GENETIC ARCHITECTURE OF YIELD AND COLD TOLERANCE IN GREENGRAM (*Vigna radiata* (L.) Wilczek)

A
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(PLANT BREEDING AND GENETICS)

By

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Dedicated to my
Beloved Parents

Dr. B. Baisakh
Professor

Bhubaneswar
Dated the 15th September, 2010

CERTIFICATE- I

This is to certify that the thesis entitled "**GENETIC ARCHITECTURE OF YIELD AND COLD TOLERANCE IN GREENGRAM (*Vigna radiata* (L.) Wilczek)**" submitted by **BINAPANI JENA,** Adm. No.24 PBG/08 to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT BREEDING AND GENETICS)** is a faithful record of *bonafide* research work carried out under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma or published in any other form. The assistance and help received during the course of investigation have been duly acknowledged.

15.9.10

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Chairman,
Advisory Committee

CERTIFICATE – II

This is to certify that the thesis entitled **"GENETIC ARCHITECTURE OF YIELD AND COLD TOLERANCE IN GREENGRAM (*Vigna radiata* (L.) Wilczek)"** submitted by **BINAPANI JENA, Adm. No.24 PBG/08** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT BREEDING AND GENETICS),** has been approved by the Student's Advisory Committee after oral examination on the same in collaboration with an External Examiner.

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ABSTRACT

Twenty five genotypes of greengram including 21 local land races and four standard varieties were evaluated in R.B.D. for yield and component traits. The genotypes showed wide and highly significant variation in all 12 traits. Seed yield of the genotypes varied from 1.26 to 4.26 g/plant and Jharsuguda leocal, Kendrapara Local and Kopergaon Local produced higher yield of 3.38-4.26 g/plant. The PCV and GCV estimates were high for response to cold of 10, 30 and 40 days old seedlings of green gram at 10°C temperature. Plant height and pods/plant had moderate to high heritability with high genetic advance indicating additive gene action. Characters like100-seed weight and days to 50 % flowering were with high-moderate heritability but low genetic advance indicating non-additive gene effect.

Pods/plant, pod length and seeds/pod showed significant positive correlation with yield. Path-analysis showed that pods/plant had highest direct positive effects on yield followed by seeds/pod and pod length. Positive correlation of most traits with yield was greatly influenced by indirect positive effect via pods/plant and pod length.

The 25 genotypes showed high genetic divergence (D^2) and tolerance to cold at 10, 40 and 30 days old seedling contributed maximum to divergence. On the basis of D^2 values using Tocher's method, the genotypes were grouped into four clusters. The 22 genotypes consisting of 18 local land races and four standard varieties was the biggest group. Considering the inter-cluster average D^2 values, cluster means for different characters including yield and character complementation in productivity traits, crosses between cluster I and III are expected to produce more transgressive in later generation. The Z_1-Z_2 scatter diagram of genotypes on basis of canonical analysis showed same four clusters as on basis of D^2 with few exchanges.

DNA isolation was made from 25 greengram genotypes using seedling leaves and molecular analysis was done using SSR markers. The gel electrophoresis figures showed that the 19 SSR primers produced 31 amplification products, seen as bands, of which 29 where polymorphic and 2 were monomorphic. The primers CEDG 088, CEDG 092, CEDG 139, VR 5 and VR 6 had better discriminating power.

Jaccard's similarity coefficient dendrogram on the basis of polymorphic bands showed that the genotypes grouped into six clusters. The multigenotype clusters included genotypes from different localities of Odisha and standard varieties developed in different states of India. Clustering/grouping of genotypes on the basis of polymorphic banding pattern was quite different from clusters analysis by D^2, and canonical. It may be due to the fact that the later mentioned methods of clusters analysis are based on morphological and yield component traits of genotypes, while polymorphic bands produced by the genotypes might be governing some quality or others traits in addition to some morphological traits.

CONTENTS

LIST OF TABLES

CHAPTER-I

Introduction

INTRODUCTION

Globally grain legumes are the second most pulses which are rich source of protein source. Greengram [*Vigna radiata* (L.) Wilczek] is an important pulse crop extensively grown in South and South East Asian countries including India under varying climatic conditions (Reddy *et al.*, 2008). They are also considered as the best choice for diversification of cereal based cropping system to sustain production. India is the largest producer of pulses in the world with 25 % share in global production and is the centre of origin of greengram as was 1st reported by de Candolle in his classical book "Origin of cultivated plants" in 1886. Because of the Indian origin and being grown in almost all the parts of India, it is known differently in different Indian languages. In hindi and urdu, it is called simply "Mung". In Tamil "Pachaipayru", in Bengali "Sonamung", in Marathi "Mug", in Gujarati "Mag", in Telgu "Pachapesalu, in Malayalam "Curupayaru" and in Kashmiri "Maung" (Tickoo *et al.*, 2006). The earlier botanical name was *Phaseolus aureus*. Recently greengram has been included in the genus *Vigna* on the basis of floral and seed morphology which are much close to genus *Vigna* than *Phaseolus*. It belongs to Subgenus ceratotropis in genus *Vigna* and is a self-pollinated diploid grain legume (2n = 22) with a geromic size of 560 mb.

With its high protein content (22 – 28 %), greengram is a major source of dietary protein for the predominantly vegetarian population of India. This also contain 1.0 – 1.5 % fat, 3.4 – 4.5 % fibre, 4.5 – 5.5 % ash and 59 – 65 % carbohydrate on dry weight basis (Tsou *et al.*, 1979) and also provide 334 – 344 k Cal. energy (Srivastav and Ali, 2004). Mungbean protein is considered to be easily digestible as it has low concentration of sulpher containing amino acid

methionine and cystein. High lysine content makes them excellent complements to cereal in terms of balanced human nutrition (Tsou *et al.*, 1979).

Greengram is the third largest pulse crop in the country after bengalgram and red gram. During last 25 years, the positive compound growth recorded in Uttar Pradesh (7.83 %), Punjab (7.58 %), Bihar (4.57 %) but negative growth rate in Odisha (-5.79 %) (Ali and Kumar, 2006).

Greengram cultivation in India during 2008-09 was about 22.4 m.ha with production of 13.4 million tonnes and of an average yield of 598 kg/ha. Although area under cultivation has increased from 1.99 m.ha in 1965 to 22.4m.ha in 2008-09, the average yield does not increase substantially. To alleviate protein energy malnutrition, a minimum of 50 g/day should available in addition to other sources of protein such milk, meat and eggs.

To make up this short fall in supply at least 23.88 million tonnes of pulses are required by 2015. India which is expected to reach 29 – 30 million tonnes by 2020. This necessitates an annual growth rate 4.2 % in pulse production. This crop also fixes 58 – 109 kg/ha nitrogen and enhance soil fertility by symbiotic associated with rhizobium bacteria. It is grown in *kharif*, *rabi* and summer in different agro-ecological regions of India. Thats why no single plant type is appropriate for all production system. In *rabi*, when this crop is grown after rice for which it is little beat delayed and crop face hard winter resulting in low production. The crop, however, has very limited genetic variability and also suffers from severe susceptibility to several diseases, pests and environmental temperature fluctuations which are directly related to plant type.

In Odisha (2008-09) greengram ranks first in acreage (7.49 lakh ha) and production (3.06 lakh tonnes) but the productivity is rather low (409 kg/ha). The estimated pulse requirement in Odisha by 2010, 2020 and 2050 are focused to be 22.3, 27.2 and 49.4 lakh tonnes, respectively (Nayak, 2009). Average productivity is very low due to obvious reasons like sowing on marginal and submarginal land under rainfed situation, lack of high yielding genotypes, negligence in plant protection, low seed replacement rate, improved high yielding varieties etc. All these factors either independently or jointly result in poor productivity of this crop. Another factor is that climate change has an adverse impact on productivity on account of reduction of total crop cycle duration for which most of pulses like greengram and blackgram, which are short duration, crop are affected more.

Though greengram grown as pre-*rabi*, *rabi* and summer crop in Odisha under residual moisture conditions after harvest of early *kharif* rice crop, the crop has rapidly been penetrating into several non-traditional area (interior districts) and cropping seasons like spring and summer and also into different cropping system. Tolerance to winter, moisture stress and early maturity can be attributed to its spread to multiple cropping system. So the varieties combining high yield potential with specific adaptation to winter condition, resistance to insect-pests and diseases, need to be developed, if greengram cultivation is to be expanded in the non-traditional areas in the state. The breeding programme for bringing about improvement in greengram of this university is being carried out under ICAR project, i.e., All India Coordinated Research Project on MULLaRP as well as under the Department of Plant Breeding and Genetics, College of Agriculture, Bhubaneswar. In Odisha, the greengram growing regions are Dhenkanal, Kalahandi, Cuttack, Balasore, Puri, Bolangir, Sambalpur whereas in the interior part of Odisha there is severe cold due to which the crop is damaged mostly and give less yield.

Low temperature is one of the major environmental factor that limits the plant growth. Many plant of tropical origin suffer cold injury, when expose to temperature below 20°C (Andrews, 1987). Cold temperature in crop plant are compared by *cold snap* – a lower than usual drop in temperature that causes crop to fail. Low temperature in growing season may reduce germination, retard vegetative growth by inducing metabolic in balance and or can delay/prevent productive e development. Each plant species has an optimum range of temperature for its normal growth and development. It varies among the genotypes within species, the specific temperature also depends on growth state and development of particular genotypes (Roy and Basu, 2009). Low temperature cold injury can be categorized into two parts (1) Chilling injury and (2) freezing injury. Chilling injury is >0°C and freezing injury is < 0°C).

Chilling injury can damage the tissue of sensitive plant, while freezing injury temperature damage most tissue during active growth. Chilling sensitive plants are typically tropical / subtropical, whereas freezing sensitive include all plants. A chilling temperature is define as a temperature low enough to cause tissue damage, but not low enough to cause freezing of tissue water (Levitt, 1980). For most chilling sensitive plant chilling temperature are between 10°C and 0°C (Lyons *et al.*, 1979). Chilling injury is perceived locally probably by each individual cell (Salpveit and Morris, 1990). So resistant varieties is needed to be developed for these region.

The choice of parents including local genotypes for crosses is a crucial factor determining success of recombination breeding for yield in autogamous crops. Local land races have their own importances in a breeding programme. These are easily adaptable to their own area as crops are grown based on certain climatic condition and the local land races are well adapted to that

environment and give satisfactory yield than other varieties. So they can be used as a donor parents for specific trait in any breeding programme. It is a common experience of plant breeders that while some crosses produce superior progeny, other prove disappointing and several generations must be grown and much effort expended before such differences become apparent. Though the problem is well recognized, an alternative method for choice of parents or crosses is yet to be found. The problem is one of prediction of the relative segregation potential of crosses that can be made among the available stock of potential parents. Assessment of divergence or similarity among the genotypes would help in identification of genotypes that could be used in cross breeding programme for producing transgressive segregants. The effectiveness of selection for a character depends upon the amount of genetic variation present in a variable population. Different multivariate analyses used for clustering or grouping of genotypes in various crop plants are genetic divergence D^2 (Rao, 1952), canonical analysis (Rao, 1952).

Assessment of diversity is traditionally done using morphological characters which are often highly influenced by the environmental factors and so are less efficient in assessing the genetic or genomic relationship. DNA based molecular marker analysis, popularly called DNA finger printing has been used for characterization and identification of commercial varieties and germplasm in many plant species and also polygenetic study in taxonomy. DNA is the ideal molecule for such analysis because of its plasticity, ubiquity and stability. DNA based markers are more powerful in genetic diversity estimation (Liu *et al.*, 1998).

Molecular marker like Random amplified polymorphic DNA (RAPD), Sample sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragmented

Length Polymorpohism (AFLP) and Sequence Tagged Micro-satellite Sizes (STMS) have used for finger printing of plant varieties or cultivars. Inter Simple Sequence Repeat (ISSR) (Tsumura *et al.*, 1996) assess the variation in numerous micro-satellite regions distributed throughout different genomes. ISSR and SSR markers were used for studying genetic diversity in different crops like *Vigna* species (Ajibade *et al.*, 2000) and cowpea (Galvan *et al.*, 2003).

The present investigation in greengram with 21 local land races and four high yielding standard varieties of diverse geographic and genetic origin was taken up with the following objectives.

(1) Characterization of the 21 local land races along with four released varieties used as standard and assessment of variability in seed yield and related traits.

(2) Study of the nature and extent of their sensitivity to cold exposure.

(3) Analysis of association among traits and assessment of direct and indirect effects of component traits on seed yield.

(4) Assessment of divergence among the 21 local land races and four released varieties through D^2 and Canonical analysis, grouping of the varieties into clusters and selection of parents for hybridization programme.

(5) Molecular characterization and DNA finger printing of genotypes using SSR primers and assessment of diversity on the basis of polymorphic banding pattern.

The result of this study will help in selecting suitable high yielding cold tolerance local strains and suitable parents for hybridization and thus will contribute towards more effective utilization of the available local cultivars.

CHAPTER-II

Review of Literature

REVIEW OF LITERATURE

Development of improved varieties with hybrid yield potential is a major objective in pulses. During last five decades breeders have been successfully in development of large number of varieties with high yield potential in different major pulse crops. Greengram is an important grain legume crop in Asian agriculture, particularly in India. Southeast and East Asia, where it complements cereal based diets with a large proportion of digestible protein.

In any crop improvement programme creation of variability in productivity traits is a pre-requisite and it can be achieved by conventional methods involving hybridization or by induction of mutations for success of breeding programme. The breeder should have adequate information on different aspects of the crop such as origin, distribution, cultivation system, cytology, cytogenetics and genomic relationship, crossability with wild relatives, available range of variability in morphological and economic traits, genetics of important traits, reaction to biotic and abiotic stress, nutritional qualities etc.

2.1 Greengram : A potential grain legume

The evolution and taxonomy of grain legumes including the genus *Vigna* have been well documented by Smartt (1990). *Vigna* falls with in tribe phaseoleae and family fabaceae. The extensive studies of Vedcourt (1970) and Marechal *et al* (as cited by Smartt 1990) are largely responsible for the radical reorganization and consideration amplification of genus *Vigna*.

The genus now includes the former Asian *Phaseolus* species and entire genus *Voandzeia*. *V. radiata* was previously known as *Phaseolus aureus* Roxb. *Vigna radiata* has been further subdivided into three subgroups, subspecies radiata, consisting of greengrams and golden grams; subspecies sublobata and subspecies glabra.

Greengram was most likely domesticated in the Indian subcontinent (Smartt, 1984), with archaeological evidence suggesting use in these regions for over 3500 years. Early in the domestication process greengram cultivation spread to other parts of Asia and into North Africa. Cultivated greengram developed through domestication and selection from *Vigna radiata sp. Sp. Radiata,* which is widely distributed through southern and eastern Asia, Africa and Austronesia.

In India the major pulse growing states are Uttar Pradesh, Madhya Pradesh, West Bengal, Orissa, Bihar and Chhattisgarh. Almost 90% of greengram production on world scale is produced in Asia, with India the worlds largest producer, accounting for more than 50% of world production. Most of Indian production is traded and consumed locally where as Thiland is the worlds largest exporter of greengram. Indian ranks second in area under greengram behind China.

Vigna species including greengram belonging to the subgenus Ceratotropis, have chromosome complements typical of the tribe phaseolae with 2n= 2x = 22 with the exception of the polyploidy. *V. glabrescens* 2n = 4x = 44 (Smartt, 1990). Greengram chromosome are small and difficult to study and as a result published reports of the greengram karyotype vary considerably. A comprehensive review of karyotype studies in greengram Poehlman showed that the haploid total chromosome length reported varied from 12-37 μ.

Greengram is a short duration (70-100 days), warm season grown legume adapted to tropical and subtropical condition (Lawn 1979 a, b, 1985). Despite of photoperiod response to short day length, greengram can be grown over a range of latitudes provided temperature exceed 15°C. Greengram crops are short stature, less than 1.25 mt. depending upon variety, plants are generally branched and habit can vary from erect to suberrect in the cultivated types. Leaves are trifoliate and root bear nodule. Flower are butterfly shape, seeds smaller. The seed appearance can vary greatly depending upon the colour. of testa and presence/absence of texture layer. In flower 10 anthers and a single style is present.

Greengram is a pulse or food legume crop primarily used as dried seed and occasionally as forage or green pods and seeds for vegetables (Lawn 1995). On a dry weight basis greengram contains 25 to 28 % protein, 1.0-1.5%, fat, 3.5 – 4.5 % fibre, 4.5 – 5.5 % ash and 60 – 65 % carbohydrate. The seed protein is rich in lysome but low in sulphur, amino acid, methionine and cystein. The seeds are also rich in ascorbic acid, potassium, iron, phosphorus and calcium but low in sodium. The crop is highly affected by YMV. The crop is a fairly drought tolerant and has the ability to do well under poor and shallow soil.

Greengram, as a pulse crop, seems to be very promising compared to other pulse crops of Indian subcontinent and rightly designated as "A potential grain legume".

2.2 Variability, heritability and genetic advance in greengram

Reddy (1997) studied genetic variability in seventy genotypes of greengram varieties of different geographical regions. Genotypic and phenotypic variation were highest for branches/plant followed by grain yield/plant and pods/plant. Days to maturity followed by plant height and pod

length had the highest heritabilities and were least influenced by the environment. Cluster/plant, pods/cluster, seeds/pod, l00 seed weight and grain yield showed high difference in phenotypic and genotypic variation, indicating that the expression of these traits was influenced by environmental components.

Byregowda *et al.* (l997) analysed genetic variability, heritability, genetic advance, phenotypic and genotypic correlation and their direct and indirect effects on grain yield and yield components. High heritability associated with high genetic advance was observed for grain yield and pods/plant, which was mainly attributed to additive gene action. The significant and positive association of grain yield was observed with pods/plant and seeds/pod. Pods per plant, seeds/pod and l00 seed weight exhibited highest direct effect on grain yield.

Vikas *et al.* (l998) investigated variability and heritability of sixty-three greengram varieties and found moderate to high genetic variation; heritability and genetic advance for plant height, number of clusters per plant, number of pods per plant, days to maturity and biological yield.

Manivannan (l999) investigated variability, heritability and genetic advance in F_1 and F_2 progenies of six greengram crosses. Among the cross Vamban l x ME 652 and VGG ll x PMB 27 recorded with GCV, heritability and genetic advance as a percentage of mean. These two crosses were recommended for further selection to obtain high yielding segregants.

Venkateswar (200l) studied genotypic coefficient of variation, heritability and genetic advance in l7 diverse genotypes of greengram. Genotypes differ significantly for 50 % flowering, days to maturity, plant height, number of clusters per plant, number of pods per plant, pod length,

number of seeds per pod except l00 seed weight. Most of the characters showed high heritability values. Seed yield expressed high genetic advance coupled with high heritability and GCV indicating the predominance of additive gene effects for this trait.

Chakraborty and Borah (200l) studied genetic variability, heritability and genetic advance and correlation at genotypic and phenotypic level in 24 greengram genotypes for 5 root characters, i.e., nodules/plant, number of secondary roots/plant, root dry weight and root:shoot ratio and seed yield. Relatively large differences between phenotypic and genotypic coefficients of variability was observed for root length, number of secondary roots per plant and root:shoot ratio indicating that the environment influences these characters. Moderately high heritability with genetic advance for seed yield/plant, nodules per plant and root dry weight suggested the partial role of additive gene effects in their inheritance. However, low heritability coupled with low genetic advance for root length, number of secondary roots per plant and root:shoot ratio indicated that these traits were predominantly governed by non-additive gene effect.

Gajraj *et al.* (200l) examined induced variability in greengram and found that the characters like number of pods/plant, number of seeds/pod and l00 seed weight showed considerable increase in heritability and genetic advance, indicating that these characters can be transmitted to future generation.

Pandey and Singh (2002) studied the genetic variability; yield correlation and performance of l0 greengram genotypes. Significant difference among the genotypes were observed in terms of plant height, number of days to 50 % flowering and maturity, number of seeds/pod, l00 seed weight and yield. Grain yield had significant and positive association with number of seed/pod and test weight.

Pandiyan *et al.* (2006) estimated variability of greengram genotypes. Phenotypic coefficient of variation was found higher than genotypic coefficient of variation in all the traits. Highest PCV and GCV were observed for number of primary branches per plant and lowest for protein content. Broad sense heritability estimates were observed high for all the traits except primary and secondary branches per plant, number of seeds per capsule and total dry matter and found high coefficient of variation for days to 50 % flowering, days to maturity and number of pods/plant. High heritability was recorded for l00 seed weight, number of seeds/pod and pods per plant.

Rao *et al.* (2006) studied variability among 60 greengram genotypes and reported high estimation of genotypic and phenotypic coefficient of variation, heritability and genetic advance for seed yield per plant, biological yield per plant, number of clusters per plant and number of pods per plant.

Pandiyan *et al.* (2006) evaluated greengram genotypes for variability, heritability and genetic advance. The PCV and GCV were higher for single plant yield, number of branches per plant, number of pods per plant, number of clusters per plant, plant height, and length of branch, indicating greater scope of selection for these traits. The estimates of heritability indicated that the number of days to full maturity, number of days to initial maturity, number of days to initial flowering, number of days to 50 % flowering, seed length, seed breadth, plant height, length of branch, l00 seed weight and length of pod were highly heritable. High GA as a percentage of mean was recorded for number of cluster per branch, length of branch, single plant yield, numbers of pods per plant, number of cluster/plant, plant height and number of branches per plant suggesting possibility of selection for these traits. High GA coupled with high heritability and GCV was observed for length of branch, number of branches per plant, number of clusters/branch, number of clusters per plant, number of pods/plant.

Wani *et al.* (2007) studied genetic variability among 20 genotypes of greengram. High heritability coupled with high GA was observed for number of pods per plant, number of pods per cluster, plant height and seed yield.

Mishra *et al.* (2008) while studying 47 genotypes of greengram found significant differences among them for 8 characteristics including yield indicating the presence of genetic variability among the genotype. Difference between PCV and GCV were low for all the characters (50 % flowering, maturity, plant height, branches, seeds/plant, seeds/pod, l00 seed weight and plant yield). High heritability but low genetic advance observed by them for seeds/pod, plant yield and maturity indicating heritability alone does not necessary mean an increase in genetic advance. High heritability along with high genetic advance for a character suggest that phenotypic selection is likely to be more efficient. They found high genetic advance as per cent of mean together with high heritability, genetic advance and GCV for pods/plant, branches/plant, plant height and seeds/pod, indicating sample directional selection could be effective for improving these characters.

Nagaral and Kajidoni (2008) evaluated 34 genotype of greengram and reported significant difference between them for all l0 characters indicating presence of genetic variance among the genotypes. The difference between PCV and GCV were low for characters like l00 seed weight, days to maturity, pod length, number of seeds/pod indicating limited role played by G x E interaction in the expression of these characters.

Singh *et al.* (2009) evaluated genetic variability in l0 diverse genotypes of greengram for yield and other economic traits. The study revealed considerable genetic variability among l0 genotypes for days to 50 % flowering, days to 75 % maturity, plant height (cm), number of productive

branches per plant, number of productive pods/plant, number of seeds/pod, 100 seed weight (g), biological yield per plant (g), harvest index (%) and seed yield per plant (g). High heritability coupled with high expected GA observed for HI, pods/plant, seed yield per plant, biological yield per plant, 100 seed weight, seed per pod, plant height and number of productive branches per plant revealed the preponderance of additive gene effects in the expression of these traits.

Samantray (2009) studied 15 crosses along with 6 parental variety of Mungbean for 4 numbers of quantitative traits and reported significant variability among them for all the characters they studied. The PCV and GCV showed wide variation indicating the influence of environment on the characters expression. The heritability estimate for yield range from 2.90 % in OBGG 12/OUM 11-5 to84.60 % of LGG 460/Pant M-4.

Rahim *et al.* (2010) evaluated 26 greengram genotypes for genetic variability study. They found that significant variations among the genotypes were observed for all characters. High heritability (broad) along with high genetic advance in per cent of mean was observed for plant height, number of pods per plant, number of seeds per pod, 1000 grain weight and grain yield per plant indicating these characters would be best for phenotypic selection.

2.3 Character association in greengram

Reddy *et al.* (1994) studied correlation on 8 yield components in 36 *Vigna radiata* genotypes. They found that pods/plant; pods/cluster and seeds/pod had a strong positive association with seed yield as well as among themselves.

Kanakamany *et al.* (1996) examined 12 genotypes of *V. radiata* and reported that number of pods/plant, productivity/day, chlorophyll and total chlorophyll content showing positive and significant association with grain yield.

Vikas *et al.* (1998) studied phenotypic correlation in segregating and non-seggregating populations of greengram. They studied eight yield related traits in six parents and F_2 progeny of 3 cross combinations and found that seed yield per plant was positively correlated with number of seeds per pod, number of pods/plant and harvest index. In segregating F_2 progeny, positively association of seed yield per plant was recorded with number of pods per plant.

Joseph *et al.* (1999) studied five *Vigna radiata* hybrid. They found that seed yield was positively correlated with plant height, branches and pods per plant.

Afiah *et al.* (2000) evaluated correlation in nine cultivars of greengram. Correlation analysis revealed that number of pods per plant, seeds per pod, 100 seed weight and seed yield per plant had the greatest influence on seed yield/plant.

Raje and Rao (2000) did association analysis for yield and its components in greengram. They studied in 200 germplasm lines along with six commercial varieties of greengram. Those were evaluated in four environmental conditions. They took observations on morphological seed yield and its components and they concluded that plant height, primary branches per plant, clusters per plant, pods per plant, pods per cluster, seeds per pod and seeds per plant were the major components of seed yield per plant. Plant height, primary branches per plant, clusters per plant, pods per plant, seeds per pod and seeds per plant were the major components of biological yield per plant and seeds per pod. Seeds per plant and seed yield per plant are the major components of harvest index.

Venkateswarlu (2001) studied correlation of 13 greengram varieties for seven characters. Association analysis revealed that pods per plant, days to maturity, plant height, 100 seed weight. Seeds per pod and pood length show

significant and positive correlation with seed yield. Pods per plant and seed per pod had maximum positive direct effect on seed yield.

Chakraborty and Borah (2001) evaluated correlation among root characters in greengram. The observation showed that seed yield was positively correlated with root length, nodules per plant and root dry weight.

Rajan *et al.* (2001) examined correlation in the F_2 generation of greengram. They studied in 7 parents and F_2 population of their 21 crosses for 13 character and found that seed yield had significant positive genotypic correlation with number of secondary roots at maturity, dry weight of plant at maturity, plant height, clusters per plant, pods per plant, seeds per pod and 100-seed weight and harvest index. Number of pods, clusters per plant and harvest index showed high positive correlation on grain yield and also with each other.

Pandey and Singh (2002) in a study of correlation of 10 greengram genotypes found that grain yield had significant positive association with number of seeds per pod and test weight.

Sreedevi and Sekhar (2004) studied correlation of morphological attributes in greengram. They took 21 F_4 progenies. Days to 50 % flowering expressed strong positive association with days to maturity. Similarly, cluster per plant displayed strong positive association with pods per plant and pods per plant with seeds per pod.

Pandiyan *et al.* (2006) reported negative association for number of seeds per pod and protein content.

Vijay-Prakash (2006) examined correlation for different traits of 64 greengram lines. Grain yield showed significant positive correlation with pods

per plant, clusters per plant, pod length, seeds per pod and 100 seed weight, while significant negative association with days to 50 % flowering.

Anil and Lokendra (2006) studied correlation in 19 greengram varieties and found that number of clusters per plant and number of productive pods per plant exhibiting significant and positive correlation with seed yield per plant.

Patel *et al.* (2006) in a correlation investigation in greengram for 30 F_3s and their parents found seed yield per plant had significant and positive genotypic correlation with days to flowering, days to maturity and number of pods/plant, while in the case of F_3s, seed yield per plant exhibited significant and positive relationship with days to maturity, number of clusters per plant, numbers of pods per plant, number of seeds per pod and leaf area at genotypic level.

Pandiyan *et al.* (2006) in a correlation study of 646 greengram genotype found pods per plant, number of clusters per plant, number of clusters per branch, number of branches per plant, length of branch, protein content and 100 seed weight exhibiting significant and positive correlation with seed yield. Plant height exhibited significant and positive correlation with branching traits, namely length of branch, number of clusters per plant and number of clusters per branch. Hundred seed weight revealed highly significant and positive correlation with seed dimension, namely length of pod, seed breadth and seed length.

Wani *et al.* (2007) while evaluating correlation in 20 genotypes of greengram varieties found that seed yield exhibited a positive and significant correlation with number of pods per plant followed by number of pods per cluster and pod length.

Saxena *et al.* (2007) studied correlation in 19 greengram genotypes. The result revealed that seed yield per plant showed positive association with

cluster per plant, pods per plant, biological yield per plant and primary branches per plant and harvest index at both phenotypic and genotypic levels.

Mittal *et al.* (2007) examined correlation in 38 genotype of greengram. They found that seed yield per plant was positively correlated with pods per plant and 100 seed weight but it was negatively correlated with days to flowering, days to maturity and plant height.

Samantray (2009) studied the correlation among segregation parameters for yield in F_3 of a six parent half diallele cross in greengram. All the segregating parameters (mean, variance, top 10 % mean of the crosses, selection differential at 10 % SI, positive transgressive segregant for yield, mean of positive transgressive segregants, maximum positive transgressive per yield; average transgression) and found significant correlation among them

Labanya and Toms (2009) studied 132 greengram genotypes for association and interrelationship among yield contributing characters. They studied 9characters and reported seed yield per plant shows positive association with all characters but positive significant association was observed with plant height, number of seeds/plant, pods/cluster, pod length and number of seeds/pod and both phenotypic and genotypic levels, respectively. Five plant height exhibited significant positive association with number of clusters/plant, pods/plant, pod length and 100 seed weight at both phenotypic and genotypic levels. Number of primary branches registered significant positive correlation with number of clusters/plant and clusters/plant had significant positive correlation with number of pods/plant. Pods/cluster recorded positive and significant association with number of pods/plant and seeds per pod. Seed/pod show significant positive association with pod length and 100 seed weight both genotypic and phenotypic level.

Khan and Wani (2009) studied the correlation heritability and genetic advance in M_2 and M_3 generation of greengram and reported yield per plant to be positively correlated with fertile branches/plant, number of pods/plant and l00 seed weight. But yield did not show any association with seed protein content.

Rahim *et al.* (2010) took 26 greengram genotypes for evaluating yield and its contributing characters and reported that the number of pods per plant, panicle length and number of seeds per pod are positively correlated with grain yield.

S. K. Singh *et al.* (2009) studied correlation among twelve quantitative characters in 80 greengram germplasm lines. Analysis of data revealed that primary branches/plant, clusters/plant, pods/cluster and pods/plant were found positively associated at genotypic, phenotypic and environmental level barring non-significant correlation between primary branches/plant at environmental level. Positive association at phenotypic and genotypic level was also recorded between pods/cluster and seeds/pod and between pods per plant and harvest index.

Atar Singh *et al.* (2009) in a correlation study for 9 economic traits of l0 diverse genotypes of greengram found seed yield per plant exhibiting positive association with harvest index followed by days to 50 % flowering at genotypic and phenotypic levels.

2.4 Path coefficient analysis in greengram

Reddy (l994) studied path analysis in 36 *Vigna radiata* genotypes on eight yield components. Path coefficient analysis revealed that the pods per plant, pods per cluster, seeds per pod had a strong positive direct effect on seed yield.

Naidu *et al.* (l994) studied path analysis of yield and yield attributes in different environments in greengram. The data revealed that shoot dry weight, shoot weight, l000 grain weight and pods per plant had positive effects on seed yield.

Niazi *et al.* (1999) investigated path coefficient analysis for 8 agronomic characters affecting seed yield. They found that pods per plant, plant height, number of columns and seeds per pod and a number of clusters per plant revealed a strong positive association with seed yield per plant.

Rajan *et al.* (2000) analysed path analysis of 7 parents and F_2 population of their 21 crosses. They found that pods per plant had the highest positive direct effect on grain yield followed by hundred gram weight on grain yield.

Sreedevi and Sekhar (2004) studied direct and indirect effect of 21 F_4 progenies. They found that pods per plant and seeds per pod contributed very high positive direct effect on yield. Critical analysis of path coefficient indicated that selection for pods per plant, seeds per pod and clusters per plant are more important and promising traits contributing to seed yield.

Sreelakshmi and Sekhar (2005) studied path coefficient analysis in F_3 generation seed of the greengram cross RMG 406 x MGG 330. They reported that the path coefficients among full-sib progenies and F_3 bulk populations revealed that intermating in F_3 was effective in shifting to direct effects of 100 seed weight, pods per plant and plant height on seed yield from a negative value in F_3 bulk population to a positive value in full-sib progenies.

Rao *et al.* (2006) in a path analysis study of 60 greengram genotypes found that the number of pods per plant, biological yield and harvest index had maximum direct contribution on seed yield.

Anil *et al.* (2006) evaluated 19 diverse genotypes of greengram for yield and yield component traits. All the traits (cluster/plant, number of productive pods/plant, plant height, number of productive branches/plant) except plant height and number of productive branches per plant had higher magnitude of

indirect effects than the direct effects on seed yield/plant. The number of productive branches per plant had a direct significant contribution to seed yield per plant.

Saxena and Singh (2007) evaluated 59 genotypes of greengram for 11 traits for path analysis. Their report that an early maturing dwarf plant with high biological yield and harvest index would be suitable for higher seed yield in mungbean.

Rahim *et al.* (2010) studied path coefficient analysis of 26 greengram genotypes for yield and its contributing characters. They reported that the number of pods per plant and number of seeds per pod are important characters.

Singh *et al.* (2009) while analysing path coefficient analysis in eighty greengram germplasm lines for 12 quantitative characters over environments found biological yield per plant, clusters per plant and seeds per pod are most important direct and indirect yield components across three environments.

Labanya and Toms (2009) studied 132 greengram genotypes for path analysis among yield attributing characters. They found seed yield provides the actual contribution of an attribute and its influence through other characters. Number of primary branches per plant, clusters/plant, pods/cluster, pods/plant, seeds/pod and 100 seed weight showed positive direct effect on seed yield/plant, plant height, days to maturity. Pods length registered negative direct effect on seed yield/plant in genotypic and phenotypic path, respectively. Number of primary branches/plant, clusters/plant, pods/cluster, pods/plant, number of seeds/pod and 100 seed weight showed positive direct effect on seed yield/plant indicating that selection for higher yield on the basis of above seed character could be reliable.

2.5 Genetic diversity in greengram

Reddy (1997) studied genetic divergence in greengram. He had taken 70 genotypes from different geographical regions in India. Genotypes were grouped into 8clusters. Days to maturity, pod length, grain yield, plant height, branches per plant and pods per cluster contributed 85 % of total divergence.

Loganathan *et al.* (2001) investigated D^2 using multivariate analysis of 10 quantitative characters. They found that seed yield per plant contributed maximum, accounting for 41.4 % of total divergence.

Granamalar *et al.* (2005) investigated genetic diversity in greengram. They evaluated 59 greengram genotypes and the genotypes were grouped into 8 clusters based on their diversity. Seed yield and 100 seed weight contributed 98 % of the total divergence.

Vijay-Prakash (2006) collected 64 greengram lines from different sources and evaluated for yield and yield components. All the genotypes irrespective of their place of collection were grouped into eight clusters each having 37, 09, 01, 07, 01 genotypes, respectively. Cluster II showed maximum genetic distance and cluster I showed maximum closeness to all other clusters.

Muhammad *et al.* (2007) analysed 40 greengram genotypes for 14 quantitative traits. All the traits were analysed using multivariate analysis technique (cluster and principal component analyses). The first four PCs with eigenvalues, 1 contributed 85.49 % of the variability amongst genotypes. Populations with high PC 1 values were high yielding and early in maturity. The populations with high PCs were late in flowering and maturity and contributed more towards vegetative growth rather than reproductive. The genotypes were categorized in four clusters based on average linkage. Cluster

I, II and IV were more clearly separated from cluster III. Cluster analysis revealed that genotypes under investigation displayed a wide range of variation for most of the traits that could be exploited in breeding programme.

Islam *et al.* (2007) studied genetic diversity of large number of genotypes. They found that the 1st and 2nd principal components of principal component analysis results accounted for 58 and 14 %, respectively. There were 7clusters distinguished in cluster analysis. The genotype in 4 and 6 clusters perform better in respect of relative total dry weight and relative root dry weight, respectively, and hence having flooding tolerance. The genotypes in clusters 7 and 1 performed very poorly and those at under cluster 3, 2 and 5 were moderate to poor. D_2 analysis indicated that the clusters differed significantly from each other.

Rahim *et al.* (2010) studied genetic divergence of 26 greengram genotypes for yield and yield contributing characters. The 26 genotypes were grouped into III clusters. Maximum number of genotypes (12) was grouped into cluster II. The maximum range of variability was observed for number of pods per plant (12.22 – 20.55) among all the characters in 3 clusters. Crosses involving cluster I and III may exhibit high heterosis for yield as well as earliness

2.6 Greengram and cold

Tropical and subtropical plants exhibit marked physiological and biochemical dysfunctions commonly referred to as chilling injury when they are exposed to temperature below 10°C to 12°C (Graham and Patterson, 1982; Wang, 1982; Guy, 1990). These dysfunctions include alteration in membrane structure and lipid composition (Lyons and Raison, 1970) metabolic modifications (Sochasnowicz and Kaniuga, 1979; Levitt, 1980; Trevanion *et al.*, 1995), changes in protein content (Marmiroli *et al.*, 1986; Bredenkamp and

Baker, 1994) and enzyme activities (Byrd *et al.*, 1995; Kumar and Tripathy, 1998), phosphorylation of thylakoid proteins (Bannett, 1991), cyclosis (Lewis, 1956), redistribution of intracellular calcium ions (Bush, 1995), cellular leakage of electrolytes and amino acids, and a diversion of electron flow to alternate pathways (Leopold and Musgrave, 1979). Dysfunctions associated with chilling stress in greengram seedlings maybe attributable to the alteration of gene expression (Guy *et al.*, 1985; Kurkela and Franek, 1990; Ouellet *et al.*, 1993; Hughes and Dunn, 1996; Kung *et al.*, 1998). However, there is still a paucity of information on the effects of cold acclimation and root temperature on chilling injury.

Chang *et al.* (2000) exposed greengram seedling to 4°C for 2 days and found induced irreversible chilling injury. The major cation in the leakage from tissues of unaculimated seedlings was K^+ the loss of which was 7 – 10 fold greater than that of Ca^{2+} or Mg^{2+}. Acclimation of seedlings at 10°C protected them from the injuries caused by 4°C treatment. Acclimation of seedling at 10°C for 2 – 3 days significantly decreased the conductivity and concentration of soluble sugars, free amino acids, and cations (K^+, Mg^{++} and Ca^{++}) in the leakage compared to the 28°C-root/28°C shoot, control seedlings. Though in the 28° C root/4° C-shoot treatment did not suffer noticeable injury, but seedlings in the 4° C-root/4° C shoot treatment did. The solute potential, water potential, and concentration of free amino acids and cations (K^+, Mg^{++}, and Ca^{++}) in the cell sap of the 28°C root/4°C shoot seedlings were similar to those of controlled seedlings.

Yu Chin-Wen *et al.* (2003) studied survival rates of greengram seedlings chilled at 4°C for 36 hour from pretreatment with 200 mMH_2O_2 increased survival rate of seedling from 30 to 70 %. These results suggest that the H_2O_2-induced chilling tolerance in these plants might be mediated by an elevation of glutathione content and is independent of the ABA mechanism of chilling protection.

2.7 SSR

Microsatelite markers are the markers of choice for genetic studies like genetic diversity assessment, genetic mapping, population genetics and marker assisted selection. Simple sequence repeats (SSRs) consist of randomly repeated units of short nucleotide motifs that are l – 6bp long, Di-, tri-, and tetra nucleotide (*e.g*, (CA)n, (AAT)n and (GATA)n, respectively) repeats are the most common and are widely distributed throughout the genomes of plant and animals (Jarne and Lagoda, l996).

2.7.1 Greengram and SSR

Conventional breeding methods have been employed in the past to solve some of the problems at a slow pace. The use of molecular marker technology can help accelerating greengram improvement process through the marker assisted selection technique. From among the markers, micro satellites offers several advantages, they are highly reproducible, highly polymorphic, PCR based and readily portable within a species. Conventional breeding methods are being deployed to address the problems in these crops at a slow pace. The breeding and selecting process for developing a new high yielding variety with resistance to cold is an arduous process, that may take up 8 – l0 years. Multienvironment testing will always be required to confirm that the identified phenotype indeep have desired agronomic characters combined with resistance. However, in current practice a significant amount of efforts is devoted to testing selections that simply do not have the genetic potential. Hence, tools for determine the genotypes of the experimental lines increase the efficiency of the selection process.

Using suitable DNA markers for selection will help in identify the right genotype that are resistant. Development of markers to identify cold resistance

in greengram and deploying them through marker-aided selection in breeding programme would fasten the process of developing resistant lines.

Choumane *et al.* (2004) while working on different taxa of leguminosae reported that transferability of SSR primers across species may potentially decrease the development cost and increase SSR markers utility.

Dikshit *et al.* (2006) studied genetic differentiation of *Vigna* species including greengram by RAPD, URP and SSR markers. They found an insight for *Vigna* species coevolution, domestication and interspecific relationship. The cluster analysis of combined data set of all the markers resulted in five major groups. The Mantel matrix correspondence test resulted in a high matrix correlation with best fit ($r = 0.95$) from combined marker data. Comparison of these markers system showed that SSR marker was more efficient in detecting genetic variability.

Chontira *et al.* (2007) studied the genetic diversity of greengram gene pool on the microsatellite analysis covering 415 cultivated, 189 wild and 11 intermediate accessions by using 19 SSR primers. These primers showed polymorphism in wild and cultivated greengram and were selected from those available for the related species azuki bean [*V. angularis* (wild) Ohwi). One or more SSR primers for each linkage group on the basis of azuki linkage map was analysed. In total, 309 alleles were detected and of these, about twice as many were detected in wild (257 alleles) as in cultivated accessions (138 alleles). The resulted show that Australia and New Guinea represent a distinct centre of diversity for wild greengram. Cultivated greengram has greatest diversity in South Asia, Asia, which supports the view that South Asia is where this crop was domesticated. SSR marker allelic diversity for cultivated greengram has distinct regional variation with high variation in South and West Asia. This study represented the first comprehensive analysis of wild and

cultivated greengram germplasms diversity by SSR marker and highlights specific genetic diversity that might be used to broaden the genetic base of currently grown greengram cultivars.

Reddy *et al.* (2008) studied genetic diversity in 30 greengram genotypes by using 19 SSR markers. From 19 SSR marker, 10 marker showed amplification. Of the 10 primers 7 showed polymorphism with 1.6 polymorphic fragments per primer. The SSR primers amplified 1 to 3 alleles of 150 bp to 300bp. Of the 16 amplified bands 12 (75 %) were polymorphic with an average of 1.2 polymorphic fragments per primer.

Dikshit *et al.* (2009) studied the molecular characterization of fixed lines from diverse cross in mungbean using SSR markers and concluded that molecular analysis is more useful in characterization of advanced breeding lines.

CHAPTER-III

Materials and Methods

MATERIALS AND METHODS

An investigation on "Genetic architecture of yield and cold tolerance in greengram (*Vigna radiata* (L.) Wilczek) was taken up under Department of Plant Breeding and Genetics and field trial was conducted at EB-II.

3.1 Materials and experimental design of field evaluation

Materials for the present study included 21 local land races and 4 released varieties of greengram (Table 4). The field trial was conducted in RBD with three replications. Trial was sown on 19th October, 2009 and each entry was represented by 3 rows of 2.8 mt length with a spacing of 30 cm x 10 cm. A fertilizer dose of 20:40:20 kg NPK/ha was applied and need based plant protection measures followed.

3.2 Observations recorded

Observations on days to 50% flowering were taken on plot basis. For other characters like plant height (cm), clusters/plant, pods/plant, pod length (cm), seeds/pod, 100-seed weight (g) and yield/plant (g) observations were recorded on 10 randomly chosen plants per plot in each replication. The observations recorded were as follows.

Days to 50 % flowering: Days from sowing to the date on which 50 % plants in the plot have at least one flower open.

Plant height (cm): Height of the plant from ground to the tip of main stem was recorded.

Clusters per plant: Number of fruit bearing bunches or a cluster in the plant was recorded.

Pods per plant: Total number of well developed pods borne in the plant was recorded.

Pod length (cm): Average length of ten random pods per plot was recorded.

Seeds per pod: Seeds per pod was calculated by dividing total number of seeds of the 10 pods by total number of pods.

100-seed weight (g): Weight of l00 random seeds was taken.

Yield per plant (g): Weight of all seeds obtained from the 10 sample plants was recorded.

Response to cold exposer was studied under controlled condition in the S.K. Sinha Molecular Breeding Laboratory where the genotypes were exposed to 10^0C temperature at 10 days, 20 days, 30 days and 40 days seedling stage. Observations were taken on number of plant wilted in each genotype every day continuously and a day to survival under cold stress was recorded.

After recording observations on 10 plants/plot, average were taken and the mean data taken for biometrical analysis.

3.3 Molecular marker study by SSR analysis

Plant materials

Twenty five greengram (*Vigna radiata*) varieties were taken for SSR analysis. Seedlings were grown in pots and fresh and young leaf samples were collected from 5 – 20 days old seedlings for isolation of genomic DNA.

Isolation of genomic DNA

Genomic DNA was isolated from tender young leaves which were harvested freshly before sunrise and washed thoroughly with cold autoclaved,

distilled water and then blotted to dry. About two grams of young leaves were excised from the upper tip portion and DNA was extracted on the same day of collection. Total genomic DNA from the leaves was isolated by using standard CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1990) with little modification. Insoluble polyvinyl polypyrrolidone (PVPP) was added to the leaf tissue prior to grinding. Two grams of fresh leaves of each land race were taken and ground to fine powder with liquid nitrogen in a mortar and pestle. The solution was then transferred to a sterile 50 ml centrifuge tube, containing preheated 10 ml of DEB (DNA extraction buffer – 10 mM Tris-HCL pH 8.3, 20 mM EDTA (pH 8.0), 2 % CTAB, 1.4 M NaCl, 0.2 % mercaptoethanol) which was preheated at 65°C in water bath. Each sample was mixed thoroughly and was incubated in water bath at 65°C for 1 hour by occasional gentle shaking. After cooling, equal volume of 24:1 chloroform isoamyl alcohol (v/v) was added and mixed gently. The centrifugation was carried out at 12,000 rpm for 20 min at 20°C. The upper supernatant was carefully transferred into another centrifuge tube. Then 0.6 volume of prechilled isopropanol was added to precipitate the DNA. The DNA was precipitated into another microcentrifuge tube and washed with 70 % ethanol twice and kept for air-drying at room temperature. The air dried crude DNA was then dissolved in sufficient amount of $T_{10}E_1$ (Tris 10 mM-1 mM EDTA, pH 8.0) buffer.

Purification of DNA

The dissolved DNA is the crude DNA and requires further purification. The RNA was removed by giving RNAse treatment. For one ml of DNA solution, 60 µg of RNAse A was added and the solution was incubated with continuous shaking in water bath at 37°C for 1 hour. After 1 hour it was removed from the water bath and equal volume of chloroform: isoamylalcohol

(24:1) was added and mixed thoroughly but gently. The solution was then centrifuged in 10,000 rpm for 10minutes with a medium speed centrifuge and upper aqueous phase was pipetted out. Starting from the addition of chloroform-isoamylalcohol, the entire process was repeated twice. For further purification, the DNA solution was washed with phenol:chloroform: isoamylalcohol (25:24:1) and subsequently thrice with chloroform: isoamylalcohol (24:1). The upper aqueous phase was separated after centrifugation as per the procedure described earlier and mixed with $1/10^{th}$ volume of 3M sodium acetate (pH 4.8). DNA was precipitated by adding 2.5 volume of chilled absolute ethanol and pelleted by spinning. The pellet was washed twice with 70 % ethanol carefully and dried under vacuum. The dried DNA was dissolved in minimum amount of $T_{10}E_1$ buffer (pH 8.0).

Test for quality and quantity of the purified DNA:

The quality as well as quantity of DNA was also checked by Uv-vis spectrophotometer (Jasco V 350, Japan). The absorbance at 260 nm wave length gave the quantity of the total DNA and the ratio of the absorbance at 260 and 280 nm indicated the quality of the purified DNA. The DNA was loaded in 2 % agarose gel along side diluted uncut lambda DNA as standard to recheck the quality and quantity and it was observed, the DNA from all the samples were qualitatively good. The quantification was done in comparison with the known standard. After quantification, the DNA was diluted in $T_{10}E_1$ buffer to a working concentration of 25 ng/µl for PCR analysis.

Simple sequence repeats (SSR) analysis

For SSR analysis 44 selected SSR primers (Chromous biotech Pvt. Ltd.) were used for PCR amplification. Out of which only 19 primers (CEDG 050, CEDG 08, CEDG 010, CEDG 020, CEDG 043, CEDG 086, CEDG 088,

CEDG 091, CEDG 092, CEDG 139, CEDG 154, CEPG 228, CEDG 156, CEDG 248, VR_1, VR_2, VR_4, VR_5, VR_6, VR_9, VR_{11} and VR_{13}) produce scorable amplification. Each amplification reaction mixture of 25 µl contain 50 ng of template DNA, 0.5 unit of *Taq* DNA polymerage, 0.1 mMi each dNTP 0.2 mM primer (each forward and reverse) in 1 x reaction buffer that contained 10 mM Tris-HCl (pH-8.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0:01% gelatin. Amplification condition were 1 cycle at 94°C for 4 min., 35 cycles at 94°C for 1 min, 47°C for 1 min., and 72°C for 1 min. followed by 1 cycle of 10 min. at 72°C. Amplification products were electrophoresed on 2 % agarose gel. SSR primers derived from mungbean (*Vigna radiata*) and Azuki bean (*V. angularis*) were tested for their ability to support amplification in greengram.

Agarose Gel Electrophoresis

The amplicons were separated in 1.5 % agarose gel. Three grams of agarose was added to 200ml of 1 x TAE buffer pH 8.0 (0.04M Tris-acetate, 0.001M EDTA) boiled for complete melting of agarose, then cooled to 50°C. Ethidium bromide (EtBr) (0.5µg/ml of gel solution) was added and casted on the gel-casting tray. Twenty-six well comb was used for formation of quality gels. After complete gelling, the gel was transferred to the submarine gel tank containing lX TAE buffer. Prior to loading the samples the comb was removed. In the submerged gel 20 µl of the PCR samples were loaded in each well along with a single well loaded with standard DNA ladder (100 bp DNA ladder plus, Chromous Biotech Ltd.). The electrophoresis was performed in a constant voltage at 55 v for 3 hours. The amplicons were visualized under the uv light and photographed. The gel was also documented by Gel doc system for scoring the bands. The sizes of the amplicons were determined by comparing them with that of the ladder.

Scoring the data

The data on presence or absence of different amplification products or bands in the 25 genotypes were scored in each SSR primer from the Gel electrophoresis photographs. All monomorphic and polymorphic band were scored. For similarity coefficient analysis for each band genotypes showing presence were given score 1 and those showing absence given score 0.

The data analysis was performed using NTSYS-pc (Numerical taxonomy system, version 2.0 (Rohlf, 1990). The SIMQUAL programme was used to calculate the Jaccard's coefficient, a common estimator of genetic identity and was calculated as follows.

$$\text{Jaccard's coefficient} = \frac{N_{AB}}{(N_{AB} + N_A + N_B)}$$

Where N_{AB} = The number of bands shared by samples

N_A and N_B = Amplified fragments in sample A and B respectively

Similarity matrices based on these indices were calculated. Similarity matrices were utilized to construct the UPGMA (Unweighted pair group method with arithmetic average) dendrograms.

3.4 Statistical methods

Observations on the 12 component characters were recorded on the 25 greengram genotypes in each of three replications. Statistical analyses carried out on the data recorded are outlined in the following paragraphs.

3.4.1 Analysis of variance

Analysis of variance for each character was carried out in RBD with plot means for partitioning of total variance into components (Table 1). The test of significance of difference among lines was done by 'F' test.

Table I. ANOVA for RBD with expectation of mean squares (MS)

Source	Df	MS	F	Expectation of MS
Replication (R)	(r-1)	MSr	MSr/MSe	$\sigma_e^2 + g\sigma_r^2$
Genotype (g)	(g-1)	MSg	MSg/MSe	$\sigma_e^2 + r\sigma_g^2$
Error (e)	(r-1) (g-1)	MSe	-	σ_e^2

The test of significance of difference between means of two lines (genotypes) were done by 't' test and critical difference (CD at 5 %) was calculated as follows.

CD (at 5 %) = $(2MSe/r)^{1/2}$ x $t_{0.05}$ at error df

3.4.2 Estimation of genetic parameters:

Variance components: The phenotypic, genotypic and environmental variance components for different characters were estimated from the mean squares in ANOVA according to Aljibouri*et al.* (1958) as follows.

Environmental variance : $(\sigma_e^2) = MS_e$

Genotypic variance : $(\sigma_g^2) = \frac{MS_g - MS_e}{r}$

Phenotypic variance : $(\sigma_p^2) = \sigma_g^2 + \sigma_e^2$

Where, MS_g and MS_e are mean squares due to genotype and error, respectively, and 'r' is the number of replication.

Coefficient of variation: The phenotypic and genotypic coefficients of variation for different characters were estimated as follows.

Phenotypic coefficient of variation (PCV) = $\frac{\sigma_p}{\bar{x}} \times 100$

Genotypic coefficient of variation (GCV) $= \frac{\sigma_g}{\bar{x}} \times 100$

Where, σ_p x σ_g are square root of phenotypic and genotypic variance, respectively and $\bar{x}$ is grand mean for the character.

Heritability : Estimation of heritability (in broad sense) of different characters was done by the following formula using the components of variance as follows.

Heritability (h^2) = $= \frac{\sigma_g^2}{\sigma_p^2}$

Genetic advance (GA): The expected genetic advance or genetic gain as a result of selection at 5 % selection intensity among the genotypes for different characters was calculated as follows:

$GA = k.h.\ \sigma_g = k.h^2.\ \sigma_p$

Where, k = standardized selection differential for specified selection intensity (k = 2.06 at 5 % selection intensity)

h = square root of heritability coefficient

σ_g = square root of genotypic variance

σ_p = square root of phenotypic variance

For comparison of GA of different characters, GA was expressed as percentage of mean of the characters.

GA (as % of mean) $= \frac{GA}{Mean} \times 100$

3.4.3 Estimation of genotypic and phenotypic correlation

The analyses of co-variance between all possible pair of 12 characters were done with plot means as in Table 2. The symbols and contents with respect to the components of co-variance in mean sum of products are analogous to that of mean squares and the components of variance as described earlier.

Table 2. Analysis of co-variance in RBD with expectation of mean sum of products (MSP)

Source	Df	MSP	Expectation of MSP
Replication	(r-1)	MSP_r	$\sigma_{e(xy)} + g\,\sigma_{r(xy)}$
Genotype	(g-1)	MSP_g	$\sigma_{e(xy)} + r\,\sigma_{gxy}$
Error	(r-1)(g-1)	MSP_e	$\sigma_{e(xy)}$

The phenotypic, genotypic and error component of co-variance between two characters were estimated according to Al-Jibouri *et al.* (1958) in similar manner as described under the components of variance.

The variance and co-variance analysis were made for the characters based on the 25 genotypes as described earlier. Utilizing the various components of variance and co-variance, the genotypic and phenotypic correlations were computed according to Al-Jibouri *et al.* (1958) by following formula.

$$\text{Genotypic correlation } (r_g) = \frac{\sigma_{g(xy)}}{\left[\sigma^2_{g(x)}.\sigma^2_{g(y)}\right]^{1/2}}$$

Where, $\sigma^2_{g(xy)}$ is the genotypic co-variance between x and y and $\sigma^2_{g(x)}$ and $\sigma^2_{g(y)}$ are the genotypic variance for the characters x and y, respectively.

$$\text{Phenotypic correlation } (r_p) = \frac{\sigma_{p(xy)}}{\left[\sigma^2_{g(x)}.\sigma^2_{g(y)}\right]^{1/2}}$$

Where, $\sigma^2_{p(xy)}$ were phenotypic co-variance between x and y and $\sigma^2_{p(x)}$ and $\sigma^2_{g(y)}$ are phenotypic variance of x and y, respectively.

Significance of correlation co-efficient was tested by t-test with (n – 2) degrees of freedom by the formula.

$$t = \frac{r}{\left[\left(1-r^2\right)/(n-2)\right]^{1/2}}$$

where, 'r' is the correlation co-efficient and 'n' us the number of genotypes.

Path co-efficient analysis

The path co-efficient analysis gives cause and effect relationship among the various correlated characters. Path co-efficient are standardized partial regression co-efficient which individually provide measures of direct effect of each causal factor on the effect variable. It permits partitioning of the correlations between the causal factors and the effect variable into components of direct and indirect effects and thus, gives a better picture of the association of causal factors with the effect variable.

In the present investigation seed yield is taken as the 'effect' and 11 growth component characters related to yield as 'causal factors'. Path co-efficient are obtained by solving simultaneous equations, which gives the basic relationship between correlations (Wright, 1921; Dewey and Lu, 1959).

$$P_{1,12} + r_{1,2}\, p_{2,12} + r_{1,3}\, p_{3,12} + \ldots\ldots\ldots\ldots + r_{1,11},\, p_{11,12} = r_{1,12}$$

$$r_{2,1}\, p_{1,12} + p_{2,12} + r_{2,3}\, p_{3,12} + \ldots\ldots\ldots\ldots + r_{2,11}\, p_{11,12} = r_{2,12}$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$R_{11,1}\, P_{1,12} + r_{11,2}\, P_{2,12} + r_{11,3}\, P_{3,12} + \ldots\ldots\ldots + P_{11,12} = r_{11,12}$$

Where, r_{ij} is the correlation co-efficient between i^{th} and j^{th} character and $p_{i,12}$ is the path co-efficient (direct effect) of i^{th} character on yield (12^{th} character).

3.4.5 Multivariate analysis of diversity among genotypes:

Multivariate analysis of divergence among 25 greengram genotypes based on the twelve productivity traits was done in two methods.

(i) D^2 analysis of genetic divergence

(ii) Canonical analysis

(i) D^2 analysis of genetic divergence

Mahalanobis' D^2 statistic (Rao, 1952) was used for estimation of genetic divergence among the 25 genotypes of greengram for twelve characters. Genetic divergence (D^2) between any two genotype is given by the formula.

$$D_p^2 = \sum_{i=1}^{p} \sum_{j=1}^{p} w_{ij}\, d_i\, d_j$$

where, w_{ij} is the inverse of the common dispersion matrix (w_{ij}), d_i and d_j are the difference in the means of the 2 genotypes for i^{th} and j^{th} characters.

The computation of D^2 using this formula is complicated and laborious when more number of mutually correlated characters is involved in the diergence analysis. So the characters means were transformed into set of uncorrelated variable using pivotal condensation of common dispersion matrix following Rao (1952). After this transformation, the formula for genetic divergence is $D_p^2 = \sum_{i=1}^{p} d_i^2$.

where, d_i is the difference between the transformed means of any two genotypes for the i^{th} character.

The relative contribution of individual characters to overall genetic divergence among the genotypes can be assessed. The contribution of each character was assessed by two methods.

(a) Rank average: In all the D^2 combinations, the characters were ranked 1 to 12 on the basis of their contribution to the D^2. Then ranks of each character are summed over the 300 D^2 combinations to get rank total and then rank average.

(b) Average D^2 : Average contribution of each character to all the 300 D^2 combinations is worked out.

Grouping of genotypes into different clusters

i) Tocher's method

Usually a cluster is defined as a group of genotypes or varieties or lines such that any two genotypes belonging to the same cluster should, on an average, show a smaller D^2 than those belonging to the different clusters. A simple device suggested by Tocher (Rao, 1952) for construction of clusters is to start with two most closely related genotypes (having the smallest D^2) and then find a third one which has smaller average D^2 from the first two and so on. At certain stage when it is felt that after adding a particular population, there is a disrupt increase in the average D^2, this population is not added to cluster. Similarly, construction of 2^{nd} and 3^{rd} and other clusters are formed till all the genotypes are included in one or the other cluster. Singh and Choudhari (1977) suggested a method for determining cut off value for addition of a genotype/population to a cluster. In that the D^2 values of each genotype with all others are to be arranged from lowest to highest values in matrix form. The highest value of the lowest column is taken as cut off value for deciding on

inclusion a genotype in the cluster. After construction of clusters, average intra-cluster and inter-cluster D^2 value were estimated.

ii) Canonical analysis

This method is an extension of multiple regression analysis and is concerned with the study of association or interdependence of two set of variables. This method can be used as a forecast model and for clustering purpose as well. The canonical analysis (Rao, l952) involve calculations of canonical vectors or canonical roots and the first two canonical root values (Z_1 and Z_2) of each genotypes/line were taken for two dimensional presentations of the genotypes in graph. This can help in clustering of genotypes or be a supplement to grouping on the basis of D^2 values.

CHAPTER-IV

Results and Discussion

RESULTS AND DISCUSSION

The present investigation on "Genetic architecture of yield and cold tolerance in greengram" among the local land races and released varieties was undertaken in the Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Bhubaneswar. The twenty-five genotypes evaluated comprised of 21 local land races and 4 high yielding varieties. Field trial was conducted in RBD with three replications at EB-II of this department where as, molecular characterization and responses to cold were studied in S. K. Sinha Molecular Breeding Laboratory of the said department.

Observation on days to 50 % flowering, plant height (cm), clusters/plant, pods/plant, pod length (cm), seeds/pod, l00-seed weight (g) and yield/plant (g) were recorded from the field trial and analysed in RBD to get information on genetic parameters of yield and its component traits, correlation among traits and direct and indirect effects of component traits on yield, which would help the breeders in selection of promising lines. Genetic divergences among the genotypes were assessed through D^2, canonical analysis and the genotypes are grouped into different clusters. It would help in selection of parents for hybridization programme.

4.1 Characterization of local land races

The 25 genotypes (21 land races and four released varieties) showed wide variation in all twelve traits including yield and the differences were highly significant (at 1 % level) for all the traits (Table 3). Mean performance of the 25 genotypes for all the 12 characters is presented in Table 4. The variation in days to 50 % flowering in the genotype was 33.33 – 47.66 days. The genotype Bijapur local, Kalamuga (Phulbani local), Nandika local, Sudhasarangi local were late, whereas OUM ll-5, Dhauli, TARM I, OBGG-52 were early. The genotype also showed wide variation in plant height

(11.86 – 31.96 cm), clusters/plant (2.60 – 4.5), pods/plant (7.46 – 15.26), pods length (6.133 – 9.167), seeds/pod (8.43 – 11.6) and 100-seed weight (2.00 – 4.11 g). maximum tolerance to cold was shown by different local land races when they were exposed to cold at different stages of seedling growth. The genotypes Nayagarh local, Kapurgaon local, Ratila local and Kalahandi local-2B showed tolerance to cold when exposed at 10, 20, 30 and 40 days of plant growth.

Venkateswarlu, O. (2001) evaluated 17 diverse genotypes of greengram and observed much wide range of variation for several morphological and agronomical traits. Similar wide range of variation for different traits in greengram with different sets of collection have been reported by Byregowda *et al.* (1997); Loganathan *et al.* (2001); Chakraborty and Borah (2001); Manivannan (2002); Khattak *et al.* (2004); Vijay-Prakash (2006); Kousar *et al.* (2007); Wani *et al.* (2007); Rozina *et al.* (2007); Rehman *et al.* (2009) and Rahim *et al.* 2010). The range of variation in different traits in the present study is wide because of their diverse origin and geographical adaptation.

The genotypes Dhauli, OUM-11-5, Nayagarh local-A, Kalahandi local-2B had more clusters/plant. Jharsuguda local, Kendrapada local-A, Sudhasarangi had more pods/plant. Jhain muga (Baragarh), Bilipara local, Kapurgaon and Sikri local had more seeds/pod. Kapurgaon local and Khadabhanga local had very bold seeds (100-seed weight > 4.00 g) and Dhauli, OUM 11-5, OBGG-52, TARM-I had moderately bold seeds. Seed yield of genotypes varied from (1.26 to 4.26 g/plant) and higher yielding genotypes were Jharsuguda local followed by Kendrapara local-A, Kapurgaon local and Kamakhya local. Yield levels of the four check varieties, viz., Dhauli, OBGG 52, OUM 11-5 and TARM-I varied from 1.643 (TARM-I) to 2.42 (OBGG-52) g/plant. High yield potential of Jharsuguda local was due to more pods/plant and more seeds/pod with moderate 100-seed weight where as, Kendrapada local-A was with highest number of clusters/plant and more number of pods/plant and seeds/pod.

Table 3. Analysis of variance for twelve characters in greengram.

Sl. No.	Character	Source	df	SS	MS	F
1	Days to 50% flowering	Replication	2	35.518	17.759	14.251 **
		Genotype	24	666.745	27.781	22.293 **
		Error	48	59.815	1.246	
2	Plant height	Replication	2	18.376	9.188	1.829
		Genotype	24	2024.215	84.392	16.788**
		Error	48	241-149	5.024	
3	Cluster / plant	Replication	2	808	0.404	3.766 *
		Genotype	24	16.509	0.688	6.416 **
		Error	48	5.146	0.107	
4	Pods / plant	Replication	2	0.297	0.148	0.063
		Genotype	24	457.022	19.043	8.123 **
		Error	48	112.524	2.344	
5	Pod length (m)	Replication	2	2.308	1.154	4.564 *
		Genotype	24	57.487	2.395	9.476 **
		Error	48	12.133	0.253	
6	Seeds / pod	Replication	2	2.109	1.054	1.352
		Genotype	24	71.586	2.983	3.828**
		Error	48	37.418	0.780	
7	100-seed weight	Replication	2	2.188	0.094	1.650
		Genotype	24	24.976	1.041	18.224 **
		Error	48	2.741	0.057	
8	Survival of 10 days old seedling at 10°C (d)	Replication	2	5.840	2.92	3.223*
		Genotype	24	4778.667	119.111	219.742**
		Error	48	43.493	0.906	
9.	Survival of 20 days old seedling at 10°C (d)	Replication	2	8.642	4.32	0.957
		Genotype	24	3532.589	147.191	32.605 **
		Error	48	216.691	4.514	
10.	Survival of 30 days old seedling at 10°C (d)	Replication	2	4.347	2.173	1.498
		Genotype	24	5131.387	213.808	147.341**
		Error	48	69.653	1.451	
11.	Survival of 40 days old seedling at 10°C (d)	Replication	2	0.721	0.360	0.351
		Genotype	24	3374.721	140.613	136.963 **
		Error	48	49.279	1.027	
12	Yield / plant	Replication	2	0.238	0.119	0.475
		Genotype	24	41.843	1.743	6.963**
		Error	48	12.020	0.250	

* Significant at 5 % level, ** Significant at 1 % level

Table 4. Mean performance of the greengram genotypes for twelve characters

Sl. No	Genotypes	Days to 50% Flowering	Plant Height (cm)	Cluster/ plant	Pods/ plant	Pod length (cm)	Seed/ pod	100 seed weight (g)	Survival of 10 days old seedling at 10°C (d)	Survival of 20 days old seedling at 10°C (d)	Survival of 30 days old seedling at 10°C (d)	Survival of 40 days old seedling at 10°C (d)	Yield (g/pl.)
1.	Dhauli	37.66	31.97	4.03	7.50	6.86	9.66	3.96	12.66	32.00	15.00	10.33	2.36
2.	OUM-11-5	33.33	19.53	4.20	7.86	6.13	10.16	3.31	6.66	38.00	16.33	21.66	2.01
3.	OBGG-52	37.00	23.70	3.23	9.00	7.03	10.33	3.43	4.00	23.33	18.33	12.33	2.42
4.	TARM-1	37.33	19.63	3.16	7.46	7.03	8.53	3.35	4.00	11.33	12.33	20.33	1.64
5.	Tigiria Local-A	39.00	15.20	3.93	11.93	8.50	10.56	2.72	2.66	34.00	11.00	19.33	2.90
6.	Kendrapara Local A	41.00	29.46	4.53	15.26	8.90	10.53	2.72	16.00	25.33	21.33	11.33	3.87
7.	Bhawanipatna Local-B	42.00	16.36	3.43	8.167	7.60	9.33	2.54	23.33	33.00	23.00	21.33	1.46
8.	Kapurgaon Local	41.00	17.33	3.93	8.46	8.93	11.10	4.11	7.33	48.0	18.00	12.33	3.38
9.	Nandika Local	45.00	15.60	3.26	8.26	9.60	9.53	3.96	6.66	29.33	3.00	12.33	2.63
10.	Sikri local	39.33	13.83	3.46	10.70	8.96	11.06	2.80	20.66	35.66	4.00	19.33	2.72
11.	Sheragarh Local	41.00	11.86	3.96	11.86	9.16	9.50	2.42	21.00	37.00	14.33	18.33	2.34
12.	Ratila local	40.00	16.30	3.66	12.63	8.53	10.83	2.37	20.33	38.00	46.32	16.00	2.79
13.	Khadabhanga local	42.66	20.10	2.70	7.90	9.56	10.76	4.02	14.00	29.66	26.66	16.00	2.73
14.	Sudhasarangi Local	44.00	26.23	3.53	14.30	8.76	9.10	2.68	22.00	35.33	4.0	17.00	2.97
15.	Jharsuguda local	41.00	19.30	3.86	16.60	9.30	10.76	2.71	24.33	40.00	15.00	11.33	4.26
16.	Kamakhya local-B	40.33	14.30	3.63	12.36	9.13	11.26	2.68	22.33	35.33	21.00	13.33	3.22
17.	Jainmuga local (Baragarh)	41.66	22.86	3.96	11.96	8.56	11.60	2.54	15.33	33.33	18.00	13.00	3.11
18.	Kalahandi local-1A	41.00	24.96	3.56	11.56	8.93	9.80	2.44	20.66	40.66	20.66	14.33	2.34
19.	Keonjhar local	40.00	17.30	2.60	8.40	8.30	10.63	2.03	6.66	42.00	20.00	15.00	1.54
20.	Banapur local-C	39.33	20.00	3.50	9.63	7.60	10.40	2.87	4.66	34.00	11.66	9.33	2.46
21.	Bilipara local	39.66	21.33	3.56	11.70	8.40	11.53	2.32	3.66	38.33	15.00	18.00	2.36
22.	Kalamungo local (Phulbani)	46.33	27.30	3.33	9.60	8.96	8.70	2.54	22.33	33.33	17.33	9.66	1.62
23.	Bijapur local	47.66	29.20	2.86	7.36	8.60	8.43	2.44	17.66	33.33	13.66	17.33	1.26
24.	Nayagarh local	40.66	20.70	4.00	9.36	8.20	8.40	2.42	27.00	34.00	15.00	19.66	1.48
25.	Kalahandi local 2B	42.33	22.56	4.26	9.73	7.96	8.70	2.50	4.00	38.66	19.33	44.00	1.95
	Mean	**40.84**	**20.67**	**3.60**	**10.51**	**8.38**	**10.02**	**2.88**	**14.00**	**34.12**	**16.81**	**16.52**	**2.48**
	CD (5 %)	**1.822**	**3.66**	**0.53**	**2.50**	**0.82**	**1.44**	**0.39**	**1.55**	**3.47**	**1.97**	**1.65**	**0.82**

4.2 Variability, heritability and genetic advance

The phenotypic and genotypic co-efficient of variability (PCV and GCV) estimates among the genotypes showed wide variation for different traits (Table 5). In general, GCV estimates for most traits were slightly lower than PCV. The characters yield/plant, plant height, pods/plant and 100-seed weight showed higher estimates for PCV and GCV (> 15 %), while these estimates were very low for days to 50 % flowering, seeds/pod, pod length and clusters/plant. Similar high estimate of PCV and GCV for seed yield, pods/plant were reported by Pandiyan *et al.* (2006), Kousar *et al.* (2007) and Rahim *et al.* (2010).

Table 5. Genetic parameters of the characters in greengram

Character	Mean	Range	PVC (%)	GCV (%)	h^2 (%)	GA	GA (% of mean)
Days to 50% flowering	40.84	33.33 – 47.66	7.45	7.28	95.51	5.98	14.64
Plant height (cm)	20.67	11.86 – 31.96	25.64	24.87	94.04	10.27	49.68
Cluster / plant	3.609	2.00 – 4.53	13.27	12.19	84.41	8.33	23.08
Pods / plant	10.513	7.36 – 16.36	23.96	22.44	87.69	4.551	43.28
Pod length (cm)	8.383	6.13 – 9.6	10.66	10.08	89.45	1.646	19.63
Seeds / pod	10.023	8.40 – 11.6	9.95	8.55	73.87	1.517	15.13
100 seed weight (g)	2.881	2.32 – 4.11	20.44	19.88	94.51	1.147	13.58
Survival of 10 days old seedling at 10°C (d)	14.00	3.66 – 27.00	58.19	58.19	99.54	16.706	119.00
Survival of 20 days old seedling at 10°C (d)	34.12	11.33 – 48.00	20.53	20.21	96.93	13.987	40.99
Survival of 30 days old seedling at 10°C (d)	16.813	3.00 – 46.33	50.21	50.04	99.32	17.273	102.73
Survival of 40 days old seedling at 10°C (d)	16.52	9.33 – 44.00	41.44	41.29	99.27	14.00	84.74
Yield / plant (g)	2.48	1.26 – 4.26	30.67	28.38	85.64	1.345	56.88

Low temperature is one of the major environmental factors that limit the plant growth. Greengram plants of tropical origin suffer cold damage when exposed to temperature below 20°C [Graham and Patterson (1983), Andrason

(1987) and Roy and Basu (2009)]. Cold temperature in crop plants are compounded *cold snap* – a lower than usual drop in temperature that causes the crop to fail due to reduce germination, retard vegetative growth by inducing metabolite imbalance and delay of prevent productive development. Each plant species has an optimum range of temperature for it's normal growth and development. It varies among the genotypes within a species, the specific temperature also depends on the growth stage and development of the particular genotype (Roy and Basu, 2009).

Land races of any crop are the important source of parental material for prevailing stress situation of any locality.

In the present case 21 local genotypes and four standard varieties of greengram were exposed to artificial low temperature in the S.K. Sinha Molecular Breeding Laboratory of this department.

ANOVA and 'F' test for all the situations revealed significant difference among the genotypes for all four stages of seedling exposed to artificial low temperature (Table 3).

The mean performance of all the local genotypes ranged from 1.26 gm/pl. to 4.26 gm/pl. in case of Bijapur local and Jharsuguda local respectively.

Local genotypes when exposed to low temperature at 10 days seedling stage (Table 6) the maximum days of survival was observed in case of Nayagarh local followed by Jharsuguda local and Bhawanipatna local B (27.00 and 23.33 days). The minimum day of survival of seedling was observed in case of Tigiria local A followed by Bilipara local and released variety TARM-1 (2.66 to 4.00 days). Tigiria local was most susceptible to the cold temperature at 10 days seedling state among all the genotypes considered together. Standard

varieties survived up to 12.66 days (Dhauli), where as TARM-I was most susceptible to the cold temperature at early stage at seedling.

Table 6. Reaction of different days old seedling of greengram to cold

Sl. No.	Genotypes	Survival duration (days)				Mean (survival days)	Yield (g/plot)
		10 days old seedling stage	20 days old seedling stage	30 days old seedling stage	40 days old seedling stage		
1	Dhauli	12.66	32.00	15.00	10.33	17.50	2.36
2	OUM-11-5	6.66	38.00	16.33	21.66	20.66	2.01
3	OBGG-52	4.01	23.33	18.33	12.33	14.50	2.42
4	TARM-1	4.00	11.33	12.33	20.33	12.00	1.64
5	Tigiria Local-A	2.66	34.00	11.00	19.33	16.75	2.90
6	Kendrapara Local A	16.00	25.33	21.33	11.33	18.50	3.87
7	Bhawanipatna Local-B	23.33	33.00	23.00	21.33	21.17	1.46
8	Kapurgaon Local	7.33	48.00	18.00	12.33	21.42	3.38
9	Nandika Local	6.66	29.33	3.00	12.33	12.83	2.63
10	Sikri local	20.66	35.66	4.00	19.33	19.91	2.72
11	Sheragarh Local	21.00	37.00	14.33	18.33	22.15	2.34
12	Ratila local	20.33	38.00	46.32	16.00	30.16	2.79
13	Khadabhanga local	14.00	29.66	26.66	16.00	21.58	2.73
14	Sudhasarangi Local	22.00	35.33	4.00	17.00	19.58	2.97
15	Jharsuguda local	24.33	40.00	15.00	11.33	22.66	4.26
16	Kamakhya local-B	22.33	35.33	21.00	13.33	22.99	3.22
17	Jainmuga local (Baragarh)	15.33	33.33	18.00	13.00	19.91	3.11
18	Kalahandi local-1A	20.66	40.66	20.66	14.33	24.08	2.34
19	Keonjhar local	6.66	42.00	20.00	15.00	20.91	1.54
20	Banapur local-C	4.66	34.00	11.66	9.33	14.91	2.46
21	Bilipara local	3.66	38.33	15.00	18.00	18.74	2.36
22	Kalamungo local (Phulbani)	22.33	33.33	17.33	9.66	20.66	1.62
23	Bijapur local	17.66	33.33	13.66	17.33	20.50	1.26
24	Nayagarh local	27.00	34.00	15.00	19.66	23.67	1.48
25	Kalahandi local 2B	4.00	38.66	19.33	44.00	26.50	1.95

When 20 days old seedlings of the local genotypes and standard varieties were exposed to artificial low temperature at 10°C maximum (48 days) of survival was noticed incase of Kapurgaon local followed by Keonjhar local (42) and Kalahandi local-1A (40.66). TARM-1 was the most susceptible variety at 20 days of seedling growth exposed to 10°C followed by OBGG-52 (23.33) and Kendrapara local-B (25.33).

When 30 days old seedlings of the local genotypes and released varieties were exposed to artificial low temperature at 10°C; Ratila local showed tolerance for 46.33 days of survival followed by Khadabhanga local, Bhawanipatna local B and Kendrapara local-A (26.66, 23.00 and 21.33 days respectively), where as Nandika local was very susceptible to cold (3 days survival) followed by Sikri local and Sudhasarangi local (4 days).

When 40 days old seedlings of greengram were exposed to artificial cold condition at 10°C, the maximum survival was noticed in Kalahandi local 2-B (44.00 days), followed by genotype OUM-11-5 (21.66 days) and Bhawanipatna local-B (21.33 days), where as the most susceptible genotype was the Banapur local-C (9.33 days) followed by Kalamuga local (Phulbani) (9.66 days) and Dhauli (10.33 days).

Considering the average of all the stages of seedling exposed to low temperature (Table 6), it was observed that most tolerant genotype was Ratila local (30.16 days of survival) followed by Kalahandi local 2-B (26.50 days of survival) and Nayagarh local (23.67 days of survival). The most susceptible genotype was a released variety (TARM-1) with 12.00 days of survival followed by Nandika local (12.83 days) and another released variety OBGG-52 (14.50 days of survival). Levitt (1939), Hughes and Dunn (1999), Chang *et al.* (2000), Yu *et al.* (2003) also reported variation of cold tolerance in different stages of greengram seedling exposed to cold for different duration.

It was observed that the highest yielder local genotype Jharsuguda local with 4.26 g/plant yield was with high tolerance to low temperature at 10 and 20 days old seedling stage where as Ratila local and Khadabhanga with moderate yield was with highest tolerance to cold temperature at 30 days old seedling stage.

Considering both yield and tolerance to cold at all stages it may be concluded that Jharsuguda local, Kalahandi local-1A and Kendrapara local-1A may be considered as good donors for early exposure to the low temperature. Kapurgaon local, Jharsuguda local and Kalahandi local-1A are good donor for the medium days old seedling exposure to low temperature. Bhawanipatna local-B, Khadabhanga local, Kalahandi local-2B and Ratila local may be considered as good donor for medium to old days seedling exposure to cold. They may be use as a donor in the breeding programme for developing desirable segregants having cold tolerance ability.

Most of the genotypes were susceptible to cold at early and late stage of their vegetative growth when exposed to the low temperature.

Mid growth seedling stage tolerance to cold was exhibited by most of the local genotypes. This may be due to high enzymatic activity of the mid stage growth of crop in comparison to young and old stage crop. Levitt (1939), Hughes and Dumn (1999), Chang *et al.* (2000), Yu *et al.* (2003) also reported variation of cold tolerance in different stages of greengram seedling exposed to cold for different duration.

Heritability estimates of the traits ranged from 73.87 to 99.54 % in field situation. The heritability of traits were high (>95 %) for days to flowering, moderate (85 – 95 %), for yield/plant, l00-seed weight, plant height, pods/plant and low (< 85 %) for seeds/pod, clusters/plant. For artificial cold conditions created in laboratory all showed high heritability, *i.e.,* highest in 10 days seedling expose to cold followed by 30 days seedling, 40 days seedling and 20 days. Vijay-Prakash (2006), Muhammad *et al.* (2006), Muhammad *et al.* (2007), Kousar *et al.* (2007), Singh *et al.* (2009) and Rahim *et al.* (2010) had reported similar kind of results earlier.

Genetic advance (as % of mean) for the traits showed wide variation ranging from 13.58 for 100-seed weight to 56.88 for yield/plant (Table 5). GA was high (> 40 %) for yield/plant, pods/plant, plant height, low (< 15 %) for days to 50% flowering, 100-seed weight and moderate, *i.e.*, (15.0 – 40.0) for clusters/plant, pod length, seeds/pod and 100-seed weight in field condition. But in laboratory condition it was lowest (40.99) in case of resistant to cold when seedling was 20 days and highest in 10 days seedling, *i.e.*, 119.00 %.

High to moderate genetic advance for yield, pods/plant, plant height, 100-seed weight and clusters/plant have been reported by Rao *et al.* (2006), Pandiyan (2006), Wani *et al.* (2007), Singh *et al.* (2009) and Rahim *et al.* (2010). Considering heritability and genetic advance jointly, plant height, pods/plant and yield/plant had moderate to high heritability accompanied with high genetic advance indicating greater role of additive gene effects on the expression of these traits which is in broad agreement with the reports of Wani *et al.* (2007), Kousar *et al.* (2007), Singh *et al.* (2009) and Rahim *et al.* (2010).

4.3 Phenotypic and genotypic correlation among traits

The phenotypic correlation (r_p) among the 12 traits in the 25 genotypes ranged from -0.405 between 100-seed weight and survival of 10 days old seedling at 10°C to 0.810 between yield/plant and pods/plant. Eight of the 66 r_p estimates were positive and significant (Table 7). The genotypic correlations (r_g) ranged from -0.419 between 100-seed weight and survival of 10 days old seedling at 10°C to 0.919 between yield/plant and pods/plant. Ten of the 66 r_g estimates were positive and significant and two (days to 50% flowering and seeds/pod) were negative and significant.

Table 7. Phenotype correlation (r_p) and genotypic correlation (r_g) among the characters in greengram

Character.		Plant height	Cluster / plant	Pods / plant	Pod length	Seeds / pod	100-seed weight	Survival of 10 days old seedling at 10°C	Survival of 20 days old seedling at 10°C	Survival of 30 days old seedling at 10°C	Survival of 40 days old seedling at 10°C	Yield/ plant
Days to 50% flowering	r_p	0.210	-0.315	0.063	0.680**	-0.370	-0.168	0.378	0.103	-0.106	-0.049	-0.090
	r_g	0.232	-0.346	0.072	-0.738**	-0.412*	-0.182	0.388	0.100	-0.110	-0.047	-0.119
Plant height	r_p		0.091	-0.061	-0.225	-0.333	0.770	0.037	-0.215	-0.026	-0.154	-0.094
	r_g		0.097	-0.093	-0.237	-0.404	0.091	0.042	-0.227	-0.028	-0.159	-0.093
Cluster / plant	r_p			0.484*	-0.151	0.141	-0.072	0.073	0.190	-0.018	0.211	0.414*
	r_g			0.552**	-0.169	0.197	-0.103	0.081	0.192	0.010	0.232	0.480*
Pods / plant	r_p				0.496**	0.461*	-0.342	0.351	0.309	0.077	-0.185	0.810**
	r_g				0.587**	0.567*	-0.354	0.375	0.341	0.080	-0.196	0.919**
Pod length	r_p					0.182	-0.143	0.421*	0.291	-0.018	-0.216	0.443*
	r_g					0.212	-0.163	0.449*	0.308	-0.019	-0.223	0.481*
Seeds / pod	r_p						0.007	-0.167	0.347	0.202	--0.353	0.669**
	r_g						0.017	-0.194	0.432*	0.249	-0.406*	0.861**
100-seed weight	r_p							-0.405	-0.251	-0.130	-0.258	0.184
	r_g							-0.419	-0.260	-0.134	-0269	0.204
Survival of 10 days old seedling at 10°C	r_p								0.181	0.166	-0.192	0.075
	r_g								0.183	0.166	-0.195	0.080
Survival of 20 days old seedling at 10°C	r_p									0.143	0.071	0.172
	r_g									0.145	0.073	0.170
Survival of 30 days old seedling at 10°C	r_p										0.027	0.027
	r_g										0.023	0.023
Survival of 40 days old seedling at 10°C	r_p											-0.352
	r_g											-0.389

Considering both r_p and r_g estimates among the component traits, clusters/plant showed positive significant correlation with pods/plant, whereas pods/plant has a strong positive correlation with pod length, days to 50 % flowering and seeds/pod. Pods/plant, pod length and seeds/pod showed positive and significant association among themselves. Similar positive association between these traits have been reported by Muhammad *et al.* (2006), Wani *et al.* (2007), Sing *et al.* (2009) and Rahim *et al.* (2010).

Considering r_p and r_g of the component traits with yield (Table 7) it was observed that pods/plant and seeds/pod showed highly significant positive association with yield/plant. Correlation of clusters/plant and pod length shows moderate significant positive association with yield/plant. Survival of different days old seedling at 10°C had very negligible positive but non-significant or negative correlation with yield. High positive and significant correlation of pods/plant, clusters/plant, pod length and seeds/pod with yield in greengram have been reported by Muhammad *et al.* (2006), Vijay-Prakash (2006), Wani *et al.* (2007), Kousar *et al.* (2007) and Rahim *et al.* (2010).

4.4 Path co-efficient analysis

The correlation of seed yield with other characters was further analysed to assess the cause and effect relationship between the component traits and yield by path co-efficient analysis. The analysis gives direct effects of the component traits on yield and their magnitude indicate the bearing of each character on expression of yield. Indirect effect of each character indicates their role in affecting correlation of other characters with yield. The phenotypic correlation co-efficient of seed yield with the eleven component traits were partitioned into direct and indirect effects of component traits on

yield by path co-efficient analysis (Table 8). The present path co-efficient analysis showed high R^2 of 170.384 and moderate residual effect of 0.839 indicating that most of the major yield components were included in this study. Pods/plant had the highest direct positive effect of 0.7472 on yield. The character seeds/pod and 100-seed weight had moderate positive direct effect of 0.6690 and 0.4430, respectively, on yield. Days to 50 % flowering (0.0301), plant height (0.0531), clusters/plant (0.0410), pod length (0.0870), survival of 10 days old seedling at 10°C (0.0743), survival of 40 days old seedling at 10°C (0.1048) showed negligible direct effect on yield. survival of 20 days old seedling at 10°C showed negative direct effect on yield, but it is counter acted by pods/plant, seeds/pod and pod length. Survival of 30 days old seedling at 10°C shows negative direct effect on yield but it is also counter acted by seeds/pod, pods/plant and days to 50 % flowering. Positive direct effect of pods/plant, seeds/pod and 100-seed weight on yield in greengram have been reported by Sreelakshmi and Sekhar (2005), Rao *et al.* (2006), Kousar *et al.* (2007), Singh *et al.* (2009) and Rahim *et al.* (2010).

Considering indirect effect of the component traits on yield *via* other traits, it was observed that correlation of pod length, seeds/pod, clusters/plant with yield were greatly influenced by indirect positive effects via pods/plant. Indirect effect of the remaining component traits on yield were generally of very low magnitude.

Rao *et al.* (2006), Wani *et al.* (2007), Rahim *et al.* (2010) and Singh *et al.* (2010) have reported that correlation of component traits with yield in greengram have been greatly influenced by positive direct effects via pod length, seeds/pod and clusters/plant and indirect effects via pods/plant.

Table 8. Direct and indirect effects of component traits on seed yield in greengram

Character	Days to 50% flowering	Plant height	Cluster / plant	Pods / plant	Pod length	Seeds / pod	100-seed weight	Survival of 10 days old seedling at 10°C	Survival of 20 days old seedling at 10°C	Survival of 30 days old seedling at 10°C	Survival of 40 days old seedling at 10°C	Yield/ plant
Days to 50% flowering	**0.0301**	0.0112	-0.0129	0.0471	0.0592	-0.1600	-0.0792	0.0281	-0.0133	0.0050	-0.0051	-0.0900
Plant height	0.0063	**0.0531**	0.0037	-0.0456	-0.0196	-0.1440	0.0363	0.0027	0.0278	0.0012	-0.0161	-0.0940
Cluster / plant	-0.0095	0.0048	**-0.0410**	0.3616	-0.0131	0.0610	-0.0339	0.0054	-0.0246	-0.0009	0.0221	0.4140
Pods / plant	0.0019	-0.0032	0.0199	**0.7472**	0.0431	0.1993	-0.1612	0.0261	-0.0400	-0.0037	-0.0194	0.8100
Pod length	0.0204	-0.0119	-0.0062	0.3706	**0.0870**	0.0787	0.0674	0.0313	-0.0377	0.0009	0.0226	0.4430
Seeds / pod	-0.0111	-0.0177	0.0058	0.3445	0.0158	**0.4323**	0.0033	-0.0124	-0.0449	-0.0096	-0.0370	0.6690
100-seed weight	-0.0050	0.0041	-0.0030	-0.2555	-0.0124	0.0030	**0.4713**	0.0301	-0.0325	0.0062	-0.0270	0.1840
Survival of 10 days old seedling at 10°C	0.0114	0.0020	0.0030	0.2623	0.0366	-0.0722	-0.1909	**0.0743**	-0.0234	-0.0079	-0.0201	0.0750
Survival of 20 days old seedling at 10°C	0.0031	-0.0114	0.0078	0.2309	0.0253	0.1500	-0.1183	0.0135	**-0.1295**	-0.0068	0.0074	0.1720
Survival of 30 days old seedling at 10°C	-0.0032	-0.0014	0.0007	0.0575	-0.0016	0.0873	-0.0613	0.0123	-0.0185	**-0.0474**	0.0024	0.270
Survival of 40 days old seedling at 10°C	-0.0015	-0.0082	0.0085	-0.1387	-0.0188	-0.1526	-0.1216	-0.0143	-0.0092	-0.0011	**0.1048**	-0.3520

R^2 = 170.384 Residual effect = 0.839

4.5 D^2 analysis of genetic divergence

Genetic divergence among 25 greengram genotypes was estimated using Mahalanobis' D^2 statistics. The D^2 estimates ranged from 41.593 between Tigiria local-A and Bilipara local to 3121.514 between Kalahandi local-2B with Kalamuga (Phulbani local). The genotypes Kamakhya local, Kalahandi local with Kalamuga, Jharsuguda local and Sudhasarangi local had high D^2 value and also had high D^2 from rest 21 genotypes indicating these genotypes to be much diverse in multi traits.

Relative contribution of 12 characters to D^2 among the genotypes was estimated by two methods – average D^2 and rank average. On the basis of average D^2 survival of 10 days old seedling at 10°C contributed maximum (28.47) to divergence followed by survival of 40 days old seedling at 10°C (28.28) and survival of 30 days old seedling at 10°C (21.25 %), while clusters/plant, pods/plant, pod length, seeds/pod contribute least to D^2 estimates (< 2 %) (Table 9). The moderate contribution (> 2 % but < 21.25 %) was by 100-seed weight, days to 50 % flowering, plant height and yield per plant. The order of contribution of characters to D^2 estimates on the basis of rank average followed by similar trend, with few minor deviations.

Maximum to moderate contribution to D^2 by 100-seed weight, seed yield, days to 50 % flowering, plant height have been reported by Reddy (1997), Gnanamalar *et al.* (2005), Vijay-Prakash (2006), Muhammad *et al.* (2007) and Rahim *et al.* (2010).

On the basis of D^2 values using Tocher's method, the 25 greengram genotypes grouped into 4 genetic clusters (Table 10). Cluster I included maximum 22 genotypes whereas rest were with single genotype each.

Table 9. Relative contribution of characters to genetic divergence

Character	Average D^2	Average D^2 (%)	Rank total	Rank average
Days to 50 % flowering	14.864	2.48 (6)	2085	8.91 (6)
Plant height	13.255	2.21 (8)	2027	8.66 (7)
Clusters / plant	4.057	0.67 (11)	1259	5.38 (11)
Pods / plant	6.860	1.14 (10)	1282	5.47 (10)
Pod length	8.606	1.43 (9)	2311	9.87 (4)
Seeds / pod	2.059	0.34 (12)	1004	4.29 (12)
100-seed weight	13.970	2.33 (7)	2173	9.28 (5)
Survival of 10 days old seedling at 10°C	170.185	28.47 (1)	2860	12.22 (1)
Survival of 20 days old seedling at 10°C	33.835	5.66 (5)	1621	6.92 (9)
Survival of 30 days old seedling at 10°C	127.042	21.25 (3)	2358	10.07 (3)
Survival of 40 days old seedling at 10°C	169.067	28.28 (2)	2635	11.26 (2)
Yield / plant	33.859	5.66 (5)	1785	7.62 (8)

Table 10. Composition of genetic clusters using D^2 value

Cluster	No. of genotypes	Name of genotype
I	22	Tigiria-A, Bilipara local, OUM 11-5, Keonjhar local, TARM-1, Banapur local-C, Kapurgaon local, OBGG 52, Nandika local, Jhainmung, Sheragarh local, Khadabhanga local, Sikri local, Bijapur local, Dhauli, Bhawanipatna local-B, Kalahandi local 1-A, Kendrapara local-A, Kamakhya local-B, Sudhasarangi, Nayagarh local, Jharsuguda local
II	1	Kalamuga (Phulbani)
III	1	Ratila local
IV	1	Kalahandi local-2B

Similar D^2 analysis and grouping of varieties and germplasm of greengram into genetic clusters have been reported by Vijay-Prakash (2006), Muhammad *et al.* (2007) and Rahim *et al.* (2010).

All the local genotypes were collected from different parts of Odisha from east to west and north to south. The 22 genotypes which coming under cluster No. I were with 18 local land races collected from different regions of the Odisha and three released varieties developed by OUAT and one variety developed by BARC. This indicates genetic clustering has no parallelism with geographic origin of genotypes, which is in agreement with reports of Vijay-Prakash (2006), Muhammad *et al.* (2007), Nath *et al.* (2005) and Rahim *et al.* (2010).

The intra and inter-cluster D^2 value for the four clusters is presented in Table 11. The intra cluster distance was maximum in cluster I and other were with one genotype each. The inter cluster D^2 values range from 496.071 to 3121.559 indicating the clusters to be wide from each other. The cluster III and IV had high inter cluster D^2 3121.559 indicating a wide gap between them and are of diversed type. Cluster III to IV divergenic was moderate indicating that these two genotypes were at average distance from each other.

Table 11. Average intra and inter cluster D^2 values for clusters of greengram genotypes

Cluster	I	II	III	IV
I	410.537	496.071	1039.838	2001.949
II		-	1095.658	3121.559
III			-	2413.092
IV				-

The cluster mean of the four clusters for the 12 characters are presented in Table 12. Cluster means gave a clear picture on character differences among the genetic clusters. Cluster III had highest yield of 2.7 g/plant. These genotypes had high cluster per plant, pods/plant and seeds/plant. Cluster I had second highest yield of 2.5 g/plant. This group had moderate values for most traits but had very high 100-seed weight. Cluster IV had the 3rd highest yield 1.9 g/plant and genotypes was of medium duration with moderate value for most traits, except high tolerant to cold. Cluster II had lowest yield (1.6 g/plant) but high days to flowering, plant height and moderately tolerant to cold.

Plant breeders often use varieties/genotype possessing high genetic divergence in cross breeding programme with an objective of getting more transgressive segregants. The scope of getting high yielding transgressive segregants from a cross between two parents with high genetic divergence is often limited if one or both parents are moderate or low yielder. Thus, for identification of crosses for getting high yielding segregants, the parental genotypes should have high D^2 value, moderate to high yield and character complementation in productivity traits. On the basis of this rational crosses between cluster III (Ratila local) and cluster I (including most of the genotypes) are expected to produce more transgressive, yield segregant in later generation.

Table 12. Character means for different genetic clusters

Character / Cluster	Days to 50% flowering	Plant height	Cluster / plant	Pods / plant	Pod length	Seeds / pod	100-seed weight	Survival of 10 days old seedling at 10°C	Survival of 20 days old seedling at 10°C	Survival of 30 days old seedling at 10°C	Survival of 40 days old seedling at 10°C	Yield/ plant
I	40.83	17.65	3.5	10.49	8.36	10.10	2.93	13.78	33.77	15.3	15.6	2.53
II	46.33	27.30	3.33	9.60	8.96	8.70	2.54	22.33	33.33	17.3	9.6	1.6
III	40.00	16.35	3.66	12.63	8.50	10.83	2.38	20.33	38.00	46.3	16.0	2.7
IV	42.33	22.56	4.26	9.73	7.96	8.70	2.50	4.00	38.66	19.3	44.0	1.9

4.6 Canonical analysis

Canonical analysis is a multivariate analysis which is an extension of multiple regression analysis. The canonical analysis (Rao, 1952) involved estimation of canonical vectors or canonical roots and the first two canonical root values (Z_1 and Z_2) of each genotype are taken for two dimensional presentation of genotypes in scatter diagram and the genotypes falling close to each other are taken to form a group/cluster.

Canonical analysis of the 25 greengram genotypes based on nine characters was done and the contribution of first two canonical roots Z_1 and Z_2 were 48.5 % and 21.1 %, respectively. The genotypes are presented as scatter of points in a two dimensional graph using Z_1 and Z_2 values of each genotype (Fig. 1). Depending on the closeness of points representing genotypes in the scatter diagram, the 25 greengram genotypes were grouped into four clusters (Table 13).

Table 13. Composition of genetic clusters using Canonical analysis

Cluster	No. of genotypes	Name of genotype
I	21	Tigiria-A, Bilipara local, OUM 11-5, Keonjhar local, TARM-1, Banapur local-C, Kapurgaon local, OBGG 52, Nandika local, Jhainmung, Sheragarh local, Khadabhanga local, Sikri local, Bijapur local, Dhauli, Bhawanipatna local-B, Kalahandi local 1-A, Kendrapara local-A, Kamakhya local-B, Sudhasarangi, Nayagarh local
II	2	Kalamuga (Phulbani), Jharsuguda local
III	1	Ratila local
IV	1	Kalahandi local-2B

Cluster I represented by 21 genotypes occupied the central position in the scattered diagram while cluster II occupied the position near to cluster I which includes 2 of the important local cultivars of Phulbani district and Jharsuguda

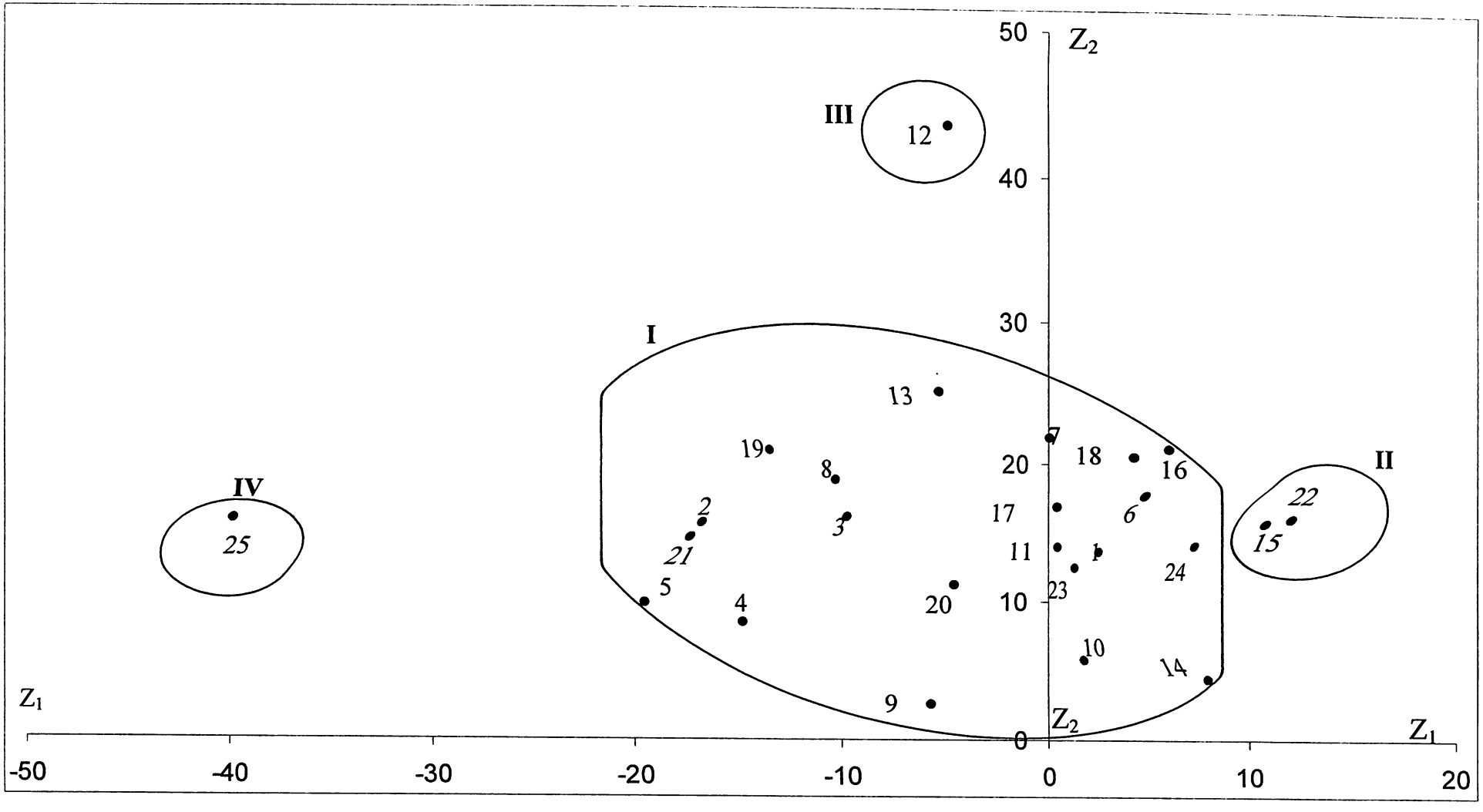


Fig.1 Clustering on the basis of canonical variates

district of Odisha and both having cold tolerance capacity. Cluster III and IV also each having one genotype but the cluster III is far away from cluster I, II and IV. Cluster IV also is at almost equal distance from cluster I, III. Clustering pattern on the basis of D^2 Tocher's method and canonical analysis were similar with few deviations. Composition of cluster III, IV were same in both methods, while in other 2 clusters there were few exchanges.

4.7 Molecular analysis

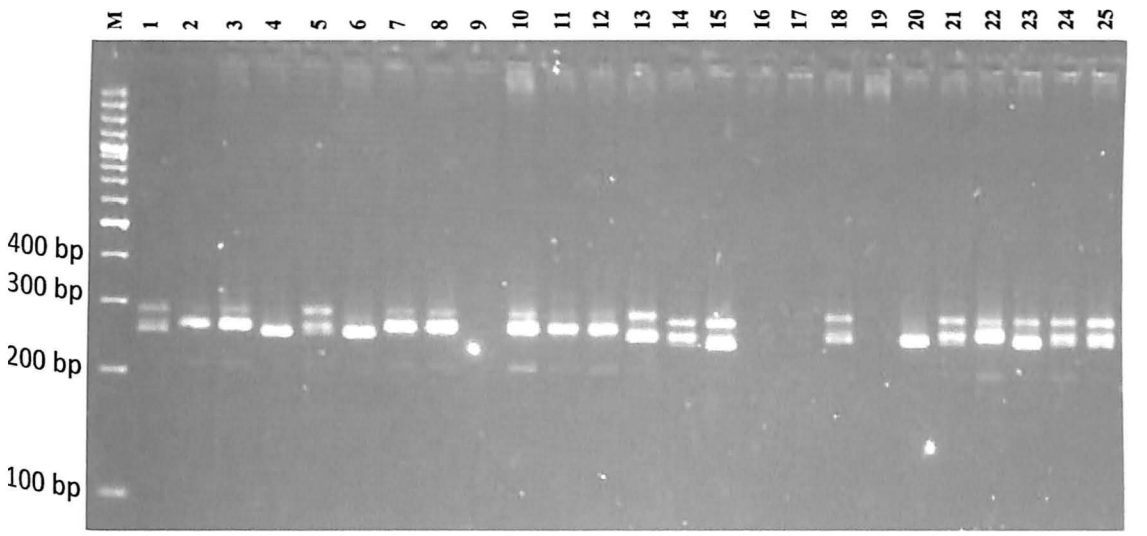
Green gram is a self pollinated deployed grain legume (2n=22) with a genomic size of 560 Mb (Arumuganathan and Earele, 1991). Majority of present day cultivars have been developed as a result of hybridization and contributed to the narrow genetic base due to some common parents in the breeding programme. To widen the narrow genetic base, parents from diversed genetic base may be used in recombination breeding programme. Hence, there is need to assess the genetic diversity for utilization in greengram varietal development. The evaluation of genetic diversity and construction of linkage maps may promote the efficient use of the vast array of genetic resources variation in breeding programme (Paterson *et al.*, 1991). DNA markers provide an opportunity to characterize genotypes and to measure genetic relationship precisely than other markers (Reddy *et al.*, 2008). A narrow genetic base has been reported for released Indian greengram cultivars using RAPD (Santalla *et al.*, 1998), Lakhanpaul *et al.*, 2000 and AFLP (Bhat *et al.*, 2005).

Microsatellite markers are the markers of choice for genetic studies like genetic diversity assessment, genetic mapping, population genetic and marker-assisted selection. Simple sequence repeats (SSRs) consist of randomly repeated units of short nucleotide motifs that are of 1-6 bp long, Di-, tri-, and tetra nucleotide [e.g, (CA)n, (AAT)n and (GATA)n respectively] repeats are

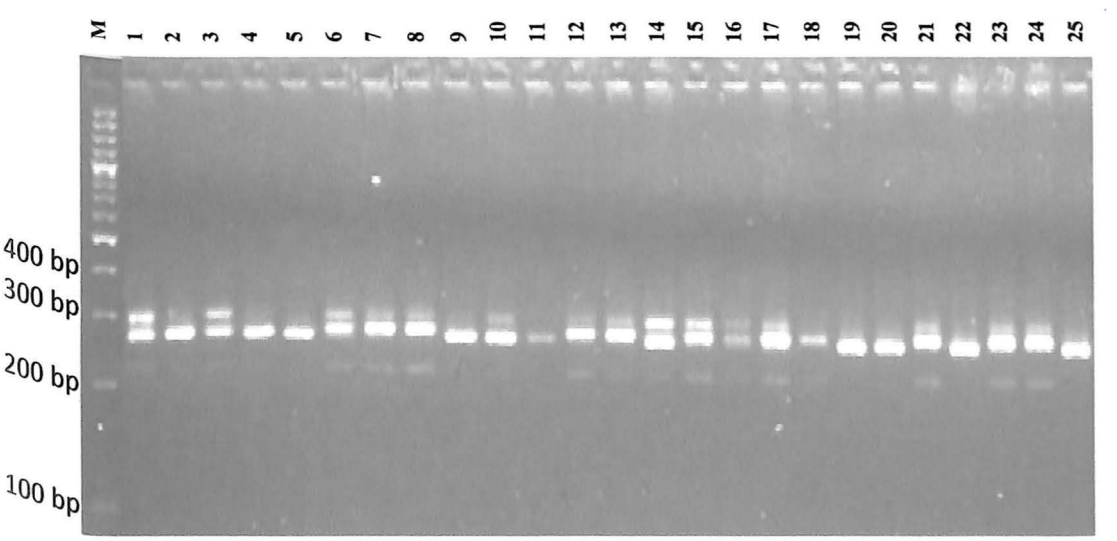
the most common and are widely distributed throughout the genome of plants and animals (Jarne and Lagoda, 1996)

DNA isolation was made from the 25 green gram genotypes using leaves from plant seedlings and molecular analysis was done using 44 SSR primaries. Amplified products were electrophoresed on agarose gel. SSR primers published for greengram (Kumar *et al.*, $2002_{(a)}$ and $2002_{(b)}$) and azukibean (Chaitieng *et al.*, 2006) were synthesized by Chromous Biotech, Bangalore and used in this study. Date analysis was performed using the NTSYS-pc (Numerical taxonomy System, version 2.0 (Rohlf, 1993). The SIMQUAL programme was used to calculate the Jaccard's coefficient.

Forty-four SSR primers were used for analyzing the polymorphism in greengram, of which 19 primers resulted in amplification. Amplification of genomic DNA of 25 genotypes, using 19 SSR primers, produced 31 fragments that could be scored with an average of 1.63 bands per primer (Table 14 and Appendix 1). The number of amplified fragments ranged from 1 (CEDG050, CEDG008, CEDG010, VR2, VR1, VR4 etc) to 3 (CEDG088, CEDG092, CDG139 VR5), which varied in size from 200 bp to 400 bp. Of the 31 number amplified band, 29 numbers (93%) were polymorphic with an average of 1.63 polymorphic fragments per primers. Amplification profile of the greengram genotypes using CEDG088, CEDG092, CEDG139, VR5, VR6, CEDG156 primers are shown in Fig. 2-7. Out of 19 primers, two numbers of primers showed monomorphic banding pattern while 17 primers were with polymorphic banding pattern. The 19 microsatellite primers pairs amplified 31 bands of which 29 were polymorphic with an average of 1.63. Twelve primers pairs produced a single allele and seven amplified more than one allele with in the predicted size range of 200 to 400 bp.



g. 2. **Gel picture of AB-SSR CEDG088 with Greengram genotypes**



ig. 3. **Gel picture of AB-SSR CEDG092 with Greengram genotypes**

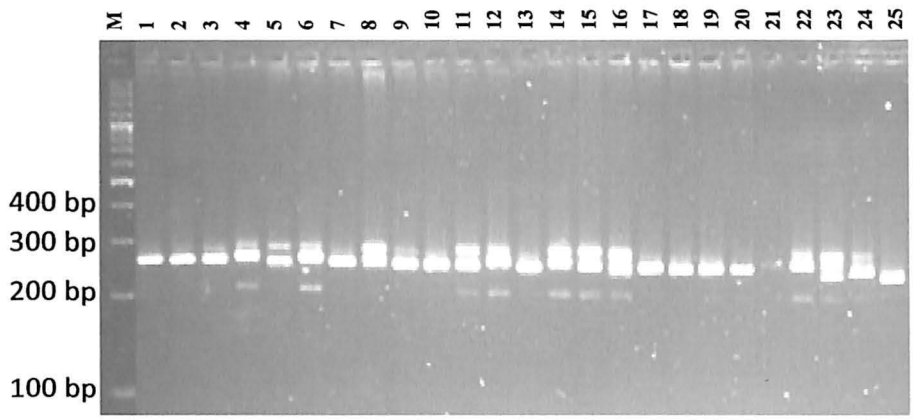


Fig. 4 Gel picture of AB-SSR CEDG139 with Greengram genotypes

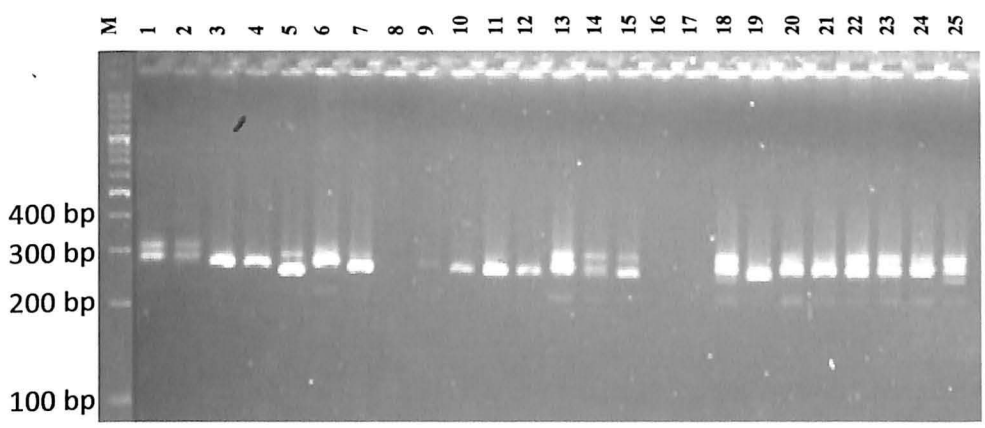


Fig. 5. Gel picture of AB-SSR OF CEDG156 with Greengram genotypes

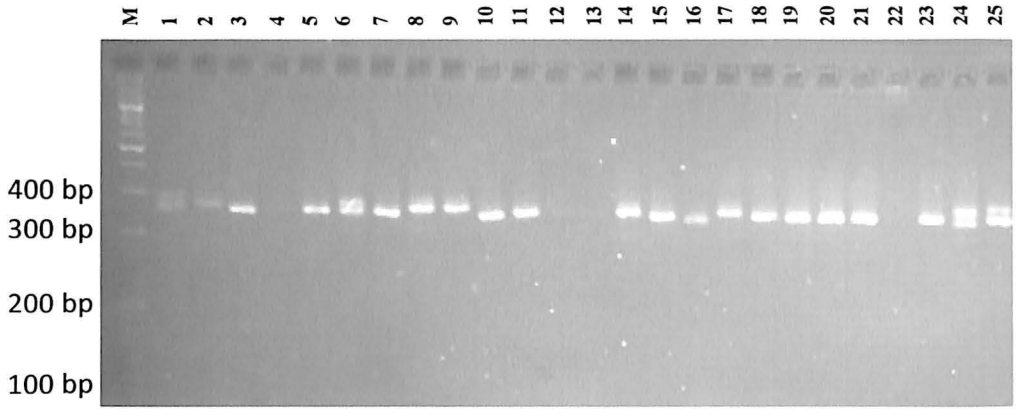


Fig. 6 Gel picture of MB-SSR OF VR5 with greengram genotype

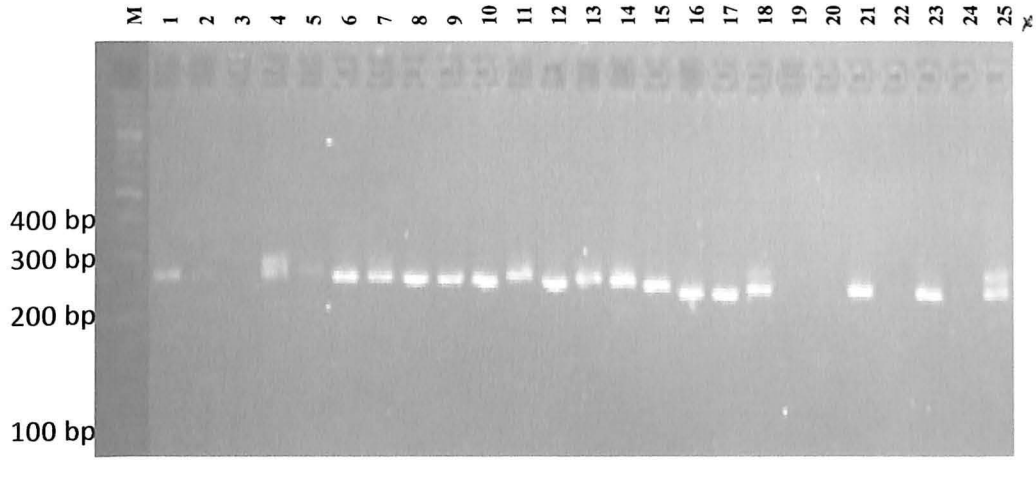


Fig. 7 Fig 4.3b: Gel picture of MB-SSR OF VR6 with greengram genotypes

Table 14. List of microsatellite primer pairs which showed amplification for greengram.

Sl. No.	Primer		Forward and reveres primer sequence	Derived from	Total numbers of bands amplified	No. of monomorphic bands	No. of polymorphic bands	% of polymorphism
1	CFDC050	F	TCCCACTTCTCCATTACCTCCAC	Azukibean	1	-	1	100
		R	GAGATTATCTTCTGGGCAGCAAGG					
2	CEDG008	F	AGGCGAGGTTTCGTTTCAAG	Azukibean	1	-	1	100
		R	GCCCATATTTTTACGCCCAC					
3	CEDG010	F	TGGGCTACCAACTTTTCCTC	Azukibean	1	-	1	100
		R	TGAGCGACATCTTCAACACG					
4	CEDG020	F	TATCCATACCCAGCTCAAGG	Azukibean	2	-	2	100
		R	CCATACCAAGAAAGAGG					
5	CEDG043	F	AGGATTGTGGTTGGTGCATG	Azukibean	1	-	1	100
		R	ACTATTTCCAATGCTGGG					
6	CEDG086	F	GAGTTTACAACAGATGGGGCTAA	Azukibean	1	-	1	100
		R	AGGTCTTGATTGACTTTCTGGGT					
7	CEDG088	F	TCTTGTCATTTAGCACTTAGCACG	Azukibean	3	-	3	100
		R	TTGTTGTTTACTAAGAGCCCGTGT					
8	CEDG091	F	CTGGTGGAACAAAGCAAAAGAGT	Azukibean	1	-	1	100
		R	TGCGTCTTTGGTGCAAAGAGAAA					
9	CEDG092	F	TCTTTTGGTTGTAGCAGGATGAAC	Azukibean	3	-	3	100
		R	TACAAGTGATATGCAACGGTTAGG					
10	CEDG139	F	CAAACTTCCGATCGAAAGCGCTTG	Azukibean	3	1	2	66.67
		R	GTTTCTCCTCAATCTCAAGCTCCG					
11	CEDG154	F	GTCCTTGTTTTCCTCTCCATGG	Azukibean	1	-	1	100
		R	CATCAGCTCTTCAACACCCTGTG					
12	CEDG228	F	GTCGTTTCCGGAAACTGTTC	Azukibean	1	-	1	100
		R	GATCCGAACCTCTTTCTGC					
13	CEDG156	F	CGCGTATTGGTGACTACTAGGTAT	Azukibean	1	-	1	100
		R	CTTTAGTGTTGGGTTGGTCCTAAGG					
14	CEDG248	F	CAGAAACACAAAAGGGTTCTCG	Azukibean	3	-	3	100
		R	GTGGATTCACTCGCTTCC					
15	VR1	F	AGCCCTTCGTGCTAGGAAAT	Greengram	1	-	1	100
		R	CCCTACCGGTTGGTTGGT					
16	VR2	F	CGCCCCTCTAGGTTGGTTGG	Greengram	1	-	1	100
		R	GGGAAAGACGAAGGGTAGAA					
17	VR4	F	TGGTTGGTTGGTTCACAAGA	Greengram	1	1	-	0
		R	CACGGGTTCTGTCTCCAATA					
18	VR5	F	TCACAAAGGGAGGGAAGAGA	Greengram	3	-	3	100
		R	CCCCAGGTTGGTTGGTTGGA					
19	VR6	F	GATGAAGACCCCTTCACAGC	Greengram	2	-	2	100
		R	GTTCACCCTCGGTTGGTTGG					
	Total				**31**	**2**	**29**	
	Average				**1.63**	-	-	**92.98**

A dendrogram constructed based on UPGMA analysis grouped the 25 greengram genotypes into six clusters (Fig.8). The Jaccard's similarity coefficient ranged from 0.42 to 0.94. Cluster 1 comprising 16 genotypes, distributed over five subgroups. The sub group I(a) was with maximum number of eight genotypes while I(c) was with three genotypes. Sub group I(b) and I(e) were with two genotypes each and sub group I(d) was with single genotype. Cluster II was with 3 genotypes, while clusters III and VI were with two genotypes each. The clusters having single genotypes each were cluster III and V. In the present study, microsatellite primers paired, designed from same and relates *sp.* (greengram and azuki bean) showed amplification in greengram.

The multi genotype clusters included genotypes developed in different states of India and different local land races of Odisha, indicating no parallelism between geographic origin of the genotype and genetic control of polymorphic banding pattern. Moreover, clustering/grouping of genotypes on the basis of this polymorphic banding pattern was quite different from cluster analysis by D^2 and canonical method. It may be due to the fact that the later mentioned two cluster analysis are based on morphological and yield component traits of the genotype, while the polymorphic bands produced by the genotypes might be governing some quality and other traits in addition to some morphological traits.

The PCR analysis using 19 SSR primers showed polymorphism for 31 amplification products in term of presence or absence of bands in greengram genotypes (Appendix I). It is interesting to note that amplification products or bands EDG 043, 300 bp, VR 5, 400 bp, CEDG 088, 250 bp, VR-6

Genotype name

1. Dhauli
2. OUM-11-15
3. OBGG-52
4. TARM-1
5. Tigiria local-A
6. Kendrapada local-A
7. Bhawanipatna-B
8. Kapurgaon local
9. Nandika local
10. Sikri local
11. Shergarh local
12. Rattila local
13. Khandabhunga local
14. Sudhasarangi local
15. Jharsuguda local
16. Kamakhya local-B
17. Jhainnuga (Baragarh)
18. Kalahandi local-A
19. Keonjhar Local
20. Barapur local-C
21. Bilipara Local
22. Kalamuga local (Phulbani)
23. Bijapur local (Baragarh)
24. Nayagarh local
25. Kalahandi local-2B

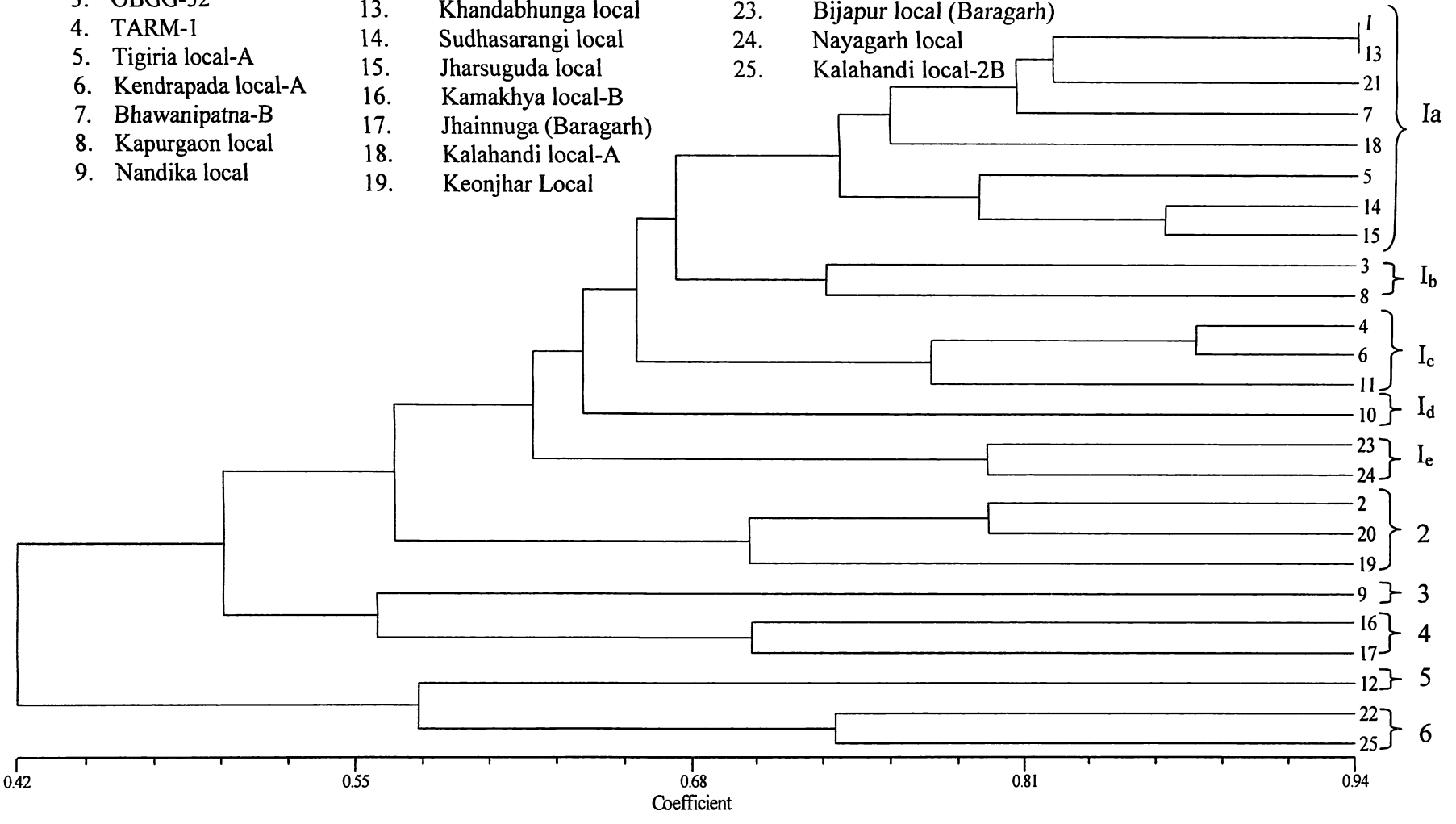


Fig.8. Dendrogram constructed using Jaccard's coefficient and UPGMA clustering for 25 greengram genotypes based on microsatellite polymorphic data

CHAPTER-V

Summary & Conclusion

SUMMARY AND CONCLUSION

The present investigation on "Genetic architecture of yield and cold tolerance in greengram [*Vigna radiata* (L.) Wilczek]" was undertaken to study the variability parameters of the productivity traits, association among the traits and for the grouping of genotypes into different clusters through multivariate analysis using productivity traits and molecular banding pattern.

Twenty-five green gram genotypes consisting of twenty one land races and four standard varieties cultivated in Odisha were evaluated in RBD during rabi, 2009 and observations on days to 50% flowering, plant height, cluster per plant, pods/plant, pod length, seeds/pod, 100-seed weight and yield/plant were recorded from field trials and response to cold was study at S. K. Sinha Molecular Breeding Laboratory of this Department.

The genotypes showed wide variation in all 12 traits and the difference were highly significant. Seed yield of the genotype varied from 1.26 to 4.26 g/plant. High yielder were Jharsuguda local, Kendrapada local and Kapurgaon local. PCV and GCV estimates were high for reaction to cold at 10, 30 and 40 days old seedling stage. Heritability and genetics advance of the traits ranged from 73.87 to 99.54 and 0.833 to 17.273, respectively. Plant height and pods/plant had moderate to high heritability accompanied with high genetics advance indicating additive gene effect. Characters like 100-seed weight and days to 50 % flowering with high to moderate heritability but with low genetics advance indicated none additive gene effects.

The phenotypic (r_p) and genotypic (r_g) correlation among 12 traits ranged from -0.405 to 0.810 and -0.419 to 0.919, respectively. Character association among component traits showed two sets strongly interrelated characters *viz*., pods/plant, pod length, seeds/pod and 100-seed weight and reaction to cold at 10 days old seedling.

The characters pods/plant, pod length and seeds/pod showed positive correlation with yield while, reaction to cold at 10 days and 30 days old seedling showed negligible correlation with yield,

Phenotypic correlation of seed yield with component traits was partition into direct and indirect effect by path analysis. Pods/plant had highest direct positive effect on yield followed by seeds/pod and pod length. Positive correlation of most traits with yield was mostly influenced by in direct positive effect *via* pods/plant and pod length.

Multivaritaes analysis of divergence among the 25 greengram genotypes based on the 8 productivity traits and 4 types of reaction to cold was done in two methods,

1) D^2 analysis
2) Canonical analysis

Genetics divergence (D^2) estimates among genotype ranged from 41.593 to 3121.514. The characters survival of 10 days old seedling at 10°C (d), survival of 40 days old seedling at 10°C (d) and survival of 20 days old seedling at 10°C (d) contributed maximum to divergence, while clusters/plant, pods/plant , pod length, seeds/pod contributed least to divergence.

Using the Tocher's method, the genotypes were grouped into 4 clusters. 22 genotypes including 4 standard varieties and 18 local land races collected from different parts of Odisha were grouped in a single cluster while other three local land races each form a separate group indicating that genetic clustering has no parallelism with genotypic origin.

Considering inter-cluster average D^2 value, cluster mean for different character including yield and character complementation in productivity traits, crosses between cluster III (Ratila local) and cluster I (including most of genotypes) are expected to produce more transgressive segregants in later generation.

The first two canonical root (Z_1 and Z_2) in the canonical analysis contributed 48.5% and 21.1% respectively of the divergence. Clustering on basis of canonical analysis was similar to that on basis of D^2, with very few exchanges.

DNA isolation was made from 25 greengram genotypes using young seedling leaves and the molecular analysis was done using SSR primers. The gel electrophoresis figure showed that 19 SSR primers produce 31 amplification products or fragments, seen as bands and size amplification products ranged from 200 bp to 400 bp of the 31 bands, 29 (93 %) were polymorphic and 2 (7 %) were monomorphic. The different SSR primers generated 0-100% polymorphism in the greengram genotypes. The no. of polymorphic band detected by the 19 SSR primers ranged from 1-3 with average for 1.58, the polymorphic information content (PIC) values indicate that primer CEDG088, CEDG092, CEDG139, CEDG156, VR5 and VR6 had high PIC value and thus posses better discriminating power.

Similarity co-efficient among genotypes on the basis of presence or absence of polymorphic band ranged from 0.42 to 0.94. The genotype grouped into 6 clusters at 0.62 phenon level. The cluster I, II, III, IV, V and VI included 16, 3, 1, 2, 1 and 2 genotypes, respectively. The multi-genotype cluster included genotypes of different location of Odisha.

Clustering/grouping of genotype on the basis of polymorphic banding pattern was quite different from cluster analysis by D^2 and canonical. It may be due to the fact that he later mentioned methods of cluster analysis are based on morphological and component traits of genotypes, while polymorphic bands produced by the genotypes might be governing some quality or other traits in addition to some morphological traits.

The amplification product or bands CEDG043, at 300 bp was present in only two genotypes of greengram followed by VR-5 at 400 bp, CEDG088 at 250 bp and VR-6 at 300 bp in three genotypes of greengram. On the other hand, CEDG091, CEDG228 and CEDG248 at 250 bp were having absence of band in only 2-3 genotypes of greengram. Such rare presence/absence of a band in any genotype would help in DNA finger printing and molecular characterization of genotypes or verities.

CONCLUSION

The 25 greengram genotypes showed wide and significant variation in all 12 traits including yield. Genetic parameters of traits, correlation among traits and path analysis revealed that selection for pods/plant, pod length and seeds/pod would be effective in isolation of high yielding genotypes.

Multivariate analysis of divergence by two methods D^2 and Canonical analysis grouped the 25 genotypes into four clusters indicating presence of

Bibliography

BIBLIOGRAPHY

Afiah, S. A. N. and Mohamed, N. A. (2000). Integrated analysis of the relative contribution of yield attributing variables by using specific statistical techniques in mungbean. *Annals. of Agricultural Science.* **38**: 1347-1362.

Ajibade, S.R., Weeden, N.F. and Chite, S.M. (2000). "Inter sample sequence repeat analysis of genetic relationships in genus *Vigna*. *Euphytica*, **111**:47-55.

Akapuram, M. (1996). Mungbean (*Vigna radiata* (L. Wilczek) In NW01010 E, Smartt, J (eds) Food and seed from legumes and oilseeds. Chapman and Hall London.

Anderson, J. A., Talliaferro, C. M. and Martin, D. L. (1993). Evaluating freeze tolerance of Bermuda grass in a controlled environment. *Hort. Science*. **28**: 955.

Andrews, C.J. (1987). Low temperature stress in field and forage crop production an overview. *Canadian Journal Plant Science*, **67**:1121-1133.

Anil S. and Lokendra-Kumar (2006). Selection criteria for genetic improvement in mungbean. *International Journal of Plant Science, Muzaffarnagar.* **1**:61-63.

Arumuganathan , K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reports*, **9**: 208-218.

Bannett, J. (1991). Protein phosphorylation in green plant chloroplasta. *Plant Mol. Biol.* **42**: 281-311.

Asifa, N.; Sadiq, M.S.; Hanif, M.; Abbas, G. and Haidar, S. (2005). Genetic parameters and path analysis in mung bean (*Vigna radiata* (L) Wilczek). *Journal of Agricultural Research, Lahore.* **43**:339-347.

Aziz Ur R., Muhammad A. A., Babar M. A., Muhammad S., Amjad. A. and Ahsan R. M. (2009). Genetic studies of yield related traits in mungbean (*Vigna radiata* L. Wilczek). *Australian Journal of Crops Science.* **3**:352-360.

Bannett, J. (1991). Protein phosphorylation in green plant chloroplasta. *Plant Mol. Biol.* **42**: 281-311.

Bhat, K.V., Lakanpaul, S. and Chadha, S. (2005). Amplified fragment length polymorphism (AFLP) analysis of genetic diversity in Indian mungbean (*Vigna radiata* L. Wilczek) cultivars. *Indian Journal of Biotechnology,* **4**:56-64.

Bredenkamp, G. J. and Baker, N. R. (1994). Temperature sensitivity of DI protein metabolism in isolated *Zea mays* chloroplast. *Plant Cell Environ.* **17**: 205-210.

Bush, D.C. (1995). Calcium regulation in plant cells and its role in signaling. Ann. Rev. Plant Physiol. *Plant Mol. Biol* **46**:95-122.

Byrd, G. T., Ort, D. R. and Ogren, W. L. (1995). The effects of chilling in the light of ribulose-1, 5-bisphosphate carboxylase/oxygenase activation in tomato (*Lycopersicon esculentum* Mill). *Plant Physiol.* **107**: 585-591.

Byregowda, M.; Chandraprakash, J.; Babu, C.S.J.; Rudraswamy, P. (1997). Genetic variability and inter relationship among yield and yield components in greengram. *Crop research, Hissar.* **13**:361-368.

Chaitieng, B, Kanga, A., Tomooka, N.T., Isemura,T. Kuroda, Y. and Vaughan,D.A. 2006. Development of a black gram (*Vigna mungo* (L.) Hepper) linkage map and its comparison with an azuki bean (*Vigna angularis* (wild) Ohwi and Ohashi) linkage map. *Theoretical and Applied Genetics,* **113**:1261-1269.

Chakraborty, S. and Borah, H.K. (2001). Genetic variability and correlations among root characters in greengram (*Vigna radiata* (L) Wilczek). *Research on crop.* **2**:213-215.

Chang, M.Y., Chen. Swu-Ling, Lee, C.F., and Chen, Y.M. (2000). Cold acclimation and root temperature protection from chilling injury in chilling sensitive mungbean (*Vigna radiata* L.) seedling. *Botanical Bulletin of Academia Sinica.* **42**:1-14.

Chontira, S., Akito, K., Norihiko, T., Duncan, V and Peerasak, S. (2007). Genetic diversity of the mungbean (*Vigna radiata* L. Wilczek) gene-pool on the basis of microsatellite analysis. *Australian Journal of Botany.* **55**: 837-847.

Choumane, W., Winter, P., Baum, M. and Kahl, G. (2004). Conservation of microsatellite flanking sequences in different taxa of leguminosae. *Euthytica.* **138**: 239-245.

Crans, H.R. and Shyter, D.J. (1970). Stimulation of solute loss from radicles of *Gossypium hiorsutum* L. by chilling, anaerobiosis and low pH. *Plant Physiol.* **40**:53-56.

Dikshit, H. K., Sharma, T. R., Singh, B. B. and Jyoti Kumari (2009). Molecular and morphological characterization of fixed lines from diverse cross in mungbean (*Vigna radiata* (L.) Wilczek). *Indian Academy of Sciences.* **88**: 341-344.

Diskhit, H.K., Jhang, T., Singh, N.K., Koundal, K.R., Bansal, K.C., Chandra, N., Tickoo, J.L. and Sharma, T.R. (2006). Genetic differentiation of *Vigna sps* by RAPD, URP and SSR. Trsharma @ nrcph.org.

Gaitan, Solis E., Dugue, M.C.,Edwards, K.J. and Tohme, J. (2002). Microsatelite repeats in common bean (*Phaseolus vulgaris*): Isolation characterization and cross-species application in *Phaseolus* ssp. *Crop Science* **42**:2128-2136.

Gajaraj, S., Sareen, P. K., Saharan, R. P. and Singh, A. (2001). Induced variability in mungbean (*V. radiata* (L.) Wilczek). *Indian J. Genet.* **61**: 281-282.

Galvan, M.Z.; Barnet, B.; Balatiti, P.A. and Branchard, M. (2003), Inter sample sequence repeat (ISSR) marker as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phasealus vulgaris* L.), *Euphytica*. **1332**:297-300.

Gnanamalar, R.P.; Vivekanandan, P. and Manickavelu, A. (2005). Genetic divergence in grengram (*Vigna radiata* (L) Wilczek). *Research on crops*. 6:106-108.

Graham, D. and Patterson, B.D. (1982). Response of plants to low, non freezing temperatures : proteins, metabolism and aulimation. *Ann. Rev. Plant Physiol.* **33**:347-372.

Graham, D. and Patterson, B.D. (1982). Response of plant to low, non-freesing temperatures Proteins, metabolism and aulomation. *Annual Review Plant Physiology,* **33**:347-372.

Guy, C.L. (1990). Cold aulimation and freezing stress tolerance: Role of protein metabolism. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **41**:187-223.

Guy, C. L., Niemi, K. J. and Brambl, R. (1985). Altered gen expression during cold acclimation of spinach. *Proc. Natl. Acad. Sci.* USA. **82**. 3673-3677.

Hughes, M.A. and Dunn, M.A. (1999). The molecular biology of plant aulimation to low temperature. *J. Exp. Bot.* **47** :291-305.

Islam, M.R.; Abdul, H.; Khaliq, Q.A.; Ahmad, J.U. Haque, M.M. and Karim, M.A. (2007). Genetic variability in flooding tolerance in mung bean (*Vigna radiata* (L) Wilczek). *Euphytica*. **105**:247-255.

J. Souframanien and T. Gopalkrishna. (2009). Cross species amplification of micro satellite loci and diversity analysis in blackgram. *Journal of Food Legumes* **22**: 11-17.

Jane, P. and Zagoda, P.S.L. (1996). Microsatellite from molecules to population and back. *Trends in Ecological Evolution*, **11**:424-429.

Jarne, P. and Lagoda, P. J. L. (1996). Microsatellite from molecules to populations and back. *Trends* in *Ecological Evolution*. **11**: 424-429.

Joseph, J. and Santosh-Kumar, A. V. (1999). Character association and cause effect analysis in some F_2 population of greengram (*Vigna radiata* (L.) Wilczek). *Legume Research*. **22**: 99-103.

Kamaleshwari; Prasad, K.D.; Verma, A.K. and Kumar, K. (2003). Genetic variability, correlation and path co-efficient analysis in greengram. *Journal of Research*. Birsa. **11**:97-101.

Khan, S. and Wani, M. R. (2009). Induced mutations for yield contributing traits in greengram. *International Journal of Agriculture and Biology*. Got from CABI International.

Kanakmany, M.T.; Mathew, G.; Leenakumary, S. and Joseph, T. (1996). Correlation and yield component analysis in greengram (*Vigna radiata* (L) Wilczek). *Journal of Tropical Agriculture*. **34**:14-16.

Khattak, G.S.S., Ashraf, M. and Khan, M.S. (2004). Assessment of genetic variation for yield and yield components in mungbean (*Vigna radiata* L. Wilczek) using generation mean analysis. *Pak. J. Bot*. **36**:583-588.

Kousar, M.; Garard, A.; Arit, J.; Singh, A.K. (2007). Genetic variability and correlations studies on yield and its components in mung bean (*Vigna radiata* (L) Wilczek). *Journal of Agriculture*, **6**:216-218.

Kumar, A. F. and Tripathy, B. C. (1998). Temperature – stressed induced impairment of chlorophyll biosynthesis reactions in cucumber and wheat. *Plant Physiol.* **117**: 851-858.

Kumar, S.V., Tan, S.G., Quab, S.C. and Yusoff, K. 2002a. Isolation of microsatellite markers in mungbean, *Vigna radiata. Molecular Ecology Notes.* **2**:96-98.

Kumar, S.V.,Tan,S.G., Quah, S.C. and Yousoff, K. (2002b). Isolation of microsatellite markers in mungbean, *Vigna radiata. Molecular Ecology Notes.* **2**: 293-295.

Kung, C. C. S., Yeh, K. W., Lin, C. Y. and Chen, Y. M. (1998). Characterization of pea gene responsive to low temperature. *Bot. Bull. Acad. Sin.* **39**: 9-15.

Kurkela, S. and Franck, M. (1990). Cloning and characterization of cold and ABA inducible. Arabiodopsis Vol.1. Johns Hopkins Press, Baltimore, pp.477-499.

Lakhanpaul, S., Chadha, S. and Bhat K.V. (2000). Random amplified polymorphic DNA (RAPD) analysis in Indian Mungbean (*Vigna radiata* (L.) Wilczek) cultivars. *Genetica* **109**:227-234.

Lavanya, G.R., Srivastava, J. and Pande, S.A.(2008). Molecular assessment of genetic diversity in mung bean germplasm. *Journal of Genetic.* **87**:65-74.

Lavanya, G. R. and Toms, B. (2009). Association and inter-relationship among yield contributing characters in mungbean. *Journal of Food Legumes.* **22**: 65-67.

Leopold, A. C. and Musgrave, M. E. (1979). Respiratory changes with chilling injury of soybeans. *Plant Physiol.* **65**: 702-705.

Lewis, D. A. (1956). Protoplasmic streaming in plants sensitive and insensitive in chilling temperatures. *Science*. **124:** 75-76.

Levitt, J. (1939). The relation of cabbage hardiness to bund water, unfrozen water, and cell contraction when foozen. *Plant Physiol.* **14**:93-112.

Levitt, J. (1980). Response of plants to environmental stress. Edition 2, Vol.1. Academic Press, New York. pp.23-64.

Loganathan, P.; Saravanan, K. and Ganesan, J. (2001). Genetic variability in greengram (*Vigna radiata* L.). *Research on crop.* **2**:213-215.

Loganathan, P.; Saravanan, K. and Ganesan, J. (2001). Genetic divergence in greengram. *Research on Crops*. **2**:386-389.

Lown, R.J. (1995). The Asiatic vigna species. *Vigna sps.* (*V. radiata, V. mungo, V. angularis, V. umbellata* and *V. acontifolia*) (Leguminosae – Papilionoideae) In Smartt, J., Simmonds, W.M. (Eds) Evolution of crop planta, 2nd eds. Logmam, London, pp:321-326.

Lu, J.J., Ambrose, M.R., Brown, M.J., M. and, J.K. and Ellis, T.H.N. (1996. "Comparative analysis of genetic diversity in pea assessed by RFLP and PCR based methods". *Theor. Appl. Genet* **93**:1103-111.

Lyons, J.M., Raison, J.K., and Steponkus, P.L. (1979). The plant membrane in response to low temperature an overview in low temperature stress in crop plants, Lyons, J.M., Graham, D., Rsison, J.K. (eds). Academic press, New York, pp.1-24.

Mace, E.S., Yuejin W, Boshou, L., Upadhyaya H, Chandra, S. and Crouch, J.H. (2007). Simple sequence repeat (SSR) based diversity analysis of groundnut (*Arachis hypogaea* L.) germplasm resistant to bacterial wilt. *Plant Genetic Resources*, **5**:27-36.

Muhammad, S. Malik, M.F.A. and Awan, S.I. (2006). Genetic divergence association and performance evaluation of different genotypes of mung bean (*Vigna radiata* (L) Wilczek). *International Journal of Agriculture and Biology*. **8**:793-795.

Muhammad, Z. Ajmal, S.U.; Mahammad, A. Haqqari, A.M. (2007). Multivariate analysis for quantitative traits in mung bean (*Vigna radiata* (L) Wilczek). *Pakistan Journal of Botany*. **3a**:103-113.

Malhotra, R.S. and Saxena, M.C. (1993). Screening for cold and heat tolerance in cool season food legumes. In breeding for stress tolerance in cool season food legumes. Singh, K.B., Saxena, M.C. (eds.). John Wiley, UK. 99:227-244.

Manivaman, N. (2002). Association analysis in segregating generations of greengram. *Legume research*. **25**(1):63-65.

Manivannan (1999). Genetic variability of seed yield and its components of greengram (*Vigna radiata* (L) Wilczek). *Agricultural Science Digest, Karnal*. **19**:96-98.

Marmiroli, N., Terzi, V., Odoardi, S. M., Lorenzoni, C. and Stanca, A. M. (1986). Protein synthesis during cold shock in barley tissues. Comparison of two genotypes with winter and spring growth habit. *Theor. Appl. Genet*. **73**: 190-196.

Miranda, G.V. Coelho, A.D.F. Shimoya, A.; Coimbra, R.R., Santhos, I.C (1999). Genetic divergence of mung bean (*Vigna radiata* (L) Wilczek). *Revista-ceres*, **46**:309-319.

Mishra, L. D., Baisakh, B. and Nayak, P. K. (2008). Genetic variability among advanced mutant lines of mungbean. *Journal of Food Legume*. **21**: 274-275.

Mittal, V. P., Singh, P. and Brar, K. S. (2007). Component characters influencing seed yield in mungbean (*Vigna radiata* (L.) Wilczek). *International Journals of Agricultural Sciences*. **3**: 80-81.

Muhammad, Z., Ajmal, S. U., Muhammad, A. and Haqqani, A. M. (2007). Multivariate analysis for quantitative traits in mungbean (*Vigna radiata* (L.) Wilczek). *Pakistan Journal of Botany*. 39: 103-113.

Mullainathan, L.; Jayamala, V.; Arulbala Chandran, D. and Velu, S. (2006). Genetic variability in quantitative traits of mung bean (*Vigna radiata* (L) Wilczek). *Research on crops*. 7:751-754.

Nagaral, I. And Kajjidoni, S. T. (2008). Estimation of genetic variability parameters among advanced breeding lines mungbean. *Journal of Food Legume*. **21**:63-64.

Naidu, N.V., Satyanarayana, A. and Rajeswari, V.R. (1994). Path analysis of yield and yield attributes in different environments in mung bean. *Indian Journal of Agricultural Research*. **28**:74-78.

Naidu, N.V. and Rosaiah, G. (1993). Association analysis in segregating and non segregating population of mung bean *Vigna radiata* (L) Wiczek. *Annals of Agricultural Research*. **14**:25-29.

Nath-D. Chowdhury and Dasgupta, B.R. (2005). Genetic divergence in some cultivated and wild species of *Vigna*. *Indian agriculturist*. **42**:27-32.

Nayak, B. C., Nayak, N. and Mohapatra, P. K. (2010). Pulse Crop in Odisha. Retrospect and Prospect. Advanced Technology for Boosting Pulse Production in NFSM District. pp. 5-14.

Niazi, I.V.K.; Khan, A.A. and Hag, A.U. (1999). Path co-efficient analysis of agronomic characters affecting seed yield in *Vigna radiata* (L) Wilczek. *Journal of Genetics and Plant Breeding*. **53**:63-65.

Nilson, P.J.; Dick, H.M.; Staden, J. V. (1994). Variation in the rooting of mung bean (*Vigna radiata* (L) Wilczek). *South Africa journal of Botany.* **60**:276-278.

Ouellet, F., Houde, M. and Sarhan, F. (1993). Purification characterization and cDNA cloning of the 200 kDa protein induced by Cud acclimation in wheat. *Plant Cell. Physiol.*, **34**: 59-65.

Pandey, D. K. and Singh, N. K. (2002). Genetic variability, correlation and performance of short duration greengram (*Vigna radiata* (L.) Wilczek) varieties in rice-wheat system. *Progressive Agriculture.* **2**: 94-95.

Pandiyan, M.; Ganeshram, S.; Babu, E. and Marimuthu, R. (2006). Correlation studies in greengram (*Vigna radiata* (L) Wilczek). *Plant Archives.* **6**:771-773.

Pandiyan, M.; Subbalakshmi, B. and Jebaraj, S. (2006). Genetic variability in grengram (*Vigna radiata* (L) Wilczek). *International Journal of Plant Sciences.* Muzaffarnagar. **1**:72-75.

Patel, G.M.; Pitha, M.S.; Bhatya, V. J. (2006). Character association between seed yield and its components in greengram (*Vigna radiata* (L.) Wilczek). *National Journal of Plant Improvement.* **8**:142-147.

Patersen, A.H., Damon, S. Hewilt, J.D., Zamir, D Rabinowitch H.D., Lincoln S.E., Lander, S.E. and Tanksley, S.P. (1991). Mendelian factrors underlying quantitative traits in tomato Comparison across species, generations and environments. *Genetics*, **127**:181-197.

Poehlman, J.M. (1991). The mung bean. Oxford and IBH, New Delhi.

Rahim, M.A., Mia, A.A., Mahmud, F, Zeba, N. and Afrin, K.S. (2010) Genetic variability, character association and genetic divergence in mungbean (*Vigna radiata* L. Wilczek). *Plant Omics Journal*, **3**:1-6.

Raje, R. S. and Rao, S. K. (2000). Association analysis for yield and its components in mungbean (*Vigna radiata* (L.) Wilczek). *Legume Research.* **23**: 42-48.

Ranjan, R.E.B.; Wilson, D.; Vijaya R.K. (2001). Correlation and path analysis in the F_2 generation of greengram (*Vigna radiata* (L) Wilczek). *Madras Agricultural Journal.* **87**:590-593.

Rao, C.R. (1952). Advanced statistical methods in biometric research. John Wiley and Sons, New Work.

Rao, C. M., Rao, Y. K. and Mohan–Reddy (2006). Genetic variability and path analysis in mungbean. *Legume Research.* **29**: 216-218.

Reddy, C.R.; Sekhar, M.R. and Reddy, K.P. (1994). Character association and path co-efficient analysis in greengram *Vigna radiata* (L) Wilczek. *Annals of Agricultural Research..* **15**:423-427.

Reddy, K.H.P. (1997). Genetic variability in greengram (*Vigna radiata* (L) Wilczek). *Annals of Agricultural Research.* **18**:554-555.

Reddy, K. S., Souframanien, J., Reddy, K.S. and Dhanasekar, P. (2008). Genetic diversity in mungbean as revealed by SSR and ISSR markers. *Journal of Food Legumes.* **21**(1):15-21.

Reddy, K.H.P. (1997). Genetic divergen in greengram (*Vigna radiata* (L) Wilczek). *Annals of Agricultural Research.* **18**:493-497.

Reddy, K.S., Souframanien J., Reddy, K.S. and Dhanasekar, P. (2008). Genetic diversity in mungbean as revealed by SSR and ISSR markers. *Journal of Food Legume*, **21**: 15-21.

Rehman, A. U., Ali, M. J., Atta, B. M., Saleem, M., Abass, A. and Mallahe, A. R. (2009). Genetic studies of yield related traits in mungbean. (*Vigna radiata* (L.) Wilczek). *Australian Journal of Crop Science.* **3**: 352-360.

Rohlf, F.J. 1993. NTSYS–pc : Numerical taxonomy and multivariate analysis system, version 2.02. *Applied Biostatistics*, New York.

Rongwen, J., Akkaya, M.S., Bhagwat, A.A., Lavi V. and Cregan, P.B. (1995). The use of microsatellite DNA markers for soybean genotype identification. *Theoretical and Applied Genetics.* **90**:43-48.

Roy, B. and Basu, A.K. (2009). Abiotic stress tolerance in crop plant. Cold tolerance. pp. 243-292.

Rozina, G, Sijid A, Hamayoon K.M., Ali, F. and Ali, I. (2007). *Journal of Agricultural and Biological Science*. **2**: 9-11.

Salpveit, M.E. Jr. and Morris, L.L. (1990). Overview of chilling injury of horticultural crops. In chilling injury of horticultural crops, Wang Cy (ed.), CRC Press, Noca Raton, pp :3-5.

Samantaray, J. (2009). Analysis of segregation potential of crosses for yield in F_3 of a half diallel crosses in greengram (*Vigna radiata* (L.) Wilczek). A Thesis submitted to OUAT.

Santalla, M., Power, J.B. and Davey, M.R. (1998). Genetic diversity in mung bean germplasm revealed by RAPD markers. *Plant breeding.* **117**:473-478.

Saxena, R.R. and Singh, P.K. (2007). Correlation and path analysis in mung bean cultivar. *Journal of Interacademicia.* **11**:143-148.

Singh, A., Singh, S.K., Sirohi A. and Yadav R. (2009) Genetic variability and correlation studies in greengram (*Vigna radiata* L. Wilczek). *Prog. Agric.* **9**:59-62.

Singh, B.B., Singh, D.P. and Singh, N.P. (1997). A Genetic variability in pigeon pea germplasm for cold tolerance. *Indian Journal of Genet.* **57**:425-430.

Singh, K.B., Malhotra, R.S. and Saxena, M.C. (1990). Source of tolerance to cold in *Cicer sp. Crop Science* **30**:1136-1138.

Singh, S.K., Singh, I.P., Singh, B.B. and Singh, O. (2009). Correlation and path coefficient studies for yield and its components in mungbean (*Vigna radiata* L.Wilczek), *Legume Research.* **32**:180-185.

Smartt, J. (1990). Grain legumes – Evolution and genetic resources. Cambridge university, p:371.

Souframanien, J. and Gopalakrishna, T. (2009). Cross species amplification of microsatellite loci and diversity analysis in blackgram. *Journal of Food Legume.* **22**:11-17.

Sreedevi, G. and Sekhar, M.R. (2004). Character association and path analysis of morphological attributes in greengram (*Vigna radiata* (L) Wilczek). *Annals of Agricultural Research.* **25**:149-152.

Sreedharan, P.N. and Rao, G.J.N. (1983). A new screening technique for isolation of donors for cold tolerance in rice. *Oryza.* **20**:156-158.

Sreekalashmi, C. and Sekhar, M.R. (2005). Path co-efficient analysis in full sib progenies and F_3 bulk population in grengram (*Vigna radiata* (L) Wilczek). *Journal of Research* – Angrau. **33**:65-67.

Srivastava, R. P. and Ali, M. (2004). Nutritional quality of Common Pulses. Kanpur. IIPR. 65p.

Ticko, J.L.; Lal, S.K.; Chandra, N; and Dikshit, H.K. (2006). Mung bean breeding in advances in mung bean and urd bean. Edt. by Ali and Shiv Kumar. IIPR Publication. pp-110-148.

Trevanion, S. J. Brooks, A. L. and Leegood , R. C. (1995). The effect of temperature on the growth and metabolism of germinating cotyledons of marrow (*Curcurbita* pep. L.) of *Plant Physiology.* **145**: 741-749.

Tsou, C. S., Hsu, M. S., Tan, S. T. and Park, H. G. (1979). Protein quality of mungbean and its improvement. *Aito. Horticulture*. **93**: 279-289.

Tsumura, Y., Ohba, K. and Strauses, S.H. (1996). Diversity and inheritance of inter simple sequence repeat polymorphium in Doughlas fir (*Pseudotsuga menzii*) and Sugi (*Cryptomeri japonica*). *Theor Appl. Genet.* **92**:40-45.

Venkateswar (2001). Genetic variability in greengram. *Legume research.* **24**(1):69-70.

Venkateswarlu, (2001). Correlation and path analysis in greengram (*Vigna radiata* (L) Wilczek). *Legume research.* **24**:115-117.

Verdcourt, B. (1970). Studies in the leguminosae – papilionoideae for the flora of tropical. East Africa. *IV Kew Bull.* **24**:507-569

Vijaya Prakash (2006). Genetic divergence and correlation analysis in mung bean (*Vigna radiata* (L) Wilczek). *Crop Improvement.* **33**:175-180.

Vikas; Paroda, V. and Singh, R.S. (1998). Genetic variability in mung bean (*Vigna radiata* (L) Wilczek) *Annals of Agril. Bioresearch.* **3**:211-215.

Vikas; Singh, S.P. and Paroda R.S. (1998). Variability of yield and its components over location during summer season in mung bean (*Vigna radiata* L. Wilczek) *Journal of the Andaman Science Association.* **14**:9-13.

Wang, C. Y. (1982). Physiological and biochemical response of plant to chilling stress. *Hort. Science.* **17**: 173-186.

Wani, B.A., Marker, S. and Lavanya, G.R. (2007). Genetic variability, correlation and path analysis in greengram (*Vigna radiata* (L) Wilczek). *Journal of Maharastra Agricultural University.* **32**:216-219.

Weir, B.S. (1996). Genetic data analysis. Methods for discrete genetic data. Sunderland M.A., Sinauer Associates.

Yaqoob, M, Khan, H.V., Malik, A.J., Nawab, K. and Malik, B.A. (1997). Path coefficient analysis in some mung bean (*Vigna radiata* (L) Wilczek). *Sarhad Journal of Agriculture*. **13**:277-281.

Yoshida, S. (1964). Low temperature-induced cytoplasmic acidosis in cultured mungbean (*Vigna radiata* L. Wilczek) cells. *Plant Physiology,* **104**:1131-1138.

Yu, C.W., Terence, M.M. and Lin, C.H. (2003). Hydrogen peroxide-induced chilling tolerance in mung bean mediated through ABA – independent glutathione accumulation. *Functional Plant Biology*, **30**:955-963.

Appendix

Appendix 1. Scores of genotypes for presence or absence of polymorphic bands in SSR analysis

Sl No.	Primer		Genotypes																								Present	Absent
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1	CFDC050	250bp	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	1	6	19
2	CEDG008	250bp	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	3	22
3	CEDG010	250bp	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	9	16
4	CEDG020	350bp	1	0	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0	1	0	0	1	1	1	1	1	12	13
		300bp	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	21	4
5	CEDG043	300bp	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	23
6	CEDG086	300bp	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	21
7	CEDG088	300bp	1	0	1	0	1	0	1	1	0	1	0	0	1	1	1	0	0	1	0	0	1	1	1	1	1	15	10
		250bp	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	21	4
		200bp	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	5	20
8	CEDG091	250bp	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	23	2
9	CEDG092	300bp	1	0	1	0	0	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	0	1	1	0	15	10
		270bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25	0
		250bp	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	1	1	0	11	14
10	CEDG139	300bp	0	0	0	1	1	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	0	13	12
		250bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25	0
		200bp	0	0	0	1	0	1	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	1	1	0	0	9	16
11	CEDG154	300bp	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	20	5
12	CEDG228	250bp	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	22	3
13	CEDG156	320bp	1	1	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	1	1	1	11	14
		300bp	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	20	5
		200bp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1	1	1	8	17
14	CEDG248	250bp	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	22	3
15	VRI	250bp	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	13	12
16	VR2	250bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25	0
17	VR4	250bp	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	5	20
18	VR5	400bp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	22
		350bp	0	1	0	0	0	1	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	8	17
		300bp	1	0	1	0	1	1	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	12	13
19	VR6	300bp	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	22
		250bp	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	19	6