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Genetic control of the Growth of Root Nodules and their Relation with yield in Black Gram (Vigna mungo L. Hepper)

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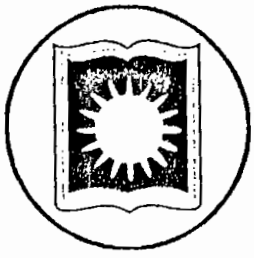

A
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Submitted By

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B. Sc. (Honours in Biochemistry)
M. Sc. (Genetics & Breeding)
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BIOMETRICAL GENETICS LABORATORY
DEPARTMENT OF GENETICS & BREEDING
UNIVERSITY OF RAJSHAHI

DEDICATED TO THE MEMORY OF
MY FATHER AND LATE MOTHER

CERTIFICATE

This is to certify that the research work embodied here for the thesis entitled " **Genetic control of the Growth of Root Nodules and their Relation with yield in Black Gram (*Vigna mungo* L. Hepper)** " has been carried out by Md. Osman Goni under our supervision.

It is further certified that the work presented here is original and suitable for submission in partial fulfillment for the degree of Master of philosophy, in the Department of Genetics and Breeding, University of Rajshahi.

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DECLARATION

I hereby declare that the thesis entitled, "**Genetic control of the Growth of Root Nodules and their Relation with yield in Black Gram (*Vigna mungo* L. Hepper)**" has been prepared by myself and the work presented here are of my own.

I further, declare that the work has not been submitted any where for any academic degree.

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ABSTRACT

For the ease of study the present work was carried out in three separate sections.

Section I: It contains the analysis of variance, components of variation, heritability and genetic advance for nine lines in black gram. The twelve quantitative characters such as, date of first flower (DFF), date of maximum flower (DMF), plant height at first flower (PHFF), plant height at maximum flower (PMFF), plant weight at harvest (PWtH), dry plant weight (DPWt), number of false pod per plant (NFPdPP), number of pod per plant (NPdPP), pod weight per plant (PdWtPP), number of seeds per plant (NSPP), seed weight per plant (SWtPP) and 100-seed weight (100-SWt). The collected lines were distinctly different from each other for these characters that justifies their inclusion in the present investigation.

The highest phenotypic variation and co-efficient of variability were found for NSPP. The genotypic variation and co-efficient of variability were observed for D50%F and PHFF respectively. The highest heritability and genetic advance as a percentage of mean (GA%) with a value of 42.717 and 16.0939 respectively, were recorded for PHFF. High error component of variation causes a low estimation of heritability. Low heritability as well as low values of genetic advance and genetic advance as a percentage of mean were noted for NSPP.

Section II: It deals with the analysis of variance with factorial analysis and correlation co-efficient of root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight (FPdWt), dry pod weight (DPdWt), number of seeds per plant (NSPP), yield per plant (YPP) and 100-seed weight (100-SWt).

Analysis of variance indicated that all lines were significant. Factorial analysis indicated that the individual effect of fertilizers is more important for root and shoot characters than yield, while yield itself showed increase response with combined fertilizer dose instead of single dose particularly nitrogen. However, all the root and shoot characters and yield per

plant except NPdPP and 100-SWt showed the importance of combined dose ie. NPK in black gram.

Correlation study indicated that RL showed positive significant correlation with NPdPP and FPdWt. SL exhibited positive significant correlation with FPWt, FRWt and NN. The correlation of FPWt was found to be positively significant with FRWt, NN, RV and NWt. FRWt showed positively significant correlation with RV. A significant positive correlation was exhibited by NN with NWt, NPdPP, YPP and 100-SWt. The correlation co-efficient of RV was found to be positively significant with NPdPP and FPdWt. NWt showed positively significant correlation with NPdPP, FPdWt, YPP and 100-SWt and negatively significant correlation with NSPP.

Section III: Factorial analysis and analyses of variance, variability, heritability, genetic advance and genetic advance as a percentage of mean for the six characters like moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C of root nodules were done in this section. In the analysis of variance for the chemical characters of root nodules the lines (L) were found to be significant in different from each other and dose (D) effects were not found at each stage (S), except reducing sugar for dose and free sugar and reducing sugar for stage. Application of the individual dose, N, K, P and combined dose, NP, PK, NK and NPK have no effect in most of the stages for all the characters. Protein showed the highest PCV and GCV among these chemical characters. All the chemical characters, except moisture and dry-matter for root nodules exhibited high heritability and genetic advance as a percentage of mean. In the calculation of molecular weight of protein and its bands it was found that lines L_{20}, L_{11}, L_{14} and L_{15} contained mostly similar types of protein in seeds and root nodules. While, similarity in regard of molecular weight of protein and its band for nodule and seeds was found in least number of cases in L_{18}. Regarding the 18 amino acids detected, in maximum two cases all the nine lines were found to be different. However, glycine, methionine, leucine, phenylalanine, lysine and arginine were common for root nodules in all the lines. The concentration of bacterial colonies all the nine lines were different. The highest concentration was found for L_{20} followed by L_{18}, L_{15} and L_{19}. With these results lines L_{20} and L_{15} may be considered for further breeding research for high yield in black gram.

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GENERAL INTRODUCTION

Food materials are the most essential commodities for the survival of human being. The essential food materials are protein, carbohydrate, fat, minerals, vitamins and water. Man is absolutely dependent on plants for food materials. The things, which he gets, are either plant materials or those derived directly or indirectly from plants. Man has been attempting since pre-historic time to improve the plants to maintain his livelihood. So, where there is human being there is plant. Man can not survive just a moment without oxygen, which comes from plants. For the survival of organism energy is necessary. This energy is trapped from the radiant energy by green plants and transferred into the chemical energy and is stored in various reserved food materials.

Black gram localy known as Mashkalai (*Vigna mungo* (L.) Hepper) belongs to the family leguminosae and sub-family Papilionaceae. Black gram is of very ancient origin. Blackgram is major crop of Chapai Nawabgonj and Rajshahi District. It is also cultivated as an important Rabi crop in Faridpur and Pabna and also in Barisal, Khushtia and Noakhali. For many centuries blackgram has been cultivated all over the world, mainly in southwestern Asia, Egypt, Europe, India, Pakistan, Bangladesh, Nepal and China as a winter crops.

With the increasing cropping intensity in the country the grain legumes should get first priority. The cropped area and production of these pulses have been on the verge of decline over the past few years mainly, because of the increased emphasis on HYV of rice and wheat. But pulses are very important because of its protein supply to the human diet and nutrition to the soil.

Nine lines of black gram were tested with three fertilizers namely, nitrogen (N), potassium (K) and phosphorus (P) mixed up to prepare eight different doses were considered as environment. Factorial analysis was followed to see the effect of N, P, and K.

Root nodule contains red pigment that is remarkable and similar in properties to the hemoglobin of red blood cells. The red pigment of the nodules is appropriately called leghemoglobin and appears to be a product of the rhizobium-legume complex. Since the pigment is not present in either organism grown alone (Allen and Allen, 1958) nodules that lack leghemoglobin are usable to fix nitrogen. Also numerous investigations (Virtanen and Miettinen, 1963) have shown a correlation between leghemoglobin concentration and rate of nitrogen fixation, which leads to us to the conclusion that leghemoglobin and symbiotic nitrogen fixation are intimately related. Leghemoglobin is an oxygen carrier, the oxygen is necessary for the electron transport chain of the rhizobium bacteriod. Because of its very high affinity for oxygen, leghemoglobin provides oxygen to the root nodule bacteria quickly, even at very low levels of free oxygen (Goodwin and Mercer, 1973). Observers also believe that the leghemoglobin keeps levels of molecular oxygen low in the bacteriod.

Protein are the chief constituents of all living matter. It is the essence of life processes, fundamental constituents of protoplasm and are involved in the hereditary transmission. Protein act as enzymatic catalysis, transport and storage, immune protection, as hormone regulator and co-ordinate motion. The amount of protein present in the root nodules of the different lines decreased with the changes of maturity

Extensive research efforts are necessary for the improvement of black gram in our country, it is known that a character i.e. its phenotype is the result of genotype and environmental interaction. So, a character is dependent on environment. Therefore, we need to measure the environmental effects on genotypes. For this reason the whole analysis of this work was done following genotype × environment interaction to select suitable genotype, which would be stable under different environments. Any development through breeding procedure depends upon the magnitude of genetic variability in the materials. All the agronomic and economic studied were analysed following biometrical technique based on mathematical models of Fisher *et al.* (1932) and as developed by Mather and also by Jinks (1971).

Most of the agronomical characters of black gram are quantitative in nature and show continuous variation. Several statistical methods have been developed for the study of inheritance of quantitative characters. Genetic information for planning in doing effective breeding programme in any crop is important. Quantitative characters are governed by a large number of genetic factors and are largely influenced by environment. Genotype-environment interaction is now recognized as an important source of phenotypic variation. Knowledge about the type of genotype-environment interaction involved in a population help the plant breeders to breed and to select better strains. Keeping this view in mind, the present investigation was undertaken to study the nature of variability and characters association to estimate magnitude of gene action and also to select the stable varieties with high yield potential. For the case of investigation the whole work under study was divided into the following section.

SECTION I: DIVERSITY STUDY OF THE AGRONOMICAL CHARACTERS

SECTION II: DIVERSITY ESTIMATE, FACTORIAL ANALYSIS AND CORRELATION STUDY OF ROOT NODULES, YIELD AND YIELD COMPONENTS

SECTION III: VARIABILITY STUDY OF THE BIOCHEMICAL CHARACTERISTICS OF ROOT NODULE AND ROOT NODULE BACTERIA

SECTION - I

DIVERSITY STUDY OF THE AGRONOMICAL CHARACTERS

INTRODUCTION

Black gram locally known as Mashkalai (*Vigna mungo* (L.) Hepper) belongs to the family leguminosae and sub-family Papilionaceae. Black grams are very ancient origin. Blackgram is major crop of Chapai Nawabgonj and Rajshahi District. It is also cultivated as an important Rabi crop in Faridpur and Pabna and also in Barisal, Khushtia and Noakhali. For many centuries black gram has been cultivated all over the world, mainly in southwestern Asia, Egypt, Europe, India, Pakistan, Bangladesh, Nepal and China as a winter crops. Recently, a number of strains of black gram are identified which can successfully be grown in the summer season by irrigation.

Black gram is one of the main edible pulse crops of Bangladesh. Black gram stands 4th in importance and rank second in respect of yield production and seed protein. It can successfully be grown without preparation of land, large labour and high capital. The seed of black gram known as dhal are one of the most highly nutritious food and is mostly low cost pulses of Bangladesh. It contains more protein than any other vegetables and is nearer to animal protein; carbohydrates and fat are also present. The dry seed contains about 9.7% water, 23.4% protein, 1% fat, 57.3% carbohydrates, 3.8% fibers and 4.5% ash (Purseglove, 1968).

Black gram pods are considered to be significant source of thiamin, riboflavin and niacin. Other vitamins of the B-complex group as well as ascorbic acid, vitamin k and tocoferols are also present. Calcium, Phosphorus, Iron and other minerals are also present in black gram. The caloric value of black gram is same as that of rice (Anon, 1966).

Black gram plays an important role in providing valuable fodder and food stuff to the cattle and poultry. All parts of black gram plants are rich in nitrogenous material and the vegetative parts provide good animal fodder. Young plants and its dry stem and husks are good sources of animal food (Rahman and Parth, 1988).

Black gram as a tropical crop, it tolerates high temperatures. It is cultivated mainly Rabi seasons and sometimes in kharif season also for greening manning of this soil. Recently

it has been cultivated in summer season in India. It is a short day plant, but day-neutral cultivars are available for cultivation in the long days of summer. The optimum temperature for better growth ranges between 25 – 35^{0}C, but it can tolerate up to 42^{0}C. Black gram increases soil fertility which is indirectly correlated with the presence of nodules on its roots, containing nitrogen fixing bacteria (*Rhizobia* sp.) living symbiotic association with the plant. Such an important crop like black gram is much neglected and little or no works has been done for the improvement of this crop in early days in our country. Whereas, it has been cultivated extensively in North and North-west zone, specially in Chapai Nawabgonj in Rajshahi division and it is a popular diet of this area. At present, some work to develop advanced lines of pulses has concentrated at the head quarter of Bangladesh pulse research institutes at Ishurdi in Pabna.

Pulse is the cheapest source of protein, which is 25% in lentil, 23% in black gram, 23.6% in mung bean, 28.2% in cowpea and 17.1% in chickpea etc (Rahman, 1981). This amount of protein is more than any other vegetables. Pulses are also play an important role in providing valuable fodder and food stuff to the cattle and poultry.

With the increasing cropping intensity in the country the grain legumes should get first priority. The cropped area and production of these pulses have been on the verge of decline over the past few years mainly, because of the increased emphasis on HYV of rice and wheat. But pulses are very important because of its protein supply to the human diet and nutrition to the soil. Since, improved technology can increase per hectare yield of pulses substantially, pulse production is projected to grow to o.85 million tons (2001 - 2002) in the terminal year of the plan as against the bench mark production of 0.53 million ton (1996/97) (Table 1 and 2). (**Reference:** The fifth five-year plan, 1997 – 2002. (Planning Commission, Ministry of Planning, Dhaka, Bangladesh)

Table 1: Projection of important crop production during fifth plan. (Area in million hectare and production in million m. tons unless otherwise noted)

Crops	1996/97 (Bench mark)		2001/2002 (Production)	
	Area	Production	Area	Production
Rice	10.4	18.88	10.11	23.4
Wheat	0.71	1.45	0.7	1.6
Sub-Total	11.11	20.33	10.81	25.0
Other coares grain	0.1	0.1	0.12	0.12
Total food grain	11.21	20.43	10.93	25.12
Potato	0.15	1.85	0.16	2.43
Sweet potato	0.05	0.5	0.05	0.66
Oil seeds	0.5	0.37	0.7	0.76
Pulses	0.65	0.53	0.78	0.85
Spices	0.15	0.33	0.22	0.5
Vegetables	0.25	1.45	0.3	.82
Fruits	0.19	2.14	0.26	3.54
Jute (million bales)	0.51	4.87	0.57	70.24
Cotton (million bales)	0.04	0.1	0.11	0.26
Sugarcane	0.18	8.10	0.18	12.37
Tea (million kg)	0.05	54.0	0.05	60.0
Tobacco	0.03	0.04	0.03	0.04

In accordance to the availability of statistics, the total area, production and yield rate of different pulses of Bangladesh are presented in the following Table 2. (**Reference**: Monthly Statistical Bulletin, Bangladesh , August 2001.)

Table 2: Average (A) production (p) and yield rate [per acre yield (p.a.y)] of different pulses (Area in acres and production in metric tons.)

Pulses	1998-99 (A) (000) (P) (000mt) (p.a.y. mt).			1999-2000 (A)(000) (P)(000mt) (p.a.y mt)			2000-01 (A) (000) (P) (000mt) (p.a.y mt).		
Chola	41	12	0.30	41	12	0.29	-	-	-
Arahar	13	3	0.20	13	3	0.21	-	-	-
Mung	137	34	0.25	136	36	0.26	-	-	-
Mosur	508	165	0.33	412	128	0.31	406	126	0.31
Mashkalai	**71**	**20**	**0.28**	**71**	**21**	**0.30**	**67**	**20**	**0.24**
Khesheri	520	166	0.32	499	166	0.33	462	155	0.33
Motor	45	13	0.30	45	14	0.31	-	-	-
others	14	3	0.21	14	3	0.24	-	-	-

Under the family leguminosae with about 600 genera the sub-family Papilionaceae, have great economic agricultural potential as a source of protein and are second only to cereals. Now this sub-family has been converted into an independent family. Grain legumes (pulse) play an important role in meeting the quantitative and qualitative protein requirements of a large part of humanity, especially in the developing countries of Asia, Africa and Latin America. Pulse is known as the poor man's meat.

Protein is the chief ingredient of life. It is the main component of brain, blood, bone, muscles and skins. Hence the importance of protein in the nutrition needs no elaboration. Protein is lacking in the diet of almost all the people of Bangladesh. Therefore, it is obvious that most of the people of Bangladesh are deprived of protein, which is urgently necessary for the proper growth of the baby.

Besides protein, there are a large amount of calorie, iron and thiamin in pulse. Pulse also is a good source of vitamin B (except Riboflavin). Rice and many pulses have no vitamin C. But in some pulses after germination vitamin C is synthesized.

Pulses also contain fair amount of minerals. The nutrition value of different pulses and other proteinacious foods are shown in Table 3. (**Source:** Black gram cultivation in Bangladesh, BARI, Joydebpur, Gazipur 1701. Publication No.19, May, 1999.)

Table 3: Nutrition value of different pulses with other proteinacious food

Foods	Energy K cal	Protein (gm)	Fat (gm)	Carbohydrate (mg)	Calcium (mg)	Iron (mg)	Thiamine (mg)	Riboflavin (mg)	β-carotene
Mashkalai	347	24	1.4	59.6	154	9.1	0.42	0.37	38
Lentil	343	25.1	0.7	59	69	4.8	0.45	0.49	270
Mung	348	24.5	1.2	59.9	75	8.5	0.72	0.15	49
Chickpea	372	20.8	5.6	59.8	56	9.1	0.48	0.18	129
Khasheri	345	28.2	0.6	56.6	90	6.3	0.39	0.41	120
Rice	356	6.40	0.4	79	9	4.0	0.21	0.09	-
Soyabean	432	43.2	19.5	20.9	240	11.9	0.73	0.76	426
Arachies	567	25.3	40.9	26.1	90	2.8	0.45	0.13	37
hypogea	181	13.5	13.7	0.8	70	3.0	0.90	0.26	540
Egg	67	3.2	4.1	4.4	120	0.2	0.12	0.19	20

Extensive research efforts are necessary for the improvement of black gram in our country, it is known that a character i.e its phenotype is the result of genotype and environmental interaction. So, a character must also be dependent on environment. Therefore, we need to measure the environmental effects of genotypes. For this season the whole analysis of this work was done following genotype × environment interaction to select suitable genotype, which would be stable under different environments. Any development through breeding procedure depends upon the magnitude of genetic variability in the materials. Most agronomic and economic characters are done by following biometrical technique based on mathematical models of Fisher *et al.* (1932) and as developed by Mather and also by Jinks (1971).

Most of the agronomical characters of black gram are quantitative in nature and show continuous variation. Several statistical methods have been developed for the study of inheritance of quantitative characters. Genetic information for planning in doing effective breeding programme in any crop is important. Quantitative characters are governed by a large number of genetic factors and are largely influenced by environment.

Diversity estimate, factorial analysis and correlation study of root nodules, yield and yield components are now recognized as an important source of phenotypic variation. Knowledge about the type of genotype-environment interaction involved in a population help the plant breeders to breed and to select better strains. The present investigation deals with the phenotypic, genotypic and within error coefficient of variability, heritability (in broad sense), genetic advance of a few developmental characters viz. DFF, PHFF, D50%F, PHMF, PWtH, DPWt, NFPdPP, NPdPP, NSPP, SWtPP and 100-SWt for nine lines of black gram.

REVIEW OF LITERATURE

Literature regarding the genetic study of some of the developmental characters in black garm (*Vigna mungo* (L.) Hepper) are scanty. In fact, papers on black gram are few and scattered. A limited number of papers have been published dealing with the problem of genetic study, on various quantitative characters in different leguminous crop and other plants. Some of these papers are reviewed below.

Johannsen (1909) explained the relationship between heredity and environment for the first time. He proposed that the environment play a significant role in determining the life situation. An investigation with beans (*Phaseolus vulgaris* L.) he showed that the phenotype was the joint product of both heritable and non-heritable effects and the phenotypic variation in any pure line was due to environmental effect.

Fisher (1918) was the first to develop statistical method to partition variance of quantitative character in segregating population into genetic and environmental components.

Mather (1949), Mather and Jones (1958) and Stevens (1959) were separately and combindly developed the techniques to measure the genotype-environment interaction based on the mathematical model of Fisher *et al.* (1932). It involved the partitioning of the variation of quantitative data into genetic and environmental effects and their interactions. Here the degree of interaction was expressed as a linear function of the effect of environment.

Weber and Moorthy (1956) studied heritability in three hybrid progenies of soybean involving four parental varieties. Genotypic and environmental variance were estimated for seven characters.

Singh (1961) undertook an investigation to estimate the relative magnitude of genotype environment interactions for material representing two quite different levels of heterozygosity. It generated scope of the study of measurements of the major agronomic characters such as yield, plant height under length and ear length of inbred lines and their

top cross progenies to determine the relative importance of line differences environmental factors and interaction.

Swarup and Chaugle (1962) worked on genetic variability in a collection of seventy divergent varieties including indigenous as well as exotic types of sorghum. A wide range of phenotypic variability was observed in most of the characters. Studies on genetic co-efficient of variation, heritability and genetic gain in various characters revealed that a large portion of the phenotypic variability was genetic and highly heritable in almost all cases.

Chandra (1968) worked on variability in gram. The estimates of components of variation for ten yield characters showed that there were wide variations in the material for all the characters and that variability was affected by environment particularly for plant height and secondary branches per plants. On the whole, heritability (broad sense) values were high but heritability for number of pods per plant was low. High heritability and high genetic advance were associated in case of setting percentage, following duration, primary branches and number of pods per plant.

Singh and Dixit (1970) studied genetic variability showed positive genotypic and phenotypic correlation between yield and the number of primary or secondary branches of the six morphological characters studied. Plant height and number of secondary branches gave the highest heritability estimates with high genetic advance that indicated that selection for more seeds per pod, more pods per plant and more secondary branches could be fruitful.

Majid *et al.* (1982) studied 40 germplasm in blackgram, growing in a randomized complete block design. Date on 10 agronomic characters were taken viz., days of flowering, days to maturity, plant height, number of primary branches per plant, number of inflorescence per plant, number of pods / pod length, number of seed / pod, 500-seed weight and seed yield / plant. The phenotypic variance was found to be larger than the genotypic variance for all the characters.

Ashutosh *et al.* (1984) worked on genetic variability and interrelationship in blackgram (*phaseolus mungo* L.). Some genetic parameters and interrelationship were studied for seven characters of eleven photosynthetic pure lines of blackgram. They reported that high

heritability along with high genetic advance was observed for plant height and days to maturity. Two important yield contribution traits, such as pods per plant and 100-seed weight showed an appreciable percentage of heritability and genetic advance.

Sarker (1984) studied some genetic parameters and interrelationship for seven characters of eleven photo insensitive pure line of blackgram. He observed high heritability along with high genetic advance for plant height and days to maturity. He recorded an appreciable percentage of heritability and genetic advance of two important yield-contributing traits, such as pods per plant and 100-seed weight. These two characters also showed significant positive association with yield.

Debnath (1990) investigate that heritability is of great importance to the breeders since it indicates possibility and extent to which improvement is possible through selection. In the present study estimates of heritability both in narrow (h^2_n) and broad (h^2_b) senses were studied in a 10×10 diallel cross of maize inbreeds over two locations.

Khalaque *et al.* (1991) studied the variability and co-relation of some chemical characteristics in chilli (*C. annum* L.). They reported that most of the chemical characteristics and yield per plant showed high GCV. All the characters except yield per plant and protein in ripe chillis under study exhibited very high heritability estimates. It was also observed that variety × season (V × S) interactions effect were highly significant. Talukder and Haque (1992) in fiber crop studied the genetic variance for harvest index (HI) and plant height (PH). For HI the genotype × location × year interaction was significant at 1% level. The estimates of heritability (in broad sense) and genetic advance were greater in biomass yield. In seed crop, except seed yield / plant and branches / plant the genotypic variance for all the traits were significant at 5% and 1% level. Heritability for branches / plant and 1000-seed weight was 2.74 and 3.15 times greater, respectively than that of seed yield / plant.

Subramani *et al.* (1997) made an investigation deals with the influence of some heavy metals (chromium, cadmium and mercury) on germination and seedling growth of blackgram [*Vigna mungo* (L.) Hepper]. The various concentrations of the heavy metal solutions were prepared and various morphometrical parameters were recorded. They

observed that growth parameters showed a gradual decline with increase in the concentrations of heavy metals.

Kannabiran *et al.* (1998) carried out a study in field under natural environmental conditions to find the effect of two concentrations (50% and 100%) of domestic sewage in the growth, biochemistry and yield of the crop plant *Vigna mungo* (L.) Hepper. The sewage used for their study showed an enhanced effect on vegetative growth at 100% concentration, whereas 50% concentration was found to inhibit vegetative growth with simultaneous increase in the yield of seeds. They suggested that 50% concentration of sewage was ideal for better yield of *Vigna mungo* (L.) Hepper.

Akanda *et al.* (1998) studied genetic variability, correlation and path co- efficient for grain yield and its component traits with 19 composites of maize where comparatively high genotype co- efficient of variation was recorded for grain yield per plant, ear height, 1000 grins weight, plant weight, ear length and days to silk. They also showed that high heritability coupled with high genetic advance in percentage of mean. Grain yield was significantly and positively associated with days to silk and maturity, plants and ear height, ear length and girth, 1000- grain weight and grains.

Choudhury (1999) studied the gene effect on seven characters viz. days of flowering, pod length, pod breadth, pod weight, seeds per pod, pod per plant and pod yield per plant in lablab bean using generation means through scaling test. Scaling test suggested that a simple genetic system, preponderantly of additive gene effects. Significant non-allelic interactions were both additive and non-additive type. He also estimates high heritability and genetic advance for most the traits including days to flowering and pod yield per plant. He suggested from this result that selection for these traits would be effective.

Amanullah and Hatam (2000) studied an experiment consisting of 11 black bean (*Vigna mungo* (L.) Hepper) germplasm, to know the yield potential of this germplasm in relation to other important agronomic characters. They observed that germplasm BB-14 ranked first by producing maximum grain yield of 844 kg/ha, followed by germplasm BB-7 (755 kg/ha). They also reported, average values of grain yield decreased in descending order from 799 kg/ha in group I to 375 kg/ha in group II and then further decreased to 243 kg/ha in group III. Similarly, the average values of days to maturity, plant height, branches and

pod per plant, 100 seeds weight, dry matter yield and harvest index decreased in descending order, and showed positive association with grain yield.

Isaacs *et al.* (2000) studied thirty-two blackgram genotypes of diverse origin for phenotypic and genotypic coefficient of variability, heritability and genetic advance. He noted sufficient values associated with high genetic advance for single plant yield, number of pod per plant and other yield-attributing traits. High heritability with medium genetic advance was obtained for 100-seed weight. They reported that yield improvement in blackgram would be achieved through selection for the above characters.

Loganathan *et al.* (2001) studied the genetic parameters of yield and related components for some metric characters from the data of a diallel cross involving seven diverse parents of green gram (*Vigan radiata* L.). Their analysis revealed the presence of both additive and non additive gene effect. They also reported that the over dominance is involved for days to first flower, plant height, number of branches per plant, number of cluster per plant, no of pods per plant, pod length and seed yield per plant. They recorded partial dominance for 100-seed weight. The preponderance of dominant gene was obsessed for expression of plant height and number of pods per plant. They also reported moderate to high magnitude of heritability for all the characters except for plant height, number of cluster per plant and seed yield per plant.

Gayen *et al.* (2002) studied the genetic variability and analysis of yield components in mung bean. He observed that high heritability and high or moderate genetic advance for all the characters that he studied except number of seed per pod. Seed yield was significantly and positively related with clusters per plant, number of pods per plant and pod length. Cluster per plant and number of pods per plant had high positive and significant association between them. Path coefficient analysis indicated that number of pods per plant, 100-seed weight and shelling percentage registered high positive and direct effect on yield. In direct effects of clusters per plant via pods per plant and pod length via 100-seed weight were high and positive. He reported from his study that the cluster per plant, pods per plant, pod length, 100-seed weight and shelling percentage are important for effective selection in mungbean.

MATERIALS AND METHODS

A. MATERIALS:

Materials used in the present study comprised of nine lines of black gram [*Vigna mungo* (L.) Hepper]. The materials (seeds of lines) were supplied from the Biometrical Genetics laboratory, Department of Genetics and Breeding, Rajshahi University, Rajshahi. The nine blackgram lines for this study are as follows:

SL.No.	No./Line	SL.No.	No./Line	SL.No.	No /Line
1	L_2	4	L_{14}	7	L_{18}
2	L_{11}	5	L_{15}	8	L_{19}
3	L_{13}	6	L_{16}	9	L_{20}

Twelve quantitative characters of blackgram were studied for the investigation of genotype-environment interaction. The characters were date of First flower (DFF), date of maximum flower (DMF), plant height at first flower (PHFF), plant height at maximum flower (PMFF), plant weight at harvest (PWtH), dry plant weight (DPWt), number of false pod per plant (NFPdPP), number of pod per plant (NPdPP), pod weight per plant (PdWtPP), number of seeds per plant (NSPP), seed weight per plant (SWtPP) and 100-seed weight (100-SWt).

The nine lines for the study of above twelve characters were put in trails with three replications in three years viz.1999, 2000 and 2001.

The experiment and analyses of data were divided into the following sub heads.

1. Collection of the experimental seeds
2. Size of experimental field
3. Preparation of the experimental field
4. Sowing of seeds and raising of seedlings
5. Maintenance of the experimental field
6. Collection of data

B. METHODS:

1.Technique of analysis of data

1. Collection of the experimental seeds: At starting of this study in 1999, the seeds of nine blackgram lines were taken from the Biometrical Genetics Laboratory, Department of Genetics and Breeding, University of Rajshahi, Rajshahi

2. Size of the experimental field: The design for this experiment was completely randomized block design. The experimental field composed an area of 450 × 740 square cm in three replications. The three replications represented nine plots. A replication was with a size of 120 × 60 cm. Each plot consisted of three rows and each row contains seven hills. Every plot was 120 cm in length and 60 cm in breath. The gap between replications were 45 cm. The gap between plots were 25 cm and between rows 30 cm and between hills were 20 cm.

3. Preparation of the experimental field: The surface soil of the field was well pulverized by ploughing before sowing of seeds. Preparation of the experimental field was ready for sowing of seeds.

4. Sowing of seeds and raising of seedlings: The seeds of nine blackgram lines were sown by randomly assigning to each plot. Each plot had one line. Three rows with 21 hills were sown randomly in each of the hills.

5. Maintenance of the experimental field: Regular weeding was done in the experimental plot. When seedlings were 7-8 cm in height, only 3 seedlings nearly in equal distance in each plot were kept and excess seedlings were removed from the experimental field.

6. Collection of data: The twelve characters measured for analysis were from taking 15 plots selected at random from each of the plots and lines in each of the treatments.

Data on different quantitative characters were collected on individual plant basis from nine lines. Data were measured and recorded from the following characters. All the measurements were done in C.G.S system.

1. Technique of analysis of data: The collected data were analysed following biometrical technique developed by Mather (1949) based on the mathematical methods

of Fisher *et al.* (1932). The technique of analysis that have been used are described in following sub-heads:

a) Sum total of three replication: Data on individual plant basis were added together to obtain subtotal.

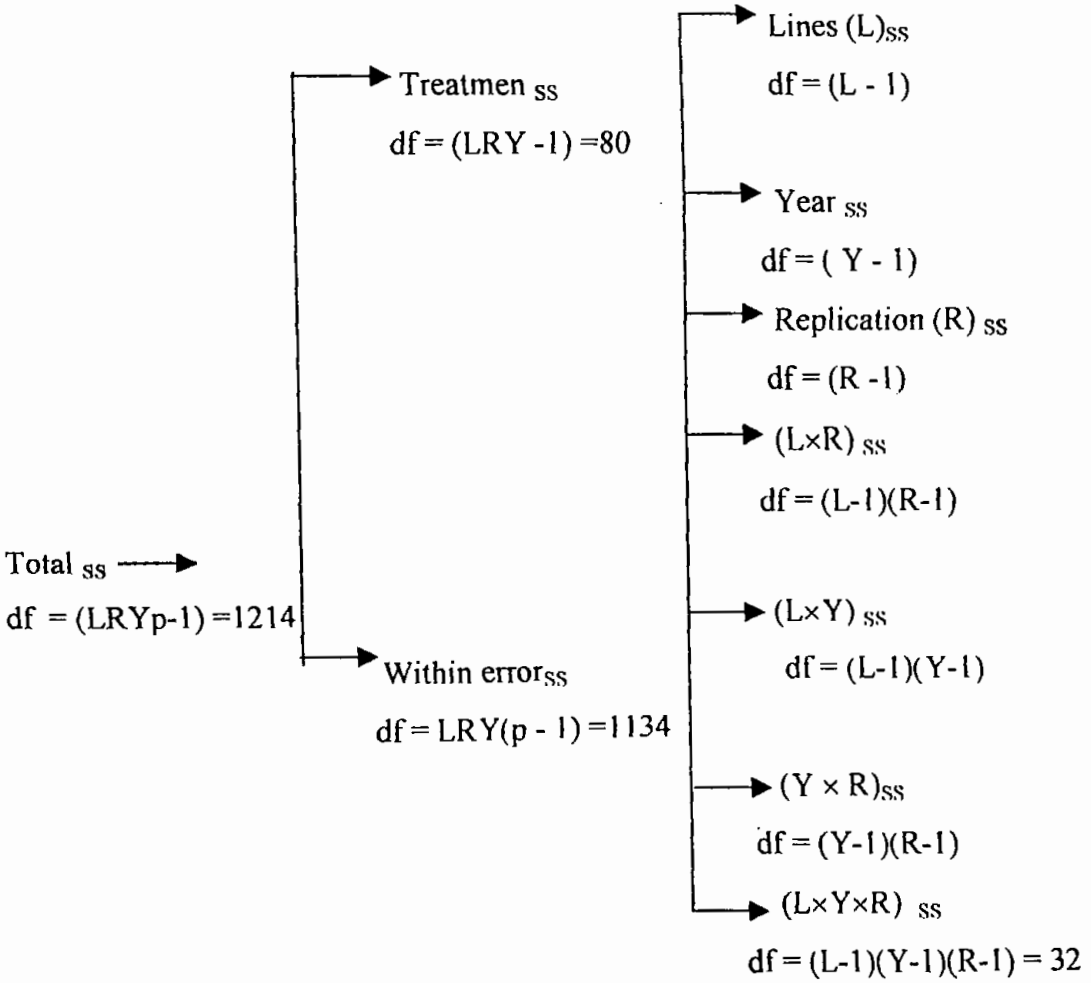
Total of 3 replication = $\sum x_i$

Where, Xi = The individual reading recorded from each plant.

$\sum$ = Summation.

b) 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 population. Variance for each of the characters was carried out separately on mean value of is plants.

The variance due to different sources such as lines (L), replication (R), years (Y), interactions (L × R), (R × Y), (L × Y), (L × R × Y) and within error in this present study were analysed according to the following skeleton.



Where,

Total SS = $\sum (PL_i R_j Y_k)^2 - CF$

Treatment SS = $\frac{\sum_{ijk} (L_i R_j Y_k)^2}{p} - CF$

Error SS = Total SS – Treatments SS

Line $_{SS}$ = $\frac{\sum_{i} L_i^2}{pRY} - CF$

Replication $_{SS}$ = $\frac{\sum_{j} R_j^2}{pLY} - CF$

Year $_{SS}$ = $\frac{\sum_{k} Y_k^2}{pLR} - CF$

L × R) $_{SS}$ = $\frac{\sum_{ij} (L_i R_j)^2}{pY} - CF - L_{SS} - R_{SS}$

(L × Y) $_{SS}$ = $\frac{\sum_{ik} (L_i Y_k)^2}{pR} - CF - L_{SS} - Y_{SS}$

(R × Y) $_{SS}$ = $\frac{\sum_{jk} (R_j Y_k)^2}{pL} - CF - R_{SS} - Y_{SS}$

(L×R×Y) $_{SS}$ = $\frac{\sum_{ijk} (L_i R_j Y_k)^2}{p} - CF - L_{SS} - R_{SS} - Y_{SS} - (L \times R)_{SS} - (L \times Y)_{SS} - (R \times Y)_{SS}$

L_i = The value of ith lines

R_j = The value of jth replication

Y_k = The value of ith year.

L_i R_j = The value of ith lines in jth replication

R_j Y_k = The value of jth replication in kth year

$L_i Y_k$ = The value of ith lines in kth year

L_i R_j Y_k = The value of ith lines in jth replication of kth year

$$CF = \text{Correction factor} = \frac{GT^2}{N}$$

GT = Grand total

N = Total number of observation = PLRY

The analysis of variance of a mixed model was uses, where line (L), replication (R) and year (Y) are fixed. The expectation in the analysis are shown in following Table 4.

Table 4: The expectation of mean square (EMS) used in the analysis of variance.

Items	d.f	MS	EMS
Line (L)	(L–1)	MS_1	$\sigma_w^2 + P\sigma^2_{LRY} + PR\sigma^2_{LY} + PY\sigma^2_{LR} + PRY\sigma^2_{L}$
Replication (R)	(R–1)	MS_2	$\sigma_w^2 + PL\sigma^2_{RY} + PLY\sigma^2_{R}$
Year (Y)	(Y–1)	MS_3	$\sigma_w^2 + PL\sigma^2_{RY} + PLR\sigma^2_{Y}$
L × R	(L–1) (R–1)	MS_4	$\sigma_w^2 + P\sigma^2_{LRY} + PY\sigma^2_{LR}$
L × Y	(L–1) (Y–1	MS_5	$\sigma_w^2 + P\sigma^2_{LRY} + PR\sigma^2_{LY}$
Y × R	(L–1) (R–1)	MS_6	$\sigma_w^2 + PL\sigma^2_{RY}$
L × R × Y	(L–1) (R–1) (Y–1)	MS_7	$\sigma_w^2 + P\sigma^2_{LRY}$
Error	(P–1) LRY	MS_8	σ_w^2

Where, L, R and Y represent the numbers of lines, replications, and years respectively

MS_1 = Mean square of line.

MS_2 = Mean square of replication

MS_3 = Mean square of year

MS_4 = Mean square of L × R

MS_5 = Mean square of L × Y

MS_6 = Mean square of R × Y

MS_7 = Mean square of L × R × Y

MS_8 = Mean square of within error

and, $PLRY\sigma^2_L$ = Variance due to lines

$PRY\sigma^2_R$ = Variance due to replications

$PLY\sigma^2_Y$ = Variance due to years

$P\,Y\sigma^2_{LR}$ = Variance due to L × R

$PR\sigma^2_{LY}$ = Variance due to L × Y

$PL\sigma^2_{RY}$ = Variance due to R × Y

$P\sigma^2_{LRY}$ = Variance due to L × R × Y

σ_w^2 = Variance due to within error.

c) Components of variation:

The components of variation were phenotypic (σ_p^2), genotypic (σ_g^2), Line × replication (L × R), line × year (L × R) and line × replication × year (L × R × Y) variance. These are calculated as follows.

Step 1:

$$\sigma_g^2 = \frac{MS_1 - MS_4 + MS_7 + MS_5}{pYR}$$

$$\sigma^2_{L\times R} = \frac{MS_4 - MS_7}{pY}$$

$$\sigma^2_{L\times Y} = \frac{MS_5 - MS_7}{pR}$$

$$\sigma^2_{R\times Y} = \frac{MS_6 - MS_8}{pL}$$

$$\sigma^2_{L\times R\times Y} = \frac{MS_7 - MS_8}{p}$$

$$\sigma_w^2 = MS_8$$

Step 2: Phenotypic variance: $\sigma_p^2 = \sigma_g^2 + \sigma^2_{L\times R} + \sigma^2_{L\times Y} + \sigma^2_{L\times R\times Y} + \sigma_w^2$

Genotypic variance, $\sigma_g^2 = \sigma_i^2$

Interaction of line × replication (L × R) variance = $\sigma^2_{L\times R}$

Interaction of line × year (L × Y) variance = $\sigma^2_{L\times Y}$

Interaction of line ×replication × year (L × R ×Y) variance = $\sigma^2_{L\times R\times Y}$

d) Co-efficient of variability:

Deviation is also expressed by the co-efficient of variation given by the formula of Burton and De vane (1953) as follow:

$$\text{Co-efficient of variability in percentage, (CV \%)} = \frac{S^2}{\bar{X}} \times 100$$

Where, S^2 = Variance

$\bar{X}$ = Mean

In present study, co-efficient of variability at different levels were calculated as follows:

i. Phenotypic co-efficient of variability. (PCV) $= \frac{\sigma^2_p}{\overline{X}} \times 100$

ii. Genotypic co-efficient of variability (GCV) $= \frac{\sigma^2_g}{\overline{X}} \times 100$

iii. Within error co-efficient of variability (ECV) $= \frac{\sigma^2_e}{\overline{X}} \times 100$

Where, $\overline{X}$ = Grand mean

σ^2_P = Phenotypic variance

σ^2_g = Gnotypic variance

σ^2_W = Within error variance.

e) Heritability, genetic advance, genetic advance as a percentage of mean:

i) Heritability (h^2_b): Heritability in broad seance was calculated by dividing the phenotypic variance by the genotypic variance and then multiplying by 100 as suggested by Warner (1952).

$$h_b^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_g = Genetypic variance

σ^2_P = Phenotypic variance.

ii) Genetic advance (GA): Genetic advance was calculated by the formula as suggested by Lush (1949).

$$GA = k\,(\sigma_P)\ \frac{\sigma^2_g}{\sigma^2_p}$$

Where,

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

σ^2_P = Phenotypic variance

σ^2_g = Genotypic variance

iii) Genetic advance as a percentage of mean (GA%): It was calculated by following formula.

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

Where, $\overline{X}$ = Grand mean for a particular character

GA = Genetic advance.

RESULTS

Results obtained for twelve characters in nine lines of blackgram (*Vigna mungo* (L.) Hepper) under three years with three replications in the present investigation are described bellow :

A.STUDY OF VARIABILITY:

1. Analysis of variance: The results of analysis of variance for the twelve characters viz. DFF, PHFF, D50%F, PHMF, PWtH, DPWt, NPdPP, NFPdPP, PdWtPP, NSPP, SWtPP & 100-SWt in nine lines of blackgram were done and are given in Table 5 (A-F). Significance test of the main items and their interactions as shown in Table 5 (A-F) was followed.

In the analysis, the line items (L) were found to be significant for the characters such as DFF, D50%F, PHFF, PHMF, HPWt, NPdPP, PdWtPP & 100-SWt and the other characters such as DPWt, NFPdPP, NSPP & SWtPP were non-significant. The year (Y) item was highly significant for all the characters except HPW & 100-SWt, which were non-significant. Replication (R) item was also appeared to be significant for all the characters except DPWt, NFPdPP & 100-SWt, which were non-significant. The lines (L) interacted differently with replications (R) as was indicated by the significant interaction item (L×R) for seven characters viz. DFF, D50%F, PHFF, HPWt, NFPdPP, PdWtPP & SWtPP and other five characters such as PHMF, DPWt, NPdPP, NSPP & 100-SWt, did not show interaction with replication as in these cases interaction items were non-significant The (L×Y) interaction item was significant for five characters viz. DFF, D50%F, PHFF, PdWtPP & 100-SWt whereas, for other some characters, such as DPWt, PWtH, NFPdPP, NPdPP, NSPP & SWtPP it was non-significant, which indicated that lines did not interact with year (Y). The interaction item (Y×R) was significant for five characters viz. D50%F, PHFF, DPWt, NFPdPP & SWtPP, while for and other seven characters viz. DFF, PHMF, HPWt, PdWtPP, NPdPP, NSPP & 100-SWt was non-significant. The second order interaction item (L×Y×R) observed to be significant for nine characters such as DFF, D50%F, PHFF, PWtH, NFPdPP, NPdPP, PdWtPP, SWtPP & 100-SWt but for other characters like PHMF, DPWt & NSPP was non-significant.

Table 5A. Analysis of variance for DFF and D50%F.

Items	df	DFF			D50%F		
		SS	MS	F	SS	MS	F
Lines	8	312.23704	39.02963	7.029***	8250.37202	1031.2965	25.11***
Year	2	4271.7481	2135.8741	384.645***	3446.53004	1723.265	41.95***
Replication	2	81.960494	40.980247	7.38**	492.327572	246.1646	5.99**
L×R	16	378.21728	23.63858	4.257**	2584.60576	161.53786	3.93**
L×Y	16	826.11852	51.632407	9.298***	2539.42551	158.7141	3.86**
Y×R	4	9.5209877	2.3802469	0.429NS	1363.58848	340.8971	8.298**
L×Y×R	32	816.79012	25.524691	4.597..	7358.54486	229.9545	5.598**
Within error	1134	6296.9333	5.5528513		46585.4667	41.081	

*, **, *** and NS indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

Table 5B. Analysis of variance for PHFF and PHMF

Items	df	PHFF			PHMF		
		SS	MS	F	SS	MS	F
Lines	8	7587.138	948.392	1169.58***	1395.2149	174.40186	4.04**
Year	2	12599.74	6299.87	7769.11***	648.06014	324.030	7.51**
Replication	2	216.869	108.4346	133.735***	405.6742	202.8371	4.698**
L×R	16	1845.279	115.3299	142.23***	1058.1104	66.131903	1.532NS
L×Y	16	4665.452	291.591	359.595***	1038.47	64.904373	1.504NS
Y×R	4	547.995	136.9988	168.949***	123.61193	30.902982	0.72NS
L×Y×R	32	2341.140	73.1607	90.23***	1510.4991	47.203095	1.093NS
Within error	1134	919.54667	0.8109		48955.707	43.170817	

*, **, *** and NS indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

Table 5C. Analysis of variance PWtH for and DPWt

Items	df	PWtH			DPWt		
		SS	MS	F	SS	MS	F
Lines	8	1304.208	163.026	4.023**	222.638	27.82975	1.29 NS
Year	2	73.14282	36.57141	0.903 NS	797.1075	398.5537	18.45***
Replication	2	256.9975	128.4988	3.171*	29.77999	14.89	0.69 NS
L×R	16	1308.786	81.79911	2.018**	336.0158	21.00099	0.97 NS
L×Y	16	658.1846	41.13654	1.015 NS	423.4514	26.46571	1.225 NS
Y×R	4	193.7309	48.43273	1.195 NS	218.9014	54.72535	2.534*
L×Y×R	32	3116.946	97.40456	2.403**	722.0907	22.56533	1.045 NS
Within error	1134	45958.9	40.52813		24494.08	21.59972	

*, **, *** and NS indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

Table 5D. Analysis of variance for NFPdPP and NPdPP.

Items	df	NFPdPP			NPdPP		
		SS	MS	F	SS	MS	F
Lines	8	25.16708	3.145885	1.15 NS	399.5967	49.94959	3.891**
Year	2	29.82881	14.9144	5.451**	2650.816	1325.408	103.243***
Replication	2	15.48807	7.744033	2.83 NS	88.87078	44.43539	3.4613**
L×R	16	139.4601	8.716255	3.185**	307.8107	19.23817	1.4986 NS
L×Y	16	46.63045	2.914403	1.065 NS	223.6428	13.97767	1.089 NS
Y×R	4	36.17613	9.044033	3.3055**	4.753909	1.188477	0.0926 NS
L×Y×R	32	177.5424	5.5482	2.028**	994.7424	31.0857	2.4214**
Within error	1134	3102.667	2.736038		14558	12.83774	

*, **, *** and NS indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

Table 5E. Analysis of variance for PdWtPP and NSPP.

Items	df	PdWtPP			NSPP		
		SS	MS	F	SS	MS	F
Lines	8	74.43277	9.3041	5.819	5859.2856	732.4107	0.4994 ^{NS}
Year	2	535.744	267.872	167.523***	46556.061	23278.030	15.867***
Replication	2	13.87119	6.936	4.3388*	23441.947	11720.974	7.9893**
L×R	16	152.911	9.5569	5.98**	15854.305	990.8943	0.6754 ^{NS}
L×Y	16	97.26243	6.0789	3.802**	11811.658	738.2286	0.504 ^{NS}
Y×R	4	3.893265	0.9733	0.609 ^{NS}	4896.5663	1224.1415	0.8344 ^{NS}
L×Y×R	32	188.7503	5.8984	3.689**	23799.004	743.7189	0.507 ^{NS}
Within error	1134	1813.211	1.59895		1663673.5	1467.084	

*, **, *** and ^{NS} indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

Table 5F. Analysis of variance for SWtPP and 100-SWt

Items	df	SWtPP			100-SWt		
		SS	MS	F	SS	MS	F
Lines	8	23.89511	2.986889	1.365 ^{NS}	48.97012	6.121265	4.147**
Year	2	39.97851	19.98925	9.1313**	5.958125	2.979063	2.0183 ^{NS}
Replication	2	20.81634	10.40817	4.755**	1.736929	0.868465	0.589 ^{NS}
L×R	16	126.8008	7.925047	3.6203**	31.85668	1.991043	1.349 ^{NS}
L×Y	16	45.54784	2.84674	1.301 ^{NS}	49.47397	3.092123	2.095**
Y×R	4	49.06487	12.26622	5.6034**	8.229105	2.057276	1.394 ^{NS}
L×Y×R	32	269.5517	8.423489	3.848**	128.922	4.028813	2.73**
Within error	1134	2482.429	2.189091		1673.807	1.47602	

*, **, *** and ^{NS} indicate significance at 1%, 5%, 0.1% level and non-significance, respectively

2. Component of variation:

The estimates of phenotypic (σ^2_p), genotypic (σ^2_g), line × replication ($\sigma^2_{L\times R}$), line × year ($\sigma^2_{L\times Y}$), replication × year ($\sigma^2_{R\times Y}$) and line × replication × year ($\sigma^2_{L\times R\times Y}$) and within ($\sigma^2_W$) error component of variation were calculated separately for all the twelve characters. The results are shown in Table 6 for nine lines of blackgram.

a) Phenotypic variation (σ^2_p): For all the characters phenotypic variation (σ^2_p) was always greater than those of σ^2_g, $\sigma^2_{L\times R}$, $\sigma^2_{L\times Y}$, $\sigma^2_{R\times Y}$, $\sigma^2_{L\times R\times Y}$ and σ^2_W component of variation as expected. The phenotype is the joint product of σ^2_g, $\sigma^2_{L\times R}$, $\sigma^2_{L\times Y}$, $\sigma^2_{L\times R\times Y}$ and σ^2_W. Table 6 shows that the greater portion of the total phenotypic variation appeared mostly due to the within error variance for all the characters. The maximum phenotypic variation was found for the character, NSPP with a value of 1436.85 and the lowest value of 1.649 for 100-SWt.

b) Genotypic variation (σ^2_g): The highest genotypic variation was observed for the character, D50%F with a value of 9.322 and the lowest genotypic variation for NFPdPP with a value of 0.0214 (Table 6).

c) L×R interaction variation ($\sigma^2_{L\times R}$): The L×R interaction variation (Table 6) was the highest with a value of 5.493 for NSPP and the lowest value of -1.5204 was recorded for D50%F.

d) L×Y interaction variation ($\sigma^2_{L\times Y}$): The estimation of the interaction variation ($\sigma^2_{L\times Y}$) was the highest for PHFF with a value of 4.854 and was the lowest with a value of -1.583 for D50%F (Table 6).

e) R×Y interaction variation ($\sigma^2_{R\times Y}$): The highest interaction variation ($\sigma^2_{R\times Y}$) with a value of 3.559 was recorded for NFPP and the lowest was recorded with a value of -0.22146 for PdWtPP (Table 6).

f) L×R ×Y interaction variation ($\sigma^2_{L\times R\times Y}$): Estimates of the interaction variation ($\sigma^2_{L\times R\times Y}$) was the highest for D50%F with a value of 12.592 and the lowest was recorded with a value of -48.22435 for NSPP.

g) Within error variation (σ^2_W): The highest within error variation (σ^2_W) was recorded for character NSPP which was 1467.08 and the lowest value of 1.47602 was recorded for 100-SWt.

3. Co-efficient of variability:

The estimates of phenotypic (PCV), genotypic (GCV), interactions $(L\times R)_{CV}$, $(L\times Y)_{CV}$, $(R\times Y)_{CV}$ & $(L\times Y\times R)_{CV}$) and within error co-efficient of variability (ECV) were computed for all the twelve characters viz. DFF, PHFF, D50%F, PHMF, PWtH, DPWt, NPdPP, NFPdPP, PdWtPP, NSPP, SWtPP and 100-SWt in nine lines of blackgram and the results obtained are shown in Table 7.

a) Phenotypic co-efficient of variability (PCV): Table 7 shows that the estimates of phenotypic co-efficient of variability was the highest for NSPP with a value of 2831.85 and the lowest phenotypic co-efficient of variability of 22.7936 was recorded for DFF.

b*) Genotypic co-efficient of variability (GCV):* The highest genotypic co-efficient of variability (GCV) with a value of 35.6059 was recorded for PHFF, which the lowest value of 1.068 was found for NFPdPP.

c) L×R interaction co-efficient of variability $(L\times R)_{CV}$: The highest value of 19.478998 was recorded for PHFF and the lowest value of -8.97706 was recorded for PWtH.

d) L×Y interaction co-efficient of variability $(L\times Y)_{CV}$: The estimates for $(L\times Y)_{CV}$ was the highest for DPWt with a value of 5.334707 and the lowest value of -2.60434 for PWtH.

e) R×Y interaction co-efficient of variability $(R\times Y)_{CV}$: For $(R\times Y)_{CV}$ the highest value of 31.006 was recorded for D50%F and the lowest value of -95.044 was recorded for NSPP.

f) L×R ×Y interaction co-efficient of variability $(L\times R\times Y)_{CV}$: Estimate for $(L\times R\times Y)_{CV}$ was the highest for NSPP with a value of 2891.428 and the lowest value of 3.254 for PWtH.

g) Withinl error co-efficient of variability (ECV): The highest within error co-efficient of variability (ECV) was recorded for NSPP with a value of 10.826 and the lowest variability -3.944 for NPdPP.

Table 6. Component of variation of nine lines for different characters in blackgram

Characters	σ^2_p	σ^2_g	$\sigma^2_{L\times R}$	$\sigma^2_{L\times Y}$	$\sigma^2_{R\times Y}$	$\sigma^2_{L\times R\times Y}$	σ^2_w
DFF	7.9367	0.686	-0.0419	0.580172	-0.17144	1.331456	5.55285
D50%F	60.713	9.322	-1.52037	-1.5831	0.8218	12.592	41.081
PHFF	20.771	8.8727	0.937095	4.85400	0.472875	4.8233169	0.8109
PHMF	45.7653	1.6324	0.420641	0.39336	-0.1207	0.2688185	43.1717
DPWt	13.751	0.5625	-0.26328	-0.38018	-0.22146	1.21653	12.8377
HPWt	43.988	1.6279	-0.34679	-1.2504	-0.36275	3.791762	40.5281
NFPdPP	2.9827	0.0214	0.070401	-0.05853	0.025895	0.187477	2.73604
NPdPP	13.751	0.5612	-0.26328	-0.38018	-0.22146	1.21653	12.8377
PdWtPP	2.0212	0.0869	0.0813	0.00401	-0.03648	0.286633	1.59895
NSPP	1436.85	9.0627	5.4928	-0.12201	3.558687	-48.22435	1467.08
SWtPP	2.5451	0.0469	-0.01108	-0.12393	0.028465	0.415627	2.18901
100-SWt	1.6488	0.0833	-0.0453	-0.0208	-0.0146	0.170186	1.47602

Table 7. Co-efficient of variability for nine lines for different characters in blackgram

Characters	PCV	GCV	ECV	$(L\times Y)_{CV}$	$(L\times R)_{CV}$	$(R\times Y)_{CV}$	$(L\times R\times Y)_{CV}$
DFF	22.7936	1.96883	-0.12037	1.666213	-0.492365	3.82385	15.9475
D50%F	149.498	22.954	-3.744	-3.898285	2.0235972	31.0056	101.157
PHFF	83.353	35.6059	3.76054	19.47898	1.8976359	19.3558	3.2540
PHMF	163.4415	5.82988	1.50223	1.4048	-0.4312	0.96003	154.17578
DPWt	500.9048	9.266	-0.7785	1.940985	5.334707	1.44159	483.7
PWtH	315.804	11.687	-2.4897	-8.97706	-2.60434	27.222	290.965
NFPdPP	148.707	1.068	3.509951	2.91803	1.291034	9.3469	136.409
NPdPP	205.98	8.4080	-3.94382	-5.69494	-3.31741	18.223	192.305
PdWtPP	71.5874	3.0759	2.879399	0.142026	-1.2921	10.1517	56.6302
NSPP	2831.85	17.862	10.82554	-0.240458	7.014	-95.044	2891.4276
SWtPP	103.635	1.90992	-0.45103	-5.04628	1.159067	16.924	89.13873
100-SWt	42.555	2.15089	-1.16869	-0.5372	-0.3769	4.392	38.09317

4. Heritability(h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%):

For all the characters heritability in broad sense (h^2_b), genetic advance and genetic advance as a percentage of mean (GA%) were computed and the results are shown in Table 8.

a) Heritability (h^2_b): The values for heritabilities in broad sense (h^2_b) were presented in Table 8. The highest heritability value was estimated for character, PHFF with a value of 42.72 and the lowest value of 0.631 was recorded for NSPP.

b) Genetic advance (GA): The highest value of genetic advance was estimated for character, PHFF with a value of 4.011 and the lowest value of 0.026 was recorded for NFPdPP.

C) Genetic advance as a percentage of mean (GA%): The highest GA% was found for PHFF with a value of 16.094 and the lowest for the same was shown by the character NSPP with a value of 0.971.

Table 8. Heritability (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%) of nine lines for different characters in blackgram.

Characters	h^2_b	G. A.	G. A.%
DFF	8.63765441	0.50128201	1.43964836
D50%F	15.353879	2.4644695	6.0685226
PHFF	42.717003	4.0104709	16.093914
PHMF	3.5669484	0.4970869	1.7752446
DPWt	1.849858	0.180227	4.035964
PWtH	3.700819	0.505628	3.630081
NFPdPP	0.718272	0.025554	1.274037
NPdPP	4.081951	0.311815	4.670878
PdWtPP	4.296736	0.12584	4.456869
NSPP	0.63073284	0.49251412	0.970685
SWtPP	1.842936	0.060566	2.466215
100-SWt	5.054563	0.133703	3.450608

DISCUSSION

An idea of diversity estimate, factorial analysis and correlation study is important in making decision concerning breeding and selection programmes and testing procedures with a crop. The study of these biometric aspect is thus important not only from genetical and evolutionary point of view but also important in aspect of the agronomical produces in general and to plant breeding in particular (Breese, 1969).

All the genetic model in the study of quantitative characters have involve certain assumptions in order to simplify statistical procedure. Fisher (1918) studied the genetic variance in relation to environmental effects and he was the first to provide statistical methods of partitioning the total variation into genetic and environmental components, with the variance and co-variance. First, Mather (1949) developed biometrical technique based on mathematical methods of Fishers *et al.* (1932).

In the present investigation, the nine lines black gram on morphological and quantitative characters such as date of first flower, plant height at first flower, date of maximum flower, plant height at maximum flower, plant weight at harvest, dry plant weight, number of false pod, number of pod per plant, pod weight per plant, number of seeds per plant, seed weight per plant and 100-seed weight were recorded.

In the analysis, the line item (L) was significant for the characters, such as DFF, D50%F, PHFF, PHMF, PWtH, NPdPP, PdWtPP& 100-SWt and for other characters such as DPWt, NFPdPP, NSPP & SWtPP it was non-significant. The year (Y) item was highly significant for all the characters except HPWt & 100-SWt, where it was non-significant. The replication (R) item also appeared to be significant for all the characters except DPWt, NFPdPP & 100-SWt, where it was non-significant. The lines (L) interacted differently with the replications (R) as was indicated by the significant interaction item for (L×R) for seven characters viz. DFF, D50%F, PHFF, PWtH, NFPdPP, PdWtPP & SWtPP and other five characters viz. PHMF, DPWt, NPdPP, NSPP & 100-SWt, where their non-significance showing that lines did not interact with replication (R). The interaction item for (L×Y) was significant for five characters viz. DFF, D50%F, PHFF, PdWtPP & 100-SWt and while other seven characters

viz. DPWt, PWtH, NFPdPP, NPdPP, NSPP & SWtPP, showed non-significance for it, which indicates that lines did not interact with year (Y). The (Y×R) interaction item was significant for five characters viz. D50%F, PHFF, DPWt, NFPdPP & SWtPP and other seven characters viz. DFF, PHMF, PWtH, PdWtPP, NPdPP, NSPP & 100-SWt showed non-significance for it, which indicated that replication did not interact with year (Y). The second order interaction item (L×R×Y) was observed to be significant for nine characters viz. DFF, D50%F, PHFF, HPWt, NFPdPP, NPdPP, PdWtPP, SWtPP & 100-SWt except three characters viz. PHMF, DPWt & NSPP which showed and non-significance and thus indicated that line (L), replication (R) and year (Y) did not interact among themselves. Which, significance for this item indicated that these lines, replications and years were distinctly different from each other which justifies their inclusion in the present investigation. Samad (1991) also recorded similar results in his investigation of certain agronomic characters in rapeseed. Similar reports were also made by Khaleque *et al.* (1991) for the chemical characters in chilli. Many reports on different type of G×E interaction in different crops were given by several workers (Ananda, 1968; Joarder *et al.* 1978; Singh *et al.* 1984; Uddin *et al.* 1987 and Henry and Daulay, 1989).

Partitioning the components of variation are shown in Table 6. A wide range of phenotypic variability for all the characters in nine lines indicated that these characters were polygenic and quantitative in nature. Ramanujam and Thirumalachar (1967) also reported the presence of wide range of phenotypic variation in a number of characters in chilli. Phenotypic variation is the joint product of genotypic, interaction (L×R, L×Y, R×Y and L×R×Y) and within error variation. In the present investigation genotypic variation is low and high phenotypic variation is due to (L×R, L×Y, R×Y and L×R×Y) interaction and within error variation. The highest phenotypic and environmental co-efficient of variability were exhibited by NSPP followed by DPWt, PWtH, NPdPP, PHMF, D50%F, NFPdPP, SWtPP, PHFF, PdWtPP, 100-SWt and DFF (Table 7). On the other hand, high genetic co-efficient value showed by PHMF followed by DFF, D50%F, PHMF, PWtH, DPWt, NFPdPP, NPdPP, PdWtPP, NSPP, SWtPP and 100-SWt (Table 7). Low genetic co-efficient of variability was found for most of the characters, which indicated that these characters were inherited with lower variability within their sibs.

Singh and Malhotra (1970) studying cowpea and found the highest genotypic co-efficient of variation for number of pod per plant. Sethi *et al.* (1972) found the highest co-efficient of variability for number of pod per plant in gram. High $(L\times R)_{CV}$, $(L\times Y)_{CV}$, $(R\times Y)_{CV}$, $(L\times R\times Y)_{CV}$ and E_{CV} were found for D50%F. Such high value suggests good scope for improvement of these characters through selection (Saha *et al.* 1981). Khurana and Sandhu (1972) obtained the highest estimation of phenotypic and genotypic co-efficient of variability for pod per plant in *Glycin max* L. Low genetic co-efficient of variability was found for maximum characters, which indicated that these characters were inherited with lower variability within their sibs.

For all the characters heritability in broad sense (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%) were computed and the results are shows in Table 8. In respect of heritability, most of the characters under study showed low values. Coyne (1968) estimated a low heritability for seed yield and yield components in field bean. Chandra (1968) observed that the heritability estimate was affected by environment in gram. High error component of variation causes a low estimation of heritability. Low heritability as well as low values of genetic advance and genetic advance as a percentage of mean were noted for NSPP. The expression of those characters may likely be conditioned by non-additive gene effect (Pance, 1957).

However in the present work, among all the characters, only PHFF showed a considerable heritability value of 42.72. For this characters, phenotypic variation was moderate with considerable genotypic variation. Within error variance was very low in comparison to the genotypic variation, indicating that the genotypic variation was significant. Bearing this point in mind, it can be saved that heritability value of 42.72 for PHFF is considerable. Increase in plant height may increase area which in term may increase yield. Therefore, this character may likely be selection breeding for high yield in blackgram.

SUMMARY

The present investigation deals with the study of diversity of twelve quantitative characters, such as date of first flower, plant height at first flower, date of maximum flower, plant height at maximum flower, plant weight at harvest, dry plant weight, number of false pod, number of pod per plant, pod weight per plant, number of seeds per plant, seed weight per plant and 100-seed weight for nine lines of black gram (*Vigna mungo* (L.) Hepper), tested in three years with three replication. The materials (seeds of lines) for the study were supplied from the germplasm stock of Biometrical Genetics Laboratory, Department of Genetics and Breeding, University of Rajshahi. Data were recorded in CGS system and analysed following standard biometrical procedure as developed by Mather (1949) based on the mathematical model of Fisher *et al.* (1932) and by De Vane (1953), Warner (1952) and Lush (1949).

The analysis of variance indicated that lines, years and replications item were significant for all the characters except DFWt, NFPdPP, NSPP & SWtPP. The significant value indicated that the lines were genetically different. The component of variation exhibited for most of the characters that major portion of the phenotypic variation was due to environmental variation. Characters exhibiting considerably low genotypic variation indicated that these characters inherited with lower variability. High phenotypic co-efficient of variability was shown by NSPP followed by DPWt, PWtH, NPdPP, PHMF, D50%F, NFPdPP, SWtPP, PHFF, PdWtPP, 100-SWt and DFF. High genetic co-efficient values were shown and PHMF followed by D50%F and NSPP.

In respect of heritability, most of the characters showed a low heritability. High error component of variation causes a low estimation of heritability. Low heritability as well as low values of genetic advance and genetic advance as $\hat{\alpha}$ percentage of mean were noted for most of the characters may likely be conditioned by non-additive gene effect. However, considerable amount of heritability with very low error variance indicated that selection of this character infurther breeding research might increase yield through the increase of shoot are in black gram.

SECTION - II

DIVERSITY ESTIMATES , FACTORIAL ANALYSIS AND CORRELATION STUDY OF ROOT NODULES, YIELD AND YIELD COMPONENTS

INTRODUCTION

The common bean plant (*Vigna mungo*) is the most important grain legume for direct human consumption in the world and can provide as 11-12% of the daily protein intake in Asian countries. Symbiotic nitrogen fixation can provide the plant with nitrogen, the major limiting nutrient, to achieve high yields in most crops plant. In addition, biological nitrogen fixation contributes to sustainable agricultural practices. Compatible interaction between rhizobia and legumes culminates in the formation of a new plant organ, called the root nodule. Within this structure, the rhizobia convert atmospheric nitrogen into ammonia for the benefit of the plant. During the last stages of the symbiotic interaction, *Rhizobium* is released in to the host plant cytoplasm surrounded by a plant-derived membrane. The bacteria, now called bacteriods, differentiate and start to fix nitrogen in a microoxic environment.

Grain legumes (pulses) constitute an important component in the farming system of Bangladesh from the point of view of crop ecology and human and animal nutrition. Pulses occupy about 7.3 lakh hectare (ha) of the total cropped area and produces 5.35 lakh metric tons of pulses (BBS 1997). Although area of pulses under cultivation is 7.3 lakh ha annually still at present there is an acute shortage of pulse production in relation to their demand. The present per capita availability of pulses is about 12 gm/capita/day (BBS 1997) against the recommended daily allowance of 45 gm/capita/day (WHO). According to the Bulletin published by the World Bank, the total population of Bangladesh was 12.58 crore in 1995, 13.7 crore in 2002 and this will be 15.3 crore in 2010. At the present rate (12 gm/capita/day) if we have to ensure the availability of pulses, the production will have to be 5.85 and 6.51 lakh metric tons for 2002 and 2010 respectively (Fig-1) and a pie chart showing production of different field crops and pulses of Bangladesh have been shown in Fig: 2 and 3.

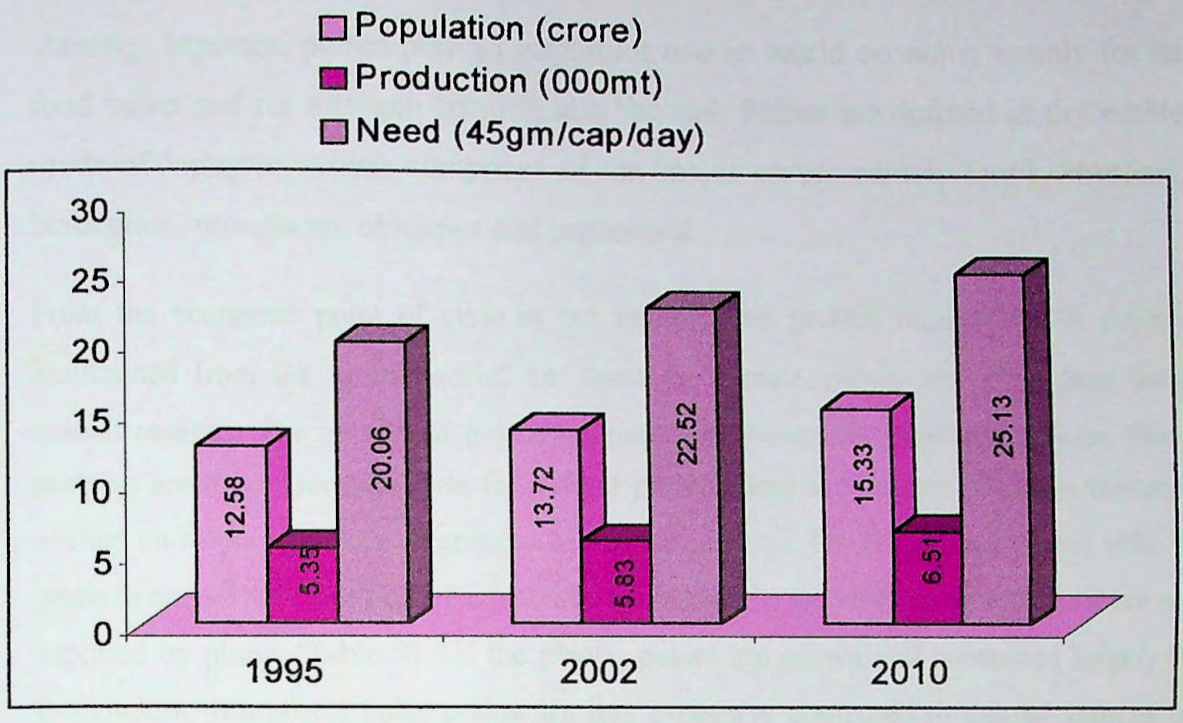


Fig 1: Population, production and need of pulses

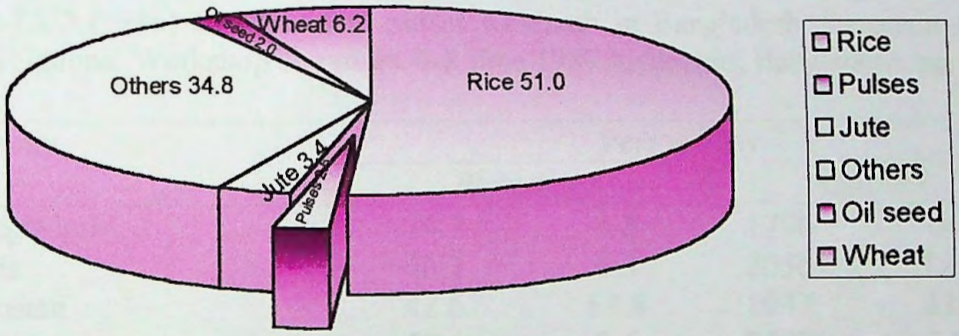


Fig. 2:

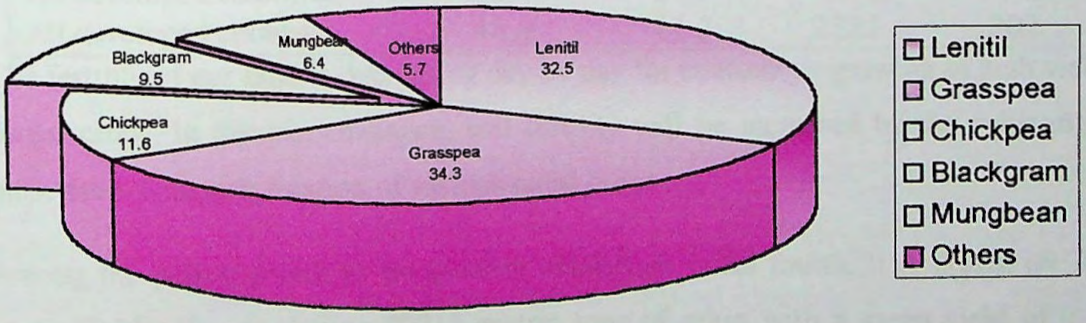


Fig. 3:

Among legumes, pulses play an important role in world economy mainly for its food value and for nitrogen fixation into the soil. Pulses are defined as dry edible seeds of legumes, which comprises of six major crops, namely lentil, kheshari, blackgram, mungbean, chickpea and pigeonpea.

From the economic point of view in our country this protein requirement is mainly maintained from the "green world" i.e. from the cereals, pulses and other than from animal sources. The protein of pulses is commonly known as vegetable protein. Plant proteins are the major substitute for animal proteins and in this context, grain legumes occupy an important place as sources of dietary proteins. On an average, about 80% of proteins and 90% of calories are consumed by man in the developing countries, which are supplied by plants (Table 9). Of the plants, pulses are grown and consumed largely in Bangladesh. Moreover, pulse grains are less expensive compared to animal sources of protein and thus considered as "poor man's meat.

Table 9: Availability of protein and calories in some Asian countries.

Source-FAO (1986) (Advances in pulses Research in Bangladesh. Proceedings of the second National Workshop on pulses. 6-8 June 1989 Joydebpur, Bangladesh. pp. 192)

Country	Person^{-1} day^{-1}			
	Protein (g)		Calories	
Bangladesh	34.3	4.3	1796	64
India	46.1	6.3	2036	125
Pakistan	42.6	13.8	1947	238
Burma	57.6	8.4	2409	1.9
Nepal	44.9	8.1	1906	142
All developed countries	42.4	54.9	2364	1010
All developed countries	46.9	11.3	2222	202

The fertility of our land is decreasing day by day for continuous growing of high yielding cereal crops. In this circumstance, soil fertility will be increased by the cultivation of pulse crop. Nitrogen fixation of various pulse crops.

Among the pulses grown in Bangladesh blackgram ranks fourth. It is grown on 70995 acres (Table 10), producing 20215 metric tons of grain with a mean yield of 0.2847 ton/acre and contributing about 10.5% of the total pulses (BARI-1999) It is one of the

main edible pulse crops of Bangladesh. About 80% of the blackgram crops are grown in two districts namely Rajshahi and Chapai Nawabganj, which is a popular diet of this area.

Table 10: Area and production of mashkalai by region, 1994-95 to 1998-99. (Area in acres and production in Metric tons.) (**Reference:** Year book of Agriculture Statistics of Bangladesh-1999, Bangladesh Bureau of Statistics).

Regions	1994-95		1995-96		1996-97		1997-98		1998-99	
	Area	Prod.	Area	Prod.	Area	Prod.	Area	Prod	Area	Prod.
Bandarban	135	45	130	45	130	45	130	45	230	70
Chittagong	280	85	275	90	275	100	270	115	15	10
Comilla	2130	585	2185	600	2185	570	2180	645	1570	445
Khagrachari	45	10	45	10	35	10	50	15	(1)	(0.30)
Noakhali	4240	1145	4420	1190	4210	1130	4425	1250	65	15
Rangamati	30	5	25	05	20	05	15	05	--	--
Sylhet	580	200	475	180	605	220	640	275	125	45
Dhaka	9620	2840	10120	3145	10140	3195	10665	3560	12105	3295
Faridpur	7745	2565	7305	2065	7135	2020	6925	2195	5070	1405
Jamalpur	2955	990	2900	1025	3095	1150	3310	1130	605	205
Kishoreganj	6905	2325	6865	2300	6945	2375	6480	1685	1165	290
Mymensingh	6975	2425	6975	2425	6875	2385	6925	2410	3450	1200
Tangail	11635	3630	14000	5055	14630	5045	14235	3685	3820	1025
Barisal	310	85	320	100	250	85	195	60	70	20
Jessore	13250	4650	13210	4650	13660	5435	12820	5015	1825	695
Khulna	535	170	415	120	500	175	555	205	40	45
Kushtia	7960	2410	7125	2135	7510	2285	7320	2260	4185	1230
Patuakhali	--	---	--	--	--	--	--	--	--	--
Bogra	3270	1075	2915	955	3160	1060	3270	1105	100	35
Dinajpur	13210	3935	11365	3195	11575	3250	11870	2955	3485	700
Pabna	14180	4905	13530	4560	12025	3585	11690	4045	11230	3270
Rajshahi	54505	15915	48910	13940	47375	13690	47380	13455	20020	5645
Rangpur	7310	2545	7080	2435	7000	2385	7410	2450	1820	600
Bangladesh	**167805**	**52540**	**160590**	**50225**	**159335**	**50200**	**158760**	**48565**	**70995**	**20215**

The importance of blackgram crop lies not only in their food value to human beings, but also it supplies the proteinacious fodder to livestock. It plays a vital role in providing fodder for farm animals, either directly or grazing or as fodder after the grain has been threshed. After dehusking, bran is also used as a quality food for animals. It has some use as a green manure and in medicine too. It is needless to emphasis that a better quality nutritious fodder is badly needed for the emaciated cattle population of the country.

In addition to the food value, blackgram also enrich impoverished soils with nitrogen in symbiosis with bacteria. By virtue of its capacity to fix atmospheric nitrogen in symbiotic association with *Rhizobium* sp. it has been playing a significant role in restoring the fertility of our soil over the past hundreds of years in absence of adequate manning. Leguminous crops not only can fix the atmospheric nitrogen towards the benefit of the crop but also save nitrate leaching during precipitation (Jones, 1939).

Factorial experiment is one in which the set of treatments consists of all possible combinations of the levels of several factors. Here the effects of a number of different factors are investigated simultaneously. Factorial experiments are used practically in almost all fields of research. They are of great value in exploratory work where little is known concerning the optimum levels of the factors, or even which ones are important.

The advantages of factorial experimentation naturally depend on the purpose of the experiment. In other words, the object is to obtain a broad picture of the effects of the factors rather than to find, that the combination of the levels of the factors that gives a maximum response.

The acquisition of knowledge of the potential yield of a crop and the understanding of factors affecting that yield are objectives central to almost all agricultural research programme. Factorial analysis has traditionally played an important role in helping to attain them. Study of different doses of fertilizers on plants can be studied by factorial analysis developed by Fisher *et al.* (1932).

Phosphorus (P) is an important nutrient in pulse crop production. It is known to enhance root development, which can result in improved drought tolerance (Walley and Hnatowich, 1999). It also improved the ability of a crop to tolerate stresses, including early frost damage. Walley and Hnatowich (1999) reported that, if P fertilizer not supplied in sufficient quantities, a P deficiency can have a negative impact on the N fixing process. They also reported that P has significant effect on seed yield in chickpea and highest yields were achieved when P was side banded at a rate of 40 kg/ha.

Response to added K has also been reported (Kay, 1979). Potassium (K) also affect the growth of pulse crop. Hossain *et al.* (1977) suggested that the presence of K in culture medium showed a long lag period of vegetative growth in blackgram. He also reported that low vegetative growth may be due to the effect of K resulting poor nodule formation in most of the growing stage, as the rapid plant growth was followed by increased nodule formation.

It is obvious from the above discussion that the use of balanced fertilizers for optimum growth and development of blackgram is necessary. But in our country use of fertilizers for pulses are not usually practiced.

Knowledge about the description, prediction and inheritance of genotype-environment interaction would provide more information and help the breeders to breed and select superior genotypes in a particular environment. The present investigation was therefore, undertaken to see the effect of fertilizers individually and in combination following factorial design. It also deals with the analysis of variance, correlation and the factorial analysis, on some of the agronomic characters like root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight (FPdWt), dry pod weight (DPdWt), number of seed per plant (NSPP), yield per plant (YPP) and 100-seed weight (100-SWt) in nine lines of black gram.

REVIEW OF LITERATURE

The literatures on the study of stability parameters and factorial analysis on agronomical characters in pulses are scanty. In fact reports on black gram are few and scattered. The limited number of papers has been published dealing with the problem of stability parameters of different quantitative characters on various leguminous crop plants. A brief review of literatures on the leguminous crops and others regarding this study are narrated below.

Fisher (1918) was the first to develop statistical method to partition variance of quantitative characters in segregating population into genetic and environmental components.

Mather (1949), Mather and Jones (1958) combinedly developed the techniques to measure the genotype-environment interaction based on the mathematical method of Fisher *et al.* (1932).

Sharma (1968) studied the response of laha (*Brassica juncea*) with the application of N, P and K. He reported that nitrogen exhibited the best response on yield as compared to P_2O_5 and K_2O application. Highest net profit was obtained at 45 kg N/ha as compared to different doses of other manures, but for balanced manuring N45, P22.5, K22.5 can be exhibited more net profits.

Buttery (1969a) exhibited the effect of plant population and N, P, K fertilizers on growth and yield of soyabean. He reported that at the early stages fertilizer in moderate quantity depressed the growth of the whole plant, but by maturity, fertilizer was associated with a small increase in weight of shoot and increase in the proportion of beans to shoot. The proportion of flowers forming mature pods reduced at high density, while fertilizer application increased it. No plant characteristics had interaction between fertilizer level and density of planting.

Buttery (1969b) made an investigation on the effect of NPK fertilizers on growth characteristics of soyabean. He found that the fertilizer increased final plant weight mainly by delaying the fall in NAR and CAR. By the fertilizer application LAI was increased slightly, presumably because of a higher relative leaf growth rate (RLGR) between 50 and 70 days from planting.

Black (1970) studied the effect of NP fertilizers on adventitious roots, tillers and grain yields of spring wheat. He reported that the samples of roots and tops taken at the end of tillering revealed that most of the effect of P and NP fertilization was accounted for by changes in plant morphology. Tillers per plant were positively correlated with adventitious roots per plant. Heads per ha were linearly related to adventitious roots per plant and to tillers per plant. Number of heads per ha accounted for 97% of the yield variance associated with fertilizer treatments. Numbers of kernels per head and kernel weights were not significantly influenced by N and P fertilization. The regression of grain yield on number of adventitious roots per plant accounted for 93% of the variations in grain yield associated with fertilizer treatments. Grain yields increased from 1984 to 2706 kg/ha when 45 kg/ha of P was added alone and to 3306 kg/ha when 45 kg/ha of N was also applied. Higher rates of P, with or without added N, failed to further increase grain yield.

Allen and Morgan (1972) described experiments on nitrogen nutrition of the spring-sown rape, showing an increase in growth and yield up to their maximum level of 210 kg N/ha. Number of pods and number of seeds per pod were all increased but seed weight was little affected by increasing nitrogen.

Hossain (1977) investigated different combinations of nitrogen, phosphorus and potassium showed different responses on the nodule formation *Phaseolus mungo* L. Plants grown on the sand culture media containing nitrogen in different combinations with phosphorus and or potassium were found to produce little amount of nodules at early stages of their growth. At later stages NP plants were found to certain significantly high amount of nodules. At the final stage NPK treated plants improved nodule content, while NK treated plants produced little amount of nodules. The plants treated separately with PK and P produced more nodules than the control plant nodule formation increased with

the increase in fresh weight and leaf surface of plants in all treatments. The total amount of sugars in the whole plant was found to be related with the amount of nodules formed.

Islam *et al.* (1988) worked on the growth and yield of rape seed (*Brassica campestris* L.) as influenced by nitrogen (N), phosphorous (P) and potassium (K) and reported that the significant effects on fertilizers were observed for relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) at all the growth stages and for relative leaf growth rate (RLGR), specific leaf area (SLA) and leaf weight ratio (LWR) at some of the growth stages. N maintained higher NAR at the later stages of the growth. LAR and SLA were increased by P and K but decreased by N. Chlorophyll content, leaf number, leaf area and dry matter yield at first flowering were increased more by N compared to P and NK treated plants had higher seed yield and number of siliquae per plant. Oil content was less affected by fertilizers, generally N decreased and P and K increased the oil content.

Saha and Paul (1988) worked on the physiological analysis of growth of the jute (*Corchorus capsularis* L.) as affected by different levels of N, P and K treatments. They reported that total dry weight and leaf area increased with increasing age as well as increasing N, P and K levels. At the early stages of growth the effects of N, P and K levels on dry matter yield and leaf area were not produced. Compared to P and K, N levels had greater effects on dry weight and leaf area at the later stages of growth. The distribution of total dry matter in the various plant parts indicated that a greater proportion of dry weight was diverted into the leaves in the early stages of growth and the proportion diverted into the stems increased in the later of growth in all the treatments, except control plants. All the growth attributes such as, RGR, NAR, LAR, RLGR, SLA, LWR index and SRR were markedly affected by N, P and K fertilizers. The analysis of variance indicated that the effects of the different levels of N, P and K on these growth attributes were significant at most of the growth stages. The different levels of P had pronounced effect on RGR, NAR and RLGR compared to N and K treatments. Generally, control plants had greater LAR, SLA and LWR than the N, P and K treated plants.

Khandakar *et al.* (1989) studied the yield stability of 10 varieties of jute tested in a wide range of environments at three zonal stations. The effect of variety had much influence whereas, the effect of environment (sowing date) was highly significant. The interaction between variety-environment was significant whereas, variety-station and station-environment were not significant. The variety 0-9897, Uganda mutant had higher yield. The varieties with higher yield (0-9897 and Uganda mutant) had less stability whereas, the variety with lower yield (0-4 and CVL-1) had higher stability across environments. The higher yield maintained an inverse relation with wider stability to environments.

Paul and Sarker (1989) made physiological analysis of the effects of N, P and K on yield of mustard and reported that effect of fertilizers were significant for leaf area, dry matter production, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and relative leaf growth rate (RLGR) at all the five harvest intervals and specific leaf area (SLA) at the first three harvests and leaf weight ratio (LWR) at all but one harvest. The effect of N on these characters were greater than those of P or K. Fertilizer had significant effect on seed and oil yield and some of the components of yield.

Campbell *et al.* (1990) worked on dynamics of dry matter, N and P accumulation in the developing kernels of four spring wheat cultivars for irrigation and dry land. They observed that N and P accumulation depended mainly on grain DM response. Duration of accumulation of DM, N and P in grain was equal under land conditions, but under irrigation the period was several ways longer for N than for DM and P. The mean rates of accumulation of DM, N and P were directly related to grain DM, N and P response.

Samad (1991) worked on genotype × environment interaction of six agronomical characters in fifteen rape seed (*Brassica campestris* L.) cultivars in six consecutive years. He showed that genotype × environment interactions were significantly operative in the experiment. He observed that all the genotypes for plant height and number of pods/plant failed to show the stable performances, while some of the genotypes like polar, Toti-9, Tori-7 and sampad were predicted to show the stable performances in regard to the agronomical characters such as number of secondary branches, number of seeds/pod and yield/plant.

Thakur *et al.* (2000) worked on the tillering pattern and productivity of wheat (*Triticum aestivum* L.) under different irrigation, seed rate and fertilizers. They observed that wheat receiving first irrigation at crown-root initiation gave 93.2% more tiller over its mother shoot (273/m^2). Whereas with holding irrigation at this stage produced only 28.2% higher tillers over initial plant stand (298/m^2). Increased seed rate recorded more seed/unit area and maximum mother shoot (380/m^2) was recorded with 200 kg seed/ha. Increasing fertility did not influence the establishment of mother shoot and their tillering ability as the total tillers at 40 and 60 days after sowing were similar with all the fertilizer levels. However, mortality of tillers decreased with increasing fertilizer level. Consequently, crop with N_{150}, $P_{32.7}$, $K_{33.2}$ and K_{180} $P_{39.3}$, $K_{33.2}$ being similar had higher total tillers than crop with N_{120}, $P_{26.2}$, $K_{33.2}$ at 80 and 100 days after sowing and maturity.

Kulapati *et al.* (2000) studied the effect of two levels of N, P and K on bunch characters in ratoon crop of banana germplasm. The cultiver Robusta registered a maximum bunch yield of 106.31 t/ha with 125% of recommended N, P and K fertilizers when two suckers per hill were retained after shooting of the main crop. Their study suggested that though Elakkibale yielded 46.89 t/ha, it gave the highest income of Rs. 245526.86 with two suckers per hill with 125% recommended N, P and K fertilizers followed by Robusta (Rs. 208584.86) which was recommended for cultivation for Bhadra Command Area.

Kumaran and Subramanian (2001b) undertaken the investigations for studying the influence of plant density and methods of nutrient application on yield of blackgram, during kharif 1995 and summer 1995. They showed that in plant population of 40 plants m-2 increased leaf area index (LAI), net assimilation rate (NAR) and grain yield during both the seasons. Highest root nodules/ plant, crop growth rate, specific leaf weight and relative growth rate were maximum at higher plant density (50 plants m-2. They also reported, treatment with ammonium molybdate 25 ppm + Zn SO_4 100 ppm + Fe So4 100 ppm and foliar application of nutrients with DAP 1%+ urea 0.5%+ $MgZnSo_4$ 0.25% recorded significantly highest leaf area index, specific leaf weight, crop growth rate, relative growth rate, net assimilation rate and grain yield of blackgram during both the seasons.

Sinha *et al.* (2002) studied the effect of ascorbic acid on amino acid translocation and nodulation in mung (*Phaseolus aureus* Roxb.) They conducted the experiment with ascorbic acid (AA) at 25mg/l and 100 mg/l for seed treatment and 500 and 1000 mg /l for sand culture. In seed treated plants 63 to 80% and in sand treated plants 100 to 130% NH_2-N was tramslocated as compared to the untreated control. They observed that the AA treated mung and pea plants showed an increase in nodule number, nodule volume, and dry weight of plants and total nitrogen content as compared to the control. Sand treatment method was more effective than seed treatment one. Nitrogen fixed per nodule showed a decrease in the treated pea plants and remained more or less unchanged in moong plants. They reported, this might be due to the increased nodule number.

Mahi *et al.* (2002) studied on Split-plot design field experiments conducted in Khartoum State, Sudan for two successive summer seasons of 1993-94 and 1994-95 to examine the effect of phosphorus and potassium fertilizers on the performance of forage sorghum [*Sorghum bicolor* (L.) Moench]. The land was chiseled down to 40 cm, harrowed and leveled. Four levels of phosphorus (P) and potassium (K) fertilizers were applied before sowing in the form of triple super phosphate and potassium sulfate, respectively. Potassium was allotted for the main plots and the sub-plots for phosphorus. The crop was grown on a highly saline-sonic soil (Natrargid) under irrigation. Phosphorus fertilization and the interaction of phosphorus and potassium significantly increased the growth attributes and the dry matter yield. Phosphorous fertilizer increased the leaf phosphorus and nitrogen contents significantly in the first season when salinity was high, whereas potassium application was not effective. Leaf phosphorus and nitrogen contents were greatly improved in the second season when salinity became low even without phosphorus fertilization. Potassium, however, caused a significant reduction in leaf sodium content. It was concluded that a large phosphorus dose in excess of 300 kg P/ha may be required by the crop in the first season, but only a maintenance dose may be required in subsequent seasons.

Hussaini *et al.* (2002) studied two-year field conducted in 1997 and 1998 dry seasons at the Kadawa Irrigation Research Station of the Institute for Agricultural Research, Samaru, Nigeria, to investigate the effect of different levels of nitrogen (0, 60, 120 and 180 kg N ha-1), phosphorus (0, 20 and 40 kg P ha-1) and irrigation regimes (based on irrigation water (IW): cumulative pan evaporation (CPE) ratios of 0.6, 0.8 and 1.0 on the productivity and water use of dry season maize. The nitrogen and irrigation were factorially combined to make the main plots, while phosphorus was assigned to the sub-plot in a split-plot arrangement using three replications. The influence of nitrogen was significant on shelling percentage, harvest index, grain yield, water use and water use efficiency, while phosphorus was significant on all but shelling percentage. Moisture regime also had a significant effect on harvest index, grain yield and water use.

MATERIALS AND METHODS

A. MATERIALS:

Materials of the present investigation comprised of nine blackgram (*Vigna mungo* (L) Hepper) lines. Seeds of these lines were supplied from the Biometrical Genetics Laboratory, Department of Genetics and Breeding, University of Rajshahi. The lines are given in the following table:

Serial No.	Lines No.	Serial No.	Lines No.	Serial No.	Lines No.
1	L_2	4	L_{14}	7	L_{18}
2	L_{11}	5	L_{15}	8	L_{19}
3	L_{13}	6	L_{16}	9	L_{20}

Thirteen agronomical characters of nine black gram lines were measured for variance, correlation, and factorial analysis. The characters were root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight per plant (FPdWt), dry pod weight (DPdWt), number of seed per plant (NSPP), 100-seed weight and yield per plant (YPP).

The process of experimentation are described under the following sub-heads:

1. Collection of the experimental seeds
2. Size of the experimental space
3. Preparation of the experimental soil
4. Sowing of seeds and raising of seedlings
5. Maintenance of the experimental bags and space
6. Collection of data

1. Collection of the experimental seeds: At the beginning of this study in 2000, theseeds of the nine lines of blackgram were supplied from the Biometrical Genetics Laboratory, Department of Genetics & Breeding, University of Rajshahi.

2. Size of the experimental space: For the present study randomized block design was followed. The seeds of blackgram were shown in polyethylene bag. The experiment was conducted in the 3rd science building of Rajshahi University during the period from the 25th September to the 23rd December, 2000. The experimental field was comprised of an area of 750 × 366 sq. cm in two replications. The size of each replication was 325× 366 sq.cm and gap between replications was 100 cm. The experimental space was under full sunny condition. The 325 × 366 sq.cm area were divided into 41×41 sq.cm by metal wire for supporting the polyethylene bags.

3. Preparation of the experimental soil: For the present experiment seeds were sown in soils prepared with the combination of 50% sand, 12.5% cowdung, 12.5% sawdust and 12.5% ash. Then these combinations were mixed up well. The prepared soil was put in to the 30×42 sq.cm polyethylene bags. Each of the polyethylene bags containing soil was treated with one of the eight fertilizer doses prepared by nitrogen, phosphorus, potassium and their combinations. The eight fertilizer doses are as follows:

a) Control (No fertilizer) (D_1)
b) Nitrogen (Urea) 1.5 gm/bag (D_2)
c) Phosphrus (TSP) 4 gm/bag (D_3)
d) Potassium (MP) 1.5 gm/bag (D_4)
e) Nitrogen 1.5 gm/bag + Phosphorus 4 gm/bag (D_5)
f) Nitrogen 1.5 gm/bag +Potassium 1.5 gm/bag (D_6)
g) Phosphorus4 gm/bag + Potassium 1.5 gm/ bag (D_7)
h) Nitrogen 1.5 gm/bag + Phosphorus 4 gm/bag + Potassium 1.5 gm/bag (D_8)

4. Sowing of seeds and raising of seedlings: The seeds of the nine lines of blackgram were sown in the polyethylene bags. Five to six seeds were sown separately in each of the polyethylene bags. The sowing date was the 25th September, 2000. After sowing, each bag was wrapped by another black polyethylene bag to make darkness, which was required for the development of roots. Bags were arranged randomly in the squares of each rectangle.

5. Maintenance of the experimental bags and space: When the seedlings were two to three inches in height, the excess seedlings were removed from the experimental bags and weeding in the experimental space was done.

6. Collection of data: Data were collected on individual plant basis. Different characters from the 9 lines of black gram of all the plants were measured in C G S system and were recorded. For statistical analysis thirteen characters were on from 3 plants from each of the polyethylene bags and lines in each of the replications.

B. METHODS:

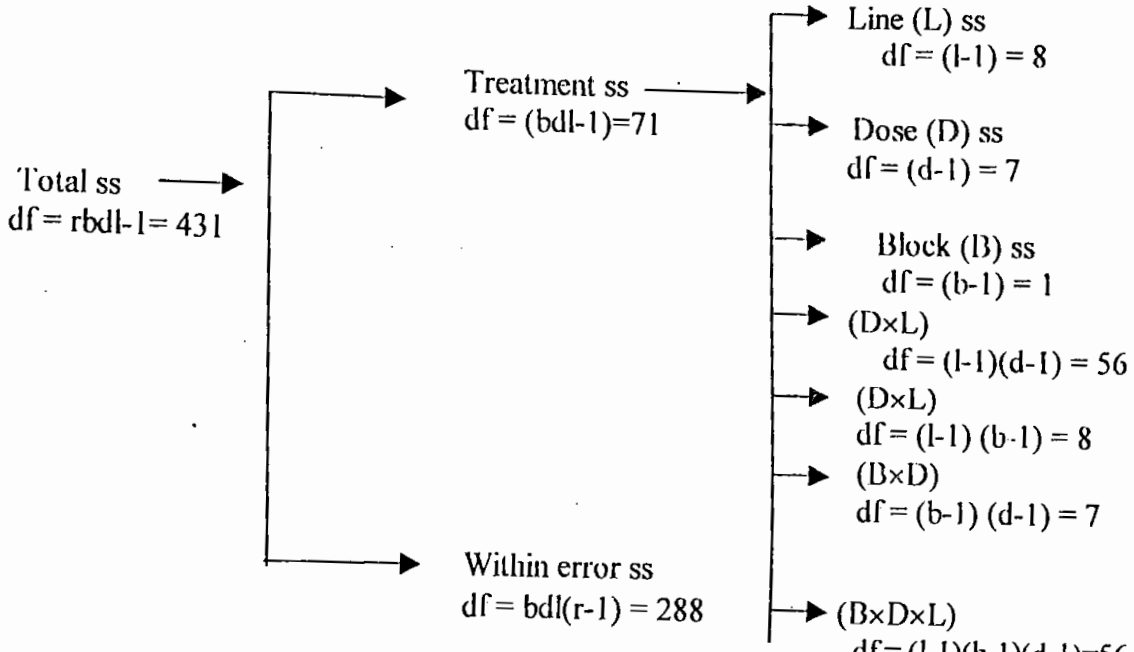
1. Technique of analysis of data: The collected data were analyzed following biometrical technique of analysis as developed and used by Eberhart and Russell (1966) and Mather (1949) based on the mathematical models of Fisher *et al.* (1932).

The techniques used are described under the following sub-heads:

a) Analysis of variance:

Variance is a measurement of dispersion of a population. Thus, the analysis of variance is done for testing the significant differences among population. Variance analysis for each of the characters were carried out separately on the mean value of 3 plants.

Variance due to different sources such as, lines (L), block (B), doses (D), interactions B×L, D×L B×D, B×D×L and within error in the present study were calculated as per the following skeleton of analysis:



Where, Total SS = $(rBDL)^2 - CF$

$$\text{Treatment SS} = \frac{\sum_{ijk}(B_j D_k L_i)^2}{r} - CF$$

$$\text{Block ss} = \frac{\sum_{j} B_j^{\,2}}{rbd} - CF$$

$$\text{Line ss} = \frac{\sum_{i} L_i^{\,2}}{rbd} - CF$$

$$\text{Doses ss} = \frac{\sum_{k} D_k^{\,2}}{rlb} - CF$$

$$\text{(D×L) ss} = \frac{\sum_{ik}(D_k L_i)^2}{rb} - CF - L_{ss} - D_{ss}$$

$$\text{(B×L) ss} = \frac{\sum_{ij}(B_j L_i)^2}{rd} - CF - L_{ss} - B_{ss}$$

$$\text{(B×D) ss} = \frac{\sum_{jk}(B_j D_k)^2}{rl} - CF - B_{ss} - D_{ss}$$

$$\text{(B×D×L) ss} = \frac{\sum_{ijk}(B_j D_k L_i)^2}{r} - CF - L_{ss} - D_{ss} - B_{ss}$$

Error SS = Total SS – Treatment SS

L_i = The number of ith lines

B_j = The number of jth blocks

D_k = The number of kth doses

B_jL_i = The value of ith lines in jth block

D_kL_i = The value of ith lines in kth doses

B_jD_k = The value of jth block in kth doses

$B_jD_kL_i$ = The value of ith lines in jth block of kth doses

r = Number of replications

L= Number of lines

B = Number of blocks

D= Number of doses

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

GT= Grand Total

N = Total number of observation = rbdl

The analysis of variance of a fixed model was used, where replication (r), line (L) and Dose (D) are fixed. Expectations in analysis are shown in Table 11.

Table 11: The expectation of mean squares (EMS) used in the analysis of variance

Items	df	MS	EMS
Block (B)	(b-1)	MS_1	$\sigma^2_w + rldK^2_b$
Line (L)	(l - 1)	MS_2	$\sigma^2_w + rbdK^2_l$
Dose (D)	(d -1)	MS_3	$\sigma^2_w + rblK^2_d$
B×L	(b - 1)(l-1)	MS_4	$\sigma^2_w + rdK^2_{bl}$
B×D	(b - 1)(d - 1)	MS_5	$\sigma^2_w + rlK^2_{bd}$
D×L	(d-1) (l-1)	MS_6	$\sigma^2_w + rbK^2_{ld}$
B×D×L	(b - 1)(b - 1)(l -1)	MS_7	$\sigma^2_w + rK^2bdl$
Within err.	bdl (r -1)	MS_8	σ^2_w
Total	(bdl-1)		

Where,

L and D represent the number of lines and doses respectively.

MS_1 = Mean square of block

MS_2 = Mean square of line

MS_3 = Mean square of Dose

MS_4 = Mean square of B×L

MS_5 = Mean square of B×D

MS_6 = Mean square of D×L

MS_7 = Mean square of B×D×L

MS_8 = Mean square of within error for expected mean square.

$rdlK^2_l$ = Variance due to block

$rbdK^2_l$ = Variance due to lines

$rblK^2_d$ = Variance due to doses

rdK^2_{bl} = Variance due to B×L

rbK^2_{dl} = Variance due to D×L

rlK^2_{bd} = Variance due to B×D

rK^2_{bdl} = Variance due to B×D×L

σ^2_w = Variance due to within error.

b) Components of variation:

The components of variation were phenotypic (σ_p^2), genotypic (σ_g^2), block × Line (B ×L), dose × line (D × L), block × dose (B × D) and block × dose × line (B × D × L) variance. These are calculated as follows.

Step 1:

$$\sigma_g^2 = \frac{MS_2 - M\,S_8}{rbd}$$

$$\sigma^2_{B\times L} = \frac{MS_4 - MS_8}{rd}$$

$$\sigma^2_{B\times D} = \frac{MS_5 - MS_8}{rl}$$

$$\sigma^2_{D\times L} = \frac{MS_6 - MS_8}{rb}$$

$$\sigma^2_{B\times D\times L} = \frac{MS_7 - MS_8}{r}$$

$$\sigma_w^2 = MS_8$$

Step 2: Phenotypic variance: $\sigma_p^2 = \sigma_g^2 + \sigma^2_{B\times L} + \sigma^2_{D\times L} + \sigma^2_{B\times D\times L} + \sigma_w^2$

Genotypic variance, $\sigma_g^2 = \sigma_j^2$

Interaction of block × line (B × L) variance = $\sigma^2_{B\times L}$

Interaction of block × dose (B × D) variance = $\sigma^2_{B\times D}$

Interaction of dose × line (D ×L) variance = $\sigma^2_{D\times L}$

Interaction of block × dose × line (B × D ×L) variance = $\sigma^2_{B\times D\times L}$

c) Co-efficient of variability:

Deviation is also expressed by the co-efficient of variation given by the formula of Burton and De vane (1953) as follow:

$$\text{Co-efficient of variability in percentage, (CV \%)} = \frac{S^2}{\overline{X}} \times 100$$

Where, S^2 = Variance

$\overline{X}$ = Mean

In present study, co-efficient of variability at different levels were calculated as follows:

i. Phenotypic co-efficient of variability. (PCV) $= \frac{\sigma^2_p}{\overline{X}} \times 100$

ii. Genotypic co-efficient of variability (GCV) $= \frac{\sigma^2_g}{\overline{X}} \times 100$

iii. Within error co-efficient of variability (ECV) $= \frac{\sigma^2_e}{\overline{X}} \times 100$

Where, $\overline{X}$ = Grand mean

σ^2_P = Phenotypic variance

σ^2_g = Genotypic variance

σ^2_w = Within error of variance

d) Heritability, genetic advance, genetic advance as a percentage of mean:

i) Heritability (h^2_b): Heritability in broad seance was calculated by dividing the phenotypic variance by the genotypic variance and then multiplying by 100 as suggested by Warner (1952).

$$h_b^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_g = Genetypic variance

σ^2_P = Phenotypic variance.

ii) Genetic advance (GA): Genetic advance was calculated by the formula as suggested by Lush (1949).

$$GA = k\,(\sigma_P)\,\frac{\sigma^2_g}{\sigma^2_p}$$

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

σ^2_P = Phenotypic variance

σ^2_g =Genotypic variance

iii) Genetic advance as a percentage of mean (GA%): It was calculated by following formula.

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

Where,

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

GA = Genetic advance.

e) Correlation co-efficient (r):

To measure the degree of association between any pair of characters, correlation co-efficient is estimated. It was described by Herzberg (1983) in the following formula.

$$r = \frac{\sum X_i Y_i - \frac{\sum X_i \sum Y_i}{n}}{\sqrt{\left\{\sum X_i^2 - \frac{(\sum X_i)^2}{n}\right\}\left\{\sum Y_i^2 - \frac{(\sum Y_i)^2}{n}\right\}}}$$

Where, $SS_{(X)}$ = Sum of square of X
$= \Sigma X^2 - (\Sigma X)^2/n$

$SS_{(Y)}$ = Sum of square of Y
$= \Sigma Y^2 - (\Sigma Y)^2/n$

n= Total number of observation

X = A variable

Y = Other variable

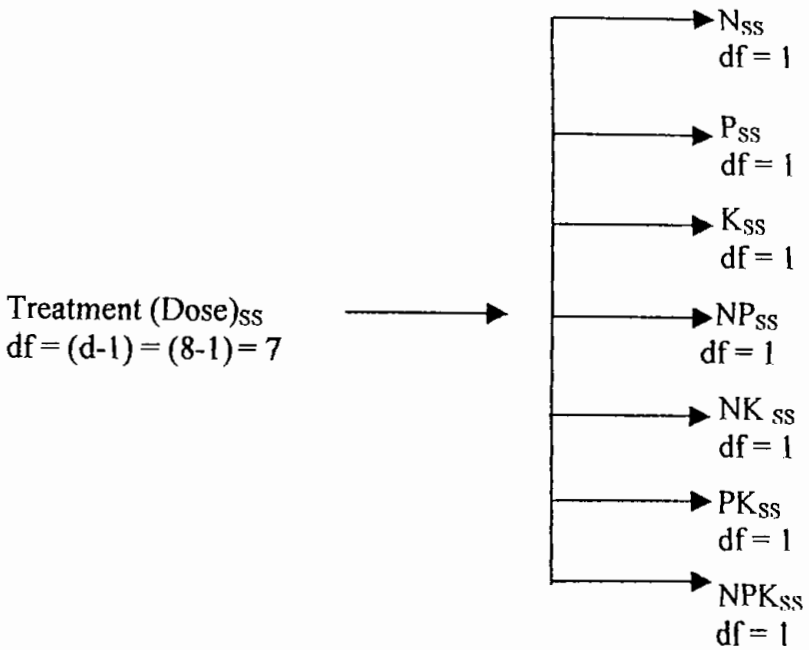
Σ = Summation

r = Correlation

Test of significance of correlation co-efficient was calculated by the following formula.

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

f) Factorial analysis: Factorial analysis was done as per of the following skeleton



In the present study the factorial analysis was done on the basis of a standard two-way analysis of variance model. The treatment items were separated into the main factors and their interactions following mutually independent, orthogonal and also valid comparisons, on the basis of following rules:

Rule 1: The function $C = \Sigma (K_i T_i)$ is a valid comparison if $\Sigma K_i = 0$,

where K is the co-efficient, either +1 or – 1.

Rule 2: If C is a comparison among the treatment totals, then $C^2/ r\Sigma K_i^2$ is a part of squares with 1 degree of freedom that is part of the treatment sum of square. Here, the numerator of this sum of squares is simply the square of the appropriate linear comparison. The denominator, on the other hand, is the product of r and ΣK_i^2. Now r is the replication factor, the number of observations which have been summed in order to obtain the treatment (dose was considered as treatment) totals. It also can be denoted as n. At last, denominator can be rewrite as 8r. So the associated sum of square will be equal to $C^2/8r$.

Rule 3: According to rule 3 for the factorial analysis seven comparisons under the study are orthogonal as sum of the cross- products of their

co-efficient is zero. That is

Treatments	$N_0P_0K_0$ T_1	$N_1P_0K_0$ T_2	$N_0P_1K_0$ T_3	$N_0P_0K_1$ T_4	$N_1P_1K_0$ T_5	$N_0P_1K_1$ T_6	$N_1P_0K_1$ T_7	$N_1P_1K_1$ T_8
N (C_1) =	$K_{11}T_1$	$K_{12}T_2$	$K_{13}T_3$	$K_{14}T_4$	$K_{15}T_5$	$K_{16}T_6$	$K_{17}T_7$	$K_{18}T_8$
P (C_2) =	$K_{21}T_1$	$K_{22}T_2$	$K_{23}T_3$	$K_{24}T_4$	$K_{25}T_5$	$K_{26}T_6$	$K_{27}T_7$	$K_{28}T_8$
K (C_3) =	$K_{31}T_1$	$K_{32}T_2$	$K_{33}T_3$	$K_{34}T_4$	$K_{35}T_5$	$K_{36}T_6$	$K_{37}T_7$	$K_{38}T_8$

Then these comparisons are orthogonal if and only if

NP (C_4)	$+K_{11}.K_{21}$	$+K_{12}.K_{22}$	$+K_{13}.K_{23}$	$+K_{14}.K_{24}$	$+K_{15}.K_{25}$	$+K_{16}.K_{26}$	$+K_{17}.K_{27}$	$+K_{18}.K_{28}$
NK (C_5)	$+K_{11}.K_{31}$	$+K_{12}.K_{32}$	$+K_{13}.K_{33}$	$+K_{14}.K_{34}$	$+K_{15}.K_{35}$	$+K_{16}.K_{36}$	$+K_{17}.K_{37}$	$+K_{18}.K_{38}$
PK (C_6)	$+K_{21}.K_{31}$	$+K_{22}.K_{32}$	$+K_{23}.K_{33}$	$+K_{24}.K_{34}$	$+K_{25}.K_{35}$	$+K_{26}.K_{26}$	$+K_{27}.K_{37}$	$+K_{28}.K_{38}$
NPK (C_7)	$+K_{11}.K_{21}K_{31}$	$+K_{12}.K_{22}K_{32}$	$+K_{13}.K_{23}K_{33}$	$+K_{14}.K_{24}K_{4}$	$+K_{15}.K_{25}K_{35}$	$+K_{16}.K_{26}K_{36}$	$+K_{17}.K_{27}K_{37}$	$+K_{18}.K_{28}K_{38}$

In the present experiment, C_1 & C_2

$(-1)(-1) + (1)(-1) + (-1)(1) + (-1)(-1) + (1)(1) + (1)(-1) + (-1)(-1) + (1)(1) = 0$

The sums of cross products of the coefficients of $C_1 C_2$, C_1C_3 and C_2C_3 are also zero. All seven comparisons and hence also their respective sum of squares are therefore, mutually orthogonal.

Dose	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
N	0	1	0	0	1	1	0	1
P	0	0	1	0	1	0	1	1
K	0	0	0	1	0	1	1	1
Total treatment								
N	-1	1	-1	-1	1	1	-1	1
P	-1	-1	1	-1	1	-1	1	1
K	-1	-1	-1	1	-1	1	1	1
NP	1	-1	-1	1	1	-1	-1	1
NK	1	-1	1	-1	-1	1	-1	1
PK	1	1	-1	-1	-1	-1	1	1
NPK	-1	1	1	1	-1	-1	-1	1

Rule 4: If among '<u>t</u>' treatment totals, t – 1 comparisons are mutually orthogonal then the sum of their associated sum of squares is equal to the treatment SS. In this case, there are eight treatments or doses so, t-1 = 8-1 = 7 comparisons have been obtained (as above mentioned)

Recalculation has been done of the above 7 items according to this rule is as follows:

$$N_{SS} = (- T_1 + T_2 - T_3 - T_4 + T_5 + T_6 - T_7 + T_8)^2/n$$

$$P_{SS} = (-T_1 - T_2 + T_3 - T_4 + T_5 - T_6 + T_7 + T_8)^2/n$$

$$K_{SS} = (- T_1 - T_2 - T_3 + T_4 - T_5 + T_6 + T_7 + T_8)^2/n$$

$$NP_{SS} = (T_1 - T_2 - T_3 + T_4 + T_5 - T_6 - T_7 + T_8)^2/n$$

$$NK_{SS} = (T_1 - T_2 + T_3 - T_4 - T_5 + T_6 - T_7 + T_8)^2/n$$

$$PK_{SS} = (T_1 + T_2 - T_3 - T_4 - T_5 - T_6 + T_7 + T_8)^2/n$$

$$NPK_{SS} = (-T_1 + T_2 + T_3 + T_4 - T_5 - T_6 - T_7 - T_8)^2/n$$

RESULTS

The present investigation deals with variance and factorial analysis and correlation, of some agronomical characters viz. root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight (FPdWt), dry pod weight (DPdWt), number of seed per plant (NSPP), yield per plant (YPP) and 100-seed weight (100-SWt). Results obtained are presented under the following sub-heads:

A. STUDY OF VARIABILITY:

1. Analysis of variance with factorial analysis of variance

The present investigation, containing factorial analysis to see the individual fertilizer effects was done. The results of the analysis of variance with factorial analysis are shows in Table 12 (A-G). The line items were highly significant for all the characters. The block effects were non-significant for the characters, except FRWt, RV, NW, and NSPP. The dose effects were significant for all the characters, except FPdWt, NPdPP and 100-SWt. The nitrogen dose (N) was also significant for all the characters, except YPP, FPdWt and NPdPP. The phosphorous (P) dose was significant for all the characters, except FRWt and NPdPP. The potassium (K) effect was found to be significant for eight characters, such as RL, FPWt, RV, NN, FPdWt, DPdWt, NSPP and YPP and the other characters, such as SL, FRWt, NWt, NPdPP and 100-SWt were non-significant. Nitrogen (N) interacted with phosphorous (P) as indicated by the significant interaction item for seven characters viz. RL, SL, FPWt, NN, RV, FPdWt, and NSPP and the other characters, such as FRWt, NWt, DPdWt, NPdPP, YPP and 100-SWt were non-significant. The (B×L) interaction item was significant for seven character, such as RL, SL, FRWt, NN, NWt, DPdWt and NPdPP, while for other six characters, viz. FPdWt, RV, FPdWt, NSPP, YPP and 100-SWt, this item was non-significant. The interaction item, (B×D) was significant for the six characters, viz. RL, SL, FRWt, NN, NWt and DPdWt, wheras for other seven

characters, such as FPdWt, RV, FPdWt, NSPP, NPdPP, 100-SWt and YPP, it was non-significant. The second order interaction item (B×D×L) was observed to be significant for the ten characters, such as RL, SL, FPWt, NN, RV, NWt, DPdWt, NSPP, NPdPP and 100-SWt but for other three characters like FRWt, FPdWt, and YPP it was non-significant.

Table 12A : Analysis of variance with factorial analysis for root length (RL) and shoot length (SL)

Items	df	RL			SL		
		SS	MS	F	SS	MS	F
Line (L)	8	1842.244	230.2805	7.467**	2430.698	303.8372	7.478**
Block (B)	1	68.80037	68.80037	2.231ns	43.38336	43.38336	1.068NS
Dose (D)	7	2026.398	289.4854	9.387**	1850.284	264.3263	6.51**
N	1	3031.503	3031.503	98.296**	6274.822	6274.822	154.45**
P	1	807.36	807.36	26.178**	619.4891	619.4891	15.245**
K	1	707.0585	707.0585	22.93**	76.56463	76.56463	1.885NS
NP	1	2242.667	2242.667	72.718**	706.335	706.335	17.386**
NK	1	70.72667	70.72667	2.293NS	6.475741	6.475741	0.16NS
PK	1	626.963	626.963	20.3291**	2438.822	2438.822	60.03**
NPK	1	8724.907	8724.907	282.90**	4679.765	4679.765	115.19**
D×L	56	8837.617	157.8146	5.118**	4842.413	86.47166	2.13**
B×L	8	1507.034	188.3793	6.108**	2186.846	273.3558	6.73**
B×D	7	1034.942	147.8489	4.794*	1195.598	170.7997	4.204**
B×D×L	56	8220.257	146.7903	4.76**	5315.438	94.91854	2.34**
Error	288	8882.127	30.84072		11700.73	40.62752	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12B : Analysis of variance with factorial analysis for fresh plant weight (FPWt) and fresh root weight (FRWt)

Items	df	FPWt			FRWt		
		SS	MS	F	SS	MS	F
Line (L)	8	2967.575	370.9469	3.64**	84.13083	9.766354	3.745**
Block (B)	1	18.82507	18.82507	0.185NS	30.5692	30.5692	12.88**
Dose (D)	7	4540.701	648.6716	6.367**	34.175	4.88214	2.056**
N	1	19314.98	19314.98	189.55**	11.95682	11.95682	5.04NS
P	1	650.5418	650.5418	6.384*	4.056296	4.056296	1.71NS
K	1	3894.635	3894.635	38.22**	6.006669	6.006669	2.53NS
NP	1	1837.36	1837.36	18.031**	3.270817	3.270817	1.378NS
NK	1	0.236811	0.236811	0.01NS	2.684474	2.684474	1.131NS
PK	1	1632.994	1632.994	16.03**	0.823869	0.823869	0.347NS
NPK	1	8994.863	8994.863	88.27**	16.97923	16.97923	7.152**
D×L	56	10075.23	179.9148	1.766*	174.7713	3.120915	1.315NS
B×L	8	1002.016	125.252	1.23NS	78.4664	9.808299	4.132*
B×D	7	1204.474	172.0678	1.69NS	51.02998	7.289997	3.071*
B×D×L	56	12235.56	218.4922	2.15**	101.019	1.803912	0.76NS
Error	288	29347.81	101.9021		683.7339	2.374076	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12C : Analysis of variance with factorial analysis for nodule number (NN) and root volume (RV)

Items	df	NN			RV		
		SS	MS	F	SS	MS	F
Line (L)	8	1870.825	233.8531	7.58**	112.5061	14.06327	5.08**
Block (B)	1	66.42676	66.42676	2.153NS	50.10866	50.10866	18.11**
Dose (D)	7	2054.574	293.5106	9.51**	252.0562	36.00802	13.01**
N	1	3046.507	3046.507	98.715**	1494.608	1494.608	540.188**
P	1	815.1119	815.1119	26.412**	15.46364	15.46364	5.589*
K	1	714.3141	714.3141	23.146**	37.00663	37.00663	13.38**
NP	1	2333.796	2333.796	75.621**	138.8134	138.8134	50.17**
NK	1	64.02667	64.02667	2.075NS	19.1757	19.1757	6.931**
PK	1	661.5	661.5	21.435**	15.01107	15.01107	5.425**
NPK	1	8801.34	8801.34	285.188**	296.3708	296.3708	107.12**
D×L	56	8858.566	158.1887	5.126**	502.0882	8.96586	3.241*
B×L	8	1516.783	189.5979	6.144*	38.89613	4.862017	1.757NS
B×D	7	1042.434	148.9192	4.825*	17.90229	2.55747	0.924NS
B×D×L	56	8188.556	146.2242	4.74**	532.6744	9.512043	3.44**
Error	288	8888.127	30.86155		796.846	2.766827	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12D : Analysis of variance with factorial analysis for nodule weight (NWt) and number of pod per plant (NPdPP)

Items	df	NW			NPdPP		
		SS	MS	F	SS	MS	F
Line (L)	8	0.457062	0.057133	3.179**	42.96719	5.370899	3.968**
Block (B)	1	0.119235	0.119235	6.64**	0.317417	0.317417	0.234 NS
Dose (D)	7	0.30942	0.044203	2.46*	5.346787	0.763827	0.564 NS
N	1	0.55551	0.55551	30.91**	4.228002	4.228002	3.124 NS
P	1	0.33182	0.33182	18.461**	1.28498	1.28498	0.949 NS
K	1	0.036973	0.036973	2.06NS	0.780002	0.780002	0.576 NS
NP	1	0.0602	0.0602	3.35NS	2.045557	2.045557	1.512 NS
NK	1	0.171479	0.171479	9.54**	33.40187	33.40187	24.68**
PK	1	0.293488	0.293488	16.329**	0.028017	0.028017	0.021 NS
NPK	1	1.025894	1.025894	57.076**	1.005869	1.005869	0.743 NS
D×L	56	2.789245	0.049808	2.771**	159.4353	2.847059	2.104*
B×L	8	0.671638	0.083955	4.671**	22.16235	2.770293	2.047*
B×D	7	0.537744	0.076821	4.274**	15.49599	2.213713	1.636 NS
B×D×L	56	2.509108	0.044806	2.493**	153.2236	2.736135	2.022**
Error	288	5.176552	0.017974		389.7599	1.353333	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12E : Analysis of variance with factorial analysis for fresh pod weight (FPdWt) and dry-pod weight (DPdWt)

Items	df	FPdWt			DPdWt		
		SS	MS	F	SS	MS	F
Line (L)	8	47.36803	5.921004	2.78*	2164.723	270.5903	6.638**
Block (B)	1	3.55486	3.55486	1.67NS	0.880208	0.880208	0.021NS
Dose (D)	7	16.78593	2.397989	1.126NS	1151.782	164.5403	4.036*
N	1	6.662686	6.662686	3.129NS	3355.935	3355.935	82.328**
P	1	32.46786	32.46786	15.246**	1007.942	1007.942	24.727**
K	1	9.315096	9.315096	4.374*	173.1646	173.1646	4.248*
NP	1	24.06938	24.06938	11.302*	59.95574	59.95574	1.471NS
NK	1	2.698763	2.698763	1.267NS	351.135	351.135	8.614**
PK	1	48.18178	48.18178	22.625**	1808.449	1808.449	44.365**
NPK	1	10.89184	10.89184	5.115*	2457.676	2457.676	60.292**
D×L	56	204.2081	3.646573	1.712*	4746.825	84.76473	2.079*
B×L	8	9.622022	1.202753	0.565NS	2116.171	264.5214	6.489*
B×D	7	28.70668	4.100954	1.926NS	1683.107	240.4439	5.898*
B×D×L	56	137.3316	2.45235	1.152NS	4528.847	80.87226	1.984*
Error	288	613.3179	2.129576		11739.69	40.7628	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12F : Analysis of variance with factorial analysis for number seed per plant (NSPP) and yield per plant (YPP)

Items	df	NSPP			YPP		
		SS	MS	F	SS	MS	F
Line (L)	8	106.6314	13.32892	4.76**	49.18838	6.148547	3.246**
Block (B)	1	46.76077	46.76077	16.69**	1.029407	1.029407	0.544NS
Dose (D)	7	237.3775	33.91107	12.106**	29.64603	4.235147	2.236*
N	1	1401.41	1401.41	500.31**	0.055552	0.055552	0.03NS
P	1	12.41953	12.41953	4.434*	41.83872	41.83872	22.09**
K	1	23.60563	23.60563	8.427*	73.79897	73.79897	38.965**
NP	1	129.3602	129.3602	46.182*	2.319646	2.319646	1.225NS
NK	1	31.40204	31.40204	11.21*	3.439303	3.439303	1.816NS
PK	1	18.34118	18.34118	6.548*	76.20307	76.20307	40.234**
NPK	1	282.4811	282.4811	100.85**	39.51298	39.51298	20.862**
D×L	56	504.6987	9.012477	3.22**	166.2385	2.968545	1.567*
B×L	8	39.25549	4.906936	1.752NS	9.101683	1.13771	0.60NS
B×D	7	19.75152	2.821645	1.01NS	9.835137	1.40502	0.742NS
B×D×L	56	529.047	9.447268	3.373*	112.7593	2.013558	1.063NS
Error	288	806.7127	2.801086		545.4675	1.893984	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

Table 12G : Analysis of variance with factorial analysis for and 100 seed weight (100-SWt)

Items	df	100-SWt		
		SS	MS	F
Line (L)	8	30.04412	3.755515	2.382*
Block (B)	1	0.511845	0.511845	0.325 NS
Dose (D)	7	7.928092	1.132585	0.718*
N	1	7.370417	7.370417	4.675*
P	1	5.060017	5.060017	3.21*
K	1	0.677824	0.677824	0.43 NS
NP	1	1.674817	1.674817	1.062 NS
NK	1	48.29898	48.29898	30.64**
PK	1	0.30375	0.30375	0.193 NS
NPK	1	0.038935	0.038935	0.025 NS
D×L	56	151.3911	2.703412	1.715*
B×L	8	18.79481	2.349351	1.49 NS
B×D	7	13.94215	1.991735	1.263 NS
B×D×L	56	159.102	2.841107	1.802*
Error	288	453.988	1.576347	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance

2. Components of variation:

The estimates of phenotypic (k^2_p), genotypic (k^2_g), block× line ($k^2_{B\times L}$), dose × line ($k^2_{D\times L}$), block × dose ($k^2_{R\times Y}$) and block × dose × line ($k^2_{B\times D\times L}$) and within (σ^2_W) error components of variation were calculated separately for all the thirteen characters. The results are shown in Table 13 for nine lines of black gram.

a) Phenotypic variation (k^2_p): For all the characters phenotypic variation (k^2_p) was always greater than those of k^2_g, $k^2_{B\times L}$, $k^2_{D\times L}$, $k^2_{B\times D}$, $k^2_{B\times D\times L}$ and σ^2_W components of variation as expected. The phenotype is the joint product of k^2_g, $k^2_{B\times L}$, $k^2_{D\times L}$, $k^2_{B\times D\times L}$ and σ^2_W. Table 13 shows that greater portion of the total phenotypic variation appeared mostly due to the within error variance for all the characters. The maximum phenotypic variation was found for the character, FPWt with a value of 162.944 and the lowest value of 0.038 for NWt.

b) Genotypic variation (k^2_g): The highest genotypic variation was observed for the character, FPWt with a value of 5.605 and the lowest genotypic variation for FRWt with a value of -0.03 (Table 13).

c) B×L interaction variation ($k^2_{B\times L}$): The B×L interaction variation (Table 13) was the highest with a value of 9.697 for SL and the lowest value of -0.037 was recorded for FPdWt.

d) D×L interaction variation ($k^2_{D\times L}$): The estimation of the interaction variation ($k^2_{D\times L}$) was the highest for NN with a value of 21.221 and was the lowest with a value of 0.005 for NWt (Table 13).

e) B×D interaction variation ($k^2_{B\times D}$): The highest interaction variation ($k^2_{B\times D}$) with a value of 7.398 was recorded for DPdWt and the lowest was recorded with a value of -0.018 for YPP (Table 13).

f) B×D×L interaction variation ($k^2_{B\times D\times L}$): The estimates of the interaction variation ($k^2_{B\times D\times L}$) was the highest for FPWt with a value of 38.863 and the lowest was recorded with a value of -0.19 for FRWt.

g) Within error variation (σ^2_W): The highest within error variation (σ^2_W) was recorded for the character, FPWt, which was 102.902 and the lowest value of 0.018 was recorded for NWt.

3. Co-efficient of variability:

The estimates of phenotypic (PCV), genotypic (GCV), interactions $(B\times L)_{CV}$, $(D\times L)_{CV}$, $(B\times D)_{CV}$ & $(B\times D\times L)_{CV}$ and within error co-efficient of variability (ECV) were computed for all the thirteen characters, viz. RL, SL, FPWt, FRWt, NN, RV, NWt, FPdWt, DPdWt, NPdPP, NSPP, YPP & 100-SWt, for nine lines of blackgram and the results obtained are shown in Table 14.

a) Phenotypic co-efficient of variability (PCV): Table 14 shows that the estimates of phenotypic co-efficient of variability were the highest for FPWt with a value of 850.25 and the lowest value of 20.394 was recorded for NWt.

b*) Genotypic co-efficient of variability (GCV):* The highest genotypic co-efficient of variability (GCV) with a value of 29.248 was recorded for FPWt, while the lowest value of –0.376 was found for FRWt.

c) B×L interaction co-efficient of variability $(B\times L)_{CV}$: The highest value of 30.899 was recorded for SL and the lowest value of -1.844 was recorded for FPWt.

d) D×L interaction co-efficient of variability $(D\times L)_{CV}$: The estimate of $(D\times L)_{CV}$ was the highest for FPWt with a value of 67.85 and the lowest value was 2.85 for NWt

e) B×D interaction co-efficient of variability $(B\times D)_{CV}$: For $(B\times D)_{CV}$ the highest value of 23.622 was recorded for DPdWt and the lowest value of -0.94 was recorded for YPP.

f) B×D×L interaction co-efficient of variability $(B\times D\times L)_{CV}$: The estimate of $(B\times D\times L)_{CV}$ was the highest for FPWt with a value of 202.791 and the lowest value of –5.648 was for FPRWt.

g) Within error co-efficient of variability (ECV): The highest within error co-efficient of variability (ECV) was recorded for FPWt with a value of 531.731 and the lowest variability was -9.66 for NWt.

Table 13 : Components of variation of nine lines for different characters in blackgram

Characters	k^2_P	k^2_g	$k^2_{B\times L}$	$k^2_{D\times L}$	$k^2_{B\times D}$	$k^2_{B\times D\times L}$	k^2_W
RL	105.706	4.155	6.564	21.162	4.334	38.65	30.841
SL	86.367	5.484	9.697	7.641	4.821	18.097	40.628
FPWt	162.944	5.605	0.9729	13.002	2.599	38.864	101.902
FPWt	2.788	-0.013	0.3098	0.1245	0.182	-0.1901	2.3741
NN	105.753	4.229	6.614	21.221	4.373	38.454	30.862
RV	6.3633	0.235	0.087	1.0332	-0.008	2.2484	2.767
NWt	0.038	0.001	0.003	0.005	0.0022	0.0089	0.018
NPdPP	2.238	0.084	0.059	0.249	0.032	0.461	1.353
FPdWt	2.2683	0.0727	-0.037	0.264	0.008	0.1113	1.849
DPdWt	82.973	4.788	9.323	7.334	7.396	13.37	40.763
NSPP	6.3596	0.219	0.0878	1.035	0.001	2.2154	2.801
YPP	2.152	0.089	-0.031	0.18	-0.018	0.04	1.894
100-SWt	2.2788	0.0454	0.032	0.188	0.0154	0.423	1.5764

Table 14 : Co-efficient of variability for nine lines for different characters in blackgram

Characters	P_{cv}	G_{cv}	$B\times L_{cv}$	$D\times L_{cv}$	$B\times D_{cv}$	$B\times D\times L_{cv}$	E_W
RL	296.984	11.674	18.442	59.4563	12.176	108.588	86.648
SL	275.202	17.473	30.899	24.346	15.3624	57.665	129.457
FPWt	850.253	29.248	5.077	67.846	13.5603	202.791	531.731
FRWt	82.837	-0.376	9.2047	3.699	5.411	-5.648	70.547
NN	297.212	11.885	18.588	59.641	12.289	108.074	86.7348
RV	192.002	7.1011	2.6342	31.174	-0.234	67.842	83.484
NWt	20.397	0.4383	1.4769	2.8503	1.171	4.805	9.6559
NPdPP	49.536	1.853	1.307	5.511	0.7054	10.203	29.957
FPdWt	124.301	3.772	-1.844	12.072	3.4862	5.1371	101.6787
DPdWt	265.022	15.293	29.779	23.424	23.622	42.704	130.1989
NSPP	190.69	6.577	2.631	31.041	0.0228	66.428	83.99
YPP	111.537	4.594	-1.633	9.283	-0.939	2.066	98.166
100-SWt	49.8564	0.993	0.705	4.1098	0.3366	9.224	34.488

4. Heritability(h^2_b), genetic advance (GA) and genetic advance as a percentage of mean(GA%):

For all the characters heritability in broad sense (h^2_b), genetic advance and genetic advance as a percentage of mean (GA%) were computed and the results are shown in Table 15.

a) Heritability (h^2_b): The values for heritabilities in broad sense (h^2_b) were presented in Table 15. The highest heritability value was estimated for the character, SL with a value of 6.349 and the lowest value of 0.454 was recorded for FRWt.

b) Genetic advance (GA): The highest value of genetic advance was estimated for the character, SL with a value of 1.216 and the lowest value of 0.016 was recorded for FRWt.

C) Genetic advance as a percentage of mean (GA%): The highest GA% was found for YPP with a value of 6.451 and the lowest for the same was shown by the character, FRWt with a value of 0.462.

Table 15 : Heritability (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%) for different characters in blackgram

Characters	h^2_b	GA	GA%
RL	3.930722	0.832508	2.338963
SL	6.349113	1.215497	3.873095
FPWt	3.439887	0.904547	4.71998
FRWt	0.45418	0.01562	0.46419
NN	3.998952	0.847147	2.380864
RV	3.698439	0.192188	5.798952
NW	2.148657	0.008625	4.633298
NPdPP	3.740205	0.115259	2.551329
FPdWtPP	3.034054	0.100846	4.815003
DPdWtPP	5.770625	1.082827	3.458616
NSPP	3.448829	0.179165	5.372216

B. CORRELATION CO-EFFICIENT:

The estimation of correlation co-efficient was done at phenotypic level of nine lines of black gram and the results obtained are shows in Table 16. The total number of characters were 13, so 78 pairs of characters and hence 78 correlation co-efficient values to be calculated.

RL showed positive significant correlation with NPdPP and FPdWt, while positive non-significant correlation was found with SL, FPWt, NN, DPdWt and 100-SWt, and negative non-significant correlation was with FRWt, RV, NWt, NSPP and YPP. SL exhibited positive significant correlation with Rv, NWt, and NN and non-significant positive correlation with RV, NWt and NPdPP, and negative non-significant correlation with FPWt, DPdWt, NSPP, YPP and 100-SWt. The correlation of FPWt was found to be positively significant with FRWt, NN, RV and NWt, while negatively non-significant with NPdPP, FPdWt, DPdWt and NSPP, and with other two characters (YPP and 100-SWt) it was positively non-significant. FRWt showed positively significant correlation with RV, while with NN, NPdPP, FPdWt and NSPP it indicated negatively non-significant correlation and positively non-significant correlation with NWt, DPdWt, YPP and 100-SWt. A significant positive correlation was exhibited by NN with NWt, NPdPP, YPP and 100-SWt, and non-significant positive significant correlation with RV, DPdWt and NSPP and negatively non-significant correlation with FPdWt. The correlation co-efficient of RV was found to be positively significant with NPdPP and FPdWt, while negatively non-significant with NWt, NSPP and YPP and with other two characters (DPdWt and 100-SWt) it was positively non-significant. NWt showed positively significant correlation with NPdPP, FPdWTt, YPP and 100-SWt and negatively significant correlation with NSPP, while negatively non-significant correlation with DPdWt. NPdPP exhibited positively significant correlation with NSPP, YPP and 100-SWt, and positively non-significant correlation with DPdWt, while negatively non-significant correlation with FPWt. FPdWt showed significant negatively correlation with NSPP and 100-SWt, while non-significant but positive correlation with YPP and non-significant negatively correlation with DPdWt. DPdWt exhibited positively significant correlation with NSPP, YPP and 100-SWt. The correlation of NSPP was found to be positively significant with YPP and 100-SWt. YPP exhibited positively significant correlation with 100-SWt.

Table 16 : Correlation co-efficient among the thirteen quantitative characters in black gram

	SL	FPWt	FRWt	NN	RV	NWt	NPdPP	FPdWt	DPdWt	NSPP	YPP	100-SWt
RL	0.102	0.342	-0.31	0.05	-0.26	-0.05	0.672*	0.721*	0.242	-0.14	-0.09	0.231
SL		0.682*	0.664*	0.71*	0.042	0.103	0.321	-0.425	-0.514	0.342	-0.14	-0.412
FPWt			0.701*	0.80*	0.912*	0.671*	-0.421	-0.245	-0.213	-0.401	0.045	0.021
FRWt				-0.01	0.661*	0.021	-0.231	-0.123	0.012	-0.001	0.321	0.256
NN					0.014	0.756*	0.683*	-0.012	0.356	.0425	0.801*	0.6672*
RV						-0.012	0.662*	0.691*	0.156	-.0531	-0.123	0.005
NWt							0.731*	0.681*	-0.124	-0.675*	0.863*	0.673*
NPdPP								-0.102	0.321	0.682*	0.721*	0.901*
FPdWt									-0.425	-0.731*	0.142	-0.665*
DPdWt										0.802*	0.673*	0.664*
NSPP											0.783*	0.692*
YPP												0.831*

df = 7

Indicated significance at 5% level, respectively.

DISCUSSION

Genetic information on the inheritance of quantitative characters is necessary for preparation of the effective meaningful breeding programme on crop for its improvement. Moreover, some reports on the inheritance of quantitative characters of blackgram (*Vigna mungo* (L.) Hepper) have been reported by different investigations. The present investigation was carried out to do the variance and factorial analysis, variability, heritability, genetic advance, and correlation among thirteen quantitative characters in nine lines of blackgram.

In the present investigation, it was found that the characters under study viz. root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight (FPdWt), dry pod weight (DPdWt), number of seeds per plant (NSPP), 100-seed weight and yield per plant (YPP) are economically important and quantitative in nature in their inheritance, because continuous gradation were found among the collected data on the characters. The quantitative nature of the characters of blackgram were also reported by Hossain (1977), Sharoar (2002), Rahman (2002) and Islam (2002). Therefore, the biometrical techniques developed to study the quantitative characters were found suitable to estimate the genetic system involved in controlling these characters. First, Mather (1949) developed biometrical techniques based on the mathematical models of Fisher *et al.* (1932). The nine lines of blackgram included in the present study showed a wide and pronounced range of variation, co-efficient of variability, genetic advance as indicated that all the thirteen characters under study were quantitative in nature and are under polygenic control. The wide range of variation emphasizes the importance of genetic combination in blackgram breeding programme. Hossain (1977) also reported the similar nature of the characters in blackgram, Chandra (1973) in gram, Bhargava *et al.* (1966) in green gram, Sharoar (2002) and Rahman (2002) both in blackgram obtained similar results.

The present investigation, containing factorial analysis to see the individual fertilizer effect was done. The results of the analysis of variance with factorial analysis are shows in Table 12 (A-G). The line items were highly significant for all the characters, which indicated that

the lines were genetically different from each other. This referred that the included lines would be suitable for further breeding research for the improvement of these characters. Similar records were also made by Samad (1981) in his investigation of certain agronomical characters in rape seed. Ali (1988) found similar result in ten lentil cultivars. Khaleqe *et al.* (1991) also obtained similar records with some chemical character in chilli. The block effects were non-significant for the characters, except FRWt, RV, NW, and NSPP. Similar records were also made by Sharoar (2002) and Islam (2002) in their investigation of some quantitative characters, except FRWt, RV, NWt and NSPP, where it was significant. While, significant block item for different characters indicated difference between blocks. The dose items were significant for all the characters, except FPdWt, NPdPP and 100-SWt, while significance indicated real effect of eight fertilizer doses on these characters. Khan *et al.* (2000) also recorded a real effect of different treatments (N, P, K and their combination) on six agronomical characters in chilli. The effect of nitrogen dose (N) was also significant for all the characters, except YPP, FPdWt and NPdPP. The dose effect for phosphorous (P) was significant for all the characters, except FRWt and NPdPP. The effect due to potassium (K) was found to be significant for eight characters, such as RL, FPWt, RV, NN, FPdWt, DPdWt, NSPP and YPP and the other characters, such as SL, FRWt, NWt, NPdPP and 100-SWt were non-significant. Nitrogen (N) interacted with phosphorous (P) as indicated by the significant interaction item for the seven characters, viz. RL, SL, FPWt, NN, RV, FPdWt and NSPP and the other characters, such as FRWt, NWt, DPdWt, NPdPP, YPP and 100-SWt, were non-significant. The (B×L) interaction item was significant for seven characters, such as RL, SL, FRWt, NN, NWt, DPdWt and NPdPP, while for other six characters, viz. FPdWt, RV, FPdWt, NSPP, YPP and 100-SWt, this item was non-significant. Paul and Sarker (1989) found significant effect of N, P and K fertilizers for leaf area, dry-matter, production of relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), and relative leaf growth (RLG), at all the five harvest intervals of mustard. Sen *et al.* (1977) and Joarder *et al.* (1979) also observed significant effect of fertilizers on seeds/pods and yield per plant of mustard. Fertilizer is an important factor, which influence germination, growth, development and yield. Not only black gram but also for other crops, fertilizer increases yield. Sharma *et al.* (2001) also recorded significant effect of P on seed yield and biological yield in green gram. The interaction (B×D) item was significant for the six characters, viz. RL, SL, FRWt, NN, NWt and DPdWt, while for other seven characters,

such as FPdWt, RV, FPdWt, NSPP, NPdPP, 100-SWt and YPP it was non-significant. The second order interaction (B×D×L) item was observed to be significant for the ten characters, such as RL, SL, FPWt, NN, RV, NWt, DPdWt, NSPP, NPdPP and 100-SWt but for other three characters like FRWt, FPdWt and YPP it was non-significant.

In the analysis of components of variance it was found that greater portion of the total phenotypic variation appeared to be mostly genotypic variation for all the characters (Table 13). Therefore, greater portion of phenotypic variation was genetic in nature. The present study the highest phenotypic variation was observed by FPWt followed by NN, RL, DPdWt, RV and NSPP and genotypic variation was exhibited by FPWt followed by SL, DPdWt, NN and RL. So, low genotypic variation was found for all the characters. Phenotypic co-efficient of variability was greater than genotypic and all other coefficient of variability. Low genetic co-efficient of variability was found for all the characters, which indicated that, these characters were inherited with lower variability within their sibs. Singh and Malhotra (1970) studying cowpea and found the highest genotypic co-efficient of variation for number of pod per plant. Sethi *et al.* (1972) found the highest co-efficient of variability for number of pod per plant in gram. High value for $(D\times L)_{CV}$, $(B\times D\times L)_{CV}$, E_{CV} and $(B\times D)_{CV}$, were found for FPWt and DPdWt, respectively. Such high value suggests a good scope for the improvement of these characters through selection (Saha *et al.* 1981). Khurana and Sandhu (1972) obtained the highest estimation of phenotypic and genotypic co-efficient of variability for pod per plant in *Glycin max* L. Low genetic co-efficient of variability was found for maximum characters, which indicated that these characters were inherited with lower variability within their sibs.

Heritability in broad sense (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%) were computed and the results are shows in Table 15. In respect of heritability, all of the characters under study showed low values. Coyne (1968) estimated a low heritability for seed yield and yield components in field bean. Chandra (1968) observed that the heritability estimate was affected by environment in gram. High error component of variation causes a low estimation of heritability. Low heritability as well as low values of genetic advance and genetic advance as percentage of mean were noted for NSPP. The expression of those characters may likely be conditioned by non-additive gene effect (Panse, 1957). So the characters under investigation may likely be conditioned by non-additive

gene effect. Low genetic advance and genetic advance as a percentage of mean values were noted for all the characters.

The estimation of correlation co-efficient was done at phenotypic level of nine lines of black gram and the results obtained are shows in Table 16. The total number of characters were 13, so 78 pairs of characters and hence 78 correlation co-efficient values were calculated. RL showed positive significant correlation with NPdPP and FPdWt, while positive non-significant correlation was found with SL, FPWt, NN, DPdWt and 100-SWt, and negative non-significant correlation with FRWt, RV, NWt, NSPP and YPP. SL exhibited positive significant correlation with FPWt, FRWt and NN, and non-significant positive correlation with RV, NWt and NPdPP, and negative non-significant with FPWt, DPdWt, NSPP, YPP and 100-SWt. The correlation of FPWt was found to be positively significant with FRWt, NN, RV and NWt, while negatively non-significant with NPdPP, FPdWt, DPdWt and NSPP and with other two characters (YPP and 100-SWt) it was positively non-significant. FRWt showed positively significant correlation with RV, while with NN, NPdPP, FPdWt and NSPP it indicated negatively non-significant correlation and positively non-significant correlation with NWt, DPdWt, YPP and 100-SWt. A significant positive correlation was exhibited by NN with NWt, NPdPP, YPP and 100-SWt, and non-significant positive significant correlation with RV, DPdWt and NSPP and negatively non-significant correlation with FPdWt. The correlation co-efficient of RV was found to be positively significant with NPdPP and FPdWt, while negatively non-significant with NWt, NSPP and YPP, and with other two characters (DPdWt and 100-SWt) it was positively non-significant. NWt showed positively significant correlation with NPdPP, FPdW, YPP and 100-SWt and negatively significant correlation with NSPP, while negatively non-significant correlation with DPdWt. Correlation studies were also done by Singh and Mathotra (1970) in mugbean, Varma and Dubey (1970) in black gram, Nandpuri *et al.* (1973) in tomato, Singh and Mehndiratra (1970) in cowpea, Salehuzzaman *et al.* (1979) in soybean, Singh *et al.* (1973) in table pea, Nandpuri and Kumar (1973) in pea and Islam *et al.* (1997) in mungbean and found that NPdPP positively correlated with YPP which is similar with present investigation. While, with NPdPP and DPdWt showed positively non-significant correlation and negatively non-significant correlation with FPWt. FPdWt showed significant negative correlation with NSPP and 100-SWt, while non-significant but positive correlation

with YPP and non-significant negative correlation with DPdWt. DPdWt exhibited positively significant correlation with NSPP, YPP and 100-SWt. The correlation of NSPP was found to be positively significant with YPP and 100-SWt. YPP exhibited positively significant correlation with 100-SWt.

In the present investigation through factorial analysis it has been perceived that the individual effect of the fertilizers is more important for root and shoot characters than yield, while yield itself showed increase response with combined fertilizer dose instead of single dose particularly nitrogen. However, all the root and shoot characters and yield per plant except NPdPP and 100-SWt showed the importance of combined dose ie. NPK in black gram. Therefore, in low dose application of this combined dose (NPK) may likely increase yield in black gram.

Further, it has been also detected through the present investigation that nodulation (NN and NWt) has increased response with the application of combined dose ie. NPK. Therefore, application of this combination of fertilizers (NPK) is likely to increase nodulation which intern will insure high measurement of yield components and high yield since nodule characters showed significant positive correlation with yield and yield contributory characters and yield components exhibited significant positive correlation among themselves and with yield per plant.

Nevertheless, due to low heritability care showed taken during selection of the black gram lines for high nodule characters, yield components and yield. Low heritability may be resulted because of high sampling and/or non-additive variation, which have been found to be prominent in the present population of black gram. However sampling variation may be controlled through rigorous field management.

SUMMARY

Variability of thirteen agronomical and economical characters of nine lines in black gram (*Vigna mungo* (L.) Hepperr) were studied during the rabi crop season from the 25th september to the 23th December, 2000. In the present investigation, it was found that the characters under study viz. root length (RL), shoot length (SL), fresh plant weight (FPWt), fresh root weight (FRWt), root volume (RV), nodule number (NN), nodule weight (NWt), number of pod per plant (NPdPP), fresh pod weight (FPdWt), dry pod weight (DPdWt), number of seed per plant (NSPP), 100-seed weight (100-SWt) and yield per plant (YPP) are quantitative in nature in their inheritance because without grouping they show continuous gradation.

In the analysis of variance all the line items were found to be highly significant for all the characters. Regarding the components of variation, it was found that through greater portion of the total phenotypic variation appeared to be mostly due to genotype, sampling variation was found to be prominent for all the characters. As a result genetic co-efficient of variability and heritability for all the characters under study showed low values of genetic advance and genetic advance as a percentage of mean also found to be for all the characters. Hence, the characters under investigation are conditioned by non-additive gene effects.

Through factorial analysis it has been detected that as individual dose nitrogen has no effect on YPP and NPdPP. On the other hand nitrogen has significant effect are NN and NWt. However, use of combined fertilizers as NPK in low dose are suggested as effect of this combined dose are significant for all the characters except, NPdPP and 100-SWt. Correlation study revealed that NN and NWt are significantly correlation with components of yield, such as NPdPP, FPdWt and 100-Swt and YPP.

However values for heritability, genetic advance and genetic advance as a percentage of mean were found to be low for almost all the characters in this population of black gram. This might be due to the higher sampling variation, which is observable from high E_w and σ^2_w for all the characters. Therefore, rigorous field management condition has been suggested in case of selection of lines for higher values for nodule characters, yield and yield components in black gram.

SECTION - III

VARIBILITY STUDY OF THE BIOCHEMICAL CHARACTERIZATION OF ROOT NODULE AND ROOT NODULE BACTERIA

INTRODUCTION

Root nodules contain bacteria which live symbiotically with the leguminous plants. The plant supply carbohydrate to the bacteria which in term provide amino acid (nitrogen) to the host plant. The root nodule bacteria may became parasitic if, for any reason the carbohydrate supply is restricted, as for example, when the plant is put in dark. The nodules may be decayed due to parasitism at the time of flowering or at the time of fruit setting.

Vigna mungo L. Hepper is a widely cultivated pulse crop of Rajshahi. The roots of this plant form very good nodules. It was found that the plants grow well in less nitrogen containing soil (Hossain, 1977; Hossain and Saha, 1979). Nodule bacteria multiply in the soil and infect the root for symbiotic association. Symbiotic association of the root nodule bacteria in leguminous plants is of great importance in agriculture. The infection of the roots by bacteria turned into the benefit of the host and the bacterium. Rhizobium is one of such root nodule bacterium. Root nodules may also be formed in non-leguminous angiosperms (Bond, 1963; Raggio and Raggio, 1962). If a bacterial strain is to have any agriculture value, it must form nodules capable of fixing appreciable amounts of nitrogen; and it is probable that there is no single strain which can form effective nodules on all plant species.

Rhizobium bacteria in the root nodules of leguminous plants are estimated to carry out 50% of the world biological nitrogen fixation, reducing approximately 20 million tones of atmospheric nitrogen to ammonia. It is also non-polluting compared to the manufacture of urea or the risk of fertilizer run-off into rivers and lakes. Leguminous plant provides sufficient nitrogen to support growth and yield in the soil. Finally leguminous are well-known colonizers of poor soils and disturbed habitats. The use of these nitrogen-fixing plants could be envisaged in areas where ground cover is required for soil stabilization, arrest of soil erosion and disidratation.

The leguminous plants establish a symbiotic relationship with the soil bacterium *Rhizobium*. The symbiosis is manifested in the formation of root nodules. The root nodules are specialised plant organs, in which, upon infection by the rhizobia, atmospheric nitrogen (N_2) is reduced to ammonia. Ammonia is then assimilated by the plant cell. This process of symbiotic nitrogen fixation permits the plants to survive on nitrogen-poor soils and thus, confers a substantial selective advantage. Likewise, the bacteria profit from the symbiotic interaction by obtaining sugar molecules produced by the plant during photosynthesis. Symbiosis between rhizobia and leguminous plants leads to the formation of N_2-fixing root nodules.

Moisture plays an important part in the growth activities of all plants. Water is indispensable for absorption and transportation of food, and to carry on photosynthesis, metabolism of materials, and the regulation of temperature. As in all other living system like the essential constituent, such as protein and carbohydrate moisture is also an essential constituent of plant. Moisture is essential for most of the physiological reactions in the plant tissues and due to its absence living system does not exist (Rangaswami, 1976). Fonseea *et al.* (1972) observed that water on the leaves improve quality and better consumption.

Proteins are the chief constituent of all living matter. It is the essence of life processes, fundamental constituents of protoplasm and are involved in the hereditary transmission. Protein, act as enzymatic catalyst, transport and storage, immune protector, hormone regulator and its helps in system of the body and also in co-ordinating motion. The amount of protein present in the root nodules of different lines decreased with the change of maturity. The decrease in protein content during maturation might be due to the increased synthesis of enzymes. Similar results have been reported in litchi (*Litchi chinensis* Sonn.) by Rahman (1989) and in kul (*Zizypus mauritiana* Lam.) by Haque (1995). Vitamin C is essential for normal growth and maintenance of living tissue and involved for protection against infection in different diseases.

In the developed countries, agriculture is dependent on manuring with emphasis more on natural manuring for achieving and maintaining the high yields that are possible with modern crop cultivars. The great challenge lies in devising more sustainable farming

systems without compromising food production levels; indeed, increased productivity will be necessary to accommodate global population growth. Synthetic nitrogen use has grown from 3 million to 80 million tons over the last 40 years. This increase occurred in both developed and developing countries. The current annual worldwide expenditure for nitrogen fertilizer exceeds $20 billion-an amount comparable to that for synthetic chemical pesticides. Modern industrial production of fertilizer nitrogen demands large inputs of energy in the form of natural gas, a finite natural resource; fertilizer constitutes a major energy cost in the production of a high-yield corn or rice crop. Moreover, carbon dioxide is released by the consumption of natural gas. Food production may thus contribute indirectly to global warming. Of the fertilizer nitrogen applied to a crop, seldom is more than 50 percent assimilated, and often the efficiency of utilization is much less. Whatever type of fertilizer nitrogen is applied, microbial action converts it to nitrate, a mobile form that is assimilated by plants and is subject to loss from surface-water movement, thereby polluting streams and rivers and eventually affecting estuarine and marine ecosystems. Furthermore, nitrate may leach into groundwater and contaminating wells and placing human health at risk. In wet soils, denitrifying bacteria convert nitrate to nitrous oxide and gaseous nitrogen. The former is a greenhouse gas that has an energy reflectivity per mole 180-fold higher than that of carbon dioxide. Thus, the use of fertilizer nitrogen may contribute to global warming. Key components of the global nitrogen cycle are being increasingly affected by the industrial conversion of atmospheric nitrogen and the accumulation of nitrous oxide. The consequences of these disequilibria are unclear, but prudence dictates that further perturbations of this major natural cycle be minimized.

The natural process of Biological Nitrogen Fixation (BNF) has a critical role in the achievement of environmentally benign, sustainable farming systems. Its increased use will mitigate the need for fertilizer nitrogen, with concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, and ground and surface-water contamination. This natural process is dependent on microorganisms, and a plant may serve as a partner.

Plants and microbes form symbiotic associations in legumes, lichens and some woody plants. The system most important for agriculture is the legume-rhizobia symbiosis: the

fixation of atmospheric nitrogen occurs within root nodules after rhizobial penetration of the root. Thus, many legumes can grow vigorously and yield well under nitrogen-deficient conditions, and may contribute nitrogen to the farming system in the vegetative residues after grain harvest, or more significantly as green manure incorporated in the soil. They have been exploited as sources of nitrogen most notably in the agricultural systems of Australia and New Zealand. The successful introduction of exotic legume crops, such as alfalfa and soybean into the United States, necessitated the simultaneous introduction of compatible rhizobia bacteria; such inoculants, in various forms, have been in use for about 100 years.

Legumes and BNF are very important in the developing world, whence much of the increases in food production must come to accommodate increasing world population. It is essential that tropical legumes be exploited to replace fertilizer nitrogen, to avoid compounding recalcitrant environmental problems of local and global proportions.

Legumes (with a few minor additions) are the only living organisms self-autonomous for organicating Carbon and Nitrogen. The carbon organication depends on photosynthesis and it is common to any plant. Nitrogen prototrophy derives from the unique association of leguminous plants with Rhizobia, a nitrogen fixing soil bacteria

Most soils can provide most essential edequately nutrients. However in agriculture fields where cropping removes nutrient eacli year, loss of some minerals can reduce soil fertility and require the addition of fertilizers. Three most common nutrients that become limiting are nitrogen, phosphorus and potassium (or N, P, K), as the three main components of fertilizers. Levels of NPK needed vary with plants, climate, soil and tissue to promote. As a result, there are a variety of fertilizers with various ratios of NPK and using the right fertilizer is essential.

The elements of nitrogen (N) are essential to all living organisms. Approximately 78% of nitrogen in our atmosphere is made up of nitrogen gas and in organic matter in the soil, plants are unable to use nitrogen in these forms. Conventional methods providing nitrogen to plants in a usable form include adding nitrogen rich fertilizers to the soil, including seed (i. e., coating the seed) with bacteria able to perform a process called

nitrogen fixing. The biological nitrogen fixation by means which atmospheric nitrogen is covered in to forms absorbed and used by plants. The biological nitrogen fixation is a problems of the highest priority in biological and agricultural sciences because it is carried out by certain plants which have involved with the genetic capabilities to fix and utilize this atmospheric nitrogen.

There are legumes plant such as black gram, soyabean, peanut, alfalfa, beans and pigeon peas can not fix nitrogen alone but need compatible genetic strain of bacteria to carry out this process. The biological nitrogen is, therefore a symbiotic relationship between bacteria and the compatible host plant.

The present investigation deals with the estimation of genetic variability, heritability, genetic advance of some of the biochemical characteristics, like moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C of root nodules in three lines of black gram. It also deals with the detection of the influence of different fertilizer doses individually and in combination following factorial analysis of those biochemical characteristics in root nodules of those three lines and nine lines of root nodules and seeds determination of molecular weight of protein. Identification of amino acids and colony counts of rhizobium bacteria were done in nine lines of root nodules.

REVIEW OF LITERATURE

Literatures regarding variance and factorial analysis with fertilizer doses of biochemical characters of root nodules and grain in black gram are scanty. However, few papers have been published dealing with the genetic study of the quantitative characters, nodule performance, and root nodule rhizobium on various leguminous plants. Therefore, brief review of them along with other crops is narrated bellow year wise.

Fisher (1918) was the first to develop statistical method to partition the variance of the quantitative character in segregating population into genetic and environmental components.

Somewhat more promising attempts were made to determine responses of species or genera of bacteria to the Rhizoshere. Starkey (1931a) worked on Agrobacterium of plant roots found that in root free soil the average population of Agrobacterium was 2×10^{10} per gram of soil, or 0.1 percent of the total bacteria count determined on the same soil samples. On root surfaces incidence of the group was as high as 1.4×10^{7} per gram of sample material, the group constituted 1 percent of the total count.

The bacterial population in Rhizoshere soil is quite commonly of the order of 500 million per gram (Clark, 1949), it rarely exceed 1 billion per gram, nor falls bellow 10 million. Rhizoshere samples, containing relatively clean roots or root surface scrapings may contain bacterial densities of one to several billion per gram of fresh sample material. Rhizobium counts of the order to and of 16×10^{12} per gram (Raicheva, 1957) 8×10^{12} per gram of rhizoshere soil are distinctly a typical.

Holding (1960) has reported Agrobacterium to constitute 1.4 percent of the microbial isolates from Rhizoshere soil. The genus was not represented among isolates from root free soil.

Hossain (1977) studied different combinations of nitrogen, phosphorus and potassium and found different responses on the nodule formation in [*Vigna radiata* (L.) Hepper]. Plants grown on the sand culture media containing nitrogen

in different combinations with phosphorus and or potassium were found to produce little amount of nodules at early stages of their growth. At later stages, NP plants were found to contain significantly high amount of nodules. At the final stage NPK treated plants improved nodule content, while NK treated plants produced little amount of nodules. The plants treated separately with PK and P produced more nodules than the control plants. Nodule formation increased with the increase in fresh weight and leaf surface of plants in all treatments.

Singh *et al.* (2000) conducted a field experiment involving three sources of nitrogen (chemical, organic and chemical + organic) in three levels of nitrogen (50, 75 and 100% of the recommended dose) using wheat as a test crop. Application of N through organic manure followed by integrated use of chemical + organic source and increasing level of N led to significant improvement in yield attributes and yield of wheat and uptake of N of by grain and straw.

Chattopadhyay *et al.* (2000) studied on the effect of nitrogen and phosphorus levels on seed production of okra during the pre-kharif seasons (spring-summer) of 1998 and 1999. Five levels of nitrogen (0, 60, 80, 100 and 120 kg/ ha) were tested against four levels of $P_2 O_5$ (0, 40, 60 and 80 kg/ha) making 20 treatment combinations and were arranged in a randomized block design with three replications. They showed that the yield of seed was significantly increased with the application of nitrogen and phosphorus at the rate of 100 and 60 kg/ha, respectively, over rest of the nutrient levels. The application of both nitrogen and phosphorus did not exhibit significant influence on 100 seed weight and germination percentage.

Hipparagi *et al.* (2000) studied the effect of two levels of N, P and K on bunch characters in ratoon crop of banana germplasm. The cultivars Robusta registered a maximum bunch yield of 106.31 t/ha with 125% of recommended N, P and K fertilizers when two suckers per hill were retained after shooting of the main crop. Their study suggested that though Elakkibale yielded 46.89 t/ha, it gave the highest income of Rs. 245526.86 with two suckers per hill with 125%

recommended N, P and K fertilizers followed by Robusta (Rs. 208584.86) which was recommended for cultivation for Bhadra command area.

Reddy *et al.* (2001) conducted a field experiment to examine and analyse the effect of phosphorus, zinc and biofertilizers on lentil cultivars in relation to dry matter production, grain yield and nutrient uptake. They used two lentil cultivars in the study viz. JL (*Microsperma*) and Lens 4076 (*Macrosperma*). Their results showed that cultivars JL 1 produced higher grain yield than Lens 4076 in 1990-91, but both the cultivars did not differ in respect of dry matter production and nutrient uptake. Application of 17.2 kg P as single superphosphate with 5 kg Zn ha^{-1} produced higher dry matter and grain yield. N, P and Zn uptake was improved by P and Zn application.

Yield and its attributes and protein content of *Vigna radiata* were studied by Shivesh *et al.* (2001) at harvest stage in kharif seasons of 1998 and 1999 under mid-hill conditions of Himachal Pradesh. The test weight (g), seed yield and biological yield (q/ha) were significantly influenced by nitrogen and phosphorus treatment. The application of 20 kg N/ha and 60 kg P_2O_5/ha brought about significantly maximum increase in these characters. Each increasing level of fertilizers N and P showed a significant difference over its previous level. The protein content in grain was significantly affected by fertilizer treatment but no treatment was able to influence the protein content in straw. However, a slight increase over control was recorded in all the treatments.

Hussaini *et al.* (2002) investigated the effect of different levels of nitrogen (0, 60, 120 and 180 kg N ha-1), phosphorus (0, 20 and 40 kg P ha-1) and irrigation regimes (based on irrigation water (IW): cumulative pan evaporation (CPE) ratios of 0.6, 0.8 and 1.0) on the productivity and water use of dry season maize. The nitrogen and irrigation were factorially combined to make the main plots, while phosphorus was assigned to the sub-plot in a split-plot arrangement using three replications. The influence of nitrogen was significant on shelling percentage, harvest index, grain yield, water use and water use efficiency, while phosphorus was significant on all but shelling percentage.

MATERIALS AND METHODS

A. MATERIALS:

Materials used in the present study comprised root nodules of three lines of blackgram as shown by the Table. The seeds of there lines were supplied from the Biometrical Genetics Laboratory, Department of Genetics and Breeding, University of Rajshahi, Rajshahi. The three blackgram lines

SL. No.	No. /Line
1	L_2
2	L_{16}
3	L_{18}

Fertilizers: In the present study fertilizers for nitrogen (N), phosphorous (P) and potassium (K) were used. Urea for N, triple super phosphate for P and murate and potash (MP) for K were used.

Either alone or in combination of these fertilizers resulting eight combinations were used in the present investigation. The fertilizes in single dose in combination as shown bellow,

(i) Control (No fertilizers)

(ii) Urea (N)

(iii) TSP (P)

(iv) MP (K)

(v) Urea (N)+ TSP (P)

(vi) Urea (N)+ MP (K)

(vii) TSP (P)+ MP (K)

(viii) Urea (N) +TSP (P) + MP (K)

1. Collection of the experimental seeds
2. Preparation of soil and filling up of polyethene bags
3. Size of the experimental space
4. Sowing of seeds and raising of seedlings
5. Maintenance of the experimental plants
6. Collection of root nodule

1. Collection of the experimental seeds: At the starting of this study in 2000, the seeds of nine blackgram lines were taken from the Biometrical Genetics Laboratory, Department of Genetics and Breeding, University of Rajshahi, Rajshahi

2. Preparation of soil: For the growing of seeds the soils were prepared by combining 50 % sand, 12.5% Cowdung, 12.5% loam, 12.5% sawdust, and 12.5% ash. The combinations were mixed up well. The prepared soil were putted in to the 30 × 44 sq.cm polyethelene bags. Each of the polyethelene bags containing soil that was treated with one of the eight fertilizer doses prepared by either sole nitrogen, phosphorus, potassium and their combination. The eight fertilizer doses are as follows:

(i) Control (No fertilizers)
(ii) Nitrogen (Urea) 1.5gm /bag
(iii) Phosphorus (TSP) 4gm /bag
(iv) Potassium (MP) 1.5 gm /bag
(v) Nitrogen (Urea) 1.5gm /bag +Phosphorus (TSP) 4 gm /bag
(vi) Nitrogen (Urea)1.5 gm /bag + Potassium (MP)1.5 gm /bag
(vii) Phosphorus (TSP) 4 gm /bag + Potassium (MP) 1.5 gm /bag
(viii) Nitrogen (Urea) 1.5 gm /bag + Phosphorus (TSP) 4 gm /bag + Potassium (MP) 1.5 gm /bag

3. Size of the experimental space: The design for this experiment was completely randomized block design. The experiment was set in the garden of 3rd science building, University of Rajshahi, during the Rabi crop season (the 25th September to the 23rd December) in 2000. A sunny place was selected at which a 457×213 sq. cm rectangle was made by splitted bamboo at a height of 15cm from the land. In the rectangle 20cm × 20cm squares were made by metal wire for supporting the polyethene bags. After sowing the polyethene bags were assign in the squares randomly.

4. Sowing of seeds and raising of seedlings: The seeds of three blackgram the lines were sown in all the polyethene bags containing prepared soil and treated with eight fertilizer doses. Since 6 bags were taken for each of the eight treatments to harvest at six times. The seeds of one blackgram line were sowed in (8×6) = 48 bags. The seeds were

sown scatardly in each of the polyethene bags. The sowing dates were the 25th September, 2000. After Sowing each bag was warped by another black polyethen bag to make darkness, which was required for the development of roots. The bags were arranged randomly to the squares in the rectangle.

5. Maintenance of the experimental bags and space: Regular weeding was done in the experimental space. When seedlings were 7-8 cm in height, only 3 seedlings nearly in equal distance in each polyethene bag were kept and excess seedlings were removed from the bag. The irrigation also done when required.

6. Collection of root nodule: Root nodule were collected in individual plant basis after every 15 days starting from seeding up to 90 days comprising six stags .The root nodules were collected on different doses from plants per bag. All root nodules collected after biochemical test.

B. METHODS: The following characters were analyzed in the present study.

a) *Moisture*

b) *Dry-matter*

c) *Protein*

d) *Free sugar*

e) *Reducing sugar*

f) *Vitamin C*

a) Moisture:

Moisture content was performed by the conventional procedure

i) Materials: a) Procelain crucible

b) Electrical balance

c) Oven

d) Desiccator

ii) Procedure:

Four grams of root nodule are weighed in a procelain crucible (which was previously cleaned, heated to 100^0 C, cooled and weighed). The crucible with the sample was heated in an electrical oven for about six hours at 100^0C. It was then cooled in a desiccator and weighed again.

Calculation:

Percent of moisture content (gm per 100 gms of root nodule)

$$= \frac{Amount\ of\ the\ moisture\ obtained}{Weight\ of\ the\ root\ nodule} \times 100$$

b) Dry-matter:

Dry matter content was calculated from the data obtained for percent of moisture content.

Calculation:

% Dry matter content = Total root nodule - % moisture content

c) Protein:

Protein content of the different lines of root nodule was determined according to Micro-Kjeldhal Method (Wong, 1923)

i) Reagents:

a) Solid potassium sulphate

b) Concentrated sulphuric acid

c) 5% $CuSO_4$, $5H_2O$ in distilled water

d) 0.01 N H_2SO_4 solution

e) Concentrated sodium hydroxide solution (5N, approximately).

f) Boric acid containing bromocresol green (receiving fluid): 10 gm of boric acid was dissolved in hot water (250 ml) and cooled. 1ml of 0.1% bromocresol green in alcohol was added and diluted upto 500 ml with distilled water.

g) Few quartz chips

h) Nitrogen determination apparatus (micromodel) according to Paranas-Warner, made of JENA Glass-all connections with interchangeable ground joints.

ii) Procedure:

Digestion: 4 to 6 ml conc. H_2SO_4, 1 gm K_2SO_4, one to two drops 5% $CuSO_4$ solution (catalyst) and some quartz chips (to avoid bumping) were added in 1-2 gms of root nodule in a Kjelhdal flask. The mixture was heated till it had become light green (2-3 hours).

Collection of ammonia: The digestion was carried out in the steam distillation chamber of the nitrogen determination apparatus. After the completion of digestion the steam distillation chamber containing the digested mixture was fitted back to the nitrogen determination apparatus. Boric acid solution (15 ml) in a small flask was so placed that the tip of the condenser outlet dipped below the surface of the boric acid solution. Sufficient amount of concentrated NaOH solution (approximately 30-40 ml) was added in the chamber containing the digest to neutralize the amount of acid present. Steam was

generated from the steam generating flask and the sample in the chamber was steam distilled untill 20 ml of distillate was collected in the boric acid solution. The condenser outlet was then rinsed with little distilled water and the receiving flask was removed.

Titrimetric estimation of ammonia: The ammonia in the boric acid solution was titrated with 0.01N H_2SO_4 till the solution had been brought back to its original yellow green colour. The titration was repeated with a control containing only 15 ml of boric acid solution diluted to approximately the final volume of the titrated sample. The volume of acid required was recorded.

Calculation:

The total nitrogen was calculated using the formula given bellow:

i) 100 ml of 1N acid =14 gm of nitrogen

ii) X gm N_2 = 6.025 × X gm of protein

Percentage of protein content (gm per 100 gm root nodule)

$$= \frac{Amount\ of\ protein\ obtained}{Weight\ of\ root\ nodule} \times 100$$

d) Free sugar:

Free sugar content of the different lines of root nodule was determined colorimetrically by the Anthrone method (Jayaraman, 1981).

i) Reagents:

Anthrone reagent: The anthrone reagent was prepared by dissolving 2 gms of anthrone in 1litre of conc. H_2SO_4.

Standard glucose solution: A standard solution of glucose (BDH chemicals Ltd., Poole, England) was prepared by dissolving 10 gm of glucose in 100 ml of distilled water.

Extraction of sugar from root nodule: Extraction of sugar from root nodule was done following the method as described by Loomis and Shull, (1937).

Four to six gms of root nodule were crushed and immediately plunged into boiling ethyl alcohol and allowed to boil for 5 – 10 minutes (5 to 10 ml of alcohol was used for every

gm of nodule). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through two layers of muslin-cloth and re-extracted the ground tissue for three minutes in warm alcohol (80%) using 2 to 3 ml of alcohol for every gm of tissue. This second extraction ensured complete removal of alcohol soluble substances. After cooling the extract has passed through muslin cloth. Both the extracts were filtered through Whatmann no. 41 filter paper.

The volume of the extract was evaporated to about $(1/4)^{th}$ the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to 100 ml volumetric flask and made upto the mark with distilled water (working standard).

ii) Procedure: 1ml aliquot of the nodule extract from each line was pipetted into test tubes 4 ml of the anthrone reagent was added to each test tube and mixed well. Glass marbles were placed on top of each test tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 minutes and then cooled. The absorbance of the blue-green solution was measured at 680 nm in a colorimeter.

The standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0.0, 10µg, 20µg, 40µg, 60µg, 80µg and 100µg of glucose, respectively and made the volume upto 1ml with distilled water. Then 4 ml of anthrone reagent was added to each test tube and mixed well.

All these solutions were treated similarly as described above. The absorbance was measured at 680 nm using the blank containing 1ml of water and 4ml of anthrone reagent.

The amount of free sugar present in the extract was calculated from the standard curve of glucose. Finally, the percentage of free sugar present in the root nodule was determined using the formula given below:

Calculation:

$$\text{The percentage of free sugar content} = \frac{Amount\ of\ sugar\ obtained}{Weight\ of\ root\ nodule} \times 100$$

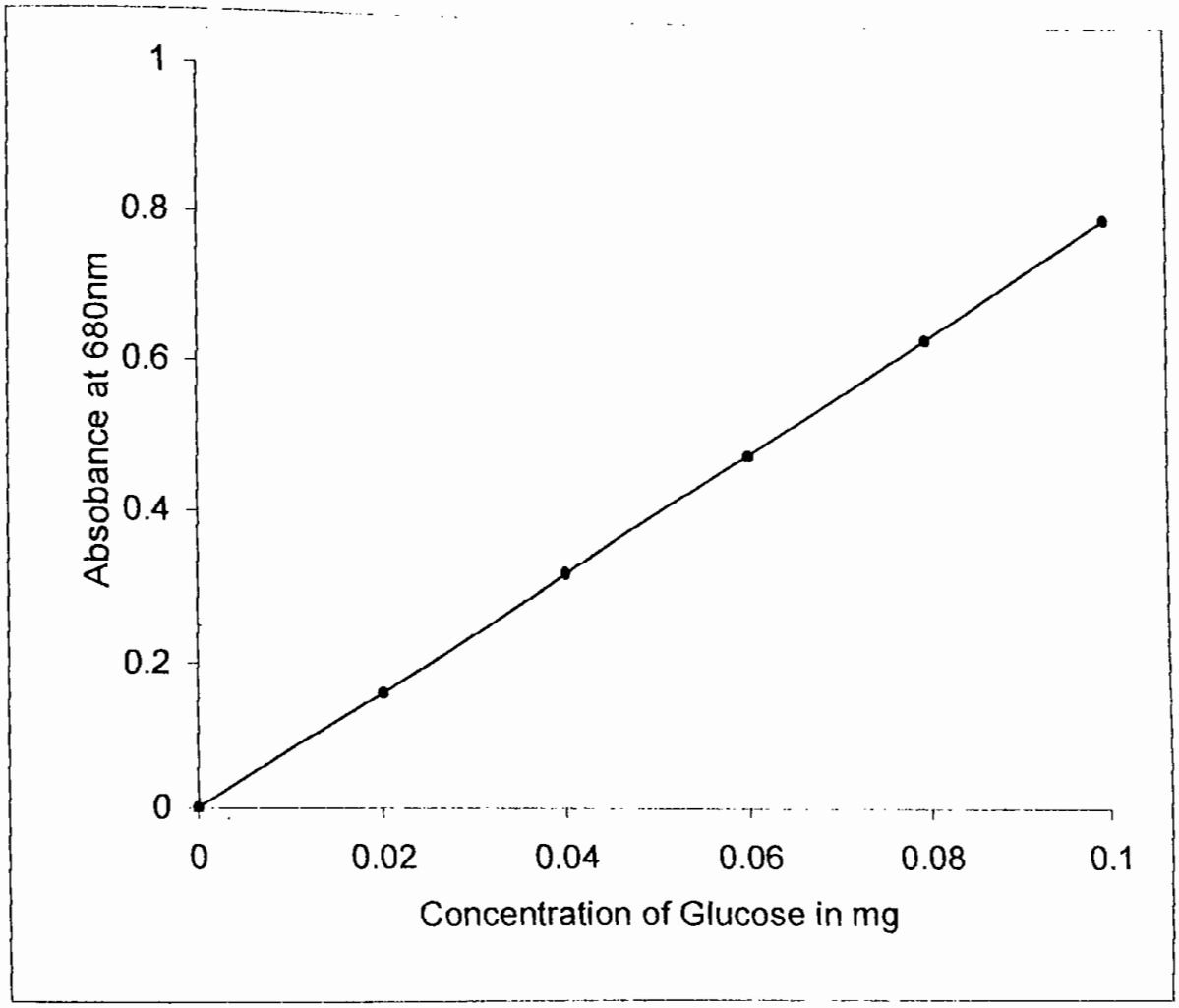


Fig. 1: Standard curve of glucose for estimation of free sugar and reducing sugar.

e) Reducing sugar:

Reducing sugar content of the different line of root nodule was determined by DNS method (Miller, 1972).

i) Reagents:

a) DNS reagent: Simultaneously, 1 gm of DNS, 200 gms of crystaline phenol and 15 gms of sodium sulphite were placed in a beaker and mixed with 100 ml of 1% NaOH solution by stirring. If it was needed to store then sodium sulphite must be added just before use.

b) 40% solution of Rochelle's salt

Extraction of sugar from root nodule:

Extraction of sugar from root nodule was done by following the method as described above

Method: Aliquots of 3 ml of the extract was pipetted into test tube and mixed well after addition of 3 ml of DNS reagent. The test tubes were heated for 5 minutes in a boiling water bath. After the color has developed, 1 ml of 40% Rochell's salt solution was added when the contents of the tubes were still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 ml of water and 3 ml of DNS reagent in tube and treated similarly. The absorbance of the solution was measured at 575 nm in a colorimeter.

The amount of reducing sugars was calculated from the standard curve of glucose (Fig. 1)

Calculation:

The percentage of reducing sugar (gm per 100 gms of root nodule)

$$= \frac{Amount\ of\ sugar\ obtained}{Weight\ of\ root\ nodule} \times 100$$

f) Vitamin C:

Vitamin C content of root nodule was determined by the Bessy's titrimetric method (1933).

i) Reagent:

a) **Dye solution:** 200 ml of 2,6 dinitrophenol indophenol (BDH chemicals Ltd.) and 210 gm of sodium bicarbonate were dissolved in distilled water, made upto 100 ml and filtered the solution.

b) **3% metaphosphoric acid reagent:** 3 gm of metaphosphoric acid was dissolved in 80 ml of acetic acid and made up to 100 ml with distilled water.

c) Stantard vitamin C solution (0.1 g/ml): 10 mg of pure vitamin C (BDH chemicals Ltd.) was dissolved in 3% metaphosphoric acid and made upto 100 ml with 3% metaphosphoric acid

ii) Procedure:

10 ml of standard vitamin C solution was taken in a conical flask and titrated against dye solution.

Four gms of root nodule were crushed thoroughly with 3% metaphosphoric acid (20ml) and filtered it through double layer of muslin cloth. The extract was centrifuged at 3000 r.p.m for 10 minutes and the clear supernatant was titrated with 2,6 dichlrophenol indophenol solution. The amount of vitamin C present in the extract was determined by comparing with the titration result of standard vitamin C solution.

2. Biochemical technique of analyses: The collected data were analysed following biometrical techniques as developed by Mather (1949) based on the mathematical models of Fisher *et al.* (1932). The techniques of analyses that have been used are described in the following sub- heads:

a) Sum total

Data on individual plant basis were added together to obtain sum total.

Total of a replication = $\sum x_i$

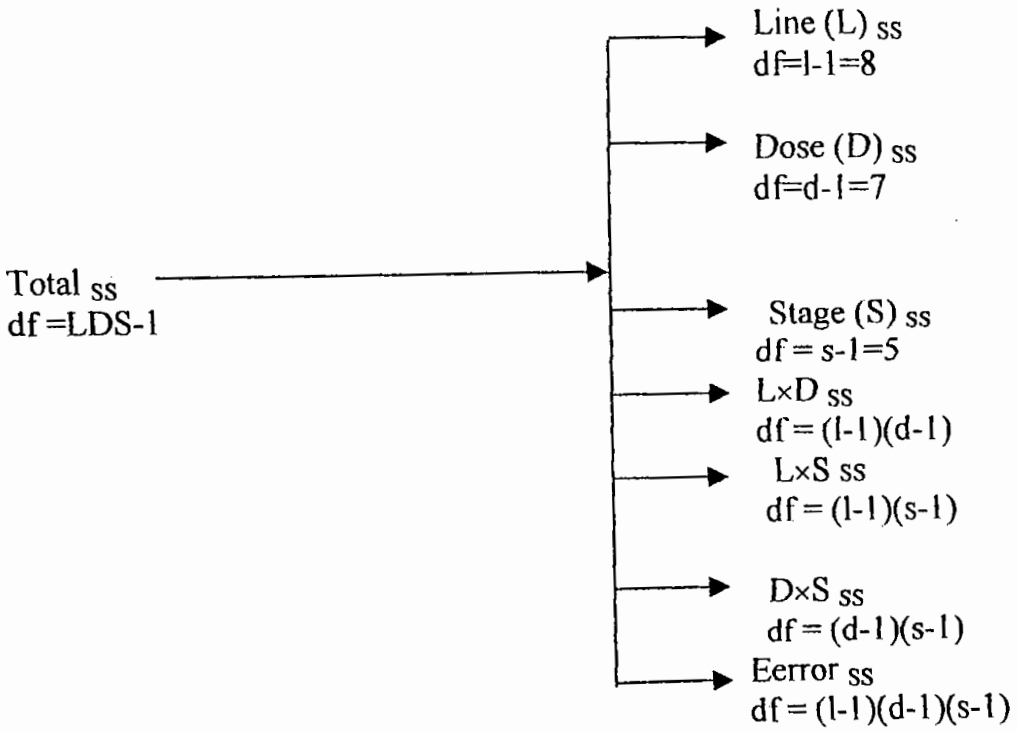
Where, Xi = The individual reading recorded from each plant.

$\sum$ = Summation.

b) 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 for each of the characters was carried out with the value of individual plant per bag, in each dose, stage and line.

The variance due to different sources such as lines (L), doses (D), stages (S), interactions (L × D, D ×S, L × S) and error (L × D × S) in this study are analysed according to following skeleton.



Where,

$$\text{Total ss} = \sum (X_{ijk})^2 - CF$$

$$\text{Line ss} = \frac{\sum X^2_{i..}}{ds} - CF$$

$$\text{Dose ss} = \frac{\sum X^2_{.j.}}{ls} - CF$$

$$\text{Stage ss} = \frac{\sum X^2_{..k}}{ld} - CF$$

$$(\text{L} \times \text{D})\ \text{ss} = \frac{\sum_{ij} (X_{jk.})^2}{s} - CF - Lss - Dss$$

$$(\text{L} \times \text{S})\ \text{ss} = \frac{\sum_{ik} (X_{i\cdot k})^2}{d} - CF - Lss - Sss$$

$$(\text{D} \times \text{S})\ \text{ss} = \frac{\sum_{jk} (X_{.jk})^2}{l} - CF - Sss - Dss$$

$$(\text{L} \times \text{D} \times \text{S})\ \text{ss} = \sum_{ijk} (L_i D_j S_k)^2 - CF - L_{ss} - D_{ss} - S_{ss} - (L \times D)_{ss} - (L \times S)_{ss} - (D \times S)_{ss}$$

X_i = The value of ith lines

X_j = The value of jth doses

X_k = The value of kth stages.

$X_{ij.}$ = The value of ith lines in jth doses.

$X_{.jk}$ = the value of jth doses in kth stages.

$X_{i\cdot k}$ = The value of ith lines in kth stage.

X_{ijk} = The value of ith lines in jth doses of kth stages.

l = Number of lines.

d = Number of doses.

s = Number of stages.

$$\text{CF} = \text{Correction factor} = \frac{\text{GT}^2}{\text{N}}$$

GT = Grand total

N = Total number of observation = lds

The analysis of variance of a fixed model was used, where line (L), dose (D) and stage (S) are fixed. The expectation in the analysis are shown in following Table 17. In the Table the second order interaction was used as error to test all other items.

Table 19: The expectation of mean square (EMS) used in the analysis of variance.

Items	d.f	MS	EMS
Line (L)	(l-1)	MS_1	$\sigma_e^2 + sk_{LD}^2 + dk_{LS}^2 + sdk_L^2$
stage (S)	(d-1)	MS_2	$\sigma_e^2 + lk_{DS}^2 + dk_{LS}^2 + ldk_S^2$
Dose (D)	(d-1)	MS_3	$\sigma_e^2 + lk^2_{SD} + sk_{LD}^2 + lsk_D^2$
L×S	(l-1) (s-1)	MS_4	$\sigma_e^2 + dk_{LS}^2$
L×D	(l-1)(d-1)	MS_5	$\sigma_e^2 + sk_{LD}^2$
D×S	(d-1)(l-1)	MS_6	$\sigma_e^2 + lk_{DS}^2$
Error (L×D×S)	(l-1)(d-1) (s-1)	MS_7	σ_e^2

Where,

L, D and S represent the numbers of lines, doses and stages respectively.

MS_1 =. Mean square of line (L)

MS_2 = Mean square of dose (D)

MS_3 = Mean square of stage (S)

MS_4 = Mean square of interaction (L × S)

MS_5 = Mean square of interaction (L × D)

MS_6 = Mean square of interaction (D × S)

MS_7 =Mean square of error (L × D × S)

and, dsk_L^2 = Variance due to line

lsk_D^2 = Variance due to doses

ldk_S^2 = Variance due to stage

sk_L^2 = Variance due to L × D

dk_{LS}^2 = Variance due to L× S

lk_{DS}^2 = Variance due to D× S

σ_e^2 = Variance due to error (L× D × S)

c) Components of variation:

The components of variation were phenotypic (k_p^2), genotypic (k_g^2), line × dose (L×D), line × stage (L×D) and dose × stage (D×S) variance. These are calculated as follows:

Step 1:

$$k_g^2 = \frac{MS_1 - (MS_5 - MS_7) - MS_4}{ds}$$

$$k^2_{L\times D} = \frac{MS_5 - MS_7}{s}$$

$$k^2_{L\times S} = \frac{MS_4 - MS_7}{d}$$

$$k^2_{D\times S} = \frac{MS_6 - MS_7}{l}$$

$$\sigma_e^2 = MS_7$$

Where,

l = Number of lines

d = Number of doses

s = Number of stages

MS_1 = Mean square of lines

MS_5 = Mean square of lines × dose (L×D).

MS_4 = Mean square of lines × stage (L×S).

MS_6 = Mean square of dose × stage (D× S).

MS_7 = Mean square of error (L × D × S).

Step 2: Phenotypic variance: $k_p^2 = k_g^2 + k^2_{L\times D} + k^2_{L\times S} + \sigma_e^2$

Genotypic variance, $k_g^2 = k_L^2$

Interaction of line × dose (L× D) variance = $k^2_{L\times D}$

Interaction of line ×stage (L × S) variance = $k^2_{L\times S}$

Interaction of stage × dose (D× S) variance = $k^2_{D\times S}$

d) Co-efficient of variability:

Deviation is also expressed by the co-efficient of variation given by the formula of Burton and De vane (1353) as follow:

Co-efficient of variability in percentage, (CV %) = $\frac{S}{\overline{X}} \times 100$

Where S = Variance

$\overline{X}$ = Mean

In the present study, co-efficient of variability at different levels were calculates as follows:

i. Phenotypic co-efficient of variability. (PCV) = $\frac{k_p^2}{\overline{X}} \times 100$

ii. Genotypic co-efficient of variability. (GCV) = $\frac{k_g^2}{\overline{X}} \times 100$

Interaction co-efficient of variability. (L× D)CV= $\frac{k^2_{L\times D}}{\overline{X}} \times 100$

Interaction co-efficient of variability. (L× S)CV= $\frac{k^2_{L\times S}}{\overline{X}} \times 100$

Interaction co-efficient of variability. (D× S)CV= $\frac{k^2_{D\times S}}{\overline{X}} \times 100$

Error co-efficient of variability, (ECV) = $\frac{\sigma_e^2}{\overline{X}} \times 100$

Where,

$\overline{X}$ = Grand mean

k^2_p = Phenotypic variance

k^2_g = Genotypic variance

σ^2_e = Error variance.

e) Heritability, genetic advance, genetic advance as a percentage of mean:

i) Heritability (h^2_b): Heritability in broad seanse was calculated by dividing the phenotypic variance to the genotypic variance and then multiplying by 100 as suggested by Warner (1952).

Where, $h_b^2 = \frac{k^2_g}{k^2_p} \times 100$

k^2_g = Genotypic variance

k^2_p = Phenotypic variance.

ii) Genetic advance (GA): Genetic advance was calculated by the formula as suggested by Lush (1949).

$$GA = K\,(k_p)\,\frac{k^2_g}{k^2_p}$$

Where,

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

k^2_p = Phenotypic variance

k_p = Square root of phenotypic variance.

k^2_g = Genotypic variance.

iii) Genetic advance as a percentage of mean (GA%): It was calculated by the following formula.

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

Where,

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

GA = Genetic advance.

3. Factorial analysis:

Factorial analysis are done to detect the individual effect of different treatments used in an experiment. In the present study the individual effect of different fertilizer doses were estimated through the factorial analysis developed by R. A. fisher (1935).

Step 1: Analysis of variance for each stage:

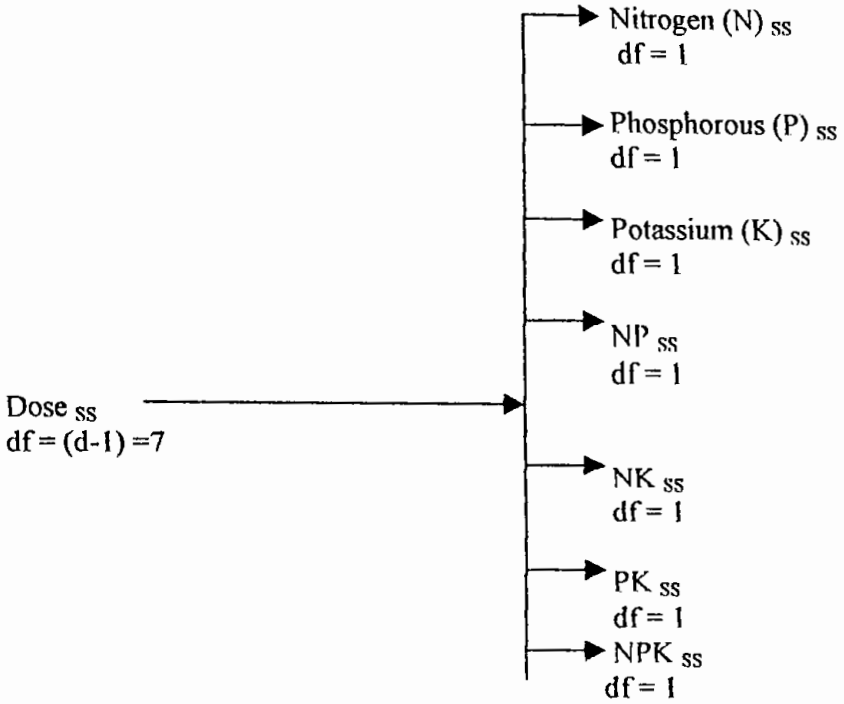
Primarily analysis of variance was done differently for each of the characters in each stage. The variance due to lines, doses, interaction and error were analyzed according to following skeleton.

Step 2: Partitioning the variance due to dose:

The variance due to different fertilizer doses further partitioned according to the following skeleton.

Step 1: Analysis of variance for each stage:

Primarily the analysis of variance was done differently for each of the characters in each stage. The variance due to lines, doses, interaction and error were analyzed according to the following skeleton.



The appropriate orthogonal comparison among the eight fertilizer doses total was set in the following way

.Dose (D)	$N_oP_oK_o$	$N_iP_oK_o$	$N_dP_iK_o$	$N_oP_oK_i$	$N_iP_iK_o$	$N_iP_oK_i$	$N_oP_iK_i$	$N_iP_iK_i$	$\sum k^2_i$	$r\sum k^2_i$
Doses total($\sum Di$)	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8		
Comparison (C_i)										
C_1	-1	1	-1	-1	1	1	-1	1	8	72
C_2	-1	-1	1	-1	1	-1	1	1	8	72
C_3	-1	-1	-1	1	-1	1	1	1	8	72
C_4	1	-1	-1	1	1	-1	-1	1	8	72
C_5	1	-1	1	-1	-1	1	-1	1	8	72
C_6	1	1	-1	-1	-1	-1	1	1	8	72
C_7	-1	1	1	1	-1	-1	-1	1	8	72

The function of C_i,

$C_1 = D_1 + D_2 - D_3 - D_4 + D_5 + D_6 - D_7$

$C_2 = - D_1 - D_2 + D_3 - D_4 + D_5 - D_6 + D_7 + D_8$

$C_3 = - D_1 - D_2 - D_3 + D_4 - D_5 + D_6 + D_7 + D_8$

$C_4 = D_1 - D_2 - D_3 + D_4 + D_5 - D_6 - D_7 + D_8$

$C_5 = D_1 - D_2 + D_3 - D_4 - D_5 + D_6 - D_7 + D_8$

$C_6 = D_1 + D_2 - D_3 - D_4 - D_5 - D_6 + D_7 + D_8$

$C_7 = - D_1 + D_2 + D_3 + D_4 - D_5 - D_6 - D_7 + D_8$

The sum square with 1 d.f of different doses.

$$N\ ss = \frac{C_1^2}{\sum k_i^2}$$

$$P\ ss = \frac{C_2^2}{\sum k_i^2}$$

$$K\ ss = \frac{C_3^2}{\sum k_i^2}$$

$$NP\ ss = \frac{C_4^2}{\sum k_i^2}$$

$$NK\ ss = \frac{C_5^2}{\sum k_i^2}$$

$$PK\ ss = \frac{C_6^2}{\sum k_i^2}$$

$$NPK\ ss = \frac{C_7^2}{\sum K^2_1}$$

Where,

D_i = Dose total

C_i = Appropriate linear comparison

K_i = Co-efficient, either + 1 or –1.

$\sum K^2_1$ = Sum square of co-efficient.

4. Determination of molecular weight of protein through sodium dodecyl sulphate polyacrylamide slab gel electrophoresis:

Different lines of root nodule and seeds in blackgram were crushed to form powder, then the powder was dissolved in 0.15 M NaCl solution. This solution was clarified by centrifugation at 4000 r.p.m.for 10 minutes and the clear supernatant solution was concentrated by the vacume pump. The concentrated extract was used for identification of protein compounds by gel electrophoresis.

a) Calibration kits for SDS-polyacrylamide Gel electrophoresis: Molecular weight of crude protein were analysed by one dimension (20 cm × 20 cm × 0.15 cm) SDS-PAGE following the modified Weber and Osborn (1969) technique using the discontinuous buffer system of Laemmli (1970). Electrophoresis was carried out on acrylamide slab gels. The marker proteins and unknown protein solutions were applied separately in the SDS-PAGE method under identical conditions

Marker protein	M.W.	Log M.W.
Ovalbumin (hen egg)	42,700	4.6304
Glutamate dehydrogenate (bovine liver)	55,500	4.7443
Ovotransferin (hen egg)	76-78,000	4.8808
Phosphorylase b (rabbit muscle)	97,400	4.988
β-Galactosidase (E.col.)	116,300	5.0655
Myosin (rabbit muscle)	200,000	5.301

Preparation of gels and sample for SDS- Slab Electrophoresis

i) Reagents and Solution:

a. 40% Acrylamide

44.4 gm acrylamide was added with 1.2 gm bis-acryamide (methyl bis-acrylamide) then heated water bath and finally made up volume upto 100ml.

b. Tris -HCl solution pH-8.8 (1.5 M):

18.7 gm Tris was dissolved in 70 ml distilled water and adjusted to pH-8.8 by adding 6N HCl, drop by drop make up to 100 ml by distilled water.

c. 0.5 M Tris-HCl (pH 6.8):

18.7 gm Tris was dissolved in 70 ml distilled water and adjusted to pH-6.8 by adding 6N HCl, drop by drop make up to 100 ml by distilled water.

d. Sodium dodecyl sulfate (10%):

10 gm SDS of analytical grade was dissolved under the volume was made upto 100 ml

e. Ammonium per sulphate solution (0.1gm/ml):

f. Chamber buffer: 6.0 gm Tris HCl 28 gm glycine and 1 gm SDS were dissolved in distilled water and then made upto 1000 ml by distilled water. The pH was adjusted in 6.8

g. Sample solution:

4% SDS solution	-- 13ml
Glycerine	-- 5ml
0.5M Tris HCl buffer, pH- 6.8	-- 7ml

h. Bromophenol blue solution:

Bromophenol blue --	10 mg l
Glycerine	2 ml
0.5M Tris HCl buffer,	0.2 ml
Distilled water	10 ml

pH -- 6.8

Preparation of sample A: Concentrated protein extract solution, prepared from root nodule and seeds (100 µ l) was added with 100 µ l sample solution and 20 µ l β- mercaptoethanol then heat the mixture at 100° C in a water bath for 2 minutes. Finally 1 drop bromophenol blue (BPL) was heated to it.

Contents	Separating gel (ml)			Staking gel (ml.)
	7.5%	10%	12.5%	
40% Acrylamide	2.55 ml	3.4 ml	4.2 ml	1.1 ml
1.5 M Tris –HCl pH- 8.8	3.75 ml	3.75 ml	3.75 ml	.....
Distilled water	8.4 ml	7.55 ml	6.75 ml	6.2 ml
10% SDS	0.15 ml			0.1 ml
APS	0.15 ml			0.1 ml
0.5 M Tris- HCl	.....			2.5 ml
TEMED	0.015 ml			0.01 ml

Application of the sample: Marker proteins (5-10 µl) and 10-20 µl of sample A solutions were applied separately in each chamber or pocket of SDS-PAGE method under identical conditions

Current supply: 10 - 20 mA current was passed and the electrophoresis completed by 2 – 205 hours.

Staining solution: The gels were transferred to a container are fixed and stained for 1 hour in a freshly prepared solution containing Coomassie brilliant blue where composition are as follows:

CBB R-250 0.25 gm

Methyl alcohol 500ml

Acetic acid 100 ml

The mixture was made upto 1000 ml by adding distilled water.

Destaining solution: Gels are destained in a solution containing 7% acetic acid and 25% methyl alcohol.

Calculations: The mobility of the marker and experimental proteins:

$$= \frac{Distance\ moved\ by\ the\ protein}{Distance\ moved\ by\ the\ traking\ dye\ (bromophenol\ blue)} \times 100$$

The molecular weight of the unknown protein was calculated from the standard graph obtained which was constructed by plotting the molecular weight of marker proteins against their relative mobility.

5.Identification of amino acids by using 'Thin Layer Chromatography' .

a) Principle:

Amino acid can be separated and identified on the basis of their R_f value, which calculated from the following formula:

$$R_f = \frac{Distance\ covered\ by\ solute}{Distance\ covered\ by\ solvent}$$

b) *Materials and Reagents*:

a) Amino acid
b) Glass plate
c) Silica gel-G
d) Acetone
e) Developing solvent: N-butanol, Glacial acetic acid and distilled water (4 : 10 : 50)
f) Spray solvent: 3% ninhydrin in N-butanol containing 3ml of acetic acid.

c) Preparation of sample (root nodule) solution: The powdered root nodule of different line (10 mg) was taken in test tube and then 3-4 ml of 6 HCl was added to it. The mixture was then heated under vacuum for 24 hours at 110°.

d) Procedure:

The glass plate was cleaned with acetone and the silica gel-G slurry was layered on the plate surface. The plate was activated by heating at 110^0 C for one hour and was allowed to cool. A line was drawn at one end of the plate for regular space. The unknown amino acid (prepared from root nodule solution by hydrolysis) mixture the standard amino acid solution were spotted at the different place on the plate.

The plate was then placed in a chromatography chamber saturated with the developing solvent and developed in ascending order with the solvent. When, the solvent had reached 1cm below the top end of the other side, the plate has removed and the distance covered by the solvent was marked by drawing a line. Then the plate was drying at room temperature.

After that the dried plate was sprayed with the ninhydrin spraying reagent. The color spot was identified by heating the plate at 110^0 C for 10 minutes. The amino acids were identified by comparing of the R_f values of the amino acids.

5. Colony counts of Rhizobium bacteria

a) Materials: Bacterial strain of Rhizobium sp. was isolated from the root nodules. This was used for the present study.

Apparatus used are as follows:

i) Autoclave	vi) Laminar flow
ii) P^H meter	vii) Water bath
iii) Refrigeration	viii) Sprite lamp
iv) Electric balance	ix) Cotton wool
v) Incubator	x) Petri dishe
	xi) Bacterial culture medium

The following are the composition and proportion in a litre.

Composition of the medium

(YEMA medium without congo red)		(YEMA medium with congo red)	
1. Mannitol	10 gm	1. Mannitol	10 gm
2. $NaCl_2$	0.1 gm	2. $NaCl_2$	0.1 gm
3. Yeast extract	0.1 gm	3. Yeast extract	0.1 gm
4. Agar	30 gm	4. Agar	30 gm
5. $MgSO_4$. $7H_2O$	0.2 gm	5. $MgSO_4$. $7H_2O$	0.2 gm
6. Distilled water	1 litre	6. Distilled water	1 litre
$P^H \longrightarrow 6.86$		7. Congo red	(1%) 2ml
		$P^H \longrightarrow 6.86$	

b) Methods:

Fresh glasswares were used for practical work. Cleaning, washing and drying of the glaswares and apparatus were done as follow:

i) Cleaning of glasswares and apparatus:

Fresh glaswares need no special treatment before being used. These were rinsed thoroughly in tap water and then dried in the sun.

ii) Used autoclave:

Used glasswares and apparatus were first sterilized by autoclaving at 120^0 C under 15 $lb/inch^2$ above atmospheric pressure, for 20 minutes to kill the discarded cultures or contaminants. After removing from the autoclave, these were washed and cleaned with brush and were soaked in chromic acid solution for overnight. Then these were rinsed in water and dried in the sun. The dirty glasswares were cleaned with washing powder 'EVA' or 'VIM' and then rinsed in hot water. After removal from the hot water, these were dried in the sun or left on the laboratory table in the room for drying. Thus, the glasswares and apparatus were made ready for use.

Iii) Preparation of medium: A beaker containing required amount of distilled water was taken. Then the required constituents (see preparation medium) were added one by one after dissolving the other. Then the container was placed in a water bath on an electric heater with constant stirring with a glass rod. Then the constituents become homogeneously mixed and boiled in the water, thus the medium was cooked. The p^H of the medium was also adjusted.

a. Sterilization

Sterilization of culture media (preparation medium), containers and other equipments are essential for microbiological work. The sterilization procedures, which were followed, are described bellow:

Sterilization by dry heat: Inoculating needles or wire loops were sterilized either by direct heat over the flame of a spirit lamp or after dipping them in the ethylalcohol and flaming over the spirit lamp.

(a) Sterilization by moist heat (autoclaving): Dry glassware and other apparatus for culturing the microorganisms were autoclaved. Before sterilization, test tubes, flasks and bottles with or without medium were plugged with non-absorbent cotton wool. The back side of the pipettes were plugged with a loose pack of cotton wool. The pipettes and petridishes were wrapped with packing papers before introducing in the autoclave. The plugged mouth of the containers containing culture media or aqueous solutions were covered with packing papers or aluminium foil to protect the plugs from excessive wetting by steam. The screw caps of the vials were loosened before autoclaving. The caps may be tightened after removing the vials from the autoclave.

Firstly, the water level in the autoclave was checked and if necessary, water was poured to make the water level upto the mark. Then the prepared materials were put into the autoclave and the lid was screwed. By this time the gas out let was loosened and the electric switch was put on. After few minutes the air and then the steam were seen to come out through the out let nozzle. White steam of air indicated the steam. Then the out let nozzle was closed tightly. Gradually the temperature was raised upto 120° C and a pressure of 15 lb/inch^2. At this situation the autoclave was left for 15 minutes. After this, the switch of the autoclave fell down at '0' position, as shown by the indicator needle of the Pressure gauge and the mercury column of the thermometer; the let nozzle was opened for releasing the residual pressure. Then the lid of the autoclave was made open and the materials were taken out. These were left on the laboratory table for cooling and for use in the experiments.

iv) Isolation of the bacterial strain- Rhizobium sp. From root nodules:

Firstly, root nodule was selected from the field in the natural condition. The surface soil was loosened around the root system and the soil was dug until the entire root system of the plant was taken out.

The procedures of isolation of Rhizobium sp. from the hosts, *Vigna mungo* L Hepper. A brief description of the process of isolation is as follows:

The collected nodulated roots were washed gently but throughly. Then a nodule was weighed by balance. Then the nodule was immersed in .2 % mercuric chloride solution and left for 5 minutes to allow surface sterilization. After this, the nodule was washed

thoroughly in sterile distilled water holding it with the help of a sterile forcep. The surface sterilized nodule was then taken in a sterilized petridish and was crushed and pressed with the help of sterile scalpel. Then these were crushed in a drop of sterile water with the help of sterile glass rod.

Five sterile test tubes each containing 9ml of sterile water was placed in the test tube stand. The tubes were arranged serially and number I, II, III, IV and V by a glass marking pencil. The crushed nodule was then taken in a test tube and gently shaken to prepare a mother suspension. Suspension ten times dilutions were prepared as shown bellow:

1. One ml of the mother suspension was added to the tube – I –10^{th} dilution.
2. One ml of the tube – I, added to the tube – II – 100^{th} dilution.
3. One ml of the tube – II, added to the tube – III – 1000^{th} dilution.
4. One ml of the tube – III, added to the tube – IV – 10000^{th} dilution.
5. One ml of the tube – IV, added to the tube – V – 100000^{th} dilution.

Thus, the serial dilution of the mother suspension was prepared for further work.

Samples from each of the suspensions poured on YMMA and congo red mannitol agar in petri dishs. Twenty-seven pairs petri dishes each with 0.2 ml suspension spread with spreader on petri dishes. These plate were incubated at 30^0 for 48 hours. During the incubation period, the bacterial cells and other micro-organisms, if present, multiplied and formed colonies. Observation was taken after 48 hours of incubation. It was observed that the plates from the 100^{th} dilution showed good growth of microbial colonies. This was indicated by the formation of well-spaced colonies. There were colonies in these plates (100^{th} dilution). Only the rhizobium colonies were isolated from the plates. Rhizobium colonies were characterized by moist and glistering with round edges. These colonies were also whitish in colour. The colonies did not absorb red dye of the congo red present in the medium. These whitish in colour colonies were selected and isolated for further work. The colonies gwoun on these plates were similar to the rhizobium colonies grown on YMMA and congo red mannitol agar. Thus, a pure form of rhizobium culture was obtained.

The suspensions both treated and untreated (control) was poured in the petridishs containing the solidified medium. One ml of suspensions was drawn in the pipette with the mouth and by holding it with the thumb, 0.2 ml of the suspension was released in each petri dish. Then the suspension was distributed on the medium with the help of a sterilized spreading glass rod. One side of a glass rod was bent twice at right angles for using it as a spreading rod. The bent portion of the rod was dipped in methylated spirit and flamed over a spirit lamp for sterilization.

The plates poured with bacterial cells were incubated at 30°C for 48 hours for the growth of the bacterial colonies.

v) Colony counting procedure:

After the incubation period, the plate were taken out from the incubator and the number of colonies grown per plate were counted. For facilitating counting of colonies, the petridish was marked with the help of a glass marking pencil under side. The number of colonies was counted and the total number of colonies in each plate were obtained. Small colonies counted under the stereo microscope for accurate counting.

Total count of colonies (number per mg of root nodules)

$$= \frac{Number\ of\ colonies\ per\ plate}{Weight\ of\ root\ nodule} \times i^{th}\ times\ dilution$$

RESULTS

A. STUDY OF VARIABILITY

1. Analysis of variance with factorial analysis for biochemical test:

For factorial analysis, first analysis of variance was done at each stage for all the six characters. The results of analysis of variance with factorial analysis for six characters of root nodule at each stage are shown Table 18 - 23.

a) Moisture: For this character line item (L) was highly significant at all the stages are shown in Table 18 (A-C). The dose (D) item was non-significant at all the stages. The stage (S) item was significant for each stage. Factor for doses viz. N, P, K, NP, NK, PK and NPK were significant at all stages except at 4th stage. The interaction (L×D, L×S and D×S) items were non-significant at all stages.

b) Dry-matter: Result obtained for this character is given in Table 19 (A-C). The line items were significant at all the stages. The dose (D) item was non-significant at all the stages. The stage (S) item was significant at all each stage. The different factor for fertilizer doses such as N, P, K, NP, NK, PK and NPK were non-significant at each stage except K and PK at 3^{rd} and 5^{th} stage, respectively. The interaction items (L×D, L×S and D×S) were non-significant at all stages.

c) Protein: For this character results are shown in Table 20 (A-C). The estimation of protein for root nodules were analyzed by factorial analysis. Analysis indicated that line (L) and stage item were significant at each stage. Dose (D) item factor due to different fertilizer dose (N, P, K, NP, NK, PK and NPK) and interactions (L×D, L×S and D×S) were non-significant at all the stages.

d) Free sugar: Result obtained for this character are given in Table 21 (A-C). The line (L) item was significant, while the dose (D) and stage (S) items were non-significant at all the stages. The different fertilizer dose items viz. N, P, K, NP, NK, PK and NPK were

non-significant except N and PK at S_1 and S_3 and NPK at S_5 stage, where they were significant. The interaction items (L×D, L×S and D×S) were non-significant at all the stages.

d) Reducing sugar: For this character result are given in Table 22 (A-C). The line (L) and dose (D) items were significant at all stages, while stage (S) item was non-significant at each stage. The factors for fertilizer doses were non-significant at all stage except 4th stage for N and PK. The interaction items (L×D, L×S and D×S) were non-significant at the stages.

e) Vitamin C: Result obtained for this character are shown in Table 23 (A-C). The line (L) item showed significance at all stages, while dose (D) and stage (S) items showed non-significance at all stages. The fertilizer doses (N, P, K, NP, PK, NK and NPK) item were non-significant at all the stages. The interaction items (L×D and L×S) were non-significant but (D×S) was significant at all the stages.

Table 18A: Analysis of variance with factorial analysis for moisture at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines (L)	8	446.8324	55.85405	2.314**	446.8324	55.85405	2.314**
Dose (D	7	334.3773	47.76819	1.979NS	334.3773	47.76819	1.979NS
Stage (S)	5	279.2099	55.84198	2.314*	279.2099	55.84198	2.314*
N	1	7.514272	7.514272	0.903 NS	42.01389	42.01389	3.542 NS
P	1	11.63227	11.63227	1.398 NS	4.400556	4.400556	0.371 NS
K	1	4.630939	4.630939	0.556 NS	2.722222	2.722222	0.229 NS
NP	1	29.72205	29.72205	3.572 NS	2.960556	2.960556	0.249 NS
NK	1	4.570272	4.570272	0.549 NS	16.15014	16.15014	1.362 NS
PK	1	24.10494	24.10494	2.897 NS	0.245	0.245	0.02 NS
NPK	1	13.57205	13.57205	1.631 NS	18.2408	18.2408	1.538 NS
L×D	56	1329.835	23.74705	0.984 NS	1329.835	23.74705	0.9838 NS
L×S	40	1308.736	32.71841	1.356 NS	1308.736	32.71841	1.356 NS
D×S	35	1137.769	32.5077	1.347 NS	1137.769	32.5077	1.347 NS
Error	280	6758.662	24.13808		6758.662	24.13808	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 18B: Analysis of variance with factorial analysis for moisture at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines	8	446.8324	55.85405	2.314**	446.8324	55.85405	2.314**
Doses	7	334.3773	47.76819	1.979NS	334.3773	47.76819	1.979NS
Stage	5	279.2099	55.84198	2.314*	279.2099	55.84198	2.314*
N	1	0.45125	0.45125	0.038 NS	139.0556	139.0556	11.465**
P	1	0.003472	0.003472	0.001 NS	278.8735	278.8735	22.993**
K	1	3.690139	3.690139	0.311 NS	89.91405	89.91405	7.41**
NP	1	6.540139	6.540139	0.552 NS	241.8534	241.8534	19.94**
NK	1	0.10125	0.10125	0.01 NS	70.01389	70.01389	5.773**
PK	1	25.56125	25.56125	2.156 NS	134.6988	134.6988	11.106**
NPK	1	0.10125	0.10125	0.01 NS	54.2535	54.2535	4.473**
L×D	56	1329.835	23.74705	0.9838 NS	1329.835	23.74705	0.9838 NS
L×S	40	1308.736	32.71841	1.356 NS	1308.736	32.71841	1.356 NS
D×S	35	1137.769	32.5077	1.347 NS	1137.769	32.5077	1.347 NS
Error	280	6758.662	24.13808		6758.662	24.13808	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 18C: Analysis of variance with factorial analysis for moisture at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines	8	446.8324	55.85405	2.314**	446.8324	55.85405	2.314**
Doses	7	334.3773	47.76819	1.979NS	334.3773	47.76819	1.979NS
Stage	5	279.2099	55.84198	2.314*	279.2099	55.84198	2.314*
N	1	0.403504	0.403504	0.03 NS	0.10125	0.10125	0.007 NS
P	1	0.036001	0.036001	0.003 NS	3.25125	3.25125	0.23 NS
K	1	3.740112	3.740112	0.271 NS	2.03347	2.03347	0.146 NS
NP	1	0.8867	0.8867	0.064 NS	3.42347	3.42347	0.246 NS
NK	1	6.47404	6.47404	0.469 NS	0.51681	0.51681	0.037 NS
PK	1	8.9535	8.9535	0.649 NS	3.16681	3.16681	0.227 NS
NPK	1	0.29262	0.29262	0.021 NS	0.25681	0.25681	0.184 NS
L×D	56	1329.835	23.74705	0.9838 NS	1329.835	23.74705	0.9838 NS
L×S	40	1308.736	32.71841	1.356 NS	1308.736	32.71841	1.356 NS
D×S	35	1137.769	32.5077	1.347 NS	1137.769	32.5077	1.347 NS
Error	280	6758.662	24.13808		6758.662	24.13808	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 19A: Analysis of variance with factorial analysis for dry-matter at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines (L)	8	469.7861	58.72326	2.146*	469.7861	58.72326	2.146*
Dose (D	7	373.2728	53.32469	1.948NS	373.2728	53.32469	1.948NS
Stage (S)	5	321.2337	64.24674	2.347*	321.2337	64.24674	2.347*
N	1	7.424089	7.424089	0.586 NS	8.164841	8.164841	0.78 NS
P	1	11.69667	11.69667	0.92 NS	5.525596	5.525596	0.52 NS
K	1	7.72245	7.72245	0.609 NS	10.10551	10.10551	0.96 NS
NP	1	5.939756	5.939756	0.468 NS	2.960556	2.960556	0.28 NS
NK	1	9.916089	9.916089	0.783 NS	7.22	7.22	0.687 NS
PK	1	3.371339	3.371339	0.27 NS	14.37016	14.37016	1.37 NS
NPK	1	3.345422	3.345422	0.264 NS	18.2408	18.2408	1.735 NS
L×D	56	1375.696	24.56599	0.898 NS	1375.696	24.56599	0.898 NS
L×S	40	1379.426	34.48564	1.26 NS	1379.426	34.48564	1.26 NS
D×S	35	1046.788	29.90823	1.093 NS	1046.788	29.90823	1.093 NS
Error	280	7663.593	27.36998		7663.593	27.36998	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 19B: Analysis of variance with factorial analysis for dry-matter at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines	8	469.7861	58.72326	2.146*	469.7861	58.72326	2.146*
Doses	7	373.2728	53.32469	1.948NS	373.2728	53.32469	1.948NS
Stage	5	321.2337	64.24674	2.347*	321.2337	64.24674	2.347*
N	1	4.029961	4.029961	0.397 NS	10.4044	10.4044	1.21 NS
P	1	0.335381	0.335381	0.033 NS	0.015312	0.015312	0.02 NS
K	1	45.9425	45.9425	4.52*	0.465613	0.465613	0.44 NS
NP	1	2.263483	2.263483	0.22 NS	2.077401	2.077401	0.196 NS
NK	1	4.054603	4.054603	0.4 NS	4.375868	4.375868	0.413 NS
PK	1	0.083096	0.083096	0.01 NS	1.487813	1.487813	0.173 NS
NPK	1	0.038921	0.038921	0.004 NS	7.980013	7.980013	0.93 NS
L×D	56	1375.696	24.56599	0.898 NS	1375.696	24.56599	0.898 NS
L×S	40	1379.426	34.48564	1.26 NS	1379.426	34.48564	1.26 NS
D×S	35	1046.788	29.90823	1.093 NS	1046.788	29.90823	1.093 NS
Error	280	7663.593	27.36998		7663.593	27.36998	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 19C: Analysis of variance with factorial analysis for dry-matter at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines	8	469.7861	58.72326	2.146*	469.7861	58.72326	2.146*
Doses	7	373.2728	53.32469	1.948NS	373.2728	53.32469	1.948NS
Stage	5	321.2337	64.24674	2.347*	321.2337	64.24674	2.347*
N	1	2.314835	2.314835	0.284 NS	1.590139	1.590139	0.21 NS
P	1	0.000501	0.00091	0.001 NS	3.929339	3.929339	0.52 NS
K	1	1.293368	1.293368	0.158 NS	2.42	2.42	0.32 NS
NP	1	3.795013	3.795013	0.46 NS	0.63845	0.63845	0.084 NS
NK	1	5.211068	5.211068	0.64 NS	9.475756	9.475756	1.25 NS
PK	1	42.4274	42.4274	5.207*	7.867222	7.867222	1.04 NS
NPK	1	9.454001	9.454001	1.16 NS	0.2738	0.2738	0.036 NS
L×D	56	1375.696	24.56599	0.898 NS	1375.696	24.56599	0.898 NS
L×S	40	1379.426	34.48564	1.26 NS	1379.426	34.48564	1.26 NS
D×S	35	1046.788	29.90823	1.093 NS	1046.788	29.90823	1.093 NS
Error	280	7663.593	27.36998		7663.593	27.36998	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 20A: Analysis of variance with factorial analysis for protein at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines (L)	8	750.259	93.78238	3.259**	750.259	93.78238	3.259**
Dose (D	7	210.7473	30.10676	1.046NS	210.7473	30.10676	1.046NS
Stage (S)	5	440.159	88.0318	3.0595*	440.159	88.0318	3.0595*
N	1	0.10125	0.10125	0.01 NS	17.405	17.405	1.363 NS
P	1	3.25125	3.25125	0.105 NS	11.84222	11.84222	0.25 NS
K	1	2.0334722	2.033472	0.174 NS	2.493889	2.493889	0.172 NS
NP	1	3.4234722	3.423472	0.257 NS	2.960556	2.960556	0.176 NS
NK	1	0.5168056	0.51681	0.043 NS	17.70125	17.70125	1.3865NS
PK	1	3.1668056	3.16681	0.264 NS	2.42	2.42	0.189 NS
NPK	1	0.2568056	0.256816	0.003 NS	18.2408	18.2408	0.21 NS
L×D	56	1355.396	24.20349	0.841 NS	1355.396	24.20349	0.841 NS
L×S	40	1241.209	31.03023	1.078 NS	1241.209	31.03023	1.078 NS
D×S	35	1026.355	29.32442	1.019 NS	1026.355	29.32442	1.019 NS
Error	280	8056.535	28.77334		8056.535	28.77334	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 20B: Analysis of variance with factorial analysis for protein at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines	8	750.259	93.78238	3.259**	750.259	93.78238	3.259**
Doses	7	210.7473	30.10676	1.046NS	210.7473	30.10676	1.046NS
Stage	5	440.159	88.0318	3.0595*	440.159	88.0318	3.0595*
N	1	17.405	17.405	1.33 NS	1.3778	1.3778	0.092 NS
P	1	11.84222	11.84222	0.28 NS	3.397356	3.397356	0.08 NS
K	1	2.493889	2.493889	0.204 NS	13.8338	13.8338	0.733 NS
NP	1	2.960556	2.960556	0.161 NS	2.960556	2.960556	0.205 NS
NK	1	17.70125	17.70125	1.355 NS	4.9298	4.9298	0.326 NS
PK	1	2.42	2.42	0.186 NS	11.17069	11.17069	0.739 NS
NPK	1	18.2408	18.2408	0.209 NS	18.2408	18.2408	0.21 NS
L×D	56	1355.396	24.20349	0.841 NS	1355.396	24.20349	0.841 NS
L×S	40	1241.209	31.03023	1.078 NS	1241.209	31.03023	1.078 NS
D×S	35	1026.355	29.32442	1.019 NS	1026.355	29.32442	1.019 NS
Error	280	8056.535	28.77334		8056.535	28.77334	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 20C: Analysis of variance with factorial analysis for protein at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines	8	750.259	93.78238	3.259**	750.259	93.78238	3.259**
Doses	7	210.7473	30.10676	1.046NS	210.7473	30.10676	1.046NS
Stage	5	440.159	88.0318	3.0595*	440.159	88.0318	3.0595*
N	1	3.25125	3.25125	0.36 NS	6.42014	6.4201389	0.792 NS
P	1	0.91125	0.91125	0.10 NS	2.84014	2.8401389	0.179 NS
K	1	0.10125	0.10125	0.01 NS	0.17014	0.1701389	0.01 NS
NP	1	16.723472	16.723472	1.84 NS	11.28125	11.28125	1.07 NS
NK	1	1.05125	1.05125	0.12 NS	0.08681	0.0868056	0.01 NS
PK	1	2.10125	2.10125	0.23 NS	0.51681	0.5168056	0.064 NS
NPK	1	0.0734722	0.0734722	0.01 NS	0.21125	0.21125	0.003 NS
L×D	56	1355.396	24.20349	0.841 NS	1355.396	24.20349	0.841 NS
L×S	40	1241.209	31.03023	1.078 NS	1241.209	31.03023	1.078 NS
D×S	35	1026.355	29.32442	1.019 NS	1026.355	29.32442	1.019 NS
Error	280	8056.535	28.77334		8056.535	28.77334	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 21A: Analysis of variance with factorial analysis for free sugar at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines	8	2080.888	260.111	6.015**	2080.888	260.111	6.015**
Doses	7	601.048	85.864	1.985NS	601.048	85.864	1.985NS
Stage	5	265.5686	53.11372	1.23NS	265.5686	53.11372	1.228NS
N	1	199.2008	199.2008	8.84**	3.042222	3.042222	0.127 NS
P	1	271.445	271.445	3.99 NS	4.702222	4.702222	0.061 NS
K	1	124.7147	124.7147	13.1 NS	1.805	1.805	0.17 NS
NP	1	2.960556	2.960556	0.14 NS	2.960556	2.960556	0.134 NS
NK	1	0.467222	0.467222	0.021 NS	0.740139	0.740139	0.031 NS
PK	1	129.5513	129.5513	5.75*	0.160556	0.160556	0.007 NS
NPK	1	18.2408	18.2408	0.2 NS	18.2408	18.2408	0.21 NS
L×D	56	856.6906	15.29805	0.354 NS	856.6906	15.29805	0.354 NS
L×S	40	1822.744	45.56861	1.054 NS	1822.744	45.56861	1.054 NS
D×S	35	1368.135	39.08957	0.904 NS	1368.135	39.08957	0.904 NS
Error	280	12109.29	43.24747		12109.29	43.24747	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 21B: Analysis of variance with factorial analysis for free sugar at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines	8	2080.888	260.111	6.015**	2080.888	260.111	6.015**
Doses	7	601.048	85.864	1.985NS	601.048	85.864	1.985NS
Stage	5	265.5686	53.11372	1.228NS	265.5686	53.11372	1.228NS
N	1	199.2008	199.2008	8.84**	0.10125	0.10125	0.01 NS
P	1	271.445	271.445	3.99 NS	3.25125	3.25125	0.105 NS
K	1	124.7147	124.7147	13.1 NS	2.0334722	2.033472	0.174 NS
NP	1	2.960556	2.960556	0.14 NS	3.4234722	3.423472	0.257 NS
NK	1	0.467222	0.467222	0.021 NS	0.5168056	0.51681	0.043 NS
PK	1	129.5513	129.5513	5.75*	3.1668056	3.16681	0.264 NS
NPK	1	18.2408	18.2408	0.2 NS	0.2568056	0.256816	0.003 NS
L×D	56	856.6906	15.29805	0.354 NS	856.6906	15.29805	0.354 NS
L×S	40	1822.744	45.56861	1.054 NS	1822.744	45.56861	1.054 NS
D×S	35	1368.135	39.08957	0.904 NS	1368.135	39.08957	0.904 NS
Error	280	12109.29	43.24747		12109.29	43.24747	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 21C: Analysis of variance with factorial analysis for free sugar at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines	8	2080.888	260.111	6.015**	2080.888	260.111	6.015**
Doses	7	601.048	85.864	1.985NS	601.048	85.864	1.985NS
Stage	5	265.5686	53.11372	1.228NS	265.5686	53.11372	1.228NS
N	1	63.7463	9.1065	0.87 NS	59.587467	8.512	1.045 NS
P	1	8.164841	8.164841	0.78 NS	2.314835	2.314835	0.284 NS
K	1	5.525596	5.525596	0.52 NS	0.000501	0.00091	0.001 NS
NP	1	10.10551	10.10551	0.96 NS	1.293368	1.293368	0.158 NS
NK	1	2.960556	2.960556	0.28 NS	3.795013	3.795013	0.46 NS
PK	1	7.22	7.22	0.687 NS	5.211068	5.211068	0.64 NS
NPK	1	14.37016	14.37016	1.37 NS	42.4274	42.4274	5.207*
L×D	56	18.2408	18.2408	1.735 NS	9.454001	9.454001	1.16 NS
L×S	40	856.6906	15.29805	0.354 NS	856.6906	15.29805	0.354 NS
D×S	35	1822.744	45.56861	1.054 NS	1822.744	45.56861	1.054 NS
Error	280	1368.135	39.08957		1368.135	39.08957	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 22A: Analysis of variance with factorial analysis for reducing sugar at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines (L)	8	1526.718	190.8398	6.237***	1526.718	190.8398	6.237***
Dose (D	7	1597.039	228.1485	7.457**	1597.039	228.1485	7.457**
Stage (S)	5	290.4535	58.0907	1.899 NS	290.4535	58.0907	1.899 NS
N	1	5.013889	5.013889	0.13 NS	0.321335	0.321335	0.013 NS
P	1	3.208889	3.208889	0.05 NS	1.872113	1.872113	0.025 NS
K	1	0.32	0.32	0.01 NS	11.05283	11.05283	0.90 NS
NP	1	2.960556	2.960556	0.09 NS	2.960556	2.960556	0.124 NS
NK	1	1.333889	1.333889	0.04 NS	1.416806	1.416806	0.07 NS
PK	1	5.12	5.12	0.14 NS	17.59233	17.59233	0.7 NS
NPK	1	18.2408	18.2408	0.21 NS	18.2408	18.2408	0.21 NS
L×D	56	433.2689	7.736945	0.253 NS	433.2689	7.736945	0.253 NS
L×S	40	1094.408	27.36021	0.895 NS	1094.408	27.36021	0.895 NS
D×S	35	711.4834	20.3281	0.665 NS	711.4834	20.3281	0.665 NS
Error	280	8567.492	30.59818		8567.492	30.59818	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 22B: Analysis of variance with factorial analysis for reducing sugar at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines	8	1526.718	190.8398	6.237***	1526.718	190.8398	6.237***
Doses	7	1597.039	228.1485	7.457**	1597.039	228.1485	7.457**
Stage	5	290.4535	58.0907	1.899 NS	290.4535	58.0907	1.899 NS
N	1	10.05014	10.05014	0.29 NS	199.2008	199.2008	8.84**
P	1	0.740139	0.740139	0.01 NS	271.445	271.445	3.99 NS
K	1	2.240139	2.240139	0.06 NS	124.7147	124.7147	13.1 NS
NP	1	2.960556	2.960556	0.087 NS	2.960556	2.960556	0.14 NS
NK	1	1.333889	1.333889	0.04 NS	0.467222	0.467222	0.02NS
PK	1	10.20014	10.20014	0.297 NS	129.5513	129.5513	5.75*
NPK	1	18.2408	18.2408	0.21 NS	18.2408	18.2408	0.2 NS
L×D	56	433.2689	7.736945	0.253 NS	433.2689	7.736945	0.253 NS
L×S	40	1094.408	27.36021	0.895 NS	1094.408	27.36021	0.895 NS
D×S	35	711.4834	20.3281	0.665 NS	711.4834	20.3281	0.665 NS
Error	280	8567.492	30.59818		8567.492	30.59818	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 22C: Analysis of variance with factorial analysis for reducing sugar at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines	8	1526.718	190.8398	6.237***	1526.718	190.8398	6.237***
Doses	7	1597.039	228.1485	7.457**	1597.039	228.1485	7.457**
Stage	5	290.4535	58.0907	1.899 NS	290.4535	58.0907	1.899 NS
N	1	0.516806	0.516806	0.021 NS	4.108889	4.108889	0.13 NS
P	1	3.083472	3.083472	0.04 NS	0.200556	0.200556	0.004 NS
K	1	0.086806	0.086806	0.01 NS	2.568889	2.568889	0.1 NS
NP	1	2.960556	2.960556	0.183 NS	2.960556	2.960556	0.144 NS
NK	1	0.760556	0.760556	0.031 NS	4.753472	4.753472	0.154 NS
PK	1	3.423472	3.423472	0.14 NS	9.975556	9.975556	0.322 NS
NPK	1	18.2408	18.2408	0.21 NS	18.2408	18.2408	0.21 NS
L×D	56	433.2689	7.736945	0.253 NS	433.2689	7.736945	0.253 NS
L×S	40	1094.408	27.36021	0.895 NS	1094.408	27.36021	0.895 NS
D×S	35	711.4834	20.3281	0.665 NS	711.4834	20.3281	0.665 NS
Error	280	8567.492	30.59818		8567.492	30.59818	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 23A: Analysis of variance with factorial analysis for vitamin C at 1st and 2nd stage (after 15 and 30 days of sowing).

Items	df	1st			2nd		
		SS	MS	F	SS	MS	F
Lines (L)	8	2021.733	252.7166	7.082**	2021.733	252.7166	7.082**
Dose (D	7	110.308	15.75829	0.4416NS	110.308	15.75829	0.4416NS
Stage (S)	5	464.431	92.8862	2.603	464.431	92.8862	2.603
N	1	0.190139	0.190139	0.01 NS	16.2355	16.2355	0.264 NS
P	1	0.66125	0.66125	0.01 NS	10.41961	10.41961	0.06 NS
K	1	0.483472	0.483472	0.055 NS	62.73867	62.73867	0.86 NS
NP	1	2.960556	2.960556	0.138 NS	2.960556	2.960556	0.04 NS
NK	1	0.5	0.5	0.021 NS	33.48347	33.48347	0.55 NS
PK	1	0.256806	0.256806	0.011 NS	77.27317	77.27317	1.26 NS
NPK	1	18.2408	18.2408	0.21 NS	18.2408	18.2408	0.21 NS
L×D	56	592.2755	10.57635	0.296 NS	592.2755	10.57635	0.296 NS
L×S	40	1466.664	36.66661	1.028 NS	1466.664	36.66661	1.028 NS
D×S	35	2705.729	77.30655	2.166 **	2705.729	77.30655	2.166 **
Error	280	9991.963	35.68558		9991.963	35.68558	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 23B: Analysis of variance with factorial analysis for vitamin C at 3rd and 4th stage (after 45 and 60 days of sowing).

Items	df	3rd			4th		
		SS	MS	F	SS	MS	F
Lines (L)	8	2021.733	252.7166	7.082**	2021.733	252.7166	7.082**
Dose (D	7	110.308	15.75829	0.4416NS	110.308	15.75829	0.4416NS
Stage (S)	5	464.431	92.886	2.603	464.431	92.8862	2.603
N	1	18.70681	18.70681	0.296 NS	13.60681	13.60681	0.183 NS
P	1	14.67014	14.67014	0.12 NS	19.95014	19.95014	0.149 NS
K	1	37.99014	37.99014	0.524 NS	30.55014	30.55014	0.325 NS
NP	1	2.960556	2.960556	0.04 NS	2.960556	2.960556	0.034 NS
NK	1	15.86722	15.86722	0.251 NS	11.20222	11.20222	0.151 NS
PK	1	3.00125	3.00125	0.05 NS	5.61125	5.61125	0.075 NS
NPK	1	18.2408	18.2408	0.2 NS	18.2408	18.2408	0.21 NS
L×D	56	592.2755	10.57635	0.296 NS	592.2755	10.57635	0.296 NS
L×S	40	1466.664	36.66661	1.028 NS	1466.664	36.66661	1.028 NS
D×S	35	2705.729	77.30655	2.166 **	2705.729	77.30655	2.166 **
Error	280	9991.963	35.68558		9991.963	35.68558	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

Table 23C: Analysis of variance with factorial analysis for vitamin C at 5th and 6th stage (after 75 and 90 days of sowing).

Items	df	5th			6th		
		SS	MS	F	SS	MS	F
Lines (L)	8	2021.733	252.7166	7.082**	2021.733	252.7166	7.082**
Dose (D	7	110.308	15.75829	0.4416NS	110.308	15.75829	0.4416NS
Stage (S)	5	464.431	92.8862	2.603	464.431	92.8862	2.603
N	1	3.042222	3.042222	0.127 NS	1.805	1.805	0.072 NS
P	1	4.702222	4.702222	0.061 NS	0.268889	0.268889	0.003 NS
K	1	1.805	1.805	0.17 NS	0.200556	0.200556	0.015 NS
NP	1	2.960556	2.960556	0.134 NS	2.960556	2.960556	0.134 NS
NK	1	0.740139	0.740139	0.031 NS	0.760556	0.760556	0.031 NS
PK	1	0.160556	0.160556	0.007 NS	0.268889	0.268889	0.01 NS
NPK	1	18.2408	18.2408	0.21 NS	18.2408	18.2408	0.21 NS
L×D	56	592.2755	10.57635	0.296 NS	592.2755	10.57635	0.296 NS
L×S	40	1466.664	36.66661	1.028 NS	1466.664	36.66661	1.028 NS
D×S	35	2705.729	77.30655	2.166 **	2705.729	77.30655	2.166 **
Error	280	9991.963	35.68558		9991.963	35.68558	

* and ** indicate significance at 5% and 1% level, respectively and NS indicates non-significance.

2. Components of variation:

The estimates of phenotypic (k^2_p), genotypic (k^2_g), line × dose ($k^2_{L\times D}$), line × stage ($k^2_{L\times S}$), dose × stage ($k^2_{D\times S}$) and error (σ^2_e) error components of variation were calculated separately for the all six characters and the results are given in Table 24.

a) Phenotypic variation (k^2_p): For all the characters phenotypic variation was always greater than those of k^2_g, $k^2_{L\times D}$, $k^2_{L\times S}$, $k^2_{D\times S}$, and σ^2_e component of variation as expected. The phenotype is the joint product of k^2_g, $k^2_{L\times D}$, $k^2_{L\times S}$, and σ^2_E. Table 3 showed that the greater portion of the total phenotypic variation was appeared mostly due to the error variation for all the characters. The maximum phenotypic variation was observed for the biochemical character free sugar with a value of 280.481 and the lowest value of 48.81231 for moisture.

b) Genotypic variation (k^2_g): The highest genotypic variation was observed for the character, free sugar with a value of 242.4918 and the lowest value of 23.52667 was recorded for moisture (Table 21).

c) D × L interaction variation ($k^2_{D\times L}$): The estimation of the interaction variation ($\sigma^2_{D\times L}$) was the highest for moisture with a value of -0.078206 and the lowest value of 0.005 was recorded for free sugar. (Table 13).

d) L × S interaction variation ($k^2_{L\times S}$): The estimation of the interaction variation ($k^2_{L\times S}$) was the highest for moisture with a value of 1.225761 and was the lowest with a value of –0.462568 for reducing sugar.

e) D × S interaction variation ($k^2_{D\times S}$): The D × S interaction variation (Table 13) was the highest with a value of 5.202621 for vitamin C and the lowest value of -1.283761 was recorded for reducing sugar.

f) Error variation (σ^2_e): The highest error variation (σ^2_e) was recorded for the character, free sugar which was 43.24747 and the lowest value of 24.13808 was recorded for moisture.

3. Co-efficient of variability:

The estimates of phenotypic, genotypic, interactions (L×D, L×S, D×S) and error co-efficient of variability were computed for all the six characters viz. moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C and the results obtained are shows in Table 22.

a) Phenotypic co-efficient of variability (PCV): Table 22 shows that the estimates of phenotypic co-efficient of variability was the highest for protein with a value of 61.83014 and the lowest value of 42.35004 was recorded for dry-matter.

b*) Genotypic co-efficient of variability (GCV):* The highest genotypic co-efficient of variability (GCV) with a value of 31.9681 was recorded for protein and the lowest value of 12.9466 was found for dry-matter.

c) L × D interaction co-efficient of variability (L×D)CV: The (L×D)CV highest value of-0.08223 was recorded for moisture and the lowest value of -5.9455 was recorded for free sugar.

d) L × S interaction co-efficient of variability (L×S)CV: The estimates for (L×S)CV was the highest for moisture with a value of 1.2866 and the lowest value of -0.4908 for reducing sugar.

e) D × S interaction co-efficient of variability (D×S)CV: For (D×S)CV the highest value of 5.528 was recorded for vitamin C and the lowest value of -0.553 was recorded for free sugar.

f) Error co-efficient of variability (ECV): The highest error co-efficient of variability (ECV) was recorded for free sugar, with value of 45.999 and the lowest variability of 25.38 was recorded for moisture.

Table 24: Phenotypic, genotypic, interactions and error variance for different characters

Characters	k^2_p	k^2_g	$k^2_{L\times D}$	$k^2_{L\times S}$	$k^2_{D\times S}$	σ^2_e
Moisture	48.81231	23.52667	-0.078206	1.225761	1.046202	24.13808
Dry-matter	54.86732	27.04161	-0.560796	1.016524	0.317281	27.36998
Protein	95.50377	67.32199	-0.91397	0.322413	0.068885	28.77334
Free sugar	280.481	242.4918	-5.589884	0.331591	-0.519737	43.24747
Reducing sugar	211.9042	186.3408	-4.572248	-0.462568	-1.283761	30.59818
Vitamin C	159.5255	128.7216	-5.021847	0.140147	5.202621	35.68558

Table 25: Phenotypic, genotypic, interactions and within error coefficient of variability for different characters.

Characters	PCV	GCV	(L×D)CV	(L×S)CV	(D×S)CV	ECV
Moisture	47.53892	20.9527	-0.08223	1.28881	1.100015	25.37964
Dry-matter	42.35004	12.9466	-0.5926	1.074162	0.335272	28.92188
Protein	61.83014	31.9681	-0.96846	0.341636	0.072992	30.48886
Free sugar	56.99592	16.5902	-5.94546	0.352684	-0.5528	45.9985
Reducing sugar	51.97652	24.8572	-4.8506	-0.4908	-1.3619	32.46072
Vitamin C	49.31878	16.5889	-5.33583	0.14891	5.52791	37.9168

4. Heritability (h^2_b), genetic advance (GA) and genetic advance as a percentage of mean (GA%):

For all the characters heritability in broad sense (h^2_b), genetic advance and genetic advance as a percentage of mean (GA%) were computed and the results are shows in Table 26.

a) Heritability (h^2_b): The values for heritability in broad sense (h^2_b) were presented in Table 26. The highest heritability value was estimated for the character, reducing sugar with a value of 87.93636 and the lowest heritability value was recorded for moisture with a value of 48.19824.

b) Genetic advance (GA): The highest value of genetic advance was estimated for the character, free sugar with a value of 2.1 and the lowest value of 0.345 was recorded for moisture.

c) Genetic advance as a percentage of mean (GA%): The highest GA% was found for reducing sugar with a value of 93.01487 and the lowest for the same was shown by the character moisture with a value of 7.186223.

Table 26: Heritability (h^2_b), genetic advance (GA) and genetic advance as a percentage as mean (GA%)

Characters	Heritability (h^2_b)	Genetic advance	Genetic advance as a % mean
Moisture	48.19824	6.936861	7.186223
Dry-matter	49.28546	7.520434	66.55251
Protein	70.49145	14.19103	60.38736
Free sugar	86.4557	29.82721	71.01716
Reducing sugar	87.93636	26.36972	93.01487
Vitamin C	80.6903	20.9944	97.19632

B. DETERMINATION OF MOLECULAR WEIGHT OF PROTEIN FOR ROOT NODULES

1. By SDS-polyacrylamide slab gel electrophoresis: Molecular weight of protein was also determined by SDS polyarylamide slab gel electrophoresis. The molecular weight of protein was calculated from the standard curve which was constructed by plotting the log 10 of molecular weight against the relative mobility of the marker protein on the gel.

The determination number of protein band and molecular weight of seeds and root nodules in different line in blackgram are also shown in Table 27 (A-B).

a) Seeds: Among these lines the highest number of protein band with a value of 11 was recorded for L_{11} and L_{19} and the lowest value of 7 for L_{16} and L_{18}. The highest molecular weight of protein with a value of 16000 for L_{19} and L_{20} and the lowest value of 41200 was recorded for L_{11} among these lines.

b) Root nodule: The highest number of protein band was recorded for L_{13} and $L_{19}$with a value of 11 and the lowest value of 7 was recorded for L_{18} among these lines. The highest molecular weight of protein with a value of 116300 for L_{13} and the lowest value of 42200 was recorded for L_{13} and L_{19} among these lines.

Table 27A: Number of protein band and molecular weight for nine line seeds in blackgram

Lines	No. band	Molecular weight										
		1	2	3	4	5	6	7	8	9	10	11
L_2	9	112200	110600	109500	86800	79300	75500	61000	54300	42400	0	0
L_{11}	11	116500	113100	110600	109500	86700	79300	74400	61200	54800	43100	41200
L_{13}	9	105500	87000	80400	77700	71400	69000	62600	54800	43000	0	0
L_{14}	10	111800	110700	109300	86000	80000	75400	59300	54400	48500	42200	0
L_{15}	8	110800	81800	79300	72800	69800	62400	54400	42800	0	0	0
L_{16}	7	80400	75400	69000	61200	54400	48400	42200				
L_{18}	7	113000	110900	86000	78500	76200	54400	43000				
L_{19}	11	116000	115100	113200	110900	107400	86000	78500	69400	54400	42200	
L_{20}	9	116000	115100	113200	86000	78500	69400	54400	42200			

Table 27B: Number of protein band and molecular weight for nine line root nodules in blackgram

Lines	No. band	Molecular weight										
		1	2	3	4	5	6	7	8	9	10	11
L_2	10	114200	111600	110900	86500	79300	76500	62000	54300	43400	41700	
L_{11}	10	115500	111600	110600	109500	86700	80300	74400	59200	54800	43100	
L_{13}	11	116300	113500	109500	87500	80400	75700	71400	67500	62600	54800	42200
L_{14}	10	114800	113700	111300	86500	80400	75700	59300	54400	48500	42200	
L_{15}	9	114800	110500	86600	79300	72800	69800	61400	54400	42200		
L_{16}	8	1109400	86400	77400	75200	69200	54400	48400	42200			
L_{18}	7	111300	110500	86400	78500	71200	54400	42200				
L_{19}	11	115900	116300	114200	111900	109400	86500	77500	69400	59300	54400	42200
L_{20}	9	116000	115100	113200	86000	78500	69400	54400	42200			

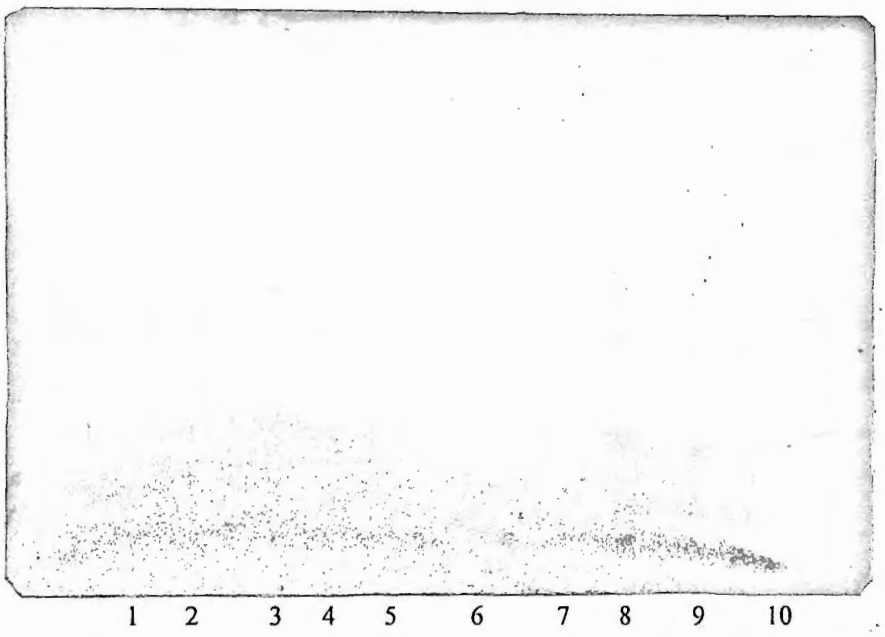


Fig. 5: SDS polyacrylamide slab gel electrophoretic pattern showing of protein bands. 1, 2, 3, 4, 5, 6, 7, 8 and 9, for lines L_2, L_{11}, L_{13}, L_{14}, L_{15}, L_{16}, L_{18}, L_{19} and L_{20}, in root nodules and 10- markers.

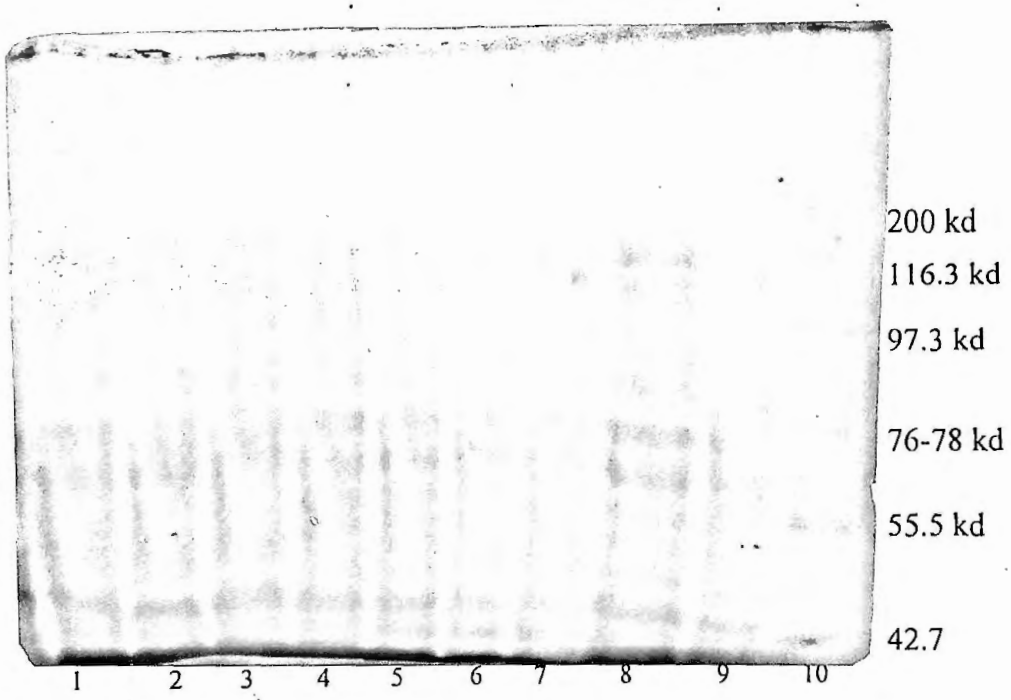


Fig. 6: SDS polyacrylamide slab gel electrophoretic pattern showing of protein bands. 1, 2, 3, 4, 5, 6, 7, 8 and 9, for lines L_2, L_{11}, L_{13}, L_{14}, L_{15}, L_{16}, L_{18}, L_{19} and L_{20}, in seeds and 10-markers.

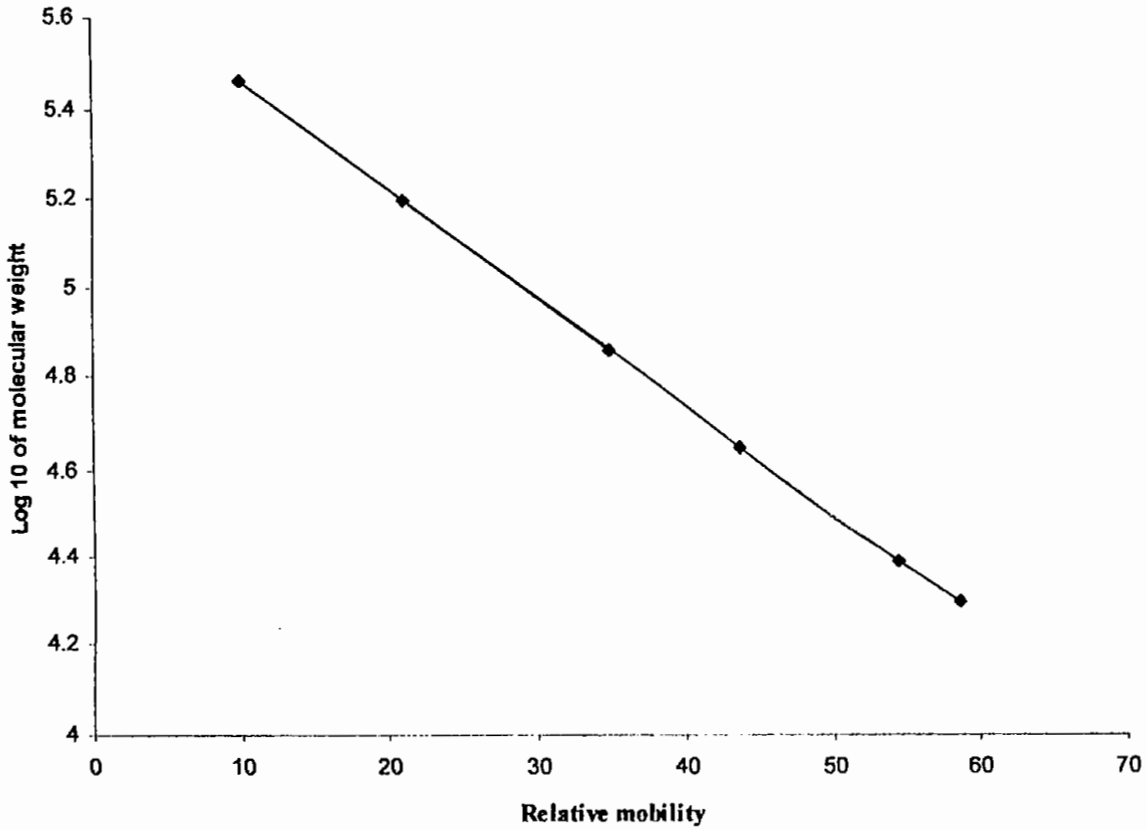


Fig. 7: Standard curve of marker protein for estimation of molecular weight of protein.

C. RESULTS OF IDENTIFICATION OF AMINO ACIDS FOR NINE LINES OF ROOT NODULE

The amino acids were calculated by comparing the R_f value of each known and unknown amino acids and the results are given in Table 28. From the table it is found that in line L_2 all the amino acids were present except threonine and aspergine. The line L_{11} contained all the amino acids except absent tyrosine. Isoleucine and glutamic acid was absent and the other amino acids were present in line L_{13}. Line L_{14} possessed all the amino acids but was absent aspertic acid. In line L_{15} except alanine and proline all the amino acids were present. Line L_{16} contained all the amino acids and line L_{18} possessed all the amino acids except isoleucine. Except serine all other amino acids were present L_{19}. Tryptophane and glutamine acid were absent and other are present in line L_{20}. On the overall found it found that glysine, methionine, leucine, phenyl alanine, lysine and arginine amino acids were contained in all the root nodule lines.

Table 28: Indicates presence and absence of amino acids in nine lines of root nodules

Amino acid	Lines no								
	L_2	L_{11}	L_{13}	L_{14}	L_{15}	L_{16}	L_{18}	L_{19}	L_{20}
Glycine	+	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	-	+	+	+	+
Methionine	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+
Isoleucine	+	+	-	+	+	+	-	+	+
Phenyl alanine	+	+	+	+	+	+	+	+	+
Threonine	-	+	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+	+	-
Serine	+	+	+	+	+	+	+	-	+
Lysine	+	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+	+
Tyrosine	+	-	+	+	+	+	+	+	+
Glutamine	+	+	+	+	+	+	+	+	-
Glutamic acid	+	+	-	+	+	+	+	+	+
Aspergine	-	+	+	+	+	+	+	+	+
Aspertic acid	+	+	+	-	+	+	+	+	+
Proline	+	+	+	+	-	+	+	+	+
Valine	+	+	+	-	+	+	+	+	+

(+) and (-) Indicate presence and absence, respectively

E. COLONY COUNTS OF RHIZOBIUM BACTERIA:

Observation was taken after 48 hours. The number of colonies were counted from each plate and data were recorded for each line. The results after calculation are put in Table 31. It was observed that the number of colonies were different in each of the lines. The highest number of colonies were found with a value of 2.7334×10^7 for L_{20} and the lowest was found to be 2.1×10^7 for L_{14}. The standard deviation (SD) and the standard error of mean (S_x) as calculated for each of the line are shown in table 29 also. Comparison of means indicated that all the means were significant.

Table 29: Total number of Rhizobium bacterium colony for ... root nodule lines

Lines	Number of colonies grown per mg			$\bar{X} \pm$ SE	SD
	R_1	R_2	R_3		
L_2	2.4×10^7	2.7×10^7	2.1×10^7	$2.4 \times 10^7 \pm 1.732 \times 10^6$	3×10^6
L_{11}	2.2×10^7	2.3×10^7	2.3×10^7	$2.267 \times 10^7 \pm 0.333 \times 10^6$	0.577×10^6
L_{13}	2.5×10^7	2.4×10^7	2.3×10^7	$2.4 \times 10^7 \pm 0.577 \times 10^6$	1×10^6
L_{14}	2.1×10^7	2.0×10^7	2.2×10^7	$2.1 \times 10^7 \pm 0.577 \times 10^6$	1×10^6
L_{15}	2.5×10^7	2.6×10^7	2.7×10^7	$2.6 \times 10^7 \pm 0.577 \times 10^6$	1×10^6
L_{16}	2.3×10^7	2.3×10^7	2.4×10^7	$2.333 \times 10^7 \pm 0.333 \times 10^6$	0.577×10^6
L_{18}	2.6×10^7	2.7×10^7	2.8×10^7	$2.7 \times 10^7 \pm 0.577 \times 10^6$	1×10^6
L_{19}	2.3×10^7	2.5×10^7	2.8×10^7	$2.533 \times 10^7 \pm 1.453 \times 10^6$	2.517×10^6
L_{20}	2.7×10^7	2.6×10^7	2.9×10^7	$2.733 \times 10^7 \pm 0.882 \times 10^6$	1.527×10^6

DISCUSSION

The present study was carried out to do the factorial and variance analyses and variability, heritability and genetic advance of six chemical characters, such as moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C of root nodule in blackgram. Identification of amino acids, and colony counts of Rhizobium bacteria in root nodules and molecular weight of protein in both root nodules and seed grains were also done.

The six chemical characters of root were found to be quantitative in nature because continuous gradation were found among the estimated data of those characters. The quantitative nature of the characters of blackgram were also reported by Hossain (1977). Therefore, the biometrical techniques as developed by Mather (1949) based on the mathematical models of Fisher *et al.* (1932) to study the quantitative characters were found suitable to dedect the genetic system involved in controlling these characters.

The results of the factorial and variance analysis for the six characters of root nodules at each stage are shown in Table 18-23. For all the characters like moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C, the line (L) items were highly significant at all the stages, which indicated that the lines were genetically different from each other. This referred the fact that the included lines would be suitable for further breeding research for the improvement of these characters. Similar records were also made by Samad (1988) in his investigation of certain agronomical characters in rape seed. Ali (1988) found similar result in ten lentil cultivars. Khaleqe *et al.* (1991) also obtained similar records with some chemical characters in chilli. Similar records were also made by Sharoar (2002) and Islam (2002) in their investigation of some quantitative characters in blackgram. The dose (D) item was non-significant at all the stages for all the characters except reducing sugar, while non-significance indicated no effect of eight fertilizer doses on these characters. Stage (S) item was non-significant at each stage for all the characters, except free sugar and reducing sugar, non-significance and significance indicated real effect and no effect of different stages, respectively, (at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}

and 6th) stages after 15, 30, 45, 60, 75 and 90 days of sowing). The effect due to nitrogen (N) was found to be non-significant at each stage for all the characters except at 4th stage for protein and reducing sugar and 1st and 3rd stage for free sugar, where it was significant. The phosphorous (P) item was non-significant at each stage for all the characters except at 4th stage for protein, while non-significance indicated real no effect of phosphorous (P) at different stages (at 1st, 2nd, 3rd, 5th and 6th stage, respectively) for all the characters. The effect of potassium (K) was found to be non-significant at each stages for all the characters except at 4th and 3rd stages of protein and dry-matter, respectively. Nitrogen (N) interacted with phosphorous (P) as indicated by the non-significant interaction item for all the characters at each stage except at 4th stage for protein. The combined dose of nitrogen (N) and potassium (K) item was observed to be non-significant at each stage for all the characters except at 4th stage for protein. The dose PK item was found to be significant at 1st, 3rd and 4th stage for reducing sugar and at 5th stage for dry-matter and other stages and characters were observed non-significant, while non-significance indicated no real effect at 1st, 2nd, 3rd, 4th, 5th and 6th stages (after 15, 30, 45, 60, 75 and 90 days of sowing) for these characters. The second order combination NPK item was non-significant at 1st, 2nd, 3rd, 4th, 5th and 6th stages (after 15, 30, 45, 60, 75 and 90 days of sowing) for these characters except at 4th and 6th stages (after 60 and 90 days of sowing) for protein and free sugar respectively, where they were significant. The interaction items (L×D, L×S and D×S) were found to be non-significant at each stage for the characters except D×S for vitamin C.

Diversity estimates are important in the selection breeding research. In this short of analysis among the chemical characters the highest genetypic and phenotypic variances were observed for free sugar following reducing sugar, vitamin C and protein. Large genotypic value is always helpful for effective selection. These results are in agreement with the findings of Main and Awal (1979). Khaleque *et al.* (1991) also obtained similar records with some chemical characters in chilli. Phenotypic co-efficient of variability was greater than genotypic and all other co-efficient of variabilities. Samad (1991) also obtained more or less similar results. Protein shows the highest phenotypic and genotypic variability among these chemical characters. It indicated that this character was inherited with higher variability within their sibs. Such high values of genotypic and phenotypic

co-efficient also suggested good scope for improvement of the character through selection (Shaha *et al.* 1981). The lowest GCV and PCV were recorded for dry-matter, which indicated lower variability within their sibs.

All the chemical characters, under study exhibited high heritability estimates except moisture and dry-matter, suggesting the possibility of selection response based on their phenotypic expression. The high heritability along with the high expected genetic gain are usually more helpful than the heritability alone in predicting the resultant effect from selecting the best individual as was suggested by Johnson *et al.* (1955). However, Swarup and Chaugle (1962) have stressed the need to evaluate the GCV together with the heritability estimate and the genetic gain. In the present work, all the chemical characters except moisture and dry-matter for root nodules exhibited a high heritability and genetic advance as percentage of mean, which indicated that the effective selection of this characters may likely improve yield as suggested by Khanum *et al.* (1981) worked in mungbean.

To study the characteristics of protein estimation of its molecular weight and calculation the number of protein bands are in important. The number of protein bands and the molecular weight as obtained were mostly different in most of the lines for seeds and root nodules. In maximum cases the molecular weight and the number of band were found to be similar for seeds and root nodules in L_{20} followed by L_{11}, L_{14} and L_{15}. The similarity in molecular weight and in number of bands in least number of cases was found in L_{18} for seeds and root nodules. As per the results obtained (Table 27), the line L_{20} contained in all 9 different protein bands and 9 different molecular weights, which were similar in seeds and root nodules. L_{11}, L_{14} and L_{15} possessed respectively 6, 4 and 4 different protein bands and different molecular weights, which were similar for seeds and root nodules. From this result it appears that protein metabolism in seeds and root nodules are similar in these lines. Further, it also indicates that similar types of enzymes are being produced through DNA template, i.e. with the concern of similar nature of genes in seeds and root nodules of the lines mostly for L_{20}, L_{11}, L_{14} and L_{15}. Significant positive correlation between the characteristics (NN and NWt), and seeds (yield) in section II of the present study confirms this relationship between root nodules and seeds. All these,

therefore, show that the lines L_{20}, L_{11}, L_{14} and L_{15} may likely be considered for the improvement of yield in blackgram through the improvement of root nodule characteristics in further breeding research. In this regard the line L_{20} is most important because all the 9 different protein bands and 9 different molecular weights are almost similar in blackgram in both root ndules and seeds.

Table 28 shows that the nine lines were different in possession of maximum two of the 18 amino acids analyzed. However, on the overall basis of the 18 amino acids analyzed only six like glycine, methionine, leucine, phenylalanine, lysine and arginine amino acids were common in all the nine lines. In consideration of these differences in different lines of blackgram, further breeding research programme may be taken for the improvement of bacterial abode in the root nodule, which in turn may influence high yield.

In the analysis of bacterial concentration it was found that the same was the highest in L_{20} followed by L_{18}, L_{15} and L_{19} (Table 29). It also appears from the table that the mean in different lines were mostly different and were highly significant since standard error of means were minimum regarding concentration of bacterial colonies in concern of nitrogen fixation which might influence higher yield in blackrgam.

Diversity estimates of the chemical characters in the present study indicated that protein, reducing and free sugar, and vitamin C was highly heritable. Regarding amino acids and concentration of the bacterial colonies the nine lines were found to be different. ...

...breeding programme may be taken for the improv... bacterial ...

... found to be different. Thus breeding programme may be taken for the improvement of seeds grain in blackgram through the improvement of bacterial abode i. e. root nodules, which with more number of bacterial colonies in turn may like to improve the yield; and this is posible because nodule number and nodule weight was significantly correlated with yield, and also for the fact that similar genes were functioning for protein synthesis in nodules and in seeds. In this regard the lines L_{20} and L_{15} would likely be good breeding materials, since most of the proteins of these lines are similar in nodules and seeds and possessed highest concentration of bacterial colonies.

SUMMARY

In the present investigation, it was found that the chemical characteristics of root nodules such as moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C are quantitative in nature in their inheritance and the biometrical techniques developed to study the quantitative characters were found suitable to evaluate the genetic system involved in controlling these characters.

In the analysis of variance of root nodules at each stage for the six characters, viz. Moisture, dry-matter, protein, free sugar, reducing sugar and vitamin C, were highly significant, which indicated that the lines were genetically different from each other. The dose (D) and stage (S) effects were not found for all the chemical characters except reducing sugar and free sugar only for stage. Application of the individual dose, N, K, P and combined dose NP, NK, PK and NPK have no effect in most of the stages for all the characters. The interaction item were found to be non-significant at each stage for all the characters except vitamin C.

Among these chemical characters, protein showed the highest PCV and GCV, which indicated that this character was inherited with higher variability within their sibs. The lowest GCV and PCV were recorded for dry-matter.

All the chemical characters, under study exhibited high heritability estimates except moisture and dry-matter, suggesting the possibility of selection response based on their phenotypic expression. In the present work, all the chemical characters except moisture and dry-matter for root nodules exhibited high heritability and genetic advance as percentage of mean.

In the analysis of molecular weight of protein and its bands it was found that lines L_{20}, L_{11}, L_{14} and L_{15} contained mostly similar types of protein in seeds and root nodules. This similarity in regard of molecular weight of protein and its band for nodule and seeds was found in least number of cases in L_{18}. The similarity in protein structure i. e. molecular

weight and band indicated that the nature of genes for protein synthesis is similar in nodules and seeds.

Of the 18 amino acids analyzed, for maximum two cases all the nine lines were found to be different. However, glycine, methionine, leucine, phenylalanine, lysine and arginine amino acids were common for root nodules in all the lines. Regarding the characteristics of bacterial colonies all the nine lines were different. The highest concentration was found for L_{20} followed by L_{18}, L_{15} and L_{19}. Selection of L_{20} and L_{15} in further breeding research for high yield in blackgram may yield good results with the higher concentration of bacterial colonies. Since the nature of genes responsible for the synthesis of protein is similar in nodules and seeds, more also the nodule characteristics are positively correlated with seed yield.

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