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Genetic Divergence, Selection Index and Genotype – Environment Interaction in Lentil (Lens culinaris Medic)

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University of Rajshahi

M. Phil Thesis

GENETIC DIVERGENCE, SELECTION INDEX AND GENOTYPE – ENVIRONMENT INTERACTION IN LENTIL (*Lens culinaris* Medic)

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Amit Kumar Dutta

A Thesis

Submitted to the University of Rajshahi in fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

by

AMIT KUMAR DUTTA

JUNE 2015

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UNIVERSITY OF RAJSHAHI

BIOMETRICAL GENETICS LAB.
DEPARTMENT OF GENETIC ENG. AND BIOTECHNOLOGY
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RAJSHAHI, BANGLADESH.

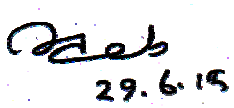
DEDICATED

TO

MY BELOVED PARENTS

CERTIFICATE

The undersigned certify that the research work embodied in this thesis was done by the author and that as to the style and contents the thesis suitable for submission. The undersigned also certify that this thesis has not already been submitted in substance for any degree and has not concurrently been submitted in candidature for any degree.



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DECLARATION

I hereby declare that the entire work submitted as a thesis towards the fulfilment for the degree of master of philosophy for the University of Rajshahi, Rajshahi, Bangladesh is the results of our own investigation.

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The Author

ABSTRACT

The whole work of the present investigation was carried out for the study of variability, selection indices, for identify of stable genotypes and for identification and characterization of genetic diversity of six lentil (*Lens culinaris* Medic) varieties under three different heads; such as part-I: genetic variability, correlation, path coefficients and selection index; part-II: genotype- environment interaction and part-III: genetic divergence using morphological and biochemical markers. For this, eleven yield and yield contributing characters viz date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF), number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP).

In part-I, the mean values were highly significant with their respective standard errors and wide range of variation among the means values were obtained, suggesting that characters studied are quantitative in nature and under polygenic control. The highest values of σ^2_P, σ^2_G, σ^2_Y, σ^2_{GY} and σ_E^2 components of variation and PCV, GCV and G□YCV were obtained for NSPP. Moderate h^2b were obtained for PHMF and high GA values were noted for NSPP, while high GA% value was recorded for PdWPP.

In the present investigation, correlation studies showed that genotypic correlation was higher than the respective phenotypic correlation in most of the cases. SWPP showed positive correlation with PWPP, PdWPP and NSPP both at phenotypic and genotypic levels. NPBFF, PHMF and PdWPP had the high direct effect on SWPP both phenotypic and genotypic levels, but NSPP showed high direct effect on SWPP at genotypic level. Considering selection index, the highest expected genetic gain was observed in NPBFF, NPdPP, PdWPP, NSPP and SWPP and with their combination. When the two yield contributing characters viz. PdWPP and SWPP were included in the indices, the expected genetic gain was increased.

In part-II, stability performances of different lines were different for different characters. Joint regression analysis revealed that genotype × environment interaction was accounted for by both linear and non-linear functions of environment. . A good number of lines showed stable performances for different characters in different environments. The genotypes which showed stable performances, i.e., adaptable to all environments are Bm1 for NPBFF, Bm2 for PHMF, NSBMF, NPdPP and NSPP, Bm3 for NPBFF, Bm4 for PHMF and PWPP, Bm5 for NPBFF and PdWPP and Bm6 for PWPP and PdWPP. The genotypes which are adaptable to favorable environment are Bm1 for most of the characters except DFF, PHFF and NPBFF, Bm3 for all of the characters except NPBFF and NPBMF, Bm4 for PHFF and NPBMF, Bm5 for NSBMF and Bm6 for all of the characters except PHMF, NSBMF and NPdPP.

In part-III, above six lentil varieties with thirteen lines of F_2 generations were analyzed through morphological markers and seed storage protein profiling for comparing the genetic divergence. In case of morphological characters, analysis of variance showed significant values for all the characters which indicating that the lines are significantly different from each other. Based on Euclidian distance, Bm1 was noted to be closely related with Bm4 × Bm3 and showed the highest dissimilarity value 0.99. Dendrogram was constructed based on the dissimilarity values of eleven characters and the lines were grouped in two clusters. In this study, seed storage protein profiling showed 70.37% polymorphism among the bands of all lines. The highest polymorphic bands were recorded in Bm5 × Bm3. Six type of seed storage protein, albumin protein was abundant in quantity in all the varieties and as well as all the varieties were polymorphic for lysozyme protein. In this analysis, close relatedness was found among cultivar Bm1 and the crosses Bm3 × Bm4 and Bm6 × Bm1 based on Jaccard's similarity coefficient and cluster analysis. The highest genetic distance showed between Bm4×Bm1 and Bm5× Bm3 F_2 lines. The results of principal component analysis is supported by the results of dendrogram, In this work, results of morphological and biochemical marker analysis suggested that the F_2 cross of Bm5Bm3 showed the highest genetic diversity among all the materials investigated and it should better for further breeding work.

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GENERAL INTRODUCTION

Bangladesh with only 55,598 square miles of land is a densely populated, agricultural based fertile deltaic land, which consists of alluvial sediments deposited by the rivers Ganges, Brahmaputra, Tista, Jamuna, Meghna and their tributaries in South Asia. Approximately 150 million highly increasing population are depending on limited agricultural land for fulfillment their food requirement. A number of crops are cultivated in different region all over the year. Major crops are Rice, Wheat, Potato and Jute, which provide greater importance. Recently staple food production in Bangladesh is reach at satisfactory level but nutrition consumption is very low. Malnutrition and blindness are the major problem for Bangladeshi people mainly for children due to malnutrition. Food security is a key issue in the developing countries, like Bangladesh. (FAO, 2014 & BBS, 2008)

Lentil (*Lens culinaris* Medic.) is the most popular and most important pulse crop in terms of both area and production, and rates the highest consumer preference in Bangladesh and it's grown extensively all over the country. It constitutes one of the main items in the daily diet of a vast majority of the people of Bangladesh. (BBS, 2007)

Cereals mainly meet up the demand of carbohydrate and pulses compensate the demand of protein. Protein is one of the main constituents of the body. It is called body building food. Bangladesh being the developing country, people have no chance to fulfill the demand of protein from animal sources such as meat, fish, milk, egg etc. Due to high price of animal protein, most of the people of our country are not able to purchase it. Pulses are the cheapest source of protein and it contains not only protein, but also contains some amounts of minerals, iron, fats, carbohydrate and vitamins. Pulses are easily available and within the purchasable capacity of the people and meet up their protein requirements in their daily diets. Thus sometimes, pulses are called 'poor men meat'. So the need of protein for the body has to obtain from the pulses. Pulses are superior food not only in Bangladesh but also in other countries of the world.

There are many type of pulses such as lentil, mung, mashkalai, chickpea, kheshari. Among them Lentil (*Lens culinaris* Medic.) is the most preferred and second most important pulse crop of the legume family, grown for its lens-shaped seeds. *Lens* is a Latin word that describes exactly the shape of the seed of a cultivated legume, which now a day's botanists call *Lens culinaris*, following the name given to it by Medikus, a German botanist-physician in 1787. Lentils contain high levels of proteins, including the essential amino acids isoleucine and lysine and are an essential source of inexpensive protein in many parts of the worlds for those who adhere to a vegetarian diet or cannot afford meat. Apart from a high level of proteins, lentils also contain dietary fiber, folate, vitamin B1 and minerals. Lentil is also one of the best vegetable sources of iron and vitamins. Iron is particularly important for adolescents and pregnant women whose requirements for it are increased.

Lentil (*Lens culinaries* Medic.) may grow as one of the first agricultural crops more than 8500 years ago. Probably it is originated in the near East and rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India and Pakistan, China and later to the new world including Latin America (Cubero, 1981; Duke, 1981). Lentil probably the oldest of grain legumes to be domesticated (Bahl *et al.,* 1993). It is now cultivated in most subtropical and also in the northern hemisphere such as Canada and Pacific Northwest regions. Lentil may have been introduced to the United States in the early 1900s.

Systematic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Sub-family: Papilionaceae

Genus: *Lens*

Species: *Lens culinaris* Medic.

Lentil adaptation and geographical distribution

Lentil is best adapted to the cooler temperate zones of the world and is predominantly grown in areas with an annual average rainfall of 300 to 400 mm (Sarker *et al*., 2007). It can grow on a wide range of soil types, however, it is sensitive to water-logging, flooding and soils with a P^H below 6.5 (Tang and Thomson, 1996). In India, Pakistan, Nepal and Bangladesh, lentil is mainly grown as a winter crop on residual moisture from monsoon rains. Moisture from melting snow provides water for lentil crop establishment early in spring, whereas timely summer rains are needed to support plant growth for rest of season. The genus *Lens* is mainly distributed in the Mediterranean region; however, individual species differ in their distribution. For example *Lens orientalis* is distributed from Turkey and Palestine to Uzbekistan. *Lens nigricans* on the other hand is mainly distributed from Palestine to Spain, Algeria and Morocco. Similarly, wild forms of *Lens ervoides* grow in Uganda and Ethiopia (Mishra *et al.,* 2007).

Nutritional values of lentil

Pulse not only contain protein twice than cereal, but also contain more than protein on weight basis than egg, fish and meat. For balanced diet optimum protein content is very much essential in our daily food with other component. It also contains a little amount of fat, carbohydrate, calcium, iron, riboflavin, carotene, vitamins and minerals.

Table 1: The Nutritional values of lentil

Energy	1,477 kJ (353 kcal)
Carbohydrates	60 g
Sugars	2 g
Dietary fiber	31 g
Fat	1 g
Protein	26 g
Water	10.4 g
Thiamine (vit. B_1)	0.87 mg (76%)
Folate (vit. B_9)	479 µg (120%)
Calcium	56 mg (6%)
Iron	7.54 mg (58%)

Source: USDA Nutrient Database.

Lentil utilization

The most valuable part of the lentil crop is seed which is primarily used as human food. Lentils are mostly used in split form as dhal or as flour for making soups, stews, bread and cakes (Williams and Singh, 1988; Aw-Hassan *et al*., 2003). Lentil products are mostly consumed in South Asia, Middle East and the Mediterranean region. Only red cotyledon type is used as food in Bangladesh, where it is boiled into soup-like dhal and eaten with rice. Khichuri is another popular dish, which is made from a mixture of split lentil seed and pounded rice, whereas lentil soup is preferred in Europe and North and South America. With endless opportunities, lentils can be used in many recipes to pack a healthy nutritional punch. Sprout seeds of lentil can be eaten as raw or cooked. They are a prominent ingredient of the raw food diet and common in Eastern Asian cuisine.

Lentil option for meeting environmental challenge

Excessive use of fertilizer in agriculture leads to a release of nitrogen and phosphorus into surface waters which results in water pollution. In fact, the use of nitrogen fertilizer contributes substantially to environmental pollution (Gard and Geetanjali, 2006).

Table 2: Nitrogen and phosphorus discharges to surface waters (in 10,000 Mg/yr) from nonpoint and point sources in the United States

Source	**Nitrogen**		**Phosphorus**
Nonpoint Sources			
Croplands	3204		615
Pastures	292		95
Rangelands	778		242
Forests	1035		495
Other rural lands	659		170
Other nonpoint sources	695		68
Total nonpoint sources	6663		1658
Total point sources	1495		330
Total discharge (nonpoint +	8158		2015
Nonpoint as percentage of	82%		84%

Source: Havens and Steinman, 1995, Gianessi *et al.,* 1986.

Note: Data were modified from the source.

Nonpoint pollution of surface water by nitrogen and phosphorus is primarily caused by agriculture and urban activities (Novotny and Olem, 1994). As shown on **Table 2**, pollution of surface water by nitrogen and phosphorus from nonpoint sources is more than point sources. Consequently, it is absolutely necessary to reduce the excessive use of fertilizer in agriculture. It has been noted that in wealthier nations, economic and environmental issues have resulted in the use of biological alternatives which can augment or replace the use of nitrogen fertilizers (Bohlool *et al.,* 1992). Reliance on the nitrogen fixing ability of legumes is one of the cheapest ways of reducing the use of nitrogen fertilizers and its attendant problems in surface waters. Studies have shown that biological nitrogen fixation (BNF) can act as a renewable and environmentally friendly source of nitrogen and can complement or replace the use of nitrogen fertilizer (Peoples *et al.,* 1995). Intercropping legumes and other species capable of symbiotic nitrogen fixation can serve as an economically attractive and ecologically viable means of reducing the external inputs of nitrogen fertilizers (Gard and Geetanjali, 2006).

Lentil and other legume crops are used in crop rotations to improve soil fertility and texture, thus increasing yields of subsequent crops (Wright, 1990; Gan *et al*., 2003). The positive effects of legume/cereal crop rotations are due to increased soil nitrogen content (Gan *et al*., 2003), elevated soil moisture levels and disease suppression (Stevenson and van Kessel, 1996). The use of lentil as a green manure crop increases soil nitrogen and carbon levels provides protection against erosion and improves soil quality compared with the traditional fallow-wheat cropping system (Biederbeck *et al*., 2005). The by-products of lentils such as leaves, stems, and bran also have a use as green manure or livestock feed (Yadav *et al*., 2007).

Lentil also plays a significant role in agriculture because of their ability to fix nitrogen from atmosphere in symbiotic association with bacterial like *Rhizobium sp*., Leguminous crops not only can fix the atmospheric nitrogen towards the benefit of crop but also save nitrate leaching during precipitation (Jones, 1939). Nitrogen fixations of various pulses in comparison with lentil are shown in **Table 3**.

Table 3: Nitrogen fixation rate of various pulses including lentil

Crops	Quantity of fixation N_2(kg/ha)	Equivalent to urea (kg/ha)
Lentil	**100**	**222**
Chickpea	115	256
Mung	105	233
Kheshari	100	222
Motor	95	221
Soybean	210	467
Arachis hypogea	150	333

Source: Satter, (1997)

Protein composition in seed of lentil

Lentil is a good source of carbohydrates, protein, most essential minerals and several vitamins but lentils seed contain great amount of proteins and amino acid. Lentil like all other pulses contains almost twice the amount of proteins as compared to cereal grains, most root crops, fruits and vegetables. The protein content of lentil seeds ranges from about 21 to 31% (Bhatty and Christison, 1984; Combe *et al*., 1991; Kavas and Nehir, 1992; Porres *et al.,* 2002; Amjad *et al*., 2003; Iqbal *et al*., 2006). Within lentil seeds, the cotyledon, embryo and seed coat contains 80-90%, 2% and 8-20% protein, respectively (Adsule *et al*., 1989; Cuadrado *et al*., 2002). Lentil proteins are generally stored in protein bodies (Wang and Daun, 2006) and mostly consist of salt-soluble globulins and water soluble albumins. The major globulins in lentils are legumins (11S) (44.8%) and vicilins (7S) (4.2%). Legumes contain higher amounts of sulphur-containing amino acids (methionine and cysteine) compared to the vicilins proteins (Bulter, 1982). Albumins constitute 16.8% of lentil proteins and are primarily composed of enzymes and protease inhibitors proteins and are contain high levels of cysteine and methionine. Glutelins and prolamins which are soluble in dilute acid/alkali detergents and alcohol constitute 11.2% and 3.5% of lentil proteins, respectively (Osborne, 1924; Gupta and Dhillon, 1993). The relative concentration of albumin to globulin and legumins to vicilins affect the amino acid profile and protein quality (Bulter, 1982). Ratios of 1:3 for albumin to globulin and 10.5:1 for legumins to vicilins have been reported for lentil (Gupta and Dhillon, 1993). Lentil proteins provide a good amino acid profile for human diet where most essential amino acids are well represented. The amino acids lysine, leucine, isoleucine,

phenylalanine, histidine, tyrosine, threonine and valine concentrations are present in higher quantities than FAO/WHO (1991) recommendations for human diet. However, lentil is deficient in tryptophan and sulphur-containing essential amino acids methionine and cysteine (Peace *et al*., 1988; Wang and Daun, 2006) **(Table 4)**. Legumes such as peanuts and soybean are often implicated in human food allergic reactions, whereas lentil allergies are less frequent (Pascual *et al*., 1999). Two allergens designated as Len c1 (12 to 16 kDa) corresponding to γ-vicilin subunits and Len c2 (66 kDa), seed-specific biotinylated protein, have been identified in boiled lentils (Sánchez-Monge *et al*., 2000).

Table 4: Amino acid composition of lentil

Essential amino acid:	**Concentration**	**FAO/WHO recommendation**
Histidine (His)	1.3 - 4.0	1.9
Isoleucine (Ile)	2.6 - 9.6	2.8
Leucine (Leu)	5.7 - 15.9	6.6
Lysine (Lys)	4.0 - 12.6	5.8
Threonine (Thr)	2.5 - 7.6	3.4
Tryptophane (Trp)	ND - 2.6	1.1
Valine (Val)	3.3 - 11.6	3.5
Phenylalanine (Phe)	3.6-10.6	
Methionine (Met) + Cysteine (Cys)	0.8 - 1.6	3.5
Arginine (Arg)	3.9 - 14.0	
Non-essential amino acid:		
Alanine (Ala)	2.4 - 39.8	
Aspartate (Asp) + Asparagine (Asn)	9.3 - 26.1	
Glutamate (Glu) + Glutamine (Gln)	12.8 - 42.3	
Glycine (Gly)	3.3 - 12.7	
Cysteine (Cys)	0.4 - 1.5	
Proline (Pro)	1.2 - 11.4	
Serine (Ser)	2.9 - 15.6	
Tyrosine (Tyr) + Phenylalanine	1.1-7.5	6.3

Source: Wang and Daun (2006), Grusak *et al.,* (2009) and Boye *et al*., (2010).

Production and consumption of lentil

Lentil production has been on the upward trend since its discovery. World lentil production increased from 2.76 million tons in 1997-1998 to 4.17 million tons in 2005-2006 (Agriculture and Agri-Food Canada, 2006). Asian countries constitute a dominant factor in lentils production. However, large volume of lentils produced in Asia never really translated into huge exports because of their high consumption rate. Meanwhile, high consumption of lentils in Asia is compensated for by a commensurate production in less consuming nations like Canada and the United States of America. There has been a sharp increase in the production of lentils in the world due in response to the growing demand. This fact is supported by a study which noted a 53% increase in the production of lentil during the 1980's until 1990 due to an increase in total area sown and overall yield per unit area (FAO, 1991). In spite of the paucity of data, it has been estimated that 75% of the world lentils production is the red type, 20% green type and 5% brown and other types . Canada and United states are the leading producers of the green lentils while red lentils are produced in other parts of the world (Agriculture and Agri-Food Canada, 2004). Lentils are mainly produced in India, Turkey, Canada, United States, Australia, Syria, Nepal, China, Bangladesh, Iran, and others. **Table 5** shows lentils production data between 2002 and 2006 for the producing countries.

Table 5: Lentil production data in thousand tones between 2002 and 2006

Country	**2002-2003**	**2003-2004**	**2004-2005**	**2005-2006**
India	974	880	1100	1000
Canada	354	520	962	1278
Turkey	565	540	540	560
USA	117	111	190	234
Australia	45	175	95	210
Syria	133	168	125	154
China	125	132	150	160
Nepal	148	150	159	161
Iran	117	120	125	125
Bangladesh	**115**	**116**	**122**	**122**
Others	197	194	199	165
Total	2890	3106	3767	4169

Source: Food and Agriculture organization, 2009.

In Bangladesh, lentil is traditionally grown during the dry winter months (rabi season) on residual soil moisture under rain fed conditions. Lentil faces serious competition with wheat, boro rice, oilseeds, potatoes and other profitable winter crops, particularly where irrigation is available. As a result, the crop has been pushed to marginal and sub-marginal lands.

The present per capita availability of pulses is only 16g daily, which is far below the recommended Bangladeshi diet of 30g. The present production of pulses is only 270000 M. tons. To meet up the demand, the government spends about 10298 million taka yearly to import 482487 M. tons pulses (BSS, 2007). If the current rate of per capita consumption of pulses is to be maintained, by the year 2015 the demand for pulses is estimated to be 670,000 tons. This means that the total production of pulses will have to increase by 25-37 per cent, a challenge that must be met either by increasing total production or increasing imports. Pulses requirement V.S. production for Bangladesh. (Fig. 1)

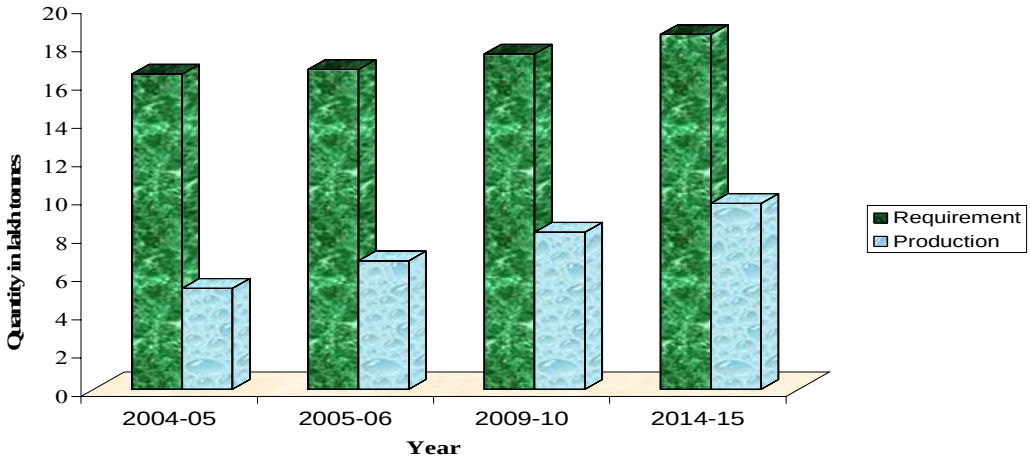


Figure 1: Pulse requirement V.S. production

Among legumes, pulses play an important role mainly for its food value and for nitrogen fixation into the soil. Leguminous crops not only can fix the atmospheric nitrogen towards the benefit of crops but also save nitrate leaching during precipitation (Jones,

1939). The crop area production and even the per hectare productivity of the major pulses in Bangladesh have been declined for quite a long time (Shaikh, 1977).

Rice is grown extensively throughout the year in Bangladesh, so all the major cropping patterns are rice-based, but vary widely depending on agro-ecological zone. The major lentil growing districts are greater Faridpur, Jessore, Kustia, Pabna, and Rajshahi **(Figure 2)**. Lentil is grown mainly as a mono-crop in Bangladesh, but mixed cropping and intercropping with wheat, mustard, linseed, sugarcane, and other crops is practiced in some areas (Miah and Rahman, 1993). In eastern Bangladesh, relay cropping in rice fields is practiced on a very small scale.

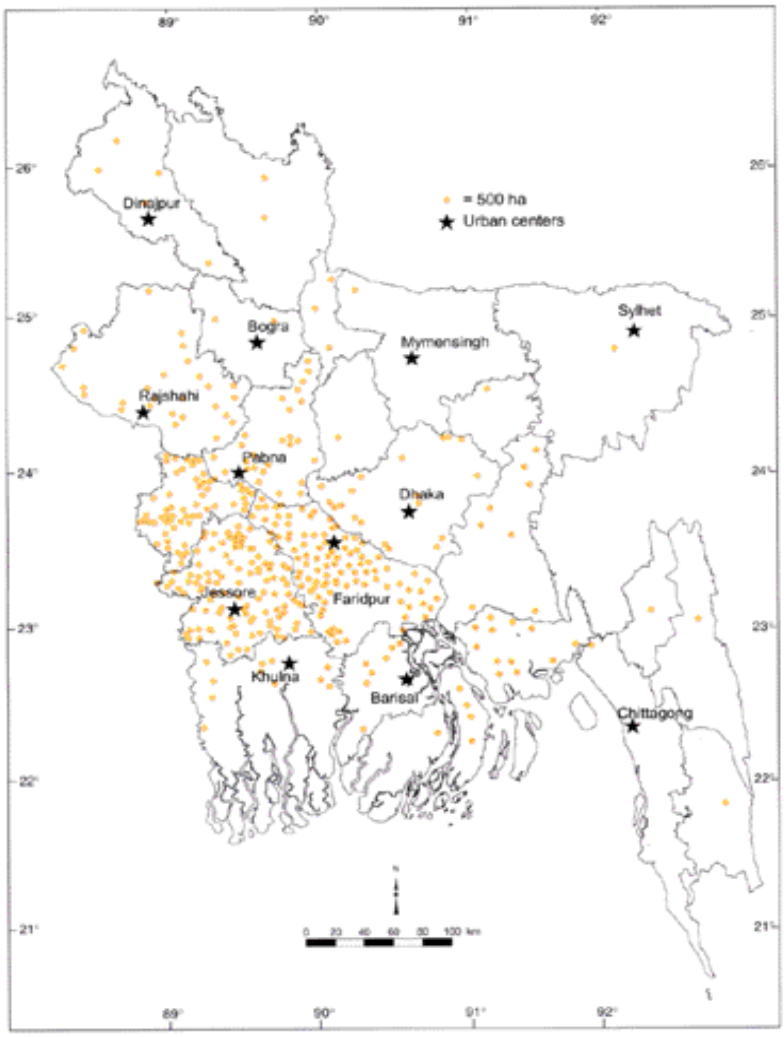


Figure 2: Lentil growing areas in Bangladesh

Germplasm collection of lentil

The International Center for Agricultural Research in Dry Areas (ICARDA, Aleppo, Syria) has a global mandate for lentil improvement and holds the largest lentil germplasm collection in the world. The ICARDA seed collection is entirely *ex situ* as seed and consists of 10,800 genotypes. Land races or cultivars collected from 70 different countries dominate the collection (8,860), followed by breeding lines (1,373), and wild accessions (583) from 24 different countries (Furman *et al.,* 2009) **(Figure 3).** Almost half of the collection (48%) comes from a region spanning Central and West Asia and North Africa, which is regarded as lentil's centre of origin and primary diversity (Zohary and Hopf, 1988; Ferguson and Erskine, 2001). A quarter of the ICARDA germplasm collection is from South Asia and the remaining 25% from the rest of the world. In addition to ICARDA collection, the lentil collections are located within the Australian Temperate Field Crops Collection (5,250 genotypes), USDA (2,797 genotypes), All-Russian Scientific Research Institute of Plant Industry collection (2,396 genotypes) and National Board of Plant Genetic Resource of India Collection (2,212 genotypes) (Diwiedi *et al.,* 2006).

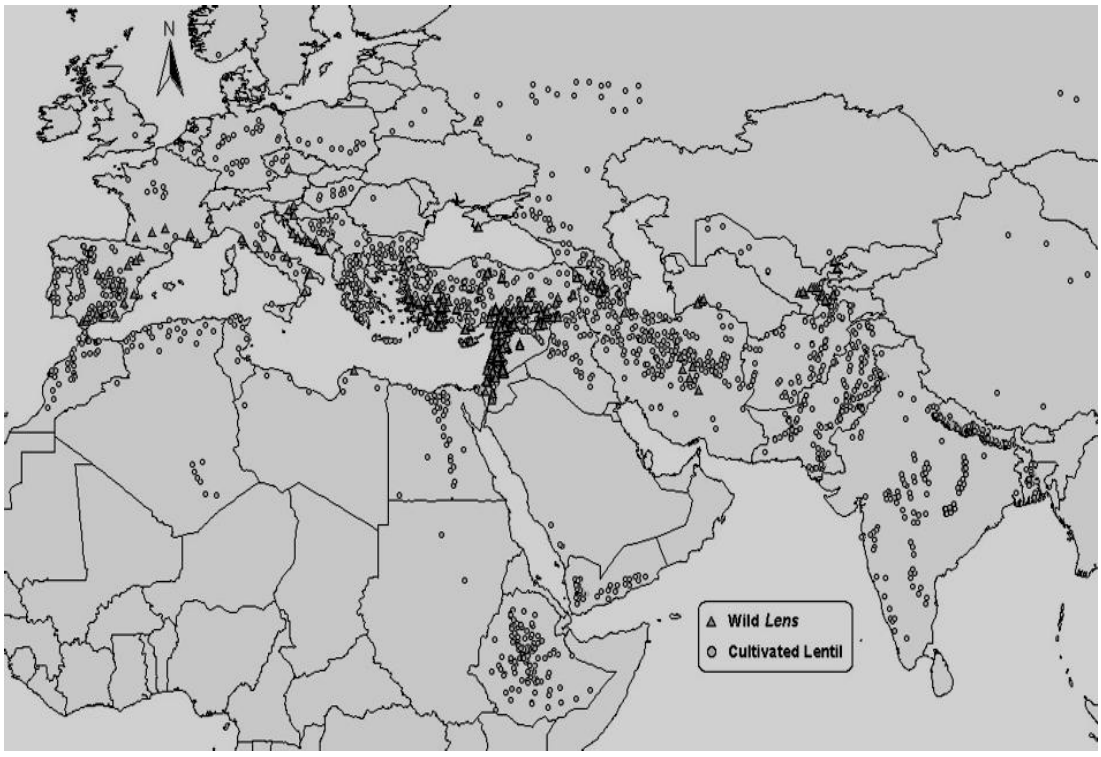


Figure 3: Geographical origin of ICARDA lentil germplasm collection

Various methods have been employed to estimate genetic diversity. An understanding of genetic variation within the lentil germplasm and of the genetic relationship between landraces is important because: (1) a broad genetic diversity can accelerate the genetic improvement of crops and (2) the identification of genetic variation is an effective method of stratifying and sampling variation in germplasm collections and can help to define priorities for conservation programs. Landraces are the most diverse populations of cultivated plants (Frankel *et al*., 1995). Besides being adapted to their environments, natural and man-made, landrace genotypes are co-adapted. Hence, genetic variation within a landrace may be considerable, but is far from random (Qualset *et al*., 1997). The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (Soleri and Smith, 1995). Knowledge of genetic variation and relationships between accessions or genotypes is important to understand the genetic variability available and its potential use in breeding program, to estimate any possible loss of genetic diversity, to offer evidence of the evolutionary forces shaping the genotypic diversities and to choose genotypes to be given priority for conservation (Thormann *et al*., 1994). Plant biodiversity must be safeguarded because it constitutes a resource of genes that may be used, for instance, in breeding programs. Quantitative characters are greatly influence by the environments with regards to phenotypic expression. Some genotypes may fair well in some environments but no so well in others (Dhillon *et al*., 1999). Selection of superior genotypes over a range of environments may be possible by stratification of environments. The present investigation was undertaken to study the nature of variability, and to select the stable lines with high yield potential. In this connection, the whole work of the present investigation was divided in to three parts as follows:

Part I: Deals with the Variability, correlation, Path-coefficient and Selection Index,

Part II: Deals with the genotype- environment interaction and

Part III: Deals with the Genetic Divergence using Morphological and Biochemical marker.

PART I

VARIABILITY, CORRELATION, PATH-COEFFICIENT AND SELECTION INDEX

INTRODUCTION

Plant breeding is one of the most important branches of research based on genetic variability. Any development throughout breeding program depends upon the magnitude of genetic variability available in the materials. Most of the economically important characters in any crops are quantitative in nature and show continuous variation which are due to the influence of polygene as well as environments. For this reason, quantitative characters cannot be studied following Mendelian classical technique of analysis and require special statistical methods. Several statistical methods have been developed for the studies of the inheritance of quantitative characters are necessary for the planning of effective breeding program in any crop.

The success of any breeding program for evolving superior genotypes depends upon the nature and magnitude of genetic variability and extent to which the desirable characters are heritable (Dudley and Moll, 1969). If the variability in the population is largely of genetic nature with least environment effect, the probability of isolating superior genotypes is high (Majid *et al*., 1982).

Study of the quantitative characters is done by the Biometrical techniques based on mathematical methods of Fisher *et al*., (1932). Partitioning the total variation into heritable and non-heritable components with the help of suitable genetic parameters, such as genotypic coefficients of variation, heritability and genetic advance under selection etc. are therefore, necessary. Quantitative characters are governed by a large number of genetic factors and are largely influenced by the environments. The genetic variability shown by plant characters can be measured by the genotypic co-efficient of variability. It is not only sufficient to determine the amount of heritable variation, the heritable portion of variation can also be measured by heritability estimates and genetic gains (Swarup and Chaugale, 1962). Some works are available regarding the variability which were done by recent past workers like Mian and Awal (1979), Singh *et al*., (1981), Sharma and Singh (1984), Mohamed *et al*., (1991),

Podder (1993) and Nahar and Khaleque (1996) in sugarcane and Deb (2002) in chickpea, Arshad *et al.,* (2003b) in chickpea, Kumar *et al.,* (2010) in mung bean.

The grain yield is the ultimate expression of various yield contributing trait and it also effected by environmental change. Therefore, direct selection for yield can be misleading. Moreover, the yield affecting characters are likely to be under much simpler genetic control and more highly heritable than total yield. For this reason, the knowledge of genotypic and phenotypic association within and between yield and yield contributing characters have great importance to plant breeders in the selection practices. It gives them more precision and accuracy in their works. The correlation coefficient measures the degree of association of yield and its components. Inclusion of more variables in correlation studies, where indirect effect become complex and important (Nandan and Pandaya, 1980). In this type of inconvenience, path-coefficient analysis further permits the partitioning of the correlation coefficient into components of direct and indirect causes of association. It also provides an efficient tool in finding out direct and indirect contributions of different contributing characters towards the yield. For simultaneous improvement of the component characters towards yield it will be helpful in effective selection. Several workers like Mannan and Ghafur (1983), Nair and Somarajan (1984), Reddy and Reddy (1986), Kang *et al.,* (1989), and Podder (1993) in sugarcane, Zubair and srinives (1986), Yaqoob *et al*., (1997) in mungbean, Hassan *et al.,* (2003) in mashbean, Ram *et al*., (1997) in green gram, Abbas (1999) in lentil, Ashok *et al*. (2000) in sunflower and Bakhsh *et al.,* (2006) and Hasanuzzaman *et al.,* (2007) in chickpea worked on correlation and path-coefficient analysis throughout their investigation.

Yield by itself is probably not an adequate criterion of economic worth as because yield is a quantitative character and is associated with other component characters which are influenced to varying degree by the fluctuations in the environmental conditions (Chaugale, 1967). Hence, selection based on that premise could easily lead to develop unsatisfactory plant type (Robinson *et al*., 1951). A complete satisfactory criterion based on discriminant function technique would be more desirable when a combination of two or more

characters with yield is studied in a selection index. The characters that show high positive genotypic correlation with yield may serve as basis for selection (Punia *et al*., 1983). The use of selection index technique would serve twofold purposes: (i) to bring about the genetic progress simultaneously in several characters and (ii) to improve the yield through selection for relatively more heritable auxiliary characters. The technique of discriminant function analysis first evolved by Fisher (1936) and adopted for plant selection by Smith (1936). Later on, different workers constructed selection indices for different crops. Such as Robinson *et al*., (1951) worked on corn; Caldwell and Weber (1965) on soybean; Paroda and Joshi (1970) on wheat; Nandan and Pandya (1980) in lentil; Samad (1991) on rape seed; Deb (2002) in chickpea; Khan (2009) in potato; Ferdous *et al*. (2010) in wheat and Ara (2010) in onion.

Objectives of the present study are as follow:

- To find out suitable lines with promising yield contributing quality through trial and then analyzing for variability, heritability and genetic advance.
- To find out direct and indirect effects through path- coefficient analysis.
- Construction of a suitable selection index using eleven quantitative characters in lentil.

REVIEW OF LITERATURE

For the future breeding research, it may be seen whether these affected the characters that in turn will influence high yield. The basic key to bring about the genetic upgrading to a crop is a sound breeding program utilizing the available genetic variability. Besides, the knowledge about yield and yield components and their relationship with each other and with yield, provide the basic information on yield improvement. For the reason, there is an effort to review of literatures on variability, heritability, genetic advance, correlation, path-coefficient and selection index has been made in lentil and also in other crops.

The development of genetics began with the rediscovery of Mendel's work in 1900. Fisher (1918) was the first to develop statistical method to partition variance due to quantitative character in segregating population into genetic and environmental components.

Robinson *et al.*, (1951) constructed a number of selection indices on corn and reported that results showing 14% more expected genetic progress in yield when selection is based entirely on ears per plant compared with selecting for yield alone. They suggested that since yield is a complex character and highly influenced by environmental variations, related character with higher heritability when properly weighted might well serve as better of indicators of the genetic yield potentialities of a progeny.

Ramanujam and Rai (1963) studied variability, correlation and path-correlation of yield and yield components in *Brassica campestris* L. var. yellow sarson. They found the largest coefficient of phenotypic and genotypic variability for secondary branches. In the analysis, most of the characters except yield per plant showed strong negative correlations among themselves. But positive correlation was found between number of pods per plant and primary and secondary branches.

Athwal and Gill (1964) made a comparative study of phenotypic and genotypic coefficients in gram and that the former values were not always the reflection of the magnitude of genetic association between characters.

Singh and Sharma (1964) studied *Cyamopsis tetragonloba* and showed that yield was highly correlated with pods per plant and branches per plant.

Hebert (1965) noticed very high correlation between stalk diameters and stalk weight and between stalk weight and yield per acre. He also obtained low negative correlation between cane yield and sucrose recovery.

Hanna and Hayes (1966) studied British bean and established correlation among number of pods, number of seeds, ratio of pods per flower and seed per flowers. They also found low heritability for all the characters except number of seeds per plant, where it was high.

Athwal and Sandhu (1967) worked on *Cicer arietinum* and found low phenotypic correlation between seed size and yield, but they noticed higher fixable genetic correlation. Seed size and seed number per pod was negatively correlated.

Chandra (1968) studied variability in gram and found that there was wide variation in ten yield component characters and that variability was affected by the environment were high. High heritability and high genetic advance were associated in case of primary branches and pods per plant indicating thereby the possibility of success for selection of these characters.

Chaudhari and Prashad (1968) made a study of genetic variability, heritability and genetic advance of the quantitative characters in *Brassica juncea* L. Czern and Cross. They observed that number of secondary branches had the highest amount of genetic variability. They obtained high heritability at the time of flowering and 1000-seed weight, while yield per plant showed the lowest heritability. The highest and the lowest genetic advances were found for 1000-seed weight and yield per plant, respectively.

Bhardwaj and Singh (1969) studied 54 varieties of brown sarson (*Brassica campestris* L. var. brown sarson) and found a wide range of variability in number of branches per plant, pods per plant, grains per pod and grain yield per plant. All the characters showed high heritability. Genetic coefficient of variation was reported to be higher for number of branches per plant, 100-grain weight and grain yield per plant.

Paroda and Joshi (1970) studied five quantitative characters and constructed selection indices for the different generation of wheat. They observed maximum gain (1950) when all the five characters were included in the discriminant function. Individually, except grain yield/plant, all of the component characters showed negative gains. When two or more characters included in a function the expected gains were positive and high when grain yield/plant was also included as an independent character.

Srivastova *et al.*, (1972) studied yield components in pea and recorded significant positive correlation between yield and days to flowering, between pod length and pod width and between pod length and seed number per pod. Significant and positive phenotypic correlations were recorded between yield and pods number or seeds number per pod. High heritability was estimated for days to flowering, pod length and pod width. Estimated genetic advance was the highest for number of seeds per pod.

Lal and Mehta (1973) studied eleven characters in 25 varieties of soybean and found medium values of heritability for number of branches, pods per plant and seeds per pod.

Singh and Singh (1974) used the discriminant function technique to construct a selection index for yield in 20 treatments in 3 crosses of Indian mustard. They reported that selection based on the number of primary branches, number of secondary branches, siliqua length and plant weight gave the highest relative efficiency. Selection based on single character, other than yield and number of primary branches was less effective than straight selection. In general, the more number of characters included in a selection index showed better performance.

Khaleque (1975) studied correlation, path-coefficient and selection index in rice and found that yield/plant correlated with most of the yield components, while negative or no correlation with yield was indicated by some of the characters .The discriminant function for selection was found to be superior over straight selection. Inclusion of yield in the function as an independent character is not essential. A combination of number of primary branches, spikelet number and kernel number may be used as selection index in the selection practices.

Miller *et al.,* (1978) constructed selection indices for four population of sugar cane and for combined data from the population. The average selection indices from combined data were applied for a test population. When selection for metric tons per ha of cane (TCH) was based on stalk length, stalk diameter and stalk number, expected genetic advance was 89% of that obtained when selection was based on TCH itself. When selection was for metric tons per ha of sucrose (THS), inclusion of brix in the index along with the above traits gave 92.1% of the expected genetic advance obtained for selection based on THS itself.

Nasker *et al.,* (1982) made a selection index analysis with the help of dispersion matrices of 10 cultivars of sunflower. They reported that maximum genetic gain was obtained when all the characters under study were considered together. Selection of component characters was found more profitable than selection for yield alone.

Kumar *et al.,* (1988) done correlation and discriminant function selection in Indian mustard. They reported that heritability estimate was found to be the lowest for yield/plant. siliqua/plant had the highest heritability (84.67) indicated the presence of additive gene action. The value of genotypic correlation was higher than the phenotypic correlation with primary branches, secondary branches and siliqua/plant. Among the yield contributing characters plant height had positive and significant correlation with primary branches and silique/plant, primary branches with secondary branches and silique/plant. The discriminant function selection showed that when two characters, silique/plant and secondary branches were considered, the maximum relative efficiency was obtained over straight selection.

Samad (1991) constructed selection indices using six agronomical characters in rapeseed (*Brassica campestris* L.) and reported that maximum expected gain was obtained when more characters were included in the discriminant function. In the discriminant function analysis seed yield per plant alone gave a negative expected gain, but in combination with two or more characters it showed the highest positive expected gain. He concluded

that seed yield was not complete character for higher yield rather it depends on other component characters for higher yield.

Paul and Nanda (1994) studied 14 varieties of rice for the construction of selection indices. The study revealed that adequate panicle density and optimum grain numbers/panicle must be achieved to obtain high yield. An appropriate number of panicles/m^2 had been as the basis of high yields. Selection indices were constructed on this assumption.

Nahar *et al.,* (2000) undertook an investigation for variability, heritability and genetic advance in ten sugarcane clones for eight quantitative characters. For heritability estimate which was found to be the highest for cane height (87.63 followed by cane diameter 77.80 and leaf area 73.29). The genetic advance as percentage of mean showed maximum value for leaf area (35.50) followed by cane height (27.47) cane yield/clump (14.96), cane diameter (12.93) and millable cane/clump (11.46).

Deb (2002) studied correlation, path-coefficient and selection index in six chickpea (*Cicer arietinum* L.) lines and found that significant correlation between PdW/P and SW/P, NS/P and SW/P. In path-coefficient analysis, he observed NPBFF, NSBFF, PWH, PdW/P and NS/P to be the most important yield component because they exhibited direct positive effect on SW/P both at phenotypic and genotypic levels. In the discriminant function analysis a combination of NPBFF, NSBFF, PHMF, NS/P and SW/P in an index gave the highest genetic gain in percent.

Ciftci *et al.,* (2004) conducted a research work to determine the relationship among yield and some of the yield components using correlation and path coefficient analysis. They used 14 chickpea cultivars designed in Randomized Block with three replications. They found positive and significant relationships among seed yield and plant height, number of branches, number of pods per plant, biological yield, harvest index and number of seeds per plant. Negative and non-significant relationship was determined between seed yield and 1000-seed weight. According to path coefficient analysis, they also found that there

were strong direct effects of the biological yield, harvest index and number of seeds per plant on the seed yield, p.c.: 0.783 and p.c.: 0.441, respectively.

Arshad *et al.,* (2004) conducted Variability, heritability, genetic advance, correlation coefficients and path coefficients for yield and its components for 24 advance lines of chickpea. He was noticed that high indirect contribution was via biological yield by most of the yield components and hence these two parameters (biological yield and harvest index) should be given more consideration while deciding about selection criteria of genotypes for rain fed conditions.

Bicer and Sakar (2004) studied the genetic variability and heritability of grain yield and yield components of 5 cultivars and 26 lines of lentil. The highest genetic variation was recorded for biological yield, grain yield and seed yield per plant. The highest heritability was recorded for seed weight and days to flowering.

Banerjee and Kole (2006) studied genetic variability in a population of 30 advance breeding lines of seasame. Phenotypic and genotypic coefficients of variability were high for plant height, branches plant^{-1}, capsules plant^{-1}, seeds capsule^{-1} and seed yield per plant and low for 1000-seed weight.

Talebi *et al.,* (2007) carried out an experiment on thirty six genotypes of chickpea for their yield performance. They suggested that selection for high seed yield should be based on biomass (biological yield) and harvest index in kabuli chickpea.

Singh *et al.,* (2007) found wide range of phenotypic (PCV) and genotypic coefficient of variation (GCV) as well as genetic advance as percent of mean for grain yield, number of nodules and number of pods per plant in lentil. Low variability and genetic advance were observed for 100-grain weight. Genetic advance for protein and methionine content ranged from 5.25 to 10.5%. Broad sense heritability ranged from 49.28 to 92.96% for the different characters. Correlation analysis revealed that number of pods per plant had positive and significant association with number of nodules per plant and grain yield per

plant. The association of protein content with methionine and grain yield was positive but not significant.

Tuncturk and Ciftci (2007) carried out an experiment to investigate the relationship between yield and some yield components of 16 oilseed rape cultivars (*Brassica napus ssp. oleifera* L.) by using correlation and path-coefficient analysis. These yield components suggested good selection criteria to improve seed yield in rapeseed breeding.

Gul *et al.,* (2008) conducted a study to determine correlation among different yield contributing traits of mungbean. Correlation was worked out among plant height, days to flowering, days to maturity, total dry weight plot^{-1}, yield plant^{-1}, 100-grain weight, harvest index and yield ha^{-1}. They found that significant differences were observed among different populations for all the parameters.

Togay *et al.,* (2008) conducted an experiment to determine the relationship among yield and some of the yield components using correlation and path-coefficient analysis. They used 12 pea genotypes in the experiment. The experiment was designed as randomized complete blocks with four replications. At the end of the study, positive and significant relationship were found among seed yield and pods per plant and biological yield in both years. The strongest and direct positive effects were the biological yield ($p = 0.6500$), numbers of pods per plant ($p = 0.3137$) and the seed yield. These were followed by first pod height ($p = 0.2398$) and number of seeds per pod ($p = 0.2227$).

Younis *et al.,* (2008) conducted a study to determine the genetic parameters and character association in elite lines of lentil (*Lens culinaris* Medik). Genetic parameters like genotypic and phenotypic variances, coefficients of variation, heritability, genetic advance, correlation coefficients and path-coefficients were estimated. Significant variation was noted for all the traits. High heritability estimates were observed for all the traits except number of primary branches per plant. In general phenotypic coefficients of variability were greater than their corresponding genotypic coefficient of variability. Higher estimates of heritability and genetic advance were observed for seed yield (97.10%, 90.71%), harvest index (96.20%, 63.29%) and maturity days (95.90%, 63.39%)

indicating that these characters are mainly controlled by additive genes and selection of such traits might be effective for the improvement of seed yield. Days to flower, plant height, number of primary branches, biological yield, harvest index and hundred seed weight had positive direct effect on seed yield. Biological yield, hundred seed weight and harvest index also had positive and highly significant genotypic and phenotypic correlation with seed yield. Hence these traits could be used for the improvement of seed yield resulting in the evaluation of high yielding varieties of lentil.

Khan (2009) studied correlation, path analysis and selection indices on twenty one yield and yield components of four high yielding varieties of potato (*Solanum tuberosum* L.). In most cases, the genotypic correlation was higher than that of corresponding phenotypic correlation suggesting that there was fairly a strong inherent relationship between the characters. Here X2 (NS/P) showed highly significant positive correlation both at phenotypic and genotypic levels with X7 (WT/P), X11 (NBST/P), X17 (WBST/P) and X18 (WNSST/P). The path-coefficient analysis indicated that the characters X6 (NT/P), X9 (NSST/P) and X13 (NSEST/P) exhibited high direct positive effect on X21 (Y/P) both at phenotypic and genotypic levels. The discriminant function for selection was found to be superior over straight selection. The highest expected genetic gain of 529800.43% was observed with six characters combination followed by five and four characters combination.

Deb *et al.,* (2009) made a study on correlation and path-coefficient to determine the contribution of different traits to seed yield in lentil (*Lens culinaris* Medic). In correlation analysis, they found that SWPP was positively correlated with all the characters but significantly correlated only with DFF, NPdPP, PdWPP and NSPP at genotypic levels. But at phenotypic level, SWPP significantly correlated only with NPdPP. Their path-coefficient analysis revealed that NPdPP and NSPP had the highest direct effect on SWPP both at phenotypic and genotypic levels. The second highest direct effect on SWPP was noted for PdWPP at phenotypic level and NPdPP at genotypic level. From this study they concluded that NPdPP and NSPP were the most important yield

components because they showed significant correlation with SWPP at genotypic level and highest direct positive effect on SWPP both at phenotypic and genotypic levels.

Ferdous *et al.,* (2010) conducted a study with twenty bread wheat genotypes at the experimental field of Bangladesh Agricultural University (BAU), Mymensingh, during the period from November 2008 to March 2009 and assessed the relationship and selection index among yield and important yield attributing characters. Days to maturity, grains per spike, 100-grain weight and harvest index showed significant and positive correlation with grain yield per plant. Path coefficient analysis suggested that grains per spike followed by 100-grain weight and effective tillers per plant contributed maximum to grain yield positively and directly. Thus, selection based on these characters might be effective for improving grain yield. Selection indices were constructed through the discriminant functions using eight characters. From the results, the highest relative efficiency was observed with the selection index based on three characters viz, plant height and grains per spike and grain yield per plant. The present investigation indicates that the selection index based on these three characters might be more effective and efficient for selecting high yielding wheat genotypes.

Jonah *et al.,* (2010) made a study on twelve cultivars of bambara groundnut those were sown for genetic correlation studies among agronomic characters and seed yield. The associations between seed yield and other quantitative characters showed positive correlation between seed yield per hectare, pod yield per plant and seed yield per plant. There was a significant genotypic and phenotypic correlation coefficient in the association between pod length and pod width, seed length and seed width during the trial, which could be a good index for selecting high yielding cultivars, as plump pods appeared to compensate for an increase in the total yield through a relatively greater weight of seeds. The path-coefficient analysis of characters showed that the seed yield per hectare indicated positive direct contribution with pod length, plant emergence at 2 WAS and stands count prior to harvest. Although these characters recorded a positive but a non-significant genotypic correlation coefficient of seed yield per hectare with other characters indicated the inefficiency of selection based on correlations alone.

Kumar *et al.,* (2010) studied by Genetic variability and character association in 23 genotypes of mung bean for different quantitative characters in *kharif* (summer or monsoon crop) 2007. In their study, analysis of variance revealed that there were highly significant differences among all the characters. Genotypes under study indicating the presence of sufficient amount of variability among the varieties. Thus there was ample scope for selection of different quantitative characters for crop improvement. They also found that the highest GCV and PCV were observed for harvest index and pods per plant, respectively. High estimates of genetic advance as percent of mean were observed for 100-seed weight and harvest index. Highly significant correlation was recorded for pods per plant and harvest index at both genotypic and phenotypic levels with seed yield per plant and plant height, primary branch per plant, clusters per branch and days to maturity had direct positive effect on seed yield.

Salehi *et al*., (2010) worked on bean (*Phaseolus vulgaris* L.) to evaluate the different effective traits on seed yield. Regression analysis indicated that the number of pods per plant was the only effective trait on seed yield. This trait explained 83.2% of total yield variations. Path analysis showed that the maximum direct and positive effects were related to number of seeds per pod and harvest index. The only direct and negative effect was related to pod length.

Sharma and Saini (2010) conducted a study with the view to elucidate the genetic variability, heritability, genetic advance, correlation and path analysis in chickpea. They found that the study revealed the presence of sufficient variability with high heritability for most of the yield components. Correlation and path analysis indicated that number of pods per plant and branches per plant could be useful as selection indices for the development of high yielding genotypes of chickpea.

Sarwar *et al*., (2010) computed genetic parameters, correlation coefficients, path-coefficients and cluster analysis in 42 true breeding lines of lentil F_5 generation of different cross combinations. Seed yield showed positive phenotypic correlation with grain filling period, plant height, branches per plant, pods per plant and harvest index.

Grain filling period, pods per plant and harvest index had positive direct effect along with positive genotypic correlation coefficient with seed yield. Hence, selection for these traits can be performed directly to improve seed yield in lentil. Maximum values of heritability were estimated for harvest index (99.40%) followed by grain filling period (87.40%) and plant height (74.80%). Higher values of genetic advance were observed for pods per plant (40.76%) and seed yield (39.74%).

Yucel and Anlarsal (2010) carried out a research work to determine selection criteria by using correlation and path-coefficient analysis in 22 genotypes of chickpea (*Cicer arietinum* L.) under Mediterranean conditions. They found positive and statistically significant relationships among seed yield and harvest index and seed number. Their study suggested that selection for high seed yield should be based on selecting plants having high harvest index in chickpea.

Ali *et al.,* (2011) conducted an experiment to estimate the correlation for quantitative traits in chickpea (*Cicer arietinum* L.). Correlation studies showed that bio-mass per plant, number of pods per plant, number of secondary branches per plant, number of seeds per pod and 100-seed weight were positive and significant at genotypic level but positive and highly significant at phenotypic level. Whereas, number of days taken to flowering, number of days taken to maturity, primary branches per plant, secondary branches per plant were positively correlated with the grain yield per plant at genotypic and phenotypic levels. Plant height was negative and non-significantly correlated with grain yield per plant at both genotypic and phenotypic levels.

Tadesse *et al.,* (2011) found the association among seed yield and related components due to lack of information on genetic diversity in Ethiopian faba bean (*Vicia faba* L.) germplasm. They grew fifteen genotypes at Sinana Agricultural Research Center and on two farmers' field at Sinja and Adaba, south Eastern Ethiopia in 2007-08 cropping season. At Sinana, they found that number of pods/plants, number of seeds/pod and plant height showed significant association with seed yield per plot. Whereas, At Adaba, thousand seed weight showed significant association with seed yield per plot. Path

analysis for seed yield per plot at Sinana indicated number of pod/plants, seeds per pod, thousand seed weight, stand percent and plant height had high positive direct effect at genotypic level. At Sinja, days to flower, days to maturity and number of pods/plant had positive direct effect on seed yield per plot whereas at Adaba stand percentage, days to flower, days to maturity, number of seeds/pod and thousand seed weight showed positive direct effect on seed yield per plot. Path analysis indicates that number of seeds/pod and thousand seed weight were the main determinants of yield per plot at Sinana and Adaba.

Tyagi and Khan (2011) carried out an experiment during winter (*rabi*) season of 2007 and 2008 to assess the correlation, path-coefficient and genetic diversity in 30 morphological diverse accessions of lentil (*Lens culinaris* Medik) under rainfed conditions. Days to 50% flowering, biological yield/plant, seed yield/plant and 100-seed weight showed significant differences and wide variations during both years. Low differences between phenotypic coefficient of variability and genotypic coefficient of variability were observed for all the descriptors during both years. Pods/plant, days to 50% flowering, biological yield/plant, seed yield/plant and 100-seed weight in both the years showed high heritability coupled with high genetic advance (per cent of mean) signifying the influence of additive gene effects. The characters viz., biological yield/plant and number of primary branches/plant showed positive and significant correlations with seed yield/plant and exerted positive and high direct effects on seed yield/plant in both years.

Zali *et al.,* (2011) studied to determine the association between genetic parameters and traits in chickpea (*Cicer arietinum* L.) genotypes. Heritability values were greater for number of days to 50% maturity (98.43%), number of days to 50% flowering (98.19%), plant height (58.87%), number of secondary branches (45.81%), number of primary branches (42.03%) and number of seeds per plant (35.42%), indicating that these traits are controlled mainly by additive genes and that selection of such traits may be effective for improving seed yield. Number of seeds per plant and 100-seed weight had a positive direct effect on seed yield. Number of seeds per plant, number of secondary branches, 100-seed weight, number of pods per plant, number of primary branches and plant height

also had positive and highly significant phenotypic correlations with seed yield. Stepwise regression analysis indicated that number of seeds per plant and 100-seed weight explained 96% of total yield variation. It can be concluded that seed yield in chickpea can be improved by selecting an ideotype having greater number of secondary and primary branches, as well as higher number of pods per plant, number of seeds per plant and 100-seed weight.

Udensi and Ikpeme (2012) worked on pigeon pea for the analysis of correlation coefficients and path-coefficients. Correlation results revealed that there was significant positive correlations between plant height plant^{-1} and number of leaves plant^{-1} (0.926**), leaf area plant^{-1} (0.574*) and number of seeds plant^{-1} (0.616*). It also showed that the number of leaves plant^{-1} was positively correlated with the pod length plant^{-1} (0.589*) and number of seeds plant^{-1} (0.682*). Leaf area plant^{-1} had a positive significant association with the number of seeds plant^{-1} (0.581*). Additionally, pod length plant^{-1} correlated positively with the number of seed plant^{-1} (0.850**) while number of nodules plant^{-1} correlated positively with 100-seed weight (0.804**). Path-coefficient results showed that 100-seed weight had the highest direct effect on yield (0.583), which was positive. This was followed by the pod length plant^{-1} (0.519), number of leaves (0.452), and leaf area (0.252) while plant height plant^{-1} had negative direct effect but very high (-0.633). Number of pod plant^{-1} had the lowest direct effect on yield (0.033). Additionally, genotypic correlation coefficient with yield showed very high coefficients, especially for pod length plant^{-1} (0.827), 100-seed weight (0.798), number of leaves plant^{-1} (0.644), plant height plant^{-1} (0.582) and leaf area plant^{-1} (0.549), respectively. Number of nodules plant^{-1} had the lowest genotypic correlation coefficient (0.042) followed by number of flowers plant^{-1} (-0.063). The two results from correlation and path-coefficient analyses strongly suggest that plant height plant^{-1}, number of leaves plant^{-1}, leaf area plant^{-1} and pod length plant^{-1} and 100- seed weight should be considered as indices for selecting high yielding pigeon pea genotypes, especially the landraces.

MATERIALS AND METHODS

A. MATERIALS

The materials for the present study comprised six genotypes of lentil. The materials were collected from Regional Agricultural Research Station, Ishurdi, Pabna, Bangladesh. The six lentil genotypes are as follows:

Serial No.	Genotype	Ac. No.
1	BARI Masur-1	Bm1
2	BARI Masur-2	Bm2
3	BARI Masur-3	Bm3
4	BARI Masur-4	Bm4
5	BARI Masur-5	Bm5
6	BARI Masur-6	Bm6

In the present investigation, six varieties of lentil were considered as plant materials. Their major characteristics are presented in below.

Table 6: Six lentil varieties with their major characteristics

Variety	Year of release	Source of origin	Crop duration (days)	Yield Potentiality (Kg/ha)	Remarks
BARI masur-1	1991	Bangladesh	110	1300-1500	Less susceptible to rust and *stemphylium* blight than the local cultivars.
BARI masur-2	1993	ICARDA	110	1800	High level of resistance to rust
BARI masur-3	1996	Bangladesh	115	2000	Resistant to rust
BARI masur-4	1996	ICARDA	116	2300	Resistant to rust and *stemphylium* blight diseases.
BARI masur-5	2006	BARI-ICARDA	110	2200	Resistant to *stemphylium* blight and Rust, Tolerant to foot rot, moderately resistant to aphid
BARI masur-6	2006	BARI-ICARDA	110	2250	Resistant to *stemphylium* blight and rust, tolerant to foot rot, moderately resistant to aphid

Source: Afzal *et al.,* 1999 and Uddin *et al.,* 2008.

Figure 4: (A, B, C, D, E and F): BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5 and BARI masur-6

B. METHODS

The methods adopted to conduct the experiment and analyse the data are divided into the following sub-heads:

1. Preparation and Design of the Experimental Field
2. Sowing of Seeds
3. Maintenance of the Experimental Plants
4. Collection of Data
5. Techniques of Analyses of Data

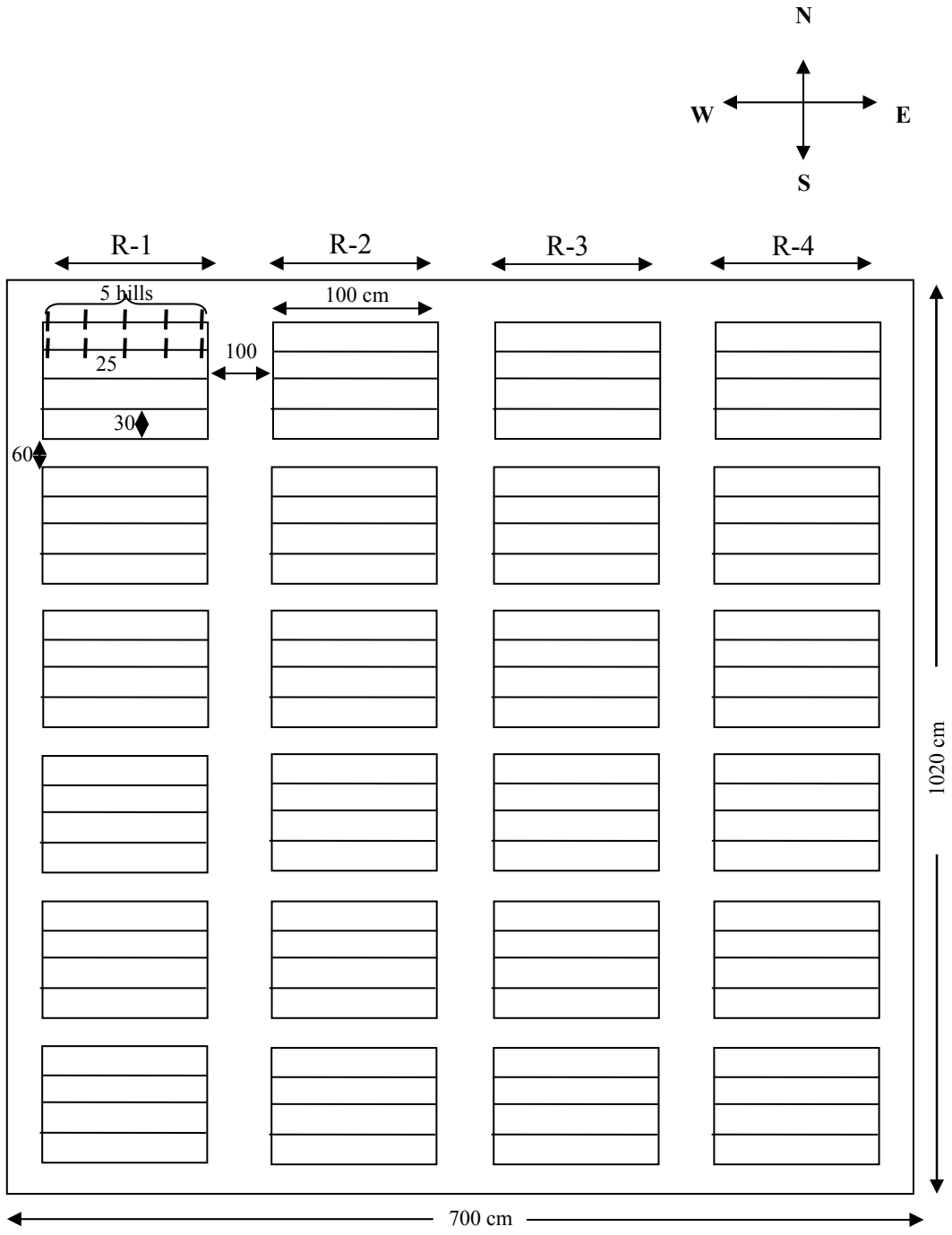
1. Preparation and Design of the Experimental Field

a. Preparation of the Experimental Field: The experimental field was set within the field behind Third Science Building of Rajshahi University, during the consecutive three Rabi crop seasons of 2009-2010, 2010-2011 and 2011-2012. The experimental field was ploughed six times repeatedly. Weeds were removed completely before layout of the field and sowing of the seeds. The field was well-pulverized and leveled properly. No chemical fertilizer was used before or after sowing of seeds. As the experimental field was sufficiently moist, no irrigation was given before sowing of the seeds. Thus, prepared experimental field was ready for sowing of the seeds.

b. Field Design: Layout of the experimental field and trial of the irradiated lines was conducted following randomized complete block design (RCBD). The experimental field was comprised an area of 700 x 1020 sq. cm. The field was consisted of four replications. Each replication contained six plots. Each plot was consist of five rows and each row having 5 hills. Space between replications were 100 cm. Gaps between plots, rows and hills were 60 cm, 30 cm and 25 cm, respectively. The seeds of six lines were randomly assigned to the rows of plots. In each hill, only one plant was maintained.

2. Sowing of Seeds

The seeds of eight genotypes were sown in the experimental field according to design on the 30th October, 2009, 30th October 2010 and 30th October, 2011.



Having 4 replication Per row having 5 hills Gap between row 30 cm.
Per replication having 6 plots Gap between block 100 cm. Gap between hills 25 cm.
Per plot having 5 rows Gap between plot 60 cm.

Figure 5: Individual plant randomization in the completely randomized block design for lentil

Plate 1: Flowering stage of lentil in research field.

Plate 2: Green pod stage of lentil in research field.

Plate 3: Semi-mature pod stage of lentil in research field.

Plate 4: Mature pod stage of lentil in research field.

3. Maintenance of the Experimental Plants

When the seedlings were 7-8 cm in heights, the excess seedlings were removed from the experimental field and regular weeding was done. As the soil of the experimental field as moist sufficiently throughout the crop season, no irrigation was given. The insecticides were sprayed at two or three times of the total life cycle of this plant whenever it was necessary.

4. Collection of Data

The data were collected and recorded on individual plant basis of six genotypes of lentil. The measurement of a character was done following C.G.S system. Eleven yield and yield contributing characters which are quantitative in nature were considered for the present investigation. The characters studied are as follows:

a) Date of first flower (DFF): Data of first flower was recorded on the opening of first flower in each of the plant.

b) Plant height at first flower (PHFF): Height of the individual plant was recorded from the base of the stem to the top of the plant at the time of first flowering stage.

c) Number of primary branches at first flower (NPBFF): The total number of primary branches at first flower per selected plant was counted and recorded.

d) Plant height at maximum flowers (PHMF): The plant height was measured in cm from the base of the stem to the tip of the plant at the maximum flowering stage.

e) Number of primary branches at maximum flowering (NPBMF): The total number of primary branches at maximum flower per selected plants was counted and recorded.

f) Number of secondary branches per plant at maximum flower (NSBMF): Secondary branches came out from the primary branches and total number of secondary branches of the individual plant at the time of maximum flowering stage was counted and recorded.

g) Plant weight per plant (PWPP): Weight of each plant was taken after completely drying then recorded.

h) Number of pods per plant (NPdPP): All the pods of the individual plant after harvesting were removed, counted and recorded.

i) Pod weight per plant (PdWPP): All the pods of the individual plant were weighted and recorded.

j) Number of seeds per plant (NSPP): All the pods of an individual plant were threshed and seeds were taken out from the pods and cleaned, then the total number of seeds was counted and recorded.

k) Seed weight per plant (SWPP): Total seeds of the individual plant were weighted and recorded.

5. Techniques of Analyses of Data

The collected data were analyzed following the biometrical techniques of analysis as developed by Mather (1949) based on the mathematical models of Fisher *et al*., (1932). The techniques used were described under the following sub-heads:

a) Mean: Data on individual plant was added together then divided by the total number of observations and the mean was obtained as follows:

$$\text{Mean } (\bar{X}) = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where,

X = The individual reading was recorded from each plant.

n = Number of observations.

i = 1, 2, 3…….n

∑= Summation.

b) Standard deviation (σ): Standard deviation is the dispersion of individuals values (*x*) around the population mean (μ).It was calculated as the square root of the variance as follows:

$$S = \sqrt{S^2}$$

Where,

S = Standard deviation

S^2 = Variance

c) Standard error of mean: Dispersion of family means around the experimental or estimated population mean is standard error of mean. The standard error of mean are determined as follows:

$$S_{\bar{x}} = \frac{S}{\sqrt{n}}$$

Where,

$S_{\bar{x}}$ = Standard error of mean

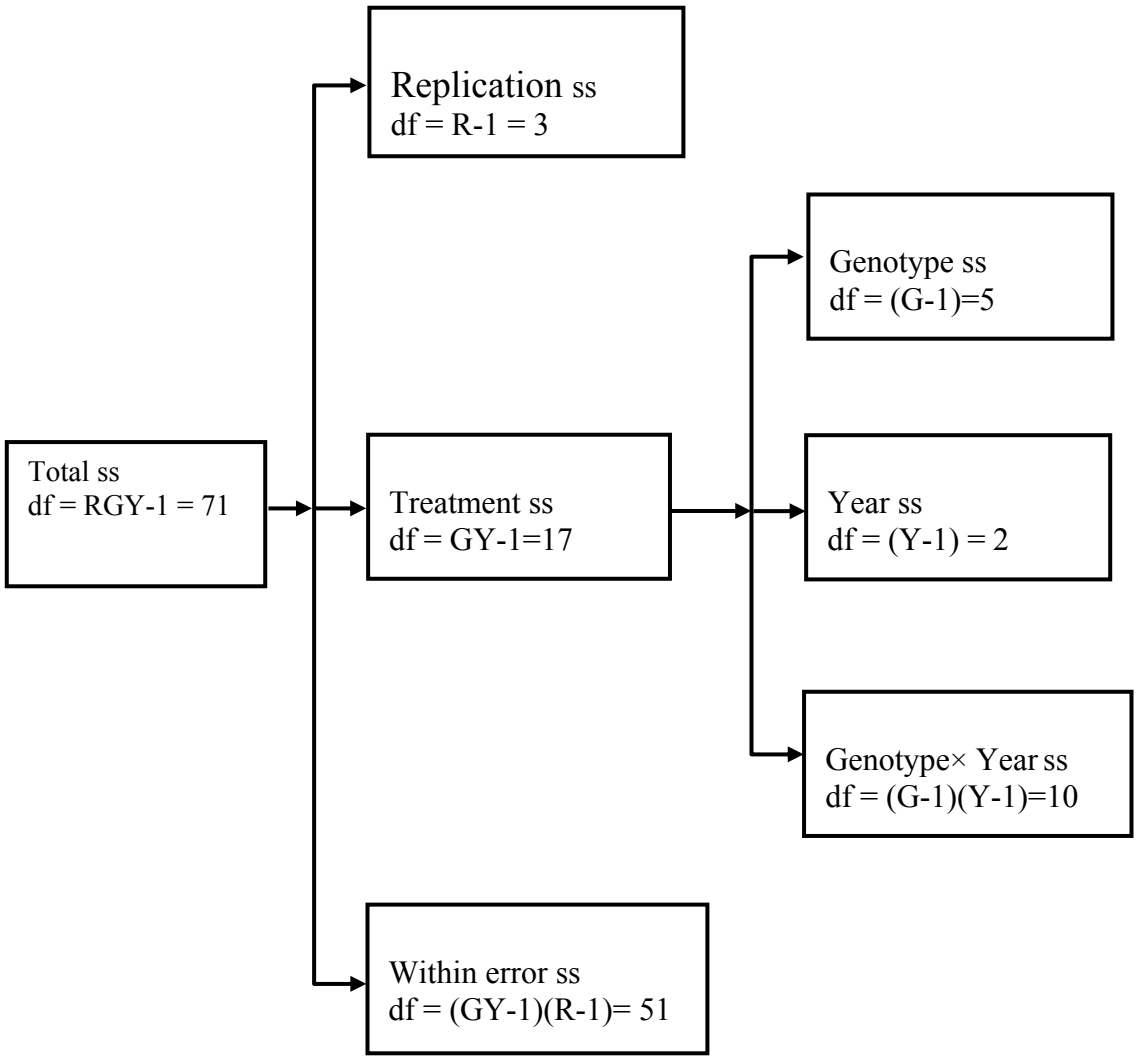
S = Standard deviation

n = Total number of individuals.

Standard error of mean gives an idea as to how any mean obtained from a sample may differ from the true hypothetical means of the population.

d) Analysis of variance: Variance is a measure of dispersion of a population. So, the analysis of variance is done for testing the significant differences among the populations. Variance analysis for each of the characters was carried out separately with raw data taken on individual plants.

The variances due to different sources such as replication (R), genotype (G), Year (Y), genotype × year (G×Y) and within error of a population were calculated as per the following skeleton of analysis.



Where,

Genotype (G) = 6

Replication (R) = 4

Year (Y) = 3

The plant to plant variance of a population was calculated according to the following formula:

$$S^2 = \frac{\sum_{i=1}^{n} Xi^2 - \left(\sum_{i=1}^{n} Xi\right)^2 /n}{n-1}$$

Where,

S^2 = Variance

X= The individual reading recorded on each plant

n = The total number of individuals.

∑= Summation

i = 1, 2, 3…….n

n-1= Degrees of freedom.

Furthermore where,

$$\text{Total}_{SS} = \sum (R_i G_j Y_k)^2 - CF$$

$$\text{Replication}_{SS} = \frac{\sum_i R_i^2}{G_j} - CF$$

$$\text{Treatment}_{SS} = \frac{\sum_{jk} (G_j Y_k)^2}{R_i} - CF$$

$$\text{Error}_{SS} = \text{Total}_{SS} - \text{Treatment}_{SS}$$

$$\text{Genotype}_{SS} = \frac{\sum_j G_j^2}{Y_k R_i} - CF$$

$$\text{Year}_{SS} = \frac{\sum_k Y_k^2}{G_j R_i} - CF$$

$$G \times Y_{SS} = \text{Treatment}_{SS} - \text{Genotype}_{SS} - \text{Year}_{SS}$$

Where,

R_i = The value of jth replication

G_i = The value of ith genotype

Y_k = The value of ith genotype

$G_j Y_k$= The value of jth genotype in kth Year

CF = Correction factor = $(GT)^2 / N$

GT = Grand total

N = Total number of observations = (RGY)

The analysis of variance of a mixed model was used, where genotype (G) was fixed and Year (Y) effect was random. The expectation of mean square (EMS) was derived

as follows.

Table 7: Analysis of variance

Item	df	MS	EMS
Replication(R)	R-1	MS_1	$\sigma^2_E + GY\sigma^2_R$
Genotype(L)	G-1	MS_2	$\sigma^2_E + R\sigma^2_{GY} + RY\sigma^2_G$
Year (Y)	Y-1	MS_3	$\sigma^2_E + RG\sigma^2_Y$
Genotype × Year (G×Y)	(G-1)(Y-1)	MS_4	$\sigma^2_E + R\sigma^2_{GY}$
Error	(GY-1)(R-1)	MS_5	σ^2_E

Where,

G = Genotype

R = Replication

Y = Year

MS_1= Represents mean square of replication.

MS_2= Represents mean square of genotype.

MS_3= Represents mean square of year

MS_4= Represents mean square of G×Y

MS_5= Represents mean square of error

$RY\sigma^2_G$ = Variance due to genotype

$GY\sigma^2_R$ = Variance due to replication

$R\sigma^2_{GY}$ = Variance due to G×Y

σ^2_E = Variance due to within error

e) Components of variation: The phenotypic (σ^2_P), genotypic (σ^2_G), interaction ($\sigma^2_{GY,}$) and error (σ^2_E) variances were determined as follows:

Step-I:

$\sigma^2_G = (MS_2 - MS_4)/RY$

$\sigma^2_Y = (MS_3 - MS_5)/RG$

$\sigma^2_{GY} = (MS_4 - MS_5)/R$

$\sigma^2_E = MS_5$

Step-II:

Phenotypic variance (σ^2_P) = $\sigma^2_G + \sigma^2_{GY} + \sigma^2_E$

Genotypic variance (σ^2_G) = σ^2_G

Genotype × replication variance = σ^2_{GY}

Error variance = σ^2_E

f) Co-efficient of variability (CV): Deviation is also expressed by coefficient of variation given by the formula of Burton and De Vane (1953) as follows:

$$CV = \frac{S^2}{\overline{X}} \times 100$$

Co-efficient of variability at different levels was calculated as follows:

a) Phenotypic coefficient of variability (PCV) $= \frac{\sigma_P^2}{\overline{X}} \times 100$

b) Genotypic coefficient of variability (GCV) $= \frac{\sigma_G^2}{\overline{X}} \times 100$

c) Error coefficient of variability (ECV) $= \frac{\sigma_E^2}{\overline{X}} \times 100$

Where,

$\overline{X}$ = Grand mean

σ^2_P = Phenotypic variance

σ^2_G = Genotypic variance

σ^2_E = Error variance

g) Heritability (h^2_b): Heritability (in broad sense) estimates was computed by dividing the genotypic variance with phenotypic variance and then multiplying by 100 as suggested by Warner (1952).

$$h^2_b = \frac{\sigma_G^2}{\sigma_P^2} \times 100$$

Where,

h^2_b = Heritability in broad sense

σ^2_P = Phenotypic variance

σ^2_G = Genotypic variance

h) Genetic Advance (GA): Genetic advance was calculated by the following formula as suggested by Lush (1949)

$$GA = K \times \sigma p \times h^2_b$$

Where,

K = The selection differential in standard units for the present study it was 2.06 at 5% level of selection (Lush, 1949).

σp = Square root of the phenotypic variance

h^2_b = Broad sense heritability

i) Genetic advance expressed as percentage of mean (GA%): It was calculated by the following formula:

$$\text{GA\% of Mean} = \frac{\text{GA}}{\overline{\overline{\text{X}}}} \times 100$$

Where,

$\overline{\overline{\text{X}}}$ = Grand mean for a particular character.

j) Analysis of covariance: For the purpose of correlation coefficients and path coefficients, the analysis of both variance and covariance are required (Miller *et al.,* 1958). Nevertheless, covariances were calculated between all possible pairs of characters separately. For the analysis of covariance the raw data of individual plant were used according to the following formula.

$$\text{COV} = \frac{\sum_{i=1}^{n} \text{XiYi} - \left\{\left(\sum_{i=1}^{n} \text{Xi}\right)\left(\sum_{i=1}^{n} \text{Yi}\right)\right\}/\text{n}}{\text{n} - 1}$$

Where,

COV = Covariance

$\sum_{i=1}^{n} \text{XiYi}$ = Sum of the X and Y

$\sum_{i=1}^{n} \text{Xi}$ = Grand total of X

$\sum_{i=1}^{n} \text{Yi}$ = Grand total of Y

n = Number of observation

n-1= Degrees of freedom

i = 1, 2, 3…….n

∑= Summation

The expectation of mean cross product (MCP) was derived as follows:

Table 8: Analysis of covariance.

Item	Df	MS	MCP
Replication(R)	R-1	MCP_1	$\sigma^2_E + GY\sigma^2_R$
Genotype(G)	G-1	MCP_2	$\sigma^2_E + R\sigma^2_{GY} + RY\sigma^2_G$
Year (Y)	Y-1	MCP_3	$\sigma^2_E + RG\sigma^2_Y$
Genotype × Year (G×Y)	(G-1)(Y-1)	MCP_4	$\sigma^2_E + R\sigma^2_{GY}$
Error	(G-1)(R-1)	MCP_5	σ^2_E

Where,

MCP_1= Mean cross product of replication.

MCP_2= Mean cross product of genotype.

MCP_3= Mean cross product of year

MCP_4 = Mean cross product of G×Y

MCP_5 = Mean cross product of error

$GY\sigma^2_R$ = Covariance due to replication

$RY\sigma^2_G$ = Covariance due to genotype

$GR\sigma^2_Y$ = Covariance due to year

$R\sigma^2_{GY}$= Covariance due to G×Y

σ^2_E = Covariance due to error

k) Components of covariation: The phenotypic (σ^2_P), genotypic (σ^2_G), interaction (σ^2_{GY}) and error (σ^2_E) covariances were measured as follows:

Step-I:

$\sigma^2_G = (MCP_2 - MCP_4)/RY$

$\sigma^2_Y = (MCP_3 - MCP_5)/RG$

$\sigma^2_{GY} = (MCP_4 - MCP_5)/R$

$\sigma^2_E = MCP_5$

Step-II:

Phenotypic variance (σ^2_P) = $\sigma^2_G + \sigma^2_{GY} + \sigma^2_E$

Genotypic variance (σ^2_G) = σ^2_G

Genotype × replication variance = σ^2_{GY}

Error variance = σ^2_E

l) Correlation coefficient: The correlation coefficient at phenotypic (r_P) and genotypic (r_g) levels were computed as follows:

$$r_P = (\sigma^2_{P12}) / (\sigma^2_{P11} \times \sigma^2_{P22})^{1/2,}$$

$$r_g = (\sigma^2_{G12}) / (\sigma^2_{G11} \times \sigma^2_{G22})^{1/2,}$$

Where,

σ^2_{P12} and $\sigma^2_{G12,}$ represent covariances at phenotypic and genotypic levels, respectively for characters 1 and 2.

σ^2_{P11} and σ^2_{G11} indicate variances at phenotypic and genotypic levels, respectively for character 1.

σ^2_{P22} and σ^2_{G22} represent variances at phenotypic and genotypic levels, respectively for character 2.

m) Path-coefficient: The path-coefficient analysis was done by using Wright's (1921 & 1923) formula as was extended by Dewey and Lu (1959). The path-coefficient analysis was carried out both at phenotypic and genotypic levels were obtained by solving a set of simultaneous equations as follows.

$$r_{xy} = P_{xy} + r_{x2} P_{2y} + r_{x3} P_{3y} + r_{x4} P_{4y} + r_{x5} P_{5y} + r_{x6} P_{6y} + r_{x7} P_{7y} + r_{x8} P_{8y} + r_{x9} P_{9y} + r_{x10} P_{10y} + r_{x11} P_{11y}$$

Where, the terms like

r_{xy} = Correlation between one component character and yield.

P_{xy} = Path coefficient between the same character and yield

$r_{x2,}$ $r_{x3.....}$ r_{xn} = Correlation between the same character and one of the remaining yield components in turn.

The relationship used in this study for yield and yield components were as follows:

1. $r_{1y} = P_{1y} + r_{12} P_{2y} + r_{13} P_{3y} + r_{14} P_{4y} + r_{15} P_{5y} + r_{16} P_{6y} + ...+ r_{111} P_{11y}$
2. $r_{2y} = P_{2y} + r_{21} P_{1y} + r_{23} P_{3y} + r_{24} P_{4y} + r_{25} P_{5y} + r_{26} P_{6y} + ...+ r_{211} P_{11y}$
3. $r_{3y} = P_{3y} + r_{31} P_{1y} + r_{32} P_{2y} + r_{34} P_{4y} + r_{35} P_{5y} + r_{36} P_{6y} + ...+ r_{311} P_{11y}$
4. $r_{4y} = P_{4y} + r_{41} P_{1y} + r_{42} P_{2y} + r_{43} P_{3y} + r_{45} P_{5y} + r_{46} P_{6y} + ...+ r_{411} P_{11y}$
5. $r_{5y} = P_{5y} + r_{51} P_{1y} + r_{52} P_{2y} + r_{53} P_{3y} + r_{54} P_{4y} + r_{56} P_{6y} + ...+ r_{511} P_{11y}$
6. $r_{6y} = P_{6y} + r_{61} P_{1y} + r_{62} P_{2y} + r_{63} P_{3y} + r_{64} P_{4y} + r_{65} P_{5y} + ...+ r_{611} P_{11y}$
7. $r_{7y} = P_{7y} + r_{71} P_{1y} + r_{72} P_{2y} + r_{73} P_{3y} + r_{74} P_{4y} + r_{75} P_{5y} + ...+ r_{711} P_{11y}$

8. $r_{8y} = P_{8y} + r_{81} P_{1y} + r_{82} P_{2y} + r_{83} P_{3y} + r_{84} P_{4y} + r_{85} P_{5y} + \ldots\ldots\ldots\ldots + r_{811} P_{11y}$

9. $r_{9y} = P_{9y} + r_{91} P_{1y} + r_{92} P_{2y} + r_{93} P_{3y} + r_{94} P_{4y} + r_{95} P_{5y} + \ldots\ldots\ldots\ldots + r_{911} P_{11y}$

10. $r_{10y} = P_{10y} + r_{101} P_{1y} + r_{102} P_{2y} + r_{103} P_{3y} + r_{104} P_{4y} + r_{105} P_{5y} + \ldots + r_{1011} P_{11y}$

11. $r_{11y} = P_{11y} + r_{111} P_{1y} + r_{112} P_{2y} + r_{113} P_{3y} + r_{114} P_{4y} + r_{115} P_{5y} + \ldots + r_{1111} P_{11y}$

Where,

y, represent seed weight per plant (SWPP). The numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 represent date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF). number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), Plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP).

xiv. Residual effect (X) = $1 - R^2$

Where,

$$R^2 = P_{1y} r_{1y} + P_{2y} r_{2y} + \ldots\ldots\ldots\ldots\ldots\ldots + P_{ny} r_{ny}$$

n) Selection index: The coefficients b_1, b_2,……b_n used in the discriminant function technique were obtained from the genotypic and phenotypic variances and covariances by solving the following equations of an index simultaneously. Similar equations were set up for each index and the values obtained for b_1, b_2, ……b_n were used in the discriminant function selection technique.

$b_1P_{11} + b_2P_{12} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + b_nP_{1n} = G_{1y}$

$b_1P_{12} + b_2P_{22} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + b_nP_{2n} = G_{2y}$

$b_1P_{1n} + b_2P_{2n} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + b_nP_{nn} = G_{ny}$

Where,

P_{11}=an estimate of the phenotypic variance of character 1

P_{12}=an estimate of phenotypic covariance of characters 1 and 2

G_{1y}, G_{2y}..... G_{ny} =an estimate of genotypic covariance of character 1 and yield (seed weight per plant), etc.

The phenotypic and genotypic variances and covariances as obtained were used for constructing discriminant functions using different character combinations according to the method developed by Fisher (1936) and Smith (1936). Later on, Hazel developed a simultaneous selection model following path analysis approach. Since then, the theory of selection index has been extended and modified in various ways by various authors to suit the requirements of practical breeders (Robinson *et al*., 1951; Singh, 1972). The expected genetic advance from strait selection [GA (S)] and from the dicriminant function [GA (D)] was calculated as follows.

$$GA(S) = \frac{Z}{P}\left(\frac{g_{yy}}{\sqrt{t_{yy}}}\right) \quad \text{and}$$

$$GA(D) = \frac{Z}{P}\sqrt{\left(b_1 g_{1y} + b_2 g_{2y} + ... + b_n g_{ny}\right)}$$

Where,

$\frac{Z}{P}$ = The selection differential in standard units, for the present study it was 2.06 at 5% level of selection (Lush, 1949)

g_{yy} and t_{yy} = The genotypic and phenotypic variances of the character y

$b_1, b_2, \ldots\ldots\ldots b_n$ = The relative weight for each character

$g_{1y}, g_{2y} \ldots g_{ny}$ = The genetic covariance of independent characters with y.

The expected gain from the discriminant function over strait selection was calculated for all the functions and studied as follows:

Expected gain (%) = [{GA (D)/ GA(S)}-1] × 100

RESULTS

Biometrical genetics is the branch of science in which the exact value of variability, components of variation, heritability, genetic advance, correlation coefficient, path-coefficient and selection index can be measured. In the present investigation, yield potential was studied for some agronomic characters, such as date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF). number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), Plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP). The results which were obtained for eleven agronomic characters are described under the following sub-heads:

A. STUDY OF VARIABILITY

Range, Mean with Standard Error and Coefficient of Variability in Percentage (CV%): The estimates of Range, Mean with standard error and CV% for each of the eleven characters were calculated separately and shown in Table (9). Tables show that range, mean with standard error and CV% are very much pronounced for all the characters. The results are described as follows:

1. Date of first flower (DFF)

Range: The highest range of variation was observed for this character in Bm3 with a value of 56.16 - 78.66, while the lowest value was recorded as 67.04 - 81.19 in Bm6.

Mean with standard error: The highest mean with standard error was 73.5409±1.4469 in Bm6, while the lowest mean with standard error was 65.7063±1.5072 in Bm1.

Coefficient of variability in percentage (CV %): The highest value for CV% was observed as 11.1427 in Bm3, while the lowest CV% was observed as 6.8155 in Bm6.

2. Plant height at first flower (PHFF)

Range: The highest range of variation was observed for PHFF in Bm1 with a value of 13.58 - 20.41, while the lowest value was 14.92 - 19.30 recorded in Bm4.

Mean with standard error: The highest and lowest means with standard errors were 19.5856±0.4581 in Bm3 and 16.5442±0.5022 in Bm6, respectively.

Coefficient of variability in percentage (CV %): The highest value for CV% was 11.7220 in Bm1, while the lowest CV% was observed in Bm3 with the value of 8.1030.

3. Number of primary branches at first flower (NPBFF)

Range: The values of 3.13 - 8.55 and 4.00 - 7.88 were noted as the highest and lowest range for this character in Bm1 and in Bm2, respectively.

Mean with standard error: The highest and lowest mean with standard errors were 7.7152±0.3643 and 6.0247±0.4962 recorded in Bm4 and Bm1, respectively.

Coefficient of variability in percentage (CV %): The values of 29.7362 and 16.3573 were noted as the highest and the lowest CV% in Bm6 and in Bm4, respectively.

4. Plant height at maximum flower (PHMF)

Range: The highest range of variation was observed for PHMF in Bm1 with a value of 22.42 - 34.77, while the lowest value was 29.08 - 33.40 recorded in Bm2.

Mean with standard error: The calculated highest mean with standard error was 32.8978±0.9390 in Bm3, while the lowest mean with standard error was 28.1652±0.8257 in Bm5.

Coefficient of variability in percentage (CV%): The highest value of CV% was 13.8911 recorded in Bm1, while the lowest value of CV% was 4.0584 noted in Bm2.

5. Number of primary branches at maximum flower (NPBMF)

Range: The highest range of variation was observed for NPBMF in Bm4 with a value of 6.18 - 14.41, while the lowest value was 5.81 - 11.40 recorded in Bm2.

Mean with standard error: The calculated highest mean with standard error was 9.9823±0.5753 in Bm5, while the lowest mean with standard error was 8.2497±0.6626 in Bm1.

Coefficient of variability in percentage (CV%): The highest value of CV% was 31.5452 recorded in Bm6, while the lowest value of CV% was 19.9638 noted in Bm5.

6. Number of secondary branches at maximum flower (NSBMF)

Range: The highest range of variation was observed for this character in Bm1 with a value of 3.08 - 20.20, while the lowest value was recorded as 6.19 - 17.90 in Bm6.

Mean with standard error: The highest mean with standard error was 15.0298±1.2071 in Bm4, while the lowest mean with standard error was 10.8555±1.6563 in Bm1.

Coefficient of variability in percentage (CV %): The highest value for CV% was observed as 52.8527 in Bm1, while the lowest CV% was observed as 27.8223 in Bm4.

7. Plant weight per plant (PWPP)

Range: The highest range of variation was observed for PWPP in Bm1 with a value of 3.57–12.86, while the lowest value was 3.90–10.04 recorded in Bm4.

Mean with standard error: The calculated highest mean with standard error was 8.3852±0.8577 in Bm1, while the lowest mean with standard error was 6.4373±0.6193 in Bm3.

Coefficient of variability in percentage (CV%): The highest value of CV% was 35.4340 recorded in Bm1, while the lowest value of CV% was 29.2049 noted in Bm4.

8. Number of pods per plant (NPdPP)

Range: The highest range of variation was observed for this character in Bm1 with a value of 52.92-180.09, while the lowest value was recorded as 84.47-173.38 in Bm2.

Mean with standard error: The highest mean with standard error was 143.0072±8.7515 in Bm5, while the lowest mean with standard error was 102.6364±11.5257 in Bm3.

Coefficient of variability in percentage (CV%): The highest value for CV% was observed as 40.9861 in Bm1, while the lowest CV% was observed as 21.1991 in Bm5.

9. Pod weight per plant (PdWPP)

Range: The highest range of variation was observed for PdWPP in Bm1 with a value of 2.30-8.13, while the lowest value was 3.99-7.43 recorded in Bm2.

Mean with standard error: The calculated highest mean with standard error was 5.7182±0.3988 in Bm2, while the lowest mean with standard error was 4.2537±0.4849 in Bm3.

Coefficient of variability in percentage (CV%): The highest value of CV% was 39.4890 recorded in Bm3, while the lowest value of CV% was 21.1882 noted in Bm4.

10. Number of seeds per plant (NSPP)

Range: The highest range of variation was observed for this character in Bm1 with a value of 73.58-331.00, while the lowest value was recorded as 152.29-312.20 in Bm2.

Mean with standard error: The highest mean with standard error was 214.4653±17.1282 in Bm2, while the lowest mean with standard error was 143.8636±15.8634 in Bm3.

Coefficient of variability in percentage (CV %): The highest value for CV% was observed as 45.9582 in Bm1, while the lowest CV% was observed as 24.0538 in Bm4.

11. Seed weight per plant (SWPP)

Range: The highest range of variation was observed for SWPP in Bm6 with a value of 1.27-6.18, while the lowest value was 2.85-5.73 recorded in Bm2.

Mean with standard error: The calculated highest mean with standard error was 4.2711±0.2508 in Bm4, while the lowest mean with standard error was 3.1280±0.3879 in Bm1.

Coefficient of variability in percentage (CV%): The highest value of CV% was 42.9577 recorded in Bm1, while the lowest value of CV% was 20.3397 noted in Bm4.

B. ANALYSIS OF VARIANCE

The results of the analysis of variance for all the eleven quantitative characters were done separately and are shown in Table 10(A-K). For significant test the main items and their interaction effects a fixed model was followed. In the analysis, the replication item (R) was non-significant for all the characters. The non-significant result indicated that the replications for this investigation were similar. The line item (L) was highly significant for DFF, PHFF, NPBFF, PHMF, NPBMF and NSBMF and rest of the characters was significant at 5% level when tested against the within replication error and pooled error. The year (E) item was highly significant both at 5% and 1% level for all of the characters except DFF, when tested against within replication error and pooled error.

In case of the L× E interactions were non-significant for all of the characters except DFF and PHFF, which were highly significant both at 5% and 1% level, when tested against within replication error and pooled error.

C. COMPONENTS OF VARIATION

The estimates of phenotypic (σ^2_p), genotypic (σ^2_G), dose (σ^2_Y), interaction (σ^2_{GY}) and error (σ^2_e) components of variation were analyzed separately for eleven characters. The results are given in the Table-11.

For all the characters, phenotypic variation (σ^2p) was greater than that of σ^2_G, σ^2_Y, σ^2_{GY} and σ^2e components of variation as expected. The phenotype is the joint product of $\sigma^2_{G,}$ and σ^2_e. NSPP showed maximum value for all of the components of variation viz, σ^2p (3381.38), σ^2_G (273.78), σ^2_Y (921.54), σ^2_{GY} (198.73) and σ^2e (2908.87). The lowest values were shown SWPP for σ^2p (1.8) and σ^2_G (0.12), DFF for σ^2_Y (0.31), PdWPP for σ^2_{GY} (0.07) and NPBFF for σ^2e (1.02), respectively.

D. COEFFICIENT OF VARIABILITY

The estimates of phenotypic (PCV), genotypic (GCV), year (YCV). Interaction (G×YCV) and within error coefficient of variability (ECV) for eleven quantitative characters of lentil were calculated separately and shown in Table 12.

In general, the phenotypic co-efficient of variability (PCV) was greater than genotypic, year, interaction and error coefficient of variability for all the characters. PCV is the joint product of GCV and ECV. In the present work, the highest PCV, GCV, G×YCV and ECV were noted for NSPP with the values of 1878.61, 152.11, 110.41 and 1616.1, respectively. The highest YCV was recorded for NPdPP with a value of 525.68. The lowest PCV for PHFF (19.64), GCV for SWPP (3.29), YCV for DFF (0.45), G×YCV for NPBMF (1.02) and ECV for PHFF (9.54), respectively.

The genetic variability shown by the character could be measured from genotypic coefficient of variability. In the present study, phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), year coefficient of variability (YCV), interaction coefficient of variability (G×YCV) and environmental coefficient of variability (ECV) were observed to be the highest for NSPP followed by NPdPP indicated that the characters were inherited with higher variability within their sibs.

E. HERITABILITY (h^2_b), GENETIC ADVANCE (GA) And GENETIC ADVANCE EXPRESSED AS PERCENTAGE OF MEAN (GA %)

Heritability (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA %) for all the eleven quantitative characters of lentil were estimated and presented in Table 13.

1. Heritability (h^2_b)

In the present investigation the lowest heritability was observed for NPdPP (8.89) and the highest heritability was estimated for PHMF (28.11).

2. Genetic advance GA

In this study, the highest value of genetic advance (9.70) was estimated for NSPP and the lowest genetic advance (0.22) was observed for SWPP.

3. Genetic advance as percentage of mean (GA %)

The highest value of genetic advance as percentage of mean was recorded as 9.79 for PWPP and the lowest genetic advance as percentage of mean was noted as 2.31 for DFF.

F. CORRELATION COEFFICIENT

Both at phenotypic and genotypic levels correlation co-efficient between pairs of characters were analyzed. In the present investigation, there were eleven characters along with 55 pairs of condition and hence 55 correlation co-efficient were obtained in each case of phenotypic and genotypic level. The results are presented in **Table-14** and **Table-15**.

In this analysis, **Table-14** and **Table-15** also showed that DFF positively correlated with NPBFF, NPBMF and NPdPP both at phenotypic and genotypic levels. But correlation value were significant for most of the characters at genotypic level. PHFF significantly correlated with PHMF at genotypic and phenotypic level. NPBFF were significantly positive correlated with SWPP at phenotypic level and with DFF at genotypic level but with NPBMF and NSBMF at both level. NPBMF showed significant relation with NSBMF at both level and NPdPP at genotypic level. NSBMF significantly correlated with SWPP at genotypic level. PWPP significantly correlated at both level with PdWPP and NSPP, but with NPdPP and SWPP at genotypic level. NPdPP showed significant relation with PdWPP, NSPP and SWPP at phenotypic level, but only with NSPP at genotypic level. PdWPP significantly correlated with NSPP and SWPP at genotypic level and phenotypic level. At phenotypic level NSPP significantly correlated with SWPP.

G. PATH-COEFFICIENT ANALYSIS

1. Phenotypic level

The results of path-coefficient analysis at phenotypic level are presented in **Table-16**. It was observed from **Table-16** that DFF had the negative direct effect of -0.2290 on SWPP. The character had indirect positive effect of 0.0523 via NPBFF followed by PHMF, NPdPP, PWPP, NSBMF, PHFF and NPBMF. The indirect effect via PdWPP and NSPP were negative. The total effect was -0.1295.

Plant height at first flower (PHFF) had the negative direct effect of -0.0827 on SWPP. The character had indirect positive effect PHMF followed by NSPP, NPBFF, NPdPP, PdWPP, DFF and NBPMF. The indirect effect via NSBMF and PWPP were negative. The total effect was 0.3134.

The positive direct effect of number of primary branches at first flower (NPBFF) on SWPP was 0.2993. The character NPBFF exhibited indirect positive effects of (0.1136) on SWPP through NSPP followed by PdWPP, NPdPP, PHMF and NPBMF. The indirect effect via DFF, NSBMF, PWPP and PHFF were negative. The total effect was 0.5169.

Plant height at maximum flower (PHMF) exhibited positive direct effect of 0.3752 on SWPP. Although it showed indirect positive effect through NSPP, PdWPP, NPdPP, NPBFF and NPBMF on SWPP but rest of the characters viz DFF, PHFF, NSBMF, and PWPP showed negative indirect effects. The total effect of PHMF on SWPP was 0.4829.

Number of primary branches at maximum flower (NPBMF) had positive direct effect of 0.0030 on SWPP. The character showed indirect positive effect on SWPP via NPBFF, NSPP, PHMF, NPdPP and PdWPP. Again, this character showed negative indirect effect on SWPP through DFF, PHFF, NSBMF and PWPP. The total effect was 0.3856.

Number of secondary branches at maximum flower (NSBMF) had the negative direct effect of -0.0421 on SWPP. This character showed positive indirect effect of 0.1860 on SWPP though NPBFF, followed by NSPP, PdWPP, NPdPP PHMF, DFF and NPBMF. This character showed negative effect via PHFF and PWPP. The total effect was 0.5601.

Plant weight per plant (PWPP) had negative direct effect of -0.0555 on SWPP. The character had indirect positive effect of 0.2051 through NSPP, PdWPP, PHMF, NPdPP NPBFF, DFF and NPBMF on SWPP. However, character PHFF and NSBMF showed indirect negative effect on SWPP. The total effect was 0.6707.

Number of pods per plant (NPdPP) had the positive direct effect 0.2045 on SWPP. This character showed positive indirect effect 0.2074 on SWPP through NSPP followed by, PdWPP, NPBFF, PHMF and NPBMF and rest of the character showed negative indirect effect on SWPP. For this trait, the total effect was 0.6480.

Pod weight per plant (PdWPP) had positive direct effect of 0.2497 on SWPP. The character showed positive indirect effect of 0.2010 on SWPP through NSPP followed by NPBFF, NPdPP, PHMF, DFF and NPBMF. The rest of characters

showed indirect negative effect through PWPP, NSBMF and PHFF. The total effect was 0.7645.

Number of seeds per plant (NSPP) exhibited positive direct effect of 0.2656 on SWPP. Although it showed indirect positive effect through PdWPP, NPdPP, PHMF, NPBFF, DFF and NPBMF, but had indirect negative effect via PHFF, NSBMF and PWPP. The total effect was 0.8085.
At phenotypic level the residual effect was noted as 0.4257.

2. Genotypic level

The result of path coefficient analysis at genotypic level presented in **Table-17**. Date at first flower (DFF) had positive direct effect of .0560 on SWPP. The character had indirect positive effects on SWPP through NSBMF followed by NPBFF, NPBMF, NPdPP and PHFF, but had indirect negative effect via, PHMF, PdWPP, PWPP and NSPP. The total effect was -0.0212.

Plant height at first flower (PHFF) had the negative direct effect of -0.0003 on SWPP. The character had indirect positive effect PHMF, followed by NSPP, NSBMF and NBPMF. The indirect effect via PdWPP, NPBFF, PWPP, NPdPP and DFF were negative. The total effect was 0.0675.

The positive direct effect of number of primary branches at first flower (NPBFF) on SWPP was 0.8364. The character NPBFF exhibited indirect positive effects of (0.1413) on SWPP through PdWPP followed by NPBMF, DFF, NPdPP and PHFF. The indirect effect via, PHMF, NSBMF, NSPP and PWPP were negative. The total effect was 0.4376.

Plant height at maximum flower (PHMF) exhibited positive direct effect of 0.8173 on SWPP. Although it showed indirect positive effect through NSPP and NSBMF on SWPP but rest of the characters viz DFF, PHFF, NPBFF, NPBMF, PWPP, PdWPP and NPdPP, showed negative indirect effects. The total effect of PHMF on SWPP was 0.4878.

Number of primary branches at maximum flower (NPBMF) had positive direct effect of 0.0792 on SWPP. The character showed indirect positive effect on SWPP via NPBFF, NSPP, DFF and NPdPP. Again, this character showed negative indirect effect on SWPP through PdWPP, PHMF, NSBMF, PWPP and PHFF. The total effect was -0.2366.

Number of secondary branches at maximum flower (NSBMF) had the negative direct effect of -0.1876 on SWPP. This character showed positive indirect effect of 1.0087 on SWPP though NPBFF, followed by NPBMF, NSPP, NPdPP and PHFF. This character showed negative effect via PHMF, DFF, PdWPP and PWPP. The total effect was 0.3001.

Plant weight per plant (PWPP) had positive direct effect of 0.1304 on SWPP. The character had indirect positive effect of 0.6613 through PdWPP followed by, NSBMF and PHFF on SWPP. However, DFF, NPBFF, PHMF, NPBMF, NPdPP and NSPP showed indirect negative effect on SWPP. The total effect was -0.0255.

Number of pods per plant (NPdPP) had the positive direct effect 0.05585 on SWPP. This character showed positive indirect effect 0.2074 on SWPP through NPBFF followed by, NPBMF, DFF and PHFF and rest of the character showed negative indirect effect on SWPP. For this trait, the total effect was -0.5540.

Pod weight per plant (PdWPP) had positive direct effect of 0.7799 on SWPP. The character showed positive indirect effect of 0.1516 on SWPP through NPBFF followed by, PWPP, NSBMF and PHFF. The rest of characters showed indirect negative effect through, NSPP, PHMF, NPBMF, DFF and NPdPP. The total effect was 0.5702.

Number of seeds per plant (NSPP) exhibited negative direct effect of -0.3080 on SWPP. Although it showed indirect positive effect through PdWPP, NPBFF, PWPP, NSBMF, NPdPP, DFF and PHFF, but had indirect negative effect via, PHMF and NPBMF. The total effect was 0.3670. At genotypic level the residual effect was noted as 0.1195.

H. SELECTION INDEX

Selection indices for yield were constructed for each set of data and different combinations were studied to identify the characters which might be useful during selection breeding. In constructing the selection indices, all the eleven agronomical characters *viz,* date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF). number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), Plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds

per plant (NSPP) and seed weight per plant (SWPP) were included of which SWPP was dependent character. In the present investigation, discriminant function analysis has been done considering individual character separately. **Table-18** also revealed that individually NPBFF showed highest positive expected gain 1072.611% this value was followed by 624.824% PdWPP.

When the index included two characters, SWPP in combination with NPBMF gave the highest positive gain of 223.040% followed by 192.401% (NPBFF + NPdPP), 163.726% (PdWPP + SWPP).

In the discriminant function analysis, when selection index included three characters, the maximum genetic gain was recorded as 87.991% (NPBFF + NSBMF + PdWPP), followed by 65.614% (NPBFF + PWPP + PdWPP).

In the same way when four characters were included in the discriminant function the highest genetic gain was 146.92 % for DFF + PHFF + PWPP + SWPP next was 127.27% for NPBFF +PWPP + PdWPP +SWPP. Similarly when five characters were included in the discriminant function, SWPP in combination with PHFF, NPBMF, PWPP and PdWPP exhibited the highest genetic gain of 285.18 % followed by 161.62 % (NPBMF+ NSBMF+ PWPP+ PdWPP + SWPP).

In case of discriminant functions when six characters were included, SWPP combination with DFF), PHFF, NPBMF, PWPP, PdWPP and SWPP showed the highest expected genetic gain of 132.84. In case of seven characters combination the characters PHFF, NPBFF, NPBMF, NSBMF, PWPP, PdWPP and SWPP showed the highest positive expected genetic gain with value of 196.07 %. The high expected gains were more frequent through the different sets of data, when more character combinations were studied in the functions.

In the present study, it was observed that individually DFF, PHFF, NPBMF, NSBMF, PWPP, NPdPP, NSPP and SWPP expressed negative genetic gain over the straight for lentil yield and NPBFF, PHMF and PdWPP expressed positive genetic gain. The negative expected gain of SWPP alone reflects that it itself is not a complete character for higher yield rather it depends on other component character for higher yield.

Table-9: Range, mean with standard error and CV% for eleven characters of lentil.

Line	DFF			PHFF		
	Range	Mean with Standard error	CV%	Range	Mean with Standard error	CV%
Bm1	58.00 - 74.88	65.7063 ± 1.5072	7.9462	13.58 - 20.41	16.5641 ± 0.5605	11.7220
Bm2	58.33 - 76.17	66.1950 ± 1.4364	7.5168	15.82 - 20.93	17.8971 ± 0.4413	8.5413
Bm3	56.16 - 78.66	67.4600 ± 2.1699	11.1427	17.39 - 22.68	19.5856 ± 0.4581	8.1030
Bm4	65.04 - 80.22	71.0458 ± 1.2489	6.0893	14.92 - 19.30	17.1425 ± 0.4372	8.8349
Bm5	64.50 - 83.00	72.6646 ± 1.4693	7.0045	15.00 - 19.65	17.1732 ± 0.4145	8.3604
Bm6	67.04 - 81.19	73.5409 ± 1.4469	6.8155	14.24 - 20.38	16.5442 ± 0.5022	10.5145
Line	**NPBFF**			**PHMF**		
	Range	Mean with Standard error	CV%	Range	Mean with Standard error	CV%
Bm1	3.13 - 8.55	6.0247 ± 0.4962	28.5283	22.42 - 34.77	29.1300 ± 1.1681	13.8911
Bm2	4.00 - 7.88	6.3387 ± 0.3212	17.5529	29.08 - 33.40	32.2198 ± 0.3775	4.0584
Bm3	4.00 - 8.04	6.0359 ± 0.4551	26.1184	28.40 - 40.56	32.8978 ± 0.9390	9.8876
Bm4	5.71 - 9.75	7.7152 ± 0.3643	16.3573	25.53 - 38.19	29.8874 ± 1.0446	12.1069
Bm5	4.78 - 9.08	6.7567 ± 0.4403	22.5734	22.55 - 30.68	28.1652 ± 0.8257	10.1554
Bm6	3.38 - 8.58	6.1189 ± 0.5253	29.7362	23.75 - 34.29	28.4147 ± 0.9576	11.6748
Line	**NPBMF**			**NSBMF**		
	Range	Mean with Standard error	CV%	Range	Mean with Standard error	CV%
Bm1	4.83 - 11.18	8.2497 ± 0.6626	27.8219	3.08 - 20.20	10.8555 ± 1.6563	52.8527
Bm2	5.81 - 11.40	8.4119 ± 0.5657	23.2970	5.94 - 18.40	11.3058 ± 1.3879	42.5243
Bm3	5.64 - 12.40	9.2381 ± 0.5829	21.8558	7.33 - 20.68	11.9237 ± 1.6250	47.2112
Bm4	6.18 - 14.41	9.6508 ± 0.7179	25.7690	10.59 - 23.60	15.0298 ± 1.2071	27.8223
Bm5	7.64 - 15.04	9.9823 ± 0.5753	19.9638	6.00 - 22.68	12.8678 ± 1.5991	43.0488
Bm6	4.88 - 12.04	8.3771 ± 0.7628	31.5452	6.19 - 17.90	11.6415 ± 1.2366	36.7958
Line	**PWPP**			**NPdPP**		
	Range	Mean with Standard error	CV%	Range	Mean with Standard error	CV%
Bm1	3.57 - 12.86	8.3852 ± 0.8577	35.4340	52.92 - 180.09	108.9163 ± 12.8866	40.9861
Bm2	3.94 - 11.85	8.3813 ± 0.7221	29.8453	84.47 - 173.38	129.4829 ± 10.7022	28.6321
Bm3	3.31 - 10.40	6.5162 ± 0.7451	39.6111	44.8 - 162.29	102.6364 ± 11.5275	38.9066
Bm4	3.90 - 10.04	7.2130 ± 0.6081	29.2049	68.53 - 188.24	126.0488 ± 10.0094	27.5082
Bm5	2.45 - 9.85	6.4373 ± 0.6193	33.3265	97.88 - 187.69	143.0072 ± 8.7515	21.1991
Bm6	4.43 - 11.47	8.0446 ± 0.7745	33.3488	53.67 - 176.60	123.0434 ± 11.2885	31.7811

Continued …….

Line	PdWPP			NSPP		
	Range	Mean with Standard error	CV%	Range	Mean with Standard error	CV%
Bm1	2.30 - 8.13	5.0896 ± 0.5530	37.6371	73.58 - 331.00	166.8133 ± 22.1311	45.9582
Bm2	3.99 - 7.43	5.7182 ± 0.3988	24.1608	152.29 - 312.20	214.4653 ± 17.1282	27.6659
Bm3	1.65 - 6.74	4.2537 ± 0.4849	39.4890	55.70 - 232.28	143.8636 ± 15.8634	38.1977
Bm4	3.95 - 7.83	5.4941 ± 0.3360	21.1882	22.21 - 269.24	193.5870 ± 13.4422	24.0538
Bm5	1.23 - 6.36	4.4276 ± 0.4740	37.0813	81.18 - 257.23	175.1758 ± 16.1509	31.9384
Bm6	2.28 - 7.18	5.1305 ± 0.4833	32.6329	93.04 - 276.65	186.0565 ± 18.0938	33.6881

Line	SWPP		
	Range	Mean with Standard error	CV%
Bm1	1.20 - 5.05	3.1280 ± 0.3879	42.9577
Bm2	2.85 - 5.73	4.2662 ± 0.3068	24.9103
Bm3	2.13 - 5.32	3.6556 ± 0.3830	36.2949
Bm4	2.99 - 6.35	4.2711 ± 0.2508	20.3397
Bm5	0.91 - 4.61	3.2280 ± 0.3504	37.5971
Bm6	1.27 - 6.18	3.7133 ± 0.4558	42.5239

Table 10(A-K): Analysis of variance of among genotypes and its interaction with year for eleven characters in lentil.

A. Date of first flower (DFF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	26.8509	8.9503	0.4242	
Genotype (G)	5	698.2166	139.6433	6.6182**	
Year (Y)	2	57.0553	28.5276	1.3520	
G × Y	10	800.9138	80.0914	3.7958**	
Within rep. Error	51	1076.0883	21.0998		
Pooled Error	61	1877.0020	30.7705		

B. Plant height at first flower (PHFF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	3.1306	1.0435	0.6259	
Genotype (G)	5	78.3590	15.6718	9.3994**	
Year (Y)	2	35.2226	17.6113	10.5626**	
G × Y	10	52.6851	5.2685	3.1599**	
With rep. Error	51	85.0334	1.6673		
Pooled Error	61	137.7186	2.2577		

C. Number of primary branches at first flower (NPBFF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	5.5368	1.8456	1.8130	1.7186
Genotype (G)	5	25.8608	5.1722	5.0808**	4.8161**
Year (Y)	2	81.9301	40.9651	40.2417**	38.1451**
G × Y	10	13.5927	1.3593	1.3353	1.2657
With rep. Error	51	51.9168	1.0180		
Pooled Error	61	65.5095	1.0739		

D. Plant height at maximum flower(PHMF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	16.3090	5.4363	0.8173	0.7351
Genotype (G)	5	238.6660	47.7332	7.1764**	6.4541**
Year (Y)	2	202.9332	101.4666	15.2550**	13.7195**
G × Y	10	111.9214	11.1921	1.6827	1.5133
With rep. Error	51	339.2207	6.6514		
Pooled Error	61	451.1421	7.3958		

E. Number of primary branches at maximum flower (NPBMF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	4.0109	1.3370	0.7407	0.7168
Genotype (G)	5	32.8865	6.5773	3.6438**	3.5264**
Year (Y)	2	215.7826	107.8913	59.7710**	57.8448**
G × Y	10	21.7173	2.1717	1.2031	1.1644
With rep. Error	51	92.0590	1.8051		
Pooled Error	61	113.7763	1.8652		

F. Number of secondary branches at maximum flower (NSBMF)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	41.4497	13.8166	1.7282	1.6653
Genotype (G)	5	137.0325	27.4065	3.4280**	3.3033*
Year (Y)	2	1149.1137	574.5568	71.8660**	69.2519**
G × Y	10	98.3574	9.8357	1.2303	1.1855
With rep. Error	51	407.7369	7.9948		
Pooled Error	61	506.0943	8.2966		

G. Plant weight per plant (PWPP)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	2.3735	0.7912	0.2238	0.2476
Genotype (G)	5	48.4341	9.6868	2.7397*	3.0313*
Year (Y)	2	220.5292	110.2646	31.1861**	34.5052**
G × Y	10	14.6107	1.4611	0.4132	0.4572
With rep. Error	51	180.3205	3.5357		
Pooled Error	61	194.9312	3.1956		

H. Number of pods per plant (NPdPP)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	1982.5975	660.8658	0.7231	0.6708
Genotype (G)	5	12728.3682	2545.6736	2.7854*	2.5841*
Year (Y)	2	32659.6054	16329.8027	17.8676**	16.5762**
G × Y	10	13482.7153	1348.2715	1.4752	1.3686
With rep. Error	51	46610.6056	913.9334		
Pooled Error	61	60093.3209	985.1364		

I. Pod weight per plant (PdWPP)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	1.9277	0.6426	0.4272	0.4412
Genotype (G)	5	20.0082	4.0016	2.6605*	2.7475*
Year (Y)	2	77.0149	38.5075	25.6020**	26.4386**
G × Y	10	12.1377	1.2138	0.8070	0.8334
With rep. Error	51	76.7080	1.5041		
Pooled Error	61	88.8456	1.4565		

J. Number of seeds per plant (NSPP)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	2651.4523	883.8174	0.3038	0.2908
Genotype (G)	5	34945.7455	6989.1491	2.4027*	2.2997*
Year (Y)	2	50051.7775	25025.8887	8.6033**	8.2344**
G × Y	10	37037.9599	3703.7960	1.2733	1.2187
With rep. Error	51	148352.3651	2908.8699		
Pooled Error	61	185390.3251	3039.1857		

K. Seed weight per plant (SWPP)

Source	DF	SS	MS	VR_1	VR_2
Replication (R)	3	0.9876	0.3292	0.3081	0.2927
Genotype (G)	5	14.3773	2.8755	2.6914*	2.5567*
Year (Y)	2	33.9871	16.9936	15.9061**	15.1096**
G × Y	10	14.1190	1.4119	1.3215	1.2554
With rep. Error	51	54.4869	1.0684		
Pooled Error	61	68.6059	1.1247		

NB: * and **, indicate significance at 5% and 1% level, respectively.

Table-11: Phenotypic (σ^2_p), Genotypic (σ^2_G), Dose (σ^2_Y), Interaction (σ^2_{GY}), and Error (σ^2_E) Components of variation of different quantitative characters in lentil.

Characters	σ^2_p	σ^2_G	σ^2_Y	σ^2_{GY}	σ_E^2
DFF	40.81	4.96	0.31	14.75	21.10
PHFF	3.43	0.87	0.66	0.90	1.67
NPBFF	1.42	0.32	1.66	0.09	1.02
PHMF	10.83	3.05	3.95	1.14	6.65
NPBMF	2.26	0.37	4.42	0.09	1.81
NSBMF	9.92	1.46	23.61	0.46	7.99
PWPP	3.70	0.69	4.45	-0.52	3.54
NPdPP	1122.30	99.78	642.33	108.58	913.93
PdWPP	1.66	0.23	1.54	-0.07	1.50
NSPP	3381.38	273.78	921.54	198.73	2908.87
SWPP	1.28	0.12	0.66	0.09	1.07

Table-12: Phenotypic (PCV), Genotypic (GCV), Dose (DCV), Interaction (L×DCV) and Error (ECV) coefficient of variability of different characters in lentil.

Characters	PCV	GCV	YCV	G x YCV	ECV
DFF	58.77	7.15	0.45	21.24	30.39
PHFF	19.64	4.96	3.80	5.15	9.54
NPBFF	21.87	4.89	25.61	1.31	15.67
PHMF	35.96	10.11	13.12	3.77	22.08
NPBMF	25.20	4.09	49.20	1.02	20.09
NSBMF	80.84	11.93	192.38	3.75	65.15
PWPP	49.39	9.14	59.32	-6.92	47.17
NPdPP	918.50	81.66	525.68	88.87	747.97
PdWPP	33.15	4.63	30.72	-1.45	29.97
NSPP	1878.61	152.11	511.99	110.41	1616.1
SWPP	34.40	3.29	17.88	2.31	28.79

Table-13: Heritability (h^2_b), Genetic advance (GA), and Genetic advance as percentage of mean (GA %) of different characters in lentil.

Characters	h^2_b	GA	GA%
DFF	12.16	1.60	2.31
PHFF	25.24	0.96	5.51
NPBFF	22.36	0.55	8.45
PHMF	28.11	1.91	6.33
NPBMF	16.22	0.50	5.59
NSBMF	14.76	0.96	7.80
PWPP	18.51	0.73	9.79
NPdPP	8.89	6.14	5.02
PdWPP	13.96	0.37	7.39
NSPP	8.10	9.70	5.39
SWPP	9.56	0.22	5.99

Table-14: Phenotypic (r_p) correlation coefficients between yield and yield contributing character in lentil.

Characters	DFF	PHFF	NPBFF	PHMF	NPBMF	NSBMF	PWPP	NPdPP	PdWPP	NSPP	SWPP
DFF	1.0000	-0.0254	0.1748	0.1251	0.1331	-0.1583	-0.1507	0.1220	-0.1325	-0.0338	-0.1295
PHFF		1.0000	0.1666	0.6312*	0.2217	0.2480	0.0867	0.1901	0.0977	0.2057	0.3134
NPBFF			1.0000	0.0343	0.5587*	0.6214*	0.2952	0.3807	0.4300	0.4278	0.5169*
PHMF				1.0000	0.2137	0.1912	0.3118	0.1759	0.2776	0.3672	0.4829
NPBMF					1.0000	0.5946*	0.2365	0.3286	0.2640	0.3330	0.3856
NSBMF						1.0000	0.3873	0.4714	0.4787	0.4955	0.5601*
PWPP							1.0000	0.5316*	0.7807**	0.7720**	0.6707**
NPdPP								1.0000	0.5914*	0.7807**	0.6480*
PdWPP									1.0000	0.7566**	0.7645**
NSPP										1.0000	0.8085**
DFF											1.0000

NB: * and **, indicate significance at 5% and 1% level, respectively.

Table-15: Genotypic (r_g) correlation coefficients between yield and yield contributing character in lentil.

Characters	DFF	PHFF	NPBFF	PHMF	NPBMF	NSBMF	PWPP	NPdPP	PdWPP	NSPP	SWPP
DFF	1.0000	-1.0759**	0.5047*	-1.1520**	0.9152**	-1.0573**	-0.6280*	0.7778**	-0.1637	0.1555	-0.0212
PHFF		1.0000	-0.4293	1.0161**	0.3989	-0.3418	-0.6720**	-1.1473**	-0.8120**	-1.1226**	0.0675
NPBFF			1.0000	-0.3394	0.8693**	1.2060**	-0.5181*	0.4623	0.1812	0.2986	0.4376
PHMF				1.0000	-0.3588	-0.3750	-0.1907	-0.9408**	-0.1352	-0.5194*	0.4878
NPBMF					1.0000	1.1038**	-1.4326**	0.8076**	-0.8691	-0.7331**	-0.2366
NSBMF						1.0000	-0.8311*	0.3034	-0.1442	-0.2466	0.3001
PWPP							1.0000	-0.4056	0.7838**	0.5287*	-0.0255
NPdPP								1.0000	-0.0790	0.5322*	-0.5540*
PdWPP									1.0000	0.9668**	0.5702*
NSPP										1.0000	0.3670
DFF											1.0000

NB: * and **, indicate significance at 5% and 1% level, respectively.

Table-16: Path-coefficient analysis showing direct and indirect effects of yield components on yield of lentil at phenotypic level.

Characters	**SWPP vs**										**Path coefficient**
	DFF	**PHFF**	**NPBFF**	**PHMF**	**NPBMF**	**NSBMF**	**PWPP**	**NPdPP**	**PdWPP**	**NSPP**	
DFF	**-0.2290**	0.0021	0.0523	0.0469	0.0004	0.0065	0.0084	0.0250	-0.0331	-0.0090	**-0.2290**
PHFF	0.0058	**-0.0827**	0.0499	0.2368	0.0007	-0.0102	-0.0048	0.0389	0.0244	0.0547	**-0.0827**
NPBFF	-0.0400	-0.0138	**0.2993**	0.0129	0.0017	-0.0256	-0.0164	0.0779	0.1074	0.1136	**0.2993**
PHMF	-0.0286	-0.0522	0.0103	**0.3752**	0.0006	-0.0079	-0.0173	0.0360	0.0693	0.0975	**0.3752**
NPBMF	-0.0305	-0.0183	0.1672	0.0802	**0.0030**	-0.0245	-0.0131	0.0672	0.0659	0.0885	**0.0030**
NSBMF	0.0362	-0.0205	0.1860	0.0717	0.0018	**-0.0412**	-0.0215	0.0964	0.1195	0.1316	**-0.0412**
PWPP	0.0345	-0.0072	0.0883	0.1170	0.0007	-0.0159	**-0.0555**	0.1087	0.1949	0.2051	**-0.0555**
NPdPP	-0.0279	-0.0157	0.1139	0.0660	0.0010	-0.0194	-0.0295	**0.2045**	0.1477	0.2074	**0.2045**
PdWPP	0.0303	-0.0081	0.1287	0.1041	0.0008	-0.0197	-0.0433	0.1210	**0.2497**	0.2010	**0.2497**
NSPP	0.0077	-0.0170	0.1280	0.1378	0.0010	-0.0204	-0.0428	0.1597	0.1889	**0.2656**	**0.2656**
Total effect	**-0.1295**	**0.3134**	**0.5169**	**0.4829**	**0.3856**	**0.5601**	**0.6707**	**0.6480**	**0.7645**	**0.8085**	

Residual effect = 0.4257

Table-17: Path-coefficient analysis showing direct and indirect effects of yield components on yield of lentil at genotypic level.

Characters	**SWPP vs**										Path coefficient
	DFF	**PHFF**	**NPBFF**	**PHMF**	**NPBMF**	**NSBMF**	**PWPP**	**NPdPP**	**PdWPP**	**NSPP**	
DFF	**0.0560**	0.0003	0.4221	-0.9415	0.0725	0.5834	-0.0819	0.0434	-0.1277	-0.0479	**0.0560**
PHFF	-0.0602	**-0.0003**	-0.3591	0.8305	0.0316	0.0641	-0.0876	-0.0640	-0.6333	0.3458	**-0.0003**
NPBFF	0.0283	0.0001	**0.8364**	-0.2774	0.0689	-0.2263	-0.0675	0.0258	0.1413	-0.0920	**0.8364**
PHMF	-0.0645	-0.0003	-0.2839	**0.8173**	-0.0284	0.0703	-0.0249	-0.0525	-0.1055	0.1600	**0.8173**
NPBMF	0.0512	-0.0001	0.7271	-0.2933	**0.0792**	-0.2071	-0.1867	0.0451	-0.6778	0.2258	**0.0792**
NSBMF	-0.1741	0.0001	1.0087	-0.3064	0.0874	**-0.1876**	-0.1083	0.0169	-0.1125	0.0760	**-0.1876**
PWPP	-0.0352	0.0002	-0.4333	-0.1558	-0.1135	0.1559	**0.1304**	-0.0226	0.6113	-0.1629	**0.1304**
NPdPP	0.0435	0.0003	0.3866	-0.7689	0.0640	-0.0569	-0.0529	**0.0558**	-0.0616	-0.1639	**0.0558**
PdWPP	-0.0092	0.0002	0.1516	-0.1105	-0.0688	0.0271	0.1022	-0.0044	**0.7799**	-0.2978	**0.7799**
NSPP	0.0087	0.0003	0.2498	-0.4245	-0.0581	0.0463	0.0689	0.0297	0.7540	**-0.3080**	**-0.3080**
Total effect	**-0.0212**	**0.0675**	**0.4376**	**0.4878**	**-0.2366**	**0.3001**	**-0.0255**	**-0.5540**	**0.5702**	**0.3670**	

Residual effect = 0.1195

Table 18: Expected gain in percent of seed weight over straight selection from the use of various selection indices in Lentil. Indices are showing up to 10 highest values of each combination only.

Selection index	Genetic gain	Selection index	Genetic gain	Selection index	Genetic gain
SWPP(x_{11})	-1143.61	$x_7+x_9+x_{11}$	-211.35	$x_5+x_6+x_7+x_8+x_9+x_{11}$	-128.76
DFF(x_1)	-201.01	$x_1+x_2+x_7+x_{11}$	146.92	$x_1+x_2+x_3+x_5+x_6+x_7+x_9$	-136.87
PHFF(x_2)	-1129.85	$x_1+x_5+x_7+x_{11}$	-174.18	$x_1+x_2+x_5+x_6+x_7+x_9+x_{11}$	-143.03
NPBFF(x_3)	1072.61	$x_2+x_4+x_5+x_9$	-178.04	$x_1+x_3+x_4+x_5+x_6+x_7+x_9$	-140.82
PHMF(x_4)	55.51	$x_2+x_5+x_7+x_{11}$	-167.18	$x_1+x_3+x_4+x_5+x_7+x_9+x_{11}$	-134.19
NPBMF(x_5)	-60.22	$x_3+x_7+x_9+x_{11}$	27.27	$x_2+x_3+x_5+x_6+x_7+x_9+x_{11}$	196.07
NSBMF(x_6)	-498.62	$x_5+x_6+x_7+x_{11}$	-212.31	$x_2+x_4+x_5+x_6+x_7+x_8+x_9$	-104.35
PWPP(x_7)	-946.25	$x_5+x_6+x_9+x_{11}$	-205.32	$x_3+x_4+x_5+x_6+x_7+x_9+x_{11}$	-167.07
NPdPP(x_8)	-82.16	$x_6+x_7+x_9+x_{11}$	-180.01	$x_3+x_4+x_5+x_6+x_8+x_9+x_{11}$	-123.32
PdWPP(x_9)	624.82	$x_6+x_7+x_9+x_{11}$	-198.65	$x_4+x_5+x_6+x_7+x_8+x_9+x_{11}$	-125.69
NSPP(x_{10})	-143.48	$x_7+x_9+x_{10}+x_{11}$	-178.44	$x_5+x_6+x_7+x_8+x_9+x_{10}+x_{11}$	-245.01
x_2+x_{11}	112.64	$x_1+x_2+x_4+x_5+x_{11}$	-140.47	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_9$	76.60
x_3+x_5	124.90	$x_1+x_3+x_5+x_7+x_9$	-170.68	$x_1+x_2+x_3+x_4+x_5+x_6+x_8+x_9$	-113.43
x_3+x_6	166.28	$x_1+x_5+x_6+x_9+x_{11}$	-158.74	$x_1+x_2+x_4+x_5+x_6+x_7+x_9+x_{11}$	-103.45
x_3+x_8	192.40	$x_1+x_6+x_7+x_9+x_{11}$	-153.13	$x_1+x_2+x_4+x_6+x_7+x_9+x_{10}+x_{11}$	-116.27
x_3+x_9	-134.04	$x_2+x_3+x_4+x_5+x_9$	-166.53	$x_1+x_2+x_5+x_6+x_7+x_8+x_9+x_{11}$	-127.54
x_5+x_{11}	223.04	$x_2+x_5+x_7+x_9+x_{11}$	285.18	$x_2+x_3+x_4+x_5+x_7+x_8+x_9+x_{11}$	-87.20
x_6+x_{11}	132.78	$x_3+x_4+x_7+x_9+x_{11}$	-153.29	$x_2+x_3+x_3+x_4+x_6+x_7+x_9+x_{11}$	-101.37
x_7+x_9	62.33	$x_3+x_5+x_7+x_9+x_{11}$	-164.99	$x_3+x_4+x_5+x_6+x_7+x_8+x_9+x_{11}$	-82.86
x_7+x_{11}	137.00	$x_4+x_6+x_7+x_9+x_{11}$	-259.09	$x_3+x_4+x_5+x_6+x_7+x_9+x_{10}+x_{11}$	-135.34
x_9+x_{11}	163.72	$x_5+x_6+x_7+x_9+x_{11}$	181.62	$x_4+x_5+x_6+x_7+x_8+x_9+x_{10}+x_{11}$	-75.92
$x_1+x_2+x_6$	-149.46	$x_1+x_2+x_3+x_5+x_7+x_9$	-143.97	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_8+x_9$	-102.33
$x_1+x_2+x_{11}$	-190.01	$x_1+x_2+x_4+x_6+x_7+x_9$	-122.83	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_9+x_{11}$	-185.46
$x_1+x_5+x_9$	-169.28	$x_1+x_2+x_5+x_7+x_9+x_{11}$	132.84	$x_1+x_2+x_3+x_4+x_5+x_7+x_8+x_9+x_{11}$	-97.53
$x_1+x_5+x_{11}$	-200.07	$x_2+x_3+x_4+x_6+x_9+x_{11}$	-133.60	$x_1+x_2+x_4+x_5+x_6+x_7+x_8+x_9+x_{11}$	-187.46
$x_1+x_6+x_{11}$	-198.33	$x_2+x_4+x_5+x_7+x_9+x_{11}$	-204.51	$x_2+x_3+x_4+x_5+x_6+x_7+x_8+x_9+x_{11}$	42.98
$x_2+x_3+x_9$	-233.01	$x_2+x_3+x_5+x_6+x_7+x_9$	-149.42	$x_2+x_3+x_4+x_5+x_6+x_7+x_9+x_{10}+x_{11}$	-140.70
$x_2+x_9+x_{11}$	--218.57	$x_3+x_4+x_5+x_7+x_9+x_{11}$	-152.53	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_8+x_9+x_{11}$	-176.53
$x_3+x_4+x_9$	-207.32	$x_3+x_5+x_6+x_7+x_9+x_{11}$	-179.67	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_9+x_{10}+x_{11}$	-67.14
$x_6+x_7+x_{11}$	-240.98	$x_4+x_5+x_7+x_8+x_9+x_{11}$	-167.26	$x_1+x_2+x_3+x_4+x_5+x_6+x_7+x_8+x_9+x_{10}+x_{11}$	-148.83

DISCUSSION

For a fruitful breeding program, plant breeders require information on nature and magnitude of variation in the existing population. The high potentiality of the genetic variability as practiced by a character is the main apprehension of a plant breeder and their magnitude can be measured from the study of genotypic coefficient of variation. Information of genetic diversity of quantitative characters is therefore, necessary for the preparation of effective and meaningful breeding program of any crop for its improvement.

Results of the investigation expected to serve as the basic information for subsequent breeding research to develop a better line which is relatively superior to the others. The present work in its biometrical aspects is important not only from genetical point of view, but also helpful to the farmers to select the appropriate line having the superior characteristics along with high yield potential. The present investigation was carried out with the eleven quantitative characters *viz*, date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower(PHMF), number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), Plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP).

In the analysis, all the characters showed a wide and pronounced range of variation indicating that the characters studied are quantitative in nature and are under polygenic in control. The wide range of variation showed that these lentil lines were good breeding materials. Similar results were obtained in chickpea by Haque (1989), Begum (1995), Haque (1997) Mannan (2001), Hasan (2001) and Deb (2002), in mustard by Paul *et al.,* (1976), Chaudhuri and Prashad (1968), Joarder and Eunus (1968), in sugarcane by Nahar and Khaleque (1996) and Nahar (1997), in chili by Husain (1977), in lentil by Azad (2008) and in chickpea by Sharma and Saini (2010).

In this investigation for all the eleven agronomical characters mean of six lentil lines as compared to their respective standard error were found to be highly significant. This indicated that the lines were different regarding the characters. The degree of coefficient of variability in percentage (CV%) was indicated by the range of variation. However, for all the lines CV% of a particular character varied from line to line. These results are in agreement with the findings of Deb (1994, 2002), Mannan (2001), Nahar (1997), Husain *et al.,* (1997) and Azad (2008).

In the ANOVA, replication item (R) was found to be non-significant for all the characters. The non-significant result indicated that the replications for this investigation were similar. The line item (L) was highly significant for DFF, PHFF, NPBFF, PHMF, NPBMF and NSBMF and rest of the characters was significant at 5% level when tested against the within replication error and pooled error. The year (E) item was highly significant both at 5% and 1% level for all of the characters except DFF, when tested against within replication error and pooled error. This referred that the included lines would be suitable for further breeding research for the improvement for those characters. Ali (1988), Salehi *et al.,* (2007), Azad (2008), Younis *et al.,* (2008), Azizi *et al.,* (2010) and Abdipur *et al.,* (2011) found similar results in different crops. Similar result was obtained by Mahmood-ul-Hasan *et al.,* (2003) and Pervin *et al.,* (2007) in blackgram; Ali *et al.,* (2009) also found similar results in chickpea.

The year (E) item was highly significant for all of the characters except DFF, when tested against within replication error and pooled error. Mannan (2001) made similar results in a number of characters in chickpea and Azad (2008) in lentil. The L × E interactions were non-significant for all the characters except DFF and PHFF, which were highly significant when tested against within replication error and pooled error. This showed that the lines and years did not interact among themselves. These results are in agreement with the findings of Bicer and Sarkar (2004), Nahar (1997), Deb (2002) and Bakhsh *et al.,* (2006).

The analysis of components of variation exhibited a wide range of phenotypic variation for all the characters in six lentil lines. Phenotypic component of variation (σ^2_P) was higher than that of genotypic (σ^2_G) year (σ^2_Y), interactions (σ^2_{GY}) and error (σ_E^2) components of variation. Ramanujam and Thirumalachar (1967) reported the presence of wide range of variation in a number of characters in chili. Khaleque *et al.,* (1991) made a similar record in a number of chemical characters in the same crop and Majid *et al.,* (1982) in blackgram. Deb (2002) in chickpea and Ara (2010) in onion. In the present research work, partitioning the components of variation showed high phenotypic variation for NSPP and NPdPP which indicated that these characters were suitable for effective selection. Swarup *et al.,* (1962) studied on sorghum and found high phenotypic variability for most of the characters. Similar results were also obtained by Singh *et al.,* (1973) in Pea, Malhotra *et al.,* (1974), Azad (2008) and Abdipur *et al.,* (2011) in lentil.

The genetic variability shown by the character could be measured from genotypic coefficient of variability. In the present study, all the variability were observed to be the highest for NSPP followed by NPdPP indicated that the characters were inherited with higher variability within their sibs. Such high values of genotypic and phenotypic coefficient also suggested good scope for improvement of character through selection Shaha *et al.,* (1981). A comparatively lower coefficient of variability were recorded for PHFF which indicated lower variability within sibs. Singh *et al.,* (1973) found high GCV for yield per plant and pod per plant in pea. Similar results were also observed by Lal and Mehta (1973) for plant height in Soybean. In the analysis, phenotypic coefficient of variability was greater than genotypic and all other coefficient of variability. The results are in agreement with the findings of Samad (1991), Deb (1994) and Nahar (1997). The difference between PCV and GCV were greater in magnitude for all the characters, which indicated that environment had considerable effect on these characters. These results are in agreement with the findings of Younis *et al.,* (2008) and Abdipur *et al.,* (2011) in lentil; Alam *et al.,* (2004) in rice and Pervin *et al.,* (2007) in blackgram.

The heritability together with genotypic coefficient of variation can give the actual picture in heritable variation because the heritable portion of variability cannot be judged

by genetic coefficient of variation alone. The heritability estimate in the present investigation was found to be low in broad sense. Comparatively higher heritability was observed for PHMF and PHFF. The lowest values of heritability indicated that the environment constituted a major portion of total phenotypic variation for the characters. Podder (1993) observed low heritability for TC and MCC in sugar cane. Begum (1995) studied in chickpea and found low heritability for all the characters except 100-SW. Johnson *et al.,* (1955), Ramanujam and Thirumalachar (1967), and Singh *et al*., (1981) suggested that heritability estimate with genetic gain are more useful for effective improvement. Genetic advance as estimated for most of the characters were low. However, genetic advance expressed as percentage of mean were recorded to be moderate. Loganathan *et al.,* (2001) found low heritability and low genetic advance for days to flowering, plant height, number of branches per plant, pod length and 100-seed weight in green gram. Different workers were obtained high h^2_b, GA and GA% for different characters in different crops viz. Deb (1994) for 100-SW in chili; Khatun (1997) for PHMF in lentil; Kabir (1997) for SWPP in lentil and Mannan (2001) for DFF in chickpea. Younis *et al.,* (2008) for grain yield, harvest index and days to maturity in lentil; Punia *et al.,* (2011) for days to flowering and plant height in lentil; Rasheed *et al.,* (2008) for harvest index, biological yield and 1000-seed weight in lentil.

In the present investigation correlation studies showed that genotypic correlation was higher than the respective phenotypic correlation in most of the cases. This situation was also marked in the path-coefficient analysis. The high genotypic correlation, indicating the strong inherent associations between pairs of characters do not always reflect nature and magnitude of phenotypic variation. Higher magnitude of genotypic correlation than phenotypic one was also found by several workers viz. Kumar *et al.,* (1988) in mustard; Nahar (1997) in sugarcane and Husain *et al.,* (1997) in chili; Sharma (1999) in lentil; Younis *et al.*, (2008) in lentil; Sharma and Saini (2010) and Ali *et al.,* (2009) in chickpea. Biabani *et al.,* (2011) observed that seed yield had a highly significant positive correlation with seed numbers and significant positive correlation with plant height and dry weight. Abdipur *et al.,* (2011) found that seed yield was associated positively ($p>0.01$) with yield contributing characters like plant height, no. of branches per plant,

no. pods per plant, no. seeds per plant and 1000-SW. Significant and positive relation between seed yield and seed number and harvest index was also obtained by Yucel *et al.,* (2006) and Yucel and Anlarsal (2010). The low phenotypic correlation due to modifying effect of environment on association of characters at genotypic level was reported by Salehuzzaman *et al.,* (1979).

It was essential that the amount of direct and indirect effect of the causal components on the effect components were determined. A path-coefficient, measuring the direct as well as indirect effects of one variable through another on the end product, was worked out separately for each set of data at phenotypic and genotypic levels. Correlation coefficient was partitioned into direct and indirect effects by path analysis. The characters NPBFF, PHMF, NPBMF, NPdPP, PdWPP and NSPP exhibited direct positive effect on SWPP at phenotypic level. This result is in close agreement with those of Togay *et al.,* (2008) in pea; Ali *et al.,* (2009), Talebi *et al.,* (2007), Sharma and Saini (2010), Yucel and Anlarsal (2010) and Ciftci *et al.* ,(2004) in chickpea. On the other hand, DFF, PHFF, NSBMF and PWPP showed negative direct effect at phenotypic level and PHFF, NSBMF and NSPP showed negative direct effects at genotypic level on SWPP. These characters also failed to contribute to yield due to its negative direct effect. Deb (2002) also observed negative direct effect to yield in chickpea both at phenotypic and genotypic levels. The path-coefficient value of DFF with SWPP was negative at phenotypic level but positive at genotypic level.

NPBFF, PHMF, NPBMF, NPdPP and PdWPP showed positive direct effect at both levels that means they contribute to yield directly. The characters showed positive and negative indirect effect via others characters at both levels. Results obtained, are in agreement with the findings of Ramanujam and Rai (1963), Ramana Rao *et al.,* (1974), Alam *et al.,* (1988), Nahar (1997), Hussain (1997), Deb (2002), and Deb *et al.,* (2009), Younis *et al.,* (2008) and Abdipur *et al.,* (2011) in lentil; Ferdous *et al.,* (2010) in wheat; Roy *et al.,* (2006) in Bush bean; Khan (2009) in potato and Ara (2010) in onion.

Above information suggested that a breeding programmer should be taken to increase the correlations magnitude between yield and its components further, to increase the yield and in that case environment should be considered. In the present investigation, SWPP showed positive correlation with PHFF, NPBFF, PHMF, NSBMF, PdWPP and NSPP both at phenotypic and genotypic level. SWPP showed significant positive correlation with NPBFF, NSBMF, PWPP, NPdPP, PdWPP, and NSPP at phenotypic level. But SWPP showed significant positive correlation with only PdWPP at genotypic level. Above information indicates that these characters are genetically related with SWPP more than those of the other yield components. The significant correlation indicated the effectiveness for directional selection for genetic improvement of lentil yield and suggested that SWPP will be increased with the increase of these characters. The significant and positive correlation was found by different workers such as Stolle (1954); Ramanujam and Rai (1963); Singh and Malhotra (1970); Gupta (1972); Ramana Rao *et al.,* (1974); Joarder (1978); Alam *et al.,* (1988); Ghafoor *et al.,* (1990); Husain *et al.,* (1997); Dash and Kole (2000); Deb (2002) and Hassan *et al.,* (2003) Talebi *et al.,* (2007), Yucel and Anlarsal (2010) in different crops.

Selection indices for seed yield were constructed to identify the character or character association over straight selection, which may be useful during selection breeding programme for higher yield. Direct selection for yield may be misleading. To ensure the high yield, the multiple selection criteria based on the selection index of most of the contributing characters to yield would be most effective. For this purpose, to estimate relative efficiency of the character combinations through discriminate function method has been successfully followed by various workers on different crop, Singh and Singh (1974); Khaleque (1975); Paul *et al.,* (1976); Joarder *et al., (*1978); Salehuzzaman and Joarder, (1979); Zuberi and Eunus (1972); Kumar *et al.,* (1988); Nahar (1997);Deb (2002); Khan (2009) and Ara (2010).

The negative expected gain of SWPP alone reelects that it itself is not a complete character for higher yield rather it depends on other component character for higher yield.

Similar result was obtained by Nahar (1997) in Sugarcane where cane yield per clump (CYC) showed negative expected genetic gain.

It is always preferable to use a discriminant function containing a minimum number of characters which may lead to the maximum genetic advance. In this investigation, the highest expected genetic gain was observed in NPBFF, NPdPP, PdWPP, NSPP and SWPP and with their combination. From this result it is also found that with the inclusion of NPBFF in an index, the values of expected genetic gain are greatly increased. It reflects that Number of primary branches at first flower is one of the important components for yield.

CONCLUSION

In the present investigation, variability, heritability, genetic advance, correlation, path-coefficient and discriminant function selection of six lentil lines for eleven quantitative characters were investigated. The eleven characters studied are date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF). number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), Plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP).

In the analysis, all the characters showed a wide and pronounced range of variation indicating that the characters studied are quantitative in nature and are under polygenic in control. In the case of eleven agronomical characters, mean of six lentil lines as compared with their respective standard error were found to be highly significant in all the cases. This indicated that the lines were significantly different regarding these characters. However, for all the lines CV% of a particular character varied from line to line.

In the analysis of variances, the line (G) item was significant for all the characters. This result indicated that genotypes were genetically different from each other. This referred that the included lines would be suitable for further breeding research for the improvement for those characters. The item year (Y) was significant for all the characters except DFF. In the present investigation, G × Y item for the characters DFF and PHFF found to be significant which indicated that line and year interacted significantly.

In the present study, for all the characters, phenotypic variation (σ^2_P) was greater than that of σ^2_G, σ^2_Y, σ^2_{GY} and σ_E^2 components of variation as expected. The phenotype is the joint product of $\sigma^2_G$and σ_E^2. NSPP showed maximum value for all of the components of variation. Again, phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), year coefficient of variability (YCV), interaction coefficient of

variability (G×YCV) and environmental coefficient of variability (ECV) were observed to be the highest for NSPP followed by NPdPP indicated that the characters were inherited with higher variability. Such high values of genotypic and phenotypic coefficient also suggested good scope for improvement of character through selection.

The heritability estimate in the present investigation was found to be low in broad sense. Comparatively higher heritability was observed for PHMF and PHFF. Genetic advance as estimated for most of the characters were low. However, genetic advance expressed as percentage of mean were recorded to be moderate.

In this investigation, correlation studies showed that genotypic correlation was higher than the respective phenotypic correlation in most of the cases. SWPP showed positive correlation with PHFF, NPBFF, PHMF, NSBMF, PdWPP and NSPP both at phenotypic and genotypic level. SWPP showed significant positive correlation with NPBFF, NSBMF, PWPP, NPdPP, PdWPP, and NSPP at phenotypic level. But SWPP showed significant positive correlation with only PdWPP at genotypic level. Above information indicates that these characters are genetically related with SWPP more than those of the other yield components.

In the path-coefficient analysis, characters such as NPBFF, PHMF, NPBMF, NPdPP, PdWPP and NSPP exhibited direct positive effect on SWPP at phenotypic level. On the other hand, DFF, PHFF, NSBMF and PWPP showed negative direct effect at phenotypic level and PHFF, NSBMF and NSPP showed negative direct effects at genotypic level on SWPP. NPBFF, PHMF, NPBMF, NPdPP and PdWPP showed positive direct effect at both levels that means they contribute to yield directly.

In the analysis of discriminant function selection, the highest expected genetic gain was observed in NPBFF, NPdPP, PdWPP, NSPP and SWPP and with their combination. From this result it is also found that with the inclusion of NPBFF in an index, the values of expected genetic gain are greatly increased. It reflects that number of primary branches at first flower is one of the important components for yield.

PART II

GENOTYPE-ENVIRONMENT INTERACTION

INTRODUCTION

The genotype-environment (G × E) interaction is the main bottleneck which can vitiate entire efforts of a plant breeder for boosting higher yield. Thus, breeding for climate or environment resilient varieties is crucial (Allard and Bradshaw, 1964). Genotype-environment interaction is a term used to describe any phenotypic effects that are due to interactions between the environment and genes. The breeder should able to determine in predicting the magnitude and extent of the effect of G × E interaction as expression of genes, which are mostly related to environmental factors. These aspects provide ideas about a particular character on which greater emphasis should be given while selecting suitable plant type (Singh *et al.*, 1981).

Genotype-environment interactions are commonly seen as one of the major complications in plant breeding and have been widely discussed, particularly in relation to the choice of the selection environment(s) (Ceccarelli, 2007)**.** In agricultural experimentation, a large number of genotypes are normally tested over a wide range of environments (locations, years, growing seasons, etc.) and the underlying statistical and genetical theories used to model this system may be rather complicated. The occurrence of the genotype × environment interaction effect further complicates the selection of superior genotypes for a target population of environments. In the absence of G × E interaction, the superior genotype in one environment may be regarded as the superior genotype in all, whereas the presence of the G × E interaction confirms particular genotypes being superior in particular environments. A variety of statistical procedures are available to analyze the results of multi-environment trials. One of the most common methods regarding G × E interaction study is to compute the simple averages across replications for a genotype in an environment and then analyzing the means. Numerous methods have been used in the search for an understanding of the causes of G × E interaction (Van Eeuwijk *et al.*, 1996). These methods can be categorized into two major strategies. The first strategy involves factorial regression analysis of the G × E matrix (*i.e.*, the yield matrix after the environment and genotype main effects are removed) against environmental factors, genotypic traits or combinations thereof (Baril *et al.*, 1995). The second strategy involves correlation or regression analysis that relates the genotypic and environmental scores

derived from principal component analysis of the G × E interaction matrix to genotypic and environmental covariates. The term G × E interaction commonly refers to yield variation that cannot be explained by the genotype main effect (G) and the environment main effect (E). For cultivar evaluation, however, both G and G × E must be considered simultaneously. Using a sites regression model (SREG), (Yan *et al*., 2000) combined G and G × E, denoted as G + G × E or GGE, and repartitioned this into non-crossover G × E interaction and crossover G × E interaction. The term G × E interaction will be hereafter used to denote this combination. Understanding the causes of non-crossover and crossover G × E interaction would help develop an understanding of the genotypic characteristics that contribute to a superior cultivar and the environmental factors that can be manipulated to facilitate selection for such cultivars.

The mean performance appeared to be associated with linear component of genotype-environment interaction (Jatasra and Paroda, 1981). The variety with higher mean yield, regression coefficient 'b_i' near to unity and deviation from regression $\overline{S}_{di}^{2}$ value close to zero would be suitable for wide range of environments (Shahid and Kabir, 1997). Stability in performance is one of the most desirable properties of a genotype to be released as a variety for wide cultivation. In dealing with instability and uncertainty of yield and in developing improved varieties for wide adaptation, genotype-environment (G × E) interaction is of major concern for crop improvement (Eberhart and Russell, 1966). The present experiment was undertaken with a view to study G × E response and to identify both high yielding and stable genotypes over different irradiation doses.

Comstock and Moll (1963) have classified the environments in two categories, (i) micro-environments, that includes physical and chemical attributes of soil, climatic variables (temperature and humidity), solar radiation, insect pest and diseases; and (ii) macro-environment which is associated with general locations and period of time and is a collection of micro-environments. Environment has been classified in predictable and unpredictable environments by Allard and Bradshaw (1964). The predictable environment includes climates, soil type and day length. It also includes controllable variables (Perkins and Jinks, 1971), such as the level of fertilizer application, sowing dates, sowing density and methods of harvesting. The unpredictable environments

include weather fluctuation, such as differences between seasons in terms of the amount and distribution of rainfall and prevailing temperatures.

An understanding of environmental and genotypic causes G × E interaction is important at all stages of plant breeding, including ideotype design, parent selection, selection based on traits, and selection based on yield (Jackson *et al*., 1996; Yan and Hunt, 1998). Analysis of quantitative characters are very much complex when more than one environments are included because change in gene expression may occur with the changes of environments. These changes are observable as genotype-environment interaction in a biometrical analysis, have long been recognized as an important source of phenotypic variation (Immer *et al.,* 1934; Yates and Cochran, 1938 and Mather, 1949).

Environmental involvement in the expression of phenotype of an individual was first recognised by Johannsen (1909) while working with dwarf bean (*Phaseolus vulgaries*). He reported that heritable and non-heritable differences were jointly responsible for the variation in seed weight of beans and were of the same order of magnitude in effect. The different analysis of continuous variation over a number of years on many plant and animal species revealed the combination of heritable and non-heritable agencies in the determination of continuous variation.

Later on, Keelble and Pellow (1910) developed Johansen's findings and subsequently Fisher (1918) for the first time provided statistical method for partitioning the variation of quantitative characters in segregating populations into genetic and environmental components. East (1915) studying the quantitative characters of *Nicotiana rustica* L., clearly showed that the quantitative character was inherited with the joint action of genetical and environmental variation and that they were inherited according to Mendel's laws of inheritance. Horner and Frey (1957), Finlay and Wilkinson (1963), Abu-EI-Fittouh *et al.,* (1969) and Shorter *et al.,* (1977) investigated the influence of test environment.

At present, it has become a challenge to breeders to understand fully the control of genetic variation due to the occurrence of genotype- environment interaction. When a set of plant genotype is grown over a range of environments the genotypes do not

behave in the same relative way in all environments and it is due to the interaction of different genotypes with different environments differently. This situation leads the breeder to face serious problems in the realization of the breeding objective for any economic crop. Genotype-environment (G × E) interactions are almost unanimously considered to be among the major factors limiting response to selection and, in general, the efficiency of breeding programs. G × E interactions become important when the rank of breeding lines changes in different environments. This change in rank has been defined as crossover G × E interaction (Baker, 1988).

Some workers have tried to solve the problem created by G × E interaction. Sprague and Fedarar (1951), Comstock and Rabinson (1952), Hanson *et al.,* (1956) and Comstock and Moll (1963) mainly developed the analysis of variance to estimate G × E interaction. It provides information on the existence and magnitude of G × E interaction only but they gave no measurement of response of individual genotype with the environment as such stability measurement of individual genotype was not tested.

In the recent past, two main approaches have been made under regression for detecting and estimating the interaction between genotypes and environments. The first is purely statistical methods originally proposed by Yates and Cochran (1938), which was later on modified by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). Finlay and Wilkinson (1963) used this method to detect and measure the magnitude of G × E interactions in barley and considered linear regression slopes as a measure of stability. Eberhart and Russell (1966) emphasized the need of considering both the linear (b_i) and non-linear $\overline{S}_{di}^2$ components G × E interaction in judging the phenotypic stability of a genotype. A cultivar with a high mean with unit regression coefficient (b =1.0) and a deviation of zero ($\overline{S}_{di}^2 = 0$) from regression is referred as stable genotype.

The second approach involves the fitting of models, which specify the contribution of genetic and environmental actions and genotype-environment interactions to the generation means and variances. It also determines the contribution of additive, dominance and non-allelic gene action to the total genotype-environment interaction components. This approach had been used by Mather (1949), Jinks (1954), and Jinks

and Mather (1955) in *Nicotiana rustica* L. followed by Bucio Alanis (1966), Bucio Alanis and Hill (1969), and Perkins and Jinks (1968).

Perkins and Jinks (1968) formed a bridge over the gap between two alternative analyses. Later, Breese (1969) and Paroda and Hayes (1971) advocated that the linear regression (b_i) could simply be regarded as measure of response of a popular genotype, whereas the deviations around the regression lines ($\overline{S}_{di}^{2}$) were considered as better measure of stability; genotypes with their lowest deviations being the most stable and vice versa. Using the above definition of the term stability, it was possible to judge the phenotypic stability and due consideration was also given to the mean performance and linear response of the individual genotype.

The joint regression analysis, a form of the analysis of variance, has been widely used in the study of G × E interaction. Its procedures and applications were reviewed by Freeman (1973) and Hill (1975). The effectiveness of the analysis in resolving the differences in genotypic response is related to the degree of linearity of response. On the other hand, successful application necessitates that a high portion of G × E interaction sum of square is attributed to the linear regression.

The study of G × E interaction in its biometrical aspects is important not only from genetic and evolutionary point of view but also necessary to the agricultural production problem in general and particularly for plant breeding problem (Breese, 1969). Kang and Miller (1984) also reported that information on cultivar stability performance across environment would help breeders to select more consistently promising cultivars.

A variety or genotype is considered to be the most adaptive or stable one if it has a high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments. It was suggested by Ruschel (1977) that plant breeders have the choice of either selecting genotypes of restricted adaptability for defined ecological condition or searching genotypes with wider adaptability capable of sustaining production in spite of wide variation in environments. It is now recognized that G × E interaction is an important source of phenotypic variations. As under the control of gene, breeders are trying to produce and select suitable cultivars which gave

maximum economic yield over a range of environments with wider adaptabilities and stabilities. In plant breeding usually many potential genotypes are evaluated in different environments before selecting certain desirable traits. Comstock and Moll (1963) reported that selection is impeded due to large effect of G × E interaction. However knowledge about the description, prediction and inheritance of genotype-environment interaction would provide more information and help the breeders to breed and select better genotypes.

In Bangladesh, no detail investigation on G × E interaction has been performed. Extensive research efforts are necessary for the improvement of lentil crop in our country. Keeping this view in mind, the present investigation was undertaken to select the stable lines with high yield potential.

The present investigation was, therefore, undertaken to study the G × E interaction for eleven quantitative characters of six genotypes in order to select the suitable genotypes having wider adaptability in different agroclimatic regions of Bangladesh.

REVIEW OF LITERATURE

Nature versus nurture arguments assumes that variation in a given trait is primarily due to either genetic variability or exposure to environmental experiences. The current scientific view is that neither genetics nor environment is solely responsible for producing individual variation and that virtually all traits show gene-environment interaction. The fundamental nature of gene action and interaction involved in the inheritance of quantitative characters were not understood until genetical assumption and biometrical methods developed in the early days of 20th century were brought together by rediscovery of Mendel's work. Johannsen (1909) for the first time put forward the idea of the relationship between heritable and non heritable (environmental) effects and that the variation in a pure line was due to environment. At present, many researchers all over the world are working on genotype-environment for different quantitative characters with various crop plants. Many literatures have already been published in various crops and a few in lentil concerning with the problem of genotype-environment interactions at different times and some of these papers are narrated below:

East (1915) showed that the continuous variation in the segregating generation was due to both genotype and environmental effects.

Fisher *et al.,* (1932) developed mathematical model which was the foundation of the Mather (1949), Mather and Jones (1958) and Stevens (1959) genotype-environmental interaction measure techniques. That involved the partitioning of the variation of quantitative data into genotypic and environmental effects and their interactions.

Fejer (1958) reported that the variations of a population were contributed not only by environmental effect but also by the genotype-environment interaction.

Finlay and Wilkinson (1963) developed statistical technique to compare the yield performance of a set of cereal varieties grown at several locations for several seasons. The regression of yield on mean yield of all varieties for each site and season when tested

for varieties and sites had a high degree of linearity. Yates and Cochran (1938) also developed similar techniques.

Gandhi *et al.,* (1964) studied the genotype-environment interaction in wheat to obtain estimates of the magnitude of the variety × location, variety × year and variety × location × year interactions. They considered the implications of these interactions for obtaining information on the optimum number and allocation of location and years and for this test were conducted over a three years period at five locations under normal sowing conditions.

Bucio-Alanis (1966) studied the genotype-environment interaction in *Nicotiana rustica.* He observed that genotype-environment interaction significantly influenced the phenotypic expression.

Eberhart and Russell (1966) recommended that a genotype with a regression coefficient (b_i) about 1.0 shows average stability over all environments tested, when b_i>1.0 there is evidence of good yielding capacity for favourable environments and when b_i<1.0 there is deficiency in yielding ability under these conditions. They again proposed that a variety with mean > grand mean, unit regression coefficient (b_i=1.0) and least deviation from regression ($\overline{S}_{di}^2$ =0) is considered as a stable genotype.

Westerman (1971) worked on the same problem and conducted that both linear and non-linear response of environment were controlled by additive and non-additive variation.

Joarder *et al.,* (1978) studied G × E interaction of some quantitative characters of four varieties of *Brassica campestries* L. They reported that G × E interaction item was highly significant for all the characters they studied and in all the six generations. The joint regression analysis showed that all the items were significant at 1% level except environment residuals for seed/siliqua and yield/plant. Both the linear and non-linear items were significant for all the characters and generations. Mean performance was

significantly correlated with $\overline{S}_{di}^2$ but bi was independent of Xi. Correlation between bi and $\overline{S}_{di}^2$ was highly significant but is negative in case of seeds/ siliqua and yield/plant.

Singh and Bejiga (1990) showed significant differences between mean of the seasons and locations and lines for all the characters in all the three years except for biological. Some lines were high yielders but sensitive to changing environments, while other lines were resistant to changing environment but average yielders.

Chowdhury *et al.,* (1998) worked on ten lentil (*Lens culinaris*) genotypes were evaluated at five cultural environments to determine the genotype × environment interactions for nodules/plant, plant height, pods/plant, individual plant biomass, 1000-grain weight and yield/plant. Significant G × E interactions were observed for all the traits. The linear portions of G × E interactions were highly significant for all the characters except 1000-grain weight. A significant non-linear component was found for pods/plant, individual plant biomass and 1000-grain weight. Genotype Bm 1052 was specially stable for nodulation. Bm 157, Bm 1052, Bm 681 and Bm 684 were most stable for different characters including yield while Bm 728, Bm 1185 and Bm 1243 were suited to unfavourable environments.

Ashraf *et al*., (2001) worked with 13 advance lines and three check varieties viz., Chakwal-86, Pak-81 and Rawal-87 of wheat were planted at nine locations to estimate genotype-environment interaction. Both the linear and non-linear (pooled deviation) components were highly significant, indicating the presence of both predictable and unpredictable components of genotype-environment interaction. The stability parameters for the individual genotype revealed that the genotype, 89R-35 and 90R-36 showed the regression closer to the unity along with low deviation from regression and thus may be stated as stable genotypes.

Islam *et al.,* (2002) worked on genotype- environment interaction on yield and some of the yield components in lentil (*Lens culinaris* Medic.). They carried out investigation for NPBFF, NSBMF, DWPP, PdWPP, NPdPP, NSPP and SWPP in twelve genotypes at

eight environments. The item genotype (G) was highly significant for all the characters, indicating that genotypes were genetically different. On the other hand, environment (E) item was significant for all the characters except NSPP. Significant G × E item indicated that the genotypes interacted with the environments differently for most of the characters under study. In the joint regression analysis, major part of G × E interaction was not due to heterogeneity however, remainder item was found to be highly significant for all the characters. The regression coefficient (b_i) exhibited above average responses for significance of regression values in different genotypes for all the characters except NSBMF, NPdPP and NSPP. The high and significant $\bar{S}^2_{di}$ values indicated the unstable performance for all the genotypes and characters under study.

Arshad *et al.,* (2003a) evaluated 25 genotypes of chickpea for stability of grain yield under twelve diverse environments within Pakistan. The interaction between the genotypes and environments was used as an index to determine the yield stability of genotypes under all the environments. The genotype-environment interaction was highly significant and both linear as well as non-linear components were equally important for determining the yield stability. Since the regressions (b_i) were not significantly different from linearity, therefore, stable performance of the varieties could not be predicted on 'b_i' alone. In this case, deviations from regression and the cultivars yield were used to judge the superior genotypes.

Hossain *et al*., (2003) studied 10 soybean genotypes across five environments to see genotype-environment interactions through different stability parameters and performance of some traits. Stability analysis revealed that the genotypes used in this study were all, more or less responsive to environmental changes. Most of the genotypes perform better in Env. 3. Based on phenotypic indices (Pi) and deviation from the regression ($\overline{S^2}_{di}$), genotype colombus was found stable for days to maturity.

Amin *et al.,* (2005) evaluated ten promising wheat genotypes for grain yield stability under varied environments at nine locations in the North West Frontier Province, Pakistan. The interaction between genotypes and environments was found significant in this study. None

of the regression coefficients (b_i) was significantly different from unity; therefore, stable performance of the genotypes could not be predicted on 'b_i' alone. In this study, deviations from regression ($\overline{\delta}^2_{di}$) and average grain yields were used to identify the superior genotypes. Above average grain yields were observed in genotypes, CT-99022, SAW-98063, CT- 99155 and Saleem-2000. Although cultivar Saleem-2000 produced high yield, on the basis of high $\overline{\delta}^2_{di}$ value seemed to be sensitive upon environmental changes. Based on grain yield performance, low deviation from regression and b_i values the genotype CT-99022 is more suitable for favourable and CT-99155 for unfavourable environments. Stable performance was expressed by SAW-98063 because of higher grain yield, regression coefficient (b_i = .983) and low deviation from regression ($\overline{\delta}^2_{di}$ = 0.065).

Mishra and Khan (2006) studied thirteen cultivars of lentil were evaluated during 1999-2000, 2000-01 and 2001-02 in Jabalpur, Madhya Pradesh, India for stability of grain yield and its component traits under rainfed conditions. Significant genotype × environment interactions was observed. The linear component of GE interaction was significant for number of branches per plant, number of pods per plant and 100-seed weight, whereas non-linear component was significant for all the traits, except for number of branches per plant. Of the 13 cultivars, JL 3 gave the highest yield, followed by PL 639, DPL 62, HPL5 and PL 4 over the years. These genotypes have, thus, wider stability for grain yield over the years.

Gupta *et al*., (2006) evaluated forty lentil genotypes for genotype × environment interaction and phenotypic stability under 8 diverse environments in Ghaziabad, Uttar Pradesh, India during 1998-99 and 1999-200. Eleven different growth and yield characters were evaluated. No single genotype was stable for all the characters in the study.

Rohman *et al*,. (2006) worked on stability analyses were carried out for days to maturity, number of fertile tillers/plant, 100-grain weight, and yield/plant using eight exotic and one local genotypes of sorghum across three locations during the rabi season of 2001-02 and 2002-03. MS for genotype, environment, and genotype × environment interaction

was significant for al the characters studied. Considering all the stability parameters, genotype BSV 16 was found to be more stable as having very low S2di with near unit regression and high yield. Genotypes BSV 34, BSV45 BSV71 BSV720 and Khagrachari local were highly responsive to favorable environment for grain yield. The genotype BSV33 was found suitable for unfavorable environment for grain yield.

Pervin *et al.,* (2007) compute genotype-environment interaction, an investigation was carried out with twenty-four lines of blackgram for five yield and yield contributing characters, such as plant height at first flower (PHFF), number of branches at maximum flower (NBMF), number of pods per plant (NPd/P), pod weight per plant (PdW/P), seed weight per plant (SW/P). Joint regression analysis revealed that G×E item was significant for PHFF and PdW/P. Line-5 for PHFF, line-4 for NPd/P and PdW/P considered as stable genotypes having unit regression coefficient and non significant ($\overline{S}_{di}^{2}$)values.

Kumar *et al.,* (2007) worked on stability analysis using 15 diverse genotypes of lentil evaluated under four environments (varying dates of sowing and fertilizer application condition) was conducted for eight characters. The ANOVA indicated significant differences for genotype, environments and most of the characters under study. Genotype × environment interaction was significant for plant height, seeds per plant, pods per plant, and harvest index thereby suggesting that genotypes differed under varying environments for these characters. Genotypes L-7357, L-7359, L-4076, L-4660 and LP-10207 were stable with high mean value, least deviation from regression and average response (bi near to unity).

Kanouni *et al*., (2007) evaluated chickpea lines under dryland conditions seven desi type chickpea genotypes together with one check cultivar (Pirouz) in three research stations (Gerize, Kharke and Ghamlu) of Kurdistan province, during three years (2001-03) in RCB design with four replications. The analyses of variance showed that there were significant differences among environments and mean yield of genotypes and the genotype-environment interaction was also significant. Stability analysis after Eberhart and Russell's method showed that there were significant variations due to genotypes,

environments and their interactions for one hundred seeds weight, and due to environment for seed yield. On the basis of stability parameters (P_i, b_i, $\bar{\delta}^2_{di}$, and CV %), genotype ICCV 91006 with the highest seed yield (821 kg/ha) was selected as the most desirable and stable genotype.

Terasawa (2008) studied to quantify environmental variation and genotype-environment effects in maize hybrids, within and between two macro-environments in the state of Paraná; and to group the environments according to the hybrid performance and determine the most adequate locations for selection in the state in the main crop season. The trials were carried out in the 2003/2004 growing season at six locations: Campo Largo (CL), Ponta Grossa (PG), Fazenda Rio Grande (FZ), Londrina (LD), Centenário do Sul (CS) and Palotina (PL). The effects of location (L), macro-environments (ME), locations within macro-environments (WME), hybrids (H), and the interactions (H × L), (H × ME), and (H × WME) were significant (at 0.1 % probability). Two clusters were formed, contrasting with the macro-environment zoning: (CL, FZ) and (PG, CS, LD). PL was excluded from both. Under the average conditions of the state, environments appropriate for high yields were most suitable for selection as well.

Acikgoz *et al*., (2009) studied to evaluate dry matter (DM) yield and seed yield of six leafed and semi-leafless pea (*Pisum sativum* L.) genotypes, and to compare them for these traits. Evaluation of genotype × environment interaction, stability and cluster analysis were also carried out at eight diverse locations with typical Mediterranean and Mediterranean-type climate during the 2001–2002 and 2002–2003 growing seasons. Significant differences were found among the pea genotypes for DM and seed yield on individual years and combined over years, and in all locations. All interactions which related to genotype × environment interaction showed significance ($P>0.001$) for DM and seed yield. The highest yield (4789 kg/ha) was obtained from the leafed genotype 'Urunlu'. However, stability analysis indicated that for DM yield, the leafed genotypes 'Golyazi' and 'Urunlu' should be grown in low yielding and high yielding environments, respectively.

Fikere *et al.,* (2009) analyzed grain yield of 16 field pea genotypes with four replications across 12 environments during 2004-2006 growing season of South Eastern Ethiopia using parametric stability measures. To quantify yield stability eight stability statistics were calculated. IFPI-1523 and IFPI-2711 were more stable genotypes which has 7 out of 8 stability statistics used in this study. Moreover, the stability analysis identified genotype IFPI-1523 (genotype 10) and IFPI-2711 (genotype 4) as most stable genotypes. Highly significant correlations were found among stability parameters implying their closer similarity and effectiveness in detecting stable genotypes and they are equivalent in measuring stability.

Atta *et al.,* (2009) developed elite chickpea genotypes through mutation breeding at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad and evaluated for stability of grain yield at four diverse locations in the Punjab province during 2003-04, 2004-05 and 2005-06. They used the genotype yield, regression coefficient (bi), deviations from regression ($\bar{S}^2_{di}$) with sustainability index to identify the stable genotypes. The analysis of variance for seed yield at individual locations showed significant to highly significant differences between genotypes. Pooled analysis of variance over locations displayed highly significant differences between genotypes, locations and genotype × location interaction. Among 14 genotypes, the maximum mean seed yield over the locations was produced by the CC119/00 (1.229 t ha^{-1}) and the highest mean seed yield producing location was NIAB (1.412 t ha^{-1}). The analysis of stability based on mean grain yield, regression coefficient and deviation from regression revealed that the genotypes; CC119/00, CC117/00 (Colchicine mutants), CM256/99, CH38/00 and K-70022 were most stable and adapted to the diverse environmental conditions of Punjab.

Mosisa and Zelleke (2009) worked on the nature and magnitude of genotype x environment interaction and phenotypic yield stability of twenty maize cultivars at nine locations with three replications for two years. Variances due to genotypes, years, locations, genotype × year, genotype × location and genotype × year × location interaction were significant ($P<0.01$). Most of the cultivars had significant deviation

mean squares ($\bar{S}^2_{di}$), implying that these cultivars had unstable performance across the testing environments.

Akhtar *et al*., (2010) tested 15 genotypes of mungbean at five locations in Pakistan in the Kharif season 2006 to study their yield stability. Pooled analysis of variance and stability analysis were performed. The genotype (G) × environment (E) interaction and both variance due to genotypes and environments were significant. The partitioning of G × E interaction into linear and non-linear components indicated that both predictable and unpredictable components shared the interaction. Three stability parameters were computed to judge the stable and superior genotype.

Choudhary and Haque (2010) studied forty-two lines of chickpea (*Cicer arietinum* L.) including two checks grown during winter season (rabi) for three years 1988-89, 1989-90 and 1990-91 in twelve environments for stability parameters of Chhotanapur regions. Pooled analysis of variance revealed that the mean sum of squares due to genotypes and environment for primary branches/plant, secondary branches/plant and grain yield/plant (g) found highly significant (except primary branches /plant) indicating presence of high variability among the genotypes and environment. Environment (linear) was found highly significant for all the characters which indicated variation in weather condition of the location. The pooled deviation (non-linear portion of the variance) was found highly significant for all the characters. The environment + (Genotypes × environment) interaction were found highly significant, showing important role of environment and genotype × environment interaction to these characters.

Karadavut *et al.,* (2010) studied with faba bean (*Vicia faba* L.) to determine genotype × environment interaction and stable cultivar(s) for grain yield in Turkey. The study was carried out during two years at six different locations around South Anatolian Region. According to stability analysis results, cultivar 1 (Eresen, 87) was the most stable for grain yield. Among the cultivars, the highest grain yield was obtained from cultivar Eresen 87 (3.21 t/ha) across environments. This genotype had regression coefficient (b_i =1) around unity and deviations from regression values ($\bar{\delta}^2_{di}$ =0) around zero. This

suggested that both these attributes were responsive to changing environments and could be recommended for favourable environments.

Duzdemir (2011) studied the influence of genotype × environment interactions on phenological characteristics of chickpea. Field experiments were carried out on four different locations, in semi-arid conditions, in complete randomized block design with four replications from 2001 to 2002. Eleven certified and 3 indigenous varieties were used. Emergency date, first flowering period, flowering period and vegetation period were examined as phonological characteristics. For all the characteristics, important changes, source of genotype × environment interactions, were determined at $P<0.01$. Stability analysis was carried out for all the characteristics according to Finlay and Wilkinson (mention year) and Eberhart and Russel's (1966) models. Stable genotypes for each characteristic were found for two parameters.

Tiawari *et al*., (2011) were evaluated sixteen early maturing and elite genotypes of sugarcane at different environmental condition for identifying the stable cultivars. The stability of genotypes was estimated by using the method of Eberhart and Russell. In this analysis sum of square due to G × E were partitioned into individual genotypes (Xi), regression of environmental means (bi) and deviation from regression ($\bar{S}^2_{di}$). The regression coefficients (bi) and mean square deviation from regression ($\bar{S}^2_{di}$) were used to define genotype stability. Significantly mean square differences among Genotypes × Environment for all the characters were observed, this is an indication of significant variability among the experimentation. The stability parameters for NMC, cane yield, sucrose % and CCS% shown by the genotype CoJ64 compared to UP05233, CoS05266, CoS05260, CoS05276 and CoS05263 indicated better adoption and less sensitive to environmental changes. They concluded that for cane yield and sucrose % in juice the genotypes UP05233 and CoS05263 performance better than rest of elite genotypes studied having high mean values of genotypes over all three environments. Therefore, these genotypes may be commercially cultivated over a wide range of environments.

Rao (2011) studied twenty one advanced breeding lines (chickpea) selected from All India Co-ordinated trials and one local popular variety "annegiri' over three years to identify high yielding stable genotypes. Genotype, environment and G × E interaction variance found to be significant. Genotypic variance over environments was significant for grain yield, pods/plant and 100 seed weight. Both linear and non-linear components were found to be important for the traits studied. Significant non-linear component for grain yield indicated the predictability of the trait. Of all the genotypes C-506 and C-527 were found to be stable.

MATERIALS AND METHODS

A. MATERIALS

The materials used in this part were same as the materials of PART-I.

B. METHODS

The methods used in this study are described under following sub-heads:

1. **Preparation and Design of the Experimental Field.**
2. **Sowing of Seeds.**
3. **Maintenance of the Experimental Plants.**
4. **Collection of Data and**
5. **Techniques of Analyses of Data**

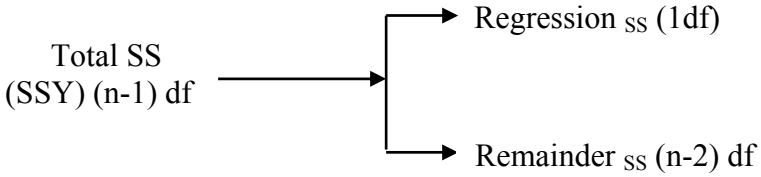
The methods from 1 to 4 are the same as those described under the methods of PART-I. The experiment was conducted in the three consecutive years, where the years considered as environment viz. (i) 2009-20010 (Y1), (ii) 2010-2011 (Y2) and (iii) 2011-2012 (Y3).

5. Techniques of Analyses of Data

a) Regression analysis

To study the genotype × environment interaction, the data were analyzed considering the following heads:

1) Regression analysis was done following Perkins and Jinks (1968) models. The primary analysis of regression was done as follows:



Where,

n = number of observation

Regression $_{SS} = (SP_{XY})^2/SS_X$

Remainder $_{SS}$ = Total $_{SS}$ (SS_Y) – Regression $_{SS}$

Where,

$$SS_X = \sum X^2 - (\sum X)^2 / n$$

$$SP_{XY} = \sum XY - \sum X.\sum Y/n$$

$$SS_Y = \sum Y^2 - (\sum Y)^2 / n$$

Regression coefficient (b_i): The responses of each genotype under different environments on the environmental means over all the genotypes are measured by regression coefficient. This was estimated as follows:

$$b_i = \frac{SP_{XY}}{SS_X}$$

The analysis of genotype × environment interaction was followed as the specification given by Mather and Jones (1958). A practical application of these specifications in inbred lines as well as in segregating generation was given by Bucio-Alanis (1966) and Bucio-Alanis and Hill (1969). Finally, the approach extended to any number of lines using the joint regression analysis by Yates and Cochran (1938) and put into a biometrical context by Perkins and Jinks (1968), was followed. The application is as follows:

In general, the Y_{ij} of the r replicates of the i^{th} genotype in the j^{th} environment is expected to be the sum of four components.

$$Y_{ij} = \mu + d_i + e_j + g_{ij} + e_{ij}$$

With 'i' varies from 1 to L, the number of lines and j varies from 1 to E, the number of environments.

μ, the overall means which is estimated as

$$Y../LE = \sum_{i=1}^{L} \sum_{j=1}^{D} Y_{ij} / LE$$

d_i is the genetical deviation of the i^{th} lines and as estimated as

$$(Y_{i.}/E) - \mu = \left(\sum_{j=1}^{D} Yij / E \right) - \mu$$

e_j is the additive environmental deviation of the j^{th} environment and is estimated as

$$(Y_{.j}/L) - \mu = \left(\sum_{i=1}^{L} Y_{ij} / L \right) - \mu$$

g_{ij} the genotype × environment interaction of the i^{th} genotype and the j^{th} environment is estimated as

$Y_{ij} - \mu - d_i - e_j$ and

Finally, e_{ij} is error associated with each observation.

Besides, the data was subjected to a standard two way analysis of variance to test the significance of the items which necessitates the inclusion of genotypes × environment

interaction model where environmental effects in each genotype are linear function of the additive environmental variance *i.e.*

$$g_{ij} = b_i e_j$$

Whether these linear function differ among the genotypes is tested by the adequacy of the models

$$Y_{ij} = \mu + d_i + (1+b_i) e_j + e_{ij}$$

by a joint regression analysis in which the sum of squares for genotype × environment (L × E) interactions are partitioned into linear and non-linear portions following Perkins and Jinks (1968) model.

In the joint regression analysis the L × E $_{SS}$ is partitioned into heterogeneity of regression sum of square (linear) and reminder sum of square (non-linear) are shown in the following skeleton and whole joint regression analysis is shown in the following:

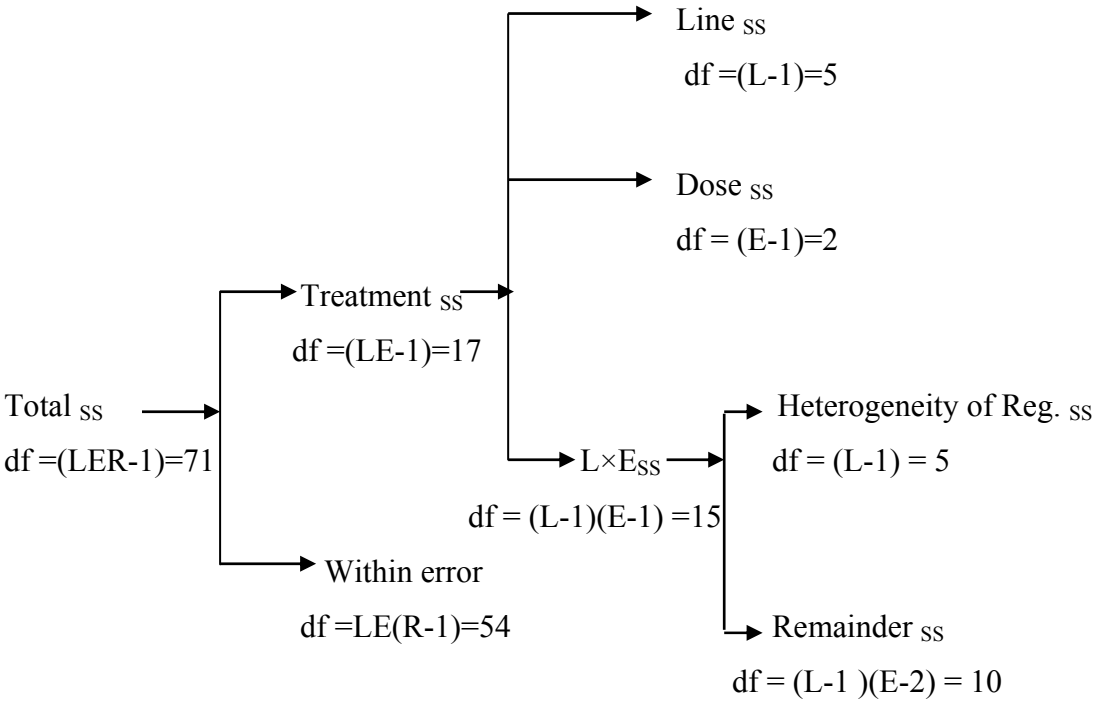


Table 19: The joint regression analysis.

Item	df	SS	MS	F1
Line (L)	L-1	SS_1	MS_1	MS_1 / MS_6
Year (E)	E-1	SS_2	MS_2	MS_2 / MS_6
L×E	(L-1)(E-1)	SS_3	MS_3	MS_3 / MS_6
Heterogeneity of Reg.	L-1	SS_4	MS_4	MS_4 / MS_6
Remainder	(L-1)(E-2)	SS_5	MS_5	MS_5 / MS_6
Within error	LE(r-1)	SS_6	MS_6	

In this approach, the regression coefficient and the deviation from regression are used as the parameters of stability. As the regression of e_j on e_j is one, and regression of g_{ij} on e_j is β, therefore, the b_i value of Eberhart and Russell's (1966) model is

$$b_i = 1 + \beta_i$$

or, $$\beta_i = b_i - 1$$

The stability parameters following Eberhart and Russell's (1966) model are calculated as follows:

$$Y_{ij} = m + \beta_i I_j + \sigma_{ij}$$

Where,

i varies from 1 to L, the number of lines and

j varies from 1 to E, the number of environments.

Y_{ij} = Mean of ith lines in jth environments,

m = Mean of all the lines over all the environments.

β_i = The regression coefficient of the ith lines on the environmental index which measures the response of this lines to varying environments.

I_j = The environmental index which is defined as the deviation of the mean of all the lines at a given environment from the over all mean.

$$= \frac{\sum_i Y_{ij}}{L} - \frac{\sum_i \sum_j Y_{ij}}{LE} \qquad \text{With } \sum_j I_j = 0$$

and σ_{ij} = The deviation from regression of the i^{th} lines at j^{th} the environment.

Two parameters of stability are calculated in the following way:

(a) The regression coefficient which is the regression of the performance of each line under different environment on the environmental mean over all the genotypes. This is estimated as follows:

$$b_i = \sum_i Y_{ij} I_j / \sum_j I_j^2$$

Where,

$\sum_j Y_{ij} I_j$ is the sum of products and

$\sum_j I_j^2$ is the sum of squares.

(b) Mean square deviations, $\overline{S}_{di}^2$ (Stability) from linear regression: It is estimated by the following formula,

$$\overline{S}_{di}^2 = \frac{\sum_j \sigma^2{}_{ij}}{(S-2)} - \frac{S_e^2}{r}$$

Where,

$$\sum_j \sigma^2{}_{ij} = \left[\sum_j Y_{ij}^2 - \frac{Y_i^2}{L}\right] - \frac{\left(\sum_i Y_{ij} I_j\right)^2}{\sum_j I^2{}_j}$$

$\sum_j \sigma^2{}_{ij}$ = The variance due to the deviation from regression, i.e., remainders sum of square.

$\sum_j Y_{ij}^2 - \frac{Y_i^2}{L}$ = The variance due to the dependent variable (SS_Y).

$\frac{\left(\sum_i Y_{ij} I_j\right)^2}{\sum_j I_j^2}$ = The variance due to regression (Reg_{SS}).

$S^2{}_e$ = the estimate of the pooled error and

r = the number of repeatitions.

The various computational steps involved in the estimation are as follows:

Computation of environmental index (I_j):

$$I_j = \frac{\sum_j Y_{ij}}{L} - \frac{\sum_i \sum_j Y_{ij}}{LE}$$

$$= \frac{\text{Total of the lines at the environment}}{\text{Number of lines}} - \frac{\text{Grand total}}{\text{Total number of observation}}$$

Computation of regression coefficient (b_i) for each line:

$$bi = \frac{\sum_j Y_{ij} I_j}{\sum_j I_j^2}$$

Where,

$\sum_j Y_{ij} I_j$ = For each lines is the sum of product of environmental index (I_j) with the corresponding mean ($\overline{X}$) of that lines at each environment.

Computation of $\overline{S}_{di}^2$: In general, it is obtained by subtracting the variance due to regression from σ_y^2. It is calculated as follows:

$$\overline{S}_{di}^2 = \left[\sum \sigma_{ij} / (S-2)\right] - \left(S_e^2 / r\right)$$

It was tested by test value. For it's testing within error was used.

Standard error of b_i was calculated as follows:

$$S_{bi} = \sqrt{\frac{Rem.ms}{SS_X}}$$

2) Graphical analysis

(i) Curve

In the graphical analysis curves were drown separately for twelve yield and yield contributing characters of lentil viz. DFF, PHFF, NPBFF, PHMF, NPBMF, NSBMF, PWPP, NPdPP, PdWPP, SNPP and SWPP. For this purpose, environmental mean were plotted along the X- axis and the genotypic mean along the Y-axis.

(ii) Regression graph

The regression graphs were drown by plotting Y_i, the genotypic values along the vertical axis against X_i, the environmental values which are independent along the horizontal axis. In the figure the straight line drown in the simple regression of Y on X, sometimes called fitted lines. The equation of regression line is as follows:

$$Y = a + b\,(X_i - \overline{X})$$

Where, Y is the estimated genotypic values given by an amount of X of the environment, and a = $\overline{Y}$, mean of all genotypes, $\overline{X}$ = environmental mean and the b, the regression coefficient is given by

$$b = \frac{SP_{XY}}{SS_X}$$

Where,

SP_{XY} = Sum of product of X and Y

SS_X = Sum of squares of X.

RESULTS

Study of genotype × environment (G × E) interaction is of much valuable in the selection of better genotypes (Islam and Newaz, 2001). The magnitude of components of genetic variation and genotype × environment interaction can help to select the better genotypes (Haque *et al.*, 2003). In presence of significant G × E interactions, estimates of stability parameters are used to determine the superiority of individual genotype across the range of environments. Although plant breeders are very much aware of the importance to genotypic difference in adaptability, they have been unable to exploit them fully in breeding programs due to lack of suitable methods of defining and measuring them.

Results obtained for eleven agronomical characters, such as date of first flower (DFF), plant height at first flower (PHFF), number of primary branches at first flower (NPBFF), plant height at maximum flower (PHMF), number of primary branches at maximum flower (NPBMF), number of secondary branches at maximum flower (NSBMF), plant weight per plant (PWPP), number of pods per plant (NPdPP), pod weight per plant (PdWPP), number of seeds per plant (NSPP) and seed weight per plant (SWPP) of six lentil lines have been described under the following sub-heads:

A. REGRESSION ANALYSIS

The data were subjected to regression analysis to get information on genotype-environment interaction and the response of individual genotype in different environments. The results of regression analysis for the present investigation were done according to Perkins and Jinks (1968) model. The result of the present research work as described under the following sub-heads:

1. Phenotypic regression (b_i)

The regression techniques for studying the genotype-environment interaction are among the most widely used methods for investigating the response pattern of the individual line. For each of the six lines, the regression analysis of the line (genotype) values (L) of g_{ij} on the corresponding e_j values was done. The results of regression coefficient (b_i),

standard error of regression coefficient (S_{bi}) and regression coefficient (β_i) for eleven quantitative characters of six lentil lines are presented in **Table 20 (A-K)**.

The regression coefficient measures the responses to increments in an improving environment. As these increments were measured by the mean of all the lines, the average response for any set of lines under consideration must have a regression coefficient of unity. As indicated by the joint regression analysis, the distribution of all the six b_i values were heterogeneous as heterogeneity of regressions were significant when tested against within error and for this, all the lines had different responses to different environments. Regression coefficient in the present investigation were $b_i = 1.0$, $b_i > 1.0$ and $b_i < 1.0$ indicated an average, above average and below average response, respectively by the lines. The character wise responses of different lines are as follows:

Date of first flower (DFF): In respect of this character, all the lines showed non-significant regression coefficients except Bm1 and Bm6. The above average responses were observed in Bm3 and Bm6. On the other hand, Bm2 and Bm5 showed below average responses to the changing environments but Bm1 and Bm4 showed negative b_i values.

Plant height at first flower (PHFF): All the lines showed non-significant regression coefficients except Bm1 and Bm6 for this trait. The above average responses were observed in Bm3, Bm4 and Bm6. While Bm2 showed average response and Bm5 showed below average responses to the changing environments. Again Bm1 showed negative b_i value.

Number of primary branches at first flower (NPBFF): For this character, Bm3 and Bm3 showed non-significant regression coefficients. The rest of the lines showed significant regression coefficients. The above average responses were observed in Bm6. Bm1 and Bm3 exhibited average responses to the environments. The rest of the lines showed below average responses to the changing environments.

Plant height at maximum flower (PHMF): In this case all the lines had significant function to the environments except Bm5. Bm1 and Bm3 exhibited above average

response while Bm4 showed average response. The rest of the lines showed below average response to the changing environments.

Number of primary branches at maximum flower (NPBMF): In respect of this character, Bm5 showed non-significant regression coefficients. The rest of the lines showed significant regression coefficients. The above average responses were observed in Bm1, Bm4 and Bm6. The average responses showed only Bm2, while rest of the lines showed below average response to the changing environments.

Number of secondary branches at maximum flower (NSBMF): For this trait all the lines exhibited significant linear responses to the environments. Bm4 and Bm6 found to exhibit below average response and the remaining lines showed average responses to the environments.

Plant weight per plant (PWPP): Regarding this character, significant regression coefficients were recorded all of the lines except Bm5. Bm1 and Bm3 exhibited above average response, while Bm4 and Bm6 showed average response to the environments. The remaining lines Bm2 and Bm5 showed below average response to the changing environments.

Number of pods per plant (NPdPP): For this case, significant regression coefficients were recorded for all the lines except Bm4. The above average response was noted for Bm1 and Bm3. Bm4, Bm5 and Bm6 showed below average response to the environments and Bm2 showed average response for the entire environments.

Pod weight per plant (PdWPP): Bm4 had non-significant function to the environments. The rest of the lines showed significant regression coefficients. Bm1 and Bm3 exhibited above average response. The below average responses to the changing environments were shown by Bm2 and Bm4 while, rest of the lines showed average response.

Number of seeds per plant (NSPP): For this character, Bm2, Bm3 and Bm6 showed significant b_i values while, rest of the lines showed non-significant b_i values. The above average response was observed in Bm1, Bm3 and Bm6, while Bm2 exhibited

average response to the environments. The rest of the lines showed below average response to the changing environments.

Seed weight per plant (SWPP): In respect of this character, Bm2, Bm3 and Bm6 showed significant regression coefficients. The rest of the lines showed non-significant regression coefficients. The above average responses were noted for Bm1, Bm3 and Bm6 and the rest of the lines *viz*. Bm2, Bm4 and Bm5 exhibited below average responses to the environments.

2. Deviation mean square ($\overline{S}_{di}^2$)

Two parameters of stability *viz.* regression coefficient (b_i) and deviation mean square ($\overline{S}_{di}^2$) are shown in Table 20 (A-K).

The regression co-efficient (b_i) is considered as parameter of response and mean square deviation ($\overline{S}_{di}^2$ **)** as the parameter of stability. The deviation mean square ($\overline{S}_{di}^2$) measures the unpredictable irregularities in response to the environments. When the deviation mean square is non-significant, performance may be predictable. This predictable performance of a line is said to be stable. The $\overline{S}_{di}^2$ values were highly heterogeneous as revealed from the significant remainder item when tested against denominator (within error) in the joint regression analysis Table 21(A-K). Again, the individual genotypic $\overline{S}_{di}^2$ were also tested with respective individual genotypic error (i.e. test value) mention in the last column in Table 20 (A-K). The results of $\overline{S}_{di}^2$ values obtained for all the eleven quantitative characters of six lines are shown in Table 20 (A-K).

For all the characters non-significant deviation mean squares were shown by all the lines, which indicated that the lines are stable for regarding the traits.

3. Joint regression

The results of joint regression analysis of six lines of lentil over three environments are shown in Table 21(A-K). The environmental effects for each of the six lines, whether a linear function of the additive environmental values or not were tested by the joint regression analysis. The regression analysis of the six lines for eleven

agronomical characters has been done separately (Table 22A-22K) before calculating the joint regression analysis. On summing over all the six lines of sum of squares for regression (Reg SS) and remainder (Rem SS) in Table 22A-22K, a total sum of squares for regression and remainder were determined. The heterogeneity of regression was calculated by subtracting total sum of squares for remainder from sum of squares for L × E interaction (joint regression). An experimental sum of square was made within the repetitions means of experiment from each environment and was termed as within error.

Table 21A-21K showed that the line (L) item was highly significant for all the character except NSBMF, PWPP, NPdPP, PdWPP, NSPP and SWPP, which showed significance at 5% level when tested against within error. The year (E) item was also showed highly significant for all of the traits except DFF, which showed non-significant result. Character DFF and PHFF were showed highly significant value for item L × E interaction but rest of the characters showed non-significant value.

In the joint regression analysis, L × E interaction sum of square was partitioned into heterogeneity of regression sum of square (linear) and remainder sum of square (non-linear) and the results are shown in Table 21A-21K. It was observed from the table that the heterogeneity of regression was highly significant only for DFF and the rest of the characters were non-significant when tested against within error. It is indicated that the major portion of genotype-environment interaction was due to the differences between the slopes of non-linear regression for these traits. The remainder item was also highly significant for PHFF and just significant (5% level) for DFF. This indicated that there were deviations from linearity in these lines for these traits, where a large portion of non-significant linearity was existed. The significant remainder item suggested the non-linear type of L × E interaction was existed in the lines.

4. Remainder mean square

To get information about the individual line involvement in the significance of remainder item, each of the remainder mean square of individual line was tested against respective individual line error as shown in Table 23. It was observed from this table that the remainder mean square of all the lines for all of the characters were find to be non-significant except Bm3 for DFF.

B. GRPHICAL ANALYSIS

The graphical analyses are described under the following sub-heads:

1. Curve

The performances of six different lines in three different environments for eleven different characters are shown by curves. For this purpose the mean performance of each of the individual line against the mean performance of each of the environments were presented in figures 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 for DEF, PHFF, NPBFF, PHMF, NPBMF, NSBMF, PWPP, NPdPP, PdWPP, NSPP and SWPP, respectively. For each figure Bm1, Bm2, Bm3, Bm4, Bm5 and Bm6 were plotted.

Date of first flower (DFF): The performances of six lines for Date of first flower (DFF) are shown in Figure 6. The figure showed that Bm1, Bm2, Bm4 and Bm5 in Year-1 and Bm3 and Bm6 in Year-3 exhibited the highest mean performances. From this figure it observed that Bm3 in Year-1 had the lowest mean performance and Bm6 in Year-3 exhibited the highest mean performances among all the environments. The figure also showed that individual curves are intersected at some points among themselves indicating the existence of genotype-environment interactions for this character. It is in agreement with the joint regression analysis.

Plant height at first flower (PHFF): The performances of this lines against 3 environments for PHFF were presented in Figure 7. It was observed from the figure that Bm1, Bm5 and Bm6 in Year-1 and Bm2, Bm3 and Bm4 in Year-3 were exhibited the highest performances. On an overall basis Bm3 showed the highest performance in Year-3 and Bm1 showed the lowest performance in Year-1 in all the environments. In these figures intersecting of the curves among themselves indicating the existence of G × E interaction which is supported by the joint regression analysis.

Number of primary branches at first flower (NPBFF): The performances of lines for this character are shown in Figure 8. The highest mean performance for Bm2 and Bm4 were observed in Year-1. On the other hand Bm1, Bm3,

Bm5 and Bm6 showed the highest mean performances in Year-2. Among all the lines, Bm4 showed the highest performance in Year-1 and Bm1 showed the lowest performance in Year-3. The intersection of curves observed for this trait also indicating the existence of G × E interaction which was supported by joint regression analysis.

Plant height at maximum flower (PHMF): The line performances for PHMF are presented in Figure 9. The Bm1, Bm2, Bm3 and Bm4 in Year-1 and Bm5 and Bm6 in Year-2 showed highest mean performances. On an overall basis performances of Bm3 in Year-1 had the highest and Bm1 in Year-3 had the lowest performances for this trait also.

Number of primary branches at maximum flower (NPBMF): The performances of six lines for this character were presented in Figure 10. Here Year-1 had the highest increasing influence in Bm2 only. On the other hand Bm1, Bm3, Bm4, Bm5 and Bm6 had the highest mean performances in Year-2.This Figure also showed that Bm4 exhibited the highest and Bm6 exhibited the lowest mean performances in Year-2 and Year-3, respectively. Intercrossing of curves in the graphs indicating the existence of G × E interaction for this trait.

Number of secondary branches at maximum flower (NSBMF): For the character NSBMF all of the lines showed the highest increasing influence in Year-2. Among all the lines, Bm4 showed the best performance in Year-2 and Bm1 in Year1 exhibited the worst performance. The Figure 11 also showed that individual lines are intersected at some points with each other which is in agreement with the joint regression analysis.

Plant weight per plant (PWPP): From Figure 12 it was observed that six lines showed slightly increasing tendency in Year-2 and performed highest among years. Here Bm1 in Year-2 showed the highest performance. On the other hand, it was noted that Bm3 at all the years showed lowest performances in Year-3.

Number of pods per plant (NPdPP): In case of this character (Figure 13) showed that all the line showed the highest performances in Year-2 and Bm5 showed top value among them. Here, Bm1 in Year-1 showed the lowest mean performance. Intersecting of the curves in the graph indicating the existence of G × E interaction for this trait.

Pod weight per plant (PdWPP): From Figure 14, it was observed that all the line exhibited better performances in Year-2, while Bm5 in Year-1 had the lowest performance. Intersecting of curves are prominent for this trait also.

Number of seeds per plant (NSPP): Line mean performances for this trait are presented in Figure 15. All of the line showed the highest mean performance in Year-2, except Bm4. Bm4 showed best performance in Year-3 while Bm1 showed the lowest performance in Year-3. Here also it was observed that Bm2 among all the lines showed the highest performance in all the environments. Prominent intercrossing indicating the existence of genotype-environment interaction.

Seed weight per plant (SWPP): The performances were presented in Figure 16 for this character. It was observed that Bm4 showed the highest performance in Year-2 while rest of the lines exhibited the highest performances in Year-2. The lowest mean performance observed in Bm1 in Year-3 and best performance of Bm2 in Year-2. Here intercrossing of curves among themselves were also observed.

2. Regression graph

The regression lines for each lentil line against the corresponding environmental mean are shown in Fig. 17-27 for DEF, PHFF, NPBFF, NSBFF, NSBMF, PAPP, PWPP, RWPP, NPdPP, PdWPP, NSPP and SWPP, respectively. To avoid confusion the individual points were not plotted in the figures. Inter-crossings of regression lines were much prominent in all the characters indicating the existence of genotype-environment interaction for these traits.

Table 20 (A-K): Mean performance ($\overline{X}_i$), regression coefficients (b_i), (β_i), standard error of b_i (S_{bi}) and stability ($\overline{S}^2_{di}$) of six lines for different characters in lentil.

Table 20A: Date of first flower (DFF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	65.7063	-1.4644	± 0.5906	-4.4644	-4.2770	0.0289
Bm2	66.1950	0.2699	± 0.4574	-0.7301	-4.6089	0.0224
Bm3	67.4600	2.7565	± 1.8592	4.7565	51.0257	0.2379
Bm4	71.0458	-0.8199	± 0.6518	-1.8199	-4.0961	0.0319
Bm5	72.6646	0.4484	± 1.4341	-0.5516	-0.2172	0.0702
Bm6	73.5409	1.8094	± 0.8253	2.8094	1.9701	0.0845

Table 20B: Plant height at first flower (PHFF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	16.5641	-0.0376	2.0239	-1.0376	5.6036	1.2396
Bm2	17.8971	1.0310	1.3198	0.0310	2.1481	0.8084
Bm3	19.5856	1.3101	0.4750	0.3101	-0.0771	0.2909
Bm4	17.1425	1.4388	0.7977	0.4388	0.5256	0.4886
Bm5	17.1732	0.6420	0.6303	-0.3580	0.1750	0.3861
Bm6	16.5442	1.6157	0.0619	0.6157	-0.4025	0.0379

Table 20C: Number of primary branches at first flower (NPBFF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	6.0247	1.3458	0.0942	0.3458	-0.2357	0.0885
Bm2	6.3387	0.7155	0.3173	-0.2845	0.0777	0.2982
Bm3	6.0359	1.0579	0.5611	0.0579	0.8087	0.5273
Bm4	7.7152	0.6101	0.3283	-0.3899	0.1019	0.3086
Bm5	6.7567	0.9733	0.0551	-0.0267	-0.2556	0.0518
Bm6	6.1189	1.2974	0.1236	0.2974	-0.2139	0.1161

Table 20D: Plant height at maximum flower (PHMF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	29.1300	2.0873	0.1798	1.0873	-1.3727	0.0273
Bm2	32.2198	0.4147	0.0304	-0.5853	-1.6382	0.0046
Bm3	32.8978	1.1492	0.4780	0.1492	0.2856	0.0726
Bm4	29.8874	1.0983	0.4007	0.0983	-0.2882	0.0609
Bm5	28.1652	0.3676	0.9467	-0.6324	5.9319	0.1438
Bm6	28.4147	0.8829	0.1421	-0.1171	-1.4751	0.0216

Table 20E: Number of primary branches at maximum flower (NPBMF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	8.2497	1.1855	0.1613	0.1855	-0.2110	0.0906
Bm2	8.4119	0.9719	0.1988	-0.0281	-0.0894	0.1117
Bm3	9.2381	0.8909	0.0790	-0.1091	-0.3886	0.0444
Bm4	9.6508	1.1378	0.0721	0.1378	-0.3981	0.0405
Bm5	9.9823	0.5057	0.3480	-0.4943	0.6438	0.1956
Bm6	8.3771	1.3083	0.0191	0.3083	-0.4415	0.0107

Table 20F: Number of secondary branches at maximum flower (NSBMF)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	10.8555	1.1066	0.3752	0.1066	4.6615	0.0451
Bm2	11.3058	1.0050	0.0043	0.0050	-2.0787	0.0005
Bm3	11.9237	1.1997	0.0742	0.1997	-1.8157	0.0089
Bm4	15.0298	0.7428	0.3445	-0.2572	3.6025	0.0414
Bm5	12.8678	1.1235	0.1849	0.1235	-0.4435	0.0222
Bm6	11.6415	0.8224	0.2241	-0.1776	0.3243	0.0269

Table 20G: Plant weight per plant (PWPP)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	8.3852	1.1347	0.1919	0.1347	-0.5076	0.0567
Bm2	8.3813	0.8981	0.1856	-0.1019	-0.5294	0.0548
Bm3	6.5162	1.1403	0.0324	0.1403	-0.8362	0.0096
Bm4	7.2130	0.9039	0.0023	-0.0961	-0.8458	0.0007
Bm5	6.4373	0.8270	0.4736	-0.1730	1.2148	0.1400
Bm6	8.0446	1.0960	0.0661	0.0960	-0.8057	0.0195

Table 20H: Number of pods per plant (NPdPP)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	108.9163	1.7741	0.6735	0.7741	392.2732	0.0007
Bm2	129.4829	1.0538	0.0102	0.0538	-224.8279	0.0000
Bm3	102.6364	1.6044	0.0917	0.6044	-213.5159	0.0001
Bm4	126.0488	0.1529	0.1643	-0.8471	-188.2506	0.0002
Bm5	143.0072	0.6114	0.1096	-0.3886	-208.6329	0.0001
Bm6	123.0434	0.8034	0.3181	-0.1966	-87.2828	0.0004

Table 20I: Pod weight per plant (PdWPP)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	5.0896	1.3199	0.2910	0.3199	-0.0923	0.1999
Bm2	5.7182	0.7166	0.0709	-0.2834	-0.3479	0.0487
Bm3	4.2537	1.2413	0.3148	0.2413	-0.0460	0.2162
Bm4	5.4941	0.6100	0.3416	-0.3900	0.0105	0.2346
Bm5	4.4276	1.0339	0.4291	0.0339	0.2268	0.2947
Bm6	5.1305	1.0784	0.2358	0.0784	-0.1857	0.1619

Table 20J: Number of seeds per plant (NSPP)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	166.8133	1.7057	1.1974	0.7057	2290.9633	0.0004
Bm2	214.4653	1.0742	0.0492	0.0742	-694.0333	0.0000
Bm3	143.8636	1.1785	0.1487	0.1785	-652.9704	0.0001
Bm4	193.5870	-0.0858	0.4595	-1.0858	-258.7562	0.0002
Bm5	175.1758	0.7870	0.9459	-0.2130	1166.9993	0.0003
Bm6	186.0565	1.3404	0.0101	0.3404	-698.8795	0.0000

Table 20K: Seed weight per plant (SWPP)

Lines	$\overline{X}_i$	b_i	S_{bi}	β_i	$\overline{S}^2_{di}$	Test value
Bm1	3.1280	1.3576	0.8964	0.3576	0.8812	0.8726
Bm2	4.2662	0.8715	0.0167	-0.1285	-0.2564	0.0163
Bm3	3.6556	1.3596	0.4138	0.3596	-0.0143	0.4028
Bm4	4.2711	0.2984	0.5472	-0.7016	0.1672	0.5326
Bm5	3.2280	0.8650	0.5746	-0.1350	0.2108	0.5594
Bm6	3.7133	1.2480	0.2052	0.2480	-0.1972	0.1998

Table 21(A-K): Results of joint regression analysis for different characters in lentil.

Table 21A: Date of first flower (DFF)

Item	df	SS	MS	F
Line (L)	5	174.5542	34.9108	6.8369**
Year(E)	2	14.2638	7.1319	1.3967
L × E	10	200.2284	20.0228	3.9213**
Heterogeneity of reg.	5	129.7946	25.9589	5.0838**
Remainder	5	70.4339	14.0868	2.7588*
Within error	54	275.7348	5.1062	

Table 21B: Plant height at first flower (PHFF)

Item	df	SS	MS	F
Line (L)	5	19.5897	3.9179	9.5989**
Year(E)	2	8.8056	4.4028	10.7868**
L × E	10	13.1713	1.3171	3.2269**
Heterogeneity of reg.	5	2.7496	0.5499	1.3473
Remainder	5	10.4216	2.0843	5.1066**
Within error	54	22.0410	0.4082	

Table 21C: Number of primary branches at first flower (NPBFF)

Item	df	SS	MS	F
Line (L)	5	6.4652	1.2930	4.8613**
Year(E)	2	20.4825	10.2413	38.5026**
L × E	10	3.3982	0.3398	1.2776
Heterogeneity of reg.	5	1.5191	0.3038	1.1422
Remainder	5	1.8790	0.3758	1.4129
Within error	54	14.3634	0.2660	

Table 21D: Plant height at maximum flower (PHMF)

Item	df	SS	MS	F
Line (L)	5	59.6665	11.9333	7.2500**
Year(E)	2	50.7333	25.3667	15.4114**
L × E	10	27.9803	2.7980	1.6999
Heterogeneity of reg.	5	16.6612	3.3322	2.0245
Remainder	5	11.3191	2.2638	1.3754
Within error	54	88.8824	1.6460	

Table 21E: Number of primary branches at maximum flower (NPBMF)

Item	df	SS	MS	F
Line (L)	5	8.2216	1.6443	3.6970**
Year(E)	2	53.9457	26.9728	60.6447**
L × E	10	5.4293	0.5429	1.2207
Heterogeneity of reg.	5	3.6454	0.7291	1.6392
Remainder	5	1.7839	0.3568	0.8022
Within error	54	24.0175	0.4448	

Table 21F: Number of secondary branches at maximum flower (NSBMF)

Item	df	SS	MS	F
Line (L)	5	34.2581	6.8516	3.2947*
Year(E)	2	287.2784	143.6392	69.0717**
L × E	10	24.5894	2.4589	1.1824
Heterogeneity of reg.	5	7.8614	1.5723	0.7561
Remainder	5	16.7279	3.3456	1.6088
Within error	54	112.2966	2.0796	

Table 21G: Plant weight per plant (PWPP)

Item	df	SS	MS	F
Line (L)	5	12.1085	2.4217	2.8632*
Year(E)	2	55.1323	27.5661	32.5916**
L × E	10	3.6527	0.3653	0.4319
Heterogeneity of reg.	5	0.8876	0.1775	0.2099
Remainder	5	2.7651	0.5530	0.6538
Within error	54	45.6735	0.8458	

Table 21H: Number of pods per plant (NPdPP)

Item	df	SS	MS	F
Line (L)	5	3182.0921	636.4184	2.8289*
Year(E)	2	8164.9014	4082.4507	18.1468**
L × E	10	3370.6788	337.0679	1.4983
Heterogeneity of reg.	5	2551.1044	510.2209	2.2680
Remainder	5	819.5744	163.9149	0.7286
Within error	54	12148.3008	224.9685	

Table 21I: Pod weight per plant (PdWPP)

Item	df	SS	MS	F
Line (L)	5	5.0021	1.0004	2.7480*
Year(E)	2	19.2537	9.6269	26.4435**
L × E	10	3.0344	0.3034	0.8335
Heterogeneity of reg.	5	1.2847	0.2569	0.7057
Remainder	5	1.7498	0.3500	0.9613
Within error	54	19.6589	0.3641	

Table 21J: Number of seeds per plant (NSPP)

Item	df	SS	MS	F
Line (L)	5	8736.4364	1747.2873	2.4994*
Year(E)	2	12512.9444	6256.4722	8.9494**
L × E	10	9259.4900	925.9490	1.3245
Heterogeneity of reg.	5	3911.6162	782.3232	1.1191
Remainder	5	5347.8738	1069.5748	1.5299
Within error	54	37750.9544	699.0917	

Table 21K: Seed weight per plant (SWPP)

Item	df	SS	MS	F
Line (L)	5	3.5943	0.7189	2.7990*
Year(E)	2	8.4968	4.2484	16.5419**
L × E	10	3.5297	0.3530	1.3744
Heterogeneity of reg.	5	1.1975	0.2395	0.9325
Remainder	5	2.3322	0.4664	1.8162
Within error	54	13.8686	0.2568	

* and ** , indicate significance at 5% and 1% level, respectively.

Table 22(A-K): Regression analysis of six lines in eleven environments for different characters in lentil.

Table 22A: Date of first flower (DFF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff. ($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	29.3618	65.7063	-3.4644	-8.2359	28.5325	0.8292
Bm2	0.6705	66.1950	0.2699	0.6417	0.1732	0.4973
Bm3	134.9106	67.4600	5.7565	13.6851	78.7787	56.1319
Bm4	2.6081	71.0458	-0.8199	-1.9490	1.5979	1.0101
Bm5	5.3669	72.6646	0.4484	1.0660	0.4780	4.8890
Bm6	41.5743	73.5409	3.8094	9.0561	34.4980	7.0763

Table 22B: Plant height at first flower (PHFF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	6.0138	16.5641	-0.0376	-0.0552	0.0021	6.0118
Bm2	4.1164	17.8971	1.0310	1.5132	1.5601	2.5563
Bm3	2.8500	19.5856	1.3101	1.9227	2.5189	0.3311
Bm4	3.9721	17.1425	1.4388	2.1116	3.0383	0.9338
Bm5	1.1880	17.1732	0.6420	0.9422	0.6049	0.5831
Bm6	3.8366	16.5442	1.6157	2.3712	3.8310	0.0056

Table 22C: Number of primary branches at first flower (NPBFF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	6.2129	6.0247	1.3458	4.5941	6.1827	0.0303
Bm2	2.0912	6.3387	0.7155	2.4425	1.7475	0.3437
Bm3	4.8951	6.0359	1.0579	3.6114	3.8204	1.0747
Bm4	1.6387	7.7152	0.6101	2.0829	1.2708	0.3679
Bm5	3.2446	6.7567	0.9733	3.3228	3.2342	0.0104
Bm6	5.7982	6.1189	1.2974	4.4290	5.7461	0.0521

Table 22D: Plant height at maximum flower (PHMF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	37.1132	29.1300	2.0873	17.6494	36.8399	0.2733
Bm2	1.4617	32.2198	0.4147	3.5062	1.4539	0.0078
Bm3	13.0986	32.8978	1.1492	9.7171	11.1670	1.9316
Bm4	11.5578	29.8874	1.0983	9.2869	10.2000	1.3577
Bm5	8.7205	28.1652	0.3676	3.1083	1.1426	7.5779
Bm6	6.7620	28.4147	0.8829	7.4654	6.5911	0.1708

Table 22E: Number of primary branches at maximum flower (NPBMF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	12.8693	8.2497	1.1855	10.6586	12.6355	0.2338
Bm2	8.8476	8.4119	0.9719	8.7381	8.4923	0.3554
Bm3	7.1917	9.2381	0.8909	8.0097	7.1355	0.0562
Bm4	11.6858	9.6508	1.1378	10.2297	11.6391	0.0467
Bm5	3.3880	9.9823	0.5057	4.5468	2.2994	1.0886
Bm6	15.3926	8.3771	1.3083	11.7628	15.3893	0.0033

Table 22F: Number of secondary branches at maximum flower (NSBMF)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	65.3736	10.8555	1.1066	52.9841	58.6325	6.7411
Bm2	48.3590	11.3058	1.0050	48.1183	48.3581	0.0009
Bm3	69.1759	11.9237	1.1997	57.4412	68.9120	0.2639
Bm4	32.1020	15.0298	0.7428	35.5665	26.4199	5.6821
Bm5	62.0699	12.8678	1.1235	53.7918	60.4338	1.6361
Bm6	34.7874	11.6415	0.8224	39.3766	32.3835	2.4039

Table 22G: Plant weight per plant (PWPP)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	12.1683	8.3852	1.1347	10.4261	11.8301	0.3382
Bm2	7.7272	8.3813	0.8981	8.2520	7.4108	0.3164
Bm3	11.9581	6.5162	1.1403	10.4781	11.9484	0.0096
Bm4	7.5077	7.2130	0.9039	8.3057	7.5076	0.0000
Bm5	8.3453	6.4373	0.8270	7.5992	6.2847	2.0606
Bm6	11.0785	8.0446	1.0960	10.0712	11.0383	0.0401

Table 22H: Number of pods per plant (NPdPP)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	4900.2994	108.9163	1.7741	2414.2198	4283.0577	617.2418
Bm2	1511.1951	129.4829	1.0538	1433.9695	1511.0545	0.1407
Bm3	3514.3727	102.6364	1.6044	2183.3078	3502.9200	11.4526
Bm4	68.5168	126.0488	0.1529	208.0202	31.7988	36.7179
Bm5	525.0697	143.0072	0.6114	832.0420	508.7341	16.3356
Bm6	1016.1264	123.0434	0.8034	1093.3421	878.4407	137.6857

Table 22I: Pod weight per plant (PdWPP)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	5.8621	5.0896	1.3199	4.2354	5.5903	0.2718
Bm2	1.6638	5.7182	0.7166	2.2994	1.6477	0.0161
Bm3	5.2628	4.2537	1.2413	3.9834	4.9448	0.3181
Bm4	1.5684	5.4941	0.6100	1.9573	1.1939	0.3745
Bm5	4.0209	4.4276	1.0339	3.3176	3.4300	0.5909
Bm6	3.9101	5.1305	1.0784	3.4605	3.7317	0.1784

Table 22J: Number of seeds per plant (NSPP)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	9057.3244	166.8133	1.7057	3557.1384	6067.2693	2990.0551
Bm2	2411.7303	214.4653	1.0742	2240.3330	2406.6718	5.0585
Bm3	2942.4235	143.8636	1.1785	2457.6841	2896.3022	46.1214
Bm4	455.7034	193.5870	-0.0858	-179.0237	15.3678	440.3356
Bm5	3157.8591	175.1758	0.7870	1641.3319	1291.7681	1866.0911
Bm6	3747.3936	186.0565	1.3404	2795.4806	3747.1814	0.2122

Table 22K: Seed weight per plant (SWPP)

Lines	SS_Y (2 df)	Mean ($\mu+ d_i$)	Linear reg. coeff.($1+\beta_i$)	SP_{XY} (2 df)	$Reg._{SS}$ (1 df)	$Rem._{SS}$ (1 df)
Bm1	3.7480	3.1280	1.3576	1.9225	2.6099	1.1380
Bm2	1.0759	4.2662	0.8715	1.2341	1.0755	0.0004
Bm3	2.8601	3.6556	1.3596	1.9253	2.6176	0.2425
Bm4	0.5501	4.2711	0.2984	0.4226	0.1261	0.4240
Bm5	1.5271	3.2280	0.8650	1.2249	1.0595	0.4676
Bm6	2.2653	3.7133	1.2480	1.7674	2.2057	0.0596

Table 23: Remainder mean squares of six lines for different quantitative characters in lentil.

Lines	DFF		PHFF		NPBFF		PHMF		NPBMF		NSBMF	
	RMS	F	RMS	F	RMS	F	RMS	F	RMS	F	RMS	F
Bm1	0.83	0.04	6.01	3.11	0.03	0.04	0.27	0.08	0.23	0.33	6.74	0.60
Bm2	0.50	0.02	2.56	2.49	0.34	0.59	0.01	0.01	0.36	0.47	0.00	0.00
Bm3	56.13	6.17*	0.33	0.18	1.07	1.25	1.93	0.27	0.06	0.03	0.26	0.03
Bm4	1.01	0.05	0.93	0.90	0.37	0.30	1.36	0.12	0.05	0.02	5.68	0.80
Bm5	4.89	0.17	0.58	0.29	0.01	0.01	7.58	1.24	1.09	0.33	1.64	0.16
Bm6	7.08	0.58	0.01	0.00	0.05	0.04	0.17	0.02	0.00	0.00	2.40	0.35

Lines	PWPP		PdNPP		PdNPP		SNPP		SWPP	
	RMS	F	RMS	F	RMS	F	RMS	F	RMS	F
Bm1	0.34	0.06	617.24	2.40	0.27	0.14	2990.06	0.95	1.14	2.10
Bm2	0.32	0.08	0.14	0.00	0.02	0.01	5.06	0.00	0.00	0.00
Bm3	0.01	0.00	11.45	0.03	0.32	0.29	46.12	0.02	0.24	0.28
Bm4	0.00	0.00	36.72	0.03	0.37	0.39	440.34	0.18	0.42	0.63
Bm5	2.06	1.08	16.34	0.02	0.59	0.39	1866.09	0.77	0.47	0.42
Bm6	0.04	0.01	137.69	0.10	0.18	0.11	0.21	0.00	0.06	0.03

* and ** , indicate significance at 5% and 1% level, respectively.

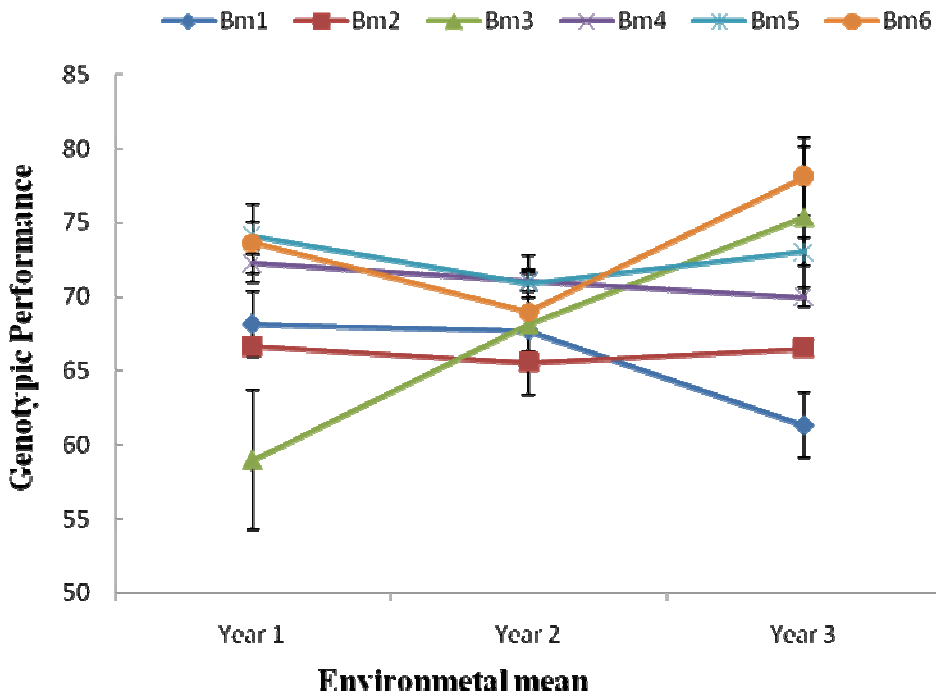


Fig. 6: Curves of individual line mean on environmental mean of six lines for DFF

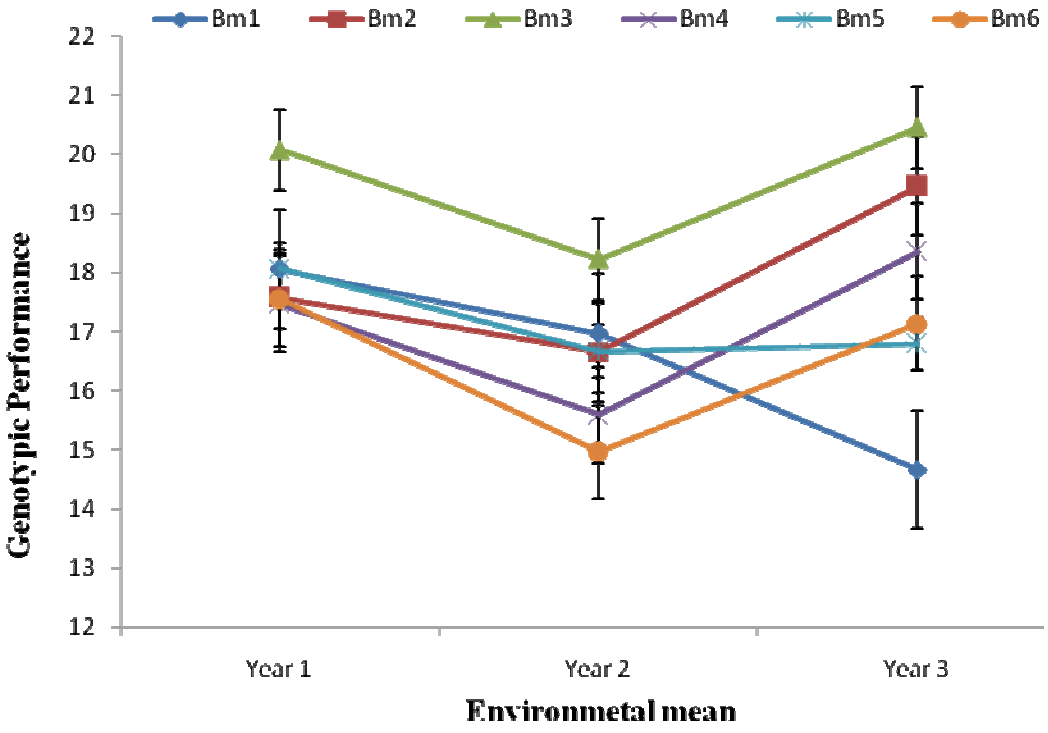


Fig. 7: Curves of individual line mean on environmental mean of six lines for PHFF

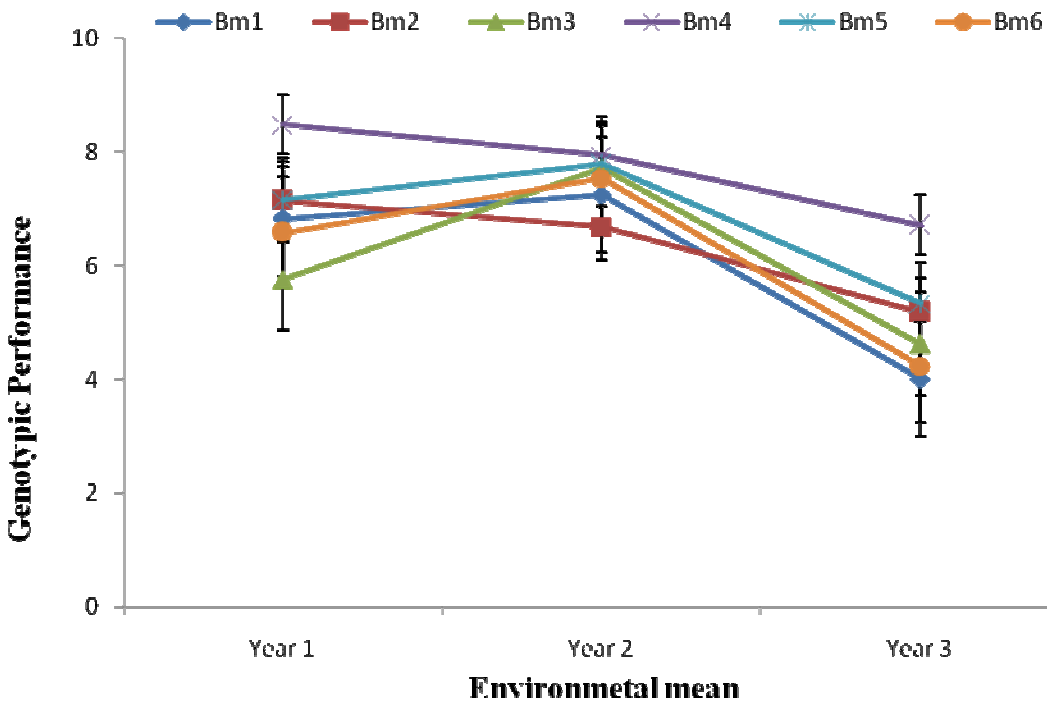


Fig.8: Curves of individual line mean on environmental mean of six lines for NPBFF

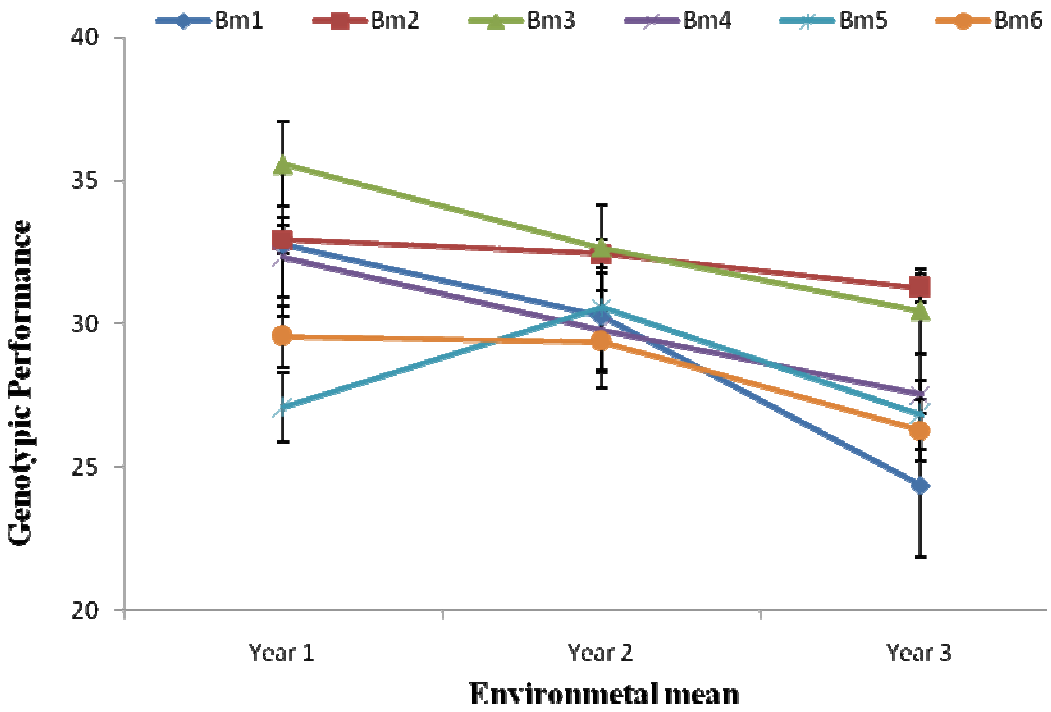


Fig.9: Curves of individual line mean on environmental mean of six lines for PHMF

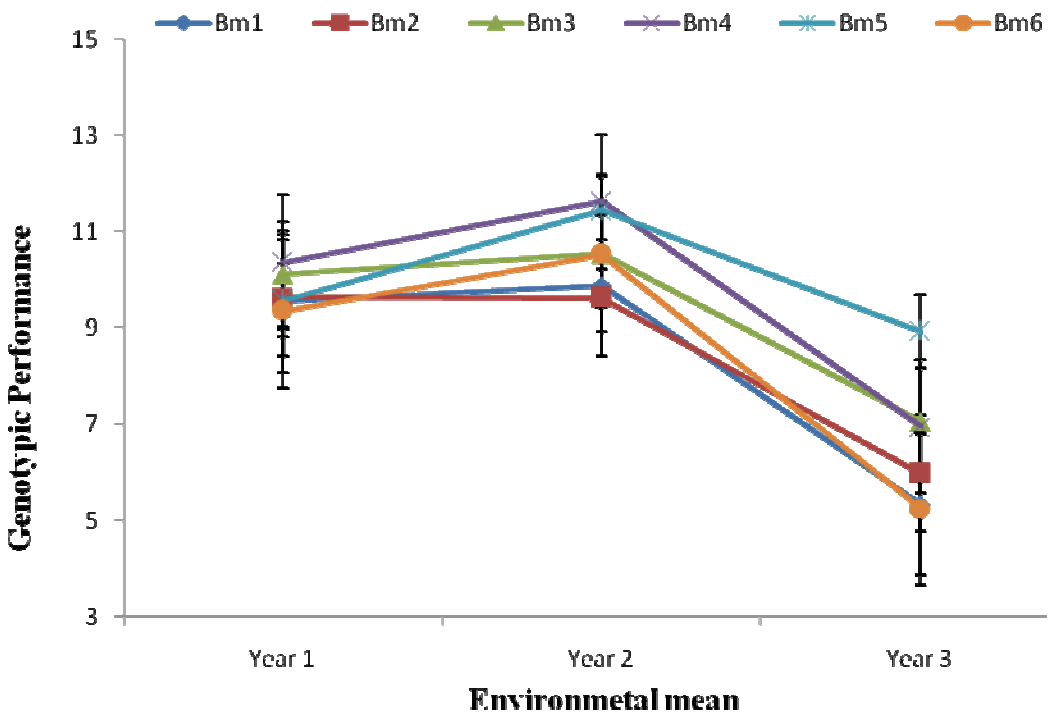


Fig.10: Curves of individual line mean on environmental mean of six lines for NPBMF

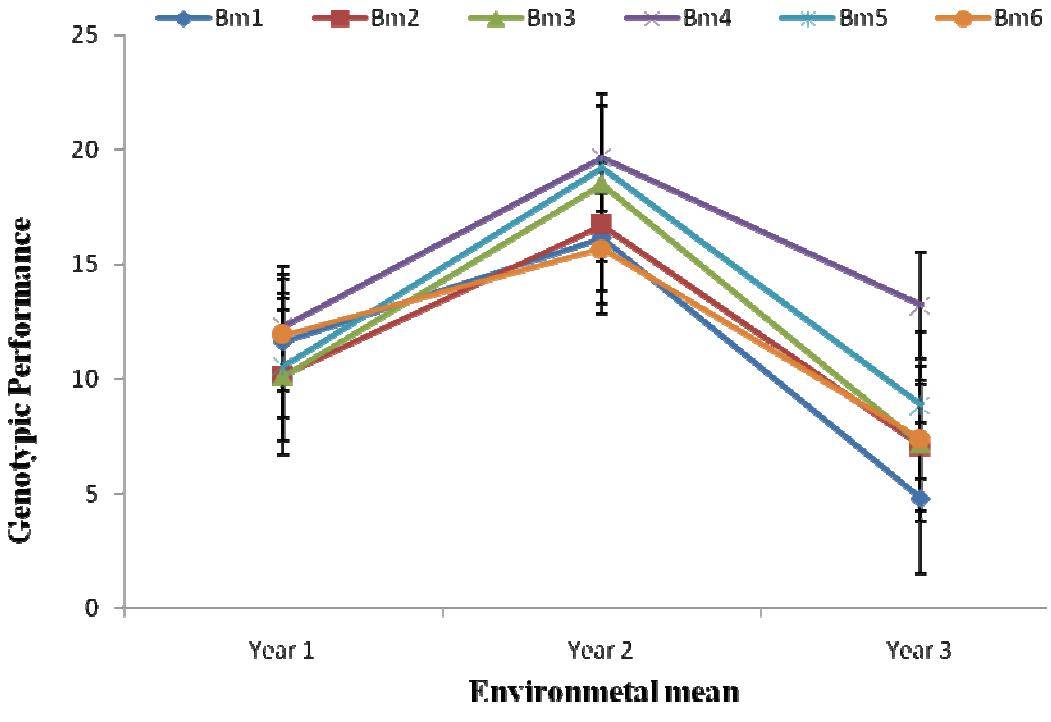


Fig.11: Curves of individual line mean on environmental mean of six lines for NSBMF

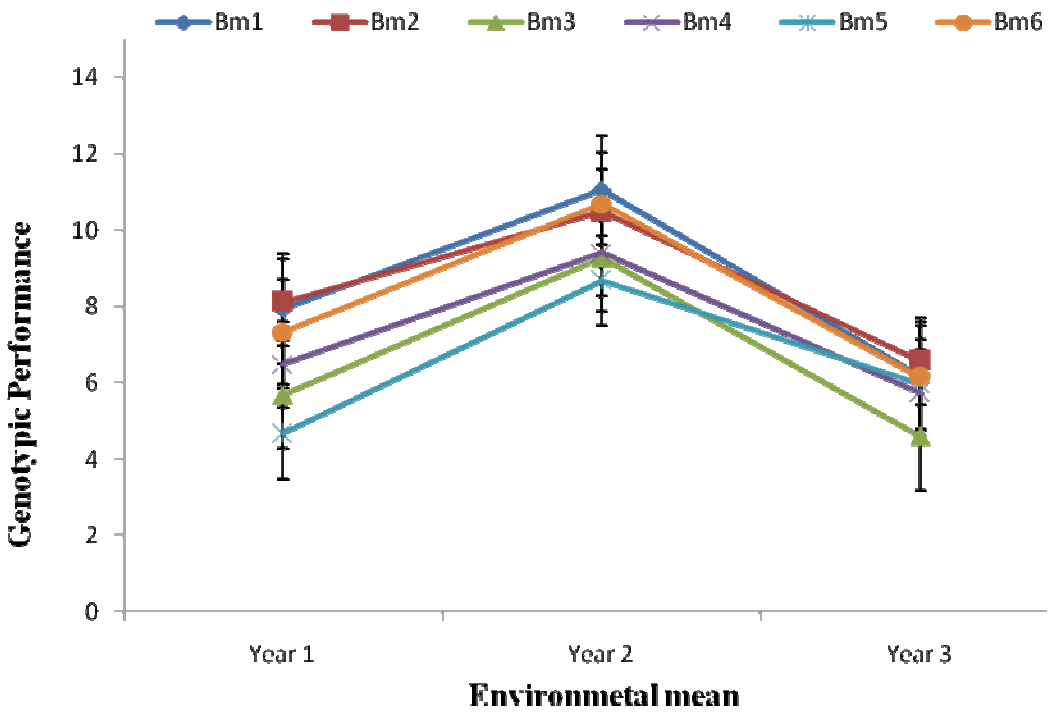


Fig.12: Curves of individual line mean on environmental mean of six lines for PWPP

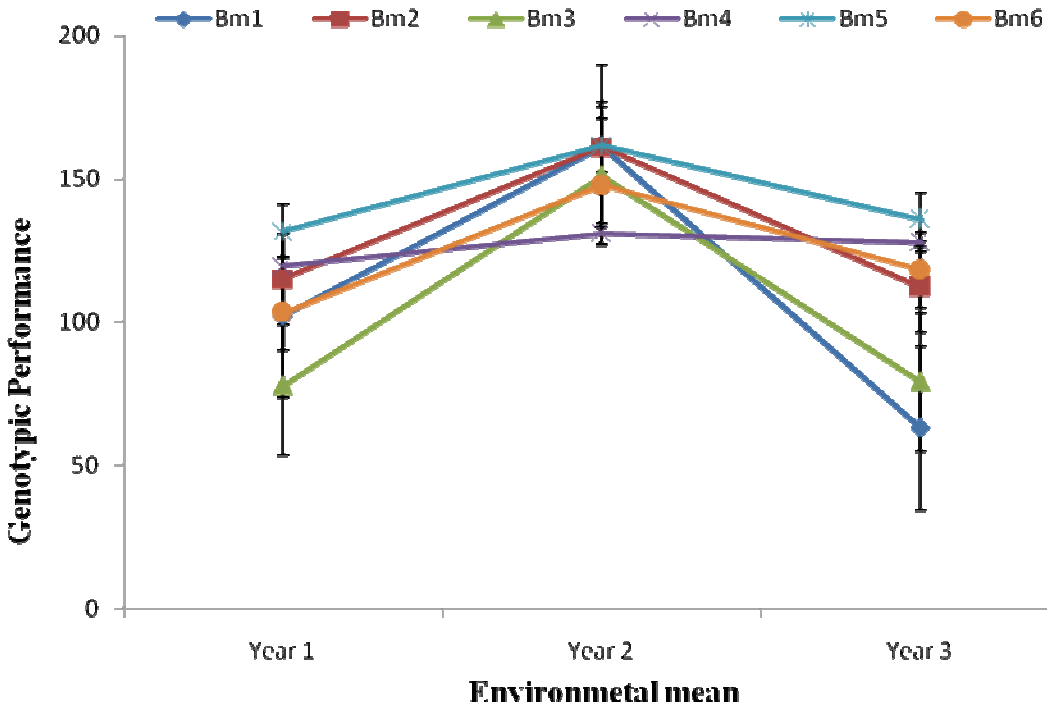


Fig.13: Curves of individual line mean on environmental mean of six lines for NPdPP

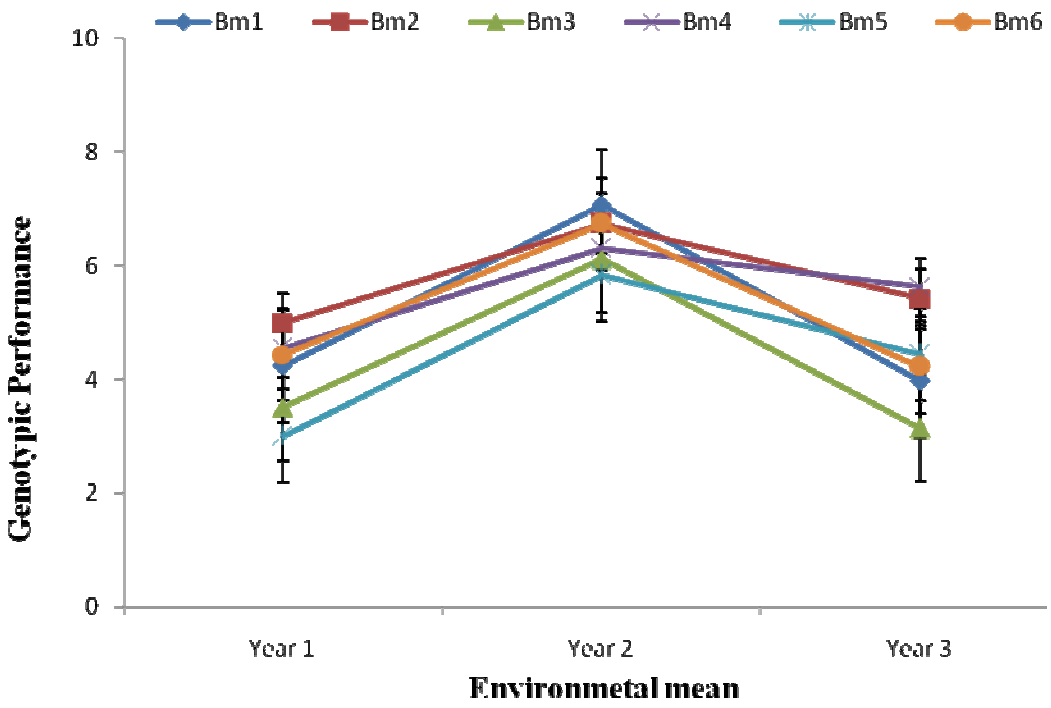


Fig.14: Curves of individual line mean on environmental mean of six lines for PdWPP

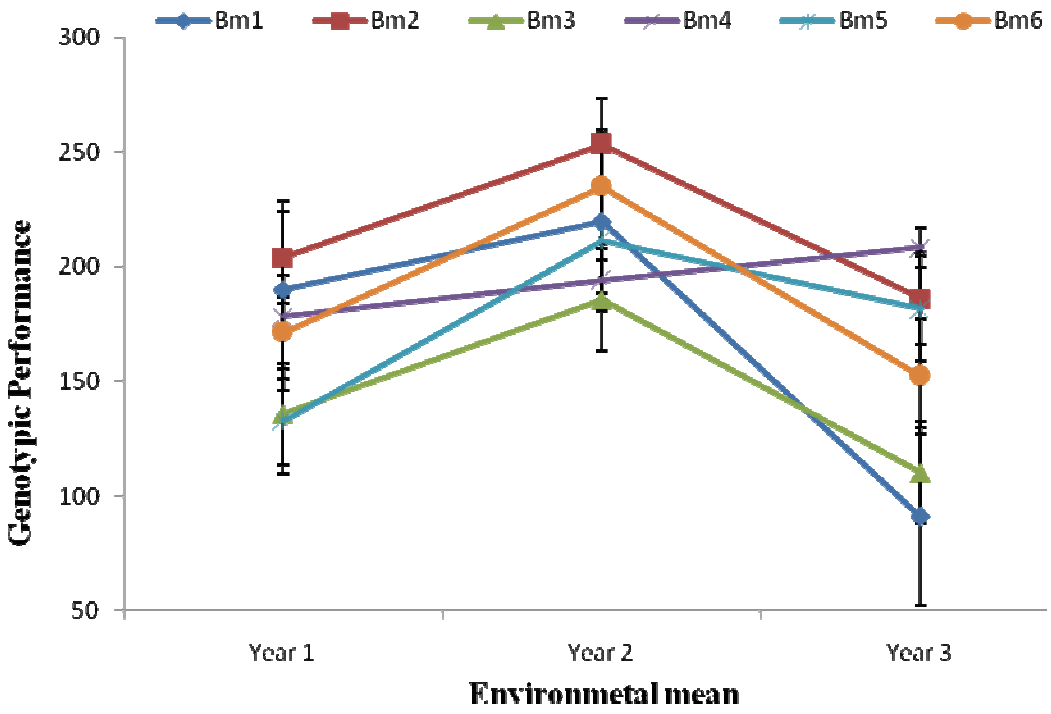


Fig.15: Curves of individual line mean on environmental mean of six lines for NSPP

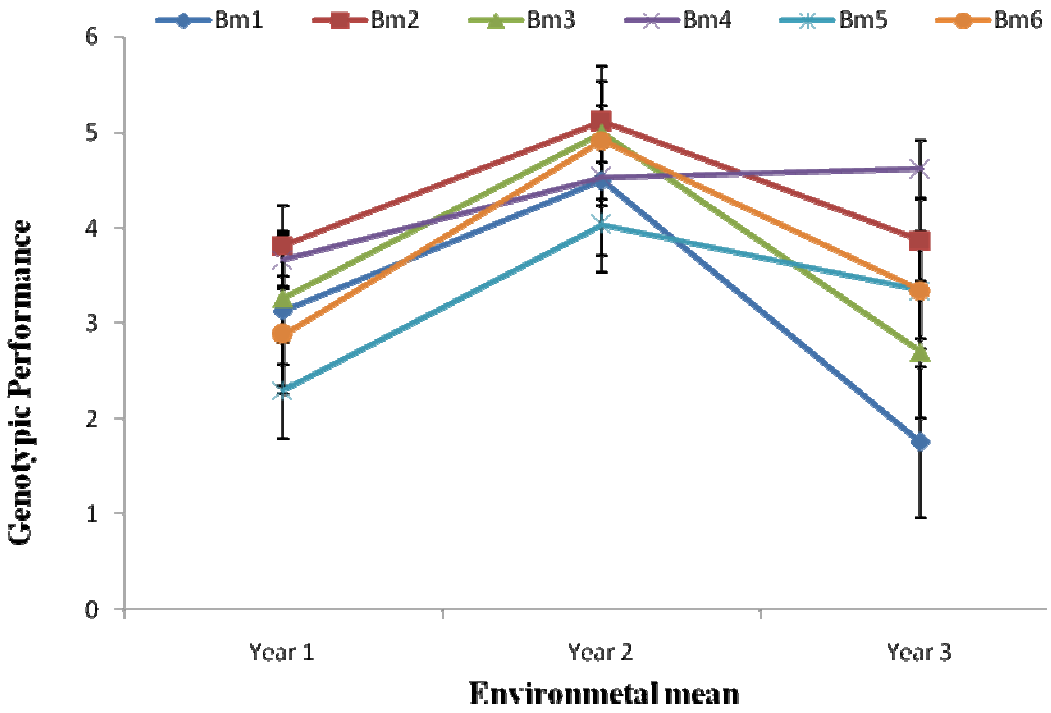


Fig.16: Curves of individual line mean on environmental mean of six lines for SWPP

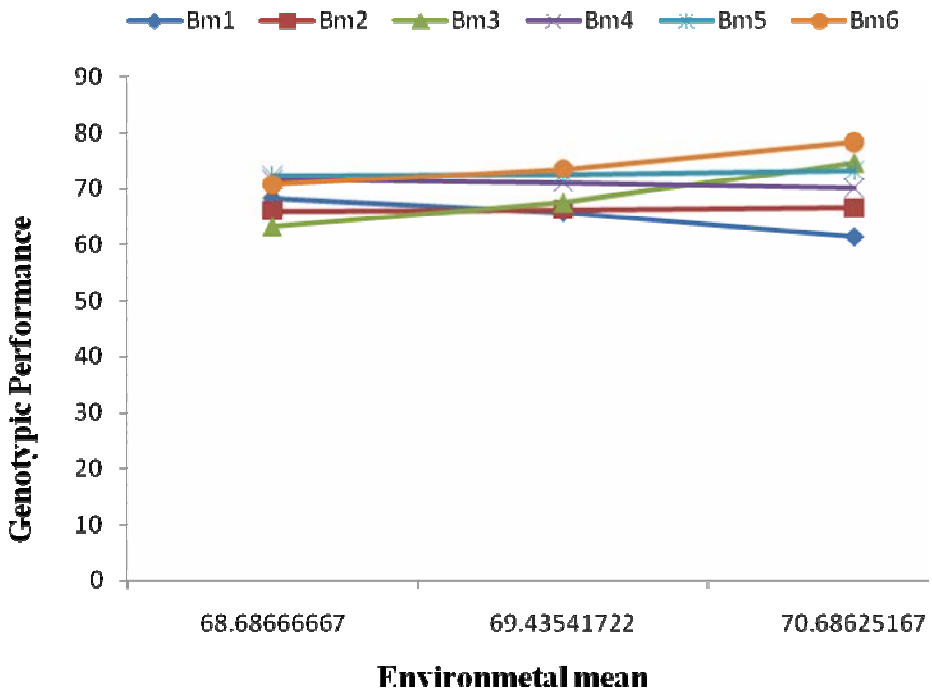


Fig. 17: Regression of individual genotypic mean on environmental mean of six lines for DFF

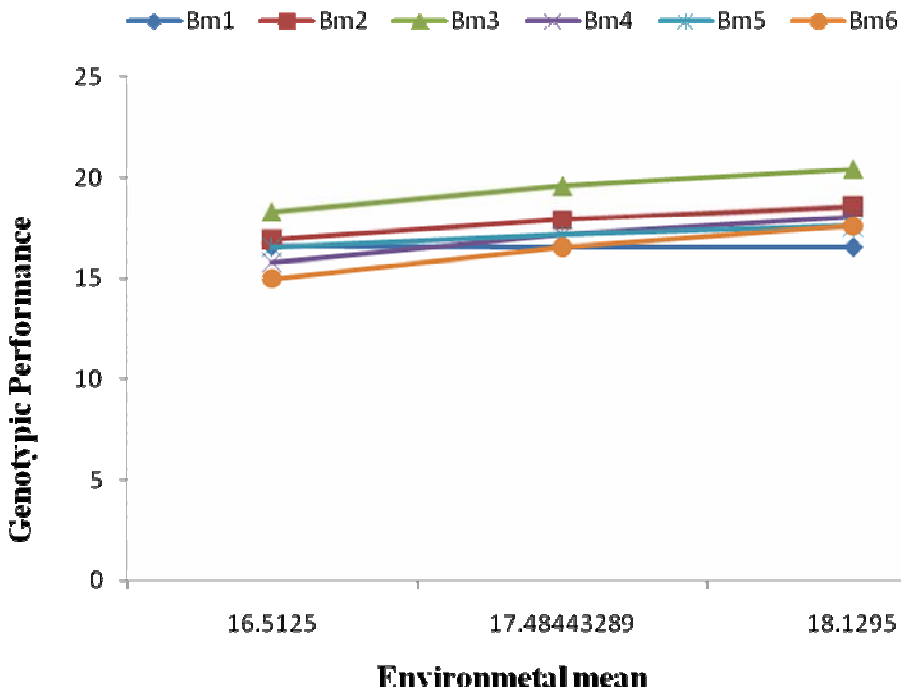


Fig. 18: Regression of individual genotypic mean on environmental mean of six lines for PHFF

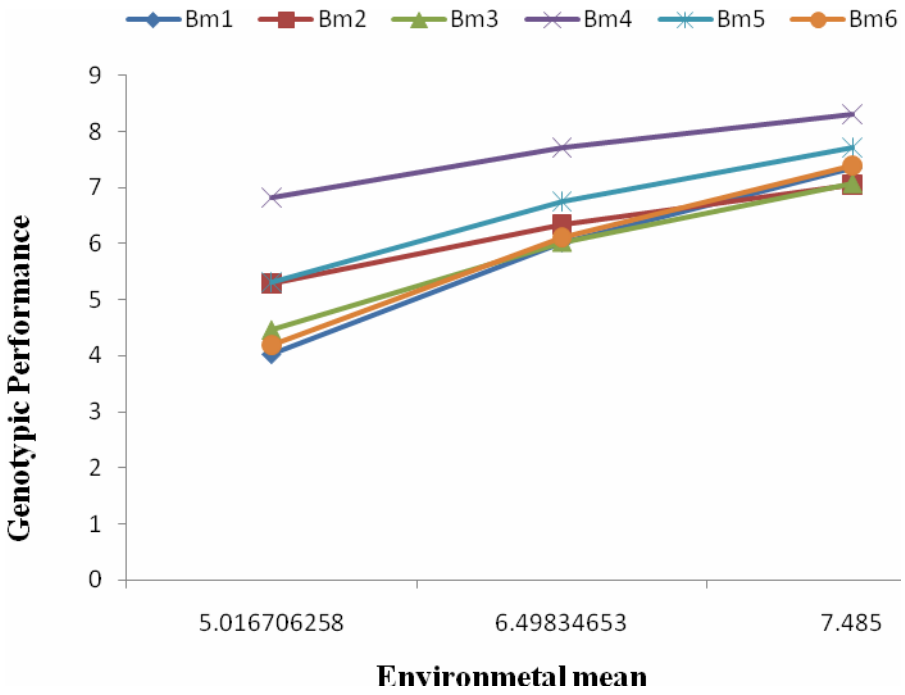


Fig. 19: Regression of individual genotypic mean on environmental mean of six lines for NPBFF

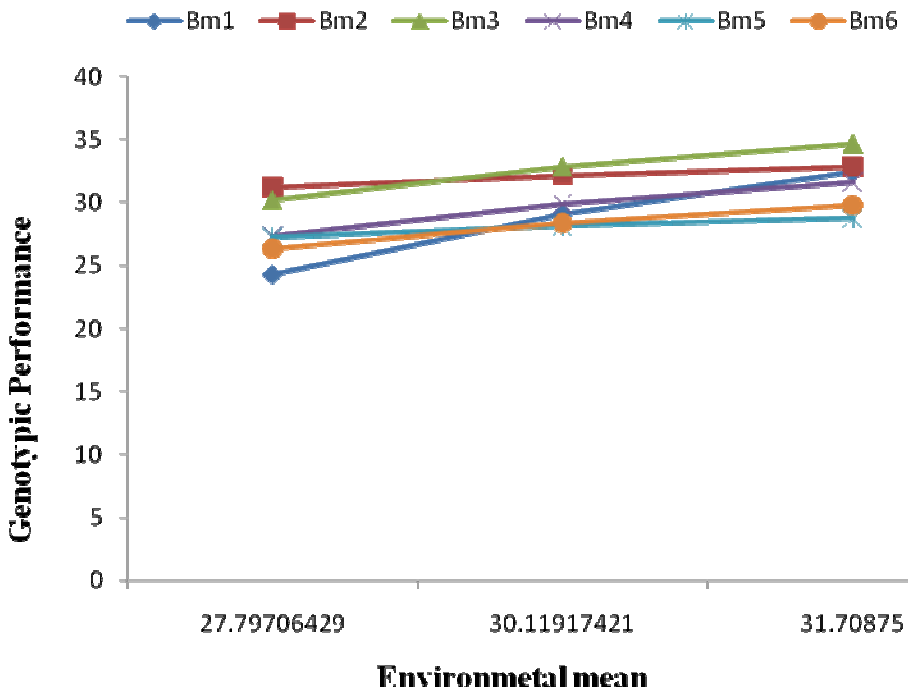


Fig.20: Regression of individual genotypic mean on environmental mean of six lines for PHMF

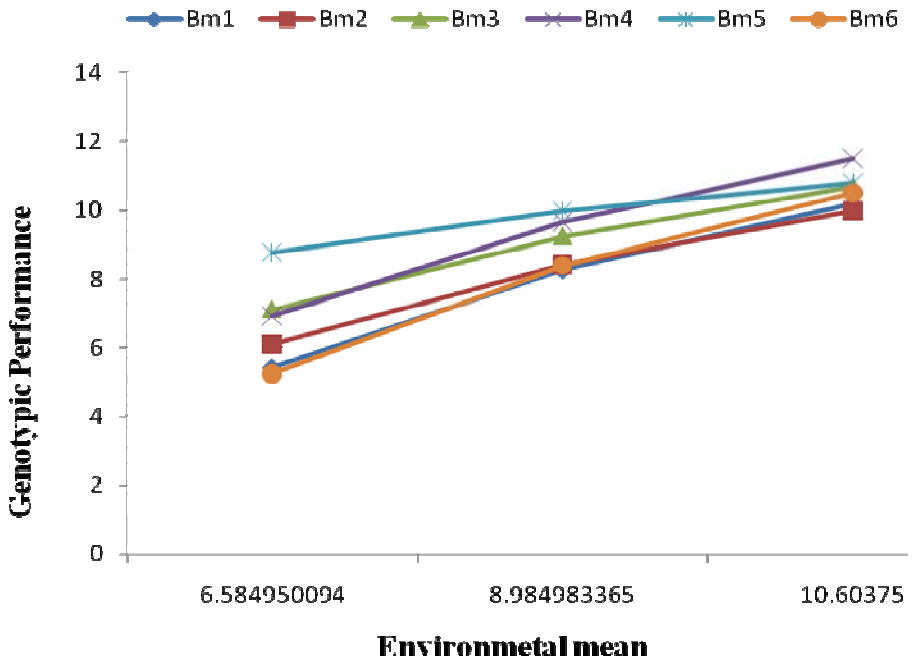


Fig. 21: Regression of individual genotypic mean on environmental mean of six lines for NPBMF

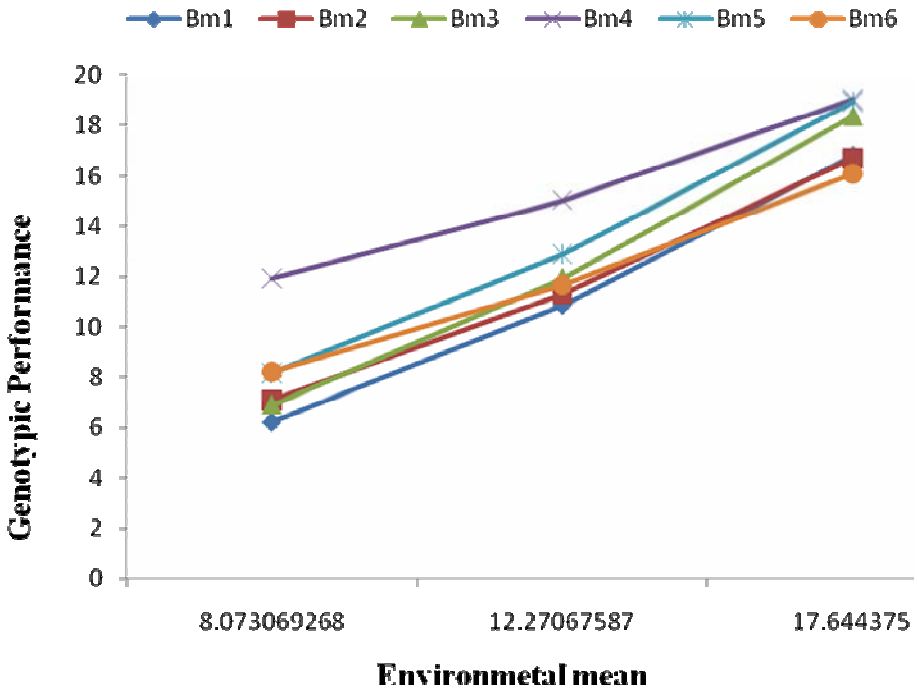


Fig. 22: Regression of individual genotypic mean on environmental mean of six lines for NSBMF

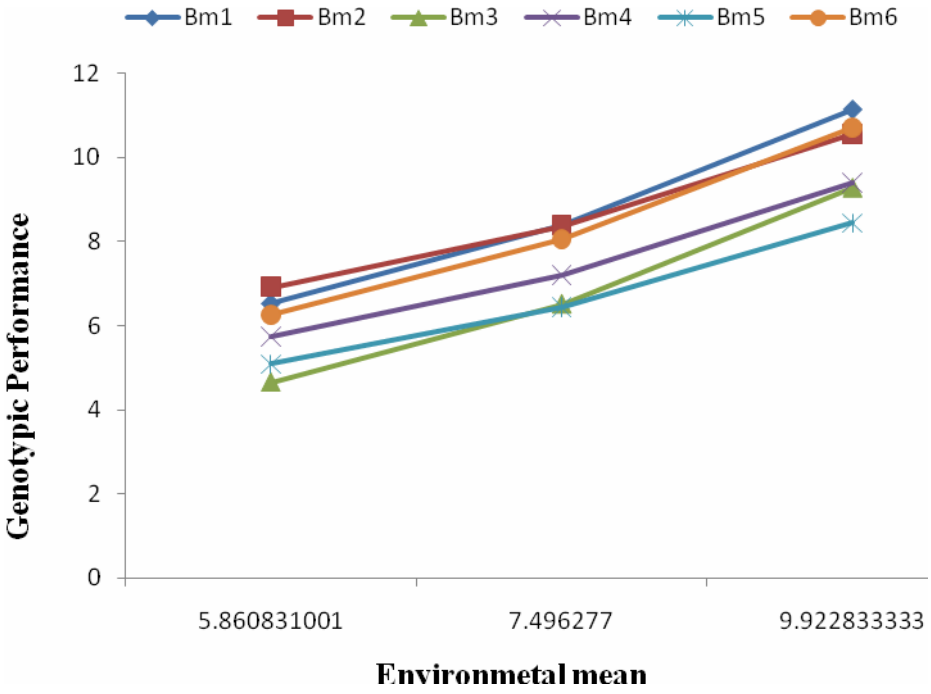


Fig.23: Regression of individual genotypic mean on environmental mean of six lines for PWPP

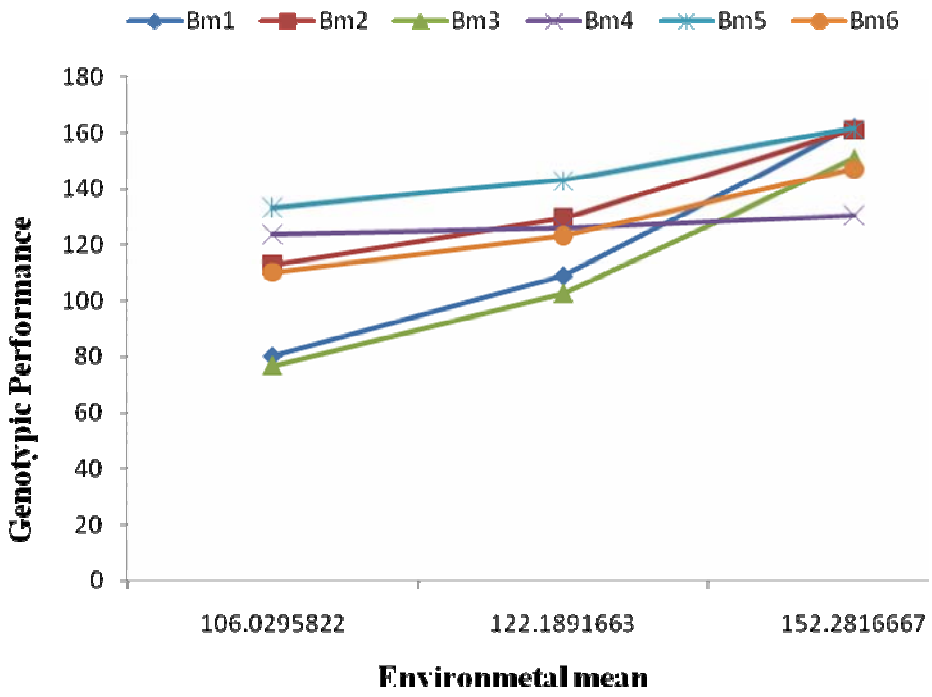


Fig. 24: Regression of individual genotypic mean on environmental mean of six lines for NPdPP

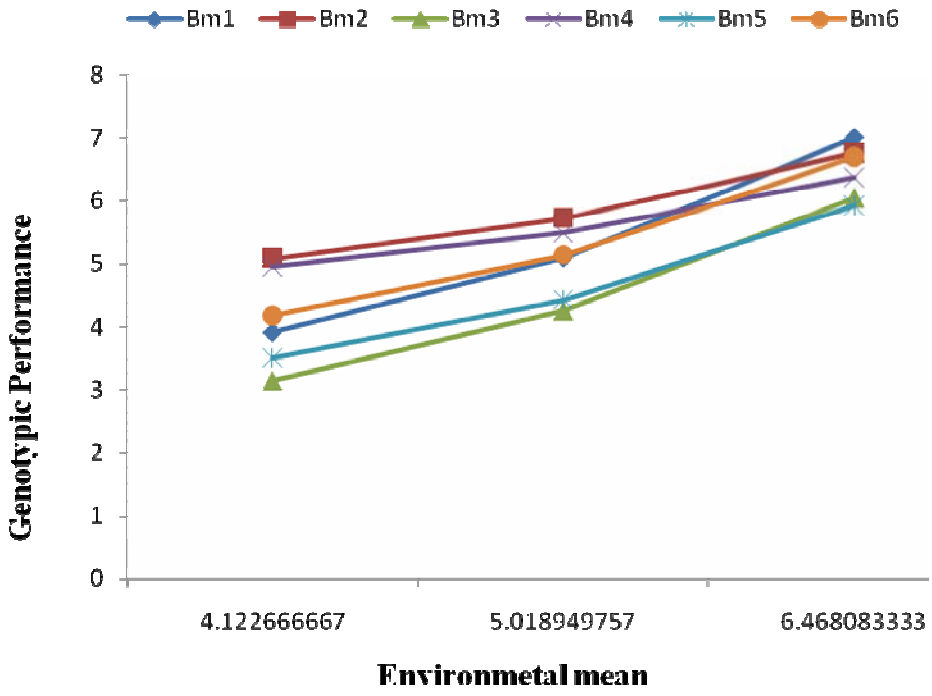


Fig.25: Regression of individual genotypic mean on environmental mean of six lines for PdWPP

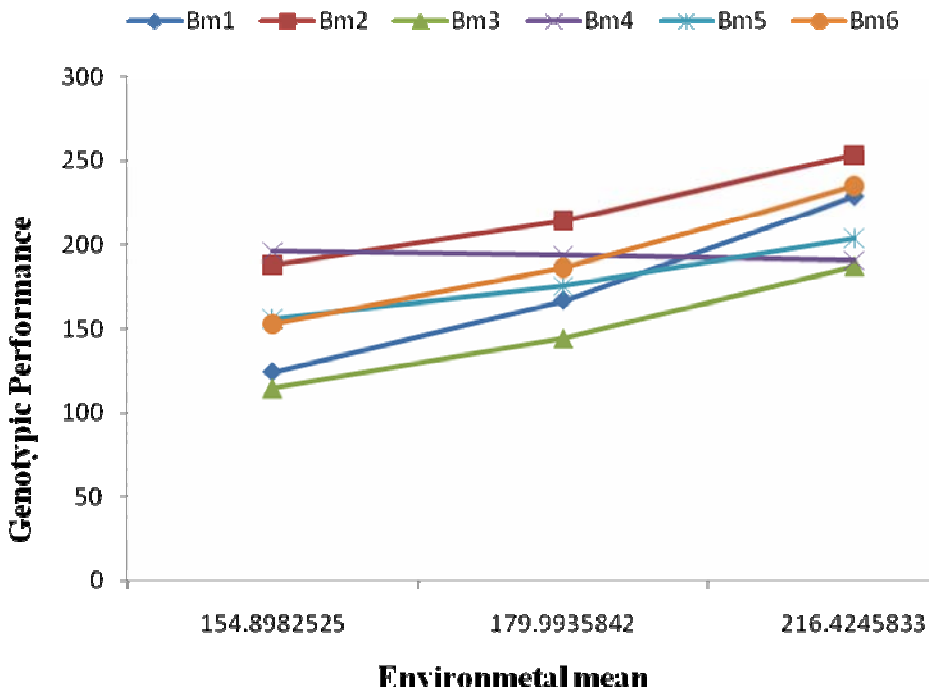


Fig.26: Regression of individual genotypic mean on environmental mean of six lines for NSPP

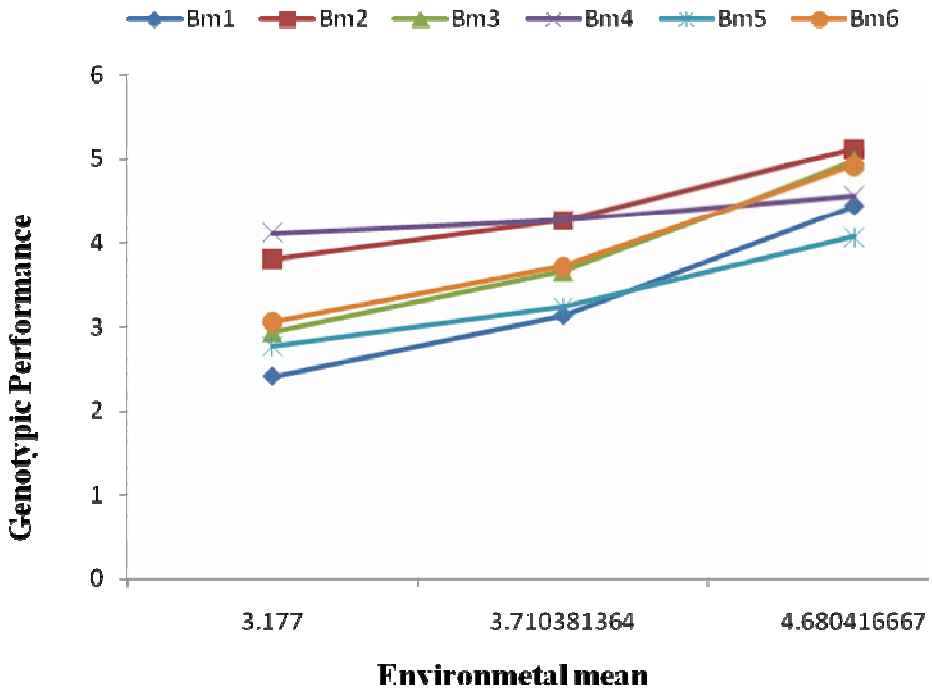


Fig.27: Regression of individual genotypic mean on environmental mean of six lines for SWPP

DISCUSSION

The knowledge of genotype-environment (G × E) interaction provides good information for selecting desirable stable and superior genotypes from a set of genotypes over a range of environment and now-a-day the importance of G × E interaction in plant breeding and variety testing is well established. The role of G × E interaction has long been of great importance to the breeders for selection of lines under different environmental conditions in future breeding program. Yield and yield contributing characters in crop plant are quantitative in nature and highly influenced by the environmental variation. Such variation confounds the selection of superior cultivars/lines by altering their relative productivity in different environments (Eagles and Fray, 1977).

Regression analysis is the only method in biometrics by which genotypic and environmental effects are simultaneously estimated. Regression analysis measures the response of a genotype over environments. Consequently, if there is any stable quality of a character in a variety over different environments, it can be measured by regression analysis. Bains and Gupta (1972) stated that the potentiality of a genotype to express greater mean over environments should be most important criterion, since the other two parameters may not have any particular utility if the genotype is potentially week.

In this Experiment, the main items line and year were significant for all the characters when tested against within error in the joint regression analysis. The significant line item indicated that the genotypes were different, which justifies their inclusion as materials in the present study. Significant year item suggested that the environments were different. Variability in environments is an important factor and in large part determines the usefulness of b_i values (Pfahler and Linskens, 1979). Statistically significant environmental effects in the present investigation indicated that variability between environments was large enough for the proper estimation of b_i values. G × E interactions were significant for all the characters except NSBMF. The significant G × E interaction item indicated that the genotypes significantly interacted with the environment in the changing environment. Thus, G × E interactions were operative in the present

investigation. These results are supported by graphical analysis. In graphical analysis intercrossing of curves and regression lines for most of the characters indicated the existence of G × E interaction. These results are in conformity with the findings of Samad (1991) in rapeseed, Ghosh and Sing (1996) and Nahar (1997) in Sugarcane, Chowdhury *et al.*,(1998) in lentil, Deb (2002) in chickpea, Hossain *et al.*, (2006) in tomato and Kumar *et al.*, (2007) in lentil. Dutta (2008) in lentil, Sharma *et al.*, (2007) and Alwawi *et al.*, (2009) and Rao (2011) in chickpea.

In the joint regression analysis, G × E interaction sum of square was partitioned into heterogeneity of regression sum of square (linear) and remainder sum of square (non-linear). Both linear and non-linear relationships with environments were reported by Khaleque (1975) in rice, Khaleque and Eunus (1977) in rice, Samad (1991) in rapeseed, Nahar (1997) in sugarcane, Deb (2002) in chickpea, Kumar *et al.*, (2007) in lentil and Dar *et al.*, (2009) in chickpea from different investigators. Their findings agreed well with the results of the present investigation.

The remainder mean square of all the lines for all of the characters were find to be non-significant except Bm3 for DFF. The significant remainder item suggested that non-linear type of L × E interaction was existed in the lines. Both linear and non-linear relationships with environments were reported by many investigators in different crops viz, Tiawari *et al.*, (2011), Khatod *et al.*, (2006), Azad (2008), Asad *et al.*, (2009), Hammed and Al-Badrany (2007) Atta *et al.*, (2009) and Choudhary and Haque (2010).

To measure the response and to find out the stable quality of a character, there are many suggestions, which are given by different researchers in different investigations in the regression analysis. Finlay and Wilkinson (1963) considered the linear regression as a measure of stability. Eberhart and Russell (1966) proposed the criteria of a stable genotype that regression coefficient (b_i) is considered as parameter of response and ($\bar{S}^2_{di}$) as the parameter of stability for a given value of independent variable, the value of dependent variable may be estimated using the regression equation, provided ($\bar{S}^2_{di}$) is not significantly different from zero. Assuming $\bar{S}^2_{di} = 0$, a high value of bi will mean more

change in Y for a unit change in *I.* In other words the variety is more responsive. Such variety may, therefore be recommended only for highly favorable environment. However, relatively lower value of bi=1 will mean less responsive to the environmental change and therefore more adaptive. If b is negative the variety may be grown only in poor environment. ($\overline{S}^2_{di}$) is significant from zero will invalidate the linear prediction. If ($\overline{S}^2_{di}$) is non-significant, the performance of a genotype for a given environment may be predicted accordingly a variety whose performance can be predicted is said to be stable. Though, the later concept merits practical consideration, the former one retain the condition of a relatively little or non-responsive genotypes in the changing environments. Further, Breese (1969), Paroda *et al.,* (1973) and Langer *et al.,* (1979) stated that regression coefficient is a measure of response to varying environments and the mean square deviation from linear regression is a true measure of stability; the genotype with the least deviation being the most stable and vice-versa.

In the present materials, the genotypes with the characters showed above average response are Bm1 for most of the characters except DFF, PHFF and NPBFF, Bm3 for all of the characters except NPBFF and NPBMF, Bm4 for PHFF and NPBMF, Bm5 for NSBMF and Bm6 for all of the characters except PHMF, NSBMF and NPdPP, because bi values of these line are more than 1. While, average response (bi=1) was exhibited by Bm1 for NPBFF, Bm2 for PHMF, NSBMF, NPdPP and NSPP, Bm3 for NPBFF, Bm4 for PHMF and PWPP, Bm5 for NPBFF and PdWPP and Bm6 for PWPP and PdWPP. Parameter of stability ($\overline{S}^2_{di}$) showed non-significant value for all the character of all the genotypes, that mean it's not different from zero.

On the basis of above criterion , if we aggregate of this two parameter, the genotypes which showed stable performances, i.e., adaptable to all environments are Bm1 for NPBFF, Bm2 for PHMF, NSBMF, NPdPP and NSPP, Bm3 for NPBFF, Bm4 for PHMF and PWPP, Bm5 for NPBFF and PdWPP and Bm6 for PWPP and PdWPP. These lines are most stable with the changing environment and could be used for the future breeding program. The results are in agreement with the findings Srivastava *et al.,*

(1999), Sharma *et al.,* (2007), Khatod *et al.,* (2006), Kanouni *et al.,* (2007), Dehghani (2010), Choudhary and Haque (2010), and Karadavut *et al.,* (2010), in various crops.

Besides this, the genotypes which are more responsive to changing environment, having non-significant $\bar{S}_{di}^{2}$ and high values of b_i are Bm1 for most of the characters except DFF, PHFF and NPBFF, Bm3 for all of the characters except NPBFF and NPBMF, Bm4 for PHFF and NPBMF, Bm5 for NSBMF and Bm6 for all of the characters except PHMF, NSBMF and NPdPP. It suggested that these lines might be recommended only for favourable environment. Similar results are obtained by Singh and Rai (1989), Singh *et al.,* (1993) and Nahar (1997) in sugarcane, Sial *et al.,* (2000) in wheat, Sharma *et al.* (2007) in chickpea, Akhtar *et al.,* (2010) and Karadavut *et al.,* (2010) in faba bean, Choudhary and Haque (2010) in chickpea.

On the other hand, the genotypes Bm1 for DFF and PHFF and Bm4 for DFF and NSPP can perform better in the poor environment (unfavorable) because of negative b_i value and non-significant stability parameter. Nahar (1997) in sugarcane, Islam (2002) in chili, Deb (2002) in chickpea, Chowdhury *et. al.* (1998), Dutta *et al.,* (2009) and Kumar *et. al.* (2007) in lentil and Karadavut *et al.* (2010) in faba bean found similar results.

While, Bm2 for all of the characters except PHMF, NSBMF, NPdPP and NSPP, Bm3 for NPBFF, Bm4 for NPBFF, NSBMF, PdWPP and SWPP, Bm5 for all of the traits except NPBFF, NSBMF, and PdWPP and Bm6 for PHMF, NSBMF and NPdPP showed poor adaptability to all the environments. Similar results are obtained by Chowdhury *et al.,* (1998) in lentil, Islam (2002) in chili, Deb (2002) in chickpea and Kumar *et al.,* (2007) and Azad *et al.,* (2008) in lentil.

As G × E interaction is under genetic control, breeders would be able to select suitable genotypes in advanced generations by growing them under different environmental conditions. The present study also revealed that yield potential can be increased by increasing the performance of the yield components in appropriate environment, since those characters are associated with yield.

CONCLUSION

Genotype - environment (G × E) interaction of six lentil lines for eleven quantitative characters at three year were investigated. Yield is a complex character and depends on some other component characters. In the present materials, eleven yield and yield components viz. DFF, PHPP, NPBFF, NSBFF, NSBMF, PAPP, PWPP, RWPP, NPdPP, PdWPP, NSPP and SWPP of six lentil lines were studied. There are several methods to test the stability of a variety but in the present materials Perkins and Jinks (1968) model was applied. According to this model, a variety with high mean and unit regression (b_i=1.0) and a deviation ($\overline{S}_{di}^2$ **=0**) from regression is referred as suitable and stable genotype, which would show constant performance over a range of environments

1. Genotypic means and environmental means were highly significant in all the cases. Environmental means also indicated that different environments had different effects on all the traits.
2. Genotype-environment interactions were found to be operative in this investigation.

3. Genotype-environment interaction accounted for by both linear and non-linear functions of the environments. A significantly greater portion was accounted for by non-linear function of the environmental mean and some portions of interactions were non-linear and independent of the linear function.

4. Both linear and non-linear components of genotype–environment interactions were under the control of different gene systems.

5. Stability performances of different lines were different for different characters. A good number of lines showed stable performances for different characters in different environments as follows:

Regarding stability the genotypes which showed non-significant $\overline{S}_{di}^2$ and average values of b_i *i.e.*, adaptable to all environment are Bm1 for NPBFF, Bm2 for PHMF, NSBMF, NPdPP and NSPP, Bm3 for NPBFF, Bm4 for PHMF and PWPP, Bm5 for

NPBFF and PdWPP and Bm6 for PWPP and PdWPP. It suggested that, these lines are most stable with the changing environment.

The genotypes which have non-significant $\bar{S}_{di}^{2}$ and high values of b_i are Bm1 for most of the characters except DFF, PHFF and NPBFF, Bm3 for all of the characters except NPBFF and NPBMF, Bm4 for PHFF and NPBMF, Bm5 for NSBMF and Bm6 for all of the characters except PHMF, NSBMF and NPdPP were more responsive to changing environment. It suggested that these lines might be recommended only for favorable environment.

From the estimation of stability parameter it was concluded that Bm2 for most of the characters showed stable performances with the changing environment and Bm1 for most of the characters showed high performances with the favorable environment and these stable lines could be used for further breeding program.

Such comparative evaluation a breeder could be able to simplify his task of experimentation in developing suitable and better performer for general or specific adaptation. The present study also expressed that the yield potentiality can be increased by increasing the performance of the yield contributing traits in an appropriate environment.

PART III

GENETIC DIVERGENCE THROUGH MORPHOLOGICAL AND BIOCHEMICAL MARKER

INTRODUCTION

Genetic diversity is the amount of heritable variability between varieties or populations of organisms. Variability occurs from differences in DNA sequences, biochemical characteristics like protein structure and physiological properties like resistance to illnesses and growth rate, and morphological characters such as leaf type and plant height. Selection, mutation, genetic drift and gene flow also affect genetic diversity in different populations by acting on the alleles in these populations. Documentation of plant genetic diversity is necessary to conserve genetic resources for plant improvement (Lane *et al.,* 2000).

The assessment of genetic variation and genetic similarities is a major concern of plant breeders and population geneticists, because it facilitates the efficient sampling and utilization of germplasm resource (Maqbool and McNeil, 1996). Main concern with lentil is low yield potential because of narrow genetic base of the local cultivars. Therefore, the key to increase lentil yield in South Asia including Bangladesh is through widening the available genetic base (Erskine and Saxena, 1993). Indian lentils are exclusively of Pilose type and show limited variations. This narrow genetic variability among indigenous germplasm has restricted breeding progress. The knowledge of genetic variation and relationships between populations is important to understand the available genetic variability and its potential use in breeding programs. Genetic variation between and within populations of crop species is a major interest of plant breeders and geneticists (Hayward and Breese, 1993). The breeders must have the idea of choosing the accession that most likely possesses the trait of interest.

Molecular marker analysis is a reliable method to study genetic diversity. A molecular marker is a readily detectable sequence of protein or DNA that are closely linked to a gene locus and/or morphological or other characters of a plant. In another word, molecular marker is a readily detectable sequence of protein or DNA whose inheritance can be monitored and associated with the trait inheritance independently from the environment.

It is can be detected by following types of markers:

1. Morphological markers
2. Molecular markers
3. Biochemical markers

Markers that associated with phenotype traits are called morphological markers. Morphological markers analysis, in which morphological characters used as marker is a traditional and one of the easiest methods which can be applied to select the desirable traits in plant species by plant breeders. Morphological characterization is the first step in the classification and description of any crop germplasm (Ghafoor *et al.,* 2001; Upadhyayav and Ortiz, 2001). Morphological trait studies are usually used parameters because they allow for a simple technique of quantifying genetic variation while assessing genotype performance simultaneously under relevant growing environments (Fufa *et al*., 2005). Morphological traits were one of the earliest markers employed in germplasm management (Stanton *et al*., 1994) but they have a number of demerits such as low heritability, low polymorphism, late expression, labor-intensive and vulnerability to environmental influences (Smith and Smith 1992 and Muthusamy *et al*., 2008) which may affect the estimation of genetic relationships successively. However, if the traits are highly heritable, morphological markers are one of the choices for diversity studies because the inheritance of the marker can be monitored visually (Yoseph, 2005). Despite the problems associated with this method, it continues to play a major role in studying and characterizing germplasm since it requires no complicated laboratory facilities and procedures.

Biochemical markers are those derived from study of the chemical compounds of gene expression such as proteins. Increasingly, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) are widely used for estimation of taxonomic relationships among inter and intra related species and assessment of genetic diversity reported by different scientist (Ghafoor *et al.,* 2002, Duran *et al.,* 2005; Iqbal *et al.,* 2006; Lioli *et al.,* 2005; Yuzbasioglu *et al.,* 2008). These techniques are reproducible and largely

independent of environmental instability/factors (Ayad *et al.,* 1995; Bretting and Widrlechner, 1995, Nisar *et al.,* 2009).

Seed storage proteins (SSPs) are a set of proteins that are synthesized in the developing seed at the stage when cell division is complete (Goldberg *et al.,* 1994) and they play a crucial role in the life cycle of higher plants since they are the major sources of nitrogen and carbon during subsequent seed germination and early seedling growth. SSPs are primarily synthesized in the late stages of seed development on rough endoplasmic reticulum (ER) as precursors, and are further converted into mature forms and accumulate in protein storage vacuoles (PSVs) of terminally differentiated cells in developing seeds (Herman and Larkins, 1999). Electrophoresis adds information to taxonomy and should not be dissociated from morphological, anatomical and cytological observations (Boutler *et al.,* 1996 and Ghafoor *et al.,* 2002). Seed proteins profiles obtained by electrophoresis have been successfully used for studying taxonomical and evolutionary relationships of several crop plants (Ladizinsky and Hymowitz, 1979; Gepts and Bliss, 1988; Sammour, 1989; Murphy *et al.,* 1990).

In Legume, many studies have been carried out based on the electrophoresis of seed proteins (Hussein and George, 2002; Hussein *et al.,* 2005). Seed storage protein profiling is most economical simple, extensively used biochemical technique and reliable method for analysis of genetic diversity of lentil especially for the cultivars levels due to the independence of environmental fluctuation (Murphy *et al*., 1990 and Yüzbaşioğlu *et al*., 2008). Genetic diversity of seed storage proteins has been reported as a more reliable method for lentil (De La Rosa and Jouve, 1992; Echeverrigaray *et al.*, 1998, Piergiovanni and Taranto, 2005). Therefore, the choice of the technique depends on the objective of the study, sensitivity level of the marker system, financial constraints, skills and facilities available (Yoseph *et al*., 2005).

According to Smith *et al.,* (1995), linkage clustering and PCA are useful for preservation and utilization of germplasm. There are several methods to detect variation. In this

investigation we have used biometrical techniques, Jaccard's similarity analysis, dendrogram for clustering analysis and lastly principal component analysis (PCA).

A dendrogram (from Greek *dendron* "tree", *gramma* "drawing") is a tree diagram frequently used to illustrate the arrangement of the clusters produced by hierarchical clustering. The dendrogram is the most important result of cluster analysis. It lists all samples and indicates at what level of similarity any two clusters were joined. The position of the line on the scale indicates the distance at which clusters were joined. The dendrogram is also a useful tool for determining the cluster number. It is a one kind of visual representation by which the differences between varieties can be detected easily. Clusters on the basis of plant descriptors have been described by Singh (1988), Caradus *et al*. (1989), Peeters and Martinelli (1989) and Clements and Cowling (1994). Although multivariate analysis on quantitative traits provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level, but according to most researchers it should not be disassociated from botanical descriptors (Muehlbauer and Slinkard, 1981; Erskine and Witcombe, 1984; Baylon and Singh, 1986; Peyghambary, 2003). The researcher 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. PCA was also performed for conforming result of dendrogram. It is a way of identifying patterns in data and expression the data in such a way as to highlight their similarities and differences. PCA is powerful tool for analyzing data as well as genetic variation.

Dendrograms, genetic similarity and principal component analysis can be done by different software's. Software's such as SPSS and NTSYSpc are the most common softwares being used for clustering based on some methods like UPGMA, COMPELETE, ELEXI, SINGLE, UPGMC, WPGMA, WPGMC and WPGMS (Rohlf, 1998). In the present investigation, NTSYSpc software version 2.11 (Rohlf, 2004) based on UPGMA method was used. NTSYSpc stands for "Numerical Taxonomy and Multivariate System for Personal **C**omputer" is a system of statistical programs that is used to find and display structure in multivariate data. The program was originally

developed for use in biology but has also been widely used in morphometrics, ecology, and in many other disciplines in the natural sciences, engineering, and the humanities (Rohlf, 1998). Perhaps the most common use of NTSYSpc is for performing various types of agglomerative cluster analysis of some type of similarity or dissimilarity matrix (Rohlf, 1998 and Tari Nezhad *et al*., 2005).

In the present investigation, eleven quantitative characters use as a morphological marker and total protein biochemical markers are used for assessment of genetic diversity. Seed storage protein profiling was done by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

REVIEW OF LITERATURE

For the genetic improvement of both quantitative and qualitative characters of lentil, plant breeders and researchers need to conduct detailed experiments to decipher the genetic structure, yield and its attributes. Morphological characterization is the first step in classification and description of any crop germplasm (Smith and Smith, 1889 and singh and Tripathi, 1985). The extent of variation present within populations of landraces could be assessed easily and rapidly by SDS-PAGE. Over the last decades considerable interest has been focused on the use of biochemical methods for reliable plant variety discrimination and identification (Sammour, 1985 and Przybylska and Ziminiak, 1995). There has been some success and several easily performed chemical methods have been developed for variety identification (AOSA, 1988). Electrophoretic procedure enables to detect differences among cultivar of various crop species (Fergunson and Grabe, 1986; Gupta and Robbelen, 1986; Moller and spoor, 1993 and Jha and Ohri, 1996). Electrophoresis adds information to taxonomy and should not be dissociated from morphological, anatomical and cytological observations (Boutler *et al.,* 1966 and Ghafoor *et al.,* 2002). In the light of this view, the literature concerning to the objectives of present investigation has been reviewed.

Barulina (1930) first recorded detailed morphological descriptions of lentil landraces and species from Asia. Ladizinsky (1979a) and Ladizinsky and Hymowitz (1979b) considered seed protein an additional approach for species identification and a useful tool for tracing back the evaluation of various groups of plants.

The traditional approach of characterization and evaluation involves cultivation of accession subsamples and their morphological and agronomic description; a procedure facilitated by the use of intentionally recognized descriptor lists (Erskine and Williams 1980).

Moore and Collins (1983) considered that monitoring of germplasm is necessary during maintenance for predicting potential genetic gain in breeding program.

Damania *et al.,* (1983) used PAGE of storage proteins (Prolamines) to screen 64 landraces of wheat and barley from Nepal and Yamen Arab Republic and two cultivars for comparison. The advantages gained by using vertical slab gel method were recognized. The extent of variation present within populations of landraces could be assessed easily and rapidly by SDS-PAGE.

Erskine and Witcombe (1984) classified the world collection of lentil germplasm on the basis of morphological variation.

Malik *et al.,* (1984) evaluated lentil germplasm for seven characters, i.e. days to flowering, days to maturity, incidence of rust, blight, plant height, pod/ plant and seed yield. Their findings revealed sufficient variability in lentil germplasms to allow selection for the character studied.

Cooke (1984) reported Cultivars and landraces are a useful source of genetic variation and higher the variation, greater the chances to select superior lines of interest to plant breeders. Polyacrylamide gel electrophoresis (PAGE), generally in sodium dodecyl sulphate (SDS) gels in currently the favoured technique for rapid analysis.

Ferguson and Grabe (1986) identified cultivars of potential Ryegrass by SDS-PAGE of seed proteins. They reported that most of the cultivars were differentiated by unique banding patterns and band patterns of protein were not affected by year and location of production, class of certified seed or viability and vigor of the seed.

Sammour (1989) studied the basic criterion of phylogenetic relationship among is gene homology, which in many cases cannot be measured directly because of reproductive barriers between species. The fractionation of "non-essential" seed storage protein by PAGE is used as an additional tool for assessing species relationship.

Bakhsh *et al.,* (1992) categorized lentil germplasm on the basis of quantitative traits and suggested the utilization of short statured lentil germplasm for crop improvement.

Ghafoor *et al.,* (1992) selected twenty-eight genotypes of mungbean on the basis of high yield potential and resistance to diseases.Genetic Variation between and within populations of crop species is a major interest of Plant breeders and geneticists (Hayward and Breese 1993).

Seventy-two land races of pea (*Pisum sativum*) evaluated for 19 morphological characters exhibited broad genetic diversity as reported by Amurrio *et al.,* (1993).

Germplasm evaluation is considered the first step in plant breeding program and it is commonly based on a simultaneous examination of large number of populations for several characters of both agronomic and physiological interest (Pezzotti *et al.,* 1994).

Smith *et al.,* (1995) conducted average linkage cluster and PCA and reported the utility of these results in preservation and utilization of germplasm.

Jha and Ohri (1996) reported phylogenetic relationships of accessions of cultivated *Cajanus cajan* and 10 wild *Cajanus* species using SDS-PAGE. According to them a considerable variation among protein profiles of different accessions *Cajanus cajan* was observed while those of wild species were very specific and distinctly different from each other.

Rabbani *et al.,* (1998) determined the extent of diversity and relation among 52 accessions of *Brassica* germplasm from Pakistan for 35 morphological characters using cluster and principal component analyses.

Tzvetelina and Pereira (1999) investigated 120 accessions of lentil for yield components and identified the most promising genotypes for inclusion in lentil breeding programme.

Shivaraj and Pandravada (2005) investigated morphological diversity among 54 accessions of bottle gourd and results of their investigation revealed immense variation in quantitative characters of fruits such as shape, blossom, luster and ridge etc. Results also suggested that a wide range of variability was observed for fruit length, fruit width, seed length, seed width etc.

Sultana and Ghafoor (2008) studied; On the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 108 accessions were homozygous and included in further analysis. In total, 55 protein bands were recorded ranging from the molecular weight (MW) of 14–66 kDa. Out of 55 protein sub-units, 13 were polymorphic in nature.

Ghafoor and Arshad (2008) observed 25 subunits in lentil and showed that among these 20 were polymorphic.

Yetisir *et al.,* (2008) collected and studied of 182 accessions of bottle gourd and their results obtained from both descriptive statistics and multivariate analysis by principle component analysis suggested that the whole collection exhibits a great deal of morphological diversity.

Yüzbaşioğlu *et al.,* (2008) used fourteen lentil cultivars to study genetic diversity. They were detected total 24 polypeptide bands with molecular masses ranging from 14.4 to 116 kDa. Out of 24 bands, five was polymorphic bands with molecular masses ranging from 35 to 116 kDa.

Koffi *et al.,* (2009) studied morphological variation of 18 *Lagenaria siceraria* germplasms accessions. They had analyzed 24 traits and found significance difference between cultivars from multivariate analysis of variance (MANOVA). The estimates of F-statistics indicated a low level of genetic differentiation between accessions (F_{ST} = 0.298). Such a value suggested that *L. siceraria* maintains about 30% of its genetic variation among accessions.

El-Nas *et al.,* (2011) recorded Twenty-nine bands in seed protein patterns with 28% polymorphism and approximately molecular mass ranging between 79.6 and 15.7 kDa. The resulted profile comprises 21 monomorphic bands and eight polymorphic ones.

Sheikh *et al.,* (2011) studied genetic diversity of lentil trough SDS-PAGE and found total 46 bands. Out of them 44 were polymorphic with 95.6% polymorphism in lentil. Two main clusters were found according to dendrogram.

Sharma and Sengupta (2013) had investigated 16 genotypes of bottle gourd for evaluating their performance for various horticultural characters and found a great deal of significant variation for all the characters among the genotypes. Their result suggested high genotypic co-efficient of variation (GCV) for fruit weight (39.48%) and phenotypic co-efficient variances were higher than the genotypic co-efficient variance, in all cases.

Srivastava *et al.,* (2014) assessed genetic diversity among ten different genotype of bottle gourd by using SDS page and performed cluster analysis of combined banding pattern of leaves and fruit proteins on SDS-PAGE; which separated from 0.68 of similarity coefficient on similarity matrix.

Yatung *et al.,* (2014) Studied on seed protein profiling in chilli (*Capsicum annuum* L) genotypes of Northeast India and reported total 92 bands from 30 genotypes of chilli. Results of the analysis showed considerable variation in banding pattern of total protein ranged from 7-19 numbers of bands. Similarity index was calculated and genotypes were grouped in three major clusters which were further sub divided in 9 sub-clusters.

MATERIALS AND METHODS

The goal of this present study was to provide useful data to lentil breeders and reveal into the evolution and dissemination of lentil. To approach the afore-said goal the following experiments were conducted during the period of 2011-2012 at the botanical research field and Professor Joarder DNA and Chromosome Research Laboratory in the Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh. The experimental method consists of Genetic diversity studies based on seed storage protein profiling.

A. MATERIALS

As a material six lentil varieties and thirteen cross material (F_2) plants were use in this investigation. In order to easily identify and describe here mention L1 to L6 for six lentil varieties and L7 to L19 for cross material (F_2). Following six lentil varieties and cross material (F_2) are uses for in the investigation:

a) Parental line

Serial Number	Variety	Ac. No.
1	BARI Masur-1 (Bm1)	L1
2	BARI Masur-2 (Bm2)	L2
3	BARI Masur-3 (Bm3)	L3
4	BARI Masur-4 (Bm4)	L4
5	BARI Masur-5 (Bm5)	L5
6	BARI Masur-6 (Bm6)	L6

b) Cross material (F_2)

Serial Number	Female	×	Male	Ac. No.
1	Bm1	×	Bm4	L7
2	Bm1	×	Bm6	L8
3	Bm2	×	Bm1	L9
4	Bm2	×	Bm4	L10
5	Bm2	×	Bm6	L11
6	Bm3	×	Bm4	L12
7	Bm4	×	Bm1	L13
8	Bm4	×	Bm2	L14
9	Bm4	×	Bm3	L15
10	Bm4	×	Bm6	L16
11	Bm5	×	Bm3	L17
12	Bm6	×	Bm1	L18
13	Bm6	×	Bm2	L19

B. METHODS

Morphological Characters

The methods followed to conduct the experiment and analyses of the data were divided into the following sub-heads:

1. Preparation and Design of the Experimental Field

A completely randomized block design with individual plant randomization was used for the experiment. The experiment was set within the field behind the third science building, University of Rajshahi, during the *rabi* crop seasons of 2011-2012. The experimental field was comprised an area of 500 x 1290 sq. cm. The field was consisted of 3 replications. Each replication contained 19 plots. The plot was consist of 2 rows and each row having 5 hills. Spaces between replications were 100 cm. Gaps between plots, rows and hills were 40 cm, 30 cm and 25 cm, respectively. The seeds of six lines were randomly assigned to the rows of plots. In each hill, only one plant was maintained. A sunny and uniform place was selected for RCBD which is shown in the **Figure 28**.

2. Sowing of Seeds

3. Maintenance of the Experimental Plants

The methods 2 and 3 are the same as those described under the methods of PART-I.

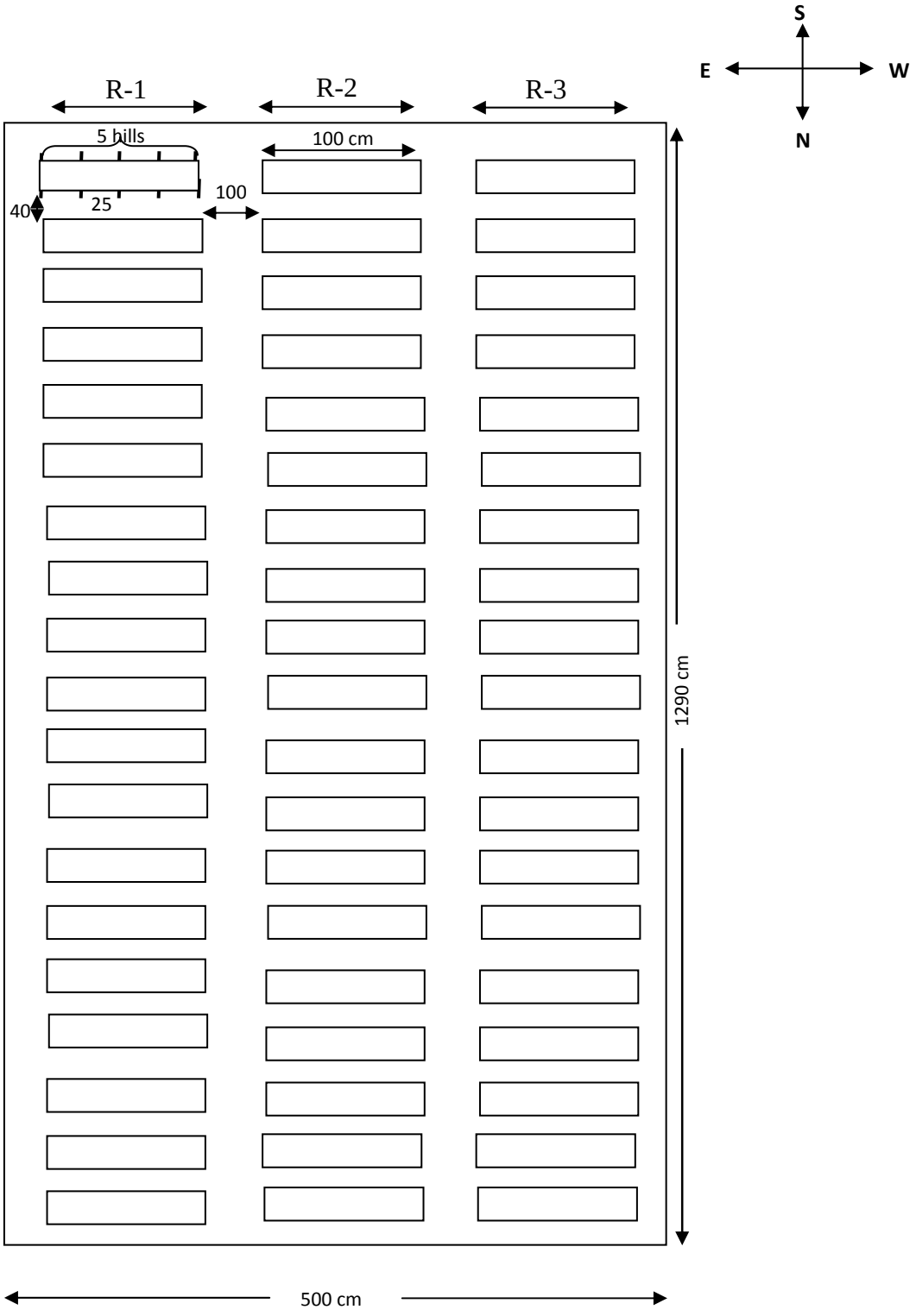
4. Collection of Data

Total eleven morphological characters were studied for morphological marker analysis on individual plant basis of lentil lines. The measurement of a character was done following C.G.S system. The characters studied are as follows:

a) Plant height at first flower (PHFF): Height of the individual plant was recorded from the base of the stem to the top of the plant at the time of first flowering stage.

b) Number of primary branches at first flower (NPBFF): The total number of primary branches at first flower per selected plant was counted and recorded.

c) Number of secondary branches at first flower (NSBFF): The total number of secondary branches at first flower per selected plant was counted and recorded.



Having 3 replications
Per replication having 19 plots
Per plot having 2 rows
Per row having 5 hills
Gap between block 100 cm.
Gap between plot 40 cm.
Gap between row 30 cm.
Gap between hills 25 cm.

Figure 28: Individual plant randomization in the completely randomized block design for lentil.

d) Plant height at maximum flowers (PHMF): The plant height was measured in cm from the base of the stem to the tip of the plant at the maximum flowering stage.

e) Number of primary branches at maximum flowering (NPBMF): The total number of primary branches at maximum flower per selected plants was counted and recorded.

f) Number of secondary branches per plant at maximum flower (NSBMF): Secondary branches came out from the primary branches and total number of secondary branches of the individual plant at the time of maximum flowering stage was counted and recorded.

g) Plant weight per plant (PWPP): Weight of each plant was taken after completely drying then recorded.

h) Number of pods per plant (NPdPP): All the pods of the individual plant after harvesting were removed, counted and recorded.

i) Pod weight per plant (PdWPP): All the pods of the individual plant were weighted and recorded.

j) Number of seeds per plant (NSPP): All the pods of an individual plant were threshed and seeds were taken out from the pods and cleaned, then the total number of seeds was counted and recorded.

k) Seed weight per plant (SWPP): Total seeds of the individual plant were weighted and recorded.

5. Techniques of Analyses of Data

The collected data were analyzed following the biometrical techniques of analysis as developed by Mather (1949) based on the mathematical models of Fisher *et al.*, (1932). The techniques used for data analysis are described under the following sub-heads:

a) Analysis of variance:

Variance is a measure of dispersion of a population. Therefore, the analysis of variance was done for testing the significant differences among the populations. Variance analysis for each of the characters was carried out separately on the mean value of a row and the preparation of ANOVA table is given in **Table 24.**

The variance due to different sources such as line (t), Replication (r), and within error of a population was calculated as per the following skeleton of analysis:

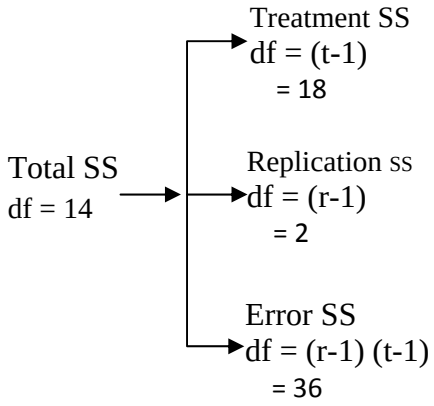


Table 24: Analysis of variance

Source	Degree of freedom	Sum of square	Mean of sum square ($MS = \frac{SS}{df}$)	$F = \frac{MS}{\text{Error MS}}$
Variety	t-1= 18	SS_1	$SS_1/df=MS_1$	MS_1/MSe
Replication	r-1= 2	SS_2	$SS_2/df=MS_2$	MS_2/MSe
Error	(r–1)(t-1)= 36	SS_3	$SS_3/df=MSe$	
Total	rt-1 = 56			

b) Genetic dissimilarity and cluster analysis

For morphological characters analysis, Euclidean distance values were used to prepare dendrogram. Euclidian distance between the varieties was calculated from the standardized trait mean values using NTSYSpc Version 2.11T (Rohlf, 2004). To group the accessions based on morphological dissimilarity, cluster analysis was conducted on Euclidian distance matrix with Unweighted Pair Group Method based on Arithmetic Averages (UPGMA) procedure of the Nested (SAHN) clustering methods (Sneath and Sokal, 1973) using NTSYSpc 2.11T.

Protein Marker

Acculadder Protein size marker (low) was used as a protein marker. It also called protein molecular weight markers. Acculadder Protein size marker (low) consists of kind proteins (6.5kDa-66 kDa). This protein size marker contains sample buffer and it can be heating

after heating at 95 ^{0}C for 5 minutes. 5 µl of marker is loaded per lane of SDS-PAGE mini-gel. Molecular weight Range (kDa): 6.5-66 (kDa) and number of bands: 6 (Table 25).

Table 25: Name of the different protein according to their molecular weight

Name of Proteins	MW (kDa)	Source
Albumin	66	Bovine serum
Ovalbumin	45	Chicken egg white
Carbonic anhydrase	29	Bovine erythrocytes
Trypsin inhibitor	20.1	Soybean
Lysozyme	14.4	Chicken egg white
Aprotinin	6.5	Bovine lung

Reagents

Equipments and reagents were nearly same for seed storage protein profiling which are given in **Table 26.**

Table 26: Name of some important reagents used for seed storage protein profiling

No.	Reagents
1	Acrylamide
2	Sodium dodecyl sulfate (For SDS)
3	Glycerol
4	TEMED
5	Glycine
6	Bromophenol blue dye
7	Methanol
8	Tris
9	Silver nitrate
10	Sodium carbonate
11	Formaldehyde
12	Hydrochloric acid
13	N-, N- methylene bisacrylamide
14	Glacial acetic acid (For SDS)
15	Sodium thiosulphate
16	2- Mercaptoethanol
17	Coomassie brilliant blue-R250 (For SDS)
18	Ethanol (For SDS)
19	Ammonium persulfate

1. Protein Profiling Methods

Seed storage protein profiling was done according to Laemmli (1970) method and following steps were followed.

Preparation of different stock solution

Preparations of different stock solutions seed storage protein profiling were nearly same which are given in below.

1.5 M Tris solution (pH 8.8) 100 ml

18.21 g Tris (hydroxymethyl) amino methane was dissolved in 60 ml distilled water and final volume was made 100 ml. pH was adjusted **8.8** by adding concentrated HCl or 1 N HCl drop-wise and stored at room temperature.

1.5 M Tris solution (pH 6.8) 100 ml

18.21 g Tris (hydroxymethyl) amino methane was dissolved in 60 ml distilled water and final volume was made 100 ml. pH was adjusted **6.8** by adding concentrated HCl or 1 N HCl drop-wise and stored at room temperature. All ingredients were dissolved and total volume was made of 100 ml and stored at 4˚C.

30% Acrylamide-bisacrylamide stock 100 ml

Acrylamide	29 g
Bisacrylamide	1 g

Two chemicals were dissolved in 60 ml of distilled water and heat at 37˚C to dissolve properly. Then final volume was made by adding distilled water.

Ammonium Persulfate (10%)

1g ammonium persulfate was dissolved in a total volume of 10 ml water and stored at 4˚C.

Protein extraction buffer

Proteins were extracted through Protein extraction buffer (50 ml) pH 6.8, which contain following chemicals

Chemicals	**Amount (gm)**
0.1 M Tris	0.605
0.25 M Sucrose	4.278
1% PVP (polyvenyl pyrrolidone)	0.5
0.1% Ascorbic acid	0.05
1 % Cystein HCl	0.05
1 mM EDTA	0.007
$MgCl_2$	0.001

Seed storage protein extraction was done by using protein extraction buffer. 0.1 M Tris HCl buffer was used as protein extraction buffer. 0.24248gm Tris HCl was dissolved in 20 ml double distilled water and pH 7.5 was adjusted.

10 % SDS

Dissolve 1g SDS in 8 ml water. Heat to solute the crystal. Adjust volume to 10 ml. Store at room temperature.

Sample loading buffer for SDS-PAGE (pH 6.8) 100 ml

Chemicals	Amount
1.5 M Tris (pH 6.8)	0.5ml
10% SDS	0.6ml
0.1% Bromophenol blue	0.75ml
2-Mercaptoethanol	10mg
15% Glycerol	1.5ml
Urea	8M

All ingredients were dissolved and total volume was made of 100 ml and stored at 4˚C.

10x SDS-PAGE running electrode buffer (pH 8.3) 1 litre (Tris-glycine buffer)

For total protein analysis, running electrode buffer was made by following chemicals

25 mM Tris base	7.6g
250 mM Glycine	36.03g
0.1% SDS	2.5g

Above chemicals were dissolved in 204 ml water and it was adjusted to a final volume of 250 ml with water and pH was also adjust 8.3 and stored at room temperature.

Commassie staining solution

After completion of electrophoresis the gel was incubated in staining and destaining solution. The commassie staining solution contain following chemicals

Name of Chemicals	Amount
Commassie blue	0.5 g
45% Methanol	90 ml
10% Glacial Acetic acid	20 ml
Double distilled water	90 ml

Adjusted volume to 250 ml with water and stored at room temperature.

Commassie destaining solution

The 50 ml commassie destaining solution contain following chemicals

Name of Chemicals	Amount
35% Methanol	20 ml
10% Glacial Acetic acid	5 ml
Add double distilled water	25 ml

Preparation of sample

1. 2 gm seeds of each variety were used for seed storage protein extraction.
2. First of all seed coats were removed.
3. Seeds were crushed with buffer in mortar pestle.
4. Transfer to a 1.5 ml tube and keep the samples in a water bath at 100^0C for 3-5 min.
5. Centrifuge it at 13000 rpm gradually for 20 min, 45 min and 60 min.
6. Collect the supernatant in a fresh 1.5 ml tube and store in a refrigerator.

Protein supernatant preparation

Proteins were quantified by Lowry assay (1951) method which is commonly used for protein assay. Stored proteins were quantification by Lowry method.

Preparation of polyacrylamide gel electrophoresis for protein

1. First 10% or 12% resolving gel for SDS-PAGE was prepared by taking the appropriate volume of solutions containing the desired concentration given in Table **27**.
2. TEMED (N, N, N, N-tetramethylenediamine) and ammonium persulfate were added and mixed quickly.

3. Acrylamide solution was poured in to the gap between the glasses leaving sufficient space for stacking gel and placed the gel in a vertical position at room temperature for 30 minutes.

4. After complete polymerization, stacking gel according to the table 10 was poured over the resolving gel and Teflon comb was inserted in to the stacking gel solution avoid trapping of air bubbles. For complete polymerization, gel was placed vertical position at room temperature for 30 minutes. Then comb was removed carefully.

5. Gel chamber was assembled and filled the buffer reservoir with 1x Tris-glycine and 10x SDS electrophoresis buffer. Gel plate was placed in electrophoresis chamber.

6. 15 µl or 15 µl for mini gel and 35 µl for big gel for SDS-PAGE of each sample with equal volume of 1x gel loading buffer was taken in wells with micropipette.

7. For SDS-PAGE, prior to load up the sample with loading dye was heated at 90˚ C for 6 minute in water bath. At the same Protein marker (5 µl for mini gel and 10 µl for big gel) was also heated at 95˚ C for 5 min.

8. Electrophoresis apparatus was attached to an electric power supply. The positive electrode was connected to the bottom buffer reservoir and supplied electricity at voltage of 8 V/cm for stacking gel and 15V/cm for resolving gel. Gel was run till the bromophenol dye front reaches 0.5 cm from the lower edge of the gel.

9. The gel plates were removed and marked the orientation of the gel by cutting a corner from the bottom of the gel that was closest to the left-most well.

10. After the completion of electrophoresis, the gel was fixed and stained for proteins.

11. After completion of gel electrophoresis, the gel can be fixed and stained for proteins. The most commonly used stain for detecting the proteins on the gel is

the ‘commasie Brilliant Blue R-250’ in methanol. Polypeptides separated by SDS-PAGE can be stained with Commasie Brilliant Blue -250 and simultaneously fixed with: glacial acetic acid.

12. Immerse the gel in at 5 volumes of staining solution and place on slowly-rotating platform gel rocker for minimum of 4 hours at room temperature. Change the destaining solution 3 or 4 times. The dye used is fairly sensitive and can detect up to 100 µg (0.1 mg) of protein in an acrylamide gel.

13. After distaining, gels may be stored indefinitely in a sealed plastic bag without any diminution in the intensity of staining or keep the gel for 10 min under UV light in a laminar airflow and then stored in sealed plastic bag at 4 ˚C.

Table 27: Amount and components used for preparation of resolving and staking gel for SDS-PAGE.

Chemical Composition	**Quantity for SDS-PAGE**			
	12% Resolving gel		**5% Stacking gel**	
	8 ml	**40 ml**	**4 ml**	**8 ml**
Double distilled water	2.6	13	2.7 ml	5.4
30% Acrylamide bis	3.2	16	0.67 ml	1.34
1.5 M Tris (pH-8.8)	2.0	10	-	-
1.5 M Tris (pH-6.8)	-	-	0.5 ml	1
10% SDS	80 µl	0.4	40 µl	80 µl
10% APS	80 µl	0.4	40 µl	80 µl
TEMED	6.4 µl	32 µl	4 µl	8 µl

2. Data analysis for seed storage protein profiling

a) Band scoring

After taking the photographs of Native-PAGE for SDS-PAGE for seed storage protein, the bands were visually scored as present (1) or absent (0) separately for the gel of SDS-PAGE which were used for the further analysis.

b) Preparation of zymograms

Zymogram is the dramatic representation of the protein band location in the strip of a gel. It is an extra plolated on the basis of relative mobility of each band in the gel. Zymograms were manually prepared for different isozymes and seed storage protein. The different steps for the preparation of zymogram are:

1. Photograph was taken by the camera of the gel.
2. Measure the total length of lane in cm on the photograph.
3. Calculate the relative mobility (Rm) of each band according to the formula.

c) Determination of relative mobility (R_m)

The proteins are allowed to migrate for a specific amount of time and then are stained with various chemicals so that the relative mobility of specific proteins can be determined. Relative mobility is a function of the size and charge of the molecule. If two proteins have different amino acid sequences, they often have different mobilities because the differences in sequence result in a change in size and/or charge of the molecule (Hedrick, 1983). Relative mobility (R_m) of each of the band was measured by the following formula.

$$\text{Relative mobility (Rm)} = \frac{\text{Migration distance of band}}{\text{Migration distance of dye front}}$$

d) Determination of polymorphism

Percentage of polymorphism is very important to study genetic variation. The determination of polymorphism of seed storage protein band was done by the following formula.

$$\text{Percentage of polymorphic bands} = \frac{\text{No. of polymorphic bands}}{\text{No. of total bands}} \times 100$$

e) Genetic similarity analysis

Based on band scoring of seed storage protein of nineteen lentil lines, Jaccrad's similarity coefficient matrix (Jaccard, 1908 and Jackson *et al.*, 1996) was done using NTSYSpc software.

f) Cluster analysis

Cluster analysis was done to detect genetic diversity among the six lentil varieties and thirteen F_2 cross lines. For cluster analysis dendrogram was constructed. Several software packages (Labate, 2000) can be used to estimate various diversity measures and genetic distances, infer population structure, test for multi-locus equilibrium, and test polymorphic loci for evidence of selective neutrality. In the present investigation, to construct dendrogram NTSYSpc version 2.11T (Rohlf, 2004) software was used

and dendrogram was constructed by using the UPGMA method (Sokal and Sneath, 1963) with SAHN module of NTSYSpc software to show a phylogenic representation of genetic relationship as revealed by the similarity coefficient. The Jaccard's genetic similarity matrix was used to build an unweighted pair-group method with arithmetic means (UPGMA) dendrogram for seed storage protein variation among the varieties.

g) Principal component analysis

Principal component analysis (PCA) was also done to confirm the results of UPGMA based clustering using EIGEN module of NTSYSpc. PCA was used for seed storage protein markers.

RESULTS

The present research was considered two stages for genetic diversity assessment in six lentil varieties and thirteen F_2 cross lines on the basis of morphological and biochemical marker analysis. Here, eleven morphological characters were studied morphological marker and total protein analyses were used for genetic diversity study among lentil lines. The results of this investigation are described as follows:

A. MORPHOLOGICAL MARKER ANALYSIS

In this investigation, eleven morphological characters of lentil lines namely plant height at first flower (PHFF); number of primary branches at first flower (NPBFF); number of secondary branches at first flower (NSBFF); plant height at maximum flowers (PHMF); number of primary branches at maximum flower (NPBMF); number of secondary branches at maximum flower (NSBMF); plant weight per plant (PWPP); number of pods per plant (NPdPP); pod weight per plant (PdWPP); number of seeds per plant (NSPP) and seed weight per plant (SWPP) were used as morphological markers. The techniques analysis of variance, genetic dissimilarity and cluster analysis was done to investigate genetic diversity of eleven morphological characters, which are described in the following subhead.

1. Analysis of Variance (ANOVA)

In this analysis, eleven morphological characters were considered for analysis of variance. Treatments were to be significant for all the characters at 5% significance level except NSBFF and PHMF, these lines showed highly significant result at 1% level and the results are shown in **Table 28(A-K)**. On the other hand, replication item was significant for the characters PWPP, NPdPP, PdWPP, NSPP and SWP at 5% level.

2. Genetic dissimilarity analysis

Genetic dissimilarity was done by Euclidian distance. Euclidian distance among six lentil varieties and thirteen F_2 crosses were calculated from the standardized trait mean values using NTSYSpc software version 2.11T (Rohlf, 2004) and presented in **Table 29**.

Based on Euclidian distance, genetic dissimilarity was ranged from 0.10 to 0.99. Where L1 (Bm1) and L15 (Bm4 × Bm3) showed the highest dissimilarity value (0.99); Second and third highest dissimilarity value were noted by between L3 (Bm3) and L14 (Bm4 × Bm2) and L16 (Bm4 × Bm6) and L19 (Bm6 × Bm2), respectively.

On the other hand, the highest genetic similarity (lowest dissimilarity coefficient) was noticed between L2 (Bm2) and L10 (Bm2 × Bm4); L2 (Bm2) and L14 (Bm4 × Bm2); L8 (Bm1 × Bm6) and L17 (Bm5 × Bm3); L9 (Bm2 × Bm1) and L15 (Bm4 × Bm3) and L13 (Bm4 × Bm1) and L19 (Bm6 × Bm2), all of them showed same value (0.10).

3. Cluster analysis

In this study, for measured the relationship of lentil lines based on morphological traits, we constructed a dendrogram of six lentil varieties and thirteen F_2 crosses based on eleven quantitative characters that divided these lentil lines into two main clusters (Cluster-I and Cluster-II). In addition, Cluster-II was grouped into two sub-clusters, which were presented in **Figure 29**.

Looking at the morphological dendrogram, it can be observed that the varieties were grouped in two main clusters: cluster-I and cluster-II. The biggest group was cluster-II that includes sixteen lines which are grouped into two sub-clusters, while cluster-I comprised only three lines.

The cluster-I was composed of L1 (Bm1), L9 (Bm2 × Bm1) and L11 (Bm2 × Bm6) where first two are closer than others and rest of the lines were belonged to cluster-II. Cluster-II was grouped into two sub-clusters; then, each of those sub clusters was divided into further sub-clusters.

Seven lentil lines are consist on sub-cluster-I in Cluster-II. L2 (Bm2) and L13 (Bm4 × Bm1) was grouped in sub-cluster-I of cluster-II and all of them Bm5 and Bm6 are very close. L4 (Bm4), L7 (Bm4 × Bm1) and L10 (Bm2 × Bm4) also presence in grouped in sub-cluster-I of cluster-II. In sub-cluster-II of cluster-II was consist rest of the lines which was divided two more sub-groups. In further, two sub-groups are belong in said

sub-groups. The results indicate that, low or high genetic distance exists respectively between varieties with similar or different origins.

B. SEED STORAGE PROTEIN PROFILING

Present investigation studied that protein profiling to detect inter-varietal genetic diversity and study phylogenetic relationship among the six varieties and thirteen F_2 crosses of *Lens culinaris* Medic.

1. Banding patterns and polymorphism analysis through total protein

In the present research, Twenty seven bands were recorded with different Rm (Relative mobility) values were identified through the SDS-PAGE photograph **(Figure 30)**, as well as scoring and zymogram **(Figure 31)** of the six lentil varieties. Among twenty-seven bands, nineteen bands were polymorphic and eight bands were monomorphic, which produced 70.37% effective polymorphism **(Table 30, Figure 30 and 31)**. The relative mobility of seed storage protein was ranging from 0.10 to 0.85. The highest polymorphic bands were recorded in L17 (Bm5 × Bm3) and number of polymorphic band eleven and the lowest polymorphic bands were recorded in L1 (Bm1) and L12 (Bm3 × Bm4), both of them have no polymorphic band.

Nine polymorphic bands were found in L10 (Bm2 × Bm4). L4 (Bm4), L5 (Bm5) and L6 (Bm) showed twenty one monomorphic bands and six polymorphic bands **(Table 31)** which were at positioning almost same **(Figure 30 and 31).** Other five lines showing same monomorphic and polymorphic in number, which was twenty two bands and five bands, respectively, but positions were different and the lines are L3 (Bm3), L7 (Bm1 × Bm4), L8 (Bm1 × Bm6), L13 (Bm4 × Bm1) and L15 (Bm4 × Bm3) **(Table 31 and Figure 31)**. L9 (Bm2 × Bm1) showed twenty three monomorphic bands and four polymorphic bands at position 0.27, 0.32, 0.41 and 0.70 **(Table 31 and Figure 30).** L2 (Bm2), L11 (Bm2 × Bm6), L14 (Bm4 × Bm2), L16 (Bm4 × Bm6) and L19 (Bm6 × Bm2) were exhibited twenty six monomorphic bands and only three polymorphic bands with different Relative mobility (Rm) values at position 0.13, 0.20 and 0.27 for L2 , 0.12, 0.58 and 0.70 for L11, 0.12, 0.13 and 0.41 for L14, 0.12, 0.13

and 0.38 for L16 and 0.70, 0.83 and 0.85 for L19. L18 (Bm6 × Bm1) shown only one polymorphic band in 0.38 position.

The bands of seed storage proteins were detected at approximately molecular weight ranging between 6.5 and 66 KDa. On the basis of banding pattern, the gel was divided into six regions with intervals of protein molecular marker. From the SDS-PAGE photograph, Region-I for albumin, Region-II for ovalbumin, Region-III for carbonic anhydrase, Region-IV for trypsin inhibitor, Region-V for lysozyme, Region-VI for aprotinin **(Figure 30)**. Region-I had seven bands of more than 66 KDa of molecular weight, of which four were polymorphic. Region-II was molecular weight ranged from 45 KDa to 66 KDa, with five protein peptides, of which four were polymorphic. Region-III ranged from 29 KDa to 45 KDa, with four protein subunits, of which three were polymorphic. Region-IV, ranging from 20.1 KDa to 29 KDa, had four protein bands, of which three were polymorphic. Region-V ranged from 14.4 to 20.1 KDa, had three bands and all were polymorphic. Region-VI, molecular weight ranging from 6.5 KDa to 14.4 KDa, had four protein bands where, two bands were polymorphic and two monomorphic.

2. Genetic similarity analysis

The similarity coefficient was calculated based on the banding patterns and it was ranged from 0.48 to 1.00. According to Jaccard's similarity coefficient **(Table 32)** L1 (Bm1) was noted to be closely related with L12 (Bm3 × Bm4) with the highest similarity coefficient as 1.00 and the same time L1 (Bm1) vs. L18 (Bm6 × Bm1) and L14 (Bm4× Bm2) vs. L18 (Bm4 × Bm6) showed same similarity coefficient 0.96.

On the other hand, L7 (Bm1 × Bm4) vs. L17 (Bm5 × Bm3) and L13 (Bm4× Bm1) vs. L17 (Bm5 × Bm3) showed lowest similarity coefficient 0.48 that means this is the highest distant relation. The second lowest similarity coefficient 0.52 could be noticed among L17 (Bm5 × Bm3) vs. L5 (Bm5), L6 (Bm6) and L9 (Bm2 × Bm1).

3. Cluster analysis

Dendrogram was constructed according to Un-weighted Pair Group Method by using Arithmetic Averages (UPGMA). Based on Cluster analysis, data placed of six varieties

and thirteen F_2 crosses of lentil into two main clusters, where Cluster-II belong only L17 (Bm5 × Bm3) and rest of the eighteen lines are in Cluster-I **(Figure 32)**. Cluster-I divided two sub-cluster where, First sub-cluster comprised eleven lentil lines which subdivided several in to groups. The dendrogram shown L1 (Bm1) and L12 (Bm3 × Bm4) are closely related.

Second sub-cluster (Sub-cluster-II) comprised seven lentil line and Sub-cluster-II further subdivided into two groups, where one group are subdivided further two sub-groups. Dendrogram also showed the highest genetic distance between L13 (Bm4 × Bm1) and L17 (Bm5 × Bm3) and L7 (Bm1 × Bm4) vs. L17 (Bm5 × Bm3).

4. Principal component analysis

The results of the principal component analysis (PCA) based on total protein are shown in **Figure 33.**The first five principal components from PCA accounted for 94.085% of the total variation among six varieties and thirteen F_2 crosses (**Table 23**). The proportions of the principal components one (PCI), two (PC2) and three (PC3) were 79.96%, 4.97% and 3.552%, respectively. Three dimensional (**Figure 33 and 34**) plots were prepared by using first three PCs, respectively. The two and three dimensional plots supported the cluster analysis and plotted nineteen lines into two main groups' *viz.* group-1 and group-2 same as cluster analysis. Group-1 composed of eighteen lines except L17 (belonging in group-2). According to PCA analysis, close relatedness was found among cultivars L1, L12 and L18 are most distantly related cultivars. Similar result has been found in cluster analysis. The highest genetic distance was found L17 from the others.

Table 28A: ANOVA of lentil lines for plant height at first flower (PHFF)

Source	df	SS	MS	F	5%	1%
Treatment	18	291.8012	16.21118	2.350205*	1.90	2.48
Replication	2	38.24208	19.12104	2.77206	3.26	5.25
Error	36	248.3199	6.897774			

Table 28B: ANOVA of lentil lines for number of primary branches at first flower (NPBFF)

Source	df	SS	MS	F	5%	1%
Treatment	18	45.96343	2.553524	2.011481*	1.90	2.48
Replication	2	4.900537	2.450268	1.930144	3.26	5.25
Error	36	45.70108	1.269474			

Table 28C: ANOVA of lentil lines for number of secondary branches at first flower (NSBFF)

Source	df	SS	MS	F	5%	1%
Treatment	18	30.92626	1.718126	2.6610**	1.90	2.48
Replication	2	1.140905	0.570453	0.883535	3.26	5.25
Error	36	23.24332	0.645648			

Table 28D: ANOVA of lentil lines for plant height at maximum flower (PHMF)

Source	df	SS	MS	F	5%	1%
Treatment	18	389.031	21.61283	2.7996**	1.90	2.48
Replication	2	28.75213	14.37607	1.862182	3.26	5.25
Error	36	277.9203	7.72001			

Table 28E: ANOVA of lentil lines for number of primary branches at maximum flower (NPBMF)

Source	df	SS	MF	F	5%	1%
Treatment	18	57.77517	3.209732	2.3165*	1.90	2.48
Replication	2	2.695312	1.347656	0.972626	3.26	5.25
Error	36	49.88106	1.385585			

Table 28F: ANOVA of lentil lines for number of primary branches at maximum flower (NPBMF)

Source	df	SS	MF	F	5%	1%
Treatment	18	170.0361	9.446448	2.1075*	1.90	2.48
Replication	2	1.443218	0.721609	0.160994	3.26	5.25
Error	36	161.3593	4.482203			

Table 28G: ANOVA of lentil lines for plant weight per plant (PWPP)

Source	df	SS	MS	F	5%	1%
Variety	5	139.32	7.740002	2.0876*	1.90	2.48
Replication	3	37.81221	18.90611	5.0992*	3.26	5.25
Error	15	133.4748	3.707633			

Table 28H: ANOVA of lentil lines for number of pods per plant (NPdPP)

Source	df	SS	MS	F	5%	1%
Treatment	18	46770.97	2598.387	1.9723*	1.90	2.48
Replication	2	11880.54	5940.272	4.5090*	3.26	5.25
Error	36	47426.68	1317.408			

Table 28I: ANOVA of lentil lines for pod weight per plant (PdWPP)

Source	df	SS	MS	F	5%	1%
Treatment	18	84.91912	4.717729	2.1159*	1.90	2.48
Replication	2	16.46274	8.23137	3.69193*	3.26	5.25
Error	36	80.26406	2.229557			

Table 28J: ANOVA of lentil lines for number of seeds per plant (NSPP)

Source	df	SS	MS	F	5%	1%
Treatment	18	84482.45	4693.469	1.9556*	1.90	2.48
Replication	2	16929.06	8464.53	3.5268*	3.26	5.25
Error	36	86400.32	2400.009			

Table 28K: ANOVA of lentil lines for seed weight per plant (SWPP)

Source	df	SS	MS	F	5%	1%
Treatment	18	53.21743	2.956524	2.2851*	1.90	2.48
Replication	2	11.44782	5.723908	4.4241*	3.26	5.25
Error	36	46.57697	1.293805			

N.B.: * and ** means significant at 1% and 5% level of significance, respectively.

Table 29: Dissimilarity coefficient matrix among six lentil varieties and thirteen F_2 crosses based on morphological characters

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19
L1	0.00																		
L2	0.35	0.00																	
L3	0.12	0.19	0.00																
L4	0.39	0.83	0.27	0.00															
L5	0.38	0.86	0.27	0.22	0.00														
L6	0.38	0.86	0.27	0.23	0.62	0.00													
L7	0.38	0.84	0.27	0.27	0.72	0.11	0.00												
L8	0.27	0.48	0.16	0.12	0.12	0.16	0.12	0.00											
L9	0.15	0.31	0.12	0.39	0.39	0.39	0.39	0.28	0.00										
L10	0.40	**0.10**	0.29	0.40	0.32	0.34	0.30	0.13	0.41	0.00									
L11	0.31	0.33	0.14	0.41	0.41	0.41	0.41	0.30	0.27	0.43	0.00								
L12	0.25	0.67	0.13	0.15	0.14	0.14	0.14	0.29	0.25	0.16	0.27	0.00							
L13	0.34	0.54	0.23	0.67	0.56	0.56	0.51	0.67	0.34	0.68	0.36	0.93	0.00						
L14	0.21	0.10	0.94	0.18	0.18	0.18	0.18	0.67	0.21	0.20	0.23	0.41	0.13	0.00					
L15	**0.99**	0.21	0.23	0.29	0.29	0.29	0.29	0.18	**0.10**	0.31	0.12	0.15	0.24	0.11	0.00				
L16	0.18	0.13	0.68	0.21	0.21	0.21	0.21	0.11	0.18	0.23	0.21	0.87	0.17	0.59	0.89	0.00			
L17	0.17	0.13	0.59	0.21	0.21	0.21	0.21	**0.10**	0.18	0.23	0.20	0.80	0.17	0.44	0.79	0.24	0.00		
L18	0.23	0.74	0.12	0.15	0.15	0.15	0.15	0.51	0.26	0.17	0.26	0.39	0.11	0.44	0.14	0.60	0.61	0.00	
L19	0.24	0.87	0.13	0.16	0.15	0.15	0.15	0.44	0.25	0.17	0.26	0.28	**0.10**	0.43	0.15	0.92	0.80	0.54	0.00

Table 30: Number of polymorphic and monomorphic loci with percentage of polymorphism for SDS-PAGE

No. of total Bands	No. of polymorphic Bands	No.of monomorphic Bands	Percentage of polymorphism (%)
27	19	8	70.37

Table 31: Number of total bands, polymorphic bands, monomorphic bands and Percentage of polymorphism

Variety	No. of total bands	No. of polymorphic bands	No. of monomorphic bands	Percentage of polymorphism(%)
L1	27	0	27	0.00
L2	27	3	24	11.11
L3	27	5	22	18.52
L4	27	6	21	22.22
L5	27	6	21	22.22
L6	27	6	21	22.22
L7	27	5	22	18.52
L8	27	5	22	18.52
L9	27	4	23	14.81
L10	27	9	18	33.33
L11	27	3	24	11.11
L12	27	0	27	0.00
L13	27	5	22	18.52
L14	27	3	24	11.11
L15	27	5	22	18.52
L16	27	3	24	11.11
L17	27	11	16	40.74
L18	27	1	26	3.70
L19	27	3	24	11.11

Table 27: Jaccard's Similarity coefficient matrix among six varieties and thirteen F_2 crosses of *Lens culinaris* Medic based on Seed storage proteins profiling

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19
L1	1.00																		
L2	0.89	1.00																	
L3	0.82	0.85	1.00																
L4	0.82	0.85	0.70	1.00															
L5	0.78	0.89	0.82	0.89	1.00														
L6	0.78	0.89	0.82	0.82	0.93	1.00													
L7	0.82	0.93	0.85	0.78	0.89	0.89	1.00												
L8	0.82	0.85	0.78	0.78	0.82	0.82	0.93	1.00											
L9	0.85	0.82	0.82	0.74	0.85	0.78	0.82	0.74	1.00										
L10	0.67	0.78	0.85	0.78	0.82	0.74	0.78	0.78	0.74	1.00									
L11	0.93	0.82	0.74	0.89	0.85	0.78	0.74	0.74	0.85	0.67	1.00								
L12	1.00	0.89	0.82	0.82	0.78	0.78	0.82	0.82	0.85	0.67	0.93	1.00							
L13	0.82	0.78	0.70	0.78	0.74	0.67	0.70	0.63	0.82	0.70	0.82	0.82	1.00						
L14	0.89	0.85	0.70	0.85	0.82	0.82	0.78	0.85	0.74	0.70	0.89	0.89	0.70	1.00					
L15	0.82	0.85	0.70	0.85	0.89	0.82	0.78	0.70	0.82	0.70	0.89	0.82	0.85	0.85	1.00				
L16	0.93	0.89	0.74	0.89	0.85	0.85	0.82	0.82	0.78	0.67	0.93	0.93	0.74	0.96	0.89	1.00			
L17	0.59	0.56	0.56	0.56	0.52	0.52	0.48	0.56	0.52	0.56	0.59	0.59	0.48	0.70	0.63	0.67	1.00		
L18	0.96	0.85	0.78	0.78	0.74	0.74	0.78	0.78	0.89	0.63	0.89	0.96	0.78	0.85	0.78	0.89	0.63	1.00	
L19	0.89	0.78	0.70	0.78	0.74	0.67	0.70	0.70	0.82	0.63	0.89	0.89	0.85	0.78	0.85	0.82	0.63	0.85	1.00

Table 28: Eigen values and percentage of variation for corresponding of six varieties and thirteen F_2 crosses of *Lens culinaris* Medic based on seed storage protein profiling

Principal component characters	Eigen values	Percentage of total variation	Cumulative percentage
1	15.194	79.964	79.964
2	0.946	4.979	84.943
3	0.675	3.552	88.495
4	0.540	2.842	91.337
5	0.522	2.747	94.085
6	0.277	1.458	95.542
7	0.220	1.158	96.7
8	0.188	0.989	97.69
9	0.135	0.71	98.4
10	0.107	0.563	98.963
11	0.078	0.411	99.374
12	0.056	0.295	99.668
13	0.025	0.132	99.8
14	0.017	0.089	99.889
15	0.013	0.068	99.958
16	0.006	0.032	99.989
17	0.002	0.011	100
18	0.000	0	100
19	0.000	0	100

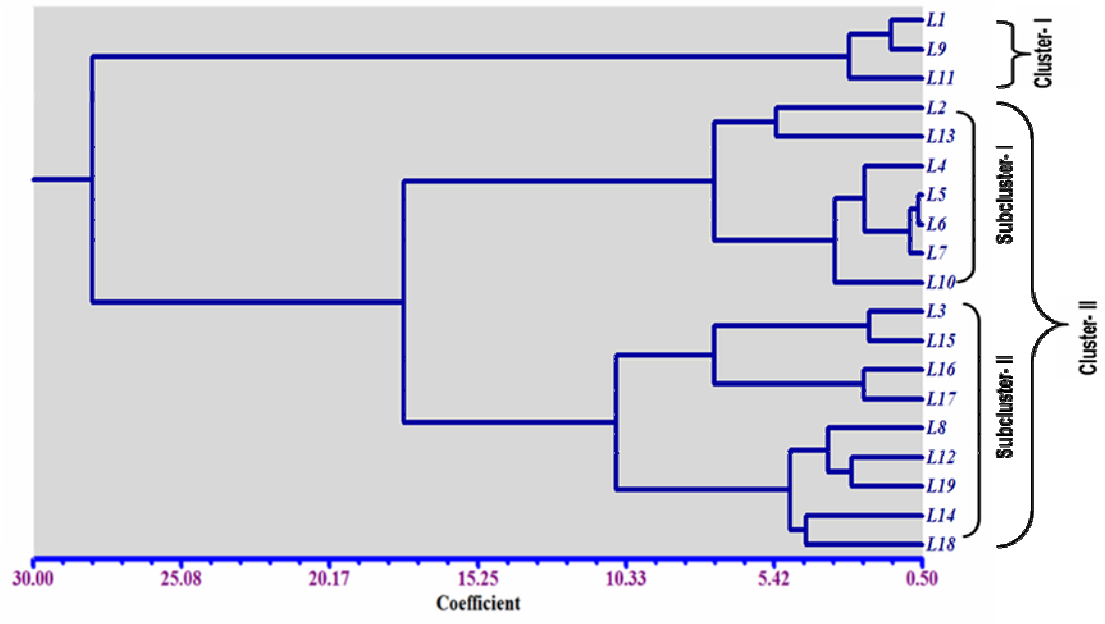


Figure 29: UPGMA Dendrogram of six varieties and thirteen F_2 Crosses lines of lentil based on eleven morphological characters using genetic distance

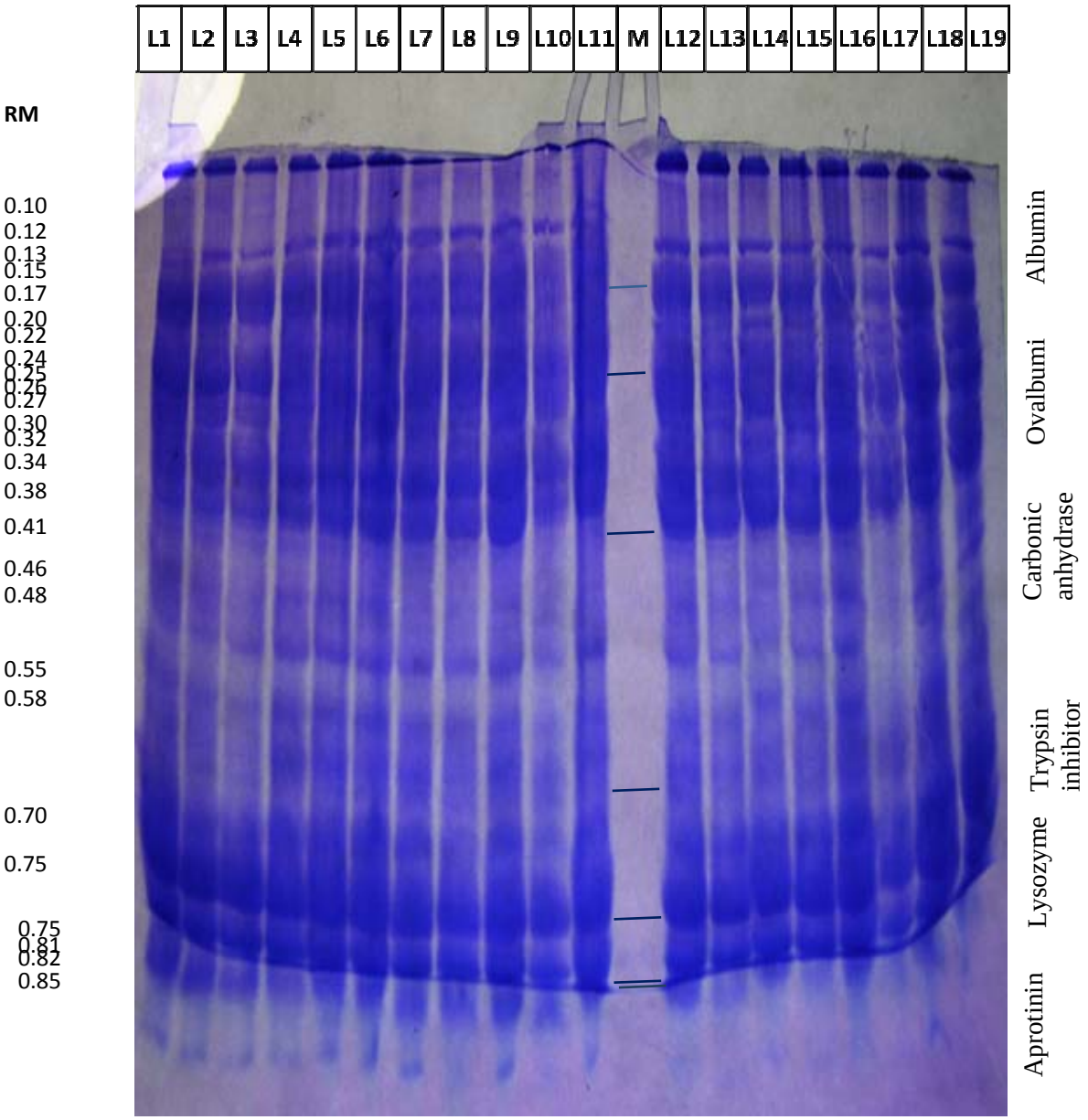


Figure 30: Photograph of SDS-PAGE with relative mobility of six varieties and thirteen F_2 crosses of *Lens culinaris* Medic

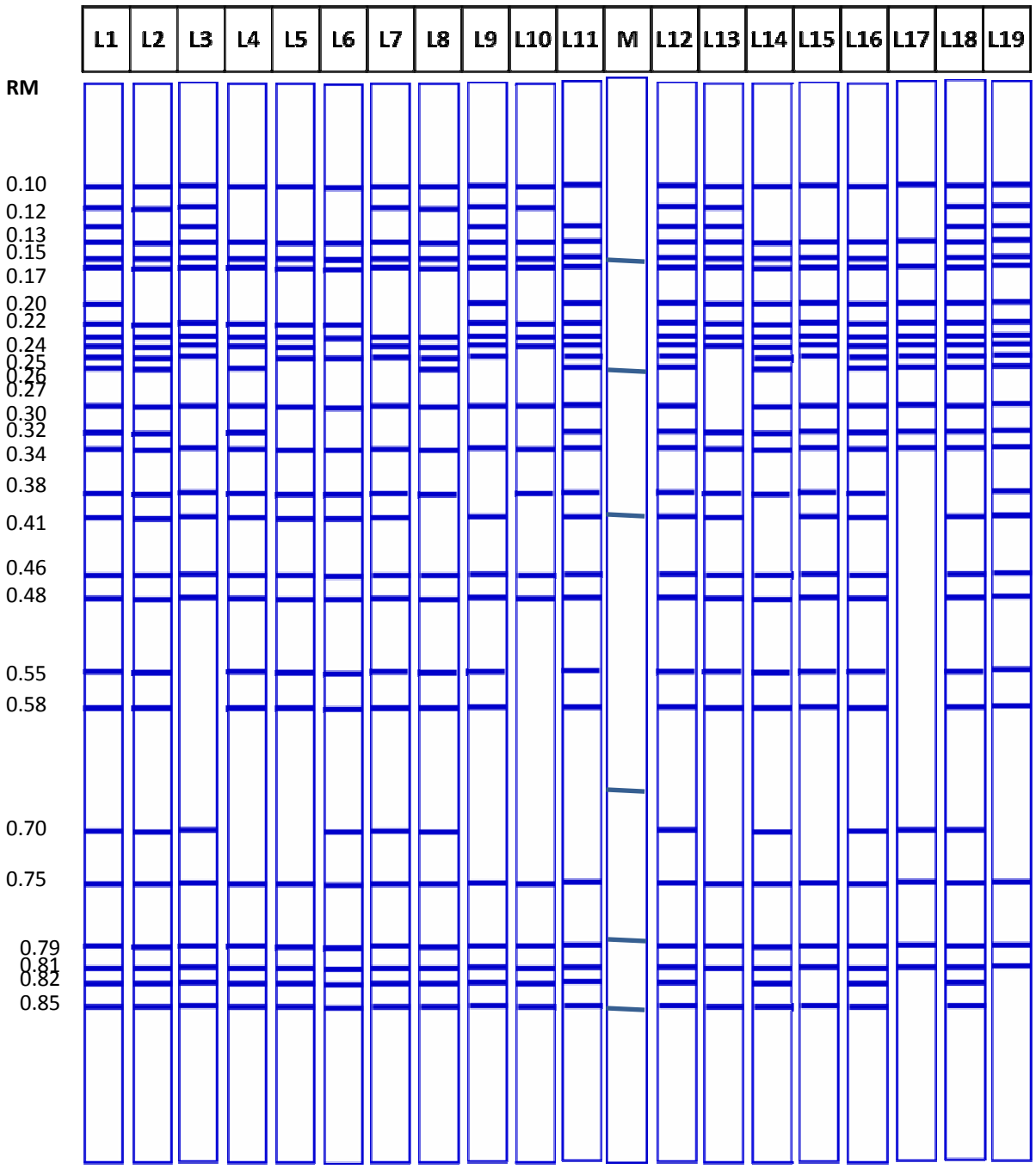


Figure 31: Zymogram of SDS-PAGE with relative mobility of six varieties and thirteen F_2 crosses of *Lens culinaris* Medic

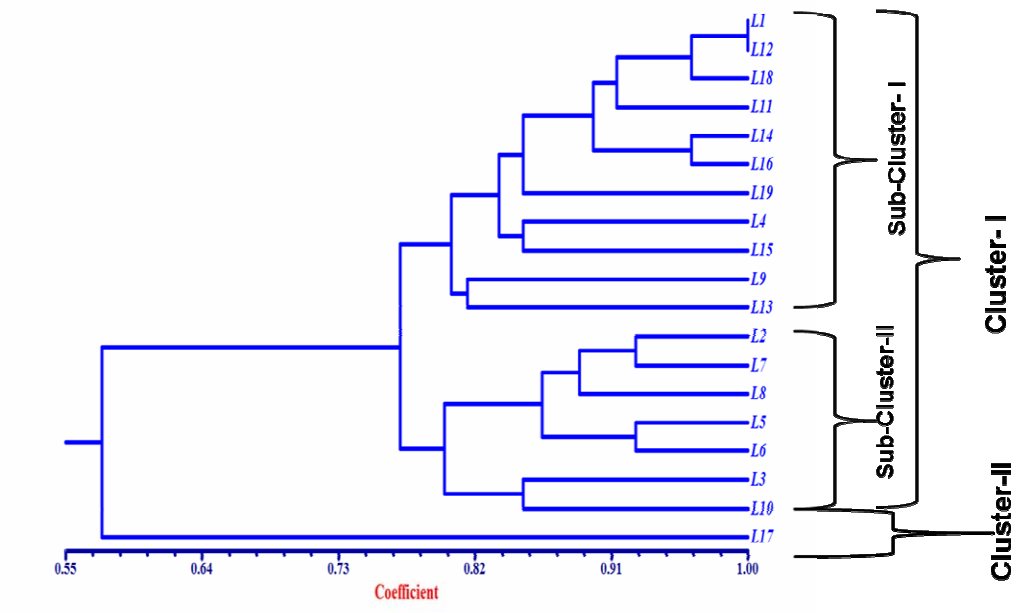


Figure 32: Dendrogram of the total seed storage protein bands from SDS-PAGE

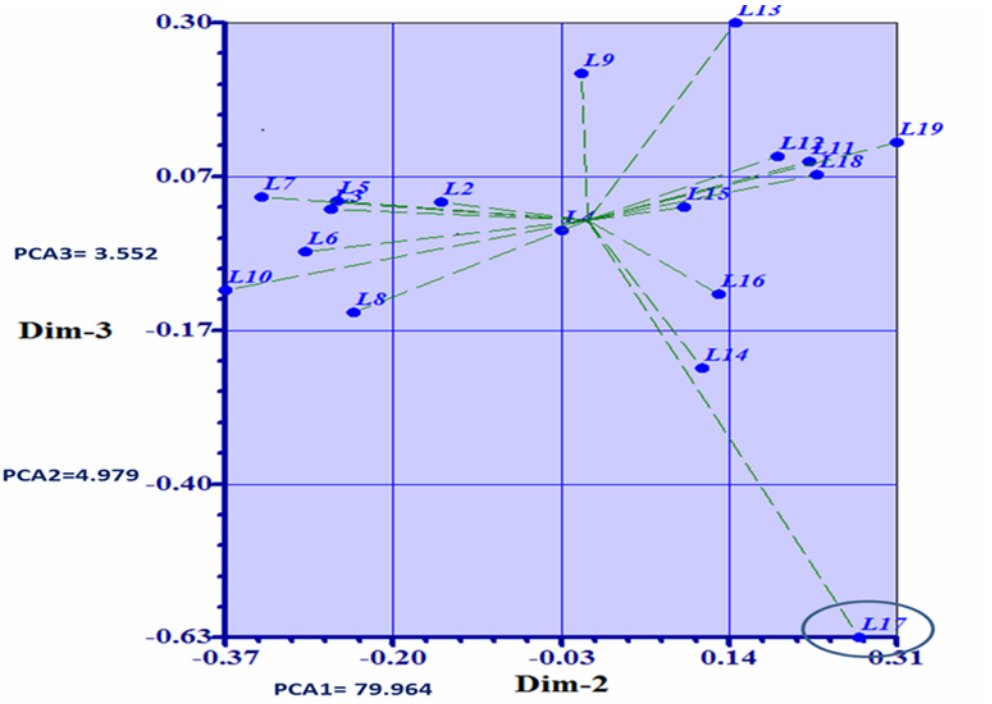


Figure 33: 2D distribution of lentil varieties revealed by first two principal components (PC1 and PC2) based on SDS-PAGE data

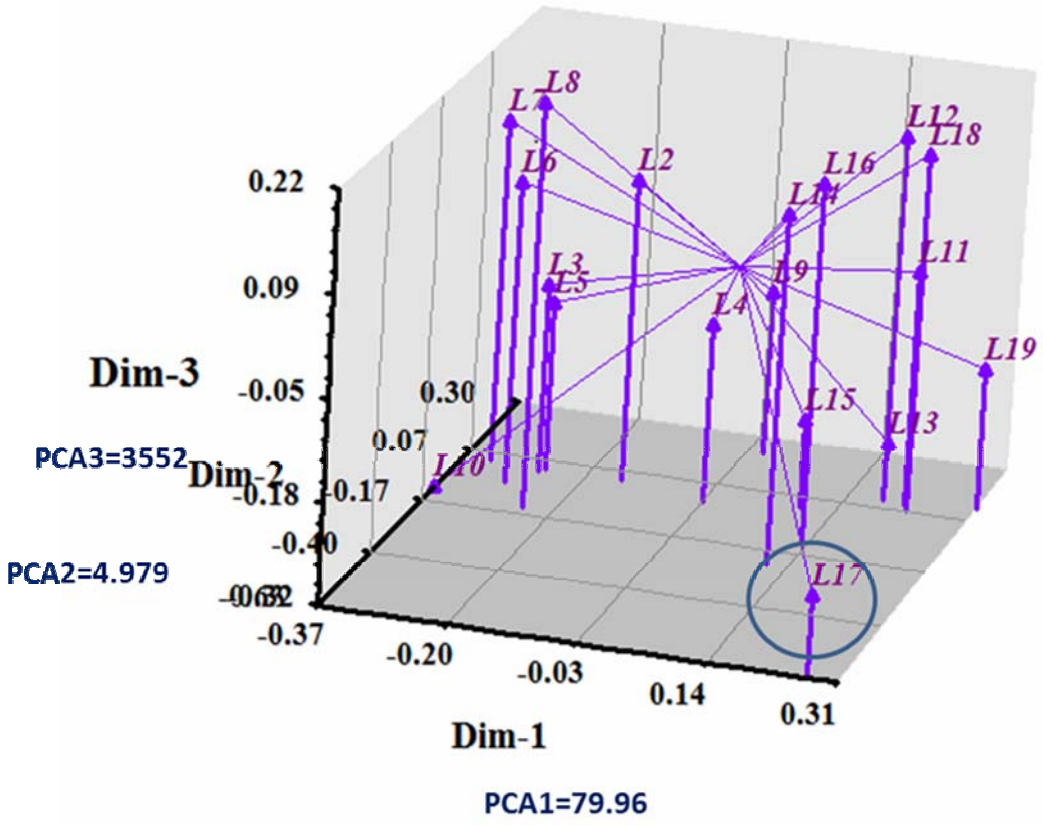


Figure 34: 3D distribution of lentil varieties revealed by first three principal components based on SDS-PAGE data

DISCUSSION

From the less land with fewer input, high-yielding crop variety are required to ensure sustainable food production. The continuing need for improved crops to meet up new environmental challenges and changing consumer's demands create a constant requirement for genetic diversity but the pool of natural. Genetic information regarding the nature, relative magnitude and type of gene action following a proper genetic model is very important in a crop for successful breeding research. Genetic characterization in different crop species has long been based on morphological traits; however morphological traits may be affected by environmental factors. Recently, biochemical and molecular techniques are emerging as a complementary strategy for characterization of the plant genome in conjunction with morphological traits aim to magnifying the level of genetic diversity for the crop improvement (Nisar *et al.,* 2011). This study was undertaken with a view to study genetic diversity in six lentil varieties and thirteen F_2 cross lines by morphological and biochemical markers. According to the results, total investigations were discussed part by part as follows:

MORPHOLOGICAL CHARACTERS ANALYSIS

Genetic progress demands more information on the inheritance of the key yield contributing traits and their associations with other plants traits according to the prevailing weather conditions of the target environment. On average, around 50% of the productivity increases have been estimated to genetic improvement (Fehr, 1984).

In the present investigation, eleven characters were used and at first analysis of variance were done. According to analysis of variance (ANOVA), significant differences were found among six lentil varieties and thirteen F_2 cross lines for all the characters for treatment item. Significant result indicating lines are significantly different from each other. Fikiru *et al.,* (2010) and Roy *et al.,* (2013) also reported significant result for all the quantitative (nine and eight) characters in lentil. Analysis of variance showed highly significant difference at the 5% significance level among the varieties for all the traits justifying the appropriateness of further analysis. Sharma and Sengupta (2013) found

significant variation in morphological and phenotypic characters among 16 genotypes of bottle gourd. Ahamed *et al.,* (2011) also observed distinct variation of morphological characters among twenty pumpkin genotypes of Bangladesh. Toklu *et al.,* (2009) reported significant variation in lentil based on Analysis of variance. Koffi *et al.,* (2009) also found significant variation for different morphological traits among cultivars of *Lagenaria siceraria.* Mondal *et al.,* (2007) reported that seed yield in lentil depends on seed size. Dewan (2005) also found significant variation in number of seeds per pod in lentil. Rahman and Ali (2004) found wide range of variability in existing lentil cultivars in 100-seed weight which was in supportive of this present study. Ercan *et al.,* (2004) and Raghuwanshi (2005) have been reported high degree of significant variation cultivated and in the wild species of sesamum. The result of morphological evaluation of the characters showed significant genetic variation of different yield and yield contributing characters in the accessions indicating the scope and their warranty to use in the breeding programs.

Crop improvement is made through generating variability in desired traits followed by selection. Genetic dissimilarity was calculated as Euclidian distance to study genetic variability among varieties for morphological characters analysis and it was ranged from 0.10 to 0.99. Based on Euclidian distance, L1 (Bm1) was noted to be most distantly related with L15 (Bm4 × Bm3) and showed the highest dissimilarity value 0.99. On the other hand, lowest genetic dissimilarity relation could be noticed between L2 (Bm2) and L10 (Bm2 × Bm4); L2 (Bm2) and L14 (Bm4 × Bm2); L8 (Bm1 × Bm6) and L17 (Bm5 × Bm3); L9 (Bm2 × Bm1) and L15 (Bm4 × Bm3) and L13 (Bm4 × Bm1) and L19 (Bm6 × Bm2), all of them showed same value (0.10). Madina *et al.,* (2013) and Fikiru *et al.,* (2010) in lentil; Burlacu *et al.,* (2011) in *Brassica nupus and* Koffi *et al.* (2009) in bottle gourd, used Euclidian distance to construct dendrogram and reported similar result.

Cluster analysis indicated the extent of genetic diversity that is of practical use in plant breeding (Sultana *et al.,* 2006). Based on morphological dendrogram it was observed that the varieties were grouped in two clusters: cluster-I and cluster-II. The biggest group was cluster-II that includes sixteen lines which are grouped into two sub-clusters, while cluster-I

comprised only three lines were composed of L1 (Bm1), L9 (Bm2 × Bm1) and L11 (Bm2 × Bm6) where first two are closer than others. Cluster-II was grouped into two sub-clusters; seven lentil lines are consist on sub-cluster-I in Cluster-II. L2 (Bm2) and L13 (Bm4 × Bm1) was grouped in sub-cluster-I of cluster-II and all of them Bm5 and Bm6 are very close. L4 (Bm4), L7 (Bm4 × Bm1) and L10 (Bm2 × Bm4) also presence in grouped in sub-cluster-I of cluster-II. In sub-cluster-II of cluster-II was consist rest of the lines which was divided two more sub-groups. Roy *et al.,* (2013) also used UPGMA dendrogram for measuring genetic diversity and reported six clusters among 110 lentil accessions. Fikiru *et al.,* (2010) found two major and four minor cluster from UPGMA dendrogram which was constructed based on genetic dissimilarity in lentil. Koffi *et al.,* (2009) also performed cluster analysis of morphological variation and reported two groups among 18 accessions of *Lagenaria siceraria.*

BIOCHEMICAL MARKERS ANALYSIS

Biochemical markers are useful for assessment and study of genetic diversity of organisms. Several studies suggested that the application of numerical analysis, coupled with the utilization of protein patterns provides an effective approach for the investigation of taxonomic relationships among crop species (Karihaloo *et al.,* 2002; Lioli *et al.,* 2005; Yuzbasioglu *et al.,* 2008). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of seed storage protein is a successful way of revealing genetic diversity and relationship among different taxa (Nisar *et al.,* 2007). We have used total protein markers for assessment of genetic diversity among six varieties and thirteen F_2 cross lines of lentil. Genetic diversity in different plant species have been carried out by using electrophoretic patterns of total seed proteins as revealed by SDS-PAGE of seed storage protein (Ladizinsky and Hymowitz, 1979a; Potokina *et al.,* 2000; Ghafoor and Arshad, 2008; Ayten *et al.,* 2009). Seed proteins have been successfully used to study the variation of seed storage protein in lentil (Sultana *et al.,* 2006 and Yuzbasioglu *et al.,* 2008). Some taxa of the genus Trifolium has been elaborated through seed storage protein using SDS-PAGE (Badr, 2000; Nikolic *et al.,* 2010). Similarly, it has been reported to study 65 germplams of muskmelon (*C. melo*) by protein profiling on SDS-PAGE (Chaudhary and Ram, 2000). To find out inter-varietal correlation between

varieties, several earlier workers *e.g*., Jha and Ohri (1996), Ladizinsky (1979b) made protein profiling study through SDS-PAGE and find almost same observations. Although the present study is one of the basic and reliable methods to find out the genetic diversity and study phylogenetic relationship among selected nineteen lentil lines by total protein analysis using SDS-PAGE.

When bands of all lines were compared, we obtained a total of twenty seven bands. Out of them nineteen were polymorphic with 70.37% polymorphism. The relative mobility of seed storage protein was ranging from 0.10 to 0.85. The highest polymorphic bands were recorded in L17 (Bm5 × Bm3) and number of polymorphic band eleven and the lowest polymorphic bands were recorded in L1 (Bm1) and L12 (Bm3 × Bm4), both of them have no polymorphic band. Nine polymorphic bands were found in L10 (Bm2 × Bm4). L4 (Bm4), L5 (Bm5) and L6 (Bm6) showed twenty one monomorphic bands and six polymorphic bands which were at positioning almost same**.** Other five lines showing same monomorphic and polymorphic in number, which was twenty two bands and five bands, respectively, but positions were different and the lines are L3 (Bm3), L7 (Bm1 × Bm4), L8 (Bm1 × Bm6), L13 (Bm4 × Bm1) and L15 (Bm4 × Bm3). L18 (Bm6 × Bm1) shown only one polymorphic band in 0.38 position. The bands of seed storage proteins were detected at approximately molecular weight ranging between 6.5 and 66 KDa and divided into six regions with intervals of protein molecular marker. In albumin protein region had seven bands of more than 66 KDa of molecular weight, of which four were polymorphic Region II was for ovalbumin protein, ranged from 45 KDa to 66 KDa, which four were polymorphic out of five. From 29 KDa to 45 KDa for Carbonic anhydrase protein, with four protein subunits, of which three were polymorphic. Region-IV was trypsin inhibitor, ranging from 20.1 KDa to 29 KDa, had four protein bands, of which three were polymorphic. Region V was for lysozyme ranged for from 14.4 to 20.1 KDa, had three bands and all were polymorphic. In Aportinin region, molecular weight ranging from 6.5 KDa to 14.4 KDa, had four protein bands where, two bands were polymorphic and two monomorphic. From six type of seed storage protein, albumin protein was abundant in quantity in all the varieties and as well as all the varieties were polymorphic for lysozyme protein. Yatung *et al.,* (2014) reported total 92

bands from 30 genotypes of chilli. Uddin (2014) found total 17 bands where eight were polymorphic in total protein analysis of ten bottle gourd cultivars and relative mobility of leaf sample protein was ranging from 0.01 to 0.94. Madina *et al.,* (2013) showed 57.12% polymorphism obtained 12 polymorphic bands out of 21 bands in lentil with ranging from 0.02 to 0.92 relative mobility. Sheikh *et al,.* (2011) found total 46 bands and out of them 44 were polymorphic with 95.6% polymorphism in lentil. Bhat and Kudesia (2011), found 100% polymorphism in different five species of Solanaceae. This little change may be due to crop change. Galani *et al*., (2010) reported 100% polymorphism in *Oryza sativa* L. Yüzbaşioğlu *et al.,* (2008) got 24 bands in lentil, in which only five bands were polymorphic with molecular masses ranging from 35 to 116 kDa. Sultana and Ghafoor (2008) were recorded 55 protein bands in lentil ranging from the molecular mass of 14–66 kDa. Out of them, 13 bands were polymorphic in nature. However they concluded that SDS–PAGE alone did not exhibit high level of intra-specific variation.

Jaccard's similarity coefficient based on banding pattern of total protein among six varieties and thirteen F_2 cross lines of lentil were ranged from 0.48 to 1.00. L1 (Bm1) was noted to be closely related with L12 (Bm3 × Bm4) with the highest similarity coefficient as 1.00 and the same time L7 (Bm1 × Bm4) vs. L17 (Bm5 × Bm3) and L13 (Bm4× Bm1) vs. L17 (Bm5 × Bm3) showed lowest similarity coefficient 0.48 that means this is the highest distant relation. The result was supported by Uddin (2014), where ranged from 0.706 to 1.00 for ten cultivars of bottle gourd and Madina *et al.,* (2013) found 0.57 to 0.90 in six lentils varieties.

The result clearly illustrated that six varieties and thirteen F_2 crosses of lentil discriminated into two main groups on the basis of their protein bands. Cluster-II belong only L17 (Bm5 × Bm3) and Remaining eighteen lines are comprised Cluster-I. Cluster-1 was further divided into two sub-clusters, where sub-cluster-I comprised eleven lentil lines indicated their relatedness by comprising. In cluster analysis, most closely relationship has been seen between L1 (Bm1) and L12 (Bm3 × Bm4) as seen in similarity coefficient analysis. But, the highest genetic variation has been found between. Second sub-cluster (Sub-cluster-II) comprised seven lentil line and Sub-cluster-II further subdivided into two

groups. Dendrogram also showed the highest genetic distance between L13 (Bm4 × Bm1) and L17 (Bm5 × Bm3) and L7 (Bm1 × Bm4) vs. L17 (Bm5 × Bm3), because of their position in different cluster and low similarity coefficient. However, it might be useful to distinguish diverse forms of lentil from one another. The findings indicated that for the discrimination of the lentil genotypes SDS-PAGE of seed proteins supplied additional banding patterns, however; the differentiations were not sufficient in distinguishing among the genotypes. Srivastava *et al., (*2014) and Uddin (2014) reported dendrogram that grouped ten bottle gourd genotypes into two groups based on leaves and fruit storage protein.

Cluster analysis was finally confirmed by principal component analysis (PCA). The first five principal components from PCA accounted for 94.085% of the total variation among six varieties and thirteen F_2 crosses. The proportions of the principal components one (PCI), two (PC2) and three (PC3) were 79.96%, 4.97% and 3.552%, respectively. Three dimensional plots were prepared by using first three PCs, respectively. The three dimensional plots supported the cluster analysis. The 2D and 3D distribution based on first three PCs plotted nineteen lines into two main groups *viz.* group-1 and group-2 same as cluster analysis. Group-1 composed of eighteen lines except L17 (belonging in group-2). According to PCA analysis, close relatedness was found among cultivars L1, L12 and L18 are most distantly related cultivars. Hojjat and Galstayan (2011), Siahsar *et al.,* (2010), Toklu *et al.,* (2009), Jeena and Singh (2002), Liu *et al.,* (2008) reported genetic diversity based on dendrogram and PCA in lentil. Similar result has been found in cluster analysis. The highest genetic distance was found L17 from the others. The results were in partial agreement with the findings of Iqbal *et al.,* (2014) in *Trifolium repens* and Madina *et al.,* (2013) in lentil.

During present proteomic assays low level of diversity was observed for each locus in lentil lines. SDS-PAGE of seed protein profiles showed that each cluster had slight discriminative protein banding. According to the SDS-PAGE results, it can be suggested that genetic variation within lentil lines show narrow genetic base. But significant genetic diversity among the six varieties and thirteen F_2 crosses of lentil on morphological analysis.

It is observed that the biochemical marker is more precise because the varieties are not influenced by the environmental conditions. So this study demonstrated that determining of genetic variability among six lentil varieties and thirteen F_2 crosses, the biochemical marker technique is more precise than the morphological markers.

Therefore, these primitive cultivated forms can be used in lentil breeding programs to broaden the narrow genetic base of existing varieties as an assurance against unpredicted biotic and abiotic threats. In our investigation of lentil, both morphological and biochemical marker analysis suggested that L17 (Bm5 × Bm3) showed the highest genetic diversity among nineteen lines lentil and therefore, can be used for further breeding program.

CONCLUSION

The present investigation was carried out for the identification of genetic diversity of six varieties and thirteen F_2 Cross lines (*Lens culinaris* Medic.) through morphological markers and seed storage protein profiling. In morphological characters, Analysis of variance of nineteen lentil lines have been done according to standard biometrical approach and varieties were found to be significant for all the characters which indicating that the lines are significantly different from each other. Based on Euclidian distance, L1 (Bm1) was noted to be closely related with L15 (Bm4 × Bm3) and showed the highest dissimilarity value 0.99. Dendrogram was constructed based on the dissimilarity values of eleven characters and the lines were grouped in two clusters. The results indicate that low or high genetic distance exists respectively between varieties with similar or different origins.

In this study, protein profiling was used as a one of the basic and reliable methods to detect inter-varietal genetic diversity and measured phylogenetic relationship among six varieties and thirteen F_2 cross lines of lentil. When bands of all lines were compared, we obtained 70.37% polymorphism. The highest polymorphic bands were recorded in L17 (Bm5 × Bm3) and number of polymorphic band eleven and the lowest polymorphic bands were recorded in L1 (Bm1) and L12 (Bm3 × Bm4). From six type of seed storage protein, albumin protein was abundant in quantity in all the varieties and as well as all the varieties were polymorphic for lysozyme protein.

Genetic similarity was analyzed based on Jaccard's similarity coefficient and it was ranged from 0.48 to 1.00. L1 (Bm1) was noted to be closely related with L12 (Bm3 × Bm4) with the highest similarity coefficient as 1.00. Cluster analysis of data placed s six varieties and thirteen F_2 crosses of lentil into two main clusters. Second cluster (Cluster-II) belong only L17 (Bm5 × Bm3) and first cluster comprised Remaining eighteen lines. In cluster analysis, most closely relationship has been seen between L1 (BARI Masur-1) and L12 (Bm3 × Bm4) as seen in similarity coefficient analysis. Dendrogram also showed the highest genetic distance between L13 (Bm4× Bm1) and L17 (Bm5 × Bm3) and L7 (Bm1 ×

Bm4) vs. L17 (Bm5 × Bm3), because of their position in different cluster and low similarity coefficient.

Finally to confirm the result of dendrogram by Principal component analysis, the first three principal components from PCA accounted for 88.48% of the total variation among lines. In this case too, the 2D and 3D distribution of PCA supported the result of dendrogram *i.e.* the highest genetic distance was found between L13 and L17 and close relatedness was found among cultivars L1, L12 and L18 are most distantly related cultivars.

From the result it was concluded that significant genetic variability was found among the varieties on both morphological and biochemical analysis, and this study demonstrated that determining of genetic variability the biochemical marker technique is more precise than the morphological markers because the SDS-PAGE results are not influenced by the environmental conditions. In our investigation of lentil, both morphological and biochemical marker analysis suggested that L17 (Bm5 × Bm3) showed the highest genetic diversity among nineteen lines lentil and therefore, can be used for further breeding program.

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