for increasing yield potential in chickpea. 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.

Baluch and Soomro (1968) and Sharma et al (1969) also reported that pods per plant and seed weight had significant positive correlations with seed yield per plant. Sandhu and Singh (1970), Rang *et al* (1980), Khorgade *et al* (1985), Setty *et al* (1977) and Singh *et al* (1978) obtained correlation between seed yield per plant and number of primary and secondary branches and pods per plant. Singh *et al* (1978) indicated that selection based on high pod number, primary branch number and a low secondary branch number would be effective to improve chickpea yield. But, the results obtained from the present experiments revealed strong associations of seed yield with number of pods per plant and branches per plant. This indicates that improving number of pods, seeds and secondary branches simultaneously will directly increase the yield per plant. Khorgade *et al* (1985) found 100-seed weight and number of total branches per plant as the most important yield determiners. Adhikari and Pandey (1982a) found a significant and

negative correlation between plant height and pods number per plant. Islam *et al* (1982) found also a negative relationship between seed yield per plant and plant height which is contrary to the results of the present study.

Similar studies were carried out by Dahiya *et al* (1986), Naidu *et al* (1982), Tomar *et al* (1982), Ram *et al* (1980) and others in a segregating population and most of the findings showed that seed yield per plant was positively correlated with the number of pods and seeds per plant. After comparing different selection criteria, Dahiya *et al* (1986), recommended to use the number of fruiting branches as the criterion to increase seed yield in chickpea. Ram *et al* (1980) found out that the number of pods per plant and seeds per plants as effective yield measures in the F2 and F3 generation of chickpea. These findings are similar to the results obtained in the present experiment.

The results regarding correlation coefficients are in agreement with the results obtained by Rani and Rao (1981), Singh *et al.* (1985) Sinha *et al.* (1986), Malik *et al.* (1987), Malik *et al* (1988), Ghafoor *et al.* (1990), Tariq (1990), Hussain *et al.* (1991), Bakhsh *et al.* (1991). The positive association between yield and these parameters revealed that increase in the height, number of branches, number of pods per plant, 100-seed weight, biological yield and increase in harvest index, would have direct and proportionate impact on grain yield in chickpea. These strong relationships between yield and these parameters would enhance the grain yield. These results get support from the findings of research workers like Bahl *et al.* (1976), Bahl and Jain (1977), Singh *et al.* (1985), Ali (1985) and Sinha *et al.* (1986). Yadava (1973), Singh and Singh (1974) also reported positive and significant correlations of grain yield with branches per plant, pods per plant in *Brassica juncea*. Katiyar and Singh (1974) also found positive association of seed yield and plant height in mustard. Katiyar and Singh (1978) reported positive correlation between yield and pods per plant. Singh *et al.* (1977) observed that plant yield was positively correlated with 100-seed weight, plant height, number of pods per plant and number of branches per plant. The reports of Johnson *et al.* (1955) for pods per plant and Malhotra *et al.* (1979) for plant height were similar to the present investigation.

Chuhan and Sinha (1982) reported positive correlation of yield with yield components. Sharma and Maloo, S.R. (1988), Sandhu *et al.* (1989), Singh *et al.*

(1989) Wadood and Yaqoob (1989), Singh *et al.* (1990), Tagore and Singh (1990), Chhina *et al.* (1991), Chaudhry *et al.* (1991) reported positive correlation between yield and yield components in cowpeas. However, Wadud and Yaqoob (1988a, 1988b) reported a negative relationship between grain yield and plant height. Hussain *et al.* (1991), Akbani *et al.* (1990), Kumbhar *et al.* (1983), Soormro and Larik (1981) reported negative association between grain yield and harvest index in chickpea, wheat and peanut respectively. Wadud and Yaqoob, (1989) reported a negative and non-significant correlation between grain yield and plant height. Chaudhry *et al.* (1991) observed negative association between yield and number of branches per plant in cowpea. This deviation may be due to differences in genotypes used and different ecological conditions.

There was a strong and highly significant correlation of biological yield with grain yield and plant height, branches, pods per plant and 100-seed weight. Singh and Malhotra (1970), Hussain *et al.* (1991) reported non-significant but negative correlation between 100-seed weight and pods per plant at both levels. However, Katiyar (1978) observed positive association. 100-seed weight showed non-significant negative association with secondary branches. Such a negative association occurs when two developing structures of plant compete for a common nutrient supply and negative correlation may arise if one structure is favoured over the other in the amount of nutrient supply (Adam, 1967). In the above discussion, it has been observed that grain yield had strong positive correlation with biological yield, plant height, branches per plant, pods per plant and 100-seed weight, so yield can be increased by increasing secondary branches, plant height, pods per plant, biological yield and seed weight. It may be concluded that yield in chickpea can be increased by improving the above positive responsive parameters. It is also suggested that branches per plant and harvest index can never be missed while making selection for high yielding varieties of gram.

The selected accessions were disease tolerant and possessed high yield potential along with medium to high harvest index. Patel & Shah, (1982) and Ghafoor *et al.* (1993b), considered 25 to 35% harvest index range best for legumes. 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 chickpea 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. 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 study, first three principal components with eigenvalues more than 1 contributed 88.58% of the variability amongst 62 genotypes evaluated for eleven quantitative traits. Other components (PC_4 to PC_{11}) were less than unity hence could not prove their importance. Principal component 1 had 60.39% of the total variation, PC_2 16.45% and PC_3 11.75% of the total variation. Only PC_1 exhibited more than half of variability, hence considered cumulative of other components. Characters that contributed more positively to PC_1 were, branches per plant, pods per plant, pods per branch, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index, whereas days to flowering contributed least to first component. Days to flowering and maturity contributed maximum genetic variance to PC_2 and plant height was assessed significant for PC_3. Days to maturity were contributed by all the factors but high effects were observed for PC_2.

All the characters under study contributed genetic variance positively towards PC_1 except days to flowering where it was negative. Eight characters (branches, pods per plant, pods per branch, seeds per pod, 100-seed weight, biological yield, grain yield and harvest index) exhibited maximum effect on PC_1 and seven characters were positive for PC_2, out of which days to flowering and days to maturity contributed maximum. In more detail, the first PC which explained 60.39% of the variance is positively associated with all the characters except one and eight important yield contributing characters exhibited more

positively, whereas days to flowering contributed least. This means that the populations with high PC_1 values are high yielding and formed by medium maturing plants characterized by high seed weight and harvest index. Seven characters contributed positively for PC_2 where days to maturity was observed with highest values for PC_2. It is evident that ten important plant characters contributed more positively to first 2 principal components and hence these could be established important for the material under investigation. The component 3 contributed maximum for plant height, although it had good share for days to maturity.

The first component is strongly associated with high yield potential and yield contributing traits, thus more related to reproductive phase. The second component is associated with days to flowering and days to maturity, contributing 17.2% of the total variance, hence the populations in this component are more likely related to vegetative traits. The population with high PC_2 values are characterized by late flowering and maturity. The populations in this component are associated negatively with plant height, seeds per pod, grain yield and harvest index which revealed that the accessions in the population failed in appropriate partitioning of economic yield which ultimately reduced harvest index. The accessions were plotted on the basis of geographic origin and source of seed collection, hence these were investigated to see whether the genetic diversity was related to geographic origin or not. The PC_1 and 2 revealed one group in the left upper half, one in the right upper half, one in between of these two groups and one consisting checks was observed in the lower half of the graph. Similar results were observed where factor 3 was plotted against y-axis instead of factor 2.

Three clusters were in the middle of the graph with similar pattern as in the graph plotted for factor 1 and 2. The only difference was that the varieties were shifted in the upper half of the graph. Results reported by Falcinelli *et al.* 1988 showed multivariate analyses to be a valid system to deal with germplasm collections. Grouping of accessions by multivariate methods in the study is of practical value to the chickpea.breeders. Representative accessions may be chosen from particular groups for hybridization programs with other approved cultivars. Several potentially important agronomic types have been identified which may be exploited for genetic potential to transfer the desirable genes and this, along with

biochemical analysis, will facilitate in assembling a core collection from the large genetic resources (Singh, 1988 and Clements & Cowling, 1994).

From the present investigation, it was concluded that chickpea germplasm displayed a wide range of diversity for most of the traits studied and that there were few accessions with unique characters. This could enable us to identify, select and combine landraces to obtain important traits in one line with a broad genetic base. Grouping of advance breeding lines in one cluster revealed that only a portion of genetic variance has been exploited for chickpea improvement in the past. If one of the goals is to bring together cultivars with genetically similar characteristics, quantitative characters may be useful for such grouping. Nevertheless, the qualitative traits must be often used for separating varieties when a limited range of quantitative traits is found.

It is estimated that in the absence of environmental stresses, known physical, chemical and biological management methodologies permit farmers of the world to produce potential grain yields of 4 t/ha. However, current average world yields are in the range of 1 t/ha and climatic catastrophes, the only unmanaged and unmanageable phenomena, does not explain the shortfall. This brief presentation examines the extent to which biotic and a-biotic stresses can be overcome by breeding for genetic resistance with emphasis on resistance to chickpea blight and drought. The important yield traits; days to flowering, maturity, branches, pods, pods/branch, biomass, grain yield and harvest index exhibited high range along with high variation which, in general revealed that the selection for these economic traits is effective in developing high yielding varieties of chickpea.

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 characters where low genetic variability seemed to restrict the scope of selection for these traits in the present germplasm collection, 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.

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 chickpea. 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 along with 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.

From the present investigation, it was determined that local chickpea germplasm displayed a wide range of diversity for most of the traits studied along with 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.

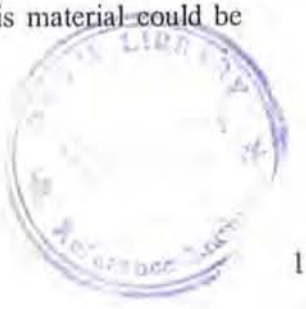
5.3. Classification Based on Geographic Distribution

To determine the distribution of diversity in germplasm, 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), Holcomb *et al.* (1977), Pezzotti *et al.* (1994), Erskine & Muehlbauer, (1991), Clements & Cowling, (1994), Perry & McIntosh, (1991) and Ghafoor *et al.*, (2001) in a variety of crop species.

All the sixty accessions included in the experiment were collected from five major chickpea growing districts of the Punjab, i.e., Bahawalnagar, Bhakkar, Khushab, Layyah and Mianwali. These accessions along with 2 checks were studied for genetic variation attributed to various collection sites. Further, these accessions were either collected from the field areas or obtained from markets. Twelve accessions represent the district Khushab, and out of these, nine were collected from farmers's fields and other as market samples. From the district Layyah, fourteen accessions were collected from the fields and 4 were collected as market samples, where two accessions were obtained from market of Mianwali. These five districts of Punjab represents the major chickpea growing areas of the country because more than 80 percent of desi types are being cultivated in these five districts.

Low to medium variance and low average performance of the accessions collected from Bahawalnagar was revealed for most of the characters which indicated that improvement from these accessions for further selection was limited. These accessions could better be utilized in the hybridization programme to create genetic variation rather than to exploit through simple selection.

Seventeen accessions were collected from Bhakkar, and out of these 14 were collected from farmers' fields and 4 were collected as market samples. This district is a major chickpea growing area and the germplasm collected from this area can be used in hybridization programme involving improved germplasm from either sources. The variation for seeds per pod was not enough for the germplasm evaluated in the present study. Twelve accessions collected from the district Khushab exhibited low to medium variation for most of the characters. Low to medium variance and low average performance for most of the characters indicated that improvement from these accessions for further selection was limited, therefore this material could be



utilized in the hybridization programme to create genetic variation. The accessions collected from Layyah consisted of maximum number, and out of 18 accessions, 14 were collected from farmer's fields and 4 were collected as market samples. This district is included in the major chickpea growing area and the germplasm collected from this area was better in evaluation as compared to the germplasm collected from other districts, therefore the selected accessions from this group can be tested under a wide range of environments or used in hybridization programme involving improved germplasm from other sources.

Two accessions were collected from the District Mianwali and both the accessions were obtained from market. This might be due to the off season or less area under chickpea cultivation during the expedition year. Both of these accessions were of low to medium importance for most of the characters under study. The varieties gave the highest mean yield along with high standard error and variance. This was followed by the accessions collected from the district Layyah which gave high mean value and variance. High standard error and variance observed in check varieties indicated the influence of environmental fluctuations. The performance under favourable environmental conditions might be better as compared to stress conditions.

The local germplasm is adaptive to various environmental stresses that could be utilized by involving these in the hybridization programme. The accessions collected from the districts of Bahawalnagar and Mianwali were of poor performance, hence could be excluded at this stage. The accessions collected from Khushab were also of poor performance but due to high number of accessions, these are preferred to be tested further for their performance. As local material is better adapted that indicated the worth for improving seed weight in chickpea, hence that could be utilized by the breeders of chickpea by involving local and exotic chickpea parents in the breeding programme.

The accessions were plotted on the basis of geographic origin and source of seed collection, hence these were investigated to know whether the genetic diversity was related to geographic origin or not. The accessions collected from Bahawalnagar were grouped together in the left side of the graph. The accessions from the district Bhakkar and Khushab were grouped in the middle of the accessions from Layyah and Bahawalnagar. Four accessions collected from the

district Layyah were grouped closer to the accessions originated from Bahawalnagar. Fourteen accessions out of 18 originated from Layyah were clearly separated from other accessions. This cluster was on the right half of the graph. Two accessions collected from Mianwali were closer to the accessions originated from Bahawalnagar and Khushab. The two checks (Punjab-91 and Paidar 91) were separated from all the germplasm and were observed in the lower half of the graph.

On the basis of seed source collection, six accessions collected as market samples were separated from others, whereas others were mixed with in the left half of the graph. In general, all the market samples except three were grouped in the left side of the graph. Twenty two accessions out of 41 collected from farmer's fields were separated in the right half of the graph, whereas others were on the right half and out of these eleven accessions were grouped together closer to the origin and eight accessions were mixed with the market samples. Both the checks included in the experiment were clearly separated in the lower half of the graph.

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 the areas of high genetic diversity. This could indicate that genetic material might have migrated from one place to new regions, followed by some degree of contamination by mixture or out crossing with other landraces. The areas with a high level of stress will present interesting tolerance to environmental stresses, but homogeneous mixtures 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.

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 in a cluster did not necessarily include all the accessions/genotypes from the same or nearby sites. The grouping pattern of landraces reflected association with geographic origin which is in contradiction to the results presented by Singh & Tripathi, (1985) and Amurrio *et al.* (1995). Further Gupta *et al.* (1991), Das *et al.* (1989), and Rabbani *et al.*, (1998) also reported no association between morphological characters and geographic origin.

In the present study, multivariate approach has proved to be very useful tool. It produced five clusters on the basis of provincial distribution much more differentiated when compared to the initial subdivision according to geographic sites of chickpea. The study confirmed the existence of a wealth of phenotypic divergence in the local chickpea germplasm. Further collecting missions to main chickpea 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 where genetic erosion takes place in cowpea along with the areas where existing genetic diversity has not yet been gathered (Padulosi, 1993).

5.4. Genetic and Path Analysis in Selected Pure-lines

Genetic variance, phenotypic variance, heritability and genetic advance revealed high proportion of genetic variation for days to flowering, days to maturity, secondary branches and 100-seed weight. The range for days to flowering, days to maturity and number of primary branches was low, but due to the adaptation of chickpea to Thall desert, the crop duration does not matter because of sole crop culture (Gull, 1995). For other characters, considerable range of the means was observed that indicated the scope of selection from these genotypes for crop improvement. Medium to high genetic variance was observed for days to flowering, maturity, secondary branches and 100-seed weight, whereas for other characters, low to medium heritability (broad sense) was observed.

Improvement of these traits through simple selection might be limited from germplasm used in the present study. For the characters like, days to flowering, days to maturity and 100-seed weight, high heritability coupled with high genetic advance revealed the presence of additive gene effects, hence crop improvement through these important traits could be possible through simple selection (Ghafoor *et al.*, 2000). Genetic advance along with heritability estimates gives an indication for gene-action and the characters with high heritability coupled with high genetic advance are supposed to be controlled additively, hence could be exploited through simple selection. Chickpea breeders should consider heritability estimates along with genetic advance because h^2 alone is not a good indicator of the amount of usable genetic variability (Ghafoor *et al.*, 1998).

The results regarding genotypic, phenotypic and environmental correlation coefficients revealed that the genotypic correlations were slightly higher than phenotypic ones for most of the characters, exhibiting high degrees of genetic association among traits under consideration. The environmental correlation coefficients were not much important in most of the cases except five combinations, i.e., primary branches Vs secondary branches, pods Vs biological yield, pods Vs grain yield, biological yield Vs grain yield where it was positive and biological yield Vs harvest index where it was negative. The significant environmental correlation indicated environmental influence which is quite expected in a crop like chickpea.

The experiment was conducted under rainfed condition and hence environments played important role to determine correlation among characters, therefore, these results could only be valid for selection under rainfed conditions. Days to flowering exhibited significantly positive correlation with primary branches, whereas negative with plant height, pods per plant and harvest index. Short duration cultivars could be selected to improve the yield potential from present material. Days to maturity gave positive correlation with primary branches and harvest index, whereas plant height had significant positive correlation with biological yield and negative with primary branches. 100-seed weight showed significant association with biological yield and grain yield. Grain yield was positively correlated with all the characters, except harvest index where it was negative, whereas it was negatively insignificant with days to flowering and

secondary branches. Genetic improvement in chickpea is mainly focused on grain yield by the breeders of the country (Bakhsh *et al.*, 1998). Grain yield is a complex character which is the final product of many (some known and others unknown) independent variables. In the present study, grain yield was positively associated with biological yield and 100-seed weight but negatively with harvest index. To improve grain yield emphasis should be given on development of chickpea cultivars with higher seed weight and biological yield. The genotypes with low grain yield and high biological yield consequently produced low harvest index and this important combination, high biological yield and harvest index could be attained using bi-parental mating to break unwanted linkage for further improvement of the crop. Positive correlation of grain yield with branches, pods and seed weight has already been reported by Gull, (1995) and Bakhsh *et al.*, (1998) that indicated the consistency of these associations in chickpea, hence could be exploited for crop improvement.

The path coefficient analysis was carried out in this study to find out the causal factors involved in determining the end product i.e., grain yield. The direct effects exhibited by secondary branches, pods and 100-seed weight were positive, whereas all the other characters gave negative direct effects. The highest direct effect was exhibited by pods per plant and it was followed by secondary branches and 100-seed weight. 100-seed weight and pods per plant also exhibited significant positive association with grain yield, hence could more confidently be exploited for crop improvement.

In the present study conducted under rainfed conditions, it is indicated that pods per plant and 100-seed weight had the maximum contribution in determining grain yield, the ultimate product in chickpea under rainfed conditions. Further, it was observed that high indirect contribution was exhibited via secondary branches and harvest index by most of the yield components, hence these two traits along with pods per plant and 100-seed weight are suggested to be given emphasis while selecting high yielding chickpea cultivars for rainfed conditions. Correlation and path coefficient analyses indicated that pods per plant and 100-seed weight were potent contributors to grain yield through direct effects. Although, biological yield had significant association but exhibited negative direct effects, whereas Singh *et al.*, (1995) reported high direct effects by biological yield, pods per plant and 100-

seed weight. The contradiction for biological yield may be related to the experimental conditions as present study was conducted under rainfed conditions. On the basis of performance, seven accessions produced higher grain yield than both the checks, hence were selected for further evaluation under a wide range of environmental conditions. These findings are in agreement with Malhotra *et al.*, (1974), Singh *et al.*, (1977), Ghani (1984), Choubery and Gupta (1986), Bakhsh *et al.* (1991), Dhumale and Mishra (1979), whereas Singh (1988), Ghafoor *et al.* (1990) and Tariq (1990) showed negative direct effect of plant height on grain yield in chickpea, mash and maize respectively. The direct effects of primary and secondary branches were also positive. These results are in agreement with Ghani (1984), Sandhu and Singh (1972), Bakhsh et al. (1991).

Number of pods per plant showed positive direct effect on grain yield as shown by Bahl et al. (1976), Das et al. (1989), whereas, Malik et al. (1987)., Singh (1988), Ghafoor *et al.* (1990), Bakhsh *et al* (1991) reported negative direct effect of pods per plant on grain yield. 100-seed weight showed moderate and positive direct effect on grain yield as reported by Singh et al. (1985) and Singh (1988), whereas Ghafoor *et al.* (1990) reported a negative direct effect of 100-seed weight on grain yield. The direct effect of biological yield on grain yield was negative. Singh (1988) also reported negative direct effect of biological yield on grain yield per plant in chickpea. Singh *et al.* (1977), Sandhu *et al.* (1980), Singh *et al.* (1990) and Hussain *et al.* (1991) reported positive direct effect of harvest index on grain yield. It can also be used as reliable criterion in the selection of high yielding chickpea genotypes with other parameters.

In improving the yield potential of chickpea, varieties under the present investigation, direct simultaneous selection based on branches, number of pods per plant, 100-seed weight and biological yield would be advantageous in parents, as the correlation between grain yield and these characters were positive and highly significant. High heritability coupled with high genetic advance for days to flowering, days to maturity, secondary branches and 100-seed weight revealed additive type of gene effects, hence simple selection could be practiced for exploitation of genetic variation to improve this crop. Partitioning of variance into its components assists the genetic resources conservation and their utilization. It enables planning for use of appropriate gene pools in crop improvement for specific

plant attributes (Pecetti *et al.* 1996, Ghafoor *et al.* 2001). Medium to high variance was observed for days to flowering, maturity, secondary branches and 100-seed weight, whereas for other characters, low to medium variance indicated the limited scope of improvement through the exploitation of present material. Genes for yield and yield components should be explored from other sources through more collections from the areas of maximum diversity or acquisition of germplasm from other sources.

In chickpea, due to breeding work and epidemics of *Ascochyta* blight, important landraces might have extinct and hence acquisition of exotic germplasm seems to be more useful for developing broad based gene-pool. Laghetti, *et al.* (1998) advocated that maximum genetic conservation would be achieved by sampling populations from as many environments as possible. Superior accessions from distinct clusters are suggested to be utilized in breeding programme as such genotypes give better hybrids (Ghafoor *et al.* 2000). Elite accessions could also be utilized directly because such cultivars give better performance under wide range of environments (Ghafoor *et al.* 1992). Cluster analysis grouped together accessions with greater genetic similarity and the clusters include accessions from same origin in one group. The group A consisted of all the accessions collected from the District Khushab except one accession and group B consisted of all the accessions originated from the Districts of Layyah and Bhakkar. These two districts are adjoining and traditionally these have similar geographic and soil features. Hence,, the grouping pattern could be related with geographic distribution of the crop and both the statistics confirmed results that indicated the validity of these two statistics for germplasm classification.

First PC was more related to days to maturity, plant height, primary branches, 100-seed weight, grain yield and harvest index, whereas the second PC contrasts variables that relate to vegetative growth. First PC was a weighted average of the characters as seven characters were positive to this PC and out of these five contributed maximum. The findings suggest that this component reflects the tendency of each accession to emphasize vegetative and reproductive growth. Although, PC_3 exhibited positive effects for all the characters but the magnitude was low except secondary branches, pods and biological yield. This suggests that the genotypes that emphasise vegetative growth tend to have low

yield, whereas those that emphasise reproductive growth tend to have lower vegetative growth. Results reported by Falcinelli *et al.* 1988 and Ghafoor *et al.* (2001) showed multivariate analyses to be a valid system to deal with germplasm collections. Grouping of accessions by multivariate methods in the study is of practical value to the breeders of chickpea. Representative accessions may be chosen from particular groups for hybridization programs with other approved cultivars. Clusters with superior agronomic types have been identified which may be exploited for genetic potential to transfer the desirable genes to improve yield potential of the crop (Singh, 1988 and Clements and Cowling, 1994).

5.5. Biochemical (SDS-PAGE) Basis of Genetic Diversity

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; Akhtar, 2000). It is a promising tool for distinguishing cultivars of a particular crop species (Cooke, 1984; Ferguson & Grabe, 1986; Gardiner & Forde, 1988; Gadgil, *et al.* 1983; Koranyi, 1989; Jha & Ohri, 1996). Studies also indicated that cultivar identification was not possible with the SDS-PAGE (Ladizinsky & Adler, 1975; Raymond *et al.* 1991; Ahmad & Slinkard, 1992; de Vries, 1996; Ghafoor *et al.*, 2001).

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. 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. Phylogenetic relationships have also been reported by Margoliash & Fitch, (1968), Sammour, (1989), Tomooka *et al.* (1992) and Akhtar, (2001) in legumes. In the present studies, intra-specific variation was limited among chickpea accessions. Similar results had already been reported by Thakare *et al.* (1987) and Iqbal, (2001) in *Vigna* and Mehrani, (2002) in *Pisum sativum* who observed low intra-specific variation within one species in their study.

The accessions evaluated for agronomic traits were also used for the analysis of SDS-PAGE through slab type gel electrophoresis using 10 samples for each accession. Although, all of these were not homozygous and polymorphism did exist for one or the other locus within various samples of the accessions. SDS-PAGE revealed that 11.25% acrylamide gel concentration, 6 µl of sample gave the best resolution as suggested by Ghafoor et al., (2001). Out of sixty two accessions, 41 were homozygous on the basis of SDS-PAGE whereas others were heterozygous hence single seed descents could be isolated from these heterogeneous lines to establish pure-lines for future breeding programme. In total, 14 protein bands were recorded ranging from the Molecular Weight (MW) of 24 to 66 KDa. Many protein subunits of lower MW were also observed but due to inconsistency 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. 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 chickpea. Many accessions which were observed similar based on protein pattern were excluded from cluster and Principal Component Analysis.

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 chickpea germplasm.

Principal component 1 had 25.30% of the total variation, PC_2 16.85% and PC_3 had 14.25% of the total variation, respectively. The protein bands which contributed more positively to PC_1, were B2, B4 and B13. Four bands (B5, B7, B12, B14) contributed maximum genetic variance to PC_2 , whereas one protein subunit (B11) exhibited the highest value for PC_3. Approved varieties were clearly separated on the basis of SDS-PAGE markers. Two accessions originated from Khushab and Bahawalnagar in each case were also clearly separated from others, whereas others were mixed together or overlapped to each other. Clear differences on the basis of origin were not observed when analysed for SDS-PAGE. Many researchers (Akhtar, 2001; Iqbal, 2001; Mehrani, 2002) did not observe any relationship between SDS-PAGE and origin or source of seed in legumes, whereas link of protein pattern has been reported by Murphy *et al.* (1990) but Moller & Spoor, (1993) could not detect any link for days to maturity, winter hardiness and disease. Mehrani, (2002) reported SDS-PAGE as a powerful tool for germplasm evaluation in peas. Cluster analysis based on SDS-PAGE markers, qualitative and quantitative traits was found independent of origin or source, hence the use of DNA markers is suggested for detailed studies.

In order to ensure the efficient and effective use of crop germplasm, its characterisation is imperative. In the present investigation, cluster analysis based on SDS-PAGE in local chickpea germplasm did not reflect any clue either for agronomic preference and/or geographic distribution. For most accessions and protein subunits, no clear observation was recorded which could facilitate selection on the basis of SDS-PAGE for improving agronomic traits in chickpea from the material under investigation. Further, 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.

Multivariate analyses provide a good evaluation of landraces by identifying those that should be further evaluated at the genetic level (Rouamba *et al.* (1996) and Ghafoor *et al.*, (2001). 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. Suggestion has been made for selecting suitable stable diverse parents so as to streamline a crossing programme for increased grain yield in chickpea. Such studies would allow more efficient enhancement and use of genetic resources with a view to introduce desirable characteristics from landraces 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 conditions. Under such circumstances, availability of genetically diverse genotypes for hybridization programme becomes imperative. Gupta *et al.* (1991), Das *et al.* (1989), Amurrio *et al.* (1993, 1995) and Rabbani *et al.* (1998) 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 chickpea, but simultaneous study for both agronomical and biochemical analysis (protein and DNA) is suggested. Multivariate analysis have been used for classifying 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 local chickpea germplasm collected from main chickpea growing areas exhibited significant variation for all the quantitative characters except seeds/pod and 100 seed weight. Although, variation was observed for total seed protein but the level was low. SDS-PAGE was not very effective for studying intra-specific genetic diversity in cultivated chickpea alone rather wild chickpea spp. could be included. Further, biochemical markers are suggested to increase by adding DNA techniques (RAPD, RFLP, ALP) for studying diversity related to germplasm collections. 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. Little geographic relationship was observed that could be enhanced by involving more diverse accessions in research material. The management of both qualitative and quantitative matrices is suggested to workout independently at the beginning and then the mixed one has advantages like, a) one gets a synthetic description of the most important characters of each cluster, b) results have a useful biological significance because some of the traits chosen are directly related to adaptability to agronomic conditions, c) clusters analysis gives a general, morphological and physiological description of the main characteristics and the possible use of each one of the groups and d) use of morphological characters give a better resolution which has more significance for varietal description.

LITERATURE CITED

LITERATURE CITED

Adams, M.W, 1967. Basis of yield components in crop plants with special reference to the field bean. Crop Sci., 7:505-510.

Adhikari, G. and M.P. Pandey, 1982a. Component analysis of seed yield in chickpea. Crop Improv., 9(1):69-74.

Ahmad, F. and A.E. Slinkard, 1992. Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor. Appl. Genet. 84: 688-692.

Ahmad, M., D.L. McNeil and J.R. Sedcole, 1997. Phylogenetic relationships in Lens species and their interspecific hybrids as measured by morphological characters. Euphytica, 94:101-111.

Akbani, A.K., A.S. Larik, and A.A Mirz, 1990. Association analysis in bread wheat (*Triticum aestivum* L.). Pakphyton, 2:53-61.

Akhtar, M. 2001. Phylogenetic relationships among *Vigna* species based on agronomic and biochemical analyses. M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan. 99pp.

Akoroda, M.O. 1983. Principal component analysis and metroglyph of variation among Nigerian yellow yam. Euphytica, 32:565-573.

Ali, N. 1985. Genetic variability and correlation studies in *Brassica juncea*. Pak. J. Bot., 17(2):297-303.

Amurrio, J.M., A. M. de Ron and M.R. Escribano, 1993. Evaluation of *Pisum sativum* landraces from the Northwest of Iberian peninsula and their breeding value. Euphytica 66:1-10.

Amurrio, J.M., A. M. de Ron and P.A. Casquero, 1991. Practical importance of numerical taxonomy as a useful tool in the classification of pea landraces for their uses. Anales de Aula Dei., 20:7-15.

Amurrio, J.M., A.M. de Ron and A.C. Zeven, 1995. Numerical taxonomy of Iberian pea landraces based on quantitative and qualitative characters. Euphytica 82: 195-205.

Anonymous, 2001. Plant Genetic Resources Institute. Annual Report, 2000. National Agricultural Research Centre, Islamabad. Pakistan.

Anonymous, 2002. Pulses Programme Annual Report, 2000. National Agricultural Research Centre, Islamabad. Pakistan.

Argikar, G.P., 1970. Gram. *In* Pulse Crops on India (Kachroo, P. and Arif, M.,eds.), ICAR, New Delhi, pp. 54-135.

Auckland, A.K. and L.J.G. van der Maesen, 1980. Chickpea. *In* (Fehr, W. R. and Hadley, H. H. eds.) Hybridization of Crop Plants. American Society of Agronomy: Crop Science Society of America, pp. 249-259.

Ayyar, V.R. and R. Balasubramanian, 1936. Inheritance of certain color characters in gram (*Cicer arietinum*). Proceedings of the Indian Academy of Science 4: 1-126.

Ayyar, V.R, and R. Balasubramanian, 1937. Inheritance of branching habit in gram (*Cicer arietinum*). Madras Agricultural Journal 25: 105-106.

Bahl, P.N. and H.K. Jain, 1977. Association among agronomic charcters and plant ideotype in chickpea (*Cicer arietinum* L.). Z. Pflanzenzuchtg, 79:154-159.

Bahl, P.N; R.B. Mehra, and D.B. Raju, 1976. Path analysis and its implications for chickpea breeding. Z. pflanzenzuchtung, 77: 67-71.

Bakhsh, A., A. Ghafoor and B.A. Malik, 1992. Evaluation of lentil germplasm. Pak. J. Sci. Ind. Res., 35(1-2):48-49.

Bakhsh, A., T. Gull, B.A. Malik and A. Sharif, 1998. Comparison between F1s and their parental genotypes for the patterns of character correlation and path coefficients in chickpea (*Cicer arietinum*). Pak. J. Bot., 30(2):209-219.

Bakhsh, A., M. Zubair and A. Ghafoor, 1991. Character correlation and path analysis in Lentil. Pak. J. Agric. Res., 12(4):246-251.

Baluch, M.A.A. and M.P.M. Soomro, 1968. Correlation studies in gram (*Cicer arietinum* L.). W. Pakistan J.Agric. Res., 6: 26-32.

Beckstrom-Sternberg, S.M. 1989. Two-dimensional gel electrophoresis as a taxonomic tool: Evidence from the Centrspermae. Biochem. Syst. Ecol., 17:573-582.

Bekele, E. 1984. Analyses of regional patterns of phenotypic diversity in the Ethiopian tetraploid and hexaploid wheats. Hereditas, 100:131-154.

Bekele, E. 1985. The biology of cereal land race population. 1. Problems of gene conservation, plant breeding selection schemes and sample size requirement. Hereditas, 103:119-134.

Benjamini, L. 1981. Effect of branching on grain production in an unirrigated chickpea. Hassadeh, 61:1266-1267.

Beuselinck, P.R. and J.J. Steiner, 1992. A proposed framework for identifying and utilizing plant germplasm resources. Field Crop Research, 29:261-272.

Bogyo, T.P., E. Porceddu and P. Perrino, 1980. Analyses of sampling strategies for collecting genetic material. Econ. Bot., 34:160-174.

Brancourt, H. M. and A.E. Slinkard, 1998. Interpreting genotype environment interaction with probe genotypes in winter wheat. Proceed. Ninth Inter. Wheat Gen. Symp. Saskatoon, Saskatchewan, Canada, 2-7 Aug., 1998. 45-48.

Broschat, T. K., 1979. Principal Component Analysis in Horticultural Research. Hortscience, 14(2): 114-117.

Brown, A.H.D. 1978. Isozymes, plant population genetic structure and genetic conservation. Theor. Appl. Genet., 52:145-157.

Brown, A.H.D. and B.S. Weir, 1983. Measuring genetic variability in plant populations. p. 219-240. In: S.D. Tanksley and T.I. Orton (eds.), Isozymes in Plant Genetics and Breeding. Vol. 1A. Developments in plant genetics and breeding 1. Elsevier, Amsterdam.

Brown, J.S. 1991. Principal component and cluster analysis of cotton cultivar variability across the U.S. cotton belt. Crop Sci. 31: 915-922.

Camussi, A., E. Ottaviano, T. Calinski and Z. Kaczmarek, 1985. Genetic distances based on quantitative traits. Genetics, 111:945-962.

Caradus, J.R., A.C. MacKay, D.R. Woodfield, J. van den Bosch and S. Wewala, 1989. Classification of a world collection of white clover cultivars. Euphytica 42: 183-196.

Celis, J.E. and R. Bravo, 1984. Two dimensional gel electrophoresis of proteins: methods and applications. Academic Press, New York.

Chandra, S. 1968. Variability in gram. Indian J. Genet., 28(2):205-210.

Chaudhry, A.R., N. Rehman, M. Akram, and M. Hussain, 1991. Analysis of yield components in cowpea. Pak.J. Agri. Res., 12(4):252-254.

Chen, N.C., L.R. Baker and S. Honma, 1983. Rice bean [*Vigna umbellata* (Thunb) Ohwi and Ohashi]. Tropical Grain Legume Bill., 10:22-25.

Chhina, B.S., M.M. Verma, H.S. Brar, and R.K. Batta, 1991. Relationship of seed yield and some morophological characters in chickpea under rainfed conditions. Tropical-Agric., 68(4):337-338.

Choubery, R.N. and S.K. Gupta, S.K. 1986. Correlation and path analysis in forage oat. Int. J. Agric. Sci., 56(9):674-677.

Chowdhry M.A. and M.A. Khan, 1974. Correlation studies in gram (*Cicer arietinum* L.). Pakistan J. Agric. Sci., 11:184-186.

Chuhan, V.S. and P.K. Sinha, 1982. Correlation and Path analysis in lentil. LENS, 9:19-22.

Clements, J.C. and W.A. Cowling, 1994. Pattern of morphological diversity in relation to geographical origins of wild *Lupinus angustifolius* from the Aegean region. Genetic Resources and Crop Evolution, 41:109-122.

Comstock, R.E. 1978. Quantitative genetics in maize breeding. p. 191-206. In: D.B. Walden (ed.). Maize breeding and genetics. Wiley, New York.

Cooke, R.J. 1984. The characterization and identification of crop cultivars by electrophoresis. Electrophoresis 5: 59-72.

Cubero, J.I. 1975. The research on chickpea (*Cicer arietinum*) in Spain. *In* Proceedings, International workshop on Grain Legumes, 13-16 Jan 1975, Hyderabad, India pp. 117-122.

Dahiya, B.S. ;I.S. Solanki and K.Ram, 1983b. F2, F3 and F4 bulk yields as indications of cross performance. International Chickpea Newsletter, 8:12-13.

Dahiya, B.S., V.S. Lather, I.S. Solanki and R. Kumar, 1984. Useful spontaneous mutants in chickpea. Intern. Chickpea Newsl., 11:4-6.

Dahiya, B.S.; K.R. Gupta, and R.S. Waldia, 1983a. Adaptation of chickpea varieties to late sowing. Indian J. Agric. Sci., 53:673-676.

Dahiya, B.S.; M.R. Naidu, R. Bakshi and M. Bali, 1986. Selection procedures in chickpea breeding. P. 63-75. In Genetic and crop improvement (Gupta, P.K. and Bahl, J.R. ed.) proceeding of the symposium on advances in genetics and crop improvement, Meerut, Dec., 1984., Rastogi and Company, India.

Dahiya, B.S.; R.S. Waldia, L.S. Kaushik and I.S. Solanki, 1984. Early generation yield testing versus visual selection in chickpea (*Cicer arietinum* L.). Theor. Appl. Genet., 68(6):525-529.

Dahiya, BS.; K.B. Singh, H.S. Brar and J.S. Brar, 1976. Identification of physiologically efficient genotypes in chickpea (*Cicer arietinum*, L). Tropical grain legume bull., 4:19-20.

Damania, A. B., E. Porceddu and M.T. Jackson, 1983. A rapid method for the evaluation of variation in germplasm collections of cereals using polyacrylamide gel electerophoresis. Euphytica, 32: 877-883.

Das, M.L., A. Rahma and J. Miah, 1989. Coefficient and Regression studies in Soybean. Bangladesh J. Agri. Res., 14(1):27-29.

Das, S. and K.K. Mukarjee, 1995. Comparative study on seed proteins of Ipomoea. Seed Sci. & Technol. 23: 501-509.

Dasgupta, T. and P.K. Das, 1984 Multivariate analysis and selection of parents for hybridization in blackgram. Philippine Agriculturist, 57(1):86-92.

Dasgupta, T. and P.K. Das, 1985. Gene pool divergence and selection of parents for hybridization in blackgram. Bangladesh J. Agric. Res., 10(1):9-15.

de Vries, I.M. 1996. Characterization and identification of *Lactuca sativa* cultivars and wild relatives with SDS-electrophoresis (Lactuca sect. Lactuca, Compositae). GRACE 43: 193-202. Genet. Resour. Crop Evol., 43:193-202.

Dhumale, D.B. and S.N. Mishra, 1979. Character association between forage yield and its components in oat. Ind. J. Agri. Soci., 49(12):918-924.

Dias, J.S., A.A. Monteiro and M.B. Lima, 1993. Numerical taxonomy of Portuguese Tronchuda cabbage and Galega kale landraces using morphological characters. Euphytica 69: 51-68.

Dixit, R.K. 1974. Interrelationship among some agronomic traits in lentil (*Lens esculentus moench*). Madras Agric. J., 61:588-591.

Donald, C.M. 1962. In search of yield. J. Aust. Inst. Agric. Sci. 28:172-178.

Dobholkar, A.R,. 1973. Yield components in *Cicer arientinum* L. JNKVV Res. J., 7:16-18.

dos Santos, J.B., J. Niehuis, P. Skroch, J. Tivang and M.K. Slocum, 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor. Appl. Genet. 87: 909-915.

Dotlacil, L., J. Hermuth, V. Tisova, J. Brindza and F. Debre, 2000. Yield potential and stability in selected winter wheat landraces and obsolete cultivars of European origin. Rostlinna Vyroba., 46(4): 153-158.

Dweikat, I., S. Mackenzie, M. Levy and H. Ohm, 1993. Pedigree assessment using RAPD-DGGE in cereal crop species. Theor. Appl. Genet., 85: 497-505.

Edwards, A.W.F. 1992. Likelihood (expanded ed.). Johns Hopkins University Press, Baltimore.

Edwards, M.D., C.W. Stuber and J.F. Wendel, 1987. Molecular markers-facilitated investigations of quantitative trait loci in maize. 1. Numbers, genomic distribution and types of gene-action. Genetics, 116:113-125.

Egawa, Y. 1988. Phylogenetic differentiation between three Asian *Vigna* species, *V. radiata, V. mungo and V. umbellata*. Bull. Natl. Inst. Agrobiol. Resour., Tsukuba, Japan. 4:189-200.

Erskine, W. and F.J. Muehlbauer, 1991. Allozyme and morphological variability: outcrossing rate and core collection formation in lentil germplasm. Theor. Appl. Genet., 83:119-125.

Falcinelli, M., F. Veronesi and S. Lorenzetti. 1988. Evaluation of an Italian germplasm collection of *Lolium perenne* L. through a multivariate approach. Proceedings of the Eucarpia Fodder Crops Section Meeting. Lusignan, France, pp. 22-24.

Ferguson, J.M. and D.F. Grabe, 1986. Identification of cultivars of perennial ryegrass by SDS-PAGE of seed proteins. Crop Sci. 26: 170-176.

Filippetti, A. and C.F. Margano, 1983. Variability for plant and seed characters in a collection of *Cicer arietinum* L. Genetica Agraria, 37:169.

Frankel, O. 1984. Genetic perspectives of germplasm conservation, p. 161-170. In: W. Arber, K. Limensee, W.J. Peacock and P. Starlinger (eds.). Genetic manipulation: impact on man and society. Cambridge Univ. Press, Cambridge.

Frankel, O. and M. Soule, 1981. Conservation and evolution. Cambridge Univ. Press, Cambridge.

Frankel, O.H., A.H.D. Brown and J.J. Burdon, 1995. The conservation of plant biodiversity. Cambridge University Press, Cambridge, UK, pp. 299.

Gadgil, J.D., K.N. Suseelan, R. Mitra, D.C. Joshua, C.R. Bhattia, M.K. Sinha, M.K. Gamil and V.K. Al-Saheal, 1983. Estimation of genetic effects for agronomic traits in wheat. Wheat Info. Serv. Minia Uni., Minia, Egypt.

Gardiner, S.E. and M.B. Forde, 1988. Identification of cultivars and species of pasture legumes by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of seed proteins. Plant Varieties & Seeds 1: 13-26.

Gepts, P. 1989. Genetic diversity of seed storage proteins in plants. In: A.H.D. Brown, M.T. Clegg, A.L. Kahler & B.S. Weir (Eds.). Plant Population Genetics, Breeding and Genetic Resources, pp. 64-82. Sinauer Associates Inc., Sunderland, Massachusetts.

Gepts, P. 1990. Biochemical evidence bearing on the domestication of Phaseolus (Fabaceae) beans. Econ. Bot., 44 (3 Suppl.):28-38.

Ghafoor, A, A. Sharif and M. Tahir. 1998. Evaluation of Blackgram (*Vigna mungo* L. Hepper) Germplasm. Pakistan Journal of Botany. 30(2):227-238.

Ghafoor, A., A. Sharif, Z. Ahmad, M.A. Zahid, and M.A. Rabbani. 2001. Genetic diversity in Blackgram (*Vigna mungo* L. Hepper). Field Crops Research, 69:183-190.

Ghafoor, A., M. Zubair and B.A. Malik, 1993b. Selection indices in *Vigna mungo* L. Pak. J. Agric., Agril. Engg. & Vet. Sci., 9:67-70.

Ghafoor, A., M. Zubair, S.M. Iqbal and M. Bashir, 1989. Evaluation, classification and usefulness of germplasm of urdbean. Pak. J. Agric. Res., 10:346-349.

Ghafoor, A., M. Zubair., B.A. Malik and S.M. Iqbal, 1992. Evaluation of selected germplasm of mungbean (*Vigna radiata* L. Wilczek). Pak. J. Bot., 24:112-118.

Ghafoor, A., Z. Ahmad, M. Afzal and A.S. Qureshi, 2000. Influence of genetic diversity and combining ability in determining hybrid vigour in *Vigna mungo* L. Hepper. Journal of Genetics & Breeding, 54:125-131.

Ghafoor, A., M. Zubair and B.A. Malik, 1990. Path analysis in mash (*Vigna mungo* L.). Pak. J. Bot., 22(2):160-167.

Ghani, A. (1984). Variability and correlation determination for various charcters in some varieties of Lentil. M.Sc. (Hons.) Thesis, Plant Breeding and Genetics. Agri. Univ., Faisalabad, Pakistan.

Gill, K.S. and D.S. Brar, 1980. Genetics of economic traits and cataloguing of genes in chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L. Millsp.). P. 406-427. In Breeding methods for the improvement of pulse crops. (Gill, K.S. ed.) Punjab Agric. Univer., Ludhiana, India.

Gilliland, T. J. 1989. Electrophoresis of sexually and vegetatively propogated cultivars of allogamous species. *Plant Varieties and Seeds*, 2, 15-25.

Goodman, M.M. 1968. A measure of "overall variability" in populations. Biometrics, 24:189-192.

Govil, J.N. 1980. Plant type in relation to protein yield and disease resistance in chickpea (*Cicer arietinum* L.). Legume Research. 3:38-44.

Govil, J.N.; B.R. Murty and B.S. Rana, 1980. Components of productivity in relationship to geographical distribution in chickpea. Indian J. Genet., 40:515-527.

Gull, T. 1995. Genetic variability, correlation and path coefficient studies in hybrid population of chickpea (*Cicer atietinum* L.). M. Phil. Thesis. Submitted to the Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan. pp-61.

Gupta, S.K., and G. Robbelen, 1986. Identification of rapeseed (*Brassica napus* L.) cultivars by electrophoresis. *Zeitschrift fur Pflanzenzuchtang*, 96, 363-370.

Gupta, S.N., S. Lal, L. Rai and Y.S. Tomer, 1983. Correlation and path analysis in mung bean (*Vigna radiata* (L.) wilczek). Haryana Agric. Univ. J. Res., 12:287-291.

Gupta, S.P., R.C. Luthra, A.S. Gill and P.S. Phul, 1972. Variability and correlation studies on yield and its components in gram. J. Res. Punjab Agric. Univ., 9:405-409.

Gupta, V.P. and S. Ramnujam, 1974. Stability of yield and its components in Bengal gram and its bearing on plant type. Indian J. Genet., 34:757-763.

Gupta, V.P., M.S. Sekhon and D.R. Satija, 1991. Studies on genetic diversity, heterosis and combining ability in Indian mustard (*Brassica juncea* L. Czern & Coss.). Indian J. Genet. 51: 448-453.

Higginbotham, J.W., J.S.C. Smith and O.S. Smith, 1991. Quantitative analysis of two-dimensional protein profiles in inbred lines of maize (*Zea mays* L.). Electrophoresis, 12:425-431.

Hoffman, D.L., D.L. Muehlbauer, and G. Ladizinsky, 1986. Isozyme polymorphism in Lens. Sys. Bot., 11:392-402.

Holcomb, J., D.M. Tolbert and K. Jian, 1977. A diversity analyses of genetic resources in rice. Euphytica, 26:441-450.

Humphreys, M.O. 1991. A genetic approach to the multivariate differentiation of perennial ryegrass (*Lolium perenne* L.) populations. Heredity 66: 437-443.

Hussain, S.A., M.B. Kumbhar, A.S. Larik and B.A. Malik, 1991. Gentic analyis of some quantitative charactes in chickpea (*Cicer arietinum* L.). Pakphyton, 3:153-163.

IBPGR, 1985. Descriptors for chickpea. International Board for Plant Genetic Resources, Rome, 15p.

Iqbal, M.S. 2001. Genetic diversity and evaluation in local and exotic cowpea [*Vigna unguiculata* (L.) Walp.] germplasm based on plant traits and SDS-PAGE. M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan. 158pp.

Islam, M.Q.; A.K. Kaul and K. Begum, 1982. Phenotypic variability and correlation studies in indigenous chickpea of Bangladesh. Bangladesh J. Agric. Res., 7:1-5.

Islam, R. and M.C. Saxena, 1981. Symbiotic nitrogen fixation in rainfed chickpeas in northern Syria. International Chickpea Newsletter 4: 22-24.

Jain, K C.; B.P. Pandya and K.Pande, 1981. Genetic diversgence in chickpea. Indian J. Genet., 41:220-225.

Jain, S.K., C.O. Qualset, G.M. Bhatt and K.K. Wu, 1975. Geographical patterns of phenotypic diversity in a world collection of durum wheat. Crop Sci., 15:700-704.

Jha, S.S. and D. Ohri, 1996. Phylogenetic relationships of *Cajanus cajan* (L.) Millsp. (pigeonpea) and its wild relatives based on seed protein profiles. GRACE 43: 275-281.

Johnson, H.W., H.E. Robinson and R.E. Comstok, 1955. Estimates of geneitc and environmental variabilty in soybean. Agron. J.47:314-318.

Kahler, A.L. and C.F. Wehrhahan, 1986. Association between quantitative traits and enzyme loci in the F2 population of a maize hybrid. Theor. Appl. Genet., 72:15-26.

Katiyar, R.P. and B. Singh, 1974. Interrelationship among yield and its components in Indian mustard. Ind. J. Agric. Sci., 44:287-290.

Katiyar, R.P. and D. Singh, 1978. Direct and indirect selection response for grain yield in chickpea (*Cicer arietinum* L.). Tropical grain legume bull., 14:32-34.

Katiyar, R.P., O.P. Sood and N.R. Kalia, 1981. Selection criteria in chickpea. Inter. Chickpea Newsletter , 4:35-38.

Khalid, M.G., D.K. Chandio, M.A. Rajput and K.H. Tahir, 1984. Genetic variability and path coefficient analysis in greengram (*Vigna radiata* L.). Pak. J. Sci. Ind. Res., 27:24-27.

Khan, I.A. and B.A. Malik, 1989. Grain and biological yield association in chickpea. Sarhad J. Agric. Res., 5(4):373-375.

Khan, M. A. 1992. Seed protein eleterophoretic pattern in Brachypodium P. Beauv. Species. Ann. Bot., 70: 61-68.

Khan, M.A.; M.A. Chaudhary and M. Amjad, 1983. Variability interrelationships and path coefficients for some quantitative characters and in gram *(Cicer arietinum* L.). Pakistan J. Agri. Res., 44(1):6-11.

Khorgade, P.W.; M.N. Narkhede and S.K. Raut, 1985. Genetic variability and regression studies in chickpea (*Cicer arietinum* L.) and their implications in selection. PKV Res. J., 9(2):9-13.

Kjaer, B. B. Haahr and J. Jensen, 1991. Association between 23 quantitative traits and 10 genetic markers in a barley cross. Plant Breeding, 106:261-274.

Kneen, B., T.A. Larue and N.F. Weeden, 1984. Genes reported to affect symbiotic nitrogen fixation by peas. Pisum Newsletter, 16:31-34.

Knott, D.R. 1972. Effect of selection for F2 plant yield on subsequent generations in wheat. Can J. P. Sci., 52:721-726.

Knott, D.R. and J. Kumar, 1975. Comparison of early generation yield testing and a single seed descent procedure in wheat breeding. Crop Sci., 15(3):295-299.

Koranyi, P. 1989. Characterization of maize (*Zea mays* L.) seed samples by the electrophoretic patterns of their protein monomers. Seed Sci. & Technol. 17: 153-159.

Krajewski, P. and T. Drzazga, 1999. Evaluation of genotype-environment interaction of spring wheat strains on the basis of 1981-1998 breeding experiments. Materialy z sympozjum grupy problemowej ds. hodowli pszenicy, Zakopane, Polska, 26-27 styczen 1999 rok. Biuletyn-Instytutu-Hodowli-i-Aklimatyzacji-Roslin. No. 211, 23-27.

Kresovich, S., J.G.K. Williams, J.R. McFerson, E.J. Routman and B.A. Schaal, 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor. Appl. Genet. 85: 190-196.

Kumamura, T., H. Satoh, N. Iwata, T. Omura, M. Ogawa and K. Tanaka, 1988. Mutants for rice storage proteins. Theor. Appl. Genet., 76:11-16.

Kumar, L. and P.P. Arora, 1992. Multivariate analyses in chickpea. Indian J. Pulses Res., 5(1):1-5.

Kumbhar, M.B., A.S. Larik, H.M.I. Hafiz, and M.I. Rind. 1983. Interrelationship of polygenic traits affecting grain yield in *T. aestivum* L. WIS., 57:42-45.

Kurlovich, B.S. 1998. Species and intraspecific diversity of white, blue and yellow lupins. Plant Genetic Resources Newsletter, 115:23-32.

Ladizinsky, G. 1975. A new Cicer from Turkey. Notes from the Royal Botanical Garden Edinburgh 34: 201-202.

Ladizinsky, G. and A. Adler, 1975. The origin of chickpea as indicated by seed protein electrophoresis. Isr. J. Bot. 24: 183-189.

Ladizinsky, G. and T. Hymowitz, 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. Theor. Appl. Genet. 54: 145-151.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Laghetti, G., B.L. Pienaar. S. Pasdulosi and P. Perrino, 1998. Ecogeographical distribution of *Vigna savi* in Southern Africa and some areas of the Mediterranean basin. Plant Genetic Resources Newsletter, 115:6-12.

Lal, S. 1967. Relationship between grain and biological yields in chickpea (*Cicer arietinum* L.). Trop Grain Legume Bull., 6:29-31.

Li, Y., S. Wu and Y. Cao, 1995. Cluster analysis of an international collection of foxtail millet (*Setaria italica* (L.) P. Beauv.). Euphytica 83: 79-85.

Malhotra, R.S. and K.B. Singh, 1971. Multivariate analyses in blackgram (*Phaseolus mungo*). Indian J. Agric. Sci., 41:757-760.

Malhotra, R.S., P.K. Gupta and N.D. Arora, 1979. Analyses of diallel cross over environments in mungbean (*Vigna radiata* L. Wilczek). Genet. Agr., 32:311-322.

Malhotra, V.V., S. Singh and K.B. Singh, 1974. Yield components in green gram (*Phaseolus aureus*). Ind. J. Agric. Sci., 44:136-141.

Malik, B.A. 1991. Genetics of resistance to *Ascochyta rabiei* in chickpea. Ph.D thesis. Quaid-i-Azam University, Islamabad.

Malik, B.A., I.A. Khan and M.R. Malik. 1988. Genetic variability and correlation among metric traits in chickpea. Pak. J. Agric. Res., 9:352-354.

Malik, B.A., M. Tahir, I.A. Khan, M. Zubair and A.H. Chaudhry, 1987. Genetic variability, character correlation and path analysis of yield components in mungbean (*Vigna radiata* L. Wilczek). Pak. J. Bot., 19(1):89-97.

Malik, B.A., M.R. Malik and I.A. Khan, 1987. Correlations and path analysis of yield and its components in pigeonpea. Pak. J. Sci. Ind. Res., 30(6):452-454.

Malik, B.P.S., V.P. Singh and M. Singh, 1983. Correlation, correlated response and relative selection efficiency in green gram. Ind. J. Agric. Sci., 53:101-105.

Malik, B.P.S. and B.B. Singh, 1982. Note on the correlations in quantitative characters in an interspecific crosses of soybean. Indian J. Agric. Sci., 52(9):608-610.

Mansur, L.M., J. Orf and K.G. Lark, 1993. Determining the linkage of quantitative trait loci to RFLP markers using extreme phenotypes of recombinant inbreds of soybean (*Glycine max* L. Merr.). Theor. Appl. Genet., 86:914-918.

Margoliash, E. and W.M. Fitch, 1968. Evolutionary variability of cytochrome "c" primary structure. Ann. N.Y. Acad. Sci., 151:359-381.

Mehrani, P. 2002. Genetic diversity in local and exotic pea (*Pisum sativum* L.) germplasm for morphological traits and SDS-PAGE markers. M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan. 104pp.

Mckenzie, R.I.H. and J.W. Lambert, 1961. A comparison of F3 lines and their related F6 lines in two barley crosses. Crop Sci., 1(4): 246-249.

Mishra, R. and S.K. Rao, 1990. Comparative study of D2 and meteroglyph analyses in chickpea (*Cicer arietinum* L). Legume Research 13(1):25-35.

Miyazaki, S. 1982. Classification and phylogenetic relationships of the *Vigna radiata*- mungo-sublobata complex. Bull. Nat. Inst. Agr. Sci. D., 33:1-61.

Mohanty, D.C. and D. Sahoo, 1974. Studies on genetic variability and correlation for yield and its components in Bengal gram (*Cicer arientinum* L.). Andhra Agri. J., 21:85-88.

Moller, M. and W. Spoor, 1993. Discrimination and identification of *Lolium* species and cultivars by rapid SDS-PAG electrophoresis of seed storage proteins. Seed Sci. & Technol. 21: 213-223.

Moolani , M. K. and S. Chandra, 1970. Gram cultivation in Haryana. Haryana Agricultural University, Hissar, India, 15 pp.

Murphy, R.W., J.W. Sites, D.G. Buth and C.H. Haufler, 1990. Protein I: isozyme electrophoresis. P. 45-126. In: D.H. Hillis and C. Moritz (eds.). Molecular systematics. Sinauer Assoc., Sunderland, MA.

Naidu, M.R.; B.S. Dahiya, P. Singh and M. Abali, 1986. Yield components as early generation selection criteria for improving seed yield in chickpea. In Poster Abst. OP. 42 (O' Keeffe, L.E. and Muehlbauer, F.J. ed.) International food legume conference. Published by College, Agric.Univ. Idaho.

Nakajima, K. 1994. Biotechnology for crop improvement and production in Japan. In: Biotechnology Applications in Agriculture in Asia and Pacific. Published by Asian Productivity Organization, p. 87-107.

Nakayama, H., M. Afzal and K. Okuno, 1998. Intraspecific differentiation and geographical distribution of Wx alleles for low amylose contents in endosperm of foxtail millet, *Setaria italica* (L.) Beauv. Euphytica, 102:289-293.

Narsinghani, V.G.; V. Goswami, Y. Mishra and S.P. Singh, 1978. Note on character correlations and path coefficients in lentil. Indian J. Agric Sci., 49(6): 404-406.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nene, Y. L. 1984. A review of ascochyta blight of chickpea (Cicer arietinum L.). In M. C. Saxena and K. B. Singh, eds. Ascochyta Blight and Winer Sowing of Chickpeas, pp. 17-34. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, The Netherlands.

Nene, Y. L., M.P. Haware and M.V. Reddy, 1981. Chickpea diseases: resistance screening techniques. Information Bulletin No. 10, ICRISAT, Patancheru, India.

Padulosi, S. 1993. Genetic diversity, taxonomy and ecogeographic survey of the wild relatives of cowpea (*Vigna unguiculata* L. Walp.). Ph. D. Thesis, Catholic University of Louvain-La-Neuve, Belgium.

Pandya, B.P. and M.P. Pandey, 1980. Chickpea improvement at Pantnagar. Pages. 197-207. In Proceeding: International Workshop on chickpea improvement. 28 Feb. -2 Mar., ICRISAT, India.

Paran, I., R. Kesseli and R.H. Michelmore, 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance gene in lettice, using near isogeneic lines. Genome. 34: 1021-1027.

Patel, S.T. and R.M. Shah, 1982. Study on genetic variability and selection indices for yield and its attributes in urdbean (*Vigna mungo* L. Hepper). Pulse Crops Newsl., 2: 29-31.

Pecetti, L., P. Annicchiario and A.B. Damania, 1992. Biodiversity in a germplasm collection of durum wheat. Euphytica 60: 229-238.

Pecetti, L., P. Annicchiario and A.B. Damania, 1996. Geographic variation in tetraploid wheat (*Triticum turgidum* spp. Turgidum convar. Durum) landraces from two provinces in Ethiopia. Euphytica, 43: 395-407.

Peeters, J.P. and J.A. Martinelli, 1989. Hierarchical cluster analysis as a tool to manage variation in germplasm collections. Theor. Appl. Genet. 78: 42-48.

Perry, M.C. and M.S. McIntosh, 1991. Geographical patterns of variation in the USDA soybean germplasm collections. I. Morphological traits. Crop Sci. 31: 1350-1355.

Pezzotti, M., C. Tomassini, M. Falcinelli and F. Veronesi, 1994. Evaluation of an Italian germplasm collection of *Dactylis glomerata* L. using a multivariate approach. J. Gene. & Breed., 48:17-24.

Przybylska, J. and Z.Z. Przybylska, 1995. Electrophoretic pattern of seed globulins in the Old-World *Lupinus* species. Genetic Resources and Crop Evolution, 42:69-75.

Rabbani, M.A., A. Iwabuchi, Y. Murakami, T. Suzuki and K. Takayanagi, 1998. Phenotypic variation and the relationships among mustard (*Brassica juncea* L.) germplasm from Pakistan. Euphytica, 101:357-366.

Raju, D.B.; R.B. Mehara and P.N. Bahl 1978. Genetic variability and correlation in chickpea. Tropical grain legume bull., 14:35-39.

Ram, C., M.S. Chowdhary, S. Chandra and D.S. Jatasra, 1980. Association in segregating populations of chickpea. Indian J. Genet., 40:117-121.

Ramana, M.V. and D.P. Singh, 1987. Genetic divergence in mungbean. Crop Improvement, Ludhiana, 14(1): 23-27.

Ramanujam, S. and V.P. Gupta, 1973. Stability of yield and its components in Bengal gram. In Second general congress of society for the advancement of breeding. Res. In Asia and Oceania. Feb. 22-28. IARI, New Delhi. P.176.

Rang, A, T.S. Sandhu and B.S. Bhullar, 1980. Variability and correlation studies on yield components and protein content and their implication in selection in chickpea. J. Res. Punjab Agri. Univ., 17:345-349.

Rani, Y.U. and J.S. Rao, 1981. Path analysis of yield components in black gram. Indian J. Agric. Sci., 51(6):378-381.

Rao, R., M. Del Vaglio, M.D.U. Paino and L. Monti, 1992. Identification of *Vigna* spp. through specific seed storage polypeptides. Euphytica, 62:39-43.

Raymond, J., V. Inquello and J.L. Azanza, 1991. The seed proteins of sunflower: comparative studies of cultivars. Phytochemistry, 30: 2849-2856.

Revilla, P. and W.C. Tracy, 1995. Morphological characterization and classification of open-pollinated sweet corn cultivars. J. Amer. Soc. Hort. Sci., 120: 112-118.

Robertson, D.S. 1989. Understanding and relationship between qualitative and quantitative genetics. p. 81-87. In.: T. Helentjaris and B. Burr (eds.), Development and application of molecular markers to problems in plant genetics. Current communications in molecular biology. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Righetti, P. G., and A.B. Bosisio, 1981. Application of isoelectric focussing to the analysis of plant and food proteins. Electrophoresis, 2: 65-75.

Rohlf, F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 1.80. Applied Biostatistics Inc., New York.

Rouamba, A., T. Robert, A. Sarr and A. Ricroch, 1996. A preliminary germplasm evaluation of onion landraces from West Africa. Genome, 39:1128-1132.

Ruiz, M., J.M. Carrillo and F. Varela, 1997. Relationships between some geographical parameters and agro/morphological and biochemical characters in a sample of Spanish landraces of barley (*Hordium vulgare* L.). Plant Genetic Resources Newsletter, 112: 86-89.

Rumbaugh, M.D. W.L. Graves, J.L. Caddel and R.M. Mohammad, 1988. Variability in a collection of alfalfa germplasm from Morocco. Crop Sci., 28:605-609.

Salih, F.A. 1982. Chickpea yield trials and selection criteria in Sudan. International Chickpea Newsleter, 7:4.

Sammour, R.H. 1989. Electrophoresis of the seed proteins of *Vicia faba* L. and its immediate progenitors: A reappraisal. Plant Breeding, 104: 196-201.

Sandhu, T.S. and N.B. Singh, 1970. Genetic variability, correlation and regression studies in gram (*Cicer arietinum* L.). J.Res., Punjab Agric. Univ., 7: 423-427.

Sandhu, T.S., B.S. Ghullar, H.S. Cheema and J.S. Barar, 1980. Path coefficient analysis for grain yield and its attributes in green gram. Ind. J. Agric. Sci., 50(7): 541-544.

Sandhu, T.S., R.K. Gumber, B.S. Bhullar and R.S. Bhatia, 1989. Genetical analysis of grain protein, grain yield and its components in chickpea (*Cicer arietinum* L.). J. Res. Punjab Agric. Univ., 26(1): 1-9.

Saxena, N. P. 1984. Chickpea: *In* The Physiology of Tropical Field Crops (Goldsworthy, P. R. and Fisher, N. M., eds.). John Wiley and Sons Ltd, UK.,pp 419-452.

Setty, A.N.; M.S. Patil and K.G. Hiremath, 1977. Genetic variability and correlation studies in *Cicer arientinum* L. Mysore. J. Agric. Sci., 11:131-134.

Shahi, V.K.; N.B. Singh, V.K. Chaudhary, C.B. Ojha and S.K. Chowdhry, 1984. Yield components and their implications in breeding Bengal gram. Indian J. Agric. Res., 18(3): 155-157.

Shanmugam, A.S. and S.R. Shreerangaswamy, 1982. Multivariate analyses of genetic divergence in blackgram (*Vigna mungo* L. Hepper). Madras Agric. J., 69: 701-706.

Sharma, A.K., R.K. Tiwari and A.S. Tiwari, 1969. Studies on genotypic, phenotypic and environmental correlations in the F2 generation of soybean crosses. Indian J. Agric. Sci., 50(1): 87-89.

Sharma, P.P. and S.R. Maloo, 1988. Correlation and path coefficient analysis in Bengal gram (*Cicer arietinum* L.). Madras Agric. J. 75: 3-4, 95-98.

Sharma, S.M., S.K. Rao and U. Goswami, 1983. Genetic variation correlation and regression analysis and their implications in selection of exotic soybean, Mysore. J. Agric. Sci., 17: 26-30.

Shoran, J. 1982. Path analysis in pigeon pea. Indian J. Genet., 42(3): 319-321.

Singh, R.B. 1983. Trends and prospects of grain legume production in Asia and the Pacific. Presented at the Consultative Group Meeting for Asian Regional Research on Grain Legumes, 11-15 Dec 1983, ICRISAT. Available from ICRISAT, Patancheru P.O., Andhra Pradesh 502 324, India.

Singh S.P. 1988. Genetic variability and path coefficient studies in chickpea. Plant physiology, 40: 48-53.

Singh, A.K., P. Subrahmanyam and S. Gurtu, 1996. Variation in wild groundnut species, *Arachis duranensis* Krapov. & W.C. Gregory. Genetic Resources and Crop Evolution, 43: 135-142.

Singh, D., R.B. Srivastava and V.P. Singh, 1982. Combing ability in bread wheat. Ind. J. Agri. Sci., 52(6): 355-359.

Singh, D.P. and D. Singh, 1974. Correlations in Indian Cloza (*Brassica campestris* L.). Ind. J. Agric. Sci., 44: 142-144.

Singh, G., H.S. Brar, M.M. Verma, J.S. Sandhu and G. Singh, 1989. Component analysis of seed yield in chickpea. Crop Improvement, 16(2): 145-149.

Singh, H.F., M.C. Saxena and J.P. Sahu, 1980. Harvest index in relation to yield in grain legumes. Trop. Grain legume Bull., 17/18: 6-8.

Singh, I.S., M.A. Hussain and A.K. Gupta, 1995. Correlation studies among yield and yield contributing traits in F2 and F3 chickpea populations. ICPN 2: 11-13.

Singh, J. V., Yadava, T.P. and Kharb, R.P. S. 1985. Correlation and path coefficient analysis in sunflower. Ind. J. Agric. Sci., 55(4): 243-246.

Singh, K.B. and R.S. Malhotra, 1970. Inter-relationship between yield and yield components in mungbean. Ind. J. Genet. and Pl. Breeding, 30(1): 244-250.

Singh, K.B., G. Bejia and R.S. Malhotra, 1990. Association of some characters with seed yield in chickpea collections. Euphytica, 49(1): 83-88.

Singh, K.B., M. Omar, M.C. Saxena and C. Johansen, 1997a. Screening of drought resistance in spring chickpea in the Mediterranean region. Journal of Agronomy and Crop Science, 178: 227-235.

Singh, K.B.; R.S. Malhotra and H. Singh, 1978. Correlation and path coefficient analysis in chickpea. Indian J. Agric. Res. 12: 44-46.

Singh, K.P. V.P. Singh and B.D. Chaudhry, 1977. Path coefficient analysis in chickpea. Z. pflanzenzuchtung, 79: 219-223.

Singh, R.K. and B.D. Chaudhry, 1985. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, Ludhiana, new Delhi, pp. 303.

Singh, R.M. and C.P. Srivastava, 1985. Evaluation, classification and usefulness of pea germplasm for quantitative characters. Legume Research, 8: 68-72.

Singh, S.B. and B.K. Tripathi, 1985. Genetic divergence in pea. Indian J. Genet. Pl. Breed., 2: 389-393.

Singh, S.P. 1988. Clustering of genotypes for selection for heterosis in yield and response to environmental variation in mungbean (*Vigna radiata* (L.) Wilczek): a proposed method. Genome, 30(6): 835-837.

Singh, S.P.; R.K. Reddy and V.G. Narsinghani, 1981. Correlation studies in F4 progenies of pigeonpea. Indian J. Agric. Sci., 51(11): 768-771.

Sinha, M.K. M.K. Guha Roy and S.K. Chaudhury, 1986. Correlation and path coefficient analysis in mesta. Ind. J. Agric. Sci., 56(2): 83-85.

Smith, J.S. and O.S. Smith, 1986. Environmental effects on zein chromatograms of maize inbred lines revealed by reversed-phase high-performance liquid chromatography. Theor. Appl. Gene., 71: 607-612.

Smith, J.S.C. and O.S. Smith, 1989. The description and assessment of distances between inbred lines of maize: The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34: 151-161.

Smith, S.E., A. Al. Doss and M. Warburton, 1991. Morphological and agronomic variation in North African and Arabian alfalfas. Crop Sci. 31: 1159-1163.

Smith, S.E., L. Guarino, A. Al. Doss and D.M. Conta, 1995. Morphological and agronomic affinities among Middle Eastern alfalfas accessions from Oman and Yemen. Crop Sci. 35: 1188-1194.

Smithson, J.B. 1985. Breeding advances in chickpeas at ICRISAT. P.223-237. In: Progress in plant breeding (Russell, G.E. ed.). Butterworths. London.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.F. Freeman & Co., San Francisco, p. 573.

Sneedon, J.L. 1970. Identification of garden pea varieties. (I) Grouping, arrangement and use of continuous characters. J. Natn. Inst. Agric. Bot., 12: 1-16.

Sokal, R.R. 1965. Statistical methods in systematics. Biol. Rev. Cambr. Phil. Soc., 40: 333-391.

Soomro, B. and A.S. Larik, 1981. Inheritance and correlation of pods and seed character in peanuts. Genet. Agric. 35: 263-274.

Steel, R. G. D. and J.H. Torrie, 1981. Principles and Procedures of statistics. 2nd Edition, Mc Graw-Hill Book Co., Inc., New York.

Stoskopf, C.N. 1985. Cereal grain crops. Reston Publishing Company, Reston, p. 7.

Stuber, C.W., M.M. Goodman and R.H. Moll, 1982. Improvement of yield and ear number resulting from selection at Allozyme loci in maize population. Crop Sci., 22: 737-740.

Sumathi, S.M and T.N. Pattabiraman, 1976. Natural plant enzyme inhibitors. Part II. Protease inhibitor of seeds. Indian Journal of Biochemistry and Biophysics 13: 52-56.

Tagore, K.R. and I.S. Singh, 1990. Character association and path analysis under two levels of management in chickpea. Crop Improvement, 17(1): 41-44.

Tahir, M. and F.J. Muehlbauer, 1995. Association of quantitative trait loci with isozyme markers in lentil (*Lens culinaris* L.). J. Genet. & Breed., 49: 145-150.

Tahir, M., A. Pavoni, G.F. Tucci and T. Turchetta, 1996. Detection and characterization of glutenin subunit with unusual high Mr at the Glu-A1 locus in hexaploid wheat. Theor. Appl. Gene., 92: 654-659.

Tanksley, S.D., H. Medina-Filho and C.M. Rick, 1982. Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific back cross of tomato. Heredity, 49: 11-25.

Tariq. M. 1990. Biometrical studies on some quantitative traits in *G. hirsutum* L. Ph.D. Thesis, Plant Breeding and Genetics. Agric. Univ. Faisalabad, Pakistan.

Tawar, M.L., A.K. Mishra, S.K. Rao and S.M. Sharma, 1988. Genetic divergence in mungbean. Legume Research, 11(3): 103-109.

Thakare, R.G., J.D. Gadgil and R. Mitra, 1987. Origin and evolution of seed protein genes in *Vigna mungo* and *V. radiata*. In: Mungbean. Proceed. IInd Inter. Mungbean Symp., 16-20 November, 1987, Bangkok, Thailand, p. 47-52.

Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang and T.C. Osborn, 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among *Cruciferous* species. Theor. Appl. Genet. 88: 973-980.

Tikka, S. B. S., Jaimini, S. N. and Goyal, S. N., 1973. Variability studies in wheat under rainfed conditions. Sci. and Culture, 39(3): 129-130.

Tinker, N. A., M.G. Fortin and D.E. Mather, 1993. Random Amlified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet., 85: 976-984.

Tolbert, D.M., C.O. Qualset, S.K. Jain and J.C. Craddock, 1979. A diversity analyses of a world collection of barley. Crop Sci., 17: 789-794.

Tomar, G.S., L. Singh and P.K. Mishra, 1973. Correlation and path coefficient analysis of yield characters in mungbean. SABRAO Newsletter, 5: 125-127.

Tomar, G.S.; Mishra, Y. and Rao, S.K. 1982. Path analysis and its implications in selection of high yielding chickpea (*Cicer arietinum* L.). Indian J. Plant Physiol, 25: 127-132.

Tomooka, N., C. Lairungreang, P. Nakeeraks, Y. Egawa and C. Thavarasook, 1992. Center of genetic diversity and dissemination pathways in mung bean deduced from seed protein electrophoresis. Theor. Appl. Genet. 83: 289-293.

Tyagi, P.S.; B.D. Singh and H.K. Jaiswal, 1982. Path analysis of yield and protein content in chickpea. Indian J. Agric. Sci. 52: 81-85.

Usha, C.T.M., J.M. Have and H.E. Clark, 1972. The complementary value of cooked Bengal gram and opaque-A corn for the growing rat. Nutrition Reports International 5: 119-129.

Van der maesen L.J.G. 1972. *Cicer arietinum* L., a monograph of the genus, with special reference to the chickpea (*Cicer arietinum* L.) its ecology and cultivation. H. Veenman and zonen N.V., Wageningen. P. 156.

Van Hintum, Th.J.L. and A. Elings, 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. Euphytica, 55: 209-215.

Vavilov, N.I. 1926. Studies on the origin of cultivated plants. Inst. Appl. Bot. Plant Breed. (Leningrad). p. 248.

Veronesi, F. and M. Falcinelli, 1988a. Evaluation of an Italian germplasm collection of *Festuca arundinacea* Schreb through a multivariate approach. Euphytica, 38: 211-220.

Veronesi, F. and M. Falcinelli, 1988b. Seed yield selection in *Medicago sativa* L. and correlated responses affecting dry matter yield. Plant Breeding, 99: 77-79.

Vierling, R.A., Z. Xiang, C.P. Joshi, M.L. Gilbert and H.T. Nguyen, 1994. Genetic diversity among elite Sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. Theor. Appl. Genet. 87: 816-820.

Virmani, S.S., K.B. Singh, K. Singh and R.S. Malhotra, 1983. Evaluation of mungbean germplasm. Indian J. Genet., 43: 54-58

Wadud, A. and M. Yaqoob, 1988a. Phenotypic and genotypic correlation analysis in gram (*Cicer arietinum* L.). Sarhad J. Agric., 3(1): 87-94.

Wadud, A. and M. Yaqoob, 1988b. Regression and correlation analysis in chickpea (*Cicer aritinum* L.). J. Agric., 5(2): 172-176.

Wadud, A. and M. Yaqoob, 1989. Regression and correlation analysis in different cultivars of chickpea (*Cicer arietinum* L.). Sarhad J. Agric. 5(2): 171-176.

Warburton, M.L. and S.E. Smith, 1993. Regional diversity in nondormant alfalfas from India and the Middle East. Crop Sci., 33: 852-858.

Weir, B. S., 1990. Genetic data analysis: Methods for discrete population genetic data. Sinauer Assoc. Sunderland, Masschusetts.

Weir, B.S. 1990. Genetic data analyses. Sinauer Assoc., Sunderland, MA.

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218.

Welsh, J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral, 1991. Parentage determination in maize hybrid using the arbitrary primed chain reaction (AP-PCR). Theor. Appl. Genet., 38: 45-59.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Yadava, T.P. 1973. Varibility and correlation studies in *Brassica juncea* L. Madras Agric. J. 60:9-12.

Yoshida, K., S. Tsurshiin, H. Fukuba, T. Tadokoro and A. Maekawa, 1997. Properties of blackmatpe globulin. J. Japan Soc. Nutr. & Food Sci., 50(1): 73-76.

Yu, L.-X. and H.T. Nguyen, 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet., 87: 668-672.

Appendix I

Sr.No.	ACC.NO.	PI No	Country	Plant name	G.H.	I.D.	D.F.	F.C.	D.M.	P.Hr.	P.P	Blight
1	PAK-53007	115449	India	NEC-2307	1	1	138	6	165	1	2	1
2	PAK-53008	193480	Ethiopia		1	1	127	6	169	1	2	1
3	PAK-53009	193482	Ethiopia		1	1	135	4	214	1	2	1
4	PAK-53010	193485	Ethiopia		1	1	137	4	214	1	2	1
5	PAK-53011	193486	Ethiopia		1	1	138	4	214	1	2	1
6	PAK-53012	193487	Ethiopia		1	1	138	4	214	1	2	1
7	PAK-53013	193767	Ethiopia		1	1	140	4	215	1	2	1
8	PAK-53014	195561	Ethiopia		1	1	139	5	215	1	2	1
9	PAK-53015	196840	Ethiopia		1	1	130	4	216	1	2	1
10	PAK-53016	203142	Jordan	ICC 9517	1	1	143	4	216	1	2	1
11	PAK-53017	207470	Afghanistan	No. 12620	1	1	134	5	217	1	1	1
12	PAK-53018	212091	Afghanistan	ICC 8167	2	1	131	4	218	1	1	1
13	PAK-53019	212595	Afghanistan	ILC 3342	1	1	127	6	163	2	1	1
14	PAK-53020	214311	India	ILC 211	2	1	128	6	164	2	2	1
15	PAK-53021	215588	India	ICC 8178	1	1	138	6	164	2	2	1
16	PAK-53022	215702	Peru	ILC 213	1	1	131	6	165	2	1	1
17	PAK-53023	219728	Pakistan	ICC 8184	1	0	135	6	165	2	2	1
18	PAK-53024	222774	Iran	ICC 9492	2	1	138	6	165	2	2	1
19	PAK-53025	223433	Afghanistan	No.1094	1	0	139	6	165	2	1	1
20	PAK-53026	244333	Ethiopia		1	0	136	6	166	2	2	1
21	PAK-53027	250143	Pakistan	ILC 219	2	1	127	6	168	2	1	1
22	PAK-53028	250144	Pakistan	ILC 220	1	0	132	6	168	2	2	1
23	PAK-53029	251024	Afghanistan	ILC 221	2	1	134	6	168	2	2	1
24	PAK-53030	251027	Iran	ILC 226	1	1	134	6	168	2	1	1
25	PAK-53031	251514	Iran	NOKHODSIAH	1	1	134	6	168	2	2	1

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26	PAK-53032	251781	Former Soviet Union	ICC 9514	1	1	134	6	168	2	2	1
27	PAK-53033	251782	Former Soviet Union	ICC 8203	1	1	127	6	169	2	1	1
28	PAK-53034	253227	Turkey	ILC 229	1	1	135	6	169	2	1	1
29	PAK-53035	253228	Turkey	Tvu 3578	1	1	127	6	170	2	1	1
30	PAK-53036	254547	Syria	ILC 232	1	1	132	6	170	2	1	1
31	PAK-53037	254548	Syria	ILC 233	1	1	134	6	170	2	1	1
32	PAK-53038	254549	Iraq	ILC 235	1	1	134	6	170	2	1	1
33	PAK-53039	254889	Spain	ILC 238	1	1	138	6	170	2	1	1
34	PAK-53040	255138	India, Uttar Pradesh	ICC 8944	1	1	138	6	170	2	1	1
35	PAK-53041	256060	Afghanistan	ICC 9515	2	0	138	6	170	2	2	1
36	PAK-53042	257584	Ethiopia	ILC 239	2	1	138	6	170	2	1	1
37	PAK-53043	257586	Ethiopia	ICC 12377	1	1	142	6	170	2	2	1
38	PAK-53044	268376	Afghanistan	ILC 240	1	1	127	6	171	2	2	1
39	PAK-53045	269883	Pakistan	CP 845	1	1	127	6	172	2	1	1
40	PAK-53046	273879	Ethiopia	ICC 8215	2	0	134	6	172	2	2	1
41	PAK-53047	288315	India	ICC 8949	2	0	136	6	172	2	2	1
42	PAK-53048	315781	India	ILC 244	1	1	138	6	172	2	1	1
43	PAK-53049	315790	India	ICC 8235	1	1	132	6	173	2	1	1
44	PAK-53050	315803	India	ICC 8240	2	1	127	6	175	2	2	1
45	PAK-53051	315810	India	ICC 8243	2	1	138	6	175	2	2	1
46	PAK-53052	315813	India	ICC 8245	1	1	138	6	175	2	2	1
47	PAK-53053	315826	India	ICC 8254	1	1	139	6	175	2	2	1
48	PAK-53054	331381	Ethiopia	SHIMBRA	2	1	127	6	176	2	1	1
49	PAK-53055	339165		ISPANYOL	1	1	141	6	176	2	2	1
50	PAK-53056	339221		ICC 9511	2	1	147	6	178	2	1	1

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51	PAK-53057	339223		ILC 263	1	1	127	6	185	2	1	1
52	PAK-53058	343014	Former Soviet Union	KUBAN'S 16	3	1	137	6	185	2	1	1
53	PAK-53059	343015	Former Soviet Union	AZERBAIDZAN'S 853	2	1	130	4	212	2	1	1
54	PAK-53060	343016	Former Soviet Union	KARASNOKUTORII 195	2	1	130	4	212	2	1	1
55	PAK-53061	343017	Former Soviet Union	K-1168	1	1	130	4	212	2	1	1
56	PAK-53062	343018	Former Soviet Union	MILYUTN'S 04	1	1	130	4	212	2	1	1
57	PAK-53063	343019	Former Soviet Union	SOVHOZ 14	2	1	130	4	212	2	1	1
58	PAK-53064	343021	Former Soviet Union	TALL 30	1	1	130	4	212	2	2	1
59	PAK-53065	347261	Italy	SULMONA	1	1	130	4	212	2	1	1
60	PAK-53066	357649	Yugoslavia	VOJNICKI	1	1	130	4	212	2	1	1
61	PAK-53067	377653	Yugoslavia	BEL KRUPEN	2	1	130	4	212	2	1	1
62	PAK-53068	357654	Yugoslavia	DOMASEN	3	1	130	4	212	2	1	1
63	PAK-53069	358914	India	RPIP 12-000-01527	1	0	130	4	212	2	1	1
64	PAK-53070	358916	India	RPIP 12-000-15335	2	1	130	4	212	2	2	1
65	PAK-53071	358922	India	RPIP 12-000-01552	2	1	130	4	212	2	2	1
66	PAK-53072	358930	Iran	ILC 267	3	1	130	4	212	2	2	1
67	PAK-53073	358935	Iran	RPIP 12-071-00475	3	1	130	4	212	2	1	1
68	PAK-53074	358938	Morroco	RPIP 12-100-00825	1	0	130	4	212	2	1	1
69	PAK-53075	359007	India	RPIP 12-069-00005	2	1	134	4	212	2	2	1
70	PAK-53076	359009	India	RPIP 12-069-00007	3	1	134	4	212	2	2	1
71	PAK-53077	359014	India	RPIP 12-069-00015	2	1	135	4	212	2	2	1
72	PAK-53078	359041	India	RPIP 12-069-00055	2	1	125	4	213	2	2	1
73	PAK-53079	359051	India	RPIP 12-069-00069	2	1	125	4	213	2	2	1
74	PAK-53080	359061	India	RPIP 12-069-00083	1	0	125	4	213	2	2	1
75	PAK-53081	359075	India	RPIP 12-069-00103	2	1	125	4	213	2	2	1

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76	PAK-53082	359085	India	RPIP 12-069-00117	2	1	126	4	213	2	2	1
77	PAK-53083	359099	India	RPIP 12-069-00140	1	0	127	4	213	2	2	1
78	PAK-53084	359100	India	RPIP 12-069-00142	2	1	130	4	213	2	2	1
79	PAK-53085	359115	India	RPIP 12-069-00163	3	1	130	4	213	2	1	1
80	PAK-53086	359127	India	RPIP 12-069-00178	2	1	131	4	213	2	2	1
81	PAK-53087	359150	India	RPIP 12-069-00212	2	1	131	4	213	2	2	1
82	PAK-53088	359159	India	RPIP 12-069-00223	2	1	131	4	213	2	2	1
83	PAK-53089	359170	India	RPIP 33-071-10885	1	1	131	4	213	2	2	1
84	PAK-53090	359179	India	RPIP 12-069-00255	1	1	131	4	213	2	2	1
85	PAK-53091	359186	India	RPIP 12-069-00264	1	1	131	4	213	2	1	1
86	PAK-53092	359213	India	RPIP 12-069-00306	2	1	131	4	213	2	1	1
87	PAK-53093	359219	India	ICC 9036	2	1	131	4	213	2	2	1
88	PAK-53094	359228	India	ILC 272	2	1	139	4	213	2	2	1
89	PAK-53095	359239	India	RPIP 12-069-00337	1	1	139	4	213	2	2	1
90	PAK-53096	359241	India	RPIP 12-069-00340	2	1	141	4	213	2	2	1
91	PAK-53097	359245	India	RPIP 12-069-00348	2	1	141	4	213	2	2	1
92	PAK-53098	359249	India	RPIP 12-069-00354	1	1	154	4	213	2	2	1
93	PAK-53099	359257	India	RPIP 12-069-00372	3	1	156	4	213	2	2	1
94	PAK-53100	359260	India	RPIP 12-069-00375	3	1	127	5	213	2	2	1
95	PAK-53101	359268	India	RPIP 12-06900387-	1	1	127	5	213	2	2	1
96	PAK-53102	359277	India	RPIP 12-069-00405	1	1	127	5	213	2	2	1
97	PAK-53103	359289	India	RPIP 12-069-00424	1	1	130	5	213	2	2	1
98	PAK-53104	359304	India	RPIP 12-069-00450	3	1	130	5	213	2	3	1
99	PAK-53105	359307	India	RPIP 12-069-00453	3	1	130	5	213	2	2	1
100	PAK-53106	359311	India	RPIP 12-069-00458	3	1	131	5	213	2	2	1

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101	PAK-53107	359313	India	RPIP 12-069-00461	2	1	131	5	213	2	2	1
102	PAK-53108	359316	India	RPIP 12-069-00464	3	1	131	5	213	2	1	1
103	PAK-53109	359329	India	RPIP 12-069-00488	2	1	131	5	213	2	2	1
104	PAK-53110	359335	India	RPIP 12-069-00495	2	1	141	5	213	2	2	1
105	PAK-53111	359348	India	RPIP 12-069-00514	2	1	141	5	213	2	2	1
106	PAK-53112	359363	India	RPIP 12-069-00538	1	1	144	5	213	2	2	1
107	PAK-53113	359372	India	RPIP 12-069-00556	2	1	144	5	213	2	2	1
108	PAK-53114	359374	India	RPIP 12-069-00558	2	1	148	5	213	2	2	1
109	PAK-53115	359406	India	RPIP 12-069-00605	1	1	148	5	213	2	2	1
110	PAK-53116	359417	India	RPIP 12-069-00620	1	1	148	5	213	2	2	1
111	PAK-53117	359429	India	RPIP 12-069-00637	1	1	127	6	213	2	2	1
112	PAK-53118	359450	India	RPIP 12-069-00663	1	1	127	6	213	2	2	1
113	PAK-53119	359460	India	RPIP 12-069-00678	2	0	127	6	213	2	2	1
114	PAK-53120	359471	India	RPIP 12-069-00690	1	1	127	6	213	2	2	1
115	PAK-53121	359481	India	RPIP 12-069-00701	2	1	135	4	214	2	2	1
116	PAK-53122	359489	India	RPIP 12-069-00712	2	1	135	4	214	2	2	1
117	PAK-53123	359498	India	RPIP 12-069-00723	2	1	135	4	214	2	1	1
118	PAK-53124	359502	India	RPIP 12-069-00727	2	1	135	4	214	2	2	1
119	PAK-53125	359525	India	RPIP 12-069-00788	2	0	136	4	214	2	1	1
120	PAK-53126	359531	India	RPIP 12-069-00869	2	1	136	4	214	2	2	1
121	PAK-53127	359544	India	RPIP 12-069-01027	1	1	137	4	214	2	2	1
122	PAK-53128	359555	India	RPIP 12-069-01088	3	1	137	4	214	2	2	1
123	PAK-53129	359560	India	RPIP 12-069-01102	1	1	138	4	214	2	1	1
124	PAK-53130	359582	India	RPIP 12-069-01135	2	1	138	4	214	2	1	1
125	PAK-53131	359588	India	RPIP 12-069-01145	2	0	138	4	214	2	1	1

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126	PAK-53132	359591	India	RPIP 12-069-01148	2	0	138	4	214	2	2	1
127	PAK-53133	359595	India	RPIP 12-069-01154	1	1	138	4	214	2	2	1
128	PAK-53134	359607	India	RPIP 12-069-01169	2	0	138	4	214	2	2	1
129	PAK-53135	359611	India	RPIP 12-069-01175	1	0	138	4	214	2	2	1
130	PAK-53136	369631	India	RPIP 12-069-01206	1	1	139	4	214	2	2	1
131	PAK-53137	369641	India	RPIP 12-069-01218	2	0	127	3	215	2	1	1
132	PAK-53138	369658	India	RPIP 12-069-01239	1	1	125	4	215	2	1	1
133	PAK-53139	359673	India	RPIP 12-069-01259	1	1	125	4	215	2	2	1
134	PAK-53140	359687	India	RPIP 12-069-01276	2	0	125	4	215	2	2	1
135	PAK-53141	359692	India	RPIP 12-069-01282	2	1	125	4	215	2	1	1
136	PAK-53142	359697	India	RPIP 12-069-01287	2	0	129	4	215	2	2	1
137	PAK-53143	359715	India	RPIP 12-069-01321	2	0	129	4	215	2	2	1
138	PAK-53144	359716	India	RPIP 12-069-01323	3	1	129	4	215	2	2	1
139	PAK-53145	359738	India	RPIP 12-069-01353	1	1	129	4	215	2	2	1
140	PAK-53146	359746	India	RPIP 12-069-01361	1	1	129	4	215	2	2	1
141	PAK-53147	359751	India	RPIP 12-069-01367	2	1	129	4	215	2	1	1
142	PAK-53148	359753	India	RPIP 12-069-01369	2	1	129	4	215	2	1	1
143	PAK-53149	359769	India	RPIP 12-069-01392	2	1	129	4	215	2	1	1
144	PAK-53150	359773	India	RPIP 12-069-01397	3	1	129	4	215	2	2	1
145	PAK-53151	359801	India	RPIP 12-069-01432	2	1	130	4	215	2	1	1
146	PAK-53152	359805	India	ICC 6778	2	0	130	4	215	2	2	1
147	PAK-53153	359815	India	RPIP 12-069-01454	2	1	134	4	215	2	2	1
148	PAK-53154	359827	India	RPIP 12-069-01468	2	0	134	4	215	2	2	1
149	PAK-53155	359830	India	RPIP 12-069-01474	1	1	134	4	215	2	2	1
150	PAK-53156	359836	India	RPIP 12-069-01481	2	1	134	4	215	2	2	1

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151	PAK-53157	359841	India	RPIP 12-069-01486	1	1	134	4	215	2	2	1
152	PAK-53158	359844	India	RPIP 12-069-01491	1	1	134	4	215	2	2	1
153	PAK-53159	359862	India	RPIP 12-069-01569	2	0	140	4	215	2	2	1
154	PAK-53160	359878	India	RPIP 12-069-01606	1	1	148	4	215	2	2	1
155	PAK-53161	359891	India	RPIP 12-069-01628	1	1	148	4	215	2	2	1
156	PAK-53162	359899	India	RPIP 12-069-01639	3	1	148	4	215	2	2	1
157	PAK-53163	359913	India	RPIP 12-069-01667	2	1	149	4	215	2	1	1
158	PAK-53164	359914	India	RPIP 12-069-01670	1	1	150	4	215	2	2	1
159	PAK-53165	359916	India	RPIP 12-069-01674	1	0	135	5	215	2	1	1
160	PAK-53166	359919	India	ICC 13132	1	1	136	5	215	2	2	1
161	PAK-53167	359922	India	RPIP 12-069-01683	2	1	138	5	215	2	2	1
162	PAK-53168	359924	India	RPIP 12-069-01687	2	1	138	5	215	2	2	1
163	PAK-53169	359944	India	RPIP 12-069-01719	1	0	139	5	215	2	2	1
164	PAK-53170	359968	India	RPIP 12-069-01765	1	0	139	5	215	2	1	1
165	PAK-53171	359969	India	RPIP 12-069-01766	2	1	141	5	215	2	1	1
166	PAK-53172	359975	India	RPIP 12-069-01773	1	1	141	5	215	2	1	1
167	PAK-53173	359986	India	RPIP 12-069-01789	1	1	141	5	215	2	2	1
168	PAK-53174	359988	India	RPIP 12-069-01792	1	1	127	4	216	2	2	1
169	PAK-53175	360010	India	RPIP 12-069-06075	1	0	127	4	216	2	1	1
170	PAK-53176	360011	India	RPIP 12-069-06076	2	1	127	4	216	2	2	1
171	PAK-53177	360029	India	RPIP 12-069-06113	2	1	127	4	216	2	2	1
172	PAK-53178	360050	India	ICC 13579	2	1	127	4	216	2	2	1
173	PAK-53179	360063	India	RPIP 12-069-06198	3	1	127	4	216	2	2	1
174	PAK-53180	360070	India	RPIP 12-069-06212	2	1	128	4	216	2	1	1
175	PAK-53181	360078	India	RPIP 12-069-06223	1	0	128	4	216	2	2	1

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176	PAK-53182	360090	India	RPIP 12-069-06245	2	1	130	4	216	2	2	1
177	PAK-53183	360095	India	RPIP 12-069-06256	3	1	130	4	216	2	2	1
178	PAK-53184	360108	India	RPIP 12-069-06278	2	1	130	4	216	2	2	1
179	PAK-53185	360111	India	RPIP 12-069-06288	1	0	130	4	216	2	1	1
180	PAK-53186	360121	India	RPIP 12-069-06302	3	0	130	4	216	2	1	1
181	PAK-53187	360122	India	ICC 13593	3	0	131	4	216	2	1	1
182	PAK-53188	360133	Iran	RPIP 12-071-00902	3	0	131	4	216	2	2	1
183	PAK-53189	360159	Iran	RPIP 12-071-01841	2	0	132	4	216	2	1	1
184	PAK-53190	360162	Iran	RPIP 12-071-01855	2	1	134	4	216	2	1	1
185	PAK-53191	360180	Iran	ICC 13158	3	0	143	4	216	2	3	1
186	PAK-53192	360189	Iran	RPIP 12-071-02021	2	1	143	4	216	2	1	1
187	PAK-53193	360193	Iran	ILC 283	1	0	143	4	216	2	2	1
188	PAK-53194	360194	Iran	ICC 6825	1	1	143	4	216	2	1	1
189	PAK-53195	360211	Iran	RPIP 12-069-02126	1	1	143	4	216	2	1	1
190	PAK-53196	360230	Iran	RPIP 12-071-02395	2	1	143	4	216	2	1	1
191	PAK-53197	360244	Iran	RPIP 12-071-02552	1	1	143	4	216	2	1	1
192	PAK-53198	360253	Iran	RPIP 12-071-02637	2	1	143	4	216	2	1	1
193	PAK-53199	360258	Iran	RPIP 12-071-02793	1	1	143	4	216	2	1	1
194	PAK-53200	360262	Iran	RPIP 12-071-02825	1	1	131	5	216	2	1	1
195	PAK-53201	360268	Iran	RPIP 12-071-07102904	2	1	131	5	216	2	1	1
196	PAK-53202	360288	Iran	RPIP 12-071-03223	2	1	131	5	216	2	1	1
197	PAK-53203	360291	Iran	RPIP 12-071-03212	1	1	134	5	216	2	2	1
198	PAK-53204	360292	Iran	ICC 13247	2	1	134	5	216	2	2	1
199	PAK-53205	360304	Iran	RPIP 12-071-03576	1	0	148	6	216	2	1	1
200	PAK-53206	360315	Iran	ILC 367	2	1	127	4	217	2	2	1

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201	PAK-53207	360328	Iran	ILC 377	2	1	139	4	217	2	1	1
202	PAK-53208	360342	Iran	RPIP 12-071-04165	2	1	139	4	217	2	1	1
203	PAK-53209	360344	Iran	RPIP 12-071-04172	1	1	139	4	217	2	1	1
204	PAK-53210	360347	Iran	RPIP 12-071-04203	1	1	139	4	217	2	1	1
205	PAK-53211	360348	Iran	ICC 6920	1	1	134	5	217	2	1	1
206	PAK-53212	360350	Iran	RPIP 12-071-04214	1	1	134	5	217	2	1	1
207	PAK-53213	360358	Iran	RPIP 12-071-04230	2	1	134	5	217	2	2	1
208	PAK-53214	360365	Iran	RPIP 12-071-04244	2	1	134	5	217	2	2	1
209	PAK-53215	360383	Iran	RPIP 12-071-04278	2	1	134	5	217	2	2	1
210	PAK-53216	360399	Iran	RPIP 12-071-04324	2	1	134	5	217	2	1	1
211	PAK-53217	360410	Iran	RPIP 12-071-04357	2	0	134	5	217	2	1	1
212	PAK-53218	360418	Iran	RPIP 12-071-04386	1	1	134	5	217	2	2	1
213	PAK-53219	360422	Iran	RPIP 12-071-04446	1	1	134	5	217	2	2	1
214	PAK-53220	360425	Iran	RPIP 12-071-04494	1	1	135	5	217	2	2	1
215	PAK-53221	360433	Iran	RPIP 12-071-04660	1	0	135	5	217	2	2	1
216	PAK-53222	360439	Iran	RPIP 12-071-04736	3	1	135	5	217	2	1	1
217	PAK-53223	360456	Iran	RPIP 12-071-04892	1	0	145	5	217	2	2	1
218	PAK-53224	360470	Iran	ICC 13514	1	0	145	5	217	2	2	1
219	PAK-53225	360472	Iran	RPIP 12-071-05017	1	1	145	5	217	2	2	1
220	PAK-53226	360485	Iran	RPIP 12-071-05116	2	0	145	5	217	2	2	1
221	PAK-53227	360493	Iran	RPIP 12-071-05203	1	1	145	5	217	2	2	1
222	PAK-53228	360505	Iran	RPIP 12-071-05282	2	0	141	4	218	2	2	1
223	PAK-53229	360517	Iran	RPIP 12-071-05345	2	0	141	4	218	2	2	1
224	PAK-53230	360530	Iran	RPIP 12-071-05401	2	0	142	4	218	2	2	1
225	PAK-53231	360545	Iran	RPIP 12-071-05432	2	1	143	4	218	2	2	1

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226	PAK-53232	360561	Iran	RPIP 12-071-05478	1	0	130	5	218	2	2	1
227	PAK-53233	360574	Iran	RPIP 12-071-05493	2	0	130	5	218	2	2	1
228	PAK-53234	360585	Iran	ICC 13601	2	0	130	5	218	2	2	1
229	PAK-53235	360596	Iran	ICC-13604	2	0	143	4	220	2	2	1
230	PAK-53236	360599	Iran	RPIP 12-071-06359	1	1	144	4	220	2	2	1
231	PAK-53237	360609	Iran	RPIP 12-071-06425	1	1	144	4	220	2	2	1
232	PAK-53238	360630	Iran	RPIP 12-071-06488	1	1	144	4	220	2	2	1
233	PAK-53239	360641	Iran	ICC 13620	2	1	144	4	220	2	1	1
234	PAK-53240	360642	Iran	RPIP 12-071-06540	2	1	144	4	220	2	1	1
235	PAK-53241	360649	Iran	RPIP-12-071-06592	2	1	144	4	220	2	1	1
236	PAK-53242	360655	Iran	ILC 410	2	1	144	4	220	2	1	1
237	PAK-53243	360657	Israel	RPIP 12-074-01013	1	0	144	4	220	2	2	1
238	PAK-53244	360658	Israel	RPIP 12-074-06623	2	0	145	4	220	2	2	1
239	PAK-53245	360659	Israel	RPIP 12-074-06625	1	1	145	4	220	2	2	1
240	PAK-53246	360660	Israel	RPIP 12-074-06626	1	0	145	4	220	2	1	1
241	PAK-53247	360662	Italy	RPIP 12-074-00858	2	1	145	4	220	2	1	1
242	PAK-53248	360663	Mexico	RPIP 12-074-00799	2	1	145	4	220	2	2	1
243	PAK-53249	360664	Mexico	RPIP 12-074-00802	3	1	145	4	220	2	1	1
244	PAK-53250	360665	Mexico	RPIP 12-074-00809	2	1	145	4	220	2	2	1
245	PAK-53251	360667	Mexico	RPIP 12-074-00811	2	1	147	4	220	2	1	1
246	PAK-53252	360669	Mexico	RPIP 12-074-00813	1	0	148	4	220	2	2	1
247	PAK-53253	360670	Morocco	RPIP 12-074-00827	1	1	130	4	221	2	2	1
248	PAK-53254	360672	Morocco	RPIP 12-074-01011	1	1	130	6	163	3	2	1
249	PAK-53255	360673	Morocco	RPIP 12-074-01015	1	0	144	6	163	3	1	1
250	PAK-53256	360674	Pakistan	RPIP 12-074-00890	1	1	132	6	168	3	2	1

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251	PAK-53257	360680	Pakistan	RPIP 12-074-00946	1	0	134	6	168	3	1	1
252	PAK-53258	360684	Pakistan	RPIP 12-074-00997	1	0	135	6	168	3	2	1
253	PAK-53259	360686	Pakistan	RPIP 12-074-01069	2	0	127	6	169	3	1	1
254	PAK-53260	360687	Sapin	RPIP 12-074-00922	2	0	138	6	169	3	1	1
255	PAK-53261	360688	Turkey	RPIP 12-074-05499	1	1	130	6	170	3	2	1
256	PAK-53262	360690	Turkey	RPIP 12-074-06011	1	0	134	6	170	3	2	1
257	PAK-53263	360691	Egypt	RPIP 12-074-00848	2	0	135	6	170	3	2	1
258	PAK-53264	360695	Former Soviet Union	RPIP 12-074-00849	1	1	137	6	170	3	1	1
259	PAK-53265	360696	Former Soviet Union	RPIP 12-074-00850	2	1	138	6	170	3	2	1
260	PAK-53266	360697	Former Soviet Union	RPIP 12-074-00851	2	1	138	6	170	3	2	1
261	PAK-53267	360698	Former Soviet Union	RPIP 12-074-00852	2	1	131	6	173	3	1	1
262	PAK-53268	368485	Yugoslavia	KRNJVESKI	2	1	131	6	173	3	2	1
263	PAK-53269	368492	Yugoslavia	LOKALEN	1	1	138	6	173	3	1	1
264	PAK-53270	370416	Yugoslavia	LOKALEN	1	1	137	6	174	3	2	1
265	PAK-53271	370417	Yugoslavia	STIPSKI	1	1	138	6	180	3	2	1
266	PAK-53272	370419	Yugoslavia	VINCENSKI	2	0	130	4	212	3	2	1
267	PAK-53273	372596	Iran	RPIP 12-069-00519	1	1	130	4	212	3	1	1
268	PAK-53274	374079	Bulgaria	OBRAZOCOV CIFLIK	2	0	134	4	212	3	1	1
269	PAK-53275	374080	Bulgaria	PLOVDOV 19	2	0	135	4	212	3	1	1
270	PAK-53276	374085	Morocco	66	1	0	125	4	213	3	1	1
271	PAK-53277	374093	Iran	BR 17	2	0	125	4	213	3	1	1
272	PAK-53278	379217	Yugoslavia	GORUBINSKI	3	1	126	4	213	3	1	1
273	PAK-53279	379220	Yugoslavia	KLISURSKI	2	0	126	4	213	3	2	1
274	PAK-53280	379221	Yugoslavia	PESAK	1	1	127	4	213	3	2	1
275	PAK-53281	420907	Jordan	9	1	1	127	4	213	3	2	1

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276	PAK-53282	420908	Jordan	40	1	0	130	4	213	3	2	1
277	PAK-53283	426190	Afghanistan	K-1001	2	0	130	4	213	3	1	1
278	PAK-53284	426193	Afghanistan	K-1037	1	1	130	4	213	3	1	1
279	PAK-53285	426194	Afghanistan	K-1056	2	1	130	4	213	3	1	1
280	PAK-53286	426195	Afghanistan	K-1057	2	1	130	4	213	3	1	1
281	PAK-53287	426196	Afghanistan	K-1067	2	1	131	4	213	3	1	1
282	PAK-53288	426535	Pakistan	K-187	2	0	131	4	213	3	1	1
283	PAK-53289	426536	Pakistan	K-202	1	1	131	4	213	3	1	1
284	PAK-53290	426546	Pakistan	K-308	1	1	131	4	213	3	2	1
285	PAK-53291	426552	Pakistan	K-343	2	0	139	4	213	3	1	1
286	PAK-53292	426554	Pakistan	K-359	2	0	139	4	213	3	2	1
287	PAK-53293	426556	Pakistan	K-367	2	1	139	4	213	3	1	1
288	PAK-53294	426561	Pakistan	K-449	3	1	140	4	213	3	2	1
289	PAK-53295	426569	Pakistan	K-516	3	1	140	4	213	3	1	1
290	PAK-53296	426571	Pakistan	K-518	3	1	140	4	213	3	1	1
291	PAK-53297	426583	Pakistan	K-599	3	1	140	4	213	3	1	1
292	PAK-53298	426586	Pakistan	K-615	4	1	141	4	213	3	1	1
293	PAK-53299	426587	Pakistan	K-616	1	0	154	4	213	3	1	1
294	PAK-53300	426591	Pakistan	K-638	2	1	154	4	213	3	1	1
295	PAK-53301	426593	Pakistan	K-646	2	0	127	5	213	3	2	1
296	PAK-53302	426608	Pakistan	K-795	2	1	127	5	213	3	1	1
297	PAK-53303	439756	India, Andhra Pradesh	182	2	1	127	5	213	3	2	1
298	PAK-53304	439779	India, Andhra Pradesh	959	1	1	128	5	213	3	2	1
299	PAK-53305	439785	India, Andhra Pradesh	1749	2	1	130	5	213	3	2	1
300	PAK-53306	439801	India, Andhra Pradesh	2835	2	0	130	5	213	3	1	1

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301	PAK-53307	439810	India, Andhra Pradesh	3426	2	1	130	5	213	3	2	1
302	PAK-53308	439829	India, Andhra Pradesh	5727	2	1	130	5	213	3	1	1
303	PAK-53309	439831	India, Andhra Pradesh	5901	2	0	130	5	213	3	2	1
304	PAK-53310	439832	India, Andhra Pradesh	6081	1	1	130	5	213	3	1	1
305	PAK-53311	439834	India, Andhra Pradesh	6462	3	1	130	5	213	3	1	1
306	PAK-53312	439847	India, Andhra Pradesh	8222	2	1	130	5	213	3	1	1
307	PAK-53313	439858	India, Andhra Pradesh	10301	2	1	131	5	213	3	1	1
308	PAK-53314	450553	Afghanistan	RPIP 12-002-04235	2	0	131	5	213	3	1	1
309	PAK-53315	450564	India	RPIP 12-069-0038	2	1	131	5	213	3	2	1
310	PAK-53316	450575	India	RPIP 12-069-00077	1	1	131	5	213	3	2	1
311	PAK-53317	450577	India	RPIP 12-069-00082	1	0	131	5	213	3	2	1
312	PAK-53318	450585	India	RPIP 12-069-00125	1	0	141	5	213	3	2	1
313	PAK-53319	450600	India	ICC 13013	1	0	141	5	213	3	2	1
314	PAK-53320	450603	India	RPIP 12-069-00219	1	0	142	5	213	3	2	1
315	PAK-53321	450615	India	RPIP 12-069-00254	2	1	142	5	213	3	1	1
316	PAK-53322	450622	India	ICC 13018	1	0	143	5	213	3	2	1
317	PAK-53323	450634	India	RPIP 12-069-00343	2	1	143	5	213	3	2	1
318	PAK-53324	450640	India	RPIP 12-069-00357	2	1	143	5	213	3	1	1
319	PAK-53325	450654	India	NP-19	2	1	144	5	213	3	2	1
320	PAK-53326	450658	India	RPIP 12-069-00434	2	1	148	5	213	3	2	1
321	PAK-53327	450669	India	RPIP 12-069-00475	2	1	148	5	213	3	2	1
322	PAK-53328	450670	India	RPIP 12-069-00490	2	1	126	6	213	3	2	1
323	PAK-53329	450684	India	ICC 13051	3	1	126	6	213	3	2	1
324	PAK-53330	450693	India	ICC 13054	3	1	127	6	213	3	2	1
325	PAK-53331	450717	India	RPIP 12-069-00711	2	0	127	6	213	3	2	1

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326	PAK-53332	450728	India	RPIP 12-069-00789	1	0	127	6	213	3	2	1
327	PAK-53333	450734	India	RPIP 12-069-00807	1	0	127	6	213	3	2	1
328	PAK-53334	450738	India	RPIP 12-069-00811	1	0	127	6	213	3	1	1
329	PAK-53335	450739	India	RPIP 12-069-00812	2	1	127	6	213	3	2	1
330	PAK-53336	450740	India	RPIP 12-069-00813	2	1	127	6	213	3	1	1
331	PAK-53337	450755	India	RPIP 12-069-00993	1	0	127	6	213	3	2	1
332	PAK-53338	450760	India	ICC 13081	2	1	127	6	213	3	2	1
333	PAK-53339	450763	India	ICC 13089	1	0	135	4	214	3	1	1
334	PAK-53340	450772	India	ICC 14459	2	0	138	4	214	3	2	1
335	PAK-53341	450778	India	ICC 13097	2	1	138	4	214	3	1	1
336	PAK-53342	450782	India	ICC 13102	1	1	138	4	214	3	1	1
337	PAK-53343	450786	India	RPIP 12-069-01191	1	0	139	4	214	3	2	1
338	PAK-53344	450787	India	ICC 13107	2	1	128	3	215	3	2	1
339	PAK-53345	450806	India	RPIP 12-069-01293	1	0	125	4	215	3	2	1
340	PAK-53346	450817	India	RPIP 12-069-01318	2	1	125	4	215	3	2	1
341	PAK-53347	450820	India	ICC 13119	2	1	128	4	215	3	2	1
342	PAK-53348	450825	India	RPIP 12-069-01350	2	1	129	4	215	3	2	1
343	PAK-53349	450832	India	RPIP 12-069-01395	1	0	129	4	215	3	2	1
344	PAK-53350	450843	India	RPIP 12-069-01490	2	1	129	4	215	3	2	1
345	PAK-53351	450851	India	RPIP 12-069-01522	2	1	129	4	215	3	2	1
346	PAK-53352	450852	India	RPIP 12-069-01524	2	1	129	4	215	3	2	1
347	PAK-53353	450867	India	RPIP 12-069-01588	2	1	129	4	215	3	1	1
348	PAK-53354	450870	India	ICC 13133	1	0	129	4	215	3	1	1
349	PAK-53355	450872	India	RPIP 12-069-04436	3	1	129	4	215	3	1	1
350	PAK-53356	450876	India	RPIP 12-069-06093	2	1	129	4	215	3	2	1

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351	PAK-53357	450884	India	RPIP 12-069-06652	2	1	129	4	215	3	2	1
352	PAK-53358	450902	Iran	ICC 13155	3	1	131	4	215	3	2	1
353	PAK-53359	450906	Iran	RPIP 12-071-01981	1	0	134	4	215	3	2	1
354	PAK-53360	450908	Iran	RPIP 12-069-01992	2	1	134	4	215	3	1	1
355	PAK-53361	450911	Iran	RPIP 12-071-02255	2	1	134	4	215	3	2	1
356	PAK-53362	450930	Iran	ICC 13198	1	0	134	4	215	3	2	1
357	PAK-53363	450955	Iran	RPIP 12-071-03099	3	1	134	4	215	3	2	1
358	PAK-53364	450965	Iran	RPIP 12-071-03151	3	1	134	4	215	3	1	1
359	PAK-53365	450975	Iran	RPIP 12-071-03249	2	1	134	4	215	3	2	1
360	PAK-53366	450977	Iran	RPIP 12-071-03258	2	1	134	4	215	3	1	1
361	PAK-52918		Pakistan		1	1	134	4	215	3	2	0
362	PAK-52919		Pakistan		1	1	140	4	215	3	2	0
363	PAK-52921		Pakistan		1	0	140	4	215	3	2	0
364	PAK-52922		Pakistan		1	1	148	4	215	3	2	0
365	PAK-52924		Pakistan		1	0	148	4	215	3	2	0
366	PAK-52925		Pakistan		1	0	153	4	215	3	2	0
367	PAK-52926		Pakistan		1	0	136	5	215	3	2	0
368	PAK-52927		Pakistan		1	1	137	5	215	3	2	0
369	PAK-52928		Pakistan		1	1	138	5	215	3	2	0
370	PAK-52929		Pakistan		1	1	138	5	215	3	2	0
371	PAK-52930		Pakistan		1	1	139	5	215	3	1	0
372	PAK-52931		Pakistan		2	1	139	5	215	3	1	0
373	PAK-52932		Pakistan		1	1	140	5	215	3	1	0
374	PAK-52933		Pakistan		2	1	140	5	215	3	2	0
375	PAK-52934		Pakistan		1	1	140	5	215	3	2	0

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376	PAK-52935	Pakistan	1	1	140	5	215	3	1	0
377	PAK-52937	Pakistan	1	1	127	4	216	3	2	0
378	PAK-52938	Pakistan	2	1	127	4	216	3	2	0
379	PAK-52939	Pakistan	1	0	127	4	216	3	1	0
380	PAK-52941	Pakistan	1	0	127	4	216	3	2	0
381	PAK-52942	Pakistan	2	1	128	4	216	3	1	0
382	PAK-52943	Pakistan	1	1	128	4	216	3	2	0
383	PAK-52944	Pakistan	2	1	128	4	216	3	2	0
384	PAK-52945	Pakistan	1	1	128	4	216	3	1	0
385	PAK-52946	Pakistan	1	0	130	4	216	3	2	0
386	PAK-52947	Pakistan	1	0	130	4	216	3	2	0
387	PAK-52948	Pakistan	1	0	130	4	216	3	1	0
388	PAK-52949	Pakistan	1	0	131	4	216	3	1	0
389	PAK-52950	Pakistan	1	1	131	4	216	3	1	0
390	PAK-52951	Pakistan	1	0	132	4	216	3	1	0
391	PAK-52952	Pakistan	1	0	134	4	216	3	1	0
392	PAK-52953	Pakistan	1	0	143	4	216	3	1	0
393	PAK-52954	Pakistan	1	1	143	4	216	3	1	0
394	PAK-52955	Pakistan	1	1	143	4	216	3	1	0
395	PAK-52956	Pakistan	2	0	132	5	216	3	2	0
396	PAK-52957	Pakistan	2	0	127	4	217	3	1	0
397	PAK-52958	Pakistan	1	1	127	4	217	3	2	0
398	PAK-52959	Pakistan	1	1	127	4	217	3	2	0
399	PAK-52960	Pakistan	1	1	139	4	217	3	1	0
400	PAK-52961	Pakistan	2	0	139	4	217	3	2	0

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401	PAK-52962	Pakistan	2	0	134	5	217	3	2	0
402	PAK-52963	Pakistan	1	1	134	5	217	3	1	0
403	PAK-52964	Pakistan	1	1	134	5	217	3	1	0
404	PAK-52965	Pakistan	2	1	134	5	217	3	2	0
405	PAK-52966	Pakistan	2	1	144	5	217	3	2	0
406	PAK-52967	Pakistan	1	1	146	5	217	3	2	0
407	PAK-52968	Pakistan	1	1	147	5	217	3	2	0
408	PAK-52969	Pakistan	2	1	141	4	218	3	1	0
409	PAK-52970	Pakistan	1	1	142	4	218	3	2	0
410	PAK-52971	Pakistan	2	1	142	4	218	3	1	0
411	PAK-52972	Pakistan	1	1	143	4	218	3	1	0
412	PAK-52973	Pakistan	3	1	130	5	218	3	1	0
413	PAK-52974	Pakistan	2	1	130	5	218	3	1	0
414	PAK-52975	Pakistan	2	1	130	5	218	3	1	0
415	PAK-52978	Pakistan	1	1	144	4	220	3	1	0
416	PAK-52979	Pakistan	1	1	145	4	220	3	1	0
417	PAK-52980	Pakistan	2	1	145	4	220	3	1	0
418	PAK-52981	Pakistan	1	1	145	4	220	3	2	0
419	PAK-52983	Pakistan	1	1	145	4	220	3	1	0
420	PAK-52984	Pakistan	1	1	148	4	220	3	1	0
421	PAIDAR-91	Pakistan	1	1	155	6	221	3	1	0
422	NOOR-91	Pakistan	1	1	130	4	223	3	1	0
423	C-44	Pakistan	1	1	127	3	224	3	1	0

GH: growth habit, i.e., (1-erect, 2-semi-erect, 3-spreading)
ID: iron deficiency, i.e., (0-tolerant, 1-susceptle)
DF: days to flowering, i.e., (from sowing to the stage when 50% of plants have begun to flower)
FC: flower colour, i.e., (1-Blue, 2-light blue; 3-Dark pink; 4-pink; 5-Light pink; 7-White, pink striped)
DM: days to maturity, i.e., (from sowing to the stage when all plants have mature pods)
PHr: plant hairiness, i.e., (1-no hairs; 2-less pubescence; 3-dense pubescence)
PP: plant pigmentation, i.e., (1-no anthocyanin, stems and leaves pale green; 2-no anthocyanin, stems and leaves green; 3-weak anthocyanin, stem and leaves partly light purple; 4-strong anthocyanin, stems and leaves predominently purple)
Blight 1-tolerant; 2-intermediate; 3-susceptle.