PROTEIN IN COWPEA : ITS VARIABILITY AND HERITABILITY, GENETIC DIVERGENCE AND ASSOCIATIONSHIP WITH YIELD COMPONENTS

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BY

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DEDICATED
TO MY
BELOVED PARENTS

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled **PROTEIN IN COWPEA : ITS VARIABILITY AND HERITABILITY, GENETIC DIVERGENCE AND ASSOCIATIONSHIP WITH YIELD COMPONENTS,** submitted by Miss Nita Kar in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Horticulture of the Bidhan chandra Krishi Viswavidyalaya, is the faithful and bonafide research work carried out under my personal supervision and guidance

The results of the investigation reported in the thesis have not so far been submitted for any other degree or diploma The assistance and help received during the course of investigation have been duly acknowledged.

Mg Som
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Dated Mohanpur,

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APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE

We the undersigned having been satisfied with the performance of Miss Nita Kar in the Viva-voce examination conducted today, the 17th December, 1997 recommended that the thesis be accepted for the award of the degree

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INTRODUCTION

INTRODUCTION

Green revolution that refers to rapid increase in yield and production of cereals (wheat, rice and maize) took place in India through the development of high yielding varieties Our soaring production of cereals is the reflection of the green revolution which has helped us achieving present self sufficiency in food front Unfortunately, comparatively little research endeavours in terms of research money also, have been channelised for the improvement of legume crops that are so important in human diets in a developing country like India In our country per capita availability of protein and calories is probably the lowest in the world The most limiting vitamins in our diet are vitamin A and riboflavin The use of vegetable protein to improve the diets of the population at nutritional risk is of particular economic and social interest specially when food as milk, meat, fish and eggs are in short supply or beyond the economic reach of such groups Plant protein is the cheapest available source of protein in countries where few can afford high value animal proteins In this situation of very low economic status, vagaries of malnutrition and vegetarian dietary habits of majority of our population, vegetable proteins are extremely and increasingly important component of our nutrition

Cowpeas (*Vigna unguiculata* (L) walp), an African Vigna and the excellent protein rich crop are considered an important species among the legumes whose dry seed, green pod, green seed, tender green leaves as well are utilized as sources of palatable and high quality inexpensive protein It is a key staple for the poorest sector of many developing countries.

Main breeding objectives of cowpea in India and other countries are high yield potential and resistance to diseases and insect pests Recently efforts are being made to frame breeding programme for improving protein content and cooking qualities (Nielsen *et al*, 1993) Although the pods and seeds of cowpea are rich in protein compared to those of other vegetables and food staples, it is important to atleast maintain or increase those levels of protein with the other changes i e increased pod/seed yield When pods/seeds are taken directly as opposed to being a source of industrial protein, protein intake usually reflects quantity of pods/seeds consumed rather than consumption until protein demands are made Since the intake quantity of pods/ seeds is usually limited by availability and inaccessibility, it is advantageous to have high concentration of good quality protein in tender pods and mature seeds of cowpea

In the present investigation different approaches have been persuaded to obtain information necessary to frame a breeding strategy using classical breeding and selection methods for improving protein content of pods and seeds concurrently with improving yield potential The investigation approaches included

1. Pod and seed characters in the three cultigroups at three different stages of pods
2. Genetic variability and heritability for different pod characters, leaf characters, pod yield and protein content of pods and seeds
3. Associationship of pod and seed protein contents with different pod and leaf characters and pod yield
4. Influence of root and nodule characters on protein content of pods and seeds

5. Gene action for pod protein content and other yield components
6. Analysis of heterosis for pod yield components, pod yield and pod protein content

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cowpeas (*Vigna unguiculata* (L) Walp) are grown widely for green pods and immature seeds as vegetable and mature dry seeds as pulse as a source of high quality inexpensive protein It also forms an important component of farming systems from the semiarid to the humid tropics Cowpeas are major source of diet~~o~~ary protein in Africa, ~~l~~Latin America and Asia The breeding objectives are primarily focussed on high yield potential and resistance to diseases and insect pests Now efforts are being made to initiate breeding programme to improve protein content and quality In this review different aspects related to chalking out breeding strategy for improving protein content have been dealt with Proteins are polymers having high molecular weight that are built from amino acid monomers of which about twenty are important Of th~~is~~ese amino acids, some can be synthesized by body chemistry and others need ~~to~~ be supplied as amino acids in diet Amino acids which need ~~to~~ be supplied as such are described as essential Important essential amino acids are Arginine, Histidine, Isoleucine, leucine, lysine, Methionine, cystine, phenylalanine, Tyrosine, Threonine, Tryptophan and Valine Polymeric saccharides are found in almost all plants whilst proteinic polymers are associated with animals and also almost all plants even if only in small doses For this reason all these storage plant proteins are associated with carbohydrate, starch in particular In grain legumes globulins, which ~~is~~ are the major storage protein, account for about 70% of the total, with gluterlins

why capital?

albumins and free amino acids making up the reminder (Norton *et al*, 1985) The total seed protein is constituted by many different proteins rather than being homogeneous Each of the protein has different chemical and physical properties

Protein content :

Protein contents of cowpea have mostly been reported in terms of seed protein and limited reports are there for the protein contents of pods and leaves Protein content of pods, leaves and seeds ha~~ve~~s been summarized in the Table 1, Table 2, Table 3 and Table 4 No reports are there to characterise the three cultigroups, Sesquipedalis, Unguiculata and Biflora with respect to their nutritional quality

In vegetable cowpea protein content in the pods varies with the days of maturity Sambandam *et al* (1965) studied the quality of pods in vegetable cowpea and advocated optimum time for picking green pods to be between 10 and 15 days of pod set, when protein and fibre contents of pods are optimum with maximum pod size and weight Kar *et al* (1995) also advocated the ideal picking stage for vegetable cowpea pod to be within 15 days of pod set, beyond which fibre content increases sharply thereby lowering the tenderness of the pods

Table 1 : Nutrient content of cowpea pods (per 100g of edible protein)

	References-		
Nutrient	Aykroyd (1963)	Purseglove (1974)	Kar (1993)
Protein (g)	4 3	3 4	3 47-4 14
Fat (g)	0 2	0 3	-
Fibre (g)	2 0	1 8	2 64-3 61
Carbohydrate(g)	8 0	7 4	-
Ash (g)	-	0 9	-
Minerals(g)	0 9	-	-
Calcium (mg)	80 0	-	-
Phosphorus(mg)	74 0	-	-
Iron (mg)	2 5	-	-
Vitamin A (IU)	9 41	-	-
Riboflavin(mg)	0 09	-	-
Thiamin (mg)	0 07	-	-
Nicotinic acid (mg)	0 9	-	-
Vitamin C (mg)	13 0	-	-
Moisture (g)	84 6	-	-

Table-2: Nutrient content of green leaf (Per 100g edible portion)

	Reference
Nutrient	Kale *et al* (1986)
Protein (g)	3 4
Fat(g)	0 7
Carbohydrate(g)	4 1
Calories	38
βcarotene(µg)	6072
Thiamin(mg)	0 05
Riboflavin(mg)	0 18
Vitamin C (mg)	4 0
Calcium (mg)	290 0
Iron (mg)	20 1
Moisture (g)	89 0

Table 3 : Nutrient content of dry seed (per 100g of edible portion)

	References			
Nutrient	Plant (1962)	Akapunam and Murakakis (1979)	Bressani (1985)	Fashakin and OJo (1988)
Protein (g)	-	23 9	24 1-25 4	20 9-26 9
Ether- extract(g)	-	-	1 1-3 0	-
Fat (g)	-	1 6	-	1 7-3 2
Fibre (g)	-	4 1	5 0- 6 9	3 2-4 9
Carbohydrate(g)	-	-	60 8-66 4	48 7-54 5
Ash (g)	-	3 3	3 4-3 9	-
Thiamin (mg)	0 9	-	0 41-0 94	-
Riboflavin(mg)	-	-	0 29-0 76	-
Niacin(mg)	2 0	-	2 15-3 23	-
Calcium(mg)	90 0	-	-	16 0-24 0
Iron(mg)	6 0-7 0	-	-	16 0-22 5
Moisture(g)	9 6	-	-	-

Table-4: Protein content of Cowpea

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Name of the Nutrients	Protein content reported
SEED PROTEIN	
Adrian and Helias-Frangne (1964)	23%
Arora and Das (1976)	17 94-27 56%
Boulter *et al* (1973)	21-34%
Bressani and Elias (1980)	17-40%
Oliveira *et al* ,(1980)	20-56%
Delrosario *et al* ,(1981)	19 96-24 4%
Ng *et al*,(1988)	20 8 -26 4%
Marconi *et al* ,(1990)	22 3 -25 5%
Nell *et al* , (1992)	24 5 -33 9%
POD PROTEIN	
Aykroyd (1963)	4 3%
Purseglove (1974)	3 4%
Kar *et al* ,(1995)	3 71-4 39%

Are these 'nutrients'?

Protein quality :

The limiting amino acid of total protein of cowpea meals are the sulphoamino acids, cystine and methionine (Boulter *et al*, 1973 , Bressani, 1985, Kachar *et al*, 1988) Lysine, leucine and phenylalanine contents are relatively high in cowpea (Bhatt, 1970 , Bressani and Elias, 1980 , Bressani, 1985) which makes cowpea an excellent improver of the protein quality of cereals (Bressani, 1985) Important Amino acid profile of cowpea has have been presented in Table - 5

Table-5 : Essential amino acid content of cowpea

	Reference
Amino acid	Brassani (1985) (mg/g)
Arginine	433-572
Histidine	169-236
Isoleucine	305-333
Leucine	434-543
Lysine	467-497
Methionine	74-82
Cystine	26-38
Phenylalanine	251-290
Tyrosine	113-137
Threonine	242-281
Tryptophan	58-82
Valine	252-368

Genetic variability and heritability :

Burton (1952) suggested that genetic variability along with heritability should be considered for assesing the maximum and accurate effect of selection Studies on the variability using genetic parameters like genotypic coeffecient of variation, heritability and genetic advance is essential for initiating an efficient breeding programme With the help of genotypic coefficient of variation alone the heritable variation can not be measured (Singh *et al*, 1974) Heritability is of interest to the plant breeder primarily as a measure of the value of selection for particular characters in various types of progenies and as an index of transmissibility (Hayes *et al* 1955) Highly heritable characters are particularly important for plant breeders as they enable~~s~~ him to base his selection reliable on phenotypic performance (Swarup and Chaugala,1962 , Sharma *et al*, 1966) Johnson *et al* (1955) suggested that heritability estimates in combinations with genetic advance would be more reliable than heritability alone for predicting the a~~e~~ffect of selection

Genetic advance or genetic gain depends on

i) The amount of genetic variability

ii) The magnitude of making effect of the genetic diversity and

iii) The intensity of selection (Comstock & Robinson, 1952)

Great variations in the seed protein expression can be tolerated in the legumes (Gepts and Bliss, 1984) Significant variability in the seed protein and some essential amino acids ~~have~~ has been identified in cowpea genotypes (Bliss *et al*, 1973 , Bliss, 1975 , Imam, 1979) High degree of variability in protein content in the segregating generation of cowpea has been attributable to genetic effects (Emebiri, 1991) Studying ten genotypes of cowpea Kar *et al* (1995) reported the range of protein content in the pods of 15 days maturity to be 3 71 to 4 39 %

Appreciably high broadsense heritability (0 70 - 0 78) ~~have~~ has been reported for seed protein content of cowpea (Emebiri, 1991) Hazra *et al* (1996) reported appreciabl~~e~~ly narrow sense heritability of 0 77 from the study of a diallel cross in cowpea Observing very high heritability estimates for seed protein content, Imam (1979) suggested that protein content might serve as selection index in any breeding programme of cowpea There was relatively small variation in the total sulphur and sulpho amino acid contents in seed protein of cowpea (Evans and Boulter, 1974)

Wide range of genetic variability was also observed in the seed protein content of other beans like, French bean (Singh and Saini, 1988 , Rutger, 1968 , Anacerbak, 1971), Fababean (El-Sherbeeny *et al*, 1992 , Griffiths and Lawes, 1978 , Eden, 1968, Bond and Toynbee - Clarke, 1968 , Clarke, 1920), Soybean (Ishige, 1984 , Mohammed *et al*, 1991)

Krober *et al* (1970),Esh *et al* (1959) and Bressani and Elias (1980) suggested that protein content of legumes are affected both by environment and genotype and similar was the view of Zebalska *et al.* (1990) in pea and Yadav and Tomer (1985) in black gram seed protein However, examining the variation in protien content in peas, mungbean and soybean, Meimers and Litzenbergers (1975) suggested that considerable variation in protein content may be achieved by selection

Associationship of the protein content with yield and yield components

Estimation of genotypic and phenotypic correlations between various characters may provide information necessary in a breeding programme when selection is based on two or more characters simultaneously Such study will help us to know the suitability of various characters for indirect traits results in correlated response in several other traits (Searle, 1965) Information could also be obtained from the studies of correlations for these charecters useful as indicators of more important ones under consideration (Liang *et al*, 1969) The intensity and direction of association among characters may #/ be measured by genotypic and phenotypic coefficients depending on the types of material under study and kind of experimental design used (Mode and Robinson, 1959)

From such linear correlations cause and effect relatioships cannot be identified In these situation, path coefficient analysis can depict more realistic interpretation of the characters involved in the study of correlations Methodology of path coefficients has been developed (Wright, 1921, 1934, 1954) Dewey and Lu (1959) for the first time applied this analysis in crops

Protein content and yield

Negative correlation between seed protein content and yield (seed or pod) has have been reported widely in cowpea (Bliss *et al*, 1973, Chauhan and Joshi, 1980 , Mak and Yap, 1980 ,Kar *et al*, 1995 & Hazra *et al*, 1996) Such negative associationships have been reported in other beans like French bean (Rutger, 1971 , Radkov and Mitranov, 1983), soybean (Hartwig, 1969), Mungbean (Yohe *et al*,1971) But often such negative correlations have been relatively small and insignificant as reported in French bean, soybean and Faba bean (Lelegi *et al*,1972 , Kelly and Bliss, 1975, Mutschler and Bliss, 1981 , Evans and Gridley, 1979 , Griffiths and Lawes,1978, El-Sherbeeny *et al*, 1992, Planchon *et al*,1992) implying that selection for protein content should not be detrimental to yield However, if the basis of this correlation is known, successful strategies can be used to prevent reduction in one trait as another is improved (Bliss and Brown, 1983)

Protein content and seed size

Increased percentage of total protein in grain legumes measured on a dry weight basis can result from decreased non protein (i e starch), increased protein on a combined change without concomitant change in seed size (Bliss, 1990) In the same manner Abdalla *et al* (1976) and El-sherbeeny *et al* (1992) reported that protein content of faba bean seemed uncorrelated with seed size but in chickpea, higher seed protein was found in smaller seeds (Kumar *et al.*, 1982) Similarly, protein content decreased with increasing seed size in French bean (Kossom, 1989) and Faba bean (Lafiandra *et al*, 1981) In pea, percentage protein content increased with increase in seed size in larger seeded genotype while for small seeded genotype there was mid range negative value (Arthur *et al*, 1991)

Leaf characters, photosynthesis and protein content

Protein synthesis in green plant is frequently closely associated with photosynthesis (Bassham *et al*, 1964 , Hall and Cocking, 1966 , Heber, 1962, Ongun and Stocking, 1965) The chlorophyll content of leaf tissues was of interest in relation to protein metabolism (Hall, 1968) The first stage pods/ of protein synthesis in fruits of French bean might have been controlled photosynthetically as rapid concurrent development of chlorophyll in the fruits was recorded (Hall, 1968) In cowpea, leaves, nodulated roots, stems plus petioles and peduncle donate mobilised nitrogen to the fruits and seeds in the relative proportion of 5 2 1 1 respectively The blossom leaves lose 70 to 77% of the nitrogen before abscission wheareas leaves at non reproductive nodes have mobilised only 44 to 57% of the nitrogen by fruit maturity and in most cases have not abcessed (Peoples *et al*, 1983)

Protein content with sulphoamino acids

In cowpea, strong negative correlation was observed, between both methionine and sulpho amino acids per 16gm of Nitrogen and percent crude protein and similarly a negative relationship was observed between total sulphur per 16 gm nitrogen and percent crude protein (Evans and Boulter, 1974) Similar associationship was reported in soybean also (Whitehead *et al*, 1989) and pea (Zebalska *et al*, 1990) In the same manner, Kossom (1989) reported that in French bean seed protein content was significantly and negatively correlated with methionine content and positively correlated with leucine content Amino acid contents was not associated with seed size

Nodulation , Nitrogen fixation and protein content

Before embarking upon any selection programme, it is imperative to have a knowledge on the magnitude of genetic variability and the extent of heritable variation in the material under study for the character to be improved Hence, such study was undertaken in cowpea for nodule number and weight and nitrogenase activity of nodules An estimate of the amount of nitrogen fixed annually by cowpea is given as between 73 and 240 kg/ha (Nutman, 1971) and more than 150 kg N_2/ha with the supply of 82 to 90% host plants nitrogen requirement (Eaglesham *et al* , 1977, Summerfield *et al* , 1977a) The actual site of nitrogen fixation is the nodules form~~s~~ed on the roots of legume plants For this reason, nodule number and nodule weight are correlated characters and are also positively correlated with nitrogenase activity of the nodules, shoot weight and shoot and root dry weight (Miller Jr. *et al* , 1986 Thompson and Dennis, 1976, Pandey *et al* , 1981, Posypanov *et al* , 1991) Posypanov *et al* (1991) concluded that in breeding for increased symbiotic activity, soybean plants to be selected should be tall, with high root and nodule weight, many leaves and well developed stem

Nitrogen fixation is essentially an anaerobic process and the nodules must have a mechanism to exclude oxygen from the bacteroid which is the site of atmospheric nitrogen fixation No oxygen is necessary for induction of nitrogenase enzyme and maintainance of high flask of oxygen at low concentration is needed for efficient nodule operation Leghaemoglobin is a pigment that limits oxygen supply and helps in providing low oxygen condition near the bacteroids with the results that not only the oxygen sensitive nitrogenous enzyme is prevented from damage but also that enough O_2 is available at the site for A T P generation (Appleby, 1984) The amount of leghaemoglobin and the extent of bacterial tissue in nodules have a direct bearing on the amount of nitogen fixed by the legumes (Bergersen and Briggs, 1958 , Chopra and Subba Rao, 1967 , Verma and Bal, 1976)

Nitrogenase, the enzyme responsible for reducing atmospheric N_2 to NH_3 and its specific reducing agent nitrogenase reductase are rather uniform both physically and functionally The exact relationship between hydrogen evolution by nitrogenase and nitrogen reduction is not clear but the two reactions are coupled closely The nitrogenase activity is measured by acetylene reduction method to assess the efficiency for fixing atmospheric N_2 and their reduction to NH_3 by the nodule In soybean, Posypanov *et al* , (1991) reported that nitrogenase activity was related closely to nodule fresh and dry weight and root dry weight, fairly closely to plant height, leaf number and dry weight and less closely to nodule and flower numbers Nitrogenase activity of nodules and leghaemoglobin/gm nodules corresponded each other in cowpea (Mandal, 1995) Singh (1989) studied root nodule formation and leghaemoglobin concentration in some important legumes including cowpea and observed wide variation in the morphology, size of nodules and leghaemoglobin content of the nodules Similar wide variability for nodule number, nodule weight and nitrogenase activity was recorded earlier in cowpea (Zary *et al* , 1978)

Genotypic variation in nitrogenase activity also existed in soybean (Planchon *et al.*, 1992). Estimates of broad sense heritability for nodule characters indicated that progress should be significant in breeding for enhanced biological nitrogen fixation in cowpea by improving nodule number, nodule weight and nitrogenase activity (Miller Jr *et al.*, 1986). Similar observation was also recorded by Mandal (1995). Predominant control of additive genes for nodule number and weight/plant (Dayap and Rasco Jr., 1988) further affirms their reliability for direct selection. So, the host plant is involved in determining when and which strain of *Rhizobi*~~*a*~~ium infects a root, the extent of nodulation and the potential for nitrogen fixation (Graham, 1982).

Works on the influence of biological nitrogen fixation and the nodule characters on protein content of legumes ~~is~~ are very much limited. In soybean, Planchon *et al.* (1992) suggested that nitrogenase activity of nodule affected seed protein content poorly. Suganuma *et al.* (1993) reported in ~~P~~pea that during early stages of nodule development, the protein compositions and activities of enzyme involved in carbon and nitrogen metabolism are not regulated by the presence or absence of nitrogenase activity. In cowpea nitrogen fixed before flowering contributed approximately 60% of the fruits' total requirement of nitrogen and the remaining 40% of the fruits' total nitrogen budget is met by nitrogen fixation after flowering (Peoples *et al.*, 1983). However, from another studies in cowpea, Eaglesham *et al.*, (1977) and Neeves *et al.* (1981) concluded that more than 75% of the fruits' nitrogen budget is met by nitrogen fixation after flowering.

Biometerial methods for genetical analysis

Protein content is a typical quantitative character and it follows normal distribution in F_2's and F_3's (Chen *et al*, 1991) Seed protein of legumes are synthesised in large quan tities in a specific tissue during a distinct developmental time period and unlike secondary products seed proteins are encoded directly by structural genes Hence can be identified easily, distinguised qualitativley and quantified (Bliss, 1990) So, proteins can be regarded as a metric trait

Yule (1906) postulated the polygenes of small and similar effects in regulation of continuous variation of quantitative characters East (1916) formulated the multiple factor hypothesis and explained the nature of inheritance of quantitative characters with many segregating genes each having small and similar effect Fisher (1918) propounded the dominance concept of multiple factors and proposed first the method of partitioning the continuous variation into three components additive effect of genes (straight line function or cumulative function of genes), dominance effects of genes (intra allelic interaction within locus) and epistatic effects of genes (non allelic interaction or inter locus interaction)

Various models depending upon the various assumption and material used have been formulated to estimates the genetic parameters Line X Tester analysis is such a model proposed by Kempthorne (1956) This design provides information about general and specific combining abilities of parents and at the same time it is helpful in estimating various types of gene effects The general combining ability (gca)in line X tester analysis refers to average performance of the parents (Lines and Testers) in a series of hybrid combinations While specific combining ability (sca) is designated to cover these cases in which certain combination do relatively better or worse as expected on the basis of the average performance of the lines and testers involved to produce the cross combinations

Genetic control of protein content

Reports, though very limited, on the inheritance of seed protein content of cowpea indicated the importance of both additive and non-additive components of variance in conditioning this characters However, inheritance of protein content of pod are yet to be reported According to Mak and Yap (1980) both additive and non-additive gene effects were involved in the genetic variation but dominance variance was more important, and seed protein appears to be associated with recessive genes There was a general trend that low yielding parents carried more recessive genes and high yielding parent carried more dominant genes On the other hand, studying a diallel~~e~~ cross, Hazra *et al* (1996) suggested predominance of additive gene effects for seed protein content and advocated to exploit this component of genetic variance to develop high yielding varieties having appreciable level of seed protein content With regard to amino acids in the proteins of cowpea, Methionine content was reported to have been controlled by recessive genes (Dassauer and Hannah, 1978) Emebiri (1991) studied the inheritance pattern of protein content in seeds of two crosses of cowpea It was observed that in both the crosses inheritance of the character inolved both additive and non-additive gene effects A significant effect of pollen source was detected in one of the two crosses In both the crosses comparision of seed protein content of reciprocal segregating generations indicated the influence of cytoplasmic factors

Genetic control of seed protein content of other legumes ~~have~~ has also been reviewed Additive genetic variance was reported to have been important in the control of seed protein content in pea (Pandey and Gritton, 1975), ~~S~~soybean (Song *et al.*, 1991, Ishige, 1984, Liu *et al.*, 1988,) ~~M~~mung bean (Pathal *et al.*, 1990) and ~~C~~chickpea (Sandhu *et al*,1984) On the otherhand,there are reports of non-additive component of variance having played major role in the control of seed protein content in French bean (Lelegi *et al*, 1972, Singh and Saini, 1988), ~~C~~chick pea (Salimath *et al*, 1988) and ~~P~~pea (Lawrence *et al*, 1986)

Importance of both additive and non-additive effects on the control of protein content ~~have~~ has also been reported in ~~P~~pea (Singh *et al*, 1989) and cluster bean (Singh *et al.*, 1992)

To estimate the number of genes controlling seed protein content more precisely, it is necessary to use materials with wider genetic variation In cowpea three groups of genes (Mark and Yap, 1980) and in soybean two to three groups of genes (Ishige, 1984) were reported to have controlled the seed protein content

Heterosis for protein content

Realisation of heterosis has been very much inconvincing for seed protein content of cowpea (Mak and Yap,1977 , Hazra, 1991) and other beans like pea (Pandey and Gritton, 1975, Lawrence *et al* ,1987) and Chick pea (Salimath *et al.*, 1988) suggesting little promise for improvement of percent protein in the F_1 hybrids of cowpea and other beans

Breeding strategies for improving seed protein content

Selection : Despite the widely held notion in cowpeas and other beans that negative relationship among percentage seed protein, yield and amino acid composition of protein preclude simultaneous improvement of the traits, changes through breeding have been made Reports of small and insignificant negative correlation between protein content and yield in some beans implies that selection for protein content should not be detrimental to yield Lower seed yield sometimes resulted in selection solely for increase percent of protein if effects of seed size or constituent seed fraction are considered (Bliss and Brown, 1983) Observing strong correlation between seed yield and protein yield, El-serbeeny *et al* (1992) supported the hypothesis that the optimal way to increase substantial protein yield per hectare was to increase and stabilize seed yield In soybean, Bliss (1990) suggested not to improve protein concentration in seed at the expense of lower yield Hazra *et al* (1996) also advocated to develop high yielding cowpea genotypes with average seed protein content Observing no significant correlation between yield per plant and protein content in Faba bean having been established, Griffiths and Lawes (1978) app~~r~~ehended that selection for types combining both high yield and average protein content was feasible Shorter *et al.* (1976) also opined that selection of soybean lines with high seed yield and high protein percentage should be possible In order to produce high protein forms in pea, Kinyapina and Khakimov (1986) recommended selection for absolute and relative protein contents of cotyledons This selection criteria together with high seed yield could lead to high protein yield Openshaw and Headly (1984) advocated direct selection for protein content alone in soybean which resulted in increase of 1 42% protein content and simultaneous selection for protein and other qualitative traits like oil and sugar was not affative

Hybridization and selection in classical breeding

In ~~P~~pea, Vetrova and Petrushina (1987) advocated inter-specific hybridization combined with individual selection as the main method for producing breeding material for high protein and amino acid Selective mating and subsequent selection from advanced generation was advocated for improving protein content in cluster bean also

In cowpea maternal genome influenced protein content strongly (Emebiri, 1991) So, in the hybridization programme high protein genotypes should be kept as a female parent

In French bean intercrossing superior inbred back cross lines produced progenies with higher level of phaseolin and increased seed protein concentration than that of the parents (Hartana, 1986) Recurrent selection was advocated as method of breeding in Soybean and French bean (Xu and Wilcox, 1992 , Delaney, 1988)

Directional selection (concentrating on high protein cross only) provides more genetic advances for protein content than random selection in soybean

Observing the conditioning of both pod yield per plant and seed protein content by additive gene action in cowpea,Hazra *et al* ,(1996) advocated pedigree method of breeding with selection in the more advanced generation Meng *et al* (1990) also advocated selection in more advanced generation on the basis of single plant protein in soybean In this method yield was not affected markedly However, Liu(1988) suggested early generation selection in pedegree method of breeding in soybean

Mutation breeding : Greater influence of nuclear gene in the control of seed protein content of cowpea seeds have been reported (Emebiri, 1991) but reciprocal difference in F_1 and its persistence in F_2 generation suggests the presence of cytoplasmic factor (Emebiri, 1991 , Singh and Hadley, 1972 , Russoke and Fatunla, 1987) The evidence which is indicative of cytoplasmic influence is of interest, particularly when one considers the possibilities of recombination that could result from the bi-parental transmission of cytoplasmic gene (Sager, 1972) and the opportunities for inducing mutations of cytoplasmic gene (Ashri and Levy, 1975)

Alteration of ploidy In pea, Mercykutty *et al* (1990) induced autotetraploidy through colchicine treatment and found that autotetraplolidy increased seed protein content of 11 1 - 21 7% compared to corresponding diploids Protein content increased at 4X level by 7 5 to 26 1 percent

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

Four different experimental approaches were followed under the present research programme Field trials were carried out at "District Seed Farm", Bidhan Chandra Krishi Viswa-vidyalaya, Kalyani, over a period of two years (1994 - 1996) Estimation of protein was done in the Department of Agriculture Chemicals and Soil Science and estimation of leghaemoglobin content and nitrogenase activity of the nodules, in the Nodule Research Laboratory of the Viswavidyalaya First, the study was conducted on changes of protein content in the pods with the age of the pods (three stages) over the cultigroups and environments The second was involved to study the genetic variability, heritability and associationship of the protein contents with different growth and physiological characters The third approach was based on the influence of biological nitrogen fixation parameters on protein content The fourth approach aimed at understanding the gene action for different pod yield components and pod protein content and an attempt was also made to study the heterosis for protein content and other yield components to identify heterotic hybrids for further use in the breeding programme

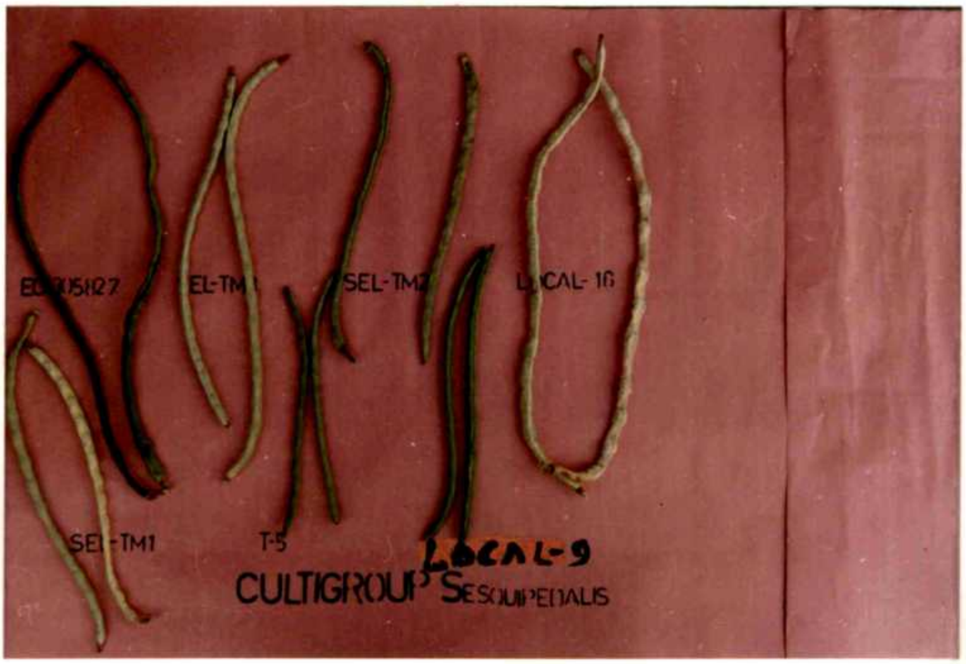
3.1 Pod and seed characters in the cultigroups at three different stages of pods

3.1.1 Materials

Twentyone genotypes of cowpea *(Vigna unguiculata* (L) walp) belonging to three cultigroups namely, Unguiculata, Biflora and Sesquipedalis were collected from West Bengal, New Delhi Ludhiana and Bangalore Brief descriptions of them have been presented in Table - 6

Table - 6 :Description of the genotypes of cowpea.

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Sesquipedalis	
C1	Sel-Tm1	Dept of Hort B C K V, Mohanpur,W B	Plants are viny, pigmentation present between stipel and at the base of primary branches Flower colour - both standard and wing are light purple Pods are medium long, tender, fleshy, pendant and light green coloured with red beak elongated reniform seed, black in colour
C2	Sel-Tm2	-do-	Plants are viny, pigmentation present between stipel, at the base of leaf stalk, at the base of primary branches and at the base of midribs of leaflets Flower colour - deep purple Pods are medium long,

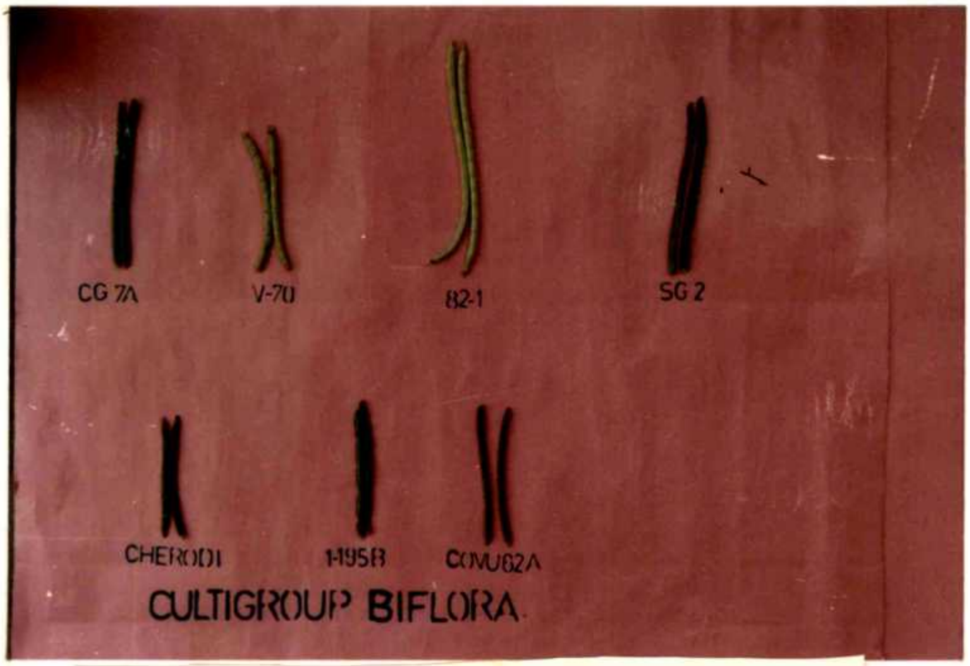

E 05127
EL-TM
SEL-TM2
L CAL-16
SEL-TM1
T-5
LOCAL-9
CULTIGROUP SESQUIPEDALIS


COWPEA 263

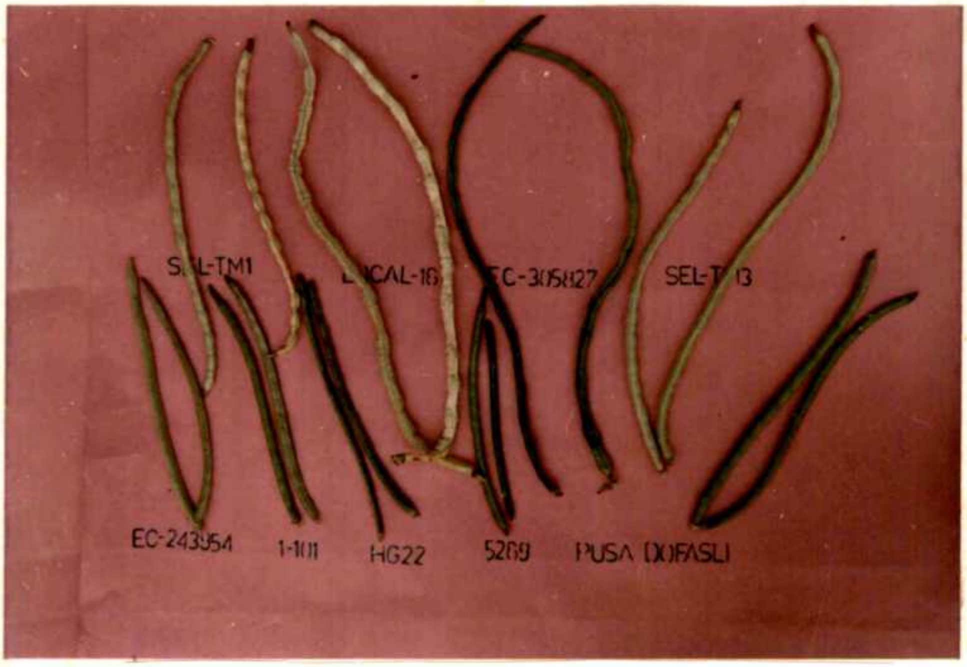

Pusa Dofasli


526

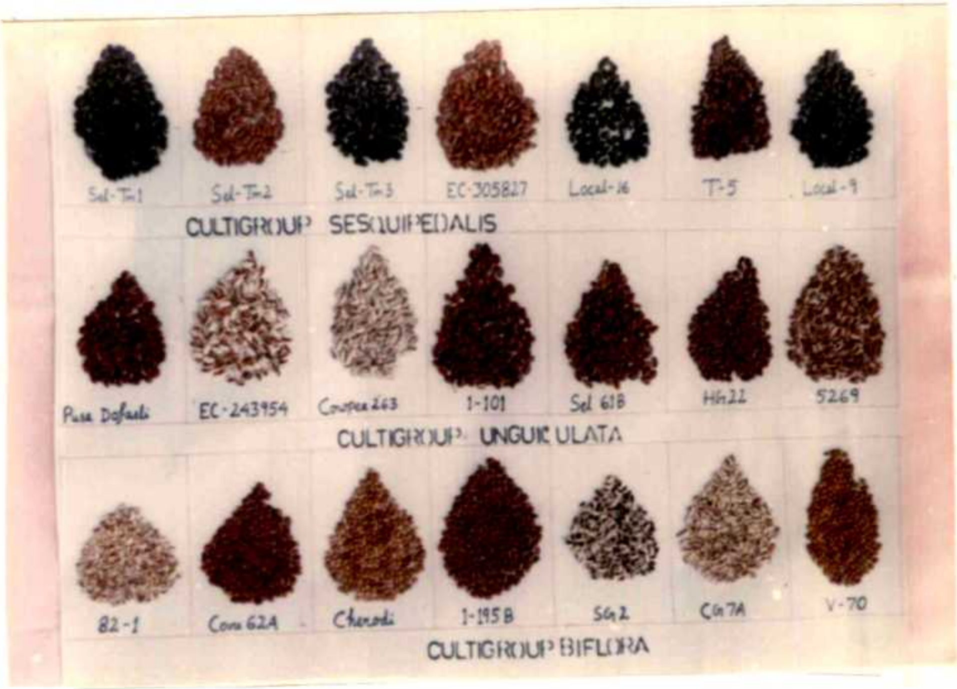

EC 243954



Variability in pod size and colour of different cowpea genotype



Variability in pod size and colour of nine parents used in line X tester analysis



Variability in seed size and colour of different cowpea genotype

Contd

Collection	Name/Name given	Place of collection	Characteristic features
Cultigroup Sesquipedalis			
			tender, fleshy, pendant and light green coloured, seeds are elongated, reniform with dark tan seed coat
C3	Sel-Tm3	-do-	Plants are viny, pigmentation present between stipel and at the base of the primary branches Flower colour - both standard and wing are deep purple Pods are long, tender, fleshy,pendant and light green coloured with red beak Seeds are elongated reniform with dark black seed coat
C4	EC-305827	N B P G R	Plants are viny, pigmentation present between stipel, at the base of primary branches, and at the base of midribs of leaflet Flower colour - both standard and wing are light purple Pods are very long, tender, fleshy, pendant

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Sesquipedalis	
			and deep green coloured with red beak Seeds are elongated, reniform with reddish brown seed coat
C5	Local-16	Madanpur	Plants are viny, pigmentation present between stipel, at the base of primary branches and midribs of leaflet Flower colour - both standard and wing are deep purple Pods are long, tender, succulent, fleshy, pendant and yellowish white with red beak Seeds are elongated reniform with black coloured seed coat
C6	T-5	N B P G R New Delhi	Plants are viny, pigmentation present between stipel and at the base of leaf stalk, primary branches, and midribs of leaf let Flower colour standard

Contd

Collection	Name/Name given	Place of collection	Characteristic features
Cultigroup Sesquipedalis			
			and wing are deep purple Pods are medium sized tender fleshy, non pendant and light green coloured Seeds are somewhat elongated, reniform with dark tan seed coat
C7	Local - 9	-do-	Plants are viny, pigmentation present between stipel and at the base of primarybranches Flower colour -both standard and wing arelight purple Pods are mediumlong, tender, fleshy, pendant and light green coloured with red beak Seeds are elongated, reniform and seed coat is black
Cultigroup Unguiculata			
C8	Pusa Dofasli	I A R I , New Delhi	Plants are semi erect and bushy, pigmentation present between stipel, at the base of primary branches and at the

Contd

Collection	Name/Name given	Place of collection	Characteristic features
Cultigroup Unguiculata			
			base of midribs or leaf let Flower colour - both standard and wing are purple Pods are medium sized tender, non fleshy, semipendant and light green coloured Seeds are small, flat and seed coat is dark tan
C9	EC-243954	NBPGR, New Delhi	Plants are semierect, dwarf and bushy, pigmentation present between stipel and at the base of primary branches Flower colour - standard is creamy white and wings are white, pods are medium sized, somewhat tough, inflated, fleshy nonpendent and creamy white coloured Seeds are bold and seed coat is creamy white with biscuit eye

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Unguiculata	
C10	Cowpea 263	P A U	Plants are semierect, dwarf and bushy Pigmentationpresent between stipel and at the base of primary branches Flower is creamish white Pods are medium sized, inflated, fleshy, nonpendant and creamy white Seeds are bold and seed coat is white with chocolate eye
C11	1-101	Pulse and Oilseed Research Station, Baharampur, West Bengal	Plants are somewhat erect, pigmentation present between stipel, at the base of primary branches and at the base of midribs of leaflet Flower colour - both standard and wing are light purple Pods are short, medium, tender, non fleshy, non pendant and light green coloured Seeds

Contd

Collection	Name/Name given	Place of collection	Characteristic features
Cultigroup Unguiculata			
			are somewhat bold with chocolate coloured seed coat
C12	Sel 61B	I I H R , Bangalore	Plants are semierect and bushy, pigmentation present between stipel and at the base of primary branches Flower colour - standard is light purple and wing is deep purple Pods are medium sized, fleshy, nonpendant and creamy (yellowish) white, Seeds are elongated, reniform with biscuit coloured seed coat
C13	HG22	Dept of Hort , B C K V , W B	Plants are some what erect and bushy, pigmentation present at the base of the leaf stalk and internodal region Flower colour is light purple, pods are medium long, fleshy, non- pendant and greenish

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Unguiculata	
			white Seeds somewhat elongated with light coloured seed coat
C14	5269	Pulse and Oilseed Research Station, Baharampore, W B	Plants are somewhat erect, pigmentation present between stipel, at the base of leafstalk and at the base of primary branches Flower colour -standard is creamy white and wing is light purple Pods are medium sized, tender, non-fleshy, nonpendant, deep green in colour Seeds are somewhat bold and seed coat is creamy white with dark tan blotch
		Cultigroup Biflora	
C15	82-1	Dept of Hort , B C K V , Mohanpur, W B	Plants are erect, bushy, pigmentation present at the base of branches and internodes Flower colour whitish stan-

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Biflora	
			dard and wing is purple Pods are medium sized nonpendant Seeds are somewhat elongated and seed coat is brownish
C16	Covu 62A	Pulse and Oil Seed Research Station, Baharampore,	Plants are semierect, dwarf and bushy Pigmentation present between stipel and at the base of the primary branches Flower colour -both standard and wing are deep purple Pod colour-deep green Semierect pod orientation, small, leathery tough , pod texture is smooth , seeds are square shaped, seed coat colour is buff with reddish brown eye
C17	Cherodi	-do-	Plants are somewhat erect, pigmentation present between stipel and primary branches, Flower colour - both standard

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Biflora	
			and wing are purple coloured Pods are green, erect, small, tough, leathery with smooth texture Seeds are bold, small and seed coat is buff coloured with brownish eye
C18	1-195B	-do-	Plants are semierect, dwarf and bushy Pigmentation is present at the base of primary branches Flower colour-standard and wing are light purple Pod colour light green, semierect, small,tough Pod texture is semi smooth, seeds are square Seed coat colour is buff with whitish eye
C19	SG 2	-do-	Plants are some what erect, pigmentation present at the base of branches and internodes Flower colour standard is white and wing are purple

Contd

Collection	Name/Name given	Place of collection	Characteristic features
		Cultigroup Biflora	
			Medium sized pod with red beak Seed coat colour is white with dark black eye
C20	CG7A	-do-	Plants are semierect, pigmentation present in the base of primary branches and leaflet Purple flower, green pod, semierect pod orientation, small leatherypods, seed coat colour is dirty cream with brownish eye
C21	V-70	-do-	Plants are erect, pigmentation present between stipel,at the base of leaf stalkand primary branches Flower colour - both standard and wing are deep purple, pod colour is green, erect pods, small, tough, leathery,smooth textured Seeds are bold, small Seed coat is buff coloured with black eye

The collected genotypes were grouped under the three cultigroups following Ng and Marechal (1985)

3.1.2. Methods

The experimental materials (genotypes) were grown in a randomised block design with three replications in two different environments as described below

Environment	Experimental materials (genotype)	Sowing date	Location
E1	C1, C2, C3, C4, C5, C6, C7, C8, C9, C10,C11,C12, C13,C14,C15,C16,C17,C18, C19,C20,C21	15 3 1994	District Seed Farm, Kalyani, B C K V
E2	-do-	9 11 1994	-do-

Physico-chemical features of 'District Seed Farm', Kalyani and mateorological data of the experimental periods ~~were~~ are presented in Appendix I and II, respectively A single row plot of 3 m length was alloted to each genotype The spacing between rows and plants were 60cm and 30cm, respectively Fertilizers were applied as basal at the rate of 20 kg N, 60 kg P_2O_5 and 40kg K_2O in the form of urea, single super phosphate and muriate of potash, respec tively Irrigation and plant protection measures were provided as and when required Observations were recorded from five randomly selected plants in each replication The characters studied were-

1 **Pod length (cm) :** Some flowers were tagged at random in the five plants from the date of flower opening Ten pods each of 10 days, 15 days and 20 days maturity from anthesis were taken per replication at random, their length were measured in centimetre and averaged

2 **Fresh pod weight (g) :** The pods which were used for measuring pod length were also used to record pod weight in gram

3 **Dry pod weight :** Collected green pods were dried in electric oven at 45°C temperature for 48 hours and dry weight were taken in gram

4. Seed number per pod : Total number of seeds from the same ten pods in each replication for three picking dates were counted and averaged

5. Protein content of the pod : Same dried pods were ground finely by grinding machine and then sieved The powdered sample were then transferred to a polythene bag and finally kept in a desicator These powdered material were used for the estimation of protein content

3.1.3 Estimation of protein content

Reagents used

Protein content was estimated by the colorimetric method of Lowry *et* *al* (1951) The following reagents were used

1 **Borate buffer (PH 10.0) :** Sol A - 0 05M solution of borax (19 05g in 1000 ml) = 0 02 M in term of sodium borate , Sol B - 0 2M NaOH (In general, 50 ml of A + 43 0 ml of B if mixed make the PH 10 00)

2 **20% Trichloro acetic acid solution (TCA)**

3 **0.1 (N) NaOH solution**

4 **Solution - A :** Prepared by dissolving 2g of Na-K-tartarate and 100g of Na_2Co_3 in 500 ml 1(N) NaOH solution and diluted to 1 litre with distilled water

5 **Solution - B :** Prepared by dissolving 2g of Na-K-tartarate and 1g of $CuSo_4$ in 90 ml distilled water and 10 ml 1(N) NaOH solution was added to it

6 **Solution - C :** Prepared by diluting 1 volume of Folin-Ciocalteu reagent (Loba) with 15 volume of distilled water

Instrument and glass goods used :

1 Centrifuge (5000 r p m) (Remi)

2 Refrigerator

3 Cyclomixer (Remi)

4 Water bath

6 Centrifuge tube, 15 ml (Borosil)

7 Graduated centrifuge tube, 10 ml (Borosil)

8 Test tube, 50 ml (Borosil)

9 Measuring cylinder

10 Volumetric Flask

11 Mortar and pestle

Extraction of protein : 50 mg of the ground sample was taken in a mortar, 5 ml of borate buffer was added to it and then the sample was crushed with the pestle vigorously to homogenise it The homogenate was transferred into a 15 ml centrifuge tube The residue still left in the mortar was washed with another 5 ml of the borate buffer and the material was again transferred into the same centrifuge tube The homogenate of 10 ml was then centrifuged at 5000 r p m for 20 minutes The supernatent was carefully decanted off into the graduated centrifuge tube Volume of the total alkaline protein was recorded 1 ml of the supernatent was taken in the 15 ml centrifuge tube, 1 ml of 20% TCA was added to it and then the mixture was kept overnight inside a refrigerator for complete precipitation Next day the centrifuge tube containing the precipitate was centrifuged at 5000 r p m for 30 minutes The supernatent was then carefully decan~~d~~ted off The residue thus obtained was dissolved in 5 ml of 0 1 (N) NaOH solution with the help of a cyclomixer 1 ml aliquot of this alkaline protein solution was taken for protein estimation

Procedure : 1 ml of the above alkaline protein solution was taken in a test tube, treated with 0 9 ml solution A and then the tube was placed in a hot water bath at 50°C for 10 minutes, cooled to room temperature and treated with 0 1 ml solution B The mixture of the solution was left at room temperature for 10 minutes, then 3 ml of solution C was forced rapidly to ensure quick mixing and proper colour development The test tube was again treated in the water bath at 50°C for 10 minutes and again cooled to room temperature Optical density (O D) value of this solution was read by U V Spectrophotometers at 750 nm wave length A blank was also run along with every estimation Original O D value was subtracted from blank O D value The amount of protein was determined from a standard curve

Preparation of a standard curve :

Bovine albumin serum (Sigma) was used for the preparation of standard curve

Stock solution : 10 mg of Bovine albumin serum was dissolved in 10 ml 0 1 (N) NaOH solution

Dilution : Dilution was done as below -

20 μg/ml = (0 2 ml stock + 9 8 ml distilled water)

40 μg/ml = (0 4 ml stock + 9 6 ml distilled water)

100 μg/ml = (1 0 ml stock + 9 0 ml distilled water)

160 μg/ml = (1 6 ml stock + 8 4 ml distilled water)

The same procedure for the estimation of protein was also employed here The original O D value was also subtracted from the blank one

Calculation : Total protein in 100 mg sample

$= X \times 10^{-3} \times 5 \times A \times 2$

$= A^{X} \times 10^{-2}$ mg/100 mg of dried sample

Where, A = Volume of total alkaline protein solution

X = Calculated value with respect to standard curve

Estimation of protein from each lot was repeated thrice and observations were recorded in a randomised block design having three replications

3.2 Leaf characters, protein contents of pod and seed and pod yield.

3.2.1 Materials - Same as chapter 3.1.1.

3.2.2. Methods - The experimental materials (genotypes) were grown in a randomised block design with three replications on 12 03 1995 at 'District Seed Farm', Kalyani Physiological features of the 'District Seed Farm' and meteorological data of the experimental period were presented in Appendix I and II respectively A single row plot of 3m length was alloted to each genotype The spacing between rows and plants were 60 cm and 30 cm, respectively Fertilizers were applied as basal at the rate 20 kg N, 60 kg P_2O_5 and 40 kg K_2O in the form of Urea, single super phosphate and muriate of potash, respectively Irrigation and plant protection measures were provided as and when required Climbing types were given support by jute stick Observations were recorded from five randomly selected plants in each replication The character studied were -

1 Fresh leaf weight (g) Fifteen fully developed trifoliate leaves (3 per plant) from the five selected plants in each replication at random at 40-45 days after seeding were sampled and their weight was measured in gram and averaged

2 Dry weight of leaf (g) · Those fifteen sampled trifoliate leaves (excluding petiole) per replication were then oven dried at 68 ±2°C for 4 days Average dry weight in mg depicted the dry weight of leaf per replication

3 Chlorophyll content Fifteen fully developed trifoliate leaves (3 per plant) from the five selected plants in each replication were sampled at random at 40-45 days after seeding and used for chlorophyll estimation

4 Pod yield per plant (g) Pod weight of the periodical harvest from five plants was taken and then averaged

3.2.3 Estimation of chlorophyll content of the leaves

Principle : Chlorophyll was extracted in 80% acetone and the absorption at 663 nm and 645 nm were read in a spectrophotometer Using the absorption co-efficients, the amount of chlorophyll ~~is~~ was calculated

Materials : 80% acetone, glass mortar, 25 ml volumatric flask, centrifuge, spectrophotometer

Procedure : Freshly harvested leaf (weighing 0 1g) was crushed in a glass mortar and pestle with 3-5 ml 80% acetone The residue was centrifuged at 2000 rpm for 10 minutes The residue was re-extracted with 80% acetone and the operation was repeated several times until the residue showed no visible green colour The pooled supernatants were combined and made upto 25 ml with 80% acetone The absorbance of the solution was read at 645 nm and 663 nm against the solvent (80% acetone) blank

Calculation :

$$\text{Total chlorophyll (mg/g)} = \frac{20\ 2 \times D645 + 8\ 02 \times D663}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll 'a' (mg/g)} = \frac{12\ 7 \times D663 - 2\ 69 \times D645}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll 'b'(mg/g)} = \frac{22\ 9 \times D645 - 4\ 68 \times D663}{a \times 1000 \times w} \times V$$

where, a = length of light path in the cell (1 cm)
b = volume of the extract in ml
w = fresh weight of the sample in g
D = optical density at consecutive nm.

3.2.4 Estimation of protein content : Same as chapter 3 2 3

3.3 Root and nodule characters and protein content of pod and seeds.

3.3.1 Materials - Same as chapter 3 1 1

3.3.2 Method - Same as chapter 3 2 2

The character studie~~s~~d were -

Root length (cm) Ten plants from each replication at flowering stage were uprooted carefully with the help of a spade and the length of tap root from the collar region were measured in centimetre and averaged

2 Fresh root weight (g) The root mass of the plants in each replication was weighed and averaged to take root weight in gram

3 Dry root weight (g) Roots were dried at electric oven at 68°C for 48 hours and weight were taken in gram

4 Nodule number per plant Ten plants from each replication at flowering stage were uprooted very carefully after flooding the field All the nodules from taproots as well as lateral roots were collected Total number of nodules of the ten plants in each replication were counted and averaged

5 Nodule weight per plant (g) All the nodules collected from ten plants in three replications were weighed in gram with the help of elec-

tronic balance

6 Leghaemoglobin content of nodule (mg/g) It was carried out following the method of Bergersen (1980) Detail procedure followed are hereunder given

Apparatus and reagents used : Test tubes, mortar and pastle, electronic balance, marker pen, spectrophotometer, phosphate buffer solution (pH 4) and a coloured reagent which was prepared by mixing 100mg Benzidine and 0 5 ml H_2O_2 together and the volume was made upto 50 ml by absolute alcohol Phosphate buffer solution (pH 4) was prepared by mixing 1 07gm of Na_2HPO_4 and 1 39gm of NaH_2PO_4 in 1 litre of water and the pH was adjusted to 4 00

Procedure : Fresh nodules were collected from the sampled plant The nodules were washed with running tap water and after drying the water film on the nodules, 100 mg lots were weighed by electronic balance The 100mg lots were then taken in the test tube filled with 4 ml of phosphate buffer If crushing of the nodules could not done immediately, test tubes containing the nodules in the phosphate buffer were kept in the refrigerator for next day's utilisation The nodules alone with phosphate buffer solution were transferred in a mortar and pastle for thorough crushing and after doing so the materials were again returned to the test tube and kept undisturbed upto 30 minutes The optical density (O D) of the material in the test tube was measured only by utilizings the clear liquid of the upper portion of the test tube About 2ml of the upper clear liquid was decanted to the measuring vial of the spectophotometer and 2ml of Berzidine colour reagent was mixed to it and after gentle shaking for through mixing optical density (O D) was recorded by using 660µm wave length light Here the O D reading was directly expressed as mg leghaemoglobin content per g of nodules

7 Nitrogenase activity in nodules (mole C_2H_2 reduced per hours)

Average number of nodules from five separate plants per replication at flowering stage were taken to estimate the nitrogenase activity of the nodules It was conducted by the Acetylene Reduction Assay (A R A) method of Turner & Gibson (1980) Detail procedure is given below

Apparatus and Reagent used : 50 ml capacity special tube, rubber septa, injection syringe, acetylene gas (C_2H_2) and gas chromatograph

Procedure : The nodules were collected at flowering stage and placed inside the 50 ml capacity specimen tubes The tubes were sealed by rubber septa The sealed samples were incubated in 10% C_2H_2 atmosphere at 30 ± 1°C for one hour After incubation, gas samples were taken (0 5 ml) from each tube by a 1ml capacity pressure lock gas syringe and the amount of ethylene (C_2H_4) formed was measured with a gas chromatograph (HP model 5730A) fitted with a glass column containing porapa KR (80-100mesh) and equipped with a flame ionisation detector The open temperature and carrier gas (N) flow rate were 80°C and 60ml minute^{-1}, respectively

8 Pod protein content of 20 days (%) - As discussed earlier in chapter 3 2

9 Seed protein content(%) As discussed earlier in chapter 3 2

10 Total protein in 100 grains (g) This was calculated by following the formula given by Singh *et al.* (1988)

$$\text{Quantity of protein in 100 seeds(g)} = \frac{\text{\% protein x 100 seed weight}}{100}$$

3.4 Gene action for pod protein content and other yield components

Gene action for pod protein content and other pod yield components ~~were~~ was studied by line x tester analysis

3.4.1 Materials : Nine genotypes belonging to the two cultigroups, Unguiculata and Sesquipedalis were used as male and female parents in line x tester analysis The characteristic features of the nine parents have been presented in Table 7

Repetition of table-6

Table 7 : Characteristic features of the parents involved in the line x tester cross.

Parent	Cultigroup	Source	Characteristic features
Sel-Tm1	Sesquipedalis	Dept of Hort , B C K V Mohanpur W B	Plants are viny, pigmentation present between stipel and at the base of primary branches Flower colour - both standard and wing are light purple Pods are medium long, tender, fleshy pendant and light green coloured with red beak, elongated reniform seed, black in colour
Sel-Tm3	-do-	- do-	Plants are viny, pigmentation present between stipel and at the base of primary branches Flower colour - both standard and wing are deep purple Pods are long, tender,fleshy, pendant and light green coloured with red beak Seeds are elongated reniform with dark black seed coat

Parent	Cultigroup	Source	Characteristic features
EC-305827	-do-	N B P G R	Plants are viny, pigmentation present between stipel, at the base of primary branches, and at the base of midribs of leaflet Flower colour - both standard and wing are light purple Pods are very long, tender, fleshy, pendant and deep green in colour with red beak Seeds are elongated, reniform with reddish brown seed coat
Local-16	-do-	Madanpur Seed market, Nadia,W B	Plants are viny, pigmentation present between stipel, at the base of primary branches and midribs of leaflet Flower colour - both standard and wing are deep purple Pods are long tender, succulant fleshy, pendant and yellowish white with red beak Seeds are elongated reniform with black coloured seed coat
5269	Unguiculata	Pulse and Oilseed	Plants are somewhat erect, pigmentation present between

Parent	Cultigroup	Source	Characteristic features
		Research Station, Baharampore,	stipel, at the base of primary branches Flower colour Standard is creamy white and wing is light purple Pods are medium sized, deep green in colour Seed coat is creamy white with dark tan blotch
HG22	-do-	Dept of Hort , B C K V, W B	Plants are some what erect and bushy, Flower colour is light purple, pods are medium long and greenish white Seeds some what elongated with light coloured seed coat
1-101	-do-	Pulse and Oil-Seed Research Station, Baharampore, W B	Plants are somewhat erect, pigmentation present between stipel, [illegible] and at the base of the mid ribs of leaf let.Flowers colour- both standard and wing are light purple Pods are short,medium, tender,non fleshy, non pendant and light green coloured Seedsare somewhat bold with chocolate coloured seed coat

Parent	Cultigroup	Source	Characteristic features
EC-243954	Unguiculata	N B P G R New Delhi	Plants are semierect,dwarf and bushy, pigmentation present between stipel and at the baseof primary branches Flower colour standard is creamy white and wing are white Pods are medium sized, somewhat tough, inflated, fleshy, non pendant and creamy white with biscuit eye
Pusa Dofasli	-do-	I A R I New Delhi	Plants are semierect and bushy, pigmentation present between stipel at the base of primary branches and at the base of primary branches and at the base of midribs on leaflet Flower colour-both standard and wing are purple Pods are medium sized, tender, nonfleshy, semipendant and light green coloured Seeds are small, flat and seed coat is dark tan

Sel-Tm1, Sel-Tm3, EC-305827, local-16 of cv-gr sesquipedalis were used as female (Lines) and genotypes of cv-gr Unguiculata namely, 5269, HG22, 1-101, EC-243954 and Pusa Dofasli were used as male parent (Testers)

Each male parent was mated to each female parent, but male parent or female parents were not crossed to each other The number of single crosses made were equal to the number of female parents (P_f) X the number of male parents (Pm), viz , P_f x Pm So, total number of twenty crosses were obtained for evaluation These crosses were evaluated along with parents in a replicated experiment

3.4.2 Methods

3.4.2.1. Hybridization technique

Cowpea flowers were found very sensitive and liable to drop off with the slightest mechanical injury For this reason, the flowers were handled with utmost care Mature and s~~o~~wollen flower buds due to open in the next morning were selected for emasculation Emasculation was done between 3 P M to 5 P M To emasculate, the bud was held between the thumb and forefinger of the left hand, with the kill side uppurmost Fine end of the forceps was run along the ridge where the two edges of the standard petal unite One side of the petal was brought down, securing its position by exposing the keel A slit was made at the juncture of the keel petals with a pair of fine foreceps The stamens were removed very carefully with a pair of pointed forceps through this slit The disturbed parts of the keel, wings and standard petals were placed in their original positions All other buds of the same inflorescence were removed The forceps were sterilized with ethyl alcohol after each operation The emasculated flower buds were enveloped with thin cotton wad to protect from pollen contamination Pollination was done within 7 A M of the next morning The cotton wad was removed and the emasculated flower buds were pollinated by gently touching their stigma with the pollen laden style of the protected flower of the desired male parent The pollinated flower was again covered with fresh cotton wad and was properly labelled indicating the specific parents and date of pollination The mature pods from each cross combination were collected separately

3.4.2.2. Raising of F_1s, lines and testers

This trial was laid out at 'District Seed Farm', B C K V Kalyani The physico-chemical features, and meteoro logical data of the experimental period were presented in Appendix I and Appendix II, respectively Twenty nine genotypes (20 F_1 hybrids, 4 lines and 5 testers) were seeded in a randomised block design with 2 replications and seeding was done on 9th March, 1996 A single row plot of 3m length was alloted to each genotype, spacing between the rows was 60cm and plants within the rows were spaced by 30 cm Fertilizers were applied as basal at the rate of 20kg N, 60kg P_2O_5 and 40kg K_2O in the form of urea, single super phosphate and muriate of potash, respectively Plant protection measures were taken as and when necessary, Viny types were trained on the support made of bamboo stripes and jute string Observations were recorded from all the ten plants in each replication The characters studied were-

1) Days to flowering - It was the time span from date of sowing to date of first flowering

2) Pod length (cm)

3) Fresh pod weight (g)

4) Dry pod weight (g)

5) Seeds per pod

6) Pods per plant (g)

7) Pod yield per plant (g)

8) Protein content of pods (%)

3.5. Analysis of Heterosis

3.5.1 Materials

F_1 hybrids of 4 x 5 Line X Tester population were employed in this study

3.5.2 Methods

The characters for which manifestation of heterosis was estimated were days to flowering, pod length, fresh pod weight, dry pod weight, seed per pod, pods per plant, pod yield per plant and protein content of pods

3.6 Statistical and biometrical analysis

The observations recorded on the various characters for pod and seed characters at three different stages, leaf characters, protein content of pods and seeds and pod yield, root and nodule characters and protein content of pod and seeds were subjected to the following statistical analysis

3.6.1. Analysis of variance

Differences between genotypes for different characters were tested for significance using analysis of variance Analysis of variance was done on the basis of the following model

$Y_{ij} = m + g_i + r_j + c_{ij}$

Where Y_{ij} = Phenotypic observation in i - th genotype and jth replication

m = general mean

g_i = effect of i-th genotype

r_j = effect of j-th replication

e_{ij} = random error associated with i-th genotype and j-th replication

Structure of analysis of variance

ANOVA

Source	d f	M S	Expected M S	F
Genotypes	(t-1)	Mt 11	$\sigma^2 e_{11} + r\sigma^2 g_{11}$	Mt11/Me11
Replication	(r-1)	Mr11	-	
Error	(r-1)(t-1)	Me 11	σe^2_{11}	

Where t = number of genotypes

r = number of replications

Standard error (S E), standard error of difference between two means (S Ed) and critical difference (C D.) were calculated as follows -

S E = (MSe/r) and S.Ed = (2 Mse/r)½

Where, MS_e = Error mean square

r = number of replications

C D = S Ed x t

Coefficient of variation (C V) was calculated as follows -

$$C\,V(\%) = \frac{\text{Environmental variance}}{\overline{X}} \times 100$$

Where, Environmental variance = Mse , $\overline{X}$ = General mean for the character Significance of means of the cultigroup for different characters have been tested by Duncan's multiple range test (DMRT) as per Gomez and Gomez (1984) The shortest significant ranges (Rp) was calculated as -

$$Rp = \frac{(rp)\ (\overline{Sd})}{\sqrt{2}} \quad \text{for } P = 2,3 \qquad t$$

Where, sd = standard error of mean difference

$$Sd = \sqrt{\frac{2s^2}{r}}$$

Where, S^2 is the error mean square in the analysis of variance and r is the number of replications

rp = tabular values of the significant studentized ranges for 5% and 1% level new multiple range test at error degrees of freedom

P = distance in rank between the pairs of treatment means to be compared i e P = 2 for two means with consecutive rankings and p = t for the highest and lowest means

In our analysis p = 2 = 3

3.6.2 Component of variances

Considering that all the genotypes tested were uniform genetically, the expected mean sum of square for error (MS_e) will be purely a random environmental variance The mean sum of squares between genotypes will consist of the variances i) attributable to genotypic differences and ii) due to environmental variation among the individuals of each genotype So, the different variances will be as follows -

Error variance $= \sigma^2 e_{11} =$ Me 11

Genotypic variance $= \sigma^2 g_{11} =$ (Mt 11 - Me 11)/r

Phenotypic variance $= \sigma^2 p_{11} = \sigma^2 g_{11} + \sigma^2 e_{11}$

Where, Mr 11, Mt 11 and Me 11 stand for mean sum of squares due to replication, genotype and error, respectively

The genotypic (G C V) and phenotypic (P C V) coefficients of variation were calculated by the formulae given by Burton (1952) and Burton and De Vane (1953) -

$$\text{G C V} = \frac{\text{Genotypic standard deviation}}{\text{Grand mean}} \times 100$$

$$\text{P C V} = \frac{\text{Phenotypic standard deviation}}{\text{Grand mean}} \times 100$$

Heritability in broad sense (h^2) was estimated by the formula suggested by Hanson *et al* (1956) -

$$h^2 (\%) = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

The expected genetic advance (G A) was calculated as per Johnson *et al.* (1955a) -

$$G A = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times K \times \sigma p$$

Where, σp = phenotypic standard deviation

K = Selection differential, a constant, 2 06 for 5% selection intensity

$$G A \text{ as percentage of mean} = \frac{G A}{\text{mean}} \times 100$$

3.6.3. Analysis of covariance.

Analysis of covariance was made for all the characters in their all possible combinations taking two variables at a time The structure of covarience table was as follows -

ANCOV

Source	d f	M P	Expected M P	F
Genotypes	(t-1)	Mt 12	$\sigma e\ 12 + r\ \sigma g\ 12$	Mt 12/ Me 12
Replication	(r-1)	Mr 12		
Error	(t-1) (r-1)	Me 12	$\sigma e\ 12$	

Where r and t are numbers of replications and genotypes, respectively

Genotypic covariance = $\sigma\ g\ 12 = (Mt\ 12 - Me\ 12)/r$

Phenotypic covariance = $\sigma p\ 12 = \sigma 12 + \sigma e\ 12$

Estimation of correlation was done as -

Phenotypic and genotypic correlation coefficient for all possible combinations were worked out by employing variance and covariance table as suggested by Aljibouri *et al,* (1958) and computation was done as follows -

$$r\ (xy) = \frac{COV\ X\ Y}{\sqrt{Var\ (X)\ \ Var(y)}}$$

Where, r (xy) is the correlation between characters x and y

Cov X Y is the covariance between x and y

Var (x) is the variance of x

Var (y) is the variance of y

To test the significance of correlation coefficient, the estimated values were compared with the table values (Fisher and Yates, 1967) at (t-2) degrees of freedom at 5% and 1% level of significance

3.6.4. Path coefficient analysis

Path coefficient were calculated to estimate the direct and indirect effects of the characters as per Dewey and Lu (1959) The following set of simultaneous equations were formed and solved for estimating various direct and indirect effects

$r_1y = P_1y + r_{12} P_2y + r_{13} P_3y + \qquad r_{1I} p_Iy$

$r_2y = r_{21}p_1y + P_2y + r_{23}p_2y + \qquad r_{2I}p_Iy$

*

*

*

** $r_{I1}P_1y = r_{I1}y + r_{12}P_2y + r_{13}P_3y + \qquad p_Iy$

Where, r1y to rIy = correlation coefficients between causal factors 1 to I and dependent character y

r_{12} to $r_{I-1,I}$ = correlation coefficients among causal factors

P_1y to P_Iy = Direct effects of characters 1 to I on character y

The avove equations were, written in a matrix form as -

$$\begin{array}{cccc} \text{A} & & \text{C} & \\ \left|\begin{array}{cc} r_1y & 1 \\ r_2y & r_{21} \\ r_3y & r_{31} \\ * & * \\ * & * \\ * & * \\ r_Iy & r_{I1} \end{array}\right| & \left|\begin{array}{cccc} r_{12} & r_{13} & & r_{1I} \\ 1 & r_{23} & & r_{2I} \\ r_{32} & 1 & & r_{3I} \\ * & * & & * \\ * & * & & * \\ * & * & & * \\ r_{12} & r_{13} & & 1 \end{array}\right| & \text{X} & \left|\begin{array}{c} P_1y \\ P_2y \\ P_3y \\ * \\ * \\ * \\ P_Iy \end{array}\right| \end{array}$$

Then $B = c^{-1}A$

$$C^{-1} = \left|\begin{array}{cccc} C_{11} & C_{12} & C_{13} & C_{1I} \\ C_{21} & C_{22} & C_{23} & C_{2I} \\ * & * & * & * \\ * & * & * & * \\ * & * & * & * \\ C_{I1} & C_{I2} & C_{I3} & C_{II} \end{array}\right|$$

Then, direct effects were calculated as follows -

$P_1y \;=\; ^{I} \quad C_{11}\, r_iy$

$P_2y \;=\; ^{I} \quad C_{21}\, r_iy$

$P_Iy \;=\; ^{I} \quad C_{Ii} r_iy$

Residual effect which measures the contribution of the character not considered in the casual scheme was obtained as -

Residual effect (PRy) $= \sqrt{1 - R^2}$

Where, $R^2 = P^2iy + 2\sum\sum_{i \neq j} Piy\ pjy\ rij$

3.6.5. Line x tester analysis.

Line x Tester analysis provides information about the general and specific combining ability of parents and at the same time it is helpful in estimating various types of gene effects Differences among the genotypes including crosses and parents for different characters were tested for significance using analysis of variance If these differences are found significant, line x tester analysis was done

3.6.5.1. Analysis of variance for simple R.B.D.

ANOVA

Source	d.f.	M.S	Expected M S	F
Genotypes	(T-1)	MT11	$\sigma^2e\ 11 + r^2\sigma g\ 11$	MT11/Me11
Replication	(r-1)	Mr11	-	
Error	(r-1) (T-1)	Me11	$\sigma^2e\ 11$	

Where T = number of genotypes

r = number of replications

To test the significance of genotypic differences treatment MS/Error MS is compared with table value of F at 5% level of significance for treatment and error degrees of freedom, respectively

3.6.5.2 Partitioning of treatment S.S.

To test the significance of the crosses and parents individually, it is possible to partition the treatment S S into various components like parents crosses and parent V_s crosses

ANOVA with parents and crosses

Source	d f	M S	F
Replications	(r-1)	Mr11	Mr11/Me11
Treatments	(T-1)	Mt11	MT11/Me11
Parents	(l + t)	M(l + t)	M (l + t)/Me
Crosses	(c-1)	Mc	Mc/Me
PV_sC	1		
Error	(r-1) (T-1)	Me11	

All the sources of variation are tested against error source of variation

3.6.5.3. Line x Tester analysis

ANOVA for Line x Tester analysis

Source	d f	M S	F
Lines	(L-1)	Ml	Ml/Me 11
Testers	(t-1)	Mt	Mt/Me11
Line x Tester	(L-1) (t-1)	Mlxt	Mlxt/Me
Error	(r-1) (T-1)	Me11	

The whole information can be compiled as follows

Anova for Line X Tester analysis including parents

Source	d f	M S	F
Replication	(r-1)	Mr11	Mr11/Me11
Treatment	(T-1)	MT11	MT11/Me11
Parents	(L+t)-1	M(L+t)	M(L+t)/Me
Parents vs. crosses 1			
Crosses	(C-1)	Mc	Mc/Me11
Lines	(l-1)	Ml	Ml/Me11
Testers	(t-1)	Mt	Mt/Me11
Line x Testers	(t-1) (t-1)	Mlxt	Mlxt/Me11
Error	(T-1) (r-1)	Me11	
Total			

M S due to lines (Ml) and testers (Ml) are to be tested against the M S due to lines x testers (Mlxt) the later is, inturn, tested against M S due to error (Me)

3.6.5.4. Estimation of GCA effects

(a) Lines $$g_i = \frac{x_i}{tr} - \frac{x}{ltr}$$

where l = no of lines

t = no of testers

r = no of replications

(b) Tester gca (tester) $$= \frac{x_j}{lr} - \frac{x}{ltr}$$

3.6.5.5. Estimaation of SCA effects

$$S_{ij} = \frac{x_{ij}}{r} - \frac{x_i}{tr} - \frac{x_j}{lr} + \frac{x}{ltr}$$

3.6.5.6. Standard errors for combining ability effects

S E (gca for line) = $(Me/r \times t)^{1/2}$

S E (gca for tester) = $(Me/r \times 1)^{\frac{1}{2}}$

S E (sca effects) = $(Me/r)^{\frac{1}{2}}$

S E (g_i-g_j) line = $(2Me/lxt)^{\frac{1}{2}}$

S E (g_i-g_j) tester = $(2Me/lxt)^{\frac{1}{2}}$

S E $(s_{ij} - s_{kl})$ = $(2Me/r)^{\frac{1}{2}}$

$$\text{Cov F S} = \frac{(Ml - Me) + (Mt - Me) + (Mlxt - Me)}{3xr}$$

$$= \text{Cov H S} = \frac{[1 + F]\,\sigma^2 A]\sigma^2 gca}{4}$$

$$\sigma^2\,sca = \frac{Mlxt - Me}{r}$$ So, σ^2A = Cov H S x 4 when F = 0

$\sigma^2\,sca$ = $[1 + F]^2\,\sigma^2$ So, $\sigma^2NA = 4 \times \sigma^2SCA$ when F = 0

Pooled data on line x tester in a two way table

Lines	Tester					Total
	5	6	7	8	9	
1	x_{ij}	-	-	-	-	x_i
2						
3						
4				X_n	j_n	X_n
Total	x_j			Xn JN		X

3.6.5.7. Genetic components

$$\text{Cov H S (line)} = \frac{M_l - Mlxt}{r \times t}$$

$$\text{Cov H S (tester)} = \frac{Mt - Mlxt}{r \times l}$$

$$\text{Cov H S (average)} = \frac{2}{r(2lt - l - tl)} \quad \frac{[l(Ml) + t(Mt)}{l + t - 2} \times ML \times t$$

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Different experimental approaches were carried out to frame the breeding strategies to elevate the protein content concurrently with improving pod yield potential

4.1 Pod and seed characters at three different stages of the pods

Twenty one genotypes,seven each of cv-gr Sesquipedalis,Unguiculata and Biflora were studied for pod length, fresh pod weight, dry pod weight, seeds per pod and protein content of pod in two years under environment 1(E1) and environment 2(E2) The mean values of the different characters belonging to the cultigroups in both the environments have been presented in Appendix- III The results are being discussed here under employing the pooled data over environments (Table - 8,9 and 10) In the earlier studies (Sambandam *et al*, 1965 and Kar *et al*, 1995) the ideal picking stage of green tender pods for vegetable purpose ranged from 10-15 days after anthesis, with 2-3 days more for sesquipedalis For these reasons, the pod and seed characters have been studied in three critical stages i e 10, 15 and 20 days after anthesis

4.1.1 10 days after anthesis

All the characters were significantly different among the cultigroups which

Table-8: Pod and seed characters 10 days after anthesis (Pooled)

Variety	Cultigroup	Pod length (cm)	Fresh pod wt (g)	Dry pod wt (g)	Seed/ pod	Protein content (%)
Sel-Tm1	Sesquipedalis	17 492	12 695	0 618	13 943	3 508
Sel-Tm2	”	16 428	10 328	1 138	14 445	3 812
Sel-Tm3	”	18 140	12 707	1 325	14 248	3 703
EC-305827	”	17 733	8 780	0 875	1 952	3 487
L - 16	”	22 093	10 750	0 955	14 958	3 498
T-5	”	14 732	8 962	0 868	12 383	3 833
L-9	”	17 728	13 043	0 985	12 580	4 235
Mean		17 76	11 04	0 9664	13 50	3 725
Pusa Dofasli	Unguiculata	16 612	4 202	0 695	14 273	4 043
EC-243954	‘’	14 777	6 942	0 642	10 140	3 370
Cowpea 263	”	15 970	4 247	0 287	11 537	3 463
1 - 101	”	14 097	2 903	0 302	7 678	3 460
Sel 61B	”	18 877	4 380	0 903	9 232	2 710
HG 22	”	8 040	3 032	0 393	8 122	3 592
5269	”	11 578	3 172	0 495	9 987	4 015
Mean		14 19	4 125	0 5310	10 14	3 522
82-1	Biflora	8 180	2 120	0 310	8 205	3 662
Covu62A	”	7 598	1 310	0 323	9 750	3 583
Cherodi	”	7 932	1 422	0 322	7 762	3 942
1 - 195B	”	7 037	2 600	0 482	9 703	3 560
SG2	”	10 577	3 170	0 860	7 807	3 367
CG7A	”	9 057	1 785	0 462	8 978	3 685
V - 70	”	7 232	1 505	0 205	7 308	3 498
Mean		8 230	1 987	0 4233	8 502	3 614
Significance of mean	S - U	*	*	*	*	*
between	U - B	*	*	*	*	*
cultigroups	B- S	*	*	*	*	*

could be demonstrated from the study of sıgnıficance of mean through 't' test The cv-gr Sesquıpedalıs have been characterısed by sıgnıficantly larger pod length (17 76 cm) followed by cv-gr Unguıculata (14 19 cm) and cv-gr Bıflora (8 23 cm) The fresh pod weıght and dry pod weıght were also hıghest ın cv-gr Sesquıpedalıs compared to cv-gr Unguıculata and cv-gr Bıflora Seeds per pod was also hıghest ın cv-gr Sesquıpedalıs followed by cv-gr Unguıculata and cv-gr Bıflora Pod proteın content also varıed sıgnıficantly among the cultıgroups wıth the hıghest ın the pods of cv-gr Sesquıpedalıs (3 72%) followed by cv-gr Bıflora (3 61%) and cv-gr Unguıculata (3 52%) (Fıg 1) In these stage of pıckıng, three genotypes, one ın cv-gr Sesquıpedalıs (L-9) and two ın cv-gr Unguıculata (Pusa Dofaslı and 5269) have regıstered more than 4% proteın ın the pods On the other hand ın most of the cases the proteın content ranged from 3 5 to 3 7 percent The genotype of cv-gr Unguıculata (Sel 61B) showed the lowest proteın (2 71%) ın thıs stage of pod

4.1.2 15 days after anthesis

In thıs stage also sıgnıficant mean dıfference exısted among the cultıgroups for all the characters (Table 9) When growth pattern ın dıfferent characters ~~were~~ was analysed cultıgroupwıse ıt was apparent that the growth was much conspıcuous ın the cultıgroup Sesquıpedalıs followed by that ın cv-gr Unguıculata and cv-gr Bıflora In pod length, as hıgh as 13 24 cm ıncrease was regıstered ın cv-gr Sesquıpedalıs compared to 4 98 cm ın cv-gr Unguıculata and 2 19 cm ın

Fig 1. Mean proteincontent of three cultigroups of cowpea in three pod stages

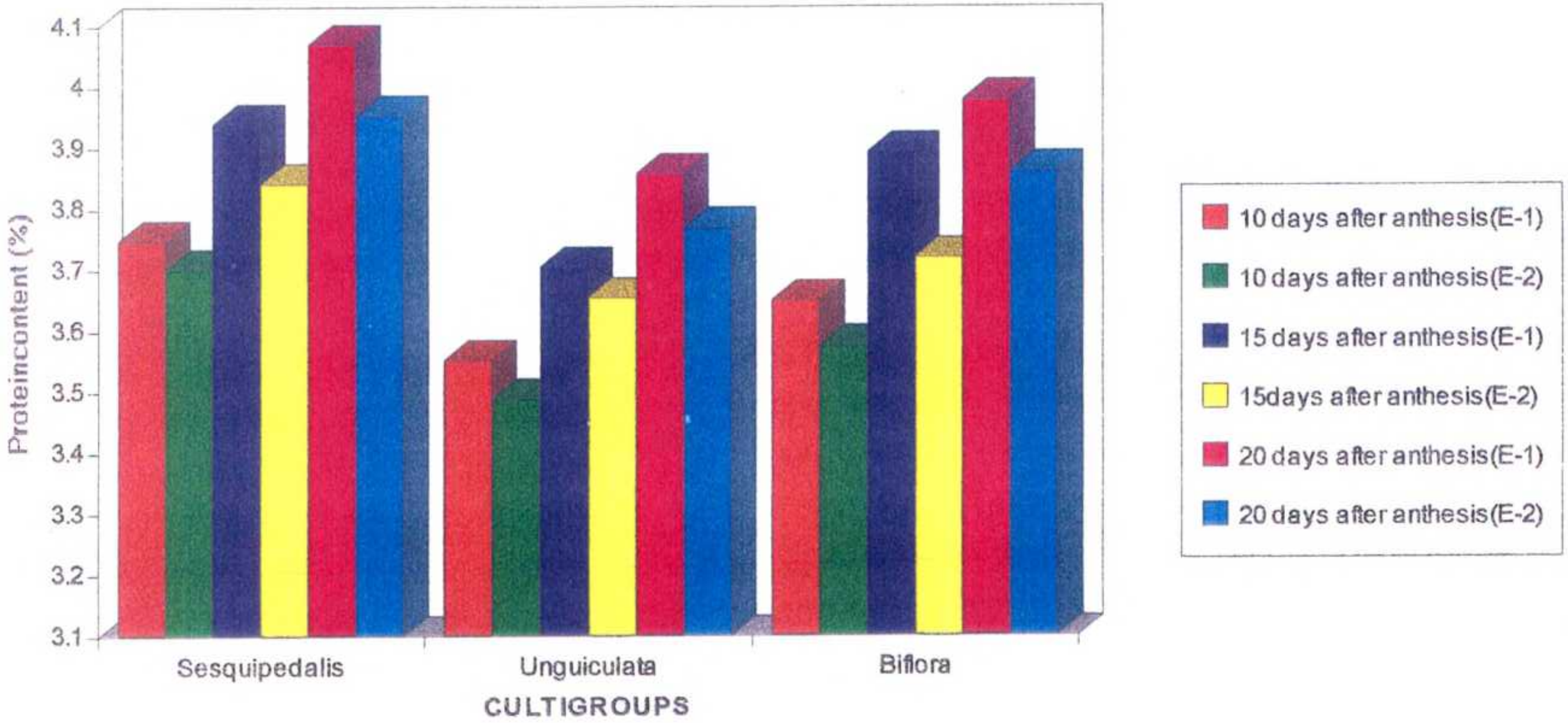


Table-9 : Pod and seed characters 15 days after anthesis (Pooled)

Variety	Cultigroup	Pod length (cm)	Fresh pod wt (g)	Dry pod wt (g)	Seeds/ pod	Protein content (%)
Sel-Tm1	Sesquipedalis	29 117	18 330	0 890	14 640	3 775
Sel-Tm2	„	24 710	13 748	1 255	14 593	3 978
Sel-Tm3	„	32 795	16 652	1 867	14 972	3 762
EC-305827	„	39 747	21 215	2 007	16 742	3 705
L - 16	„	35 040	14 398	1 298	14 620	3 623
T-5	„	24 598	11 268	1 432	14 352	4 032
L-9	„	0 985	18 388	1 030	13 743	4 360
Mean		31 000	16 291	1 397	14 81	3 891
Pusa Dofashi	Unguiculata	21 888	5 128	1 015	16 098	4 118
EC-243954	„	22 920	7 838	0 953	11 057	3 655
Cowpea 263	„	20 992	5 105	0 947	11 782	3 522
1 - 101	„	16 168	4 400	0 530	10 520	3 605
Sel 61B	„	21 308	4 980	0 987	10 527	2 815
HG 22	„	13 393	4 272	0 592	7 950	3 907
5269	„	17 492	4 515	0 718	10 947	4 137
Mean		19 17	5 177	0 820	11 27	3 680
82 -1	Biflora	11 098	3 572	0 425	9 927	3 753
Con u 62A	„	11 135	3 100	0 510	9 587	3 720
Cherodi	„	7 868	1 762	0 350	9 102	4 008
1 - 195B	„	11 460	2 400	0 832	10 363	3 642
SG2	„	12 250	3 588	0 852	7 963	3 497
CG7A	„	10 742	3 227	0 630	8 887	4 147
V-70	„	8 360	2 088	0 390	8 213	3 687
Mean		10 42	2 820	0 5698	9 149	3 779
Significance	S - U	*	*	*	*	*
of mean						
between	U - B	*	*	*	*	*
cultigroups	B - S	*	*	*	*	*

cv-gr Biflora in five days Similarly gain in fresh pod weight was 5 25 g in cv-gr Sesquipedalis, 1 05 gm in cv-gr Unguiculata and 0 83 gm in cv-gr Biflora In dry weight of pod the increase in five days was 0 43 gm in cv-gr Sesquipedalis, 0 29 gm in cv-gr Unguiculata and 0 15 gm in cv-gr Biflora Seeds' ~~number~~ per pod also increased but not so conspicuously It was interesting to note that increase in protein content in the pod was almost similar in all the cultigroups, 0 166% in cv-gr sesquipedalis, 0 165% in cv-gr Biflora and 0 158% in cv-gr Unguiculata

In this stage also, highest pod length, fresh pod weight, dry pod weight, seeds per pod and pod protein content was observed in the cultigroup Sesquipedalis Maximum pod length of 39 74 cm was recorded in the genotype EC-305827 The genotype L-9 which registered maximum protein content of 4 23% in the earlier stage also showed highest protein of 4 36% in this stage In this stage apart from L-9 of cv-gr Sesquipedalis four other genotypes, two each of cv-gr Unguiculata (Pusa Dofasli and 5269) and two each of cv-gr Biflora (Cherodi and CG7A) showed more than 4% protein in the pod (Table 9)

4.1.2 20 days after anthesis

In this stage also the characters under consideration differed significantly among the cultigroups Growth in the characters at the next five days interval have been drastically reduced and the rate of increase also changed with the cultigroups For pod length, fresh pod weight and dry pod weight cultigroup

Table - 10 : Pod and seed characters 20 days after anthesis (Pooled)

Variety	Cultigroup	Pod length (cm)	Fresh pod wt.(g)	Dry pod wt(g)	Seed/pod	Protein content(%)
Sel-Tm1	Sesquipedalis	31.662	20.317	1.113	15.675	3.907
Sel-Tm2	"	25.942	15.377	1.320	15.410	4 100
Sel-Tm3	"	36.743	17.960	2.168	16.698	3 812
EC-305827	"	42.318	23.677	2.117	18.647	3.823
L-16	"	37.903	15.670	1.485	14.548	3.707
T-5	"	25.512	12.293	1.540	14.757	4.185
L-9	"	33.368	19.190	1.162	14.220	4.557
Mean		33.35	17.78	1.558	115.71	4.013
Pusa Dofasli	Unguiculata	23.293	7.102	1.222	17.283	4.208
EC-243954	"	24.598	8.922	1.100	13.862	3.792
Cowpea 263	"	22.623	6.463	1.090	12.917	3.647
1-101	"	17.205	4.878	0.667	13.015	8.757
Sel 61B	"	23.502	5.413	1.113	1.130	3.020
HG 22	"	14.595	5.033	0.770	9.963	4.047
5269	"	18.840	5.145	0.813	11.692	4.225
Mean		20.67	6.137	0.9679	12.84	3.814
82-1	Biflora	11.482	4.342	0.527	11.388	3.873
Convu 62A	"	12 732	3.745	0.582	11.662	3.795
Cherodi	"	8.932	2.588	0.468	10.1118	4.100
1-195B	"	12.827	2.913	0.928	12.228	3.777
SG 2	"	12.805	3.888	0.940	10.033	3.735
CG 7A	"	11.410	3.593	0.680	10.227	4.412
V-70	"	8.800	2.747	0.477	9.657	3.753
Mean		11.28	3.402	0.6574	10.77	3.921
Significance of mean between cultigroups	S-U	*	*	*	*	*
	U-B	*	*	*	*	*
	B-S	*	*	*	*	*

Table-11: Increase in the pod and seed characters at 5 days interval

Cultigroups	Pod length (cm)	Fresh pod wt(g)	Dry pod wt(g)	Seeds perpod	Protein content (%)
Increase over 10 days old pod					
Sesquipedalis	13.24	5.25	0.431	1.31	0.166
Unguiculata	4.98	1.05	0.289	1.13	0.158
Biflora	2.19	0.83	0.146	0.64	0.165
Increase over 15 days old pod					
Sesquipedalis	2.35	1.49	0.161	0.90	0.122
Unguiculata	1.50	0.96	0.147	1.50	0.134
Biflora	0.86	0.58	0.087	1.62	0.142

Sesquipedalis registered the maximum increase (2 35 cm, 1 49 g and 0 161 g, respectively) followed by cv-gr Unguiculata (1 50 cm, 0 96 g and 0 147 g respectively) and cv-gr Biflora (0 86 cm, 0 58 g and 0 087 g, respectively) Rate of increase in the seeds per pod was highest in cv-gr Biflora (1 62) followed by in cv-gr Unguiculata (1 57) and Sesquipedalis (0 90) Similar was trend for the increase in pod protein percentage where the rate of increase was highest in cv-gr Biflora (0 142%) followed by in cv-gr Unguiculata (0 134%) and cv-gr Sesquipedalis (0 122%) (Table 11) Genotype of cv-gr Biflora are determinate and short duration where as those of Sesquipedalis are indeterminate and long duration For the reason, rate of protein deposition might have been faster in Biflora in the advanced stage of pod growth

In this stage, highest pod length, fresh pod weight (Fig 2) dry pod weight, seeds per pod (Fig 3) and protein content were observed in the cultigroup Sesquipedalis Maximum pod length (42 32 cm), fresh pod weight(23 67 gm), dry pod weight (2 11 g) and seeds per pod (18 64) was revealed in the genotype EC-305827 which also showed the same trend in the earlier stages Highest pod protein content both among the genotypes of the cultigroup Sesquipedalis and among the total genotypes of the three cultigroups was registered in L-9 The other Sesquipedalis genotypes revealing more than 4% protein were T-5 (4 18%) and Sel- Tm2 (4 1%) Cultigroup Biflora ranked second and Ungluiculata third interms of protein content of the pod considering all the three stages of pods In the 20 days stage the second highest protein content was revealed in the cv-gr Biflora genotype CG 7A (4 41%) The other genotype showing more than 4%

Fig 2. Mean fresh pod weight of three cultigroups of cowpea in three pod stages

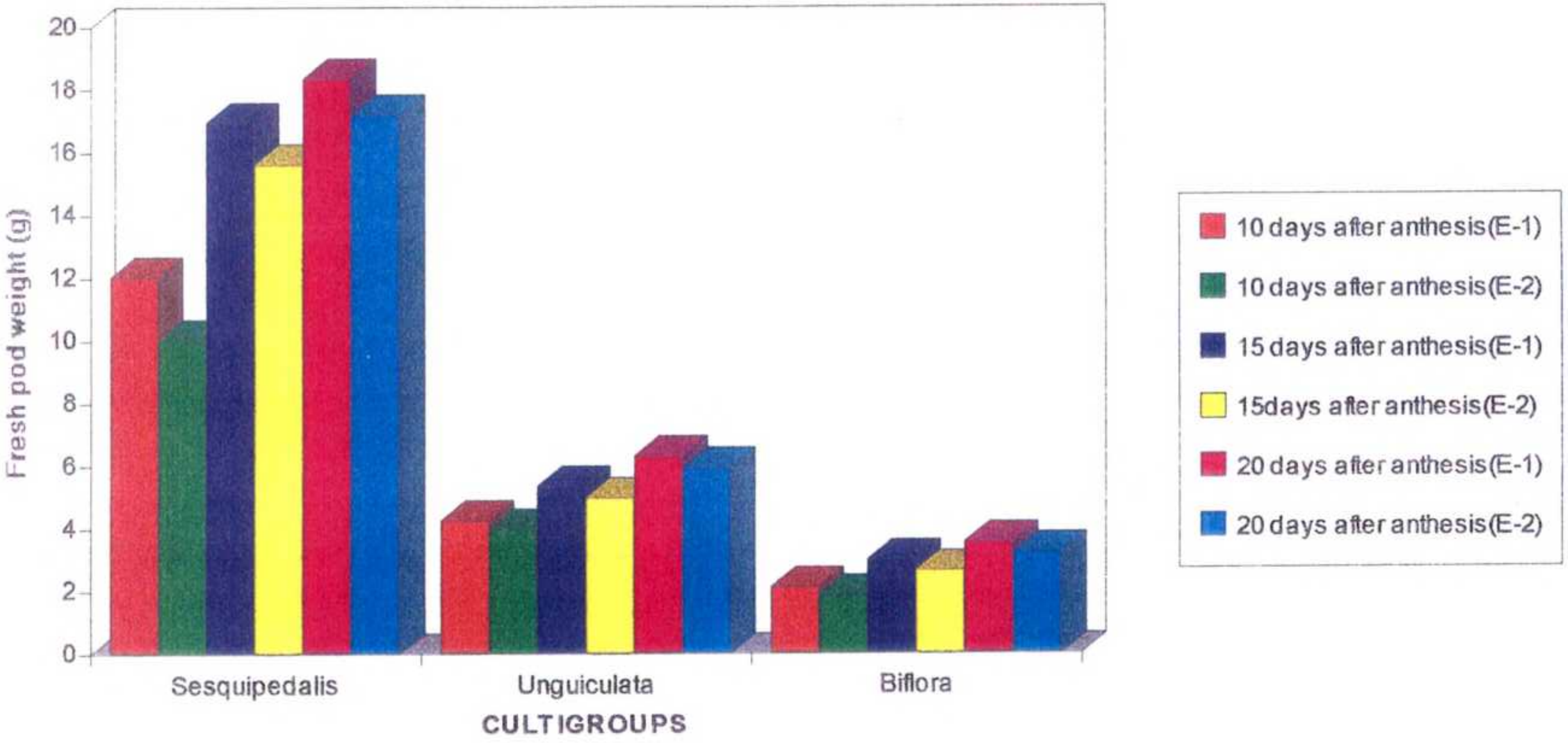
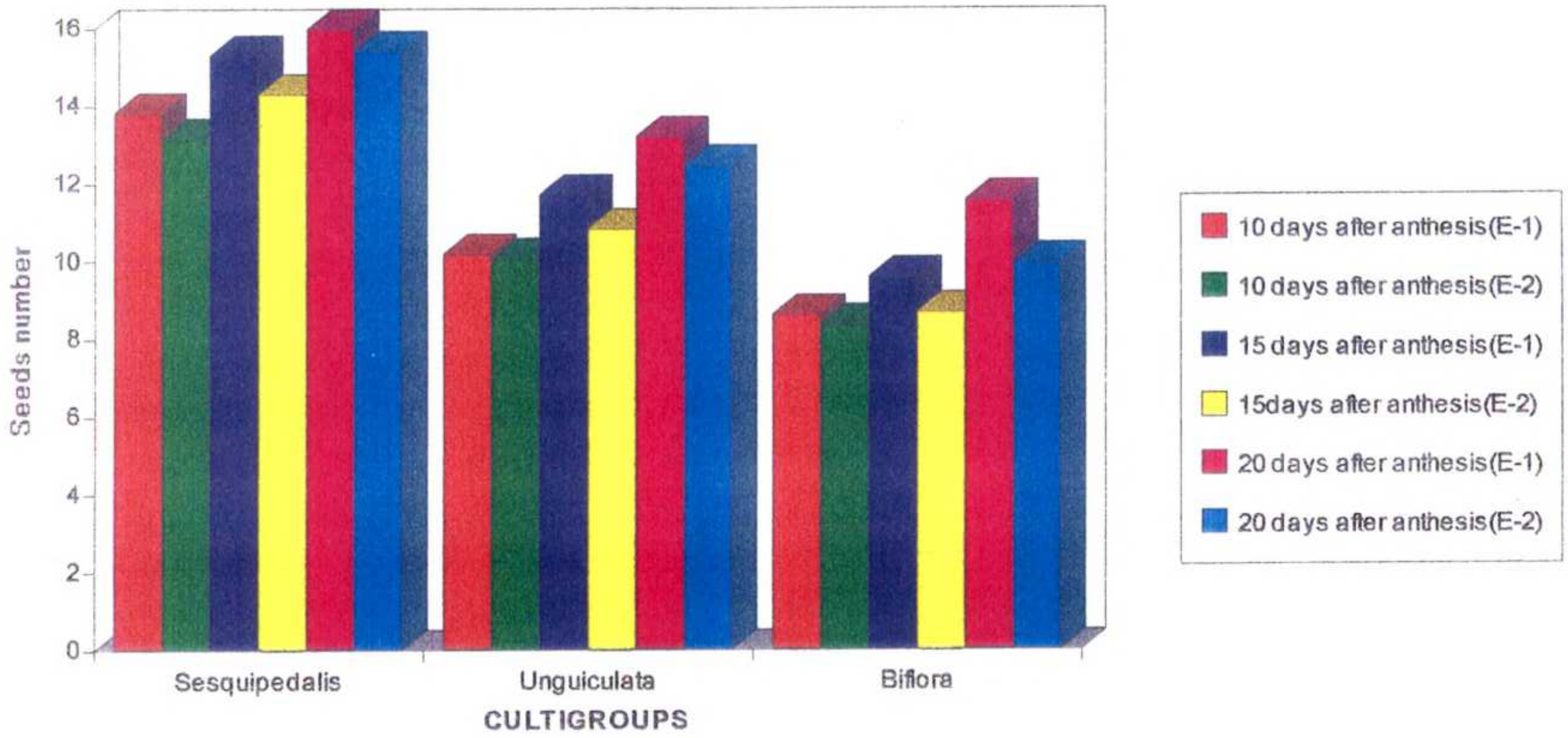


Fig 3. Mean seeds number of three cultigroups of cowpea in three pod stages



protein in the pod in this cultigroup was Cherodi (4 1%) In the 20 days stage three genotypes of this cultigroups (Pusa Dofasli, HG22 and 5269) showed more than 4% protein in their pods

The characterization studies of Ng and Marechal (1985) and Hazra *et al* (1993)indicated cultigroup Sesquipedalis as indeterminate viny plant having maximum pod length (even upto 90 cm) and weight, with maximum number of seeds In the present study the cultigroup Unguiculata has taken an intermediate position in between cultigroup Sesquipedalis and cv-gr Biflora for pod length, pod weight (fresh as well as dry) and seeds per pod In other words, cv-gr Sesquipedalis and cv-gr Biflora seemed to have occupied dimetrically two extreme positions (Highest for cv-gr Sesquipedalis and lowest for cv-gr Biflora) in which the cultigroup Unguiculata came in between for the characters concerned This proposition on the relative position of the three cultigroups was earlier put forward by Hazra *et al* (1993) This situation was not entirely unexpected because the typical cowpeas of Africa (Unguiculata forms) after reaching South-East Asia including Indian subcontinent were subjected to human selection according to specific needs since long which resulted in the culmination of two more cultigroups - the short poded fodder type Biflora cultigroup at one end and the fleshy long poded vegetable type at the other end of these selection scale (Smart, 1985) Porbably cv-gr Unguiculata is a compromise between two extrteme selections

Protein content in the pods containing immature seeds increased with the age irrespective of the cultigroups due to translocation of amino acids from the site of synthesis (mostly leaves) via stem to the fruits and seeds This proposition of translocation of amino acids in the other beans was put forward by Boulter (1965), Hall (1968),Pasala and Umadevi (1989) and Qiu *et al* (1991) Increase in protein content in the pods with the age was also observed in cowpea (Kar *et al*, 1995) Hall (1968) observed rapid increase in the amino acid level in the fruits in the early stages of the fruit development with the aparent ceasation of protein accumulation in the leaves at that time Dry weight of pods also increased along with the fresh weight with age of the fruit because along with the translocation of amino acids for the conversion of proteins in the pods and ultimately to seeds, low molecular weight compounds are also incorporated in the pods as reserve materials Carbohydrate conversion are of great significance in the pod and seed development of bean (Culpepper, 1936) Loewenberg (1955)stressed the importance of respiratory path ways in providing much of the energy and intermediatory compound during fruit development of beans In cowpea fibre content increases with decreasing sugar content during ageing of pods (Bautista and Dutta 1976 , Kar *et al*, 1995) which is also responsible for the increase in fresh and dry weight of the pods with the age

It is interesting to note that the genotypes irrespective of cultigroups which showed high protein content in the pod in the earliest statge revealed the consistancy in protein contents in other two stages also which indicated the genotypic control over protein synthesis and translocation to the sink Protein content is a typical quantitative character and that in legumes are affected both by genotype and environment (Krober *et al*, 1970 , Yadav and Tomer, 1985 , Zebalska *et al*, 1990) From the study, it can be suggested that the screening of the genotypes for pod protein content can effectively be done in the early stages of the pod development also The genotypes namely L-9 and T-5 of cv-gr sesquipedalis, Pusa Dofasli and 5269 of cv-gr Unguiculata, CG 7A and Cherodi of cv-gr Biflora can be regarded as outstanding for having registered maximum pod protein content in all the three stages These genotypes can be selected and used for further breeding programme Among these genotypes, L-9 an indeterminate type, was the highest yielder (343 66 gm/plant)

4.2 Analysis of genetic variability

This study was carried out employing different characters of twentyone genetypes belonging to three cultigroups

4.2.1 Pod and seed characters of three stages

Ananlysis of variance for pod length, fresh pod weight, dry pod weight, seeds per pod and pod protein content of 10, 15 and 20 days old pods have been computed seperately for two environments (E1 and E2) as well as pooled over mean of the cultigroups and over environments (Table 12 and 13) All the characters showed highly significant differences among the genotypes in each analys~~e~~is of variance Estimates for coefficients of phenotypic and genotypic variation (P C V and G C V), heritability in broad sen~~c~~se (h^2), genetic advance (G A) and genetic advance as percentage of mean (G A %) for ~~this~~ these characters were computed for two environments separately as well as pooled over

Table-12: Analysis of Variance for pod and seed characters in the genotypes of three cultigroups

CHARACTERS

Mean Sum of Square

Source	d.f	Pod length (cm)		Fresh pod wt.(g)		Dry Pod wt.(g)		Seeds/pod		Protein Content (%)	
		E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
For 10 days after pod setting											
Replication	2	6.769	0.411	0.015	0.632	0.003	0.004	4.703	0.959	0.005	0.004
Genotype	20	74.073	59.376	63.637	42.257	0.345	0.277	21.659	19.613	0.292	0.312
Error	40	2.926	1.051	0.789	0.461	0.005	0.013	0.780	0.664	0.010	0.002
S.E.		0.9876	0.592	0.5128	0.3922	0.0404	0.0655	0.51	0.4705	0.0564	0.0256
C.D.(at 5%)		2.821	1.692	1.465	1.120	0.1154	0.1872	1.457	1.344	0.1611	0.0731
C.D.(at 5%)		3.701	2.263	1.9609	1.499	0.1544	0.2504	1.950	1.799	0.2156	0.0978
C.V.		11.85	8.30	14.47	12.38	10.71	18.12	8.12	7.73	2.67	1.23
For 15 days after pod setting											
Replication	2	5.210	0.561	0.367	0.050	0.004	0.008	8.192	1.341	0.053	0.003
Genotype	20	267.609	262.831	130.274	111.665	0.634	0.584	25.999	22.677	0.342	0.293
Error	40	0.910	1.282	0.261	0.331	0.005	0.004	1.530	0.719	0.006	0.002
S.E.		0.5509	0.6537	0.2949	0.3321	0.0395	.0377	0.7141	0.4897	0.0464	0.0288
C.D. (at 5%)		1.574	1.868	0.8428	0.9491	0.1128	0.1077	2.040	1.399	0.1326	0.0823
C.D. (at 1%)		2.106	2.499	1.127	1.269	0.1510	0.1441	2.730	1.872	0.1774	0.1101
C.V.		4.62	5.73	6.05	7.42	7.18	7.21	10.13	7.52	2.10	1.34
For 20 days after pod setting											
Replication	2	2.799	0.174	0.768	0.051	0.024	0.007	4.342	0.561	0.008	0.001
Genotype	20	311.61	305.026	145.370	130.013	0.686	0.683	18.519	24.420	0.322	0.304
Error	40	2.171	0.509	0.350	0.217	0.010	0.015	1.312	0.249	0.006	0.002
S.E.		.8507	0.4121	0.3414	0.2687	0.0581	0.0713	0.6614	0.2884	0.0460	0.0246
C.D.(at 5%)		2.431	1.177	0.9757	0.7679	0.1660	0.2037	1.890	0.8242	0.1314	0.0703
C.D.(at 1%)		3.253	1.575	1.305	1.027	0.2221	0.2726	2.529	1.102	0.1759	0.940
C.V.		6.62	3.35	6.28	5.29	9.17	12.06	8.43	3.96	2.01	1.10

S.E. = Standard error
C.D. = Critical difference
C.V. = Coefficient of variation

Table-13: Analysis of variance for pod and seed characters in the genotypes of three cultigroups (pooled)

Source	d.f	Mean Sum of Square									
		Pod length (cm)		Fresh pod Wt.(g)		Dry pod Wt.(g)		Seeds /pod		Protien content (%)	
		Over Mean	Over Environment	Over Mean	Over Environment	Over Mean	Over Environment	Over Mean	Over Environment	Over Mean	Over Environment
For 10 days											
Replication	1	19.49	136.46	3.20	22.46	0.003	0.023	0.519	3.63	0.017	0.117
Treatment	20	139.194	131.18	134.26	104.18	0.496	0.613	38.984	40.50	0.062	0.595
Error	80	0.73	1.98	0.09	0.62	0.001	0.009	0.175	0.72	0.000	0.006
S.E.		0.34	0.57	0.12	0.32	0.014	0.038	0.170	0.34	0.084	0.309
C.D.(5%)		1.390	2.289	0.488	1.281	0.051	0.154	0.680	1.380	0.000	0.126
C.D.(1%)		1.845	3.036	0.647	1.700	0.068	0.204	0.903	1.832	0.000	0.167
C.V.		6.40	10.53	5.43	13.83	5.66	14.73	3.91	7.93	0.61	2.09
For 15 days											
Replication	1	3.52	24.64	2.10	14.763	0.01	0.072	3.90	27.30	0.03	0.249
Treatment	20	640.21	529.43	310.30	241.33	1.079	1.211	49.062	48.054	0.067	0.621
Error	80	0.12	1.09	0.02	0.29	000	0.004	0.063	1.125	0.000	0.004
S.E		0.14	0.42	0.58	0.22	0.006	0.027	0.102	0.432	0.073	0.027
C.D(5%)		0.563	1.698	0.230	0.876	000	0.267	0.408	1.725	0.000	0.102
C.D(1%)		0.748	2.225	0.305	1.162	000	0.354	0.542	2.290	0.000	0.136
C.V.		1.73	5.19	1.76	6.72	1.59	7.20	2.13	9.03	0.48	1.77
For 20 days											
Replication	1	4.22	29.60	1.72	12.10	0.025	0.17	4.24	29.68	0.052	0.361
Treatment	20	735.81	616.11	349.92	274.72	1.255	1.364	36.950	41.84	0.060	0.621
Error	80	0.05	1.34	0.04	0.28	0.001	0.013	0.096	0.78	0.000	0.004
S.E.		0.09	0.47	0.08	0.21	0.093	0.046	0.126	0.36	0.085	0.026
C.D.(5%)		0.363	1.883	0.325	0.861	0.051	0.185	0.504	0.143	0.000	0.102
C.D.(1%)		0.482	2.499	0.431	1.142	0.068	0.245	0.669	1.907	0.000	0.136
C.V.		1.09	5.32	2.39	5.84	2.15	10.62	6.74	2.36	1.63	0.53

S.E. = Standard Error
C.D. = Critical difference
C.V. = Co-efficient of variation

environment irrespective of the cultigroups and over mean of the cultigroups (Table - 14, 15) In each of the growth stages the G C V and P C V agreed clearly indicating lower influence of the environments for the expression of characters in the genotypes In the estimation of genetic parameters pooled over environment consistantly high G C V for all the three stages of pod growth was recorded for fresh weight of pod followed by dry weight of pod Very low G C V was recorded for pod protein content (8 63% for 10 days, 8 21% for 15 days and 7 93% for 20 days old pod) The heritability in broad sense was very high (over 90%) for all the characters including pod protein content in all the three stages of pod growth These broad sense heritability values were likely to be over estimated as in this calculations it was not possible to exclude variation due to different genetic components and their interaction, hence the present heritability estimates have to be considered with these limitations in view The estimates of genetic advance (both GA and GA%) increased with the stages of pod growth The highest genetic advance as percentage of mean was revealed for pod fresh weight Protein content of pods in all the three stages recorded very low G A and G A% of mean Our observation on high heritability and high genetic advance for pod weight and pod length corroborated the earlier observations of Sahoo *et al* (1991), Lakshmi and Goud (1977), Dharmalingam and Kadambavana sundaram (1986), Hazra (1991) and Chattopadhyay (1995) In most of the cases variability of seed protein content of different legumes including cowpea have been studied Such variability study for pod protein content is scarce The earlier study of Kar (1993) revealed that protein content of pods after 15 days of anthesis varied significantly among the cowpea genotypes Very low G C V and P C V were recorded for pod protein but the heritability estimate in broad sense was very high with a very low estimate of genetic advance as percentage of mean

Table 14 : Estimation of genetic parameters for pod and seed characters at three stages of pod

CHARACTERS	MEAN		h^2		G.C.V.		P.C.V.		G.A.		G.A.(%)	
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
For 10 days after pod setting												
Pod length(cm)	14.43	12.35	89.01	94.87	33.73	35.68	35.75	36.63	9.16	8.84	63.48	71.59
Fresh pod wt(g)	6.13	5.29	96.37	96.87	74.55	69.10	75.94	71.64	9.25	7.55	150.75	142.58
Dry pod wt (g)	0.65	0.62	98.56	87.12	89.58	47.31	90.23	50.68	1.19	0.57	181.95	90.90
Seeds per pod	10.88	10.54	90.02	90.48	24.23	23.83	25.55	25.05	5.15	4.92	47.31	46.66
Protein content (%)	3.65	3.59	90.38	98.10	8.39	8.95	8.83	9.03	0.60	0.65	16.44	18.24
For 15 days after pod setting												
Pod length(cm)	20.63	19.75	98.98	98.55	45.63	47.27	45.87	47.62	19.30	19.09	93.52	96.65
Fresh pod wt(g)	8.43	7.75	99.40	99.11	78.03	78.58	78.27	78.93	13.52	12.49	160.26	161.11
Dry pod wt (g)	0.95	0.90	24.84	97.97	48.03	48.58	96.37	49.08	0.46	0.89	49.30	99.04
Seeds per pod	12.20	11.27	84.20	91.05	23.39	23.99	25.49	25.15	5.34	5.39	44.21	47.08
Protein content (%)	3.82	3.73	24.66	97.97	8.74	8.32	17.60	8.41	0.34	0.63	8.93	16.98
For 20 days after pod setting												
Pod length(cm)	22.25	21.28	89.01	94.87	21.88	20.70	23.19	21.27	9.46	8.84	42.51	41.53
Fresh pod wt(g)	9.41	8.79	96.35	96.79	48.60	42.42	49.51	43.12	9.25	7.57	98.22	86.06
Dry pod wt (g)	1.09	1.02	95.77	87.12	30.65	28.96	31.32	31.03	0.67	0.57	61.79	55.69
Seeds per pod	13.58	12.61	89.92	90.48	19.41	19.91	20.47	20.93	5.15	4.92	37.92	39.02
Protein content (%)	3.96	3.86	90.38	98.10	19.52	8.32	8.12	8.40	0.60	0.65	15.12	16.96

h^2 = heritability in broad sense.
G.C.V. = Genotypic coefficient of variation.
P.C.V. = phenotypic coefficient of variation.
G.A. = Genetic advance.
G.A.(%) = Genetic advance as percentage of mean.

Table-15: Estimation of genetic parameters for pod and seed characters at three stages of pod (pooled)

CHARACTERS		MEAN			h^2			G.C.V			P.C.V.			G.A.			G.A. (%)		
		10 Days	15 Days	20 Days	10 Days	15 Days	20 Days	10 Days	15 Days	20 Days	10 Days	15 Days	20 Days	10 Days	15 Days	20 Days	10 Days	15 Days	20 Days
Pod length(cm)	POOLED	13.39	20.19	21.76	97.47	99.90	99.95	35.90	51.15	50.87	36.37	51.17	50.88	9.65	21.26	22.80	72.10	105.31	104.77
Fresh pod wt(g)		5.71	8.09	9.10	97.50	61.36	99.76	82.27	124.94	83.80	83.31	125.04	83.90	9.56	12.79	15.70	167.34	158.06	172.37
Dry pod wt(g)	OVER	0.64	0.92	1.06	98.97	98.83	99.66	63.39	45.60	42.94	63.77	45.68	43.01	0.83	0.87	0.93	132.02	93.94	88.31
Seeds per pod		10.71	11.74	13.10	97.63	99.45	96.47	23.75	24.35	18.81	24.04	24.41	19.15	5.18	5.87	4.98	48.34	50.02	38.06
Protein content (%)	MEAN	3.62	3.78	3.96	94.87	94.87	100.00	2.76	2.78	6.17	2.76	2.85	6.17	0.20	0.21	0.50	5.69	5.58	15.78
Pod length(cm)	POOLED	13.39	20.19	21.76	91.16	98.87	99.12	34.60	46.47	46.55	36.23	46.73	46.75	9.11	19.22	20.78	68.05	95.18	95.47
Fresh pod wt(g)	OVER	5.71	8.09	9.10	94.54	99.02	99.10	72.28	78.25	74.19	74.34	78.64	74.53	8.27	12.98	13.85	144.77	160.42	152.15
Dry pod wt (g)	ENVIRON-MENT	0.64	0.92	1.06	100.00	97.34	95.60	47.47	47.47	44.85	49.72	48.11	45.87	0.65	0.89	0.95	102.84	96.48	90.34
Seeds per pod		10.71	11.74	13.10	81.64	89.28	93.88	24.06	24.06	28.12	26.58	25.46	29.02	4.78	5.43	7.35	44.70	46.32	56.13
Protein content (%)		3.62	3.78	3.96	88.67	85.00	95.63	8.21	8.21	7.93	9.26	8.88	8.11	0.61	0.58	0.62	16.93	15.56	15.98

h^2 = heritability in broad sense.
G.C.V. = Genotypic coefficient of variation.
P.C.V. = phenotypic coefficient of variation.
G.A. = Genetic advance.
G.A.(%) = Genetic advance as percentage of mean.

4.2.2 Leaf characters, protein contents of pod and seed and pod yield

We also studied leaf fresh weight, leaf dry weight, chlorophyll content of leaf, pod protein content, seed protein content and pod yield of the twenty one genotypes belonging to three cultigroups in the light of variation within genotypes and cultigroups for the characters concerned (Appendix IV) Different genetic parameters for these characters were also computed to show the extent of variability existed that could be ascribed due to genotype

4.2.2.1 Mean difference among cultigroups

The cultigroup Unguiculata exhibited significantly higher leaf fresh weight as compared to both cv-gr Sesquipedalis and cv-gr Biflora However, leaf dry weight did not vary significantly between cv-gr Sesquipedalis and cv-gr Unguiculata, although lowest leaf fresh and dry weight were revealed in the cultigroup Biflora Earlier study of Hazra *et al* (1993) gave somewhat different picture with regard to variation in leaf dry weight among cultigroups In this study lowest leaf dry weight was recorded in cultigroup Biflora which agreed to our findings However, they recorded highest leaf dry weight in the cultigroup Sesquipedalis which was not in conformity to our findings These variations might have been resulted due to the difference in genotypes under the studies Chlorophyll content was highest in cultigroup Unguiculata (601mg/gm) followed by in cv-gr Sesquipedalis (524mg/gm) and cv-gr Biflora (491/mg/ gm) Chlorophyll content varied significantly between cv-gr Unguiculata and cv-gr Sesquipedalis and between cv-gr Unguiculata and cv-gr Biflora but it did not vary between cv-gr Biflora and cv-gr Sesquipedalis This finding clearly indicated the variation in photosynthetic capacity of the genotypes belonging to different cultigroups

Variation in the pod protein content at different stages of pod have already been discussed in the chapter 4 1 It was clear from the study that pod protein content of all the three stages (10, 15 and 20 days) were highest in cultigroup Sesquipedalis followed by cv-gr Biflora and cv-gr Unguiculata Seed protein content varied significantly among the cultigroups It ~~is the~~ was highest in the cultigroup Biflora (22 96%) followed by that in cv-gr Sesquipedalis (20 85%) and cv-gr Unguiculata (19 97%) Highest seed protein content in the cv-gr Biflora was also recorded in the earlier study (Hazra, 1991)

4.2.2.2 Differences in genetic parameters

Genetic parameters for these leaf characters, pod and seed protein content and pod yield were computed both irrespective of cultigroups and over mean of the cultigroups (Table 16) The results have been presented following the analysis irrespective of the cultigroups However, both the studies indicated closeness of P C V and G C V revealing less influence of the environments Among the leaf characters,the highest G C V and P C V was recorded for leaf dry weight Protein content of pods in all the three stages and that of seed showed very low G C V indicating less variability among the genotypes for protein content Although, these variability in protein content of pods and seeds were significant as revealed from the analysis of variance (Table - 17) Significant variability in

Table 16 : Analysis of variance for leaf characters; pod yield and protein content

SOURCE	d.f.	Leaf fresh wt.(g)	Leaf dry wt.(g)	Chlorophyll content (mg/g)	Pod yield (g)	Protein content in 10 days(%)	Protein content in 15 days(%)	Protein content in 20 days(%)	Seed protein (%)
Replication	2	4.67	0.002	0.027	314.41	0.004	0.039	0.000	0.005
Genotype	20	54.08	0.544	0.049	45484.04	0.298	0.294	0.313	11.63
Error	40	1.68	0.031	0.001	451.28	0.002	0.012	0.002	0.041
S.E.		0.75	0.101	0.020	12.26	0.028	0.063	0.027	0.116
C.D.(at 5%)		2.14	0.290	0.059	35.05	0.080	0.181	0.077	0.332
C.D.(at 1%)		2.86	0.388	0.079	46.90	0.108	0.108	0.104	0.445
C.V.		8.63	11.90	6.66	9.55	1.35	2.91	1.2	0.95
Pooled									
Replication	2	0.66	0.000	0.004	44.91	0.001	0.006	0.000	0.001
Genotype	2	19.58	0.085	0 010	47766.09	0.031	0.034	0.029	7.055
Error	4	0.22	0.003	0.000	37.98	0.000	0.001	0.000	0.008
S.E.		0.27	0.029	0.009	3.55	0.007	0.021	0.009	0.050
C.D.(at 5%)		1.06	0.116	0.038	13.96	0.030	0.084	0.036	0.197
C.D.(at 1%)		1.77	0.194	0.063	23.16	0.050	0.139	0.061	0.326
C.V.		3.13	3.49	3.12	2.77	0.37	0.98	0.42	0.41

S.E. = Standard error
C.V. = Co-efficient of variation
C.D. = Critical difference

Table-17 : Estimation of genetic parameters for leaf characters, pod yield and protein content

Characters	Mean	h^2	G.C.V.	P.C.V.	GA	GA(%)
Pooled over cultigroup						
Leaf fresh wt (g)	15.05	91.18	27.76	29.07	8.22	54.61
leaf dry wt(g)	1.47	84.65	27.99	30.42	0.78	53.06
Chlorophyll content (mg/g)	0.53	94.11	23.46	24.18	0.25	46.88
Pod yield (g)	222.39	97.08	55.09	55.91	248 67	111.81
Protein Content (10 days) (%)	3.62	98.01	8.66	8.75	0.64	17.67
Protein content (15 days)(%)	3.77	88.67	8.11	8.62	0.59	15.74
Protein content (20 days)(%)	3.91	98.10	8.21	8.29	0.65	16.76
Seed Protein(%)	21.26	98.94	9.24	9.29	4.02	18 93
Pooled Over mean						
Leaf fresh wt(g)	15.05	96.65	16.87	17.16	5.14	34.15
Leaf dry wt(g)	1.47	90.09	11.18	11.78	0.32	21.86
Chlorophyll content (mg/g)	0.53	100.00	10.65	10.65	0.11	21.94
Pod yield (g)	222.39	99.76	56.71	56.78	257.88	111.95
Protein content (10 days)(%)	3.62	100.00	2.76	2.76	0.02	0.71
Protein content (15 days)(%)	3.77	100.00	2.77	2.77	0.21	5.80
Protein content (20 days)(%)	3.91	100.00	2.42	2.42	0.20	5.17
Seed Protein(%)	21.26	99.66	7.20	7.21	3.15	14.81

h^2 = Heritability in broad sense.
G.C.V. = Genotypic co-efficient of variation.
P.C.V. = Phenotypic co-efficient of variation
G.A = Genetic advance.
G.A(%) = Genetic advance as percentage of mean.

seed protein content and some essential amino acids have been identified in cowpea by several workers (Bliss *et al*, 1973 , Bliss, 1975 , Imam, 1979 , Emebiri, 1991) Genotypic coefficient of variation was very high for pod yield Heritability in broadsense was very high (more than 95%)for all the characters Very high heritability for leaf dry weight was reported earlier (Hazra *et al* ,1994) High heritability for pod yield was also reported by Chandrappa *et al* (1974) , Jana *et al* (1982), Hazra (1991), Chattopadhyay (1995) Appreciably high broad sense heritability for seed protein content of cowpea was also reported earlier (Emebiri,1991) Genetic advance as percentage of mean was the highest in pod yield per plant (115 95) and very low for pod and seed protein percentage Genetic advance for all the leaf characters were also low indicating lower genetic gain for the character concerned in the next cycle of selection under fixed selection intensity

The genotypic coefficient of variation along with heritability estimates would give a clear picture regarding the extent of genetic advance for further selection (Burton,1952) The G C V helps to measure the range of genetic variability in the character and provides a measure to compare the genetic variability present in various characters, however, with the help of G C V alone heritable variation can not be measured (Singh *et al*, 1974) Highly heritable characters are particularly important for plant breeder as they enable him to base his selections reliably on the phenotypic performance (Swarup and Chaugala, 1962 , Sharma *et al*, 1966) In the present study, all the characters including protein content of pods as well as seeds showed high heritability indicating their reliability as selection index Observing very high heritability estimates for seed protein content of cowpea,Imam (1979) suggested that protein content might served as a selection index in any breeding programme This proposition was put forward by several workers in other beans like pea, french bean, faba bean and soyabean (Eden, 1968 , Bond and Toynbee-Clarke, 1968 , Meiners and Litzenbergers, 1975 , Griffiths and Lawes, 1978)

Johnson *et al* (1955) however suggested that heritability estimates in combination with genetic advance was more reliable for predicting the effect of selection Although, according to Hanson (1961), heritability and genetic advance were complimentory aspects Association of high G C V , high heritability and high genetic advance is attributed to the conditioning of additive gene action for the concerned characters (Panse,1957) According to this proposition the pod characters namely pod length, fresh and dry weight of pod and pod yield might have been under the control of loci with additive gene action So these characters are most reliable for phenotypic selection On the other hand, association of high heritability and low genetic advance is attributable to the conditioning of non-additive action for the concerned characters So, the leaf characters mainly leaf fresh wt, leaf dry weight, chlorophyll content, protein content of pods in all the stages of growth and protein content of seeds, might have been under the control of non-additive gene action Hence, they can be regarded as less reliable for direct selection on the basis of phenotypic performence Predominance of non-additive gene affects in the genetic variation for seed protein content of cowpea have been reported earlier (Mak and Yap,1980) Such nature of inheritance for seed protein have also been observed in French bean (Singh and Saini, 1988) In our study the range of seed protein content was quite wide, 19 27% in HG 22 (Unguiculata) to 26 57% in Cherodi (Biflora) These variability can be realised to a greter extent with the assemblage of more number of diverse genotypes under study For exemple, from a study employing 840 pure line faba beans (*Vicia faba*), El-Sherbeeny *et al* (1992) recorded genetic variation for protein content ranging from 180 to 311 gm/kg with a standard deviation of 18 gm/kg Although the reliability of protein contents (both pod and seed, seeds in particulars) has not been realised fully in the present investigation it can not be ignored altogther particularly with the view of very high heritability for protein content So, considerable variation in protein content in favourable direction may be achieved by selection if suitable and diverse gene pool is created In this study, however, pod characters namely pod length, pod fresh weight and dry weight and pod yield per plant emerged as reliable components for selection due to their probable control under the loci having additiive gene action

4.3 Character associationship

Association analyses of different characters were investigated through the study of genotypic and phenotypic correlations and partitioning of direct and indirect effects employing phenotypic correlations In all the analyses for different character constellation, all the genotypes as a population irrespective of cultigroups were considered In the present correlation study it was not possible to draw conclusions on pleiotrophy, linkage and developmental balance because

Table-18 : Phenotypic (P) and Genotypic (G) correlation co-efficient for pod and seed characters at 10 days of pod

Characters	Pod length (cm)	Fresh pod wt(g)	Dry pod wt(g)	Seeds per pod	Protein content (%)
Pod length(cm)	1	.810	724	795	-.124
Fresh pod wt (g)	.738**	1	792	833	.180
			G		
Dry pod wt.(g)	.665**	.758**	1	.701	.042
		P			
Seeds per pod	697**	.783**	.657*	1	.291
Protein content(%)	-.097	.176	.038	.267	1

* = Significant at 5% level
** = Significant at 1% level

Table-19 :Phenotypic (P) and Genotypic (G) correlation co-efficient for pod and seed characters at 15 days of pod

Characters	Pod length (cm)	Fresh pod wt(g)	Dry pod wt(g)	Seeds per pod	Protein content (%)
Pod length(cm)	1	.929	.876	.887	.020
Fresh pod wt. (g)	.921**	1	.795	.825	.177
			G		
Dry pod wt. (g)	.860**	.785**	1	.814	-0.37
		P			
Seeds per pod	.823**	.767**	.759**	1	.211
Protein content(%)	.019	.178	-.035	196	1

* = Significant at 5% level
** = Significant at 1% level

Table-20 :Phenotypic (P) and Genotypic (G) correlation co-efficient for pod and seed characters at 20 days of pod

Characters	Pod length (cm)	Fresh pod wt(g)	Dry pod wt. (g)	Seeds per pod	Protein content(%)
Pod length(cm)	1	.930	.891	.863	-.023
Fresh pod wt. (g)	.922**	1	.810 G	.835	.156
Dry pod wt.(g)	.870**	.791**	1 P	.845	-.068
Seeds per pod	.799**	.770**	.772**	1	.119
Protein content(%)	-.018	.163	-.052	.130	1

* = Significant at 5% level
** = Significant at 1% level

Table - 21 : Path coefficient analysis for pod and seed characters after 10 days of pod setting

	Direct and indirect effect via				
Characters	Pod length (cm)	Fresh pod wt.(g)	Dr~~t~~y pod wt.(g)	Seeds per pod	Phenotypic correlation with protein content
Pod length (cm)	**-.60498**	.231046	- 10342	.380372	-.097
Fresh pod wt (g)	-.44647	**.313071**	-.11789	.42730	.176
Dry pod wt (g)	-.40231	.237307	**-.15553**	.358543	.038
Seeds per pod	-.42167	.24514	-.10218	**.545728**	.267

Residual effect = 0.8639
Bold figure denotes direct effect

seperation of these factors required study of variance and covariance among the individuals with the generations

4.3.1 Character associationship of pod and seed characters of three stages of pod.

In all the three stages of pod growth, pod length,fresh pod weight, dry pod weight and seeds per pod registered significantly positive correlation among themselves (Table 18,19 and 20) Such positive interrelationship between pod length and weight ha~~ve~~s also been reported by earlier workers (Sharma *et al* 1988, Hazra, 1991, Kar *et al*, 1995 and Chattopadhyay, 1995) It is to be mentioned that pod length and weight have already been exhibited as important component of vegetable cowpea by several workers (Jana *et al., 1983* , *Sharma* et al ,1988 , Hazra, 1991) In all the growth stages no significant correlations have been registered between pod and seed characters and protein contents of the pods The magnitude of such correlations were also low So, from the study of correlations no pod and seed characters could be identified that influenced pod protein content in a convincing manner These correlations have further been analysed to find out the direct and indirect effects of the pod and seed characters on protein content of the pods of three different stages (Table 21, 22, 23) In all the three path analysis for three different stages, the residual effects were found to be very high indicating that the pod, and seed characters under study were not sufficient to justify their implications on protein content of the pods However, in all the three growth stages,maximum positive direct effects on protein content of pods were registered by fresh pod weight followed

Table-22: Path co-efficient analysis for pod and seed character after 15 days of pod setting

	Direct and indirect effect via				
Character	Pod length (cm)	Fresh pod wt (g)	Dry pod wt (g)	Seed per pod	Phenotypic corelation with
protein content					
Pod length (cm)	**-1.14181**	0.930426	-.24337	.473768	.019
Fresh pod wt(g)	-1.05160	**1.01023**	-.22214	.441531	.178
Dry pod wt(g)	-0.98195	.793034	**-.28299**	.436925	-.035
Seed per pod	-0.93970	.774850	-.21478	**.7660**	.196

Residual effect = 0.8596
Bold figure denotes direct effect

Table - 23 : Path co-efficient analysis for pod and seed characters after 20 days of pod setting

	Direct and indirect effect via				
Characters	Pod length (cm)	Fresh pod wt.(g)	Drt pod wt.(g)	Seeds per pod	Phenotypic correlation with protein content
Pod length (cm)	**-1.13128**	1.020445	-.17979	.272642	-.018
Fresh pod wt (g)	-1.04304	**1.106774**	-.16346	.262747	.163
Dry pod wt (g)	- .98421	.875458	**-.20666**	.263429	-.052
Seeds per pod	-.90389	.852215	-.15954	**.341230**	.130

Residual effect = 0.8626
Bold figure denotes direct effect.

by seed number per pod The amino acids after their synthesis in different sites (leaves, roots, petioles,etc) are translocated mainly to the seeds for conversion in the protein form In the beans,seeds have been reported to be the site of extremely rapid protein accumulation (Hall, 1968, Boulter, 1965) So, increase in no of seeds per pod must have got a direct implication on the protein percentage in the immature pods as a whole In this study, fresh pod weight also registered positive direct effect on protein content of the pods but this effect was exerted mainly via high positive indirect effect through seed number per pod So, seed number per pod can be considered as the main influencing character for protein content of the pods, obviously within the limitation of the present investigation

4.3.2 Leaf characters, protein content of pods and seed and pod yield.

Leaf fresh weight registered significantly positive correlation with leaf dry weight and chlorophyll content (Table 24) No leaf characters like leaf fresh weight, dry weight and chlorophyll content registered any significant correlation with pod protein content (three stages), seed protein content and pod yield per plant Their correlations with protein content of pods and seeds were very low in magnitude Protein synthesis in green plant is frequently and closely associated with photosynthesis (Bassham *et al.*, 1964 , Hall and Cocking, 1966 , Heber, 1962 , Ongun and Stocking, 1965) and the chlorophyll content of the tissue was of interest in relation to protein metabolism (Hall,1968) In our study on correlations, direct bearing of the photosynthetic parameters of leaf on the protein content of pods and seeds could not be established because such relationships had not be drawn through the ontogeny of the genotypes

Table 24 : Phenotypic (P) and Genotypic (G) correlation of co-efficient for leaf characters, pod Yield and Protein content

Characters	Leaf fresh wt (g)	Leaf dry wt (g)	chlorophyll content(mg/g)	Pod yield(g)	Pod protein 10 days(%)	Pod protein 15 days(%)	Pod protein 20 days(%)	Seed protein(%)
Leaf fresh wt (g)	1	.807	.535	.023	-.159	-.212	-.219	-.258
Leaf dry wt(g)	.787**	1	.369	.076	-.172	-.173	-.053	-.147
Chlorophyll content (%)	.469 *	.302	1	.093	-.082	-.173	-.207	-.469
Pod yield (g)	.010	-.090	.083	1	G .156	.177	.092	-.382
Pod protein 10 days (%)	-.151	-.178	-.073	P .152	1	1.0	.915	.984
Pod protein 15 days (%)	-.190	-.131	-.143	.159	.908**		.984	.889
Pod protein 20 days(%)	-.20	-.046	-.194	.090	.896*	916**	1	.924
Seed protein(%)	-.243	-.139	-.433*	-.378	.119	.084	.137	1

* = Significant at 5%
** = Significant at 1%

Pod protein contents of 10 days, 15 days and 20 days stage registered very high correlations among themselves meaning pod protein contents of any stage under study could imply the protein contents of the pods in other stages

Interestingly, pod protein content of any stage did not register any significant correlations with the seed protein content However, the correlations were positive in nature, although very low in magnitude Seed protein content i e protein content of the ultimate sink depends both on protein synthesis in different sites as well as their efficient translocation to the seeds In the later stages of ontogeny of french bean, Hall (1968) suggested that translocation of protein precursors as amino acids via stem was more likely to be of importance in the seed protein development In cowpea, mobilised nitrogen is donated by leaves, nodulated roots, stem, petioles and peduncles towards the nitrogen requirement for the development of fruits and seeds (Eagleshaman *et al.*, 1977, Neeves *et al* , 1981, Peoples *et al* ,1983) So, active translocation of the nitrogen which depends on the efficiency of the genotypes for the same actually signifies the protein content of the seeds

In our study, pod yield did not register any significant correlation with the protein content of the pods at three stages and the seeds It's correlations with the pod protein contents of three stages was very low but positive in nature Such was the associationship between pod yield per plant and seed protein contents but the correlation was negative in nature Significant negative correlation between seed protein content and yield (both seed and pod) have been reported widely in cowpea (Bliss *et al,* 1973 , Chauhan and Joshi, 1980 , Mak and Yap, 1980 , Kar *et al*, 1995, Hazra, *et al*, 1996) Such negative associationship between yield and seed protein content have been reported in other beans like French bean (Rutger, 1971 , Adams, 1967 , Radkov and Mitranov 1983), soybean (Hartwig, 1969) and Mungbean (Yohe *et al*, 1971) But often such negative correlations have been reported to be relatively small and insignificant in the beans like, French bean, soybean and Faba beans (Lelegi *et al*, 1972 , Kelly and Bliss, 1975 , Mutschler and Bliss, 1981 , Evans and Griddley, 1979 , Griffiths and Lawes, 1978 , El-sherbeeny *et al*, 1992 and Planchon *et al*, 1992) Our findings supported with these reports implied that selection for protein content should not be detrimental to yield, may it be seed or pod yield because seed yield and pod yield is highly correlated traits However, if the basis of this correlation is known, successful strategies can be used to prevent reduction in one traits as another is improved (Bliss and Brown, 1983)

Study of path coefficients

Path coefficient analyses revealed interesting picture Pod protein percentage of 10 days exhibited highest positive direct effect on seed protein percentage (0 521) followed by leaf dry weight (0 124) If we analyse the direct effect of pod protein of 10 days it appeared that this positive direct effect have been exerted mainly via positive indirect effect through chlorophyll content of leaf Indirect effects of chlorophyll content of leaf via pod protein of 15 days and 20 days were also positive (Table 25) which established the implication of photosynthetic parameters on protein content of fruit and seed particularly in

Table- 25: Path coefficient analysis for leaf charecters, pod yield and protein content

	Direct and indirect effect via							
Characters	Leaf fresh wt. (g)	Leaf dry wt (g)	Chlorophyll content(mg/g)	Pod yield(g)	Pod protein 10 days(%)	Pod protein 15 days(%)	Pod protein 20 days(%)	Phenotypic corre-lation with seed protein
Leaf fresh wt (g)	**-.15164**	.097702	-.18835	-.00358	-.07879	.042102	.039576	-.243
Leaf dry wt(g)	-.11934	**.124145**	-.12128	.032242	-.09288	.029028	.009102	-.139
Chlorophyll content (mg/g)	-.07111	.037491	**-.40162**	-.02973	-.03809	.031687	.038388	-.433
Pod yield (g)	-.00151	-.0117	-.03333	**-.35825**	.079321	-.03523	-.01780	-.378
Pod protein 10 days (%)	.022897	-.02209	.029318	-.05445	**.52185**	-.20120	-.17730	.119
Pod protein 15 days (%)	.028811	-.01626	.057431	-.05696	.47383	**-.22159**	-.18125	.084
Pod protein 20 days(%)	.030328	-.00571	.077914	-.03224	.46757	-.20297	**-.19788**	.137

Residual effect = 0.8091
Bold figure denotes direct effect.

the early stages Aassociationship between protein synthesis and photosynthesis ha~~ve~~s been put forward earlier (Bassham *et al*, 1964 , Hall and Cocking, 1966 , Heber, 1962 , Ongun and Stocking, 1965 and Hall, 1968) The other character revealing positive direct effect on protein content of seed was also a leaf character that influence photosynthesis Several leaf characters including leaf dry weight are known to infleuence different physiological functions of plant either directly or indirectly and thereby exercise control on different growth and production output In our study, indirect effect of leaf dry weight via pod yield was positive In an earlier study on the influence of leaf characters on pod yield of cowpea, Hazra *et al.* (1992) regarded leaf dry weight as a pod yield component From our study of associationship it can be suggested that during screening of the genotypes for seed protein content in a selection programme the leaf characters namely leaf dry weight and chlorophyll content of the leaf and protein content of 10 days pod should be taken into consideration simultaneously for realisation of considerable variation in positive direction in the cycles of selection

4.4 Influence of root and nodule characters on protein content of pods and seeds.

A feature unique to the legumes is the symbiotic relationship that exist between them and certain bacteria that are capable of fixing atmospheric nitrogen This biological nitrogen fixation is mostly provided by the symbiotic association between the roots of the legumes and the *Rhizobium* bacteria and the symbiosis is easily recognised by the apprearence of root nodules which harbour the nitrogen fixing microsymbionts Cowpea,the important protein source of many developing countries is one of the best plant species for plant breeding attention in the area of biological nitrogen fixation because such symbiotic association in many legumes is known to influence yield and protein content Important component of the symbiotic nitrogen fixation process that can be realised quantitatively are nodule number and weight (nodulation) and nitrogenase activity of the nodule (nodule function) In this investigation, variability of root and nodule characters of 21 genotypes of cowpea and there influence on protein content of pods and seeds have been studied to have an understanding on these biological nitrogen fixation parameters in the light of improvement of protein content of pods and seeds This study was carried out with indegenous microbial symbiont (Appendix -V)

4.4.1 Mean difference among cultigroups

Root characters: The cultigroup Sesquipedalis exhibited significantly higher root length (18 93cm) as compared to both cv-gr Biflora and cv-gr Unguiculata However, root length did not vary significantly between cv-gr unguiculata and cv-gr Biflora Highest root fresh weight and dry weight have also been exhibited in the cultigroup Sesquipedalis followed by in cv-gr Unguiculata and cv-gr Biflora Mean difference for root fresh weight between cv-gr Sesquipedalis and cv-gr Unguiculata and for root dry weight between cv-gr Unquiculata and cv-gr Biflora were not significant

Nodule characters: Nodule number was reported to be the highest in the flowering stage of cowpea (Anthony Raj *et al*, 1989 , Mandal 1995 , Chattopadhyay, 1995) In our study nodule number and weight per plant have been taken in the flowering stage of the plant Parameters of nodule function like leghaemoglobin content and nitrogenase activity have also beeen estimated from the nodules of this stage of the plant.

Average nodule number was highest in cultigroup Biflora (31 34) followed by in cv-gr Unguiculata and cv-gr Sesquipedalis respectively. Mean difference for this character among cultigroups was also significant Nodule number in cv-gr Biflora ranged from 22 46 to 34 94, in cv-gr Unguiculata from 21 43 to 37 97 and in cv-gr Sesquipedalis from 20 15 to 25 37 The highest number of nodules were exhibited ~~in~~ by the genotype HG22 of cultigroup Unguiculata This result indicated positive genotype x inherent rhizobium interaction which resulted varied incitation of the symbiosis Many factors such as, genotype of the host plant, quality and quantity of available rhizobium and numerous environmental factors influence nodulation and thereby biological nitrogen fixation in cowpea (Doku, 1970 , Miller Jr. and Fernandez, 1985) Nodule weight per plant also varied significantly among the cultigroups It was highest in cv-gr Unguiculata followed by that in cv-gr Biflora and cv-gr Sesquipedalis Very high nodule weight of 1 707 gm in the genotype 1-101 and 1 62 gm in HG22 (both of cultigroup Unguiculata) was observed in our study which acceded to the previous findings of Chattopadhyay (1995) on same genotype Significant variation in nodule number and weight among the cultigroups in the present and earlier studies (Mandal, 1995 , Chattopadhyay, 1995) indicated that cowpea genotypes reacted differentially during the infection of indegenous rhizobium strain of the soil

Nitrogen fixation is essentially an anaerobic process and the nodules must have a mechanism to exclude oxygen from the bacteroid which is the site of nitrogen fixation Low oxygen is necessary for induction of nitrogenase and the maintainance of high flux of oxygen at low concentration is needed for efficient nodule operation This is accomplished by leghaemoglobin around bacteroids enclosed by membranous envelopes of host origin Leghaemoglobin content was highest in Biflora (840 mg/g) which was significantly higher than that of cv-gr Unguiculata and cv-gr Sesquipedalis Leghaemoglobin content between cv-gr Sesquipedalis and cv-gr Unguiculata did not vary significantly Highest leghaemoglobin content of 0 920 mg/gm was exhibited by the genotypes HG22 of Unguiculata and SG2 of Biflora

Nitrogenase, the enzyme responsible for reducing Nitrogen into to NH_3 and its specific reducing agent nitrogenase reductase are rather uniform both physically and functionally when compared among diversed organisms Thus nitrogenase activity measured by acetylene reduction method would help to asses the efficiency of fixing atmospheric N_2 and their reduction to ammonia by the nodule Nitrogenase activity was significantly higher in cv-gr Biflora (12 308 µm C_2H_2/ plant/hr) as compared to cv-gr Unguiculata (6 847 µm C_2H_2/pl/hr) and cv-gr Sesquipedalis (3 45 µm C_2H_2/pl/hr) This result indicated the involvement of the host plant in determining the extent of nodulation and the potential nitrogen fixation (Graham, 1982)

The mean difference among the cultigroups for pod and seed protein percentage have already been discussed in earlier chapters In this study total protein content in 100 grains have been calculated taken into consideration the seed weight as per Singh *et al.*(1988) Comparison on the basis of protein content (percentage) in legumes sometimes may give misleading pictures because the genotypes with high protein content might have lower grain weight as reported in french bean, faba bean and pea (Lafiandra *et al* , 1981 , Arthur *et al.*, 1991) This indicates that high protein content is not a result of increase in absolute protein content but because of reduction of seed weight So, total amount of protein in 100 seeds may be used for variability study

The seed protein percentage was highest in Biflora followed by cv-gr Sesquipedalis and cv-gr Unguiculata But total protein content in 100 grains was markedly high in cv-gr Sesquipedalis due to more seed weight and it fell low sharply in cv-gr Biflora due to less seed weight in the genotypes of this cultigroup The characterization study of Faris (1965), Ng and Marechal (1985) and Hazra *et al.* (1993) indicated the cultigroup Sesquipedalis as heaviest seeded type and seed weight was generally lowest in the cultigroup Biflora

4.4.2 Differences in genetic parameters

Analysis of variance for root and nodule characters and protein content over the genotypes irrespective of the cultigroups indicated significant differences among the genotypes for these characters The genetic parameters

Table-26 : Analysis of variance for root, nodule and protein content in the genotypes of three cultigroups

Source	d.f	Root length (cm)	Root fresh wt(g)	Root dry wt(g)	Nodule no./ plant	Nodule wt(g)	Leghaemo-globin content (mg/g)	Nitrogenase activity mole C_2H_2 red/pl./hr.	Pod protien 20 days (%)	Seed Protein (%)	Protein in 100 seeds (g)
Replication	2	2.63	0.03	0.009	40.80	0.042	0.002	.025	.000	.007	.010
Genotype	20	10.68	2.76	0.355	48.83	0.144	0.018	97.734	.269	11.612	.818
Error	40	0.60	0.12	0.016	5.54	0.014	0.001	0.314	.011	0.040	.011
S.E.		0.44	0.02	0.073	1.36	0.069	0.020	0.323	.061	0.116	.059
C.D.(at 5%)		1.28	0.57	0.021	3.88	0.198	0.058	0.924	.174	.331	.170
C.D.(at 1%)		1.71	0.76	0.281	5.20	0.265	0.078	1.237	.233	.443	.228
C.V.		4.17	6.50	11.20	8.62	9.99	4.39	7.44	2.73	0.95	4.67
Pooled											
Replication	2	0.376	0.005	.001	5.829	.000	.000	.004	.000	.001	.001
Genotype	2	0.190	0.719	.069	53.105	.065	.003	59.873	.026	7.029	.843
Error	4	0.024	0.007	.002	0.535	.000	.000	.028	.001	.008	.001
S.E.		0.089	0.049	.026	0.422	.010	.006	.096	.021	.050	.022
C.D.(at 5%)		0.351	0.193	.103	1.658	.040	.020	.378	.084	.455	.087
C.D.(at 1%)		0.583	0.320	.171	2.750	.067	.040	.627	.139	.328	.144
C.V.		0.83	1.60	4.02	2.68	1.49	1.32	2.21	0.96	0.41	1.73

S.E. = Standard Error
C.D. = Critical difference
C.V. = Co-efficient of variation.

for this characters have been computed both irrespective of cultigroups and over mean of the cultigroups (Table 26) In both the analyses P C V and G C V agreed closely which indicated that the characters expressed were basically ascribed to the genotypes The details of the implication of the genetic parameters for this characters is presented following the analysis irrespective of the cultigroups (Table 27) The root characters showed low to medium G C V indicating somewhat narrow extent of genetic variability Of these root characters, the highest G C V was shown by root dry weight (29 56%) and the lowest of 9 83% by root length Of the four nodule characters, nodule number and weight per plant exhibited moderate G C V of 20 41% and 17 32%, respectively The lowest G C V of 9 26% was shown by the leghaemoglobin content/gm nodule, Singh (1989), however, observed wide variation in the morphology, size of nodule and leghaemoglobin content of the nodule On the other hand, very high G C V and P C V was recorded for nitrogenase activity of the nodules, the prime character for nodule function Similar wide variability for nitrogenase activity of the nodules was recorded earlier in cowpea (Zary *et al*, 1978 and Mandal, 1995) We have already examined the revealation of very low G C V for pod and seed protein content in terms of percentage But in this analysis protein or seeds? content in 100 grams have been employed in addition to protein content in percentage to study the variability Interestingly, protein content in 100 seeds revealed satisfactorily high G C V of 23 41% indicating a good extent of variability in terms of total protein content in an unit seed number Heritability estimates in broad sense for all the root and nodule characters and protein content of pods and seeds were very high indicating their high transmissibility to the

Table-27 : Estimation of genetic parameters for root, nodule and protein content

Characters	Mean	h^2	G C.V	P.C.V.	G.A.	G.A (%)
Root length (cm)	18.64	84.74	9.83	10.68	3.47	18.64
Fresh root wt (g)	5.33	88.04	17.58	18.74	1.81	33.98
Dry root wt (g)	1.13	87.59	29.56	31.58	0.64	56.99
Nodule number per plant	27.31	84.85	20.41	22.16	10.58	38.73
Nodule wt per plant(g)	1.20	75.56	17.32	19.93	0.37	31.01
Leghaemoglobin content(mg/g)	0.80	84.84	9.26	10.05	0.14	17.56
Nitrogenase activity(mole C_2H_2 red/pl./hr.)	7.53	99.04	75.67	76.04	11.68	155.11
Pod protein (20 days)(%)	3.87	88.65	7.57	8.13	0.56	14.68
Seed protein content(%)	21.26	98.97	9.23	9.28	4.02	18.92
Protein in 100 seeds(g)	2.21	96.07	23.41	23.88	1.04	47.26
Pooled						
Root length (cm)	18.64	69.73	1.26	1.51	0.40	2.16
Fresh root wt (g)	5.33	97.13	9.25	9.25	0.98	18.52
Dry root wt (g)	1.13	91.66	13.04	13.62	0.29	25.72
Nodule number/plant	27.31	97.03	15.32	15.55	8.49	31.09
Nodule wt per plant(g)	1.20	100.00	12.06	12.01	0.29	29.85
Leghaemoglobin content(mg/g)	0.80	100.00	3.91	3.91	0.06	8.05
Nitrogenase activity(mole C_2H_2 red/pl./hr.)	7.53	99.88	59.31	59.34	9.19	1.22
Pod protein (20 days)(%)	3.87	89.24	2.35	2.49	0.17	4.57
Seed protein content(%)	21.26	99.65	7.19	7.20	3.14	14.79
Protein in 100 seeds(g)	2.21	99.64	23.91	23.95	1.08	49.16

h^2 = Heritability in broad sense.
G.C.V. = Genetypic co-efficient of variation
P.C.V. = Phenotypic co-efficient variation
G.A = Genetic advance
G.A(%) = Genetic advance as percentage of mean.

progenies However, the maximum heritability of 99 04% was registered by nitrogenous activity of the nodules This finding indicated that progress should be significant in breeding for enhanced biological nitrogen fixation in cowpea by improving the important components of biological nitrogen fixation like nodule number and weight per plant, leghaemoglobin content and nitrogenous activity of the nodules This findings acceded to the earlier proposition of Millar Jr *et al* (1986) The genetic advance expected from selecting the top 5% of the genotypes expressed as percentage of mean ranged from 18 64 to 56 99 percent for root length, root fresh weight and root dry weight , from 17 56 to 155 11% for nodule number per plant, nodule weight per plant, leghaemoglobin content and nitrogenase activity of the nodules We have already seen the revealation of low genetic advance for seed and pod protein content in terms of percentage but moderate genetic advance of 47 26% was revealed with respect to protein content in 100 seeds Association of high G C V, high heritability and high genetic advance is attributed to the conditioning of the additive gene action for the concerned character (Panse, 1957) Such combination of genetic parameters is attributable to nitrogenase activity of the nodules in our investigation which might have been under the control of loci with additive gene action Genetic advance for nodule number and nodule weight per plant in our investigation were below moderate though heritability estimates for these characters were very high This combination according to Liang and Walter (1968) may be attributable to non-additive gene action This proposition of conditioning of non-additive gene action for nodule number and weight did not find support to the earlier reports of Dayap and Rasco Jr (1988) and Mandal (1995) From this study, three different characters namely root dry weight, nitrogenase activity seeds ? and protein content in 100 gms emerged as reliable indices for phenotypic selection among diverse genotypes

4.4.3 Character associationship

Study on genotypic and phenotypic correlation

The study of correlationship will help us to know the suitability of various characters for direct selection because selection of one or more traits results in correlated responses in several other traits (Searle, 1965) In our study, the phenotypic and genotypic correlation among the root and nodule characters and protein content of pods and seeds have been computed jointly irrespective of the cultigroups (Table 28) The significance of the correlation coefficiants for the character combinations have been restricted to phenotypic level only Root length did not register any significant correlation with other root and nodule characters and pod and seed protein content It's correlation with root dry weight was positve but not significant. Pod protein and seed protein percentage revealed very low negative correlations with both root length and weight (fresh and dry) Correlations of protein content in 100 grains with root length and weight (fresh and dry) were though positive yet very low in magnitude It emerged from the correlation study that no root characters had significant bearing on the nitrogen fixation parameter and protein content of pods and seeds Chattopadhyay (1995) also recorded positive but insignificant correlation between root length and weight Significant and positive correlation was recorded between nodule number and weight which was agreeable to the findings of Thompson and Dennis (1976)

Table - 28 : Phenotypic (P) and Genotypic(G) correlation co-efficient for root, nodule and protein content

Character	Root length (cm)	Fresh root wt. (g)	Dry root wt. (g)	Nodule number per plant	Nodule wt./ plant (g)	Leghaemo- globin content (mg/g)	Nitroge- nase activity (mole C_2H_2 red/pl./hr.)	Pod protein 20 days (%)	Seed protein (%)	Protein in 100 seeds (g)
Root length (cm)	1	0.111	0.123	-0.153	-0.072	-0.358	-0.135	-0.082	-0.103	0.059
Fresh root wt (g)	0.129	1	0.419	-0.008	0.248	0.187	-0.022	-0.261	-0.442	0.350
Dry root wt.(g)	-0.082	0.425	1	0.059	0.174	0.150	-0.123	0.100	-0.343	0.332
Nodule no./pl.	-0.129	0.003	0.067	1	0.705	0.796	0.889	-0.189	0.112	-0.681
Nodule wt./pl. (g)	-0.051	0.225	0.140	0.598**	1	0.430	0.655	-0.100	-0.184	-0.480
Leghaemaglobin content (mg/g)	-0.305	0.168	0.163	0.669**	0.293	1	0.734	0.032	0.044	-0.492
								G		
Nitrogenase activity(mole C_2H_2 red/pl./hr.)	-0.124	-0.017	-0.118	0.798**	0.575**	0.648**	1	-0.035	0.166	-0.759
							P			
Pod protein 20 days (%)	-0.104	-0.217	0.052	-0.145	-0.075	0.033	-0.030	1	0.151	0.129
Seed protein(%)	-0.078	-0.405	-0.320	0.097	-0.159	0.045	0.164	0.139	1	-0.216
Protein in 100 seeds (g)	0.070	0.341	0.315	-0.611	-0.385	-0.427	-0.738	0.116	-0.216	1

* = Significant at 5% level.
** = Significant at 1% level.

, Pandey *et al* (1987) and Sinclair *et al* (1991) and contrary to that of Chattopadhyay (1995) In the biological nitrogen fixation, the nodulation stage is realised with nodule number and weight which are always expected to be highly correlated Nodule number also registered significantly positive correlations with leghaemoglobin content per gram nodule and nitrogenase activity of the nodule Positive correlation between nodule number and leghaemoglobin content is entirely expected because leghaemoglobin, the pigment are present around bacteroids enclosed by membranous envelopes of host origin Significant positive correlation between nodule number and weight and nitrogenase activity of the nodule corroborated to the findings of Posypanov *et al* (1991) in soybean Nodule weight per plant and leghaemoglobin content also registered signifgicant correlation with nitrogenase activity Several workers reported that the amount of leghaemoglobin and the extent of bacteroid tissues in the nodules have a direct bearing on the amount of nitrogen fixed by the legumes Bergersen and Briggs (1968), Chopra and Subba Rao (1967), Verma and Bal (1976), opined that the exact relationship between hydrogen evoluation by nitrogenase and N_2 reduction is not clear but two reactions coupled closely From the correlation study it clearly appeared that nodule function as determined quantitatively by leghaemoglobin content and nitrogenase activity was amply influenced by nodulation i e nodule number and weight So in breeding for improved symbiotic activity the plant should have high nodule number and weight which would give increased nitrogense activity in the nodule concomitantly The correlation between nodule number per plant and protein content of the pods (20 days) was negative but very low in magnitude It had no correlation with protein % in seed but its correlation with protein content in 100 grains was significantly negative Such was the association between protein content in pod and seed and nodule weight per plant Similar was the findings of Chattopadhyay (1995) Nitrogenase activity and leghaemoglobin content registered in significant and very low negative corrrelation with percent protein in the both pods (20 days) and mature seeds but correlation between nitrogenase activity and protein content in 100 grains was significantly negative Protein content in 100 seeds depend on protein % and seed weight Seed weight in turn is an important yield component Planchon *et al.* (1992) recorded that seed protein content was less affected by nitrogenase activity Suganuma *et al.* (1993) reported in pea that during early stages of nodule development, the protein composition and activity of enzymes involved in carbon and nitrogen metabolism are not regulated by the presence or absence of nitrogenase activity In cowpea, nitrogen fixed before flowering contributes approximately 60% of the fruits' total requirement of nitrogen

and within this amount, leaves, nodulated roots, stems plus petioles and peduncles donate mobilised nitrogen in the relative proportion of 5 2 1 1, respectively The blossom leaves loss 72-77% of the nitrogen before abscission where as lower leaves at non-reproductive nodes have mobilised only 44-57% of the nitrogen by fruit maturity and in most cases, have not abscissed The remaining 40% of the fruits nitrogen budget is met by nitrogen fixation after flowering (Peoples *et al*, 1983) From other studies in cowpea Eaglesham *et al* (1977) and Neeves *et al.*, (1981) concluded that more than 75% of the fruits nitrogen budget is met by nitrogen fixation after flowering So, it is clear that nitrogen fixation before and after flowering is more important in unveiling intricate relationship between nitrogen fixation and protein content of pod and seed

Study of path coefficients

Path coefficient analysis helps to elucidate the direct and indirect contributions of the characters influencing end product, seed protein percentage in our study Phenotypic correlations of the root and nodule characters and protein content of pods and seeds were partitioned through path co- efficient analysis (Table-29) Highest positive direct effect on seed protein percentage ha~~ve~~s been shown by nitrogenase activity (0 267) followed by nodule number per plant (0 156), protein content in the pods of 20 days (0 091) and protein content in 100 seeds (0 084) Phenotypic correlation between nitrogenase activity of the nodules and seed protein percentage was positive but low in magnitude (r = 0 164) The high direct effect of nitrogenase activity on seed protein percentage happened through the positive indirect effects mainly via nodule number per plant Similarly, appreciably high positive direct ~~a~~effect of nodule number per plant on seed protein percentatge was revealed mainly through high positive indirect effect via nitrogenase activity In this manner, strong associationship between nodule number per plant and nitrogenase activity as emerged from phenotypic correlation (r = 0 798) has again been fortified in the partitioning study of correlation Pod protein content (20 days) registered insignificant positive correlation with seed protein percentage and its direct effect was also

Table-29 : Path coefficient analysis for root, nodule and protein content

Character	Root length (cm)	Fresh root wt(g)	Dry root wt(g)	Nodule number/ plant	Nodule wt./ plant	Leghaemo-globin content (mg/g)	Nitrogen-ase activity (mole C_2H_2 red/pl./hr)	Pod Protein 20 days (%)	Protein in 100 seeds (g)	Phenotypic correlation with seed Protein
Root length (cm)	**-.03352**	-.03368	.014328	-.02016	.013679	.018080	-.03319	-.00943	.005908	-.078
Fresh root wt(g)	-.00432	**-.26111**	-.07426	.000469	-.06035	-.00995	-.00455	-.01967	.028782	-.405
Dry root wt.(g)	.002748	-.11097	**-.17474**	.010474	-.03755	-.00966	-.03158	.004715	.026587	-.320
Nodule no. /pl.	.004324	-.00078	-.01170	**.15634**	-.16040	-.03965	-.213617	-.01314	-.05157	.097
Nodule wt./ pl.(g)	.001709	-.05874	-.02446	.093491	**-.26823**	-.01736	.153922	-.00680	-.03249	-.159
Leghaemaglobin content(mg/g)	.010223	-.04386	-.02848	.104591	-.07859	**-.05928**	.173463	.002992	-.03604	.045
Nitrogenase activity(mole C_2H_2 red/pl./hr)	.004156	.004438	.020619	.124759	-.15423	-.03841	**.267691**	-.00272	-.06229	.164
Pod protein 20 days (%)	.003486	.056660	-.00908	-.02266	.020117	-.00195	-.00803	**.090685**	.009791	.139
Protein in 100 seeds(g)	-.00234	-.08903	-.05504	-.09552	.103268	.025312	-.19755	.010519	**.084406**	-.216

* = Significant at 5%

** = Significant at 1%

Bold figure denotes direct effect

low in magnitude though positive Intrestingly, pod protein percentage and seed protein content in 100 grains revealed positive indirect effect via nodule weight per plant So, nodule number, nodule weight and nitrogenase activity emerged as main influencing characters for seed protein percentage in this study At the same time, considering the positive direct effect of pod protein content and protein content of 100 seed on the seed protein percentage, these two parmeters can also be considered at the time of breeding for seed protein content in cowpea Heritability in broad sense was found very high for all these characters discussed indicating that the progress should be significant in the next cycle of selection employing these characters Among these characters, nitrogenase activity of the nodules deserves special emphasis because of its probable conditioning by additive gene action

4.5 Gene action for pod protein content and other yield components

Systematic recombination within a set of parental genotypes normally provides considerable variability among the cross combinations Such variation provides estimates of the combining ability variances for the characters to be improved by breeding Genetic nature of the quantitative characters can be determined from the combining ability variances which will enable the breeder to choose the most appropriate breeding methods and selection procedures for the improvement of the crop Several biometrical models are available for the estimation of the gene action and we attempted a 4 x 5 Line x Tester model of Kempthorne (1957). Most of the Cowpea genotypes for vegetable purpose belong either to cultigroup Sesquipedalis or cultigroup Unguiculata Intermediate types between cv-gr Unguiculata and cv-gr Sesquipedalis are also used for vegetable purpose (Steele and Mehera, 1980) In our study, the four lines used namely, Sel-Tm1, Sel-Tm3, EC-305827, L-16 are of cv-gr Susquipedalis and the five Testers namely, 5269, HG22, 1-101, EC-243954, Pusa Dofasli of cv-gr Unguiculata Among the Testers, 5269 and Pusa Dofasli have been identified as outstanding parent for pod protein content from our earlier studies From this Line x Tester model nature of gene action for eight characters namely, ~~D~~days to flowering, pod length, fresh pod weight, dry pod weight, seeds per pod, ~~P~~pods per plant (pod yield compoents), pod yield per plant and protein content of pods ha~~ve~~s been studied (Appendix-VI)

Analysis of combining ability

The analysis of variance for genotypes i e parents (Lines and Testers) and cross combinations were significant for all the characters except in seeds per pod (Table 30) The components of gca and sca variances (Mean squares) were highly significant for all the characters excepting seeds per pod and pods per plant which indicated that significant differences existed among the parents and cross combinations regarding the gca and sca effects for days to flowering, pod length, fresh pod weight, dry pod weight, pod yield per plant and protein content of pods. The combining ability is determined mainly by two types of gene actions, additive and non-additive The additive effects are mainly due to polygenes which act in additive manners producing fixable effects The non-additive gene action results from dominance, epistasis and various other interaction effects which are not fixable The combining ability analysis measures these effects interms of general and specific combining ability The gca in general

Table-30 : Analysis of variance for parents (lines and testers) and cross combinations for different characters

Characters	MEAN SQUARES FOR Replication	 Genotypes(Parents/cross combinations)	 Error
1. Days to flowering	1.268	41.96**	0.514
2. Pod length (cm)	1.198	143.00**	1.483
3. Fresh pod weight (g)	0.063	50.456**	0.518
4. Dry pod weight (g)	3.391	40.004**	1.334
5. Seeds / pod	267.735	366.687	297.742
6. Pods / plant	1.450	167.445**	28.571
7. Pod yield / plant (g)	85.251	20385.449**	456.893
8. Protein content of pods(%)	45.425	1409. 411**	31.437

***,** Significant at 0.05 and 0.01 levels, respectively.**
Mean squares for replication, Genotype and Error are based on 2, 28 and 56 degree of freedom, respectively.

is a function of additive genetic effects, but may partialy include some dominance effects when gene frequencies are not equal to one half in the parents that are included in the analysis to estimate variances (Singh and Paroda, 1984) On the other hand sca is the function of non-additive genetic effects In our analyses following the model of Kempthorne(1957) gca variances were higher than sca variances for days to flowering, pod length, pods per plant and protein content of pods On the other hand, the reverse situation existed for fresh pod weight, seeds per pod and pod yield per plant The estimated components of genetic variance worked out from the variances of gca and sca demonstrated that additive genetic variance (σ^2a) ~~were~~ was greater than non-additive genetic varience in magnitude for days to flowering, pod length, fresh pod weight, seeds per pod, pods per plant and protein content of pods On the otherhand, non-additive genetic variances were higher in magnitude for dry pod weight and pod yield per plant The relative importance of additive and nonadditive genetic effects for these characters were further analysed in the view of predictability ratio as per Baker (1978) The predictability ratio was close to unity more than (0 8) only for pod length indicating the predominance of additive gene effects for this character (Table 31) The predictability ratio for pod length further fortified the determination of the genes contribution to the additive variation Earlier reports corobroate the present findings (Singh and Jain, 1972 , Lal *et al.*, 1976 , Mak and Yap, 1980 , Hazra *et al.*, 1994 and Chattopadhyay, 1995) For the characters, days to flower, pod weight, pods per plant and protein content of pods both additive and non-additive genetic effects were important in the control of these characters as the predictability ratios were less then 0 8 for these characters

Table-31 : **Analysis of variance for combining ability, estimates of genetic variance (additive and non-additive) and predictability ratio for different characters**

	Variance for			Estimates of genetic variance		
Characters	gca	sca	error	Additive	Non-additive	Predictability ratio
1. Days to flowering	6.742**	3.937**	0.171	13.484	3.937	0.77
2. Pod length (cm)	12.285**	4.819**	0.494	24.570	4.819	0.83
3. Fresh pod weight (g)	0.911**	1.134**	0.172	1.822	1.134	0.62
4. Dry pod weight (g)	1.304**	2.938**	0.444	2.609	2.938	0.47
5. Seeds / pod	30.049	44.206	99.247	60.099	44.206	0.57
6. Pods / plant	7.052	5.002	9.523	14.104	5.002	0.73
7. Pod yield / plant(g)	1378.616**	4683.006**	152.297	2757.233	4683.006	0.37
8. Protein content of pods(%)	2.603**	1.787**	0.1047	5.206	1.787	0.74

** = Significant at 0.01 level

Some workers (Mak and Yap, 1980 , Singh and Dabas, 1986 , Hazra *et al* , 1994) suggested non-additive genetic effects to be of major importance for pods per plant On the other hand, preponderance of additive genetic effects for pods per plant ~~have~~ has been reported by Aryeetey and Laing (1973) and Chattopadhyay (1995) In our study though gca and sca variances were not significant but the estimates of genetic variances indicated the importance of both additive and non-additive genetic variances for this trait, although, the estimate of additive genetic variance was higher than that of non-additive genetic variance Such disparaties in the genetic control of pods per plant might have arose from differences in the genetic constitution of parental materials studied, Variation in the environment, techniques used in analysing the data and the precision of the experiment

Predictability ratio was very low (0 37) for pod yield per plant and dry pod weight indicating the overwhelming importance of non-additive genetic variance for the control of these characters Although both gca and sca variances were significant for these characters In this respect, importance of both additive and non-additive genetic variance should be taken into consideration Earlier reports also suggested the importance of both additive and non-additive genetic variance for the control of pod yield per plant (Mak and Yap, 1980 , Singh and Dabas, 1986 , Chattopadhyay, 1995 , Hazra, *et al* , 1996) In our study predictability ratio was much lesser than 0 8 for fresh pod weight (0 62) which indicated the importance of both additive and non additive genetic variance for this character However, the estimates of additive genetic variances (σ^2a) was higher than that of non additive genetic variance Earlier report suggested the preponderance of additive genetic effects in the control of fresh pod weight (Hazra *et al*, 1994, Chattopadhyay, 1995)

For pod protein content predictability ratio was somewhat closer to 0 8 (0 74) indicating the importance of both additive and non-additive genetic variance for the control of this trait, although, the significance of additive genetic variance was more than the non-additive genetic variance in the genetic control of pod protein content Gene action for pod protein content has so far not been reported in cowpea However, reports on the genetic control of the seed protein content revealed disperities Reports of the predominance of additive gene effects for seed protein content (Hazra *et al.*, 1996) did not support the earlier report of Mak and Yap (1980) where non-additive gene action was reportedly predominant

General combining ability effects

Among the lines, the highest positive gca effects (gi) for days to flowering was shown by Sel-Tm3 followed by L-16 of cv-gr Sesquipedalis and among the testers the only parent showing positive gi was EC-243954 of cv-gr Unguiculata (Table 32) Among the parents highest positive gi for pod length was shown by EC-305827 of cv-gr Sesquipedalis, the line parent followed by 5269 and Pusa Dofasli of cv-gr Unguiculata, both tester parents The *per se* performence of EC-305827 was also the highest (42.83cm pod) With regard to pod weight EC-305827 of cv-gr Sesquipedalis registered significant positive gi and revealed highest pod weight of 21 60 gm. Although, highest positive gi was registered by EC-243954 of cv-gr Unguiculata, a tester parent in this respect No parent either of line or tester revealed significantly positive gi for seeds per pod For

Table-32: General combining ability effects of parents for different characters

	Characters							
Parents	Days to flowering	Pod length (cm)	Fresh Pod wt (g)	Dry pod wt (g)	Seeds/ pod	Pods/ plant	Pod yield plant (g)	Protein content of pods (%)
Lines								
Sel-Tm1	-1.028**	3.194 **	-0.198	1.928**	10.862	-1.164	-23.850**	.1215
Sel-Tm3	3.478**	-2.387 **	-0.112	-0.032	-3.324	4.563*	55.172*	-.0885**
EC-305827	-3.602**	4.839 **	0.795 **	-0.385	-2.391	-0.610	14.455*	-.2125**
L - 16	1.152**	0.741	-0.485	-1.512**	-5.147	-2.788	-45.766**	.1795**
Testers								
5269	-0.833**	2.694 **	0.160	1.008*	-2.906	1.847	35.731**	.0958**
HG 22	-2.167**	-5.286 **	-1.373 **	-0.308	-4.586	2.459	-16.696	0.150
1 - 101	-0.717*	-1.339 **	-1.065 **	-1.042*	-3.444	1.858	-7.012	-.1666**
EC-243954	2.967**	1.528 **	1.968 **	-0.075	-1.569	-3.265	18.209*	-.0966**
Pusa Dofasli	0.750*	2.403 **	0.310	0.417	12.506	-2.899	-30.232 **	.1528
S.E. (lines)	0.2618	0.4447	0.2629	0.4218	6.3007	1.9518	7.8051	.0204
S.E. (testers)	0.2927	0.4972	0.2940	0.4716	7.0444	2.1822	8.7263	.0228

S.E. = Standard Error
* = Significant at 5% level.
** = Significant at 1% level.

pods per plant only one parent (line), Sel-Tm3 of cv-gr Sesquipedalis registered significantly positive general combining ability effects However, significantly negative gi for this character was not revealed in any of the parent Three parents registered significantly positive gi for pod yield per plant Of these, the highest was shown by Sel-Tm3 of cv-gr Sesquipedalis (line) followed by 5269 and EC-243954 (Tester) of cv-gr Unguiculata The highest yielding genotype EC-305827 (yield 459 90 gm/plant) revealed positive but insignificant gi Ranking of the parents for gi and *per se* performance did not match altogether. In general, it may be suggested that the parents with high *per se* performance may record high gca effects but the situation may not be one to one in all the cases Similar observation was recorded in a number of grain legumes including cowpea (Hazra, 1991) , Pigeon pea (Mehera, 1982), Black gram (Dasgupta, 1983)

For pod protein content four parents, two each of cv-gr Sesquipedalis (Line) and cv-gr Unguiculata (tester) revealed positive gca effects The highest gi was shown by L-16 of cv-gr Sesquipedalis but it did not register the heighest protein content In fact, the parent (EC-243954) showing highest protein content in the pod (4 11%) registered significantly negative gi High protein content in the pod along with significantly positive gi was shown by 5269 of cv-gr Unguiculata, a tester parent Among the parents showing significantly positive gi for protein content in the pod, L-16 of cv-gr Sesquipedalis is the highest yielder followed by Sel-Tm1 of cv-gr Sesquipedalis, 5269 and Pusa Dofasli both of cv-gr Unguiculata

The two most outstanding genotypes found in combining ability analysis

With the consideration of gca effects and performance of the parents for pod yield, it's important components (pods per plant, pod length and pod weight) and pod protein content, the tester parent 5269 of cv-gr Unguiculata emerged as out standing because it revealed significantly positive gi for pod length, positive but insignificant gi for pod weight, positive but insignificant gi for pods per plants, significantly positive gi for pod yield per plant and pod protein content With the consideration of pod yield, The parent EC-305827 of cv-gr Sesquipedalis (line) proved to be outstanding revealing highest pod yield and positive gi for yield and yield components This parent (line) contained comparatively low protein content in the pods It also revealed significantly negative gi for this character If a compromise between yield and protein content is considered then Sel-Tm3 of cv-gr Sesquipedalis (line) emerged as promising because it revealed the highest positive gi for pods per plant and pod yield per plant Though, it showed significantly negative gi for pod protein content yet it's *per se* performance is quite high (3 95%)

Specific combining ability effects

The specific combining ability (sca) effects results mostly from dominance and interaction effects between the hybridizing parents The two parents, if carry different genes for the character in question, will tend to complement each other and produce hybrids of superior genetic constitution In self pollinated crop like cowpea the non-additive component of genetic variation is not fixable but the crosses with high sca effect involving good general combiner parent can be used in a future breeding programme

In the present analysis, sca effects (Sij) for the characters have been presented in the Table 33 Earliness is an important character for all the crops including cowpea In days to flowering significantly negative sca effects is of importance Five cross combinations, namely Sel-Tm1 x 5269, Sel-Tm 1 x Pusa Dofasli, Sel-Tm3 x Pusa Dofasli, EC-305827 x 1-101 and L-16 x EC- 243954 showed significantly negative sij for days to flowering Only three cross combinations namely Sel-Tm1 x 5269, L-16 X EC— 243954 and L-16 x Pusa Dofasli presented significantly positive sij for pod length These cross combinations produced medium long pods (33 26 - 33 83 cm) The cross combination namely, L-16 X Pusa Dofasli registered the highest significant sij for fresh pod weight and its *per se* performance was also quite high (14 03 gm) The cross combination having the heaviest pod of 14 47gm (EC-305827 X 5269) also showed significantly positive sca effect for pod weight No cross combination registered significant specific combining ability effect for seed number per pod and pod number per plant

Though significantly positive sij could not be realised in most of the cross combinations for the yield components, yet significantly positive sij was realised in six cross combina tions for pod yield per plant This might have happened due to mutual cancellation and balancing of the yield components towards the expression of yield Of the six cross combinations, the highest sij was registered by L-16 x Pusa Dofasli The others in the descending order in this respect were

Table-33 : Specific combining ability effects of the cross combinations for different characters

Cross Combinations	Days to flowering	Pod length (cm)	Fresh pod wt(g)	Dry pod wt(g)	Seeds per pod	Pods/plant (%)	Pod yield/ plant(g)	Protein content of pods(%)
Sel-Tm1x5269	-3.147**	4.619**	-1.060	3.672	-7.867	1.726	-15.598	.2068**
Sel-Tm1xHG22	0.520	-0.067	0.040	1.322	-8.021	-0.453	-14.840	-.0709
Sel-Tm1x1-101	2.003**	0.019	0.698	-0.812	-7.996	-1.851	2.859	.0106
Sel-Tm1xEC-243954	2.820**	-1.681	0.032	-1.945**	-8.171	-0.961	-10.319	.0393
Sel-Tm1xPusa-Dofasli	-2.197**	-2.890**	0.290	-2.237*	32.054	1.539	37.899**	-.1565**
Sel-Tm3x5269	1.680**	-2.254*	0.353	-0.935	3.319	4.966	71.210**	-.0498
Sel-Tm3xHG22	-0.587	1.726	0.620	0.115	2.699	0.787	26.027	.1110**
Sel-Tm3x1-101	0.363	0.746	1.045	0.015	2.558	2.755	61.810**	.0493
Sel-Tm3xEC-243954	-0.220	-0.321	0.045	0.282	3.249	-4.355	- 54.207**	.0126
Sel-Tm3xPusa-Dofasli	-1.237*	0.104	-2.063**	0.523	-11.826	4.155	-104.840**	-.1231**
EC-305827x5269	1.127	-1.148	1.313**	-1.248	3.619	1.306	60.550**	-.1958**
EC-305827xHG22	0.327	1.133	-0.153	0.635	1.933	-0.873	- 5.669	-.0350
EC-305827x 1-101	-2.490**	-0.848	-0.695	0.635	2.158	-1.504	-36.780**	-.0333
EC-305827x EC-243954	-1.073	-0.415	-0.228	0.035	2.083	1.286	11.599	-.0100
EC-305827x Pusa Dofasli	2.110**	1.277	-0.237	-0.057	-9.792	-0.215	-29.700	.2741**
L-16x5269	0.340	-1.216	-0.607	-1.488	0.929	-7.999	-116.162**	.0388
L-16xHG22	-0.260	-2.792**	-0.507	-2.072*	3.389	0.538	- 5.518	.0030
L-16x1-101	0.123	0.084	-1.048	0.162	3.280	0.600	- 27.889*	-.0053
L-16xEC-243954	-1.527*	2.417*	0.152	1.628	2.839	4.030	52.927**	-.0420
L-16xPusa-Dofasli	1.323*	1.508*	2.010**	1.770	-10.436	2.830	96.641**	.0055
S.E.(Sij)	0.5855	0.9943	0.5879	0.9432	14.0888	4.3644	17.4527	.0457

S.E. = Standard error
* = Significant at 5% level.
** = significant at 1% level.

Sel-Tm3 x 5269, Sel-Tm3 X 1-101, EC-305827 X 5269, L-16 X EC-243954 and L-16 x EC-243954 The *per se* performance and revealation of sij in the cross combinations for pod yield/plant did not match For this reason, the cross combination L-16 X Pusa Dofasli with 396 80 gm pod yield stood first but Sel-Tm3 X 5269 with the highest pod yield of 538 28 gm ranked second with regard to sij effect for pod yield So, both sca effects and *per se* performance must be considered in selecting the cross combinations for further breeding programme

With regard to pod protein content, only three cross combinations namely Sel-Tm1 X 5269, Sel-Tm3 X HG 22 and EC-305827 X Pusa Dofasli registered significant positive sca effects Of the parents of these crosses, the testers 5269 and Pusa Dofasli have been identified of having high pod protein content (more than 4%) from our earlier studies (chapter 4.1 2) From gca studies, 5269 of cv-gr Unguiculata (tester) emerged as out standing parent for protein content of pods However most of the cross combinations showed insignificant sij effect for protein content of the pods Only two cross combinations out of six revealing significantly positive sij for pod yield exhibited significantly negative sij for pod protein content The other four cross combinations exhibited insignificant sij for pod protein content This picture seemed to be encouraging in selec ting the gentoypes with high pod yield potential and appreciable pod protein content

In view of the specific combining ability effects of cross combinations, gca effects of the parents involved and the *per se* performance of the cross combinations, the following combinations emerged as promising

Cross combinations	Pod yield (g)	Protein content in pods (%)	Sij for pod yield/plant	Sij for protein content in pod
Sel-Tm3 X HG 22	440 67	3 81	P	P*
Sel-Tm1 X 5269	372 45	4 19	N	P*
Sel-Tm3 X 5269	538 28	3 73	P*	N

P = Positive but insignificant
P* = Positively significant
N = Negative but insignificant and low in magnitude

From the joint analysis of gca and sca effects and *per se* performance of the cross combinations some important findings emerged

a) The cross combinations showing significantly positive sij for the character concerned involved at least one parent having significant gca effect for the concerned character In these crosses additive and additive x additive interactions are likely to be predominant Such croses are likely to throw large number of desirable segregates in the advanced generations In this respect, our identified cross combinations, namely Sel-Tm3 X 5269, Sel-Tm3 x HG22 and Sel-Tm3 x 5269 are expected to give desirable segregates for selection of the genotypes having high yield potential with appreciable pod protein content

(b) In another situation the cross combination showing significantly positive sij for the character concerned involved the parents with significantly negative gca effect for that character For eg, Sel-Tm1 x Pusa Dofasli for pod yield per plant In such situations, dominance X dominance interaction played major role in the expression of the character in these cross combinations

4.6 Heterosis for pod yield components, pod yield and pod protein content

From the study of heterosis for pod yield components (days to flowering, pod length, fresh pod weight, dry pod weight, seeds per pod and pods per plant), Pod yield per plant and pod protein content (Table 34), it revealed that ten cross combinations recorded significantly negative heterosis for days to flowering with respect to mid parent Out of eight cross combinations exhibiting significantly positive heterosis for pod length, the maximum of 31 4% over mid parent (MP) was shown by Sel-Tm1 X 5269 This cross combination also revealed highest sij for this character Most of the cross combinations recorded significant heterotic depression for pod weight Of the nine cross combinations showing significantly positive heterosis for pod yield over MP three (Sel-Tm3 X 5269, Sel-Tm3 X 1-101 and EC- 305827 X 5269) revealed significantly positive sij, five (Sel-Tm 1 x HG 22, Sel-Tm1 X 5269, Sel-Tm1 X 1-101, Sel-Tm3 X HG 22 and EC-305827 X HG 22) showed insignificant sij and only one (Sel-Tm3 X EC-243954) recorded significantly negetive sij for pod yield per plant Most of the parents involved in these cross combinations showed significantly positive gca effect for pod yield per plant So, there is reasonable ground to suggest that additive genetic variances were also involved in the mainfestation of heterosis in the self pollinated crops like cowpea Singh *et al*

Table 34 : Manifestation of heterosis (%) for different characters over mid parent (MP), Line(L) and Tester(T)

CHARACTERS

Cross combination	Days of flowering			Pod length (cm)			Fresh pod weight (g)			Seeds per pod		
	M P	L	T	M P	L	T	M P	L	T	M P	L	T
Sel-Tm1 X 5269	-18.53 **	-26.04 **	-9.33 **	31.40 **	13.80 **	55.45 **	-11.32 *	-41.88 **	87.08 **	-1.10	-5.25	3.44
Sel-Tm1 X HG 22	-12.77 **	-21.28 **	-2.20	-2.62	-29.53 **	57.53 **	-8.71	-44.15 **	149.80 **	9.24	-16.81	59.04
Sel-Tm1 X 1-101	-6.74 **	-15.30 **	3.75 *	6.18	-15.74 **	43.50 **	0.29	-39.09 **	183.74 **	8.56	-9.45	35.53
Sel-Tm1 X EC-243954	-6.25 **	-6.12 **	-6.37 **	-0.58	-11.74 **	13.82 **	0.12	-26.70 **	57.89 **	17.99	1.26	41.35
Sel-Tm1 X Pusa-Dofasli	- 16.56 **	-20.87 **	-11.75 **	-2.18	-12.88 **	11.53 *	-11.06**	-34.03 **	36.46 **	337.97**	343.49**	332.58
Sel-Tm3 X 5269	12.13 **	10.32 **	14.00 **	-9.83 **	-30.14 **	27.10**	13.00**	-23.01 **	112.36 **	-25.34	-32.02	-17.20
Sel-Tm3 X HG 22	3.96 **	1.61	6.42 **	-10.79 **	-40.41 **	77.42 **	9.84	-30.75 **	165.42 **	-25.13	-45.01	17.27
Sel-Tm3 X 1-101	9.14 **	7.42 **	10.91**	-6.71*	-32.79 **	52.43 **	17.92**	-26.27 **	194.31 **	-24.15	-39.36	1.26
Sel-Tm3 X EC-243954	4.97 **	14.92 **	-3.39 **	-9.20 **	-28.17 **	23.38 **	11.76 **	-13.85 **	59.02 **	-8.49	-24.86	17.01
Sel-Tm3 X Pusa-Dofasli	3.79 **	7.10 **	0.68	-5.23	-24.83 **	28.18 **	-19.27 **	-36.86 **	11.91	-27.58	-30.51	-24.39
EC-305827 X 5269	-3.67**	-2.21	-5.08**	10.64**	-17.04**	66.04**	5.08	-33.02 **	143.82 **	-18.44	-26.30	-8.72
EC-305827 X HG 22	-8.46 **	-7.71 **	-9.21 **	6.72 **	-30.35 **	128.14 **	-11.35 **	-44.91 **	168.54 **	-24.71	-45.00	19.28
EC-305827 X 1-101	12.59 **	-11.23 **	-13.91 **	6.00*	-25.76 **	85.24 **	-12.58 **	-47.99 **	173.98 **	-21.21	-37.41	6.29
EC-305827 XEC243954	-10.07 **	1.91	19.53 **	7.18 **	-18.05 **	54.85 **	-3.28	-31.79 **	66.17 **	-11.01	-27.41	14.96
EC-305827 X Pusa-Dofasli	-2.09	7.81**	7.81 **	14.72**	-12.06**	14.72 **	-15.24**	-39.51**	41.52**	-10.89	-15.19	-6.15
L-16 X 5269	-0.55	-5.41 **	4.83 **	6.09 *	-16.87 **	6.09 *	-17.06 **	-46.94 **	89.89**	-47.16	-48.10	-46.19
L-16 XHG 22	-4.53 **	-9.77 **	1.35	-14.15**	-42.20 **	-14.15**	22.89**	-53.69 **	130.29 **	26.39	-42.92	3.61
L-16 X1-101	-0.83	5.64 **	4.50 **	4.31	-24.12 **	4.31	-24.21 **	-54.79 **	134.15 **	-24.94	-36.06	-9.12
L-16 XEC-243954	-6.17 **	-1.05	-10.78**	12.03 **	-10.34 **	12.03 **	-8.08 *	-34.85 **	56.02 **	-16.27	-26.55	-2.64
L-16 XPusa Dofasli	0.79	0.38	1.21	11.61 **	-10.42 **	11.61 **	-7.08 *	-33.91 **	51.99 **	-24.26	-21.24	-27.05
S.E. (M P)	0.5070	-	-	0.8611	-	-	0.5091	-	-	12.2013	-	-
S.E. (L/T)	-	0.5855	0.5855	-	0.9943	0.9943	-	0.5879	0.5879	-	14.0888	14.08

Contd.

CHARACTERS

Cross combination	Pod Per Plant			Pod yield/Plant			Dry pod weight (g)			Protein (%)		
	M P	L	T	M P	L	T	M P	L	T	M P	L	T
Sel-Tm1 X 5269	4.57	88.74**	-27.68**	18.87**	9.75	29.66**	72.30**	70.57**	74.06**	.0665	.1083**	.0278
Sel-Tm1 X HG 22	0.42	79.92**	-30.36**	20.64**	-5.48	66.70**	70.94**	33.78**	136.69**	.0039	.0114	-.0035
Sel-Tm1 X 1-101	1.01	68.67**	-27.91*	34.45**	2.59	95.02**	41.76**	5.02	118.06**	.0031	-.0185	.0258*
Sel-Tm1 X EC-243954	-5.80	44.84	-30.20*	6.51	6.14	6.88	7.11	3.34	11.15	.0231*	.0132	.0332**
Sel-Tm1 X Pusa-Dofasli	34.48	60.98*	15.48	6.30	6.08	6.52	1.94	5.35	-1.25	-.0148	.0273*	-.0535**
Sel-Tm3 X 5269	32.61**	139.66**	-8.34	86.46**	85.55**	87.38**	-9.67	-21.75**	6.83	-.0721**	-.0573**	-.0865**
Sel-Tm3 X HG 22	22.37	119.55**	-15.18	82.65**	51.90**	129.00**	-7.21	-23.75**	80.47**	-.0231*	-.0371**	-.0087
Sel-Tm3 X 1-101	35.92**	127.26**	-3.05	107.47**	67.58**	172.30**	2.94	-30.00**	94.44**	-.0589**	-.0986**	-.0156
Sel-Tm3 X EC-243954	2.81	58.27*	-23.87*	26.08**	36.28**	17.30**	-6.49	-20.75**	14.03	-.0613**	-.0901**	-.0305*
Sel-Tm3 X Pusa-Dofasli	34.75	61.47*	15.61	-5.65	2.13	-12.33*	-5.70	-15.25*	6.27	-.0793**	-.0615**	-.0965**
EC-305827 X 5269	0.99	65.79**	-27.39**	30.33**	5.87	69.50**	44.35**	-61.45**	0.00	-.1102**	-.0632**	-.1527**
EC-305827 X HG 22	-3.03	58.07**	-30.07**	12.90**	-19.93**	91.37**	-33.26**	-59.21**	83.43**	-.0606**	-.0415**	-.0789**
EC-305827 X 1-101	-0.23	52.00*	-25.74*	8.65	-24.59**	94.27**	-36.28**	-62.11**	100.00**	-.0815**	-.0903**	-.0727**
EC-305827XEC-243954	-0.19	40.51	-22.60	5.51	-8.58*	24.75**	-42.39**	-60.66**	7.00	-.0675**	-.0650**	-.0070**
EC-305827 X Pusa-Dofasli	21.58	34.93	10.63	-17.10**	-28.10**	-2.14	-42.35**	-59.08**	-2.51	.0218*	.0794**	.0300**
L-16 X 5269	-41.93**	-26.14	-52.16**	-29.32**	-40.49**	12.98*	-26.42**	-35.71**	-13.99	.0656**	.1394**	.0008
L-16 XHG 22	-17.47	4.33	-31.74**	0.63	-26.64**	60.15**	-30.48**	-50.26**	15.38	.0686**	.1069**	.0330**
L-16 X1-101	-13.99	2.53	-25.92*	-1.27	-29.66**	65.51**	-10.45	-38.78**	66.67**	.0485**	.0539**	.0432**
L-16 XEC-243954	-13.00	-3.11	-21.07	6.07	-4.41	19.14**	-6.57	-20.15**	12.59	.0447**	.0632**	.0269*
L-16 XPusa Dofasli	3.16	-5.88	14.13	4.70	-5.54	17.42**	-6.61	-15.31*	4.08	.0680**	.1459**	.0000
S.E.(M P)	3.7797	-	-	15.1144	-	-	0.8168	-	-	.0396	-	-
S.E.(L/T)	-	4.364	4.364	-	17.452	17.452	-	0.9432	0.9432	-	.0457	.0457

S.E. = Standard Error,

* = Significant at .5% level.

** = Significant at .1% level.

(1992) also suggested that larger proportion of non-additive effects seemed to be due to additive x additive interaction effect Seven cross combinations exhibited significantly positive heterosis, though very low in magnitude, for pod protein content Poor realisation of heterosis for seed protein content was earlier reported in cowpea (Mak and Yap,1977 , Hazra *et al* 1996), Pea (Pandey and Gritton, 1975 , Lawrence *et al.,* 1987) and Chickpea (Salimath *et al*, 1988)

Our study also suggested little promise for improvement of protein percentage in the F_1 hybrids of cowpea The highest protein content of 4 08% was recorded in the cross combination L-16 x 5269 which also registered significantly positive sij for this character Interestingly, the two parents involved in this cross combination were good general combiners for pod protein content This finding~~s~~ also indicated the importance of additive genetic variance in the heterotic expression for pod protein content

4.7 Breeding strategies for developing high yielding types with appreciable protein content ; Selection

We did not realize the widely held notion of strong negative correlation between

yield and protein content of both pods and seeds Several suggestions are there for not improving protein content at the expense of yield So the strategy should be to increase protein yield per unit area In this view, selection of high yielding genotypes with appreciable protein content in pods and seeds should be encouraged From our observation on character association, simultaneous improvement of pod yield and protein content may be possible For direct selection, a wide gene pool for both pod yield and protein content need to be assembled or created by hybridization During selection of genotypes for protein content, some characters like, leaf dry weight, chlorophyll content and nitrogenase activity of nodules should be given weightage

Hybridization and selection

Selective mating and subsequent selection from advanced generations will prove successful for getting desirable segregates with high pod yield and appreciable protein content Maternal inheritance is widely reported for protein content in legumes For this reason, the high pod protein parents like 5269, Pusa Dofasli, etc. should be kept as female parent in hybridization programme Som and Hazra (1993) suggested the combination of the genotype of cv-gr Unguiculata and Sesquipedalis for the development of relatively short statured determinate / semi determinate plant type with medium long, succulent pods In this proposition, our identified cross combinations (Unguiculata x Sesquipedalis) namely, Sel-Tm3 X HG22, Sel-Tm1 X 5269 and Sel-Tm3 X 5269 will prove to be worthy in the persuation for getting segregates having ideal plant frame, high pod yield and appreciable protein content Importance of both additive and non additive effects in the genetic control of pod yield per plant and pod protein content was revealed in our study However, additive genetic variance was more important for pod protein content while non-additive genetic variance was predominant for pod yield Exploitation of non-additive genetic effects in the form of using F_1 hybrids in cowpea is not feasible because of poor crossing success and less number of seeds per pod However, homogygous lines equal to or better than the F_1 hybrids have been reported from highly heterotic crosses in some self-pollinated crops like tomato (Williams, 1959), barley (Aesaviet, 1964), and Chickpea (Singh, 1989) So, the possibility of deriving pure lines performing better than or as well as F_1 hybrids suggest that a large proportion of non-additive gene effects in self-pollinated crops seems to be due to additive x additive effects and that selection should be deferred to later generations (Singh *et al*, 1992) So, hybridization of cv-gr Unguiculata X cv-gr Sesquipedalis genotypes and pedigree method of selection is advocated for developing desirable plant type The selection should be deferred to later generation for identifying desirable segregates Simultaneous selection for pod yield and protein content of pod/ seed need to be administered to get a proper balance between pod yield and protein content in the segregates

CHAPTER V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was undertaken with the objective of obtaining information on plant architecture needed for initiating a rational approach for elevating protein content of pods and seeds of cowpea (*Vigna unguiculata* (L) walp)

Twenty one genotypes of cowpea, 7 each of cultigroup Sesquipedalis, Unguiculata and Biflora were collected and field trials were carried out at District Seed Farm,Bidhan Chandra Krishi Viswavidyalaya, Kalyani

The investigation approaches included

1 Pod and seed characters in the three cultigroups at three different stages of pods

2 Genetic variability and heritability for different pod characters, leaf characters,pod yield and protein content of pods and seeds

3 Associationships of pod and seed protein contents with different pod and leaf charcters and pod yield

4 Influence of root and nodule characters on protein content of pods and seeds

5 Gene action for pod protein content and other yield components

6 Analysis of heterosis for pod yield components,pod yield and pod protein content

Important findings of the present study and conclusion drawn from the investigation have been sum~~e-rized~~ marised here topic wise

1. **Pod and seed characters in the three cultigroups at three different stages of pods:** Protein content in the pods increased with the age of the pod irrespective of the cultigroups However, the rate of increase was maximum from 10 days to 15 days stage From 10 days to 15 days stage, rate of increase in protein content was almost similar in all the cultigroups but from 15 to 20 days stage it was highest in cv-gr Biflora, followed by in cv-gr Unguiculata and cv-gr Sesquipedalis In all the three stages however, maximum protein content was exhibited by the genotypes of cultigroup Sesquipedalis It is suggested that screening of genotypes for pod protein content can be effectively done in the early stages of pod development because the genotypes irrespective of cultigroups which showed high protein content in the pod in the earliest stage revealed the consistancy in protein content in other two stages also Six genotypes namely L-9 and T-5 of cv-gr Sesquipedalis, Pusa Dofasli and 5269 of cv-gr Unguiculata CG7A and Cherodi of cv-gr Biflora emerged as outstanding having registered maximum pod protein content in all the three stages

2. **Genetic variability and heritability for different pod characters, leaf characters, pod yield and protein content of pods and seeds** Protein content of pods in all the three stages and that of seeds along with all the pod and leaf characters revealed very high broadsense heritability But G C V of the protein content of pod and seeds were low in all the analysis Seed protein content was highest in cv-gr Biflora followed by in cv-gr Susquipedalis and cv-gr Unguiculata With the consideration of heritability and genetic advance together, the leaf fresh weight and dry weight, chlorophyll content , protein content of pods in all the three stages and protein content of seeds have been proposed to be under the control of non-additive gene action On the other hand,pod length, pod weight and pod yield might have been under the cotrol of loci having additive action which indicated their reliability for direct selection Seeing the very high heritability for protein content it is suggested that considerable variation in protein content in favourable direction may be achieved by selection if suitable and

diverse gene pool is created

3.Associationships of pod and seed protein content with different po and leaf character and pod yield: These studies were carried out employin both correlation coefficient (phenotypic and genotypic) and path coefficient I all the growth stages no significant correlation have been registered betwee pod and seed characters (pod length, fresh pod weight, dry pod weight and seed per pod) and protein content of pods In the path analysis of all the three stages maximum positive direct effect on protein content of pods were registered b fresh pod weight followed by seed number per pod Although in all the pat analyses the residual effects were very high still influence of seed number pe pod in particular on pod protein content could be identified examining all th direct and indirect effects

No leaf characters (leaf fresh weight, dry weight and chlorophyll content registered any significant correlation with pod protein content (three stages and seed protein content Pod protein content of three stages showed very hig correlation among themselves but pod protein content of any stage did nc register significant correlations with seed protein content. Pod yield per plar did not register any significant correlation with protein content of pods an seeds The magnitude of correlations were also low However, the correlatio between pod yield and seed protein content was negative in nature From th path coefficient analysis it revealed that pod protein percentage of 10 days exerte maximum positive direct effect on seed protein percentage which was followe by leaf dry weight Examining both the direct and indirect effects chlorophy content of leaf along with leaf dry weight and protein content of pods in 10 day have been considered as influencing characters for seed protein content It i suggested that during screening of genotypes for seed protein content in selection programme leaf dry weight, chlorophyll content of leaf, seed numbe per pod and protein content in 10 days pod should be taken into consideratio simultaneously for realisation of best results in selection

4. Influence of root and nodule characters on protein content of pods and seeds: Root characters (root length, root fresh weight and dry weight) and nodule characters (nodule number, nodule fresh weight, leghaemoglobin content and nitrogenase activity) varried significantly among the cultigroups which indicated the involvement of the host plant in determining the extent of nodulation and potential nitrogen fixation The root characters exhibited somewhat narrow extent of genetic variability Of the nodule characters, nodule number and weight exhibited moderate G C V and the highest G C V was recorded for nitrogenase activity of nodules The heritability estimates in broad sense were very high for all the root and nodule characters For nitrogenase activity, high heritability in combination with high genetic advance were revealed which indicated its control by additive gene action Genetic advance for nodule number and weight were below moderate though heritability estimates for these characters were very high which is attributable to conditioning of non-additive gene action for these two characters In this study, along with pod and seed protein percentage, protein content of 100 seeds were also taken into consideration Low genetic advance and high heritability were recorded for pod and seed protein percentage but high heritability and moderate genetic advance was, for protein content of 100 seeds Root characters did not register any significant correlation with pod and seed protein content Significant correlations were recorded between nodule number per plant and nodule weight per plant, leghaemoglobin content per g nodule and nitrogenase activity of the nodule Nodule weight and leghaemoglobin content also registered significant correlations Nodule number, nodule weight, leghaemoglobin content and nitrogenase activity did not show any significant correlation with pod and seed protein percentage However, correlation between nitrogenase activity of nodules and protein content in 100 seeds was significantly negative Path coefficient analysis revealed that highest positive direct effect on seed protein percentage was shown by nitrogenase activity followed by nodule number per plant, pod protein content and protein content in 100 seeds It is proposed from the study that at the time of breeding for seed protein content in cowpea the root and nodule characters need to be given weightage Three important biological nitorgen fixation parameters namely nodule number and weight per plant and nitrogenase activity of nodule along with pod protein content and protein content of 100 seeds deserve due importance in the improvement programme

5.Gene action for pod protein content and other yield components: We attempted a 4 x 5 Line x Tester analysis to understand the gene action for days to flowering, pod length, fresh pod weight, dry pod weight, seeds per pod, pods per plant, pod yield per plant and protein content of pods The components of gca and sca variances were highly significant for all the characters excepting seeds per pod and pods per plant The estimated genetic variance indicated the overwhelming importance of additive genetic variance for pod length and that of non-additive genetic variance for pod yield and dry pod weight For other characters including protein content of pods, both additive and non-additive genetic variance were significant but additive genetic variances were more in magnitude than non-additive genetic variance With the consideration of gca effects for pod yield, yield compoents and pod protein content, the Tester parent 5269 of cv-gr Unguiculata emerged as outstanding With the consideration of pod yield and pod yield components the line parent Sel-Tm3 of cv-gr Sesquipedalis proved to be the best In view of joint consideration of pod yield and protein content in the light of sca effects of the cross combination and the gca effects of the parents involved, three cross combinations namely Sel-Tm3 x HG22, Sel-Tm3 x 5269 and Sel-Tm1 x 5269 emerged as promising

6.Analysis of heterosis From the study of heterosis it revealed that eight cross combinations exhibited significantly positive heterosis for pod length Most of the cross combinations showed heterotic depression for pod weight Nine cross combinations showed significantly positive heterosis for pod yield per plant Most of the parents involved with these cross combinations showed significantly positive gca effects for pod yield per plant suggesting the involvement of additive genetic variance in the manifestation of heterosis for pod yield Though seven cross combinations exhibited significantly positive heterosis for pod protein content, yet the realisation of heterosis is very low Importance of additive genetic variance in the expression of heterosis for pod protein content have also been realised

CHAPTER VI

FUTURE SCOPE OF RESEARCH

FUTURE SCOPE OF RESEARCH

Some important studies may be taken up in future with a view to improve protein content of pods and seeds in combination with high pod yield

1 Study on physiological traits of plants and biological nitrogen fixation parameters in relation to pod and seed protein content with the ontogeny of cowpea employing different cultigroups to identify the growth stages with prominent interrelationships among the parameters

2 Combination of genotypes of cultigroup Unguiculata and Sesquipedalis following pedigree method of breeding with differed selection of the segregates

3 Protein content is reported to have been influenced much by environment So, a stability analyse of the selected genotype in different environments and preferably across locations should be conducted to identify stable genotypes for both pod yield and protein content

4 With the view of the reported maternal influence on the protein conten of the other grain legumes, mutation breeding may be tried in cowpea

5 Limiting amino acids in the seed protein of cowpea are methio - nine , cystine and tryptophan So , at the time of improving total protein content in the seeds, screening of the genotypes for this amino acids directly or indirectly by estimating sulpher content of seeds need to be carried out

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APPENDIX

Appendix I. Physico-chemical featuers of the soil in the experimental plots

	District Seed Farm, Kalyani, B.C.K.V	Method
Clay	24.36	International Pipette method (piper,1966)
Silt %	36.42	-do-
Sand %	41.40	-do-
Total N %	0.054	Modifield kjeldahl Method (Jackson, (1967b)
Available P_2O_5 (Kg/ha)	34.00	Olsen's method (Chopra and kanwal, 1986c)
Available K_2o (Kg/ha)	97.00	Flame photometric method (Jackson, 1967c)
Organic Carbon(%)	0.49	Walklay and Black's method (Chopra and Kanwas, 1986a)
pH of the soil	6.8	Potentiometric method with glass electrode (Chopra and kanwar, 1986b).

Appendix II. Materological data of the experimental period

Year month	Temperature (0° C)		Total Rainfall(mm)	Relative humidity (%)	
	Max	Min		Max	Min
1994					
March	33.84	18.24	8.00	92.34	47.52
April	35.02	20.55	106.70	86.59	55.7
May	36.04	24.21	148.90	90.43	43.50
June	33.05	24.27	350.40	93.70	54.41
July	32.31	26.63	213.10	95.13	57.50
Aug	32.01	26.16	202.90	96.10	80.44
Sept	32.98	25.19	58.60	93.30	80.81
Oct	32.82	22.85	41.20	90.68	82.90
Nov	29.48	17.68	11.50	92.63	50.17
Dec	27.14	11.24	Nil	40.64	66.96
1995					
Jan	24.1	10.1	13.0	94.9	48.1
Feb	27.94	14.63	55.9	95.11	49.56
March	33.0	19.56	6.6	89.67	36.07
April	37.81	24.81	29.8	85.82	36.97
May	36.0	27.6	95.67	92.0	57.32
June	30.0	26.56	80.4	93.9	75.0
July	32.6	25.95	344.19	96.0	82.58
Aug	33.2	26.1	358.6	98.0	79.0
Sept	31.9	25.43	437.493	97.0	81.25
Oct	32.04	23.66	93.699	94.94	68.41
Nov	28.42	18.4	384.0	94.96	55.1
Dec	26.5	12.60	Nil	96.77	47.1
1996					
Jan	25.14	12.22	8.79	96.29	52.57
Feb	27.93	14.61	64.6	95.66	50.64
March	33.52	21.81	33.7	93.16	42.03
April	35.60	29.5	123.198	90.7	46.24
May	35.16	25.9	108.0	90.9	53.9
June	33.16	25.53	359.194	94.5	71.67

Appendix III : Mean values of genotypes belonging to three cultigroups for pod and seed characters in E1 and E2 for 10 days,15 days and 20 days after anthesis

Name of the variety	Pod length (cm)		Fresh pod wt(g)		Dry pod wt(g)		Seeds per pod		Protein content(%)	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Sesquipedalis										
Sal-Tm1	18.417	16.567	14.170	11.220	0.667	0.570	14.350	13.537	3.587	3.430
sel-Tm2	17.193	15.663	11.503	9.153	1.177	1.100	14.667	14.223	3.853	3.770
Sel-Tm3	18.843	17.437	13.577	11.837	1.413	1.237	15.407	13.090	3.750	3.657
EC- 305827	18.910	16.557	9.450	8.110	0.940	0.810	12.373	11.530	3.500	3.473
L-16	23.377	20.810	12.599	8.903	1.000	0.910	14.897	15.020	3.510	3.487
T-5	15.907	13.557	8.990	8.933	0.843	0.893	12.283	12.483	3.900	3.767
L-9	18.717	16.740	13.993	12.093	1.010	0.960	12.843	12.317	4.140	4.330
Mean	18.766	16.761	12.040	10.036	1.001	0.926	13.831	13.171	3.749	3.702
Unguiculata										
Pusadofasli	16.943	15.080	4.267	4.137	0.640	0.750	14.127	14.420	4.123	3.963
EC-243954	16.740	12.813	7.140	6.743	0.677	0.607	9.993	10.287	3.400	3.340
Cowpea 263	16.643	15.297	4.390	4.103	0.310	0.263	12.097	10.977	3.500	3.427
1-101	15.533	12.660	3.097	2.710	0.320	0.283	7.610	7.747	3.477	3.443
Sel 61B	21.667	16.087	4.467	4.293	0.963	0.843	8.783	9.680	2.730	2.690
HG 22	8.443	7.637	3.077	2.987	0.407	0.380	8.640	7.603	3.617	3.567
5269	12.430	10.727	3.410	2.933	0.463	0.527	10.140	9.833	4.027	4.003
Mean	15.486	12.900	4.264	3.987	0.540	0.522	10.199	10.078	3.553	3.490
Biflora										
82-1	9.093	7.267	2.247	1.993	0.323	0.297	8.223	8.187	3.697	3.627
Covu62A	8.780	6.417	1.300	1.320	0.320	0.327	9.337	10.163	3.617	3.550
Cherodi	8.317	7.547	1.683	1.160	0.300	8.343	8.013	7.510	4.027	3.857
1-195B	7.660	6.413	2.637	2.563	0.523	0.440	9.843	9.563	3.593	3.527
SG 2	12.567	8.587	3.457	2.883	0.820	0.900	8.110	7.503	3.407	3.327
CG7A	9.623	8.490	1.903	1.667	0.433	0.490	9.207	8.750	3.667	3.703
V-70	7.360	7.103	1.567	1.443	0.180	0.230	7.613	7.003	3.547	3.450
Mean	9.057	7.403	2.113	1.861	0.414	0.432	8.621	8.383	3.650	3.577

Contd.

Name of the variety	Pod length (cm)		Fresh pod wt(g)		Dry pod wt(g)		Seeds per pod		Protein content(%)	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
After 15 days										
Sesquipedal										
Sel-Tm1	29.697	28.537	19.697	16.963	0.970	0.810	15.107	14.173	3.913	3.637
sel-Tm2	25.503	23.917	14.553	12.943	1.257	1.253	15.337	13.850	4.023	3.933
Sel-Tm3	33.590	32.000	17.337	15.967	1.937	1.797	16.207	13.737	3.793	3.730
EC- 305827	40.063	39.430	21.867	20.563	2.067	1.947	17.237	16.247	3.747	3.663
L-16	35.870	34.210	14.863	13.933	1.350	1.247	15.063	14.177	3.620	3.627
T-5	24.253	24.943	11.580	10.957	1.380	1.483	14.420	14.283	4.147	3.917
L-9	30.727	31.243	18.747	18.030	1.080	0.980	13.807	13.680	4.333	4.387
Mean	31.386	31.611	16.949	15.622	1.434	1.360	15.311	14.307	3.940	3.842
Unguiculata										
Pusadofasli	22.680	21.097	5.323	4.933	1.010	1.020	17.090	15.107	4.147	4.090
EC-243954	23.420	22.420	8.243	7.433	0.930	0.977	11.653	10.460	3.647	3.663
Cowpea 263	22.367	19.617	5.197	5.013	0.980	0.913	12.517	11.047	3.523	3.520
1-101	16.703	15.633	4.770	4.030	0.543	0.517	10.480	10.560	3.667	3.543
Sel61B	21.630	20.987	5.070	4.890	1.010	0.963	10.617	10.437	2.817	2.813
HG 22	13.617	13.170	4.367	4.177	0.607	0.577	8.317	7.583	3.967	3.847
5269	17.717	17.267	4.633	4.397	0.703	0.733	11.273	10.620	4.167	4.107
Mean	19.733	18.599	5.372	4.982	0.826	0.814	11.707	10.830	3.705	3.655
Biflora										
82-1	11.463	10.733	3.747	3.397	0.430	0.420	10.570	9.283	3.790	3.717
Covu62A	12.120	10.150	3.273	2.927	0.547	0.473	9.627	9.547	3.743	3.697
Cherodi	8.190	7.547	1.917	1.607	0.333	0.367	9.407	8.797	4.070	3.947
1-195B	12.073	10.847	2.690	2.110	0.870	0.793	10.980	9.747	3.637	3.647
SG 2	12.270	12.230	3.777	3.400	0.927	0.777	8.417	7.510	3.557	3.437
CG7A	10.890	10.593	3.377	3.077	0.687	0.573	9.517	8.257	4.313	3.980
V-70	8.510	8.210	2.137	2.040	0.393	0.387	8.720	7.707	3.760	3.613
Mean	10.788	10.044	2.988	2.651	0.598	0.541	9.605	8.692	3.893	3.720

Contd.

Name of the variety	Pod length (cm)		Fresh pod wt(g)		Dry pod wt(g)		Seeds per pod		Protein content(%)	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
After 20 days										
Sesquipedal										
Sel-Tm1	31.950	31.373	21.627	19.007	1.173	1.053	16.287	15.063	4.023	3.790
sel-Tm2	26.493	25.390	16.450	14.303	1.383	1.257	15.523	15.297	4.143	4.057
Sel-Tm3	37.600	35.887	18.350	17.570	2.163	2.173	17.537	15.860	3.853	3.770
EC-305827	42.787	41.850	23.910	23.443	2.187	2.047	18.543	18.750	3.897	3.750
L-16	38.477	37.330	16.377	14.963	1.490	1.480	15.170	13.927	3.773	3.640
T-5	25.170	25.853	12.293	12.293	1.547	1.533	14.473	15.040	4.233	4.147
L-9	34.080	32.657	19.507	18.873	1.217	1.107	14.377	14.063	4.587	4.527
Mean	33.794	32.906	18.359	17.208	1.594	1.521	15.987	15.429	4.071	3.954
Unguiculata										
Pusadofasli	24.317	22.270	7.317	6.887	1.313	1.030	17.510	17.057	4.247	4.170
EC-243954	25.053	24.143	9.060	8.783	1.187	1.013	14.270	13.453	3.830	3.753
Cowpea 263	23.353	21.893	6.697	6.230	1.110	1.070	13.410	12.423	3.673	3.620
1-101	18.010	16.400	5.250	4.507	0.710	0.623	12.667	13.363	3.800	3.713
Sel61B	24.013	22.990	5.530	5.530	1.123	1.103	11.440	10.820	3.073	2.967
HG 22	14.820	14.370	5.263	4.803	0.790	0.750	10.513	9.413	4.120	3.973
5269	19.280	18.400	5.180	5.110	0.860	0.767	12.653	10.730	4.260	4.190
Mean	21.264	20.067	6.328	5.946	1.013	0.922	13.209	12.466	3.858	3.770
Biflora										
82-1	11.937	11.027	4.550	4.133	0.553	0.500	11.947	10.830	3.930	3.817
Covu62A	13.240	12.223	3.903	3.587	0.623	0.540	12.657	10.667	3.833	3.757
Cherodi	9.533	8.330	2.697	2.480	0.460	0.477	10.507	9.730	4.150	4.050
1-195B	13.067	12.587	3.097	2.730	0.937	0.920	13.447	11.010	3.827	3.727
SG 2	12.953	12.657	4.080	3.697	0.990	0.890	11.047	9.020	3.743	3.727
CG7A	12.110	10.710	3.800	3.387	0.733	0.627	11.183	9.370	4.553	4.270
V-70	9.027	8.573	2.830	2.663	0.513	0.440	10.213	9.100	3.817	3.690
Mean	11.695	10.872	3.565	3.240	0.687	0.628	11.571	9.961	3.979	3.862

Appendix IV: Mean of leaf characters, pod yield, protein content of three cultigroups

Cultigroups	Variety	Leaf fresh wt(g)	Leafdrywt (g)	chlorophill content (mg/g)	Pod yield (g)	Pod protein 10 days (%)	Pod protein 15 days (%)	Pod protein 20 days (%)	Seed protein (%)
Sesquipedalis	Sel-Tm1	12.493	1.403	0.465	334.370	3.510	3.777	3.910	20.910
	Sel-Tm2	15.510	1.837	0.384	255.280	3.813	3.980	4.100	20.667
	Sel-Tm3	15.980	1.660	0.638	354.227	3.703	3.763	3.813	19.730
	EC-305827	12.463	1.150	0.386	469.637	3.490	3.710	3.827	21.530
	L-16	21.170	2.023	0.605	306.990	3.500	3.623	3.673	23.677
	T-5	12.567	1.213	0.576	324.020	3.837	4.033	4.187	19.337
	L-9	17.127	1.470	0.617	343.663	4.237	4.363	4.560	20.137
Mean		15.330	1.537	0.524	341.170	3.727	3.893	4.010	20.855
Unguiculata									
	Pusa Do-fasli	15.360	1.433	0.825	319.170	4.047	4.120	4.210	19.553
	EC-243954	11.830	1.037	0.473	335.390	3.373	3.657	3.793	20.463
	Cowpea 263	12.487	1.163	0.563	334.737	3.463	3.527	3.650	19.713
	1-101	19.157	1.813	0.437	175.073	3.463	3.607	3.760	19.803
	Sel 6113	25.527	2.310	0.830	151.523	2.710	2.820	3.020	19.550
	HG 22	15.963	1.567	0.505	129.320	3.597	3.910	4.047	19.270
	5269	21.863	1.933	0.577	207.353	4.017	4.140	4.227	21.480
Mean		17.455	1.608	0.601	236.081	3.524	3.683	3.815	19.976
Biflora	82-1	17.110	1.427	0.564	75.610	3.667	3.757	3.877	20.523
	Covu 62A	15.107	1.263	0.552	67.977	3.587	3.723	3.797	22.157
	Cherodi	11.333	0.890	0.375	87.803	3.943	4.010	4.100	26.567
	1-195 B	10.130	0.813	0.461	152.140	3.560	3.643	3.777	24.033
	SG 2	9.867	1.243	0.386	97.920	3.367	3.500	3.737	22.390
	CG 7A	14.097	2.273	0.479	70.450	3.690	3.950	4.413	24.420
	V-70	8.937	1.100	0.616	77.547	3.500	3.690	3.753	20.633
Mean		112.369	1.287	0.491	89.921	3.616	3.753	3.922	22.960
Significance of mean between cultigroup	S-U	*	N.S	*	*	*	*	*	*
	V-B	*	*	*	*	*	*	*	*
	B-S	*	*	N.S	*	*	*	*	*

Appendix V: Mean of root and nodule and protein content of three cultigroups

Varieties	Cultigroup	Root length (cm)	Root fresh wt.(g)	Root dry wt(g)	Nodule number/ plant	Nodule wt.per plant (g)	Leghaemoglobin content mg/100g	Nitrogenase activity umole C_2H_2	Pod protein of 20 days	Seed protein content (%)	Total protein 100 grains (%)
Sel-Tm1	Sesquipedalis	18.560	4.830	1.180	23.630	1.077	0.857	4.097	3.923	20.910	2.650
Sel-Tm2		18.333	7.170	1.333	25.370	1.067	0.917	4.550	4.090	20.667	2.800
Sel-Tm3		17.967	6.213	1.907	23.310	1.183	0.770	3.130	3.777	19.730	2.877
EC-305827		20.993	4.813	0.943	21.557	1.143	0.677	2.147	3.787	21.530	2.837
L-16		18.863	5.177	1.053	22.923	1.003	0.683	2.507	3.677	23.677	2.917
T-5		21.377	6.297	1.143	20.157	0.820	0.753	3.320	4.117	19.337	2.870
L-9		16.443	4.887	1.600	23.670	1.020	0.837	4.420	4.353	20.137	2.270
Mean		18.934	5.627	1.309	22.945	1.045	0.785	3.453	3.960	20.855	2.746
Pusa-Dofasli	Unguiculata	20.433	6.457	1.603	25.610	1.513	0.727	4.223	4.200	19.553	2.460
EC-243954		19.717	5.477	0.817	29.223	1.090	0.790	4.093	3.663	20.463	2.580
Cowpea 263		22.273	4.500	0.790	24.777	1.147	0.747	3.990	3.667	19.713	1.880
1-101		20.050	6.790	1.123	28.847	1.707	0.813	12.627	3.697	19.803	1.867
Sel 61B		15.857	6.660	0.963	25.723	1.170	0.820	5.617	3.020	19.550	2.017
HG 22		16.280	4.887	1.133	37.970	1.620	0.920	13.740	4.053	19.270	1.877
5269		14.840	4.537	1.130	21.433	1.103	0.750	3.637	4.133	21.480	2.807
Mean		18.493	5.615	1.080	27.655	1.336	0.795	6.847	3.776	19.976	2.212
82-1	Biflora	18.000	4.027	0.813	23.170	1.063	0.720	4.780	3.867	20.523	1.757
Covu 62A		19.133	6.053	0.940	34.943	1.330	0.913	18.980	3.753	22,157	1.753
Cherodi		19.193	4.057	0.690	22.467	0.993	0.783	4.467	4.147	26.567	1.807
1-195 B		18.137	5.033	1.473	34.210	1.210	0.860	7.353	3.667	24.020	1.973
SG 2		18.943	5.517	1.577	38.857	1.443	0.920	17.527	3.667	22.367	1.683
6G 7A		16.737	4.520	0.545	32.040	1.323	0.863	17.500	4.280	24.420	1.533
V-70		19.387	4.207	1.120	33.687	1.203	0.857	15.547	3.780	20.633	1.293
Mean		18.504	4.773	1.023	31.339	1.224	0.845	12.308	3.880	22.955	1.686
Significance	S-U	*	N.S	*	*	*	N.S	*	*	*	*
of Mean	U-B	N.S	*	N.S	*	*	*	*	*	*	*
between											
cultigroups	B-S	*	*	*	*	*	*	*	N.S	*	*

Appendix VI. Mean values of parents and F$_1$'s

Parents and combinations	Days to flowering	Pod length (cm)	Green pod wt(g)	Dry pod wt (g)	Seeds per pod	Pods/ plant	Pod yield per plant(g)	Protein content(%)
Lines								
Sel-Tm1	40.03	29.23	19.10	9.96	15.86	17.76	339.36	3.78
Sel-Tm3	41.33	38.93	16.36	13.33	17.70	17.73	290.10	3.95
EC-305827	38.82	42.83	21.60	25.33	18.80	20.30	459.90	3.69
L-16	44.33	37.73	21.23	13.06	15.06	30.03	420.06	3.58
Testers								
5269	40.00	21.40	5.93	9.76	14.53	46.36	287.26	4.08
HG 22	39.46	13.07	4.27	5.63	8.30	45.90	192.43	3.84
1-101	40.03	17.16	4.10	4.80	10.60	41.56	178.53	3.62
EC-243954	49.16	22.66	8.86	9.26	11.36	36.86	337.03	3.71
Pusa Dofasli	43.96	22.83	9.23	10.63	16.26	24.76	337.93	4.11
Crosses								
Sel-Tm1X5269	36.26	33.26	11.10	17.0	15.03	33.53	372.45	4.19
Sel-Tm1XHG 22	38.60	20.60	10.66	13.3	13.20	31.96	320.78	3.83
Sel-tm1X1-101	41.53	24.63	11.63	10.46	14.36	29.96	348.16	3.71
Sel-Tm1XEC--243954	46.03	25.80	14.00	10.30	16.06	25.73	360.20	3.83
Sel-Tm1XPusa-Dofasli	38.80	25.46	12.60	10.50	70.36	28.60	359.98	3.89
Sel-Tm3X5269	45.60	27.20	12.60	10.43	12.03	42.50	538.28	3.73
Sel-Tm3XHG22	42.00	23.20	11.33	10.16	9.73	38.93	440.67	3.81
Sel-TM3X1-101	44.40	26.16	12.06	9.33	10.73	40.30	486.13	3.56
Sel-Tm3XEc-243954	47.50	27.96	14.10	10.56	13.30	28.06	395.34	3.60
Sel-Tm3XPusa-Dofasli	44.26	29.26	10.33	11.30	12.30	28.63	296.26	3.71
EC-305827X 5269	37.96	35.53	14.46	9.76	13.26	33.66	486.90	3.46
EC-305827X HG22	35.83	29.83	11.46	10.33	9.90	32.10	368.25	3.54
EC-305827X1-101	34.46	31.80	11.23	9.60	11.26	30.86	346.83	3.36
EC-305827X EC243954	39.56	35.10	14.73	9.96	13.06	28.53	420.43	3.45
EC-305827X Pusa Dofasli	40.53	37.66	13.06	10.36	15.26	27.40	330.69	3.98
L-16X5269	41.93	31.36	11.26	8.40	7.82	22.18	249.96	4.08
L-16XHG22	40.00	21.80	9.83	6.50	8.60	31.33	308.17	3.97
L-16X1-101	41.83	28.63	9.60	8.00	9.63	30.79	295.49	3.78
L-16xEC-243954	43.86	33.83	13.83	10.43	11.06	29.10	401.52	3.81
L-16XPusa-Dofasli	44.50	33.80	14.03	11.06	11.86	28.26	396.80	4.11