GENETIC DIVERGENCE ANALYSIS IN LENTIL (*Lens culinaris* Medik.)

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CERTIFICATE

This is to certify that the thesis entitled ***"GENETIC DIVERGENCE ANALYSIS IN LENTIL (Lens culinaris Medik)"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in GENETICS AND PLANT BREEDING,*** *embodies the result of a piece of bona fide research work carried out by* ***Syed Abu Siam Zulquarnine****, Roll No. 00281, Registration No. 25134/00281 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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ABSTRACT

BY

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A field experiment was conducted with 60 lentil genotypes at Sher-e-Bangla Agricultural University experimental Farm, Sher-e-Bangla Nagar, Dhaka, to study their diversity based on different morphological characteristics during November 2005 to March 2006. Different multivariate analysis techniques were used to classify 60 lentil genotypes. Diversity was estimated by cluster distance. All the genotypes were grouped into six clusters. Principal Component Analysis, Cluster Analysis and Canonical Variate Analysis exhibited similar results. Significant variations were observed among the lentil genotypes for all the parameters under study. Cluster V had the maximum (22) and cluster I had the minimum (6) number of genotypes. The highest intra-cluster distance was observed in cluster IV followed by II. The highest inter-cluster distance was observed between cluster II and VI and the lowest inter-cluster distance was found between the clusters IV and I. Plant height and dry matter weight contributed maximum towards divergence among the lentil genotypes. Genetic divergence related to geographical diversity was not observed. The genotypes which had moderate inter-cluster distance coupled with medium to high yield could be utilized for screening suitable materials from large population. Considering diversity pattern, genetic status and other agronomic performances some of the materials viz. BD 5969 & BD 5983 from cluster I; BD 5988, BD 4085 & BD 4091 from cluster II; BD 5991 & BD 5980 from cluster III; BD 3861, BD 4103 & BD 4110 from cluster IV; BD 4074, BD 3853, BD 5961 & BD 5973 from cluster V and BD 5977, BD 5958, BD 5967, BD 5990, BD 5970, BD 5981 & BD 5966 from cluster VI, could be used as superior parents for lentil improvement programme.

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SOME COMMONLY USED ABBREVIATIONS

Abbreviations		Full Word
AEZ	=	Agro- Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BARI	=	Bangladesh Agricultural Research Institute
FAO	=	Food and Agricultural Organization
DAS	=	Days After Sowing
SAU	=	Sher-e- Bangla Agricultural University
HI	=	Harvest Index
%	=	Percent
g	=	gram (s)
kg	=	kilogram (s)
DM	=	Dry Matter
cv.	=	Cultivar (s)
t/ha	=	Tonnes per hectare
CV %	=	Percentage of Coefficient of Variation
hr	=	Hour (s)
ppm	=	Parts per million
^{0} C	=	Degree Celsius
m^2	=	meter square
NS	=	Non significant
cm	=	Centi-meter
No.	=	Number
var.	=	Variety
et al.	=	And others
etc.	=	Etcetera
RCBD	=	Randomized Complete Block Design
m	=	Metre
G	=	Genotype
GN.	=	Genotype Number
BD	=	Bangladesh
	=	Ministry of Agriculture

MOA		
df	=	Degrees of Freedom
SE	=	Standard Error
CEC	=	Cation Exchange Capacity
ppm	=	Parts per million
Univ.	=	University
J.	=	Journal
Sci.	=	Science
Agric.	=	Agriculture
Agron.	=	Agronomy
Agril.	=	Agricultural
Res.	=	Research
ICARDA	=	International Centre for Agricultural Research in Dry Areas
BSMRAU	=	Bangabandhu Sheikh Mujibur Rahman Agricultural University
IARI	=	Indian Agricultural Research Institute
NARS	=	National Agricultural Research Institute

Chapter 1
INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the major legume crops in Bangladesh, which ranks third among the lentil growing countries of Asia Pacific region. It is the second most important pulse crop in area and production, but stands first in the consumer's preference in this country. In 2005-2006 it was grown on about 134,642 ha of land producing 115,370 tonnes of grain, with an average yield of 857 kg ha^{-1} and contributes about 33% to the total pulses production (BBS, 2006). In the humid tropical countries including Bangladesh, leguminous food crops are of special significance because of the low protein content of the major food crops such as cereals and animal protein (Miah, 1976).

In Bangladesh its cultivation is mostly concentrated in the Gangetic flood plain of western part of the country. Lentil is cultivated during winter (rabi or post rainy season; November-March). Domestic pulse production satisfies less than half of the country's needs. The rest, near about 140,000 tonnes, need to import at a cost of about US$ 32.2 million per annum. The resulting high prices have led to widespread protein malnutrition especially among vulnerable groups, such as rural children and the aged.

L. culinaris, the only cultivated species (Sindhue and Shinkard, 1985), is further divided into two major groups: microsperma with small seeds and macrosperma with bold seeds. In Bangladesh all the indigenous landraces and varieties are microsperma with orange cotyledons, whereas the exotic macrosperma varieties posses both yellow and orange cotyledons.

Lentil plays an important role in the agro-economy and national health of Bangladesh. Nutritionally, lentil is very rich and complementary to any cereal crops including rice. It supplies about four times as much protein and eight times as much riboflavin as does rice; the caloric value of it is

equal to rice (Anonymous, 1966). Moreover, it is known as poor man's meat. It is a versatile source of nutrients for man, animal and soil (Miah, 1976). After analyzing 1985 germplasm lines Erskine and Witcombe (1984), reported a mean seed protein content of 25.78%. Lentil also contains 59% carbohydrate, 0.5% fats, 2.1% minerals (Gowda and Kaul, 1982). Sufficient amount of vitamins viz. vitamin A 16 IU; thiamine 0.23 mg and vitamin C 2.5 mg (Anonymous, 1976) are available from a gram of lentil. Because of its high lysine contents, the most limiting amino acid in several cereals, lentil can form a balanced diet when supplemented with cereals (Abu-Shakra and Tannous, 1981).

In spite of so many advantages, lentil in Bangladesh is generally grown under minimum fertility and management practices. The development of high potential genotypes with good, stable yield and higher protein content is important to improve yield status of the crop. The average yield of lentil in Bangladesh is gradually declining. Several factors are responsible for low yield of lentil, such as, less attention on cultural practices, little use of fertilizers, lack of pest control measures, postharvest losses, over and above, the use of traditional varieties or landraces with low genetic potential and instability of yield. The existing varieties in Bangladesh are mostly poor yielding. The development of high yielding and high protein containing lines with other desirable characters is badly needed to improve the yield status of this crop. The research work in this direction is only limited and fragmentary in Bangladesh. More work is needed for making a tangible improvement of this crop. Reportedly, an extensive genetic erosion of lentil occurred in Bangladesh as elsewhere in the world and the need for influx of exotic germplasms into the country has been stressed (Mia *et al*., 1986).

In crop improvement programme, genetic diversity has been considered as an important factor, essential to meet the diverse goals in plant breeding such as producing cultivars with increased yield, (Joshi and

Dhawan, 1966) wider adaptation, desirable quality and pest resistance (Nevo *et al*., 1982). Diversified genotypes are also a pre-requisite for hybridization programme to develop desirable genotypes.

Information on genetic divergence among the plant materials is vital to a plant breeder for an efficient choice of parents for hybridization. It is an established fact that genetically diverse parents are likely to contribute desirable segregates and/or to produce high heterotic crosses. More diverse the parents, greater are the chances of obtaining high heterotic F_1s and broad spectrum of variability in segregating generations (Arunachalam, 1981). The parents identified on the basis of divergence analysis would be more promising in selecting genotypes with desirable character combinations from the segregating generations obtained through hybridization. Furthermore, genetic divergence as a function of heterosis, is one of the criteria of parent selection. Therefore, the availability of transgressive segregants in any breeding programme depends upon the divergence of test parents. Precise information on the nature and degree of genetic divergence of the parents is the prerequisite of an effective breeding programme.

The quantification of genetic diversity through biometrical procedures (Anderson, 1957; Rao, 1952) has made it possible to choose genetically diverged parents for a successful breeding programme. The importance of genetic diversity in the improvement of a crop has been stressed in both self and cross-pollinated crops (Griffing and Lindstrom, 1954; Murty and Anad, 1966; Gaur *et al*., 1978). Moreover, evaluation of genetic diversity is important to know the sources of genes for a particular trait within the available germplasm (Tomooka, 1991).

In Bangladesh, information on genetic diversity in lentil germplasm is scanty. Therefore, the present investigation is undertaken with the following objectives:

(a) To estimate the nature and magnitude of genetic divergence among the lentil genotypes.

(b) To identify the most divergent parents or genotypes for further breeding programme.

(c) To find out the different gene pool or clustering pattern among the material.

(d) To find out the relationship of genetic diversity with their geographic or ecological background.

Chapter 2

REVIEW OF LITERATURE

2.1 Origin and distribution

The lentil was grown from early times throughout the eastern Mediterranean region as well as in the Nile Valley. Today, it is cultivated throughout the world (Aykroyt and Doughty, 1964). The mountainous region between Hindukush and Himalayas was suggested earlier as the centre of origin but evidence acquired later supported the Near Eastern origin (Zohary, 1972). On the basis of examination and evaluation of archaeological remains and on the identification of the world progenitors and delimitation of their geographic distribution Zohary and Hopt (1973), concluded that pea and lentil should be regarded as founder crops of old world Neolithic agriculture; they were domesticated in the Near East, simultaneously with wheat and barley. Lentil used by the ancient-dwellers and is thought to be one of the earliest domesticated crops (Zohary and Hopt, 1973; Cubero, 1984). Archaeologically, lentil was established as one of the primary domesticant that founded the Neolithic agricultural revolution of wild species *L. orientalis* that is centered in the Near East. The geographic distribution of wild sp. and *L. orientalis* is centered in the Neolithic nuclear area of the Near East arc, i.e. northern Israel, Syria, South Turkey, North Iraq and Western Iran. Ladiginsky (1979) reported that lentil originated in Southern Turkey. Cubero (1984), reported that the region between Western Turkey and Kurdistan could be it's place of origin. According to Azad *et al*. (1991) lentil is thought to have originated in Asia Minor. It spreads quickly to Greece, central and Southern Europe, Egypt, Mediterranean, Afghanistan, Indian subcontinent and China. Lentil is now also cultivated in Argentina, Canada, Colombia, Mexico, Peru and the USA. It is a temperate crop, but is also cultivated in the subtropics during winter months and at high altitudes in the tropics during colder months.

2.2 Cytotaxonomy

Lentil is essentially a self-pollinated crop although natural cross-pollination occurs through insect (Poehlman and Borthakur, 1969). The crop belongs to the family Fabaceae (Leguminosae), sub-family Papilionaceae. Taub and tribe vicineae Bron (Barulina, 1980).

The *Lens* comprises five annual species of which only *L. culinaris* is cultivated (Sindhue and Slinkard, 1985). Lentil is diploid in nature, cytologically containing 7 pairs of chromosomes (2n=14). Previously lentil was included in the genus *Ervum*. In the year 1987 Medikus suggested the botanical name, *Lens culinaris,* for lentil. Moench called it *Lens esculentus* in 1978. Both the nomenclature can be found in the literature but the name given by Medikus is now internationally accepted and approved. Other important species under the genus *Lens* are: *Lens ervoids*, *Lens montbretti*, *Lens nigricans* and *Lens orientalis.*

2.3 Genetic Divergence

Genetic divergence means the nature and degree of variability existing among the genotypes under studies, which is measured by range, mean, standard deviation, variance, standard error, coefficient of variation, etc.

Genetic divergence analysis used to identify specific parents for realizing heterosis and recombination in breeding programme. Several workers have followed the technique of Mahalanobis D^2 statistics on wide range of crops spices to measure the genetic distance among the breeding materials and to identify the character(s) responsible for such type of divergence.

The utility of multivariate analysis for measuring the degree of divergence and for assessing the relative contribution of different characters to the total divergence in self-pollinated crops has been established by several workers (Golakia and Makne, 1992; Natarajan *et al*., 1988; Das and Gupta, 1984; Sindhu *et al*., 1989).

Genetic diversity analysis is mainly based on multivariate techniques. During last decade different multivariate techniques are developed through the development of computer programme. However, literature related to efficient multivariate techniques for diversity analysis are reviewed in the following paragraphs.

Lentil (*Lens culinaris* M.) is one of the most important pulse crops under the family Leguminosae & sub-family Papilionaceae grown in both tropical and arid regions of the world. Research effort on diversity analysis of lentil seems to be limited in world literature especially in Bangladesh. Therefore, information related to the diversity of lentil and some other self-pollinated oil and pulse crops available in the literature are reviewed in this section. Beside these, literatures pertaining to the efficient multivariate technique for diversity analysis are also reviewed.

Adhikari and Pandey (1983) by using D^2 analysis in chickpea reported that in native types seed per pod, pod per plant and in kabuli types primary branches per plant and 100 seed weight contributed maximum towards diversity. In addition to this, Angadi *et al.* (1979) through multivariate analysis in cowpea reported that the characters 100 seed weight and pod length contributed maximum to the genetic diversity.

Agrawal and Lal (1985) evaluated 500 lentil accessions and reported substantial variations for time to flowering, time to maturity, plant height, 100-seed weight and seed yield. On the other hand, Katiar and Singh (1979) observed in chickpea that 250-grain weight and primary branches per plant contributed major portion of the total genetic diversity.

An investigation was carried out for the divergence in eight genotypes of mungbean and their 15 hybrids by Natarajan and Palanisamy (1990). They utilized generalized distance and canonical analysis and found five clusters. The canonical analysis confirmed to a large extent the clustering pattern obtained by multivariate analysis.

Analyzing the data on pod yield/ plant and 12 related traits, using the Mahalanobis`s D^2 statistic, Reddy *et al.* (1987) found that 20 germplasms of groundnut, investigated for two years divided into six clusters in both the years. They also observed that genetic diversity was not related to geographical distribution.

Badigannavar *et al.* (2002) studied on genetic base and diversity in groundnut and reported that cluster analysis of groundnut indicated no relationship between clustering pattern and subspecies among genotypes during rainy or summer seasons. Despite this narrow base, greater diversity could be possible following judicious use of mutation and recombination breeding to bring about genetic improvement.

Bartual *et al.* (1985) grouped 125 soybean genotypes by PCA, where maximum likelihood factor analysis and cluster analysis were based on morphological and physiological characters. The identified groups were quite stable in their performance through change in environments. Some genotypes were identified as parents for future use.

Chowdhury *et al*. (1998) observed D^2 analysis of yield components of 30 groundnut genotypes classified them into 5 clusters. Cluster III had the maximum (10) and cluster V had the minimum (1) number of genotypes. Maximum inter cluster distance was observed between cluster I and V. Metroglyph analysis with a few exception, showed similar types of clustering patterns. In 1996, Varman and Raveendran also studied genetic diversity in groundnut cross combinations and grouped them into 5 clusters. Cluster V recorded the highest values for 100-pod weight, 100 kernel weight, pod yield and oil content. Cluster IV recorded the highest values for maturing index and recovery percentage.

Dixit (1980) in the investigation in lentil observed that primary branches per plant and yield per plant contributed a large to the total genetic diversity. In the same crop Sharma and Luthra reported that pods per plant, seeds per plant and yield per plant contributed maximum towards diversity in 1987.

Genetic divergences were studied by Malik *et al.* (1985) in mungbean. They observed days to flowering, seed yield and plant height-contributed maximum towards divergence. However, genetic diversity in blackgram was studied by Das and Gupta (1984). They observed 100-grain weight and branches per plant were the main components of diversity. Sagar *et al.* studied the same experiment in 1976 through Mahalanobis`s D^2 in blackgram and found days to flowering, plant height, 100 seed weight and pod length contributed maximum towards diversity.

Godshalk and Timothy (1988) in their study reported comparisons of index selection with principal component analysis, principal factor analysis, and maximum likelihood factor analysis. Multivariate analysis was accomplished on both simple and genotypic correlation matrix for three sets of characters (5 characters per set) in Switch grass (*Panicum virgatum*). Comparisons were made by computing Spearman's rank correlations between selection index plant scores computed from multivariate analysis and by determining the number of plants selected in common for the selection methods. Among the multivariate analysis method PCA had the highest correlation with the index selection. They also suggested that PCA is more economic than the other analysis.

Golakia and Makne (1992) investigated diversity in 35 genotypes of Virginia runner groundnut using Mahalanobis`s D^2 statistic. These genotypes were grouped into seven clusters, but there was no relationship between genetic and geographical diversity.

Golakiya and Makne (1991) and Nadaf *et al.* (1986) found that grouping of genotypes in indifferent clusters were not related to their geographical origin. It was indicated that the geographical isolation might not be the only factor for genetic diversity. The same authors (1992) found that the genotypes of common geographic origin or same location were grouped into different clusters that suggested lack of relationship between genetic and geographic

diversity. In 1991, Katule *et al*. suggested that geographic diversity was not related to genetic diversity. Reddy *et al.* (1987) also found similar result.

Golakiya and Makne (1991) carried out divergence analysis and revealed that the 23 genotypes of groundnut were grouped into six clusters. Same authors (1992) analyzed genetic diversity with 27 varieties of groundnut over two years and divided them into 6 clusters in both the years. Katule *et al.* (1991) studied genetic divergence among eighteen geographically diverse genotypes of semi-spreading groundnut and reported eight different groups of clusters.

In 60 early maturing genotypes of pigeon pea, Murty and Dorairaj (1990) studied genetic diversity through D^2 and canonical analysis from different origin. The genotypes were grouped into three clusters. Genetic diversity was found independent of genotypic origin also.

In cowpea, days to flowering, maturity, pod length, pod girth and 100 grain weight contributed considerably towards diversity reported by Kumar *et al.* (1982). On the contrary, in pigeon pea, Bainiwal and Jatastra (1980) observed through D^2 analysis that plant height, pod length, and days to flowering were the principal component of diversity.

Islam *et al*. (1995) studied genetic divergence among 90 genotypes of groundnut using D^2 and principal components analysis and grouped the varieties into 5 clusters. The inter-cluster distances were larger than the intra cluster distance suggesting wider genetic diversity among the genotypes of different groups. The intra-cluster value was maximum in cluster IV and minimum in cluster III. Cluster III showed the lowest mean values for days to first flowering, days to fifty percent flowering, days to maturity, primary branches per plant and highest shelling percentage, while cluster IV revealed the highest mean values for days to first flowering, days to 50 percent flowering, days to maturity and branches per plant. Germplasms much in use of the above mentioned four characters both in cluster III and IV would offer a good scope of improvement of the crop through rational selection.

Joel and Mylsamy (1998) studied Mahalanobis D^2 statistics to assess the genetic diversity of 26-groundnut genotype of diverse origin and to find out best parents for pod yield and rust resistance breeding. The genotypes were grouped into 3 clusters. Cluster I had the maximum of 22 genotypes, while cluster II and III had 3 and 1 respectively. It is suggested that the genotypes from cluster I and III may be utilized in crossing to create a wide spectrum of variability and to select from segregants with high pod yield with rust resistance.

Juned *et al.* (1988) investigated genetic diversity in 22 accessions of wild potato from Paraguay and Argentina. They observed a close relationship between the geographical groups using Principal Component Analysis (PCA), Cluster analysis and genetic diversity.

Katiar and Singh (1990) investigated the genetic diversity of 40 indigenous and exotic strains of fababean *(Vicia faba L*) using Mahalanobis's D^2 statistic. The strains were grouped into 12 different clusters. They found no direct association between geographic distribution and genetic divergence.

Malik *et al*. (1984) in an evaluation-cum-observation trial with 55 lentil accessions collected from Sind and Panjab province of Pakistan, found that the time to flowering varied from 117-150 days with mean value of 124.3 days; time to maturity varied from 130-165 days with a mean of 151.3 days; plant height ranged from 29.0-45.5 cm and the mean was 35.6 days. Pod/ plant and yield/ plant ranged between 22-154.8 and 0.48-3.95g with the coefficients of variation 47.3% and 45.2%, respectively. Variability for these traits in lentil germplasm was also reported by Tiwari and Singh (1980).

Mishra and Rao (1990) reported that metroglyph analysis did not show similar type of clustering pattern as observed in D^2 analysis carried out in a comparative study of D^2 and metroglyph analysis with 117 genotypes of chickpea. Similarly, Kotaiah *et al*. (1986) compared the Mahalanobis D^2 and metroglyph analysis in 26 genotypes of groundnut and observed deviation between D^2 and metroglyph method regarding the number of clusters formed

and number of genotypes in the clusters. It was suggested that the metroglyph analysis would be suitable for preliminary grouping before taking up D^2 analysis.

Muchlbauer (1974) conducted an experiment to findout the variability and association of characters in 45 lentil cultivars and found the greater variability in three characters viz. yield (kg/ha), seeds/plant and pods/plant with the standard high variation (31.37%) was found for yield/plant and number of pods/plant (23.88%). Todorov (1980) found in his study that plant height, number of pods/plant, seeds/ plant, seed weight/ plant and pod length has got greater variation among the 35 lines and 18 initial populations.

Multivariate analysis using Mahalanobis D^2 statistic was used to group 83 genotypes from 18 countries on the basis of yield/plot and six other agronomic characters of bunch groundnut by Nadaf *et al.* (1986). They found nine clusters, which were not related to the grouping formed by geographical origin. They also observed that variation in pod yield accounted for 88% of the total variation between clusters but number of developed pods. Days to 50% flowering and 100 seed weight were important in accounting for divergence with clusters.

Natarajan *et al*. (1988) studied genetic association and diversity using D^2 analysis among 45 genotypes of diverse origin of green gram. 45 genotypes were grouped in to four clusters. They reported that, in selected materials seed weight contributed maximum followed by days to flower towards the genetic divergence.

Payne *et al.* (1989) reported that the hierarchical nature of the grouping into various number of classes can impose undue constrains and the statistical properties of the resulting groups are not at all clear. Therefore, they have suggested non-hierarchical classification, as an alternative approach to optimize some suitabilities choosing criteria directly from the data matrix. They also reported that the squared distance between means are Mahalanobis's

D^2 statistics when all the dimensions are used can be computed using Principal Coordinate Analysis (PCO). They also recommended the Canonical Variate Analysis (CVA) for discriminatory purposes.

Pod length and 100 seed weight contributed maximum towards divergence in mungbean reported by Gupta and Singh (1970). Whereas Ramanujam *et al.* (1974) investigated diversity in mungbean using D^2 and found flowering time, maturity, seed density and 100 seed weight contributed considerably.

Reddy and Reddy (1993) reported on forty-eight genotypes of groundnut, which were grouped into 11 clusters. Cluster I was the largest with 23 genotypes followed by cluster V1 and III with 9 and 7 genotypes respectively. Genetic diversity indicated that 100 pod weight (36%) number of branches /plant (31%) and harvest index (15%) accounted for more than 80% of the total divergence. These 3 characters may be considered in future breeding programme.

Reddy *et al.* (1986) analyzed the data on pod yield and 12 related traits using Mahalanobis D^2 statistic in 20-groundnut genotypes for two years. He reported that genetic diversity was not related to geographical distribution of the varieties. The greater inter cluster distance, occurred between clusters I (with 10-11 varieties) and II (with 4-6 varieties) and between clusters I and IV (1 variety), depending on year.

Sangha and Sandhu (1973) studied twenty spreading groundnut varieties from diverse sources in respect of secondary branches, number of pods, pod yield and 100 kernel weights. Highly significant differences were observed among the varieties, when tested by multivariate dispersion analysis. The varieties were grouped into six and spatial pattern of groups was not corresponding to geographical diversity.

Shahi *et al*. (1986), from a study involving 57 accessions of lentil germlasm from different parts of Madhya Prodesh, India, reported that wide

range of variability for seed size with the range 1.4-3.4 g/100-seed (mean 2.4), seed permeability 5.0-55.8 (mean 26.4%) as well as for germination, 44.2-89. 46 (mean 72.9%).

Shanmugam and Rangasamy (1982) observed that the characters yield per plant and pod cluster per plant contributed considerably towards diversity in black gram. Again the same authors in 1982 assigned 45 genotypes of blackgram to ten clusters by analyzing data on yield and nine yield components using Mahalanobis`s D^2 statistic and stated that geographical diversity was not the only factor for determining genetic diversity. The clustering pattern more or less confirmed the canonical (vector) analysis. They found that yield per plant contributed most to genetic divergence. Furthermore, Sindhu *et al.* (1989), investigated diversity in 20 strains of blackgram from different agro-ecological zones of India using Mahalanobis's D2 statistic. They observed no parallelism between geographical and genetic diversity.

Singh and Singh (1969), in a study comprising 20 indigenous and 20 exotic lines of lentil; found that pod number, bunch numbers and days to flowering had high variability. They also observed that the characters, which had high phonotypic variability, also exhibited high genotypic variability and wide ranges. Number of bunches and number of pods had very wide ranges and also had very high phenotypic variability. Practically exotic lines had very small number of bunches and pods per plant whereas the indigenous lines had very high no. of bunches and pods, and these wide differences accounted for larger phenotypic variability.

Singh and Singh (1989) studied the genetic diversity and stability in chickpea entries. They suggested crossing among the 14 selected genotypes on the basis of intra/inter cluster distances to recombine the genes for stability and high yield.

Swarup and Lal (1987) evaluated 28 high yielding and bold seeded (22.5g/100 seed) for time to 50 flowering, time to maturity, plant height, and 100-seed weight at Sehore, India. They observed the time to 50% flowering raged from 55-69

days, time to maturity ranged from 113 to 134 days after sowing in SL-904 and SL- 397 respectively. Plant height varied from 28.7 cm (SL 945) to 33.9 cm (SL 598) and 100-seed weight from 2.90 g (SL 666) to 4.30g (SL 143).

Teng and Hor (1994) reported the analysis of 15 agronomic characters in 35 groundnut varieties that were divided into 6 clusters of different genetic divergences. Little variation was found within clusters but large differences were observed between clusters. It was suggested that single plant productive capacity, quality and quantity of branches, and shelling percentage were the primary characters influencing yield.

The clustering and ordination methods used often cannot deal explicitly with the computational consequences of large data sets with incomplete information. However, it is shown that the ordination technique of principal component analysis and the mixture maximum likelihood method of clustering can be employed to achieve such analysis (Harch *et al*., 1999). Genotypes within the cluster are having a smaller D^2 value among themselves than those from group belonging to two different clusters. On the other hand the inter cluster distance is the criterion used for selecting genotypes as parent for hybridization. The genotypes those in clusters with maximum inter cluster distance are genetically more divergent. Variation within the cluster is measured by inter cluster distance. The inter and intra cluster values (D) of groundnut were reported to be ranged from 9.50 to 22.20 and 5.18 to 8.45 (Katule *et al.*, 1991), 3.84 to 7.35 and 4.24 to 4.81 (Golakiya and Makne, 1991) and 4.95 to 7.09 and 3.61 to 4.51 (Golakiya and Makne, 1992).

The coordinates obtained from the PCA are used as input of PCO analysis to calculate distances among the points reported by Digby *et al.* (1989). PCA is used for graphical representation of the points while PCO is used to calculate the minimum distance in a straight line between each pair of points.

The genetic divergence among 7 parents and their 12 hybrids of cowpea were studied by Thiagarajan *et al.* (1988) using Mohalanobis's D^2 statistics.

They observed that the characters namely 50% flowering, 100-grain weight and plant height contribute maximum toward genetic divergence. Similar reports were made by Ramanujam *et al*. (1974) in the study of 10 parents and their 25 F_1s in mugbean.

The genetic diversity of 40 newly developed soybean lines and ten parents were studied by Singh and Ram (1985). The cultivars were grouped into nine clusters by D^2 analysis. They observed lines originating from one or related crosses tended to be included in the same cluster and potential crosses based on inter cluster distance.

The range of variability was studied in some ICARDA collections grown at Tel Hadya, Syria during 1978-79 seasons (Solh and Erskine, 1984). They observed that the range of 100-seed weight (g), Crude protein %, time to maturity, plant height (cm), lowest pod height (cm) and pod number per peduncle were reported as being 1.1-3.6, 20.6-35.6, 154-197, 10-45, 6-30 and 1.0-1.7 respectively, with the corresponding mean value of 3.2, 28.1, 170.3, 25.5, 14.1 and 1.1.

Thinking about magnitude of genetic variability for yield and its component characters has been of considerable interest to the plant breeders for planning and execution of genetic improvement programme. A large number of such investigations have been carried out in different crops including Lentil (Malhotra *et al*., 1974), Groundnut (Reddy *et al.*, 1987), Soybean (Singh and Ram, 1985, Mishra *et al*., 1987, Broich and Palmer, 1980), Black gram (Singh *et al.*, 1973; Das, 1978; Singh and Mishra, 1983), Mungbean (Gupta and Singh, 1969; Yohe and Poehlman, 1972; Malik *et al*., 1983, Chickpea, (Chandra, 1968; Dumber and Deshmukh, 1983), pigeon pea (Heermath and Talwar, 1971; Dumbre and Deshmukh, 1983) and Pea (Singh *et al*., 1973; Singh, 1985). All these studies were on the basis of simple analysis of variance, which enabled to compute genetic variance for different characters. But total genetic diversity among different natural populations of these crops could not be obtained, which is important from evolutionary and breeding point of view. Under these circumstances, multivariate analysis is of great importance.

Through Mahalanobis`s D^2 analysis in pea (*Pisum sativum* L.), Narshighani *et al.* (1978) found that seed size, plant height and days to maturity contributed a major portion to the total diversity whereas Ranalli (1982) found a major role of days to flowering. Moreover, Singh *et al.* (1976) reported that pod length, days to flowering and seed yield contribute maximum towards divergence in mungbean through D^2 analysis.

Two hundred and seventy lentil lines were evaluated by Sinha and Chowdhary (1984) at Bihar, India for different morphological and quantitative characteristics. Lines varied little from each other in growth habit, flower color and seed color. Enough variability was found providing scope for selection in quantitative characters such as plant height (cm), time to flowering (days), 100-seed weight (g) and seed yield (g) per meter row within the range of 20-25, 51-80, 1.02-2.66 and 7.2-71.5 respectively. Nandan and Pandey (1980) found the range of 100 seed weight within 1.52-3.62 g.

Using PCA Mian *et al.* (1991) studied the genetic divergence in 128 germplasms of pea. They reported that the whole population divided into 16 broad based groups and random distribution of genotypes in the clusters suggested no parallel relationship between genetic and geographical diversity in pea.

Working with two hundred early maturing exotic lentil lines Mia *et al.* (1986) found very low coefficient of variation for time to maturity (3.94%) with a mean value 122.3 days, time to flowering (9.65%) with a mean value 74.7 days, and plant height (109%) with a mean value 55.5 cm, but high for seed yield per plant (43.9%) and 1000 seed weight (29.02%) with mean value of 0.96 g and 22.8 g respectively.

Chapter 3

MATERIALS AND METHODS

Agricultural research uses a large number of procedures and techniques for successful conduction of field experiment. The techniques to be adopted depend on the nature of the research trial and its objectives. Success of field experiment largely depends on the appropriateness of establishment. This means how precisely different aspects of field plot techniques are considered and adopted to maximize non-treatment variations or errors.

3.1 Site of experiment

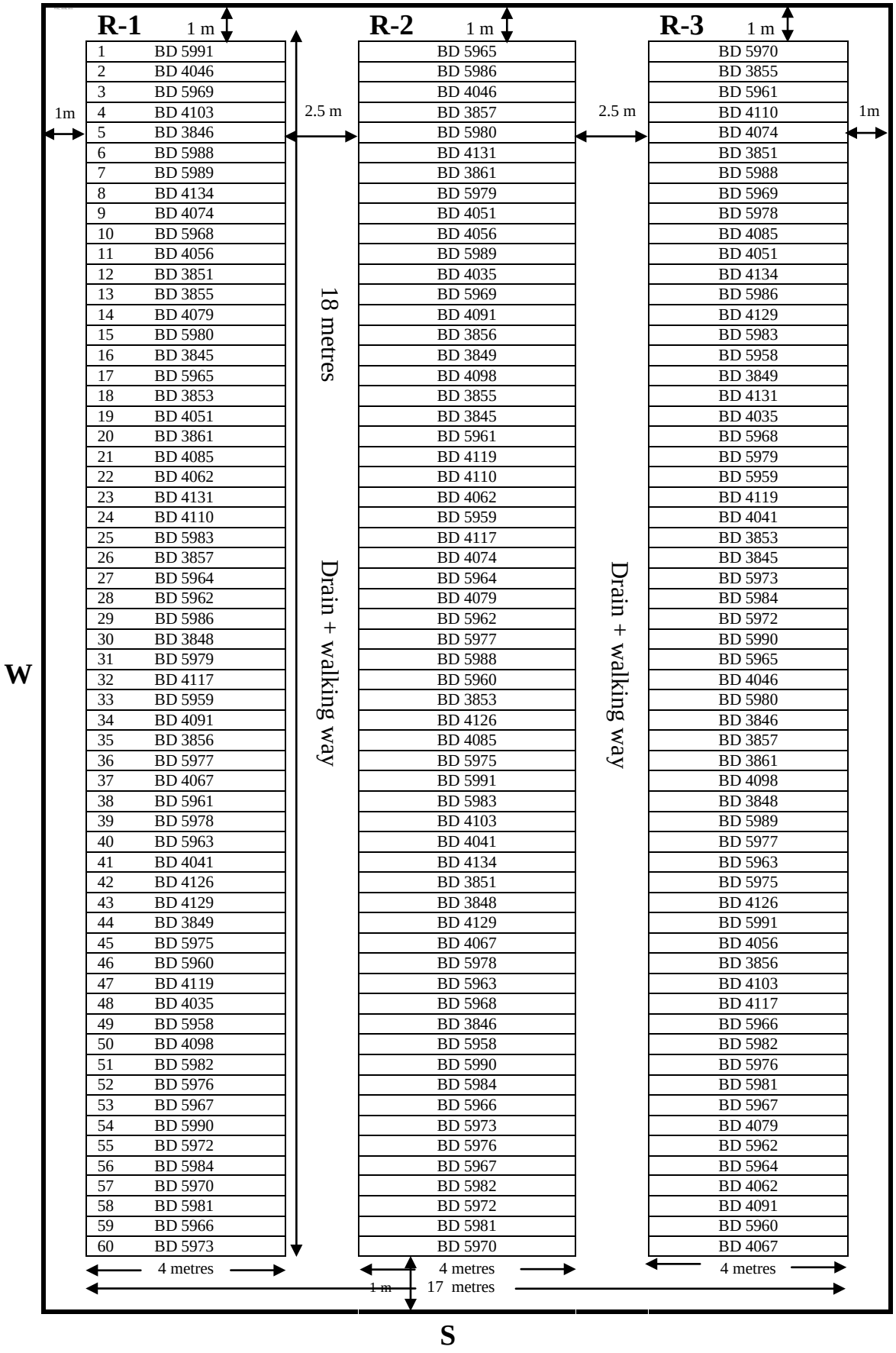
The experiment was conducted at the field laboratory of the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka during the period from November 2005 to March 2006. The experimental site was at $90^0$22" E longitude and $23^0$41" N latitude at an altitude of 8.6 meters above the sea level. The physical and chemical characteristics of the soil have been presented in Appendix III.

3.2 Materials

A total of sixty genotypes (60) of lentil (Table 1), originated from different places of Bangladesh were used in this experiment. The materials were collected from Jessore, Rajshahi, Noagaon, Nowabgonj, Kurigram and Genetic Resources Centre at BARI in Gazipur.

3.3 Soil and climate

The land belongs to Agro-ecological region of 'Madhupur Tract' (AEZ 28) of Nodda soil series. The soil was sandy loam in texture having pH 5.47-5.63. The mean temperature of the growing period was 24.36^o C with average maximum and minimum being 30.0^0 C and 18.67^0 C respectively. The monthly total rainfalls, average sunshine hour, temperature during the study period are shown in Appendix IV.



	R-1	R-2	R-3
1	BD 5991	BD 5965	BD 5970
2	BD 4046	BD 5986	BD 3855
3	BD 5969	BD 4046	BD 5961
4	BD 4103	BD 3857	BD 4110
5	BD 3846	BD 5980	BD 4074
6	BD 5988	BD 4131	BD 3851
7	BD 5989	BD 3861	BD 5988
8	BD 4134	BD 5979	BD 5969
9	BD 4074	BD 4051	BD 5978
10	BD 5968	BD 4056	BD 4085
11	BD 4056	BD 5989	BD 4051
12	BD 3851	BD 4035	BD 4134
13	BD 3855	BD 5969	BD 5986
14	BD 4079	BD 4091	BD 4129
15	BD 5980	BD 3856	BD 5983
16	BD 3845	BD 3849	BD 5958
17	BD 5965	BD 4098	BD 3849
18	BD 3853	BD 3855	BD 4131
19	BD 4051	BD 3845	BD 4035
20	BD 3861	BD 5961	BD 5968
21	BD 4085	BD 4119	BD 5979
22	BD 4062	BD 4110	BD 5959
23	BD 4131	BD 4062	BD 4119
24	BD 4110	BD 5959	BD 4041
25	BD 5983	BD 4117	BD 3853
26	BD 3857	BD 4074	BD 3845
27	BD 5964	BD 5964	BD 5973
28	BD 5962	BD 4079	BD 5984
29	BD 5986	BD 5962	BD 5972
30	BD 3848	BD 5977	BD 5990
31	BD 5979	BD 5988	BD 5965
32	BD 4117	BD 5960	BD 4046
33	BD 5959	BD 3853	BD 5980
34	BD 4091	BD 4126	BD 3846
35	BD 3856	BD 4085	BD 3857
36	BD 5977	BD 5975	BD 3861
37	BD 4067	BD 5991	BD 4098
38	BD 5961	BD 5983	BD 3848
39	BD 5978	BD 4103	BD 5989
40	BD 5963	BD 4041	BD 5977
41	BD 4041	BD 4134	BD 5963
42	BD 4126	BD 3851	BD 5975
43	BD 4129	BD 3848	BD 4126
44	BD 3849	BD 4129	BD 5991
45	BD 5975	BD 4067	BD 4056
46	BD 5960	BD 5978	BD 3856
47	BD 4119	BD 5963	BD 4103
48	BD 4035	BD 5968	BD 4117
49	BD 5958	BD 3846	BD 5966
50	BD 4098	BD 5958	BD 5982
51	BD 5982	BD 5990	BD 5976
52	BD 5976	BD 5984	BD 5981
53	BD 5967	BD 5966	BD 5967
54	BD 5990	BD 5973	BD 4079
55	BD 5972	BD 5976	BD 5962
56	BD 5984	BD 5967	BD 5964
57	BD 5970	BD 5982	BD 4062
58	BD 5981	BD 5972	BD 4091
59	BD 5966	BD 5981	BD 5960
60	BD 5973	BD 5970	BD 4067

Figure 1. Layout of the experimental land

R- Replication, m- metre, BD- Bangladesh, N- North, S- South, W- West and E- East.

Plate 1: The author is working at his experimental field

Plate 2: The overall view of the experimental field

Plate 3: Experimental field at maximum pod bearing stage

Plate 4: The best genotype (GN 54 - BD 5990) of the experiment based on yield performance

3.4 Experimental design and layout

The study was laid out in Randomized Complete Block Design (RCBD) with three (3) replications (Figure 1). The plant to plant distance was 10 cm and line to line distance was 30 cm. The total land size was 19m X 20m. There were three long plots measuring 4 metres width and 18 metres length. The plot to plot distance was 2.5 m. The genotypes were randomly distributed to each row within each line.

3.5 Land preparation

The experimental plot was prepared by ploughing with tractor followed by harrowing and laddering by cows. Weeds and stubbles were removed. Manures and fertilizers were applied as per the recommended dose before the final land preparation. Irrigation channels were made around each plot. The final land preparation was done on 14 November.

3.6 Manure and fertilizer

Due to its ability of nitrogen fixation from the atmosphere lentil require less nitrogen application. But for initial establishment of plant up to the stage of nodule formation a starter dose of 20-40-20 NPK respectively was applied.

In this study fertilizer was applied as per the recommendation of Bangladesh Agricultural Research Institute (BARI). The following doses of fertilizers and manures were applied to the plot for lentil cultivation.

Fertilizers/ Manures	**Dose (Kg)**	
	Applied in the plot	**Quantity/ha**
Urea	1.71	45
TSP	3.23	85
MP	1.33	35
Cow dung	Applied earlier	1.5 ton

Urea, TSP, MP and Gypsum were applied at the time of final land preparation. Cow dung was applied two weeks before sowing during the land preparation.

3.7 Sowing of seeds and intercultural operation

The seeds of 60 lentil genotypes were sown in the field on 15th November 2005. Intercultural practices were done uniformly for all the genotypes. Thinning was done 25 days after sowing and wedding was done twice-the first during thinning and the second after about two months of sowing.

3.8 Harvesting

Different genotypes matured at different times. The harvesting was completed by 11 March 2006. Ten plants from each plot were randomly selected to collect data and these were harvested by uprooting. Border plants were discarded to avoid border effect.

3.9 Recording of Experimental Data

Data on the following characters were recorded on individual plant basis from 10 randomly selected plants per genotypes in each replicate. Out of 12 characters, days to 50% flowering and days to maturity were recorded in the field condition and the data on the other characters were recorded in the field Laboratory after harvest.

3.9.1 Plant height: The height of plant from the ground level to tip of the plant was measured in centimeter as plant height.

3.9.2 Days to 50% flowering: Data on days to 50% flowering was recorded from the date of sowing to date when 50% of plants within a line had flowered.

3.9.3 Days to maturity: Data on days to maturity was recorded from date of sowing to date of pod maturity.

3.9.4 Pod per plant: The total number of pods in individual plants was recorded.

3.9.5 Branches per plant (primary and secondary): The total number of primary branches and secondary branches including the main stem was counted.

3.9.6 Yield pert plant: Weight of the total seeds from each of the sample plant was recorded in gram (g).

3.9.7 Harvest index: This was measured as the ratio of grain yield to the biomass or biological yield expressed as percentage.

3.9.8 Seed per pod: Total number of seed in each pod within the individual plants was counted.

3.9.9 Weight of 100 seed: One hundred clean sun dried seeds were randomly taken from each line and weighed in gram (g).

3.9.10 Dry matter weight: Sun dried plants were weighted by electrical balance, randomly counting ten plants from each of the line.

3.9.11 Seed per plant: Total number of seed in each plant was counted.

3.10 Analysis of data

Genetic diversity was estimated following Mahalanobis's (1936) generalized distance. Selection of parents in hybridization programme based on Mahalanobis's D^2 statistic is more reliable as requisite knowledge of parents in respect of a mass of characteristics is available prior to crossing**.** Statistical analysis such as Mahalanobis D^2 and Canonical Variate Analysis (CVA), which quantify the differences among several quantitative traits are efficient method of evaluating genetic diversity. Mean data of each quantitative character were subjected to both univariate and multivariate analysis. For univariate analysis of variance, analysis was done individually and least of significance was done by F- Test (Panse and Shukhatme, 1978). Mean, range, co-efficient of variation (CV) and correlation was estimated using MSTAT computer programme. Multivariate analysis viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CLU) and Canonical Variate Analysis (CVA) were done by using GENSTAT programme.

3.10.1 Principal Component Analysis (PCA)

Principal Component Analysis, one of the multivariate techniques, is used to examine the inter-relationships among several characters. It can be done from the sum of squares and products matrix for the characters. Thus PCA finds linear combinations of a set variety that maximize the variation contained within them; they are expressed by displaying most of the original variability in a smaller number of dimensions. Therefore, principal components were computed from the correlation matrix and genotype scores obtained for the first components (which has the property of accounting for maximum variance) and succeeding components with latent roots greater than unity (Jeger *et al*., 1983).

3.10.2 Principal Coordinate Analysis (PCO)

Principal coordinate analysis is equivalent to PCA but it is used to calculate inter unit distances. Through the use of all dimensions of P it gives the minimum distance between each pair of the N points using similarity matrix (Digby *et al*., 1989).

3.10.3 Clustering

To divide the genotypes of a data set into some number of mutually exclusive groups clustering was done using non- hierarchical classification. In GENSTAT, algorithm was used to search for optimal values of chosen criteria.

3.10.4 Canonical Variate Analysis (CVA)

Canonical Variate Analysis, complementary to D^2 statistic, is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes respectively and derived. Canonical Variate Analysis computed linear combination of original variability that maximized the ratio between ground and within group variations, thereby giving functions of the original variables that could be used to discriminate between the groups. Thus in this analysis, a series of orthogonal transformation was done sequentially for maximizing the ratio of the groups to within group variations.

Table 1. List of lentil genotypes with their sources and origin

Genotype No.	Name/Acc No. (BD)	Place of origin	Source
1.	BD 5991	Jibannagar, Chudanga	BARI
2.	BD 4046	Shibjganj, Nowabganj	BARI
3.	BD 5969	Bagherpara, Jessore	BARI
4.	BD 4103	Unknown	BARI
5.	BD 3846	Unknown	BARI
6.	BD 5988	Kaliganj, Jhenaidah	BARI
7.	BD 5989	Kaliganj, Jhenaidah	BARI
8.	BD 4134	Unknown	BARI
9.	BD 4074	Dhamoirhat, Noagaon	BARI
10.	BD 5968	Sadar, Jessore	BARI
11.	BD 4056	Bagha, Rajshahi	BARI
12.	BD 3851	Unknown	BARI
13.	BD 3855	Unknown	BARI
14.	BD 4079	Rajarhat, Kurigram	BARI
15.	BD 5980	Keshobpur, Jessore	BARI
16.	BD 3845	Unknown	BARI
17.	BD 5965	Sadar, Jessore	BARI
18.	BD 3853	Unknown	BARI
19.	BD 4051	Putia, Rajshahi	BARI
20.	BD 3861	Unknown	BARI
21.	BD 4085	Ulipur, Kurigram	BARI
22.	BD 4062	Puthia, Rajshahi	BARI
23.	BD 4131	Unknown	BARI
24.	BD 4110	Unknown	BARI
25.	BD 5983	Keshobpur, Jessore	BARI
26.	BD 3857	Unknown	BARI
27.	BD 5964	Sadar, Jessore	BARI
28.	BD 5962	Chougacha, Jessore	BARI
29.	BD 5986	Keshobpur, Jessore	BARI
30.	BD 3848	Unknown	BARI
31.	BD 5979	Kaloroa, Khulna	BARI
32.	BD 4117	Unknown	BARI
33.	BD 5959	Sadar, Jessore	BARI
34.	BD 4091	Rajarhat, Kurigram	BARI
35.	BD 3856	Unknown	BARI
36.	BD 5977	Sharsha, Jessore	BARI
37.	BD 4067	Sadar, Natore	BARI
38.	BD 5961	Sadar, Jessore	BARI
39.	BD 5978	Kalaroa, Khulna	BARI
40.	BD 5963	Kaliganj, Jhenaidah	BARI
41.	BD 4041	Shibjganj, Nowabganj	BARI
42.	BD 4126	Unknown	BARI
43.	BD 4129	Unknown	BARI
44.	BD 3849	Unknown	BARI
45.	BD 5975	Sharsha, Jessore	BARI
46.	BD 4035	Sadar, Jessore	BARI

Table 1. Cont'd.

Genotype No.	Name/Acc No. (BD)	Place of origin	Source
47.	BD 4119	Unknown	BARI
48.	BD 4035	Godagari, Rajshahi	BARI
49.	BD 5958	Sadar, Jessore	BARI
50.	BD 4098	Unknown	BARI
51.	BD 5982	Keshobpur, Jessore	BARI
52.	BD 5976	Sharsha, Jessore	BARI
53.	BD 5967	Sadar, Jessore	BARI
54.	BD 5990	Jibannagar, Chuadanga	BARI
55.	BD 5972	Bagherpara, Jessore	BARI
56.	BD 5984	Monirampur, Jessore	BARI
57.	BD 5970	Bagherpara, Jessore	BARI
58.	BD 5981	Keshobpur, Jessore	BARI
59.	BD 5966	Sadar, Jessore	BARI
60.	BD 5973	Bagherpara, Jessore	BARI

3.10.5 Computation of average intra-cluster distances

When the clusters are formed, the average intra-cluster distance for each cluster was calculated by taking possible D^2 values within the members of a cluster obtained from the principal Coordinate Analysis (PCO). The formula used was D^2/n, where D^2 is the sum of distances between all possible combinations (n) of the genotypes included in a cluster. The square root of the average D^2 values, represent the distance (D) within cluster.

3.10.6 Cluster diagram

Cluster diagram was drawn using the intra and inter cluster distance. It gives a brief idea of the pattern of diversity among the genotypes included in a cluster.

Chapter IV
RESULTS AND DISCUSSION

Diversity is the function of parent selection and also heterosis. The availability of transgressive segregants in a breeding programme depends upon the divergence of parents. Thus, the accurate information on the nature and degree of diversity of the parents is the pre-requisite of an effective breeding programme. The knowledge of genotypic variation within genotypes in relation to morphology, phenology and yield would help to screen better genotypes for hybridization programme. Therefore, to generate information in the degree of diversity, sixty lines of lentil were raised in the growing season of 2005-2006 at the field of Sher-e-Bangla Agricultural University, Dhaka. The data on days to 50% flowering, days to maturity, plant height (cm), dry matter weight (g), 100 seed weight (g), pod per plant, seed per pod, seed per plant, primary branches, secondary branches, yield per plant (g) and harvest index etc. were recorded, analyzed and presented in this chapter.

Genetic diversity was analyzed using GENSTAT software programme. Genetic diversity analysis involves several steps, i.e., estimation of distance between the varieties, clustering and analysis of inter-cluster distance. Therefore, more than one multivariate techniques were required to represent the results more clearly and it was obvious from the results of many researchers (Bashar, 2002; Uddin, 2001; Juned *et at.*, 1988 and Ario, 1987). In the analysis of genetic diversity in lentil multivariate techniques were used.

4.1 Construction of Scatter Diagram

Based on the values of principal component scores 2 and 1 obtained from the principal component analysis, a two dimensional (Z_1-Z_2) scatter diagram was constructed, using component score 1 as X-axis and component score 2 as Y-axis, which is presented in Figure 2. The positions of the genotypes in the scatter diagram were random, which indicated the considerable diversity among the genotypes. The scatter diagram gives a brief idea of the pattern of diversity among the genotypes included in a cluster. Some

distantly located genotypes of different clusters were the genotype number 54, 36, 8, 3, 24, 1, 6, 11, 16, 37, 41, 44 and so on.

4.2 Principal Component Analysis (PCA)

Principal components were computed from the correlation matrix and genotype scores obtained from first components (which has the property of accounting for maximum variance) and succeeding components with latent roots greater than the unity (Jeger *et al.,* 1983). Contributions of the different morphological characters towards divergence were discussed from the latent vectors of the first two principal components.

The principal component analysis yielded eigen values of each principal component axes of coordination of genotypes in which the first axes totally accounting for the variation among the genotypes, whereas four of these eigen values above unity accounted for 78.81%. The first two principal axes accounted for 55.64% of the total variation among the 12 characters describing in 60 lentil genotypes (Table 2). Based on principal component axes I and II, a two dimensional chart (Z_1-Z_2) of the genotypes are presented in (Figure 2). The scattered diagram (Figure 4) represents that apparently there were mainly six clusters and the genotypes were distantly located from each other.

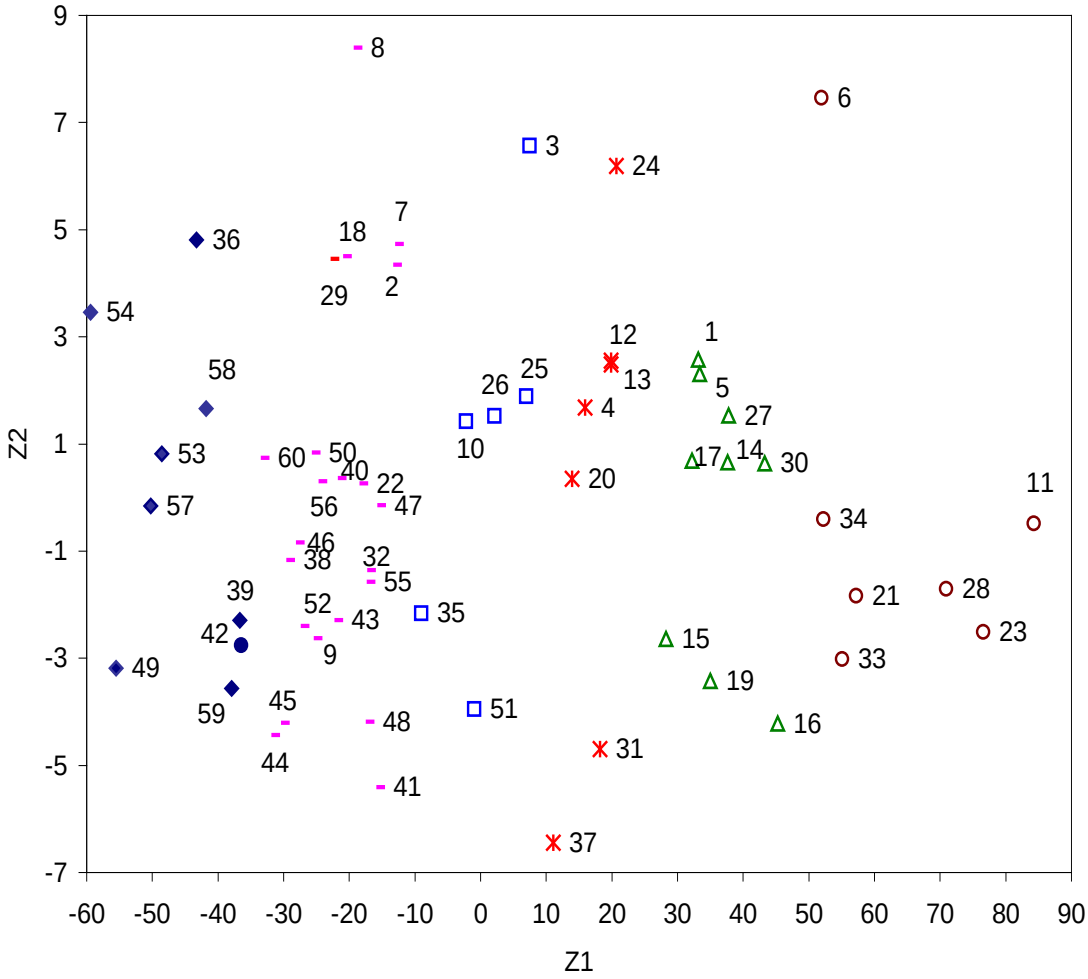


Figure 2. Scatter diagram of lentil genotypes based on their principal component scores

4.3 Principal Coordinate Analysis (PCO)

Inter-genotypic distances obtained from principal coordinate analysis for selective combination, showed that the highest distance (1.2664) was observed between the genotypes number 11 and 49, followed by 11 and 44 (1.2390) and the lowest distance was observed between 35 and 51 (0.0731) followed by 41 and 43 (0.1085), 49 and 57 (0.1096) (Table 3).

By using these inter-genotypic distances intra-cluster genotypic distances were calculated (Table 6) as suggested by Sinha and Chowdhary (1984). Cluster IV that showed the highest intra-cluster distance (0.2412) composed of seven genotypes and cluster VI showed the lowest intra-cluster distance (0.0827) composed of nine genotypes, which indicated within group diversity of the genotypes, was maximum in cluster IV and minimum in cluster VI. Intra-cluster distances between II (0.2311) to IV (0.2412) and III (0.1371) to V (0.1502) were more or less similar.

Table 2. Eigen values and percentage of variation in respect of twelve characters in lentil

Principal component axis	Eigen values	% of total variation accounted for	Cumulative percent
I	4.0793	33.99	33.99
II	2.5977	21.65	55.64
III	1.4681	12.23	67.87
IV	1.3123	10.94	**78.81**
V	0.8200	6.83	85.64
VI	0.8029	6.69	92.33
VII	0.5490	4.57	96.90
VIII	0.3447	2.87	99.77
IX	0.0188	0.16	99.93
X	0.0061	0.05	99.98
XI	0.0009	0.01	99.99
XII	0.0003	0.01	100.00

Table 3. Inter genotypic distances (D^2) of 15 highest and 15 lowest genotypes of different clusters of lentil

Serial number	Between Genotype (G)	Distance (Highest)	Serial number	Between Genotype (G)	Distance (Lowest)
1	**11-49**	**1.2664**	1	**35-51**	**0.0731**
2	11-44	1.2390	2	41-43	0.1085
3	11-57	1.2385	3	49-57	0.1096
4	11-53	1.2373	4	42-45	0.1106
5	11-54	1.1810	5	52-55	0.1128
6	11-41	1.1718	6	39-45	0.1138
7	11-59	1.1436	7	40-43	0.1148
8	28-49	1.1227	8	35-40	0.1160
9	11-39	1.1225	9	41-55	0.1170
10	8-11	1.1167	10	35-43	0.1175
11	28-44	1.1144	11	52-60	0.1177
12	11-45	1.1122	12	41-51	0.1204
13	2-11	1.1107	13	56-60	0.1204
14	11-42	1.0928	14	40-41	0.1216
15	28-53	1.0921	15	20-26	0.1224

4.4 Non – hierarchical Clustering

The computation from co-variance matrix gave non-hierarchical clustering among 60 genotypes. By application of non- hierarchical clustering and using covariance matrix, the 60 lentil genotypes were grouped into six different clusters. Mishra *et al*. (1985) reported similar number of clustering in 75 soybean genotypes. Shunmugam *et al.* (1982) reported ten clusters; Nadaf *et al.* (1986) nine clustering, Golakia and Make (1992) seven clustering; Reddy *et al.* (1987) six clusters in groundnut. These results confirmed the clustering pattern of the cultivars according to the Principal Component Analysis. So, the results obtained through PCA were confirmed by non-hierarchical clustering. Compositions of different clusters with their corresponding genotypes including the clusters are presented in Table 4.

Composition of different clusters with their corresponding genotypes is presented in Table 4 and in Figure 4. Cluster V had maximum twenty-two genotypes followed by cluster III, VI, II, IV and I, which had nine, nine, seven, seven and six genotypes, respectively. Cluster I composed of six genotypes namely BD 5969, BD 5968, BD 5983, BD 3857, BD 3856 and BD 5982. Cluster II was composed of seven genotypes namely BD 5988, BD 4056, BD 4085, BD 4131, BD 5962, BD 5959 and BD 4091. Cluster III was constituted of nine genotypes namely BD 5991, BD 3846, BD 4079, BD 5980, BD 3845, BD 5965, BD 4051, BD 5964 and BD 3848. Cluster IV constituted of seven genotypes namely BD 4103, BD 3851, BD 3855, BD 3861, BD 4110, BD 5979 and BD 4067. Cluster V consisted the highest number (twenty-two) of genotypes namely BD 4046, BD 5989, BD 4134, BD 4074, BD 3853, BD 4062, BD 5986, BD 4117, BD 5961, BD 5963, BD 4041, BD 4129, BD 3849, BD 5975, BD 5960, BD 4119, BD 4035, BD 4098, BD 5976, BD 5972, BD 5984 and BD 5973. Cluster VI contains nine genotypes namely BD 5977, BD 5978, BD 4126, BD 5958, BD 5967, BD 5990, BD 5970, BD 5981 and BD 5966.

Table 4. Distribution of 60 genotypes of lentil genotypes in different clusters

Cluster	Members	Genotypes
I	6	BD 5969, BD 5968, BD 5983, BD 3857, BD 3856, BD 5982
II	7	BD 5988, BD 4056, BD 4085, BD 4131, BD 5962, BD 5959, BD 4091
III	9	BD 5991, BD 3846, BD 4079, BD 5980, BD 3845, BD 5965, BD 4051, BD 5964, BD 3848
IV	7	BD 4103, BD 3851, BD 3855, BD 3861, BD 4110, BD 5979, BD 4067
V	22	BD 4046, BD 5989, BD 4134, BD 4074, BD 3853, BD 4062, BD 5986, BD 4117, BD 5961, BD 5963, BD 4041, BD 4129, BD 3849, BD 5975, BD 5960, BD 4119, BD 4035, BD 4098, BD 5976, BD 5972, BD 5984, BD 5973
VI	9	BD 5977, BD 5978, BD 4126, BD 5958, BD 5967, BD 5990, BD 5970, BD 5981, BD 5966

Table 5. Cluster means for twelve characters in lentil

Characters	Clusters					
	I	II	III	IV	V	VI
Days to 50% flowering	65.34	69.29	66.41	68.24	65.55	64.15
Days to maturity	98.83	99.95	98.59	99.81	98.74	98.15
Plant height	36.82	35.89	36.32	37.51	37.47	37.81
Pod/ plant	109.87	78.64	94.18	102.68	122.12	133.01
Seed/ pod	1.81	1.82	1.78	1.79	1.79	1.80
Primary branches	4.59	4.07	4.61	5.12	4.47	3.98
Secondary branches	13.94	12.40	13.97	15.66	13.52	12.12
Seed/ plant	198.62	143.77	166.84	184.03	218.02	238.61
100 Seed weight	2.63	2.60	2.61	2.63	2.61	2.69
Dry matter weight	12.65	10.17	10.65	12.68	13.69	14.44
Yield/ plant	5.22	3.87	4.35	4.83	5.69	6.21
Harvest index	0.44	0.42	0.43	0.40	0.44	0.46

However, if we consider the yield contributing characters of the experiment then the following scenario will capture our attention:

Day to 50% flowering: It is observed that minimum days required in the cluster group VI (64.15 days). It reveals that most of the early flowering materials are laying in this group. On the other hand late flowering materials are present in the cluster group II (69.29 days).

Days to maturity: In this experiment days to maturity is not significantly different from each other. It depicts that all the materials are more or less early mature. However, nearly a two days difference is observed between the cluster groups VI (98.15) and II (99.95).

Pod per plant: The highest pod per plant is found in the cluster group VI (133.01) and the lowest value is observed in the cluster II (78.64). This is an important character that contributes towards yield.

Seed per pod: This is also a yield contributing character. The highest value is observed in the cluster II (1.82) and the lowest value is found in the cluster groups IV and V (1.79). It reveals that small seeds are laying in the cluster group II.

Seed per plant: The highest number of seeds is found in the cluster VI (238.61) and the lowest value is observed in the cluster II (143.77).

100 Seed weight: The highest 100 Seed weight is observed in the cluster group VI (2.69 g) and the lowest mean is found in the cluster II (2.60 g). It means that most of the bold seeded genotypes were present in cluster VI.

Yield per plant: The highest mean is observed in the cluster group VI (6.21 g) and the lowest value is found in the cluster group II (3.87 g). It reveals that the high yielding genotypes are belonging to this cluster group.

According to the above discussion it could be recommended that the materials preset in the cluster VI are early maturing and simultaneously high yielding as other yield contributing characters are also high in this group.

From the class mean values it was observed that all the cluster mean values for days to 50% flowering, days to maturity, plant height, seed per pod, no. of primary branches, no. of secondary branches, 100 seed weight, dry matter weight, yield per plant and harvest index were more or less similar. The maximum range of variability was observed for the character seed per plant

(143.77 to 238.61) and pod per plant (78.64 to 133.01) among all the characters in six clusters.

Cluster II and IV included mainly late flowering and late maturing genotypes with low yield, but they were highly heterogeneous in nature. The high yielding lines belonged to early flowering and early maturing groups, VI and V. Bartual *et al.* (1985) also reported similar relationship in soybean. To develop high yielding varieties/lines, genotypes of these groups could be used in hybridization programme.

4.5 Canonical Variate Analysis (CVA)

Canonical Variate Analysis was performed to compute the inter-cluster Mahalanobis's values. Statistical distances represent the index of genetic diversity among the clusters. The average intra and inter-cluster distance (D^2) values are presented in Table 6. Results indicated that the highest inter-cluster distance was observed between II and VI (19.967), followed by II and V (16.048), III and VI (13.741), I and II (12.214) and IV and VI (10.729). The lowest inter-cluster distance was observed between the cluster I and IV (3.077) followed by I and V (3.970), III and IV (3.975) and V and VI (4.25), whereas a similar type of distance was found between I and VI (7.993), II and III (7.885) and I and V (3.970), III and IV (3.975), suggesting a close relationship among those clusters (Figure 3). The inter-cluster distances were larger than the intra-cluster distances suggesting wider genetic diversity among the genotypes of different groups (Table 3 and Figure 3). Islam (1995) obtained larger inter-cluster distances than the intra-cluster distances in a multivariate analysis.

However, the maximum inter-cluster distance was recorded between cluster II and VI followed by between II and V. Genotypes from these clusters can use in hybridization programme.

The intra-cluster divergence varied from 0.0827 to 0.2412, maximum for cluster IV, which was comprised of seven genotypes of diverse origin, while the minimum distance was observed in cluster VI that comprised nine genotypes.

Table 6. Average intra and inter-cluster distances (D^2) for lentil genotypes

Cluster	I	II	III	IV	V	VI
I	**0.1900**					
II	12.214	**0.2311**				
III	5.981	7.885	**0.1371**			
IV	3.077	10.070	3.975	**0.2412**		
V	3.970	16.048	9.727	6.658	**0.1502**	
VI	7.993	19.967	13.741	10.729	4.125	**0. 0827**

*Underlined bold figures denote intra-cluster distances.

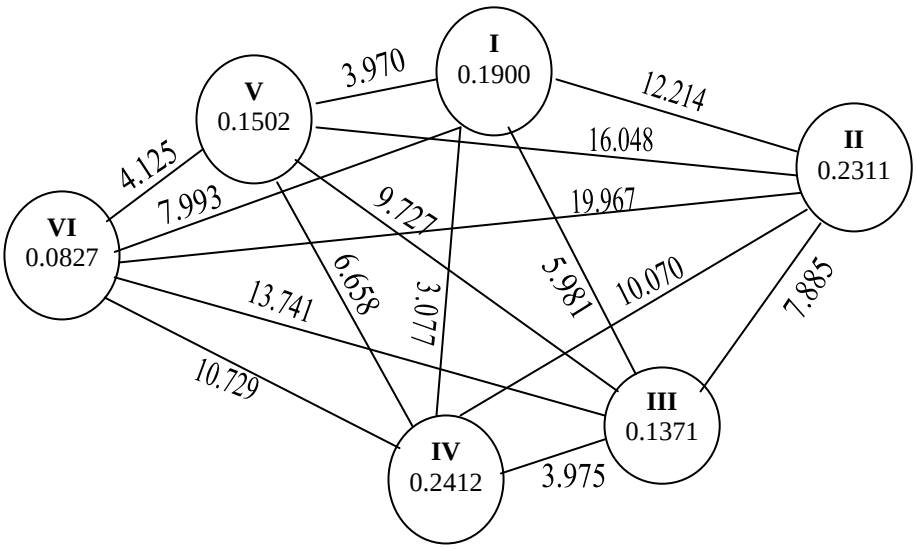


Figure 3. Diagram showing inter-cluster (outside the circle) and intra-cluster (inside the circle) distances of lentil genotypes

Results obtained from different multivariate techniques were superimposed in Figure 3 from which it may be concluded that all the techniques gave more or less similar results and one technique supplemented and confirmed the results of another one.

The clustering pattern of the genotypes revealed that varieties/lines originating from the same places did not form a single cluster because of direct selection pressure. This indicated that geographic diversity was not related to genetic diversity that might be due to continuous exchange of genetic materials among the countries of the world. Same results have been reported by Shewe *et al.* (1972) in groundnut; Verma (1970) in groundnut and soybean; Murty and Anand (1966); Anand and Rawat (1984) in brown mustard; Das and Gupta (1984) in black gram; Natarajan *et al*. (1988) green gram, Patel *et al.* (1989) in sunflower; Mian and Bhal (1989) in chickpea.

It had been observed that geographic diversity is not always related to genetic diversity and therefore, it is not adequate as an index of genetic diversity. Murty and Arunachalam (1966) studied that genetic drift and selection in different environment could cause greater diversity than geographic distance.

Furthermore, there is a free exchange of seed material among different region, as a consequence, the characters constellation that might be associated with particular region in nature, loose their individuality under human interference, and however, in some cases effect of geographic origin influenced clustering that is why geographic distribution was not the sole criterion of genetic diversity.

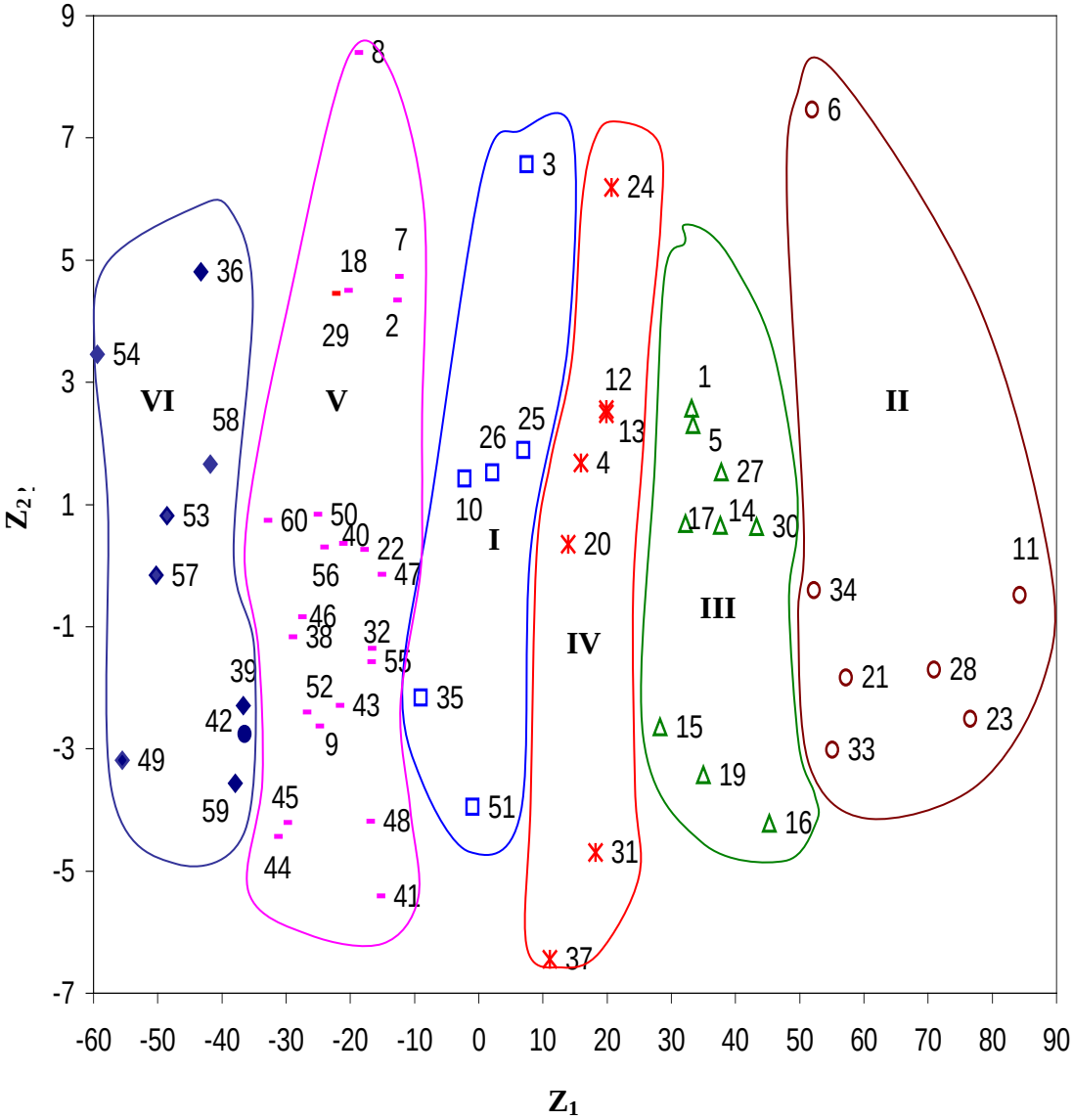


Figure 4. Scatter diagram with clustering pattern of sixty lentil germplasms

In the Diagram the number indicates the germplasm numbers 1=BD 5991, 2=BD 4046, 3=BD 5969, 4=BD 4103, 5=BD 3846, 6=B 5988, 7=BD 5989, 8=BD 4134, 9=BD 4074, 10=BD 5968, 11=BD 4056, 12=BD 3851, 13=BD 3855, 14=BD 4079, 15=BD 5980, 16=BD 3845, 17=BD 5965, 18=BD 3853, 19=BD 4051, 20=BD 3861, 21=BD 4085, 22=BD 4062, 23=BD 4131, 24=BD 4110, 25=BD 5983, 26=BD 3857, 27=BD 5964, 28=BD 5962, 29=BD 5986, 30=BD 3848, 31=BD 5979, 32=BD 4117, 33=BD 5959, 34=BD 4091, 35=BD 3856, 36=BD 5977, 37=BD 4067, 38=BD 5961, 39=BD 5978, 40=BD 5963, 41=BD 4041, 42=BD 4126, 43=BD 4129, 44=BD 3849, 45=BD 5975, 46=BD 5960, 47=BD 4119, 48=BD 4035, 49=BD 5958, 50=BD 4098, 51=BD 5982, 52=BD 5976, 53=BD 5967, 54=BD 5990, 55=BD 5972, 56=BD 5984, 57=BD 5970, 58=BD 5981, 59=BD 5966 and 60=BD 5973 respectively.

The free clustering of the genotypes suggested dependence upon the directional selection pressure applied for realizing maximum yield in different regions; the nicely evolved homeostatic devices will favour constancy of the associated characters will thus indiscriminate clustering. This would be suggested that it was not necessary to choose diverse parents for diverse geographic regions for hybridization.

4.6 Contribution of characters towards divergence of the cultivars

The character contributing maximum to the divergence were given greater emphasis for deciding on the cluster for the purpose of further selection and choice of parents for hybridization (Jagadev *et al*., 1991). The PCA revealed that in vector I (Z_1) the important characters responsible for genetic divergence in the major axis of differentiation were pod per plant (0.4385), seed per plant (0.4209), yield per plant (0.4016), dry matter weight (0.4014) and plant height (0.2285) (Table 7).

In vector II (Z_2) that was the second axis of differentiation, plant height (0.2273), days to 50% flowering (0.2118), dry matter weight (0.2025) and days to maturity (0.1578) were important but days to 50% flowering, days to maturity, seed per pod, primary branches, secondary braches, 100 seed weight and harvest index played only a minor role in the first axis of differentiation. The role of seed per pod, primary branches, secondary branches, 100 seed weight and harvest index had a minor role in the genetic divergence. The role of plant height and dry matter weight in both the vectors were positive across two axes indicating the important components of genetic divergence in those materials.

Table 7. Latent vectors for 12 morphological characters in lentil

Characters	Vector-I	*Vector-II*
Days to 50% flowering	-0.2393	0.2118
Days to maturity	-0.1049	0.1578
Plant height	0.2285	0.2273
Pod/ plant	0.4385	-0.2501
Seed/ pod	-0.1590	-0.2252
Primary branches	-0.2192	-0.3944
Secondary branches	-0.2268	-0.3989
Seed/ plant	0.4209	-0.2941
100 Seed weight	-0.2106	-0.0884
Dry matter weight	0.4014	0.2025
Yield/ plant	0.4016	-0.3160
Harvest index	-0.1357	-0.4642

4.7 Comparison of different multivariate techniques

The clustering pattern of D^2 analysis through non- hierarchical clustering had taken care of simultaneous variation in all the characters under study. However, the distribution of genotypes in different clusters of the D^2 analysis had followed more or less similar trend of the Z_1 and Z_2 vector of the principal component analysis. The D^2 and principal component analysis were found to be alternative methods in giving the information regarding the clustering pattern of genotypes. However, the principal component analysis provides the information regarding the contribution of characters towards divergence of lentil.

4.8 Selection of cultivars for future hybridization

Genotypically distant parents are able to produce higher heterosis (Falconer, 1960; Moll *el al*., 1962; Ramanujam *et al.,* 1974; Chauhan and Singh, 1982; Arunachalam *et al.,* 1981; Ghaderi *et al.,* 1984; Mian and Bhal, 1989). Beside this, Arunachalam *et al.* (1981) reported in groundnut that the higher heterosis for yield and its components could be obtained from the crosses between the intermediate divergent parents than extreme ones. Mian and Bhal (1989) also reported the same in chickpea that medium divergent genotypes showed higher heterosis in crosses for different yield contributing characters. Srivastava and Arunachalam (1977) reported in triticale that very high or very low parental divergent failed to result in heterosis. Mian and Bhal (1989) also reported the same concept in chickpea that medium divergent genotypes showed higher heterosis in crosses for different yield contributing characters.

Considering this idea and other agronomic performances (Appendix III and IV), the genotypes BD 5969 & BD 5983 from cluster I; BD 5988, BD 4085 & BD 4091 from cluster II; BD 5991 & BD 5980 from cluster III; BD 3861, BD 4103 & BD 4110 from cluster IV; BD 4074, BD 3853, BD 5961 & BD 5973 from cluster V and BD 5977, BD 5958, BD 5967, BD 5990, BD 5970, BD 5981 & BD 5966 from cluster VI were selected as promising germplasms for higher yield, number of seed per plant, early maturity and greater dry matter

weight. Therefore, considering group distance, genetic distance and other agronomic performances, the inter-genotypic crosses between 2-11, 8-11, 11-39, 11-44, 11-49, 11-53, 11-57, 28-44, 28-49 etc. (Table 3) might be suggested to use for future hybridization programme.

Chapter 5

SUMMARY AND CONCLUSION

An experiment with 60 lentil genotypes was conducted in the field of Sher-e-Bangla Agricultural University, Dhaka to study diversity pattern based on 12 characters during November 2005 to March 2006. Seeds were sown in the main field in the month of November 2005 in RCBD with three replications. Data on plant height, days to 50% flowering, days to maturity, primary branches, secondary branches, pod per plant, seed per pod, seed per plant, 100 seed weight, yield per plant and harvest index were recorded on plant basis.

Significant differences among the clusters were observed through Multivariate analysis, Cluster analysis, and Canonical Variate analysis by using GENSTAT programme at BSMRAU computer centre. The first four components with eigen value greater than unity contributed a total of 78.81% variation towards the divergence. As per PCA, D^2 and Cluster analysis, the genotypes were grouped into six different clusters. Cluster I, II, III, IV, V and VI composed of six, seven, nine, seven, twenty two and nine genotypes respectively. The highest inter-cluster distance was observed between II and VI (19.967) followed by II and V (16.048). The lowest inter-cluster distance was observed between I and IV (3.077) followed by I and V (3.970). The highest and lowest intra-cluster distances were observed in cluster IV (0.2412) and in cluster VI (0.0827) respectively. Genotypes included in cluster IV were important for primary (5.12) and secondary (15.66) branches whereas plant height (37.81 cm), pod per plant (133.01), seed per plant (238.61), dry matter weight (14.44 g),100 Seed weight (2.69 g), yield per plant (6.21 g) and harvest index (0.46) were remarkable features for cluster VI. The clustering pattern of the genotypes revealed that germplasms collected from the same places did not form a single cluster. In addition to this, genotypes which had moderate inter-

cluster distance comprised with medium to high yield could be utilized in screening suitable materials from large population.

Considering diversity pattern, genetic status and other agronomic performances, BD 5969 & BD 5983 from cluster I; BD 5988, BD 4085 & BD 4091 from cluster II; BD 5991 & BD 5980 from cluster III; BD 3861, BD 4103 & BD 4110 from cluster IV; BD 4074, BD 3853, BD 5961 & BD 5973 from cluster V and BD 5977, BD 5958, BD 5967, BD 5990, BD 5970, BD 5981 & BD 5966 from cluster VI might be considered better parents for efficient hybridization programme.

Results of the present study indicated significant variation among the genotypes for all the characters studied (Table 7 and Appendix III). The characters plant height and dry matter weight contributed maximum towards divergence among the lentil genotypes. Number of primary and secondary branches, days to fifty percent flowering, days to maturity, plant height, pod per plant, seed per plant, dry matter weight and yield per plant contributed moderately towards genetic diversity as well as yield improvement.

Sixty lentil genotypes formed six different clusters. PCA, PCO and Cluster analysis gave similar results. The morphological characters manifested the diversity (Appendix IV), whereas distribution of the genotypes had no impact on it. Involvement of such diverse genotypes in crossing programme may produce desirable segregants. So, divergent genotypes are recommended to use as parent in hybridization programme.

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Appendix I. Mean sum of squares from the ANOVA of lentil genotypes for 12 characters

Source of variation	df	Parameters											
		Days to 50% flowering	Days to maturity	Plant Height	Pod per Plant	Seed per pod	Primary Branches	Secondary ranches	Seed per Plant	100 Seed weight	Dry matter weight	Yield per Plant	Harvest Index
Replication	2	120.689**	69.072**	1.825^{NS}	152.169^{NS}	0.017^{NS}	1.364*	16.327**	558.156^{NS}	0.054**	62.107**	1.110^{NS}	0.139**
Genotype	59	27.732**	14.713**	15.065**	940.971**	0.008^{NS}	1.815**	16.693**	2876.896**	0.010**	23.193**	1.890**	0.021**
Error	118	4.598	7.942	5.839	233.586	0.008	0.320	2.812	788.899	0.006	5.827	0.534	0.009

** = Significant at 1% level of significance, * = significant at 5% level of significance, and ^{NS} = non-significant.

Appendix II. Range, Mean and Standard Error with Coefficient of Variation

(CV) for 12 morphological characters

Characters	Minimum	Maximum	Mean ± SE	CV%
Days to 50% flowering	62.33	77.67	66.19 ± 0.39	3.24
Days to maturity	95.00	105.67	98.91 ± 0.29	2.86
Plant Height	31.88	42.70	37.10 ± 0.29	6.51
Pod/ plant	68.70	141.30	111.00 ± 2.29	13.77
Seed/ pod	1.67	1.93	1.79 ± 0.01	4.96
Primary Branches	3.63	6.60	4.46 ± 0.10	12.69
Secondary Branches	11.10	20.00	13.54 ± 0.30	12.38
Seed/Plant	126.11	252.66	198.86 ± 4.00	14.12
100 Seed weight	2.44	2.76	2.62 ± 0.01	2.84
Dry matter weight	8.13	19.45	12.71 ± 0.36	18.99
Yield/Plant	3.37	6.56	5.21 ± 0.10	14.02
Harvest Index	0.28	0.59	0.43 ± 0.01	21.79

SE = Standard Error
CV = Co- efficient of Variation

Appendix III. Principal component scores for 60 lentil genotypes

Genotype no.	Z_1	Z_2
1	33.18	2.57
2	-13.14	4.34
3	7.45	6.57
4	15.90	1.68
5	33.38	2.31
6	51.90	7.46
7	-12.85	4.73
8	-19.13	8.39
9	-25.26	-2.63
10	-2.19	1.42
11	84.28	-0.48
12	19.90	2.48
13	19.89	2.55
14	37.61	0.66
15	28.22	-2.64
16	45.26	-4.22
17	32.18	0.69
18	-20.77	4.50
19	35.00	-3.42
20	13.92	0.35
21	57.19	-1.84
22	-18.27	0.26
23	76.55	-2.51
24	20.72	6.19
25	6.99	1.89
26	2.15	1.52
27	37.76	1.53
28	70.93	-1.71
29	-22.68	4.45
30	43.26	0.64
31	18.18	-4.70
32	-17.11	-1.36
33	55.11	-3.02
34	52.22	-0.41
35	-9.00	-2.16
36	-43.29	4.81
37	11.09	-6.44
38	-29.43	-1.17
39	-36.70	-2.29
40	-21.53	0.36
41	-15.69	-5.41
42	-36.43	-2.76
43	-22.05	-2.29
44	-31.71	-4.44
45	-30.19	-4.21
46	-27.97	-0.84
47	-15.52	-0.15
48	-17.30	-4.19
49	-55.49	-3.19
50	-25.54	0.84
51	-0.93	-3.95
52	-27.20	-2.40
53	-48.56	0.82
54	-59.42	3.46
55	-17.15	-1.58
56	-24.52	0.30
57	-50.23	-0.16
58	-41.80	1.66
59	-37.92	-3.56
60	-33.27	0.74

Appendix IV. Mean performances of 60 lentil genotypes for 12 characters

Genotype number	Days to 50% flow	Days to maturity	Plant Height	Pod/ plant	Seed/ pod	Primary Branches	Secondary Branches	Seed /Plant	100 Seed wt	Dry matter	Yield /Plant	Harvest Index
1	63.00	99.00	31.88	97.30	1.73	5.50	16.60	168.60	2.65	9.21	4.47	0.50
2	65.33	100.00	41.20	114.97	1.83	6.60	19.90	211.74	2.63	12.26	5.57	0.45
3	64.33	95.33	34.26	105.70	1.83	6.17	18.63	193.45	2.67	9.28	5.17	0.57
4	66.33	98.67	35.01	102.63	1.80	4.93	15.17	185.38	2.60	12.28	4.82	0.39
5	65.00	99.67	35.93	94.70	1.80	5.70	17.23	169.79	2.62	10.74	4.45	0.42
6	64.67	98.33	31.91	80.77	1.93	5.70	17.17	156.50	2.67	8.88	4.18	0.49
7	64.67	100.67	33.54	115.47	1.83	4.93	14.90	211.25	2.62	10.69	5.53	0.52
8	66.00	97.00	33.44	117.90	1.87	6.57	20.00	217.20	2.72	10.21	5.87	0.59
9	64.67	96.33	33.27	130.30	1.67	4.80	14.40	216.93	2.72	13.78	5.90	0.43
10	64.67	97.67	34.25	111.70	1.80	4.03	12.37	201.01	2.66	11.51	5.35	0.47
11	69.33	95.00	37.00	68.70	1.80	4.00	12.00	126.11	2.69	8.33	3.37	0.40
12	66.33	99.00	37.88	102.17	1.77	6.30	19.30	181.11	2.66	10.85	4.79	0.45
13	67.33	98.33	36.77	102.23	1.77	5.77	17.70	181.18	2.66	9.74	4.82	0.49
14	75.33	101.00	36.91	92.57	1.80	4.40	13.67	166.62	2.59	8.69	4.32	0.50
15	64.33	95.33	37.87	99.53	1.73	4.07	12.30	172.80	2.66	11.50	4.60	0.43
16	64.67	98.33	36.66	92.63	1.70	3.97	11.97	157.14	2.57	11.82	4.03	0.35
17	64.67	96.33	35.80	96.33	1.80	4.10	12.50	170.28	2.57	8.13	4.39	0.54
18	69.00	99.33	36.20	115.37	1.90	4.13	12.50	220.43	2.71	12.41	5.97	0.55
19	70.33	100.33	39.54	94.90	1.77	4.43	13.23	167.81	2.61	13.41	4.38	0.33
20	70.00	100.00	38.00	102.53	1.83	4.83	14.60	187.77	2.67	13.48	5.00	0.38
21	74.67	101.00	39.12	82.97	1.80	3.80	11.50	149.42	2.76	8.71	4.13	0.48
22	68.67	98.33	38.59	121.27	1.77	5.00	15.17	214.19	2.61	12.15	5.58	0.47
23	67.00	99.33	37.07	72.17	1.83	3.87	12.13	132.76	2.67	12.97	3.58	0.28
24	70.33	100.33	36.19	97.80	1.87	6.23	19.10	182.91	2.60	9.51	4.76	0.50
25	66.67	103.00	36.37	106.13	1.83	4.43	13.23	193.75	2.66	10.60	5.16	0.49
26	65.67	100.33	38.02	108.10	1.83	4.80	14.80	198.03	2.50	12.73	4.93	0.39
27	63.67	96.67	34.01	91.33	1.83	4.03	12.33	166.58	2.62	10.14	4.38	0.43
28	64.67	99.00	38.10	76.27	1.80	3.63	11.10	136.99	2.70	8.65	3.70	0.44
29	66.67	100.33	35.49	118.57	1.87	4.33	13.07	220.83	2.60	10.84	5.74	0.53
30	66.67	100.67	38.30	88.33	1.83	5.27	15.87	161.98	2.57	12.21	4.16	0.37
31	67.00	103.33	37.12	104.57	1.73	3.83	11.83	181.52	2.60	15.19	4.72	0.31
32	64.67	96.67	36.86	117.30	1.83	3.80	11.47	214.66	2.57	17.28	5.51	0.32
33	67.00	102.67	34.64	83.80	1.80	3.80	11.60	150.84	2.64	14.67	3.98	0.29
34	77.67	104.33	33.42	85.77	1.80	3.70	11.33	153.80	2.69	8.96	4.13	0.53
35	65.00	99.00	38.99	114.87	1.80	4.10	12.43	206.79	2.65	15.76	5.48	0.36
36	64.00	95.67	35.35	129.83	1.83	4.23	13.23	238.03	2.61	10.54	6.21	0.59
37	70.33	99.00	41.61	106.80	1.77	3.97	11.93	188.31	2.61	17.68	4.91	0.29
38	65.33	97.67	40.01	126.60	1.77	4.53	13.63	223.76	2.69	13.53	6.02	0.45
39	64.00	97.67	36.44	132.23	1.73	4.33	13.00	228.87	2.44	15.12	5.58	0.37
40	65.67	98.67	39.47	118.83	1.83	4.30	13.23	219.03	2.50	15.05	5.47	0.38
41	66.00	97.67	38.33	123.37	1.70	4.13	12.60	209.70	2.54	16.06	5.32	0.34
42	64.33	96.67	38.29	130.03	1.77	4.00	12.20	229.69	2.55	16.68	5.85	0.38
43	63.67	99.00	37.61	123.07	1.77	4.27	12.97	217.13	2.67	15.97	5.79	0.36
44	67.33	98.33	38.67	127.60	1.77	4.10	12.53	225.64	2.58	19.45	5.83	0.32
45	65.00	98.33	35.89	130.73	1.70	4.03	12.00	222.25	2.58	15.42	5.73	0.38
46	66.67	102.33	36.74	127.97	1.73	3.80	11.73	221.61	2.63	10.05	5.82	0.59
47	63.33	96.00	37.51	119.97	1.77	3.97	13.17	211.53	2.55	11.80	5.36	0.46
48	65.00	100.00	42.70	120.70	1.77	4.57	12.77	212.99	2.60	15.79	5.52	0.36
49	67.33	100.33	37.59	141.30	1.73	4.00	11.90	245.44	2.65	15.97	6.51	0.42
50	70.00	105.67	38.40	123.13	1.80	4.37	13.43	221.64	2.59	11.67	5.75	0.51
51	65.67	97.67	39.04	112.70	1.77	4.00	12.20	198.72	2.64	16.04	5.23	0.33
52	64.67	97.33	39.47	125.63	1.77	4.13	12.10	221.67	2.59	14.45	5.74	0.41
53	63.00	98.33	40.21	130.47	1.87	3.83	12.20	243.33	2.64	16.57	6.43	0.39
54	63.67	99.00	37.36	136.57	1.87	3.70	11.30	252.66	2.60	12.25	6.56	0.58
55	63.67	99.33	38.18	118.70	1.80	4.00	11.73	213.98	2.60	15.33	5.57	0.36
56	63.00	96.33	36.02	122.60	1.80	3.80	11.67	220.32	2.59	13.36	5.70	0.45
57	65.33	100.67	38.79	133.00	1.83	3.73	11.37	243.97	2.67	16.18	6.48	0.40
58	63.33	98.00	37.90	130.90	1.80	4.20	12.63	235.59	2.67	11.78	6.27	0.56
59	62.33	97.00	38.40	132.73	1.73	3.83	11.23	229.87	2.61	14.89	6.02	0.41
60	63.00	97.00	36.72	126.70	1.80	4.17	12.47	228.06	2.62	13.66	5.98	0.48

Appendix V. Morphological, physical and chemical characteristics of initial soil

(0-15 cm depth)

A. Physical composition of the soil

Soil separates	%	Methods employed
Sand	36.90	Hydrometer method (Day, 1915)
Silt	26.40	Do
Clay	36.66	Do
Texture class	Clay loam	Do

B. Chemical composition of the soil

Sl. No.	Soil characteristics	Analytical data	Methods employed
1	Organic carbon (%)	0.82	Walkley and Black, 1947
2	Total N (kg/ha)	1790.00	Bremner and Mulvaney, 1965
3	Total S (ppm)	225.00	Bardsley and Lanester, 1965
4	Total P (ppm)	840.00	Olsen and Sommers, 1982
5	Available N (kg/ha)	54.00	Bremner, 1965
6	Available P (kg/ha)	69.00	Olsen and Dean, 1965
7	Exchangeable K (kg/ha)	89.50	Pratt, 1965
8	Available S (ppm)	16.00	Hunter, 1984
9	Ph (1:2.5 soil to water)	5.55	Jackson, 1958
10	CEC	11.23	Chapman, 1965

Appendix VI. Monthly average of Temperature, Relative humidity, Total Rainfall and sunshine hour of the experiment site during the period from October, 2005 to February, 2006

Year	Month	Air temperature			Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
		Maximum	Minimum	Mean			
2005	October	30.6	24.6	27.60	77	326	142.20
	November	29.1	19.8	24.45	70	03	197.63
	December	27.1	15.7	21.4	64	Trace	217.03
2006	January	25.3	18.2	21.75	68	0	165.10
	February	31.3	19.4	25.35	61	0	171.01

Source: Bangladesh Metrological Department (Climate division), Agargaon. Dhaka-1212.