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

A
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THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
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(PLANT BREEDING AND GENETICS)

By

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2012

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Dedicated
to my

Beloved
Parents

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF AGRICULTURE
OUAT, BHUBANESWAR-3

Dr. B. Baisakh
Professor

July 31, 2012

CERTIFICATE- I

This is to certify that the thesis entitled "**GENETIC ARCHITECTURE OF YIELD AND COLD TOLERANCE IN MUTANT LINES OF GREENGRAM (*Vigna radiata* (L.) Wilczek)**" submitted by **SUBASH CHANDRA SWAIN,** Adm. No.05 PBG/10 to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT BREEDING AND GENETICS** is a faithful record of *bona fide* 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.

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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 MUTANT LINES OF GREENGRAM (*Vigna radiata* (L.) Wilczek)”** submitted by **SUBASH CHANDRA SWAIN,** Adm. No.05 PBG/10 to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for degree of **MASTER OF SCIENCE (AGRICULTURE)** in **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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I apologize for my omission, which of course not deliberate.

Subash Chandra Swain

Bhubaneswar
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Title of the thesis	:	**GENETIC ARCHITECTURE OF YIELD AND COLD TOLERANCE IN MUTANT LINES OF GREENGRAM (*Vigna radiata* (L.) Wilczek)**
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ABSTRACT

Thirty genotypes of greengram including 22 mutant lines, two parents and two standard varieties along with four land races 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 1.610 to 3.590 g/plant. OE2-3, ON3-1 and OGN2-3 produced higher yield of 3.590, 3.350 and 3.200 g/plant. Maximum tolerance to cold was exhibited by SG1-1 followed by OE1-2 with average yield of 2.090-2.917 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 high heritability with high genetic advance indicating additive gene action. Characters like100-seed weight and seed/pod were with high-moderate heritability but low genetic advance indicating non-additive gene effect.

Plant height, cluster/plant, 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 plant height. Positive correlation of most traits with yield was greatly influenced by indirect positive effect via pods/plant and plant height.

Among the different mutagenic treatment, T5E2 was found to be most effective as it was able to change six out of twelve characters in the positive direction followed by T9N3 which change four characters in the positive direction.

The 30 genotypes showed high genetic divergence (D^2) and tolerance to cold at 30, 40 and 10 days old seedling contributed maximum to divergence. On the basis of D^2 values using Tocher's method, the genotypes were grouped into 09 clusters. The 14 genotypes consisting of 12 mutants, one parent and one standard variety 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 IV and IX are expected to produce more transgressive in later generation. The Z_1-Z_2 scatter diagram of genotypes on basis of canonical analysis showed seven clusters almost as on basis of D^2 with few exchanges.

Seed protein analysis (albumin and globulin) through SDS-PAGE was done to assess the status of genotypes for protein quality and molecular characterization of 30 greengram genotypes. Electrophoresis results revealed 12 albumin and eight globulin polypeptide bonds with different molecular weights. . The polypeptide band AL3 was medium thick but sharp in all genotypes except genotype SN3-3 , SG3-3, SE2-3, SM1-3, SE2-2, SG1-2, OGN2-3 and OGM2-3 indicating absence of gene responsible for production of that polypeptide in them which can be used as marker for those particular genotype. Similarly GL1 and GL3 which were faint and thin in nature were absent in few genotypes and can be used as molecular marker for genotypes OG3-2, SN1-2 and SE3-2 respectively for their identification.

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Introduction

INTRODUCTION

Pulses own a strategic position in intensive as well as sub intensive agriculture as they are excellent source of dietary protein for millions of people, nutritious feed for livestock, and mini nitrogen plant having profound ameliorative effect on soil (Ali & Kumar, 2009). An equally important role is played by pulses in rainfed and irrigated agriculture by improving physical, chemical and biological properties of soil and consider excellent crop for viable and sustainable agriculture. Pulses are also considered as important source of minerals, micro & macro nutrients as well as health promoting secondary metabolites (White & Brown, 2010) and considered poor man's only source of protein (Reddy, 2009).

India grows such a variety of grain legumes which none of the country in the world grows. In India, more than a dozen of pulses crop namely chickpea, pigeon pea, urudbean, greengram, lentil, lathyrus, cowpea, commonbean, mothbean, horsegram, ricebean are cultivated under varying agroecological condition (Nandarajn and Chaturvedi, 2010). India is the largest producer of pulses accounting for about 25% of global share. During 2010-11, 1.80 million tonnes of pulses were produce on 3.55 million ha. area in India with an average grain yield of 512 Kg/ha.(Project Coordinator's Report, 2011-12: Annexure-VI). Pulses can grown on marginal and sub marginal soil hence due to change in the global agriculture during last century it has been push from high productivity long growing cool environment to short low yielding and relatively warm environment. Due to this shift of pulses area from North to Central and South, the potential of high yielding variety could not be realize to the level of satisfaction. That's why no single plant type is appropriate for all production system (Singh, 1997).

Greengram, (*Vigna radiate* (L.) Wilczek) is an important pulse crop which is an annual legume. It has diploid chromosomes no. 2n=22 (Karpechenko, 1925). This crop is cultivated in tropical and subtropical temperate zone of Asian including Bangladesh, India, Pakistan, Myanmar, Indonesia, Philippines, Srilanka, Nepal, China, Japan. It is also cultivation in Australia, East Africa and USA. It's seeds contain about 20-25% protein having major source of 10 amino acids that are essential in the diet of non-ruminant animals, including human. It also serves as an excellent source of easily-digestible protein and particularly nutritious when combined with cereals because, while the cereals compensate for greengram low levels of sulfur amino acid, the beans compensate the shortage of lysine in cereal. Most developing countries of the world today face the problem of inadequate protein intake by a large section of their population. However, utilization of legume protein is below their potential partly due to the deficiency of some essential amino acids in their proteins and also due to the presence of some anti-nutritional factors associated with their protein (Kavas and Nehir, 1992).

Sulfur containing amino acids like cysteine and methionine are the most nutritionally limiting amino acids in greengram protein. Unlike other leguminous seed crops, there is a dearth of information on the seed storage protein profiles and protein subunits of greengram. Improvement of the nutritional quality of greengram by increasing its protein content will contribute to the improvement of the nutritional quality of the diet for people. Breeding and molecular genetic methods are new tools for improving nutritional quality of greengram.

India is the center of origin of greengram (originated from North eastern Indian Myanmar region of Asia) as was first reported by de Candolle in his classical book "Origin of cultivated Plants" in 1886 and placed within the subspecies- Radiata, Species-Radiata, Subgenus-Ceratotropis, Genus-Vigna, Tribe-Phaseoleae, Family-Fabaceae, Order-Fabales, Subclase-Rosidae, Class-Magnoliopsida, Division-Magnoliophyta, Subkingdom-Tracheobionta, Kingdom-Plantae is one of the important pulse crop grown in different season in India and its rank 3rd after the chickpea and pegeonpea in area and production (Lvanya *et al*, 2011).

In Odisha, the greengram growing regions are Dhenkanal, Kalahandi, Cuttack, Balasore, Puri, Bolangir, Sambalpur. This crop rank first in acreage 836.03 thousand hectare and production of 362.79 thousand matric tonnes and with average yield of 434 kg per ha. The low productivity of crop is mainly due to cultivation in marginal and sub marginal land, non-conventional areas, poor crop husbandry, diseases and lack of suitable variety for different seasons and different agro-climatic situation including inner district with cold hazards. Due to autogamous nature and problem of flower drop, lack of synchronous maturity, susceptible diseases like MYMV, improvement through hybridization and recombination become difficult. Since genetic variable is essential for crop improvement, induction of mutation provide a powerful means of creating new and useful variability in both qualitative and quantitative traits (Kumar *et al.*, 2008).

The efficiency of isolating desirable mutant for quantitative character is low because of different in identifying micro mutants. Techniques that could provide quantitative measures of genetic divergence with regard to multiple characters induced by mutation would prove valuable in breeding. Mahalanobis' D^2-Statistics and Canonical Variate Analysis (CVA) are such multivariate measure of divergence which predicts classification of population into groups on the basis of genetic affinity or diversity with regard to several characters (Rao, 1952, 1960). The utility of D^2 analysis in grouping from different mutagenic treatment and the undesirable one from the yield point of view but improved in one or few agronomic characters to be utilized as parents in a hybridization programme has been emphasized. It has been demonstrated in wheat (Bhatt, 1973) that selection of parents for hybridization on the basis of D^2 analysis was more effective for improvement of yield than that based on other methods.

The effectiveness of selection for a character depends upon the amount of genetic variation present in a variable population. The variability of a biological population is an outcome of genetic contribution of individuals making of that population in relation to prevailing environment. A survey of genetic variability with helps of suitable parameters such a genotypic coefficient variation, heritability, genetic advance are absolutely necessary to start an efficient breeding programme. The coefficient of correlation between yield and its contributing traits shows a complex relationship; Path coefficient analysis, partitions the component of correlation coefficient into direct and indirect effects and visualizes the relationship in more meaningful way (Rahim *et al*, 2010). Genetic diversity is one of the criteria of parent selection in hybridization programme. Secondly, knowledge of association of yield with its components, especially at the genotypic level, is of considerable importance in selection for yield. Information about the interrelationship among these yield components themselves is also equally important for selection of an optimum combination of the characters which would improve yield. Thirdly, in a system of closely associated characters, such as yields and its component, it is of use to study the cause and effect relationships in terms of nature and relative contribution of the characters component to yield. With the increasing in financial status of the individual, people have become more cautious about quality aspect of the pulses for which the seed storage proteins component is important.

Keeping the above point in view, the present mutation breeding project in green gram was under taken for micro-mutational improvement of yield of two varieties namely Sujata and OBGG-52. The mutagenic treatment included three doses of physical mutagen i.e. gamma ray and three chemical mutagen i.e. EMS, Nitroso guanidine and Malic Hydrazide and three combination of physical and chemical mutagen at the intermediate dose of such mutagen. The population was advance to M_7 generation and the present study was undertaken with 22 numbers of induced mutants, 02 parents, 04 locals and 02 high yielding standard varieties of diverse geographic and genetic origin with the following objectives

1. To evaluate the genotypes for their yield and yield contributing traits *per se* including cold sensitivity;
2. To study the nature and extent of variation in these characters and examine the scope of selection of the traits;
3. To evaluate these lines for components of productivity and asses genetic divergence among them by multivariate analysis;
4. To examine the genetic changes induced by different mutagenic treatments by analyzing the clustering pattern of mutant clusters;
5. To examine the association of different characters by correlation studies;
6. To analyze the protein profile by SDS-PAGE in M_7 lines of greengram to reveal out polymorphism in seed storage protein.

The result of this study will help in selecting suitable high yielding, cold resistance/tolerant mutant greengram genotype(s) with good quality seed proteins for their direct commercial cultivation by farming community as well as suitable parents for future hybridization programme of Odisha.

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CHAPTER-II

Review of Literature

REVIEW OF LITERATURE

The earliest record of mutation dates back to 1791, when an English farmer named Seth Wright discovered a male lamb with unusual short legs, which was a source of development of Ancon breed of sheep. The term mutation was introduced by Hugo de Vries (1901) for sudden heritable changes in different characters in evening primrose (*Oenothera lamarckiana*) Hugo de Vries (1901) in his classical work "Die Mutations Theorie" heralded the remarkable statement "Knowledge of the principles of mutation will certainly some time in future enable a fully planned artificial induction of mutation *i.e.*, the creation of new properties in plants and animals. Moreover, man will be able to produce superior varieties of cultivated plants and animals by commanding the origin of mutations".

2.1 Induction of mutations

Induction of mutations by use of physical and chemical mutagens have been proved as efficient methodology of creating useful variability in both qualitative and quantitative characters in various crop plants. Efficient mutagenesis involves the production of desirable changes often free from other associated undesirable ones. From plant breeding point of view, mutation frequency induced by different mutagenic treatment could be taken as a measure of comparison for mutagenic efficiency (Kawai, 1969).

2.1.1 Induction of micromutations

Scossiroli (1965) extensively reviewed all the previous work on effect of irradiation on quantitative traits in several crops, such as soybean, white mustard, peanut, tobacco, *Arabidopsis,* tomato, barley, rice, wheat, maize and cotton and summarized that (i) there was a conspicuous shift in the mean and variance of a polygenic trait after mutagenic treatment, (ii) the mean value of quantitative traits in most instances were lower in mutagen treated populations than untreated control populations, but the difference between means of treated and untreated populations decreased in subsequent generations, (iii) the symmetry of variation sometimes depended on the history of previous selection of the trait and selective forces during reproduction, (iv) the phenotypic variability for different morphological and productivity traits were larger in treated populations than control and the increase in variability was mainly due to increase in genetic component of variability (evolution of new gene constellations) indicating greater scope of improvement by selection, (v) the relationship between dose of mutagen and the variability was not linear and in most cases intermediate doses induced largest increase in variability, (vi) the genetic nature of the increased variability through physical and chemical mutagens and effectiveness of selection for improvement in yield was stressed and (vii) in some instances yield improvement achieved by mutation breeding was not previously achieved by conventional method of plant breeding. So it is clearly demonstrated that mutation breeding was an efficient as hybridization in generating genetic variation in polygenic traits and selection was effective in bringing out improvement.

2.2 Mutagens

A variety of mutagens are available which can be broadly classified as physical mutagen and chemical mutagen.

2.2.1 Physical mutagens

The physical mutagens namely X-rays, γ-rays, β-rays, ultraviolet rays, fast neutrons and thermal neutrons have been frequently used for induced mutagenesis (Elliot, 1958). Except ultraviolet rays, all radiation types were found to ionize atoms in a tissue by detaching electron from the atoms (Anonymous, 1977).

Among the physical mutagens, gamma ray was found to be effective and most widely used mutagen for induction of mutations in crop plants. A great majority of mutant varieties (64%) were developed by the use of gamma-rays (Ahloowalia *et al.,* 2004). Gamma-rays are sparsely ionizing electro-magnetic radiation with a shorter wave length of 0.001 to 0.1 A^0.

There are scores of reports on its efficiency for induction of both macro- and micro-mutations in various crop plants. Induction of mutations in greengram through gamma irradiation have been reported by several workers, to cite a few, Auti *et al.* (2007), Singh (2007), Singh *et al.* (2008), Tah (2008), Khan and Goyal (2009), Tah and Saxena (2009) Kumar *et al.* (2009) Thilagavathi and Mullainathan (2011).

2.2.2 Chemical mutagens

During fifties and sixties, increasing popularity of chemical mutagens led to identification of very large array of chemical mutagens. Of these mutagens, so far only a few were really found to be more effective and useful in crop improvement programmes and most of which belong to the group of alkylating agents and are found to be the most potent mutagens (Rapoport *et al.,* 1966).

Alkylating agents have one or more functional alkyl groups, which can be transferred to other molecules at a position of very high electron density. They react with DNA by alkylating the phosphate group as well as purine and pyrimidine bases, often leading to backbone break, thereby causing deletion, duplication or mistakes in incorporation of base leading to gene or point mutation. The best known and most widely used alkylating agents are Ethyl Methane Sulphonate (EMS) and Nitrosoguanidine (NG).

Ethyle Mathane Sulphonate (EMS)

Ethyl Methane Sulphonate ($CH_3.SO_2.O.C_2H_5$) is a monofunctional alkylating agent and mutagenic properties of EMS was reported by Loveless (1958) in T_2 bacteriophase. It has long been known that EMS reacts preferentially with the base guanine of DNA (Bautz and Freese, 1960; Freese, (1963). Krieg (1963) has shown that EMS reacts with three of four bases of DNA in the order of guanine, adenine and cytosine. The action on guanine base is associated with ethylation of N-7 position thereby inducing mutations and chromosomal breaks. Lawley and Brooks (1963) have reported that a secondary effect of the N-7 alkylation of guanine is loosening of the bond between the base and the sugar-phosphate chain leading to gradual leaching out of guanine from alkylated DNA. The "apurine gaps" thus created, if filled up wrongly, would produce transition or transversion mutations. If not filled up, it might result in frame-shift mutations. EMS induces C- to T- changes resulting in C/G to T/A substitution (Krieg, 1963; Kovalchuk *et al.,* 2000; Greene *et al.*, 2003). Bautz and Freese (1960) had observed that EMS treatment resulted in more of transition from GC to AT, while transition of AT to GC, transversion and frame-shift mutations were relatively less and chromosomal breaks were rare.

EMS induced mutagenesis in greengram was reported by Singh and Singh (2007), Auti *et al.*(2007), Singh *et al.* (2008), Singh and Nalinikanta (2008), Balai and Krishna (2009), Khan and Goyal (2009), Kumar *et al.* (2009) and Kozgar *et al.*(2010) and Mondal *et al*, (2011)

Nitroso Guanidine (NG)

The nitroso compound, N-methyl-N-nitrosoguanidine (CH_3. NHO. CNH. NH. NO_2), commonly called "Nitroso Guanidine" (NG), is an alkylating agent containing methyl group. Mutagenicity of NG was first reported in *E. coli* by Mandell and Greenberg (1960) and subsequently, its mutagenic activity was established in various test materials. Swaminathan *et al.* (1968) in a study with rice, barley and wheat observed that NG and NMU were more potent mutagens than EMS and qualify to group under the so called "supermutagens" class of Rapoport *et al.* (1966).

Mandell and Greenberg (1960) reported that mutagenicity of NG was more due to its structure, rather than to its breakdown product. Eisenstark *et al.* (1965) and Baker and Tessman (1968) reported that NG produces equal proportion of GC to AT and AT to GC transition. Singer *et al.* (1968) have observed that methyl group of NG reacts with purine bases and produces more of methylated guanine than methylated adenine. Cerda-Olmedo *et al.* (1968) observed that NG acts specifically at the replication fork causing mutations. Dong (1982) working on barley observed that NG treatments induced structural chromosome and chromatid rearrangement and the effect increased with concentration. Flora *et al.* (1982) reported that NG induced more of chromosomal aberrations than EMS. NG treatments were reported to have drastic effect on germination survival, seedling growth, pollen and seed fertility in M_1 generation (Kempanna *et al.*, 1969; Sahoo, 1971; Levy and Ashri, 1973; Weirsbinski,1984).

Reports on induction of macro- and micro-mutation in greengram by NG are quite few *viz*. Sahoo (1971), Prasad (1972),Grover and Virk (1984), Saikrishna (1991) Mishra (1995) Misra and Momin (2004), Mishra *et al.* (2004), Das and Misra (2005), Das *et al.* (2006) and Singh and Nalinikanta (2008), Das and Baisakh (2009,2010) and Das (2011).

Maleic hydrazide (MH)

Maleic hydrazide (CH.CH. CO. NH. NH. CO), a derivative of hydrazine (N_2H_4) with similar mode of action of hydrazine. It is a growth regulator and auxin antagonist. Darlington and Mc Leish (1951), Graf (1957), Lewis and John (1963) have reported that MH is an effective chromosome breaker and acts preferentially on heterochromatin region. Since heterochromatin is the site of majority of the polygenes (Mather's hypothesis) and MH reacts on the heterochromatin, it can be effectively utilized for induction of micromutation in quantitative traits (Ghose and Chatterjee (1989) and Rieger *et al.* (1990) had observed that hydrazine treatment induced chromosome abnormalities like stickyness, fragments, laggards, bridges and also sterile mutations Sinha and Acharya (1986) reported induction of poly-embryony in rice following MH treatment and observed haploid plants. Induction of mutations by MH treatments were reported by Reddy and Smith (1984), Ghose and Chatterjee (1989), Misra (1990), Senapati (2007), Baisakh *et al* (2011) and Das (2011).

2.3 Induction of mutations In greengram

Singh and Rao (2007a) investigated the induced morphological mutation in two varieties of greengram (Sujata and TARM-1) by gamma irradiation. Results indicated induction of a wide spectrum of morphological mutations in both varieties. The frequency of morphological mutation increased with increase indose of the radiation. Variety Sujata was found to be more sensitive to gamma irradiation in comparison to variety TARM-1.

Kumar *et al.* (2008) induced the mutation in greengram (var. PS16 and Sona) by different doses of gamma rays (10, 20, 30, 40 and 50Kr), EMS (0.1, 0.2 and 0.3%) and their combinations (all gamma-rays treatments with 0.2% EMS) and studied the induced genetic variability for eight quantitative characters.

A study on mutagenic effectiveness and efficacy of EMS and NG in mungbean was undertaken by Singh and Nalinikanta (2008). They observed higher effectiveness of NG treatments in comparison to EMS treatments. The reverse trend was observed in case of efficacy. Both effectiveness and efficacy of both chemicals decreased with increase in mutagenic dose. The possible reason for this could be less damaging effect of lower doses of both chemicals on the genetic materials.

Khan and Goyal (2009) conducted an experiment by treating mungbean seeds with EMS, SA and gamma-rays to reduce maturity period of two genotypes (K 851 and PS-16). High heritability coupled with high GA recorded for days to maturity indicated that genetic progress to be expected from selection..

Kozgar *et al*. (2010) studied the effect of EMS induced variability in *Vigna radiata* at different doses (0.1 to 0.4 %). Decrease in mean value of various quantitative traits at higher concentration of EMS and a non-linear relationship was observed between the yield and doses of mutants.

Das (2011) studied physical and chemical mutagens impacts on two popular greengram genotypes and generated useful mutants.

Balai and Krishna (2012) assed the chemical mutagen induced polygenic variability in M_3 progenies of mungbean along with standard varieties and found significant differences among the mutants for different traits.

2.4 Genetic variability, heritability and genetic advance in quantitative traits in greengram

Induced variability, character association and path coefficient in mutant cultures of greengram variety Pusa Vishal were studied by Momin and Misra (2004) in M_4 generation. As per their findings, the range of variation among the mutant culture was quite wide in both positive and negative direction compared to parent variety showing mutagenic treatment to be effective in inducing micro-mutations in polygenic traits. Both PCV and GCV estimates were high for plant height, pods/plant, 100-seed weight and yield. High heritability was observed for days to flowering and maturity with low genetic advance indicating non-additive gene action. Plant height, pods/plant, 100-seed weight and yield show moderate to high heritability and high GA indicating additive gene action and selection for these GA would be effective. Pods/plant, 100-seed weight and seeds/pod show significant positive association with yield. Associations among these traits were of low magnitude.

Presence of substantial variability was reported for the eleven numbers of quantitative character studied in 20 mutant liens of mungbean variety Pusa 9072 in M_4 generation by Babu *et al.* (2007).The GCV for all characters were smaller than that of PCV indicating some influence of environment on the character. GCV was moderate for seed protein content, pods per clusters, clusters per plant and pods per plant, indicating the scope for selection to bring about genetic improvement in these traits. Traits like protein content, seed yield per plant, pods/plant, seeds/pod, plant height and primary branches exhibited high heritability, indicating that a major part of the phenotypic variability might be attributed to genetic causes through the induction of mutation.

Presence of substantial variability was reported for the eleven numbers of quantitative characters studied in 20 mutant liens of mungbean variety Pusa 9072 in M_4 generation by Babu *et al.* (2007). Four doses each of gamma-rays, EMS and their combiantions in T_{44} and PDM-54 cultivars of mungbean by Singh and Singh (2007).

Mishra *et al.* (2008) while studying 47 genotypes of mungbean 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, 100 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 suggests 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.

Kumar *et al.* (2008) induced mutation in greengram (var. PS16 and Sona) by different doses of gamma rays and EMS and their combinations (all gamma-rays treatments with 0.2% EMS) and studied the induced genetic variability for eight quantitative characters. Highest heritability was recorded for number of pods per plant, number of seeds per pod, grain yield per plant and plant height. The maximum genetic variability was noticed for 30 kR and 40 kR doses of gamma-rays, 0.2 % EMS and 30 kR + 0.2 % EMS and 40 kR + 0.2 % EMS for most of the yield component characters. The study revealed that mutagenesis is a potent tool for the improvement of polygenic characters, provided large populations are raised and rigorous selections are followed.

Khan and Goyal (2009) conducted an experiment by treating mungbean seeds with EMS, SA and gamma-rays to reduce maturity period of two genotypes (K 851 and PS-16). High heritability coupled with high GA recorded for days to maturity indicated that genetic progress to be expected from selection.

Genotypic and phenotypic variance, coefficient of variance, heritability, genetic advance, correlation and path coefficient for yield and its contributing characters in 26 Mungbean genotypes was evaluated by Rahim (2010). Significant variations among the genotypes were observed for all the characters. High heritability (broad) along with high genetic advance in percent 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.

Tabasum *et al.* (2010) studied ten mungbean genotypes to assess variability and degree to which various plant traits associate with seed yield.Primary and secondary branches, pods per cluster and pod length showed lesser variability whileclusters per plant, 100 seed weight and harvest index exhibited intermediate range of variability.Sufficient genetic variability was observed for plant height, pods per plant, total plant weight andseed yield. Moderate to high heritability estimates were found for all traits.

Variability, heritability and correlations among agronomic and physiological characters of fifty six mungbean accessions were determined by Parinya and Piyada (2011). They found that genotypes differed significantly for all characters studied. The highest heritability values were recorded on days to flowering and pod length.

Reddy *et al. (*2011) evaluated thirty five divergent genotypes of greengram [*Vigna radiata*. (L.) Wilczek] for yield and yield attributes. Genotypes differed significantly for all the characters studied. High genetic advance coupled with high heritability was observed for characters *viz*., plant height, and number of pods/plant, shoot dry matter/plant and seed yield/plant, indicating there to determine the variability, heritability and correlations among agronomic and physiological characters of 56 mungbean (*Vigna radiata* (L.)

Wilczek) and to identify their direct and indirect effects on seed yield. It was found that genotypes differed significantly for all characters studied. The highest heritability values were recorded on days to flowering and pod length.

Twenty diverse mungbean (*Vigna radiata* L. Wilkeezek) genotypes were evaluated for the estimation of genetic variability, heritability, genetic advance, correlation coefficient and path coefficient analysis for ten quantitative characters by Makeen *et al.* (2011). The genotypes differed significantly for all characters studied. Higher genotypic and phenotypic coefficient of variation was observed for seed yield and number of pods per plant. Maximum heritability values were recorded in seed protein content, plant height and test weight. High heritability coupled with high genetic advance was observed in pods per plant, plant height and test weight indicating the importance of additive gene effect for expression of these characters.

Begum *et al.* (2012) examined the parameters like branches plant-1, plant height, pods plant-1, pod length, number of grains pod-1, number of grains plant-1, grain yield plant-1and 100-grain weight. Significant variation were observed among the yield and related traits. Pak-22 produced maximum number of branches plant-1 (4.25). NM-51 had maximum pod length (7.82 cm) followed by NM-93 (7.42 cm). Maximum grain yield was recorded for genotype Ramzan (45.35) while maximum 100-grain weight was recorded for NM-93 (5.85). High heritability was recorded for days 100-grain weight (77.17%), and number of branches plant-1 (68.63%). Low heritability was recorded for grains pod-1 (33.33%), pods plant-1 (29.67%), pod length (35.42%), grain plant-1 (30.50%), and grain yield plant-1 (33.50%).

2.5 Correlation in greengram

Genetic parameter and path-coefficient analysis in M_4 generation of mungbean were carried out by Idress *et al.* (2006). High heritability coupled with moderate to high GA for primary branches, pods per plant, 100-seed weight and seed yield indicated additive gene mainly controlled these characters. Cluster per plant, pods per plant, pod length had positive highly significant correlation with seed yield. Plant height, primary branches and cluster/plant showed strength positive direct effect on seed yield.

Pandey *et al.* (2007) evaluated twenty mungbean genotypes to study the genetic variability, correlation and path analysis. Character association studies revealed significant and positive correlation of harvest index, pod number, 100-seed weight and pod length with seed yield. Hence, selection for genotypes with higher harvest index and biological yield, taller height and longer duration could facilitate augmentation of seed yield in mungbean.

Hakim (2008) studied 350 mungbean accessions for Correlation and path coefficient analyses to quantify the magnitude of the relationship between yield components and grain yield. The variability among the accessions was significant for most of the characters studied, especially for days to maturity, plant height, pods per plant, and seed size. Among the yield components, the number of pods per plant and plant height positively correlated with the grain yield, but the seed size negatively correlated with grain yield. Therefore, these parameters (number of pods per plant and plant height) can be used as the selection criteria in mungbean breeding program. These criteria can be visualized during bulk selection on the early generation stage of F2 to F4, and subsequently on line development of individual plant (pedigree) of F5.

While studying correlation in local greengram Jena (2010) found pods/plant, pod length and seeds/pod had positive correlation with yield.

26 Mungbean genotypes was examined by Rahim (2010) and reported that the number of pods per plant, panicle length and number of seeds per pod to be positively correlated with grain yield.

Khajudparn and Tantasawat (2011) conducted study to determine the variability, heritability and correlations among agronomic and physiological characters of mungbean (*Vigna radiata* (L.) Wilczek) and to identify their direct and indirect effects on seed yield in fifty six mungbean accessions. Seed yield was significantly and positively correlated with pods per plant, clusters per plant, total dry matter (TDM), seeds per pod, seeds per plant, biomass, leaf area index and branches per plant, and negatively correlated with days to maturity.

In (2011) Mondal *et al* took study on 45 mungbean genotypes. Genotypic correlation study among the traits investigated indicated leaf area index (LAI) was the most important source that determined total dry mass (TDM) yield, and reproductive characters like numberof racemes, flowers and pods plant-1 were the most important sinks that determined seed yield. Contrarily, reproductive efficiency (RE, % pod set to opened flowers) did not show significantrelationship with pod number and seed yield, indicating that selection of high yield based on RE may bemisleading.

Correlation analysis by Reddy *et al*. (2011) in greengram indicated that seed yield/plant was positive and significantly associated with days to maturity, plant height, number of pods/plant, number of seeds/pod, 100-seed weight, shoot nitrogen, seed protein and shoot dry matter/plant.

Twenty diverse mungbean (*Vigna radiata* L. Wilkeezek) genotypes were evaluated by Makeen *et al*, (2011) for correlation coefficient for ten quantitative characters. Character association indicated that pods per plant and plant height have significant positive correlation with seed yield.

Begum *et al* , (2012) conducted research on genotypic correlations among traits of 10 mungbean genotypes for branches plant-1, plant height, pods plant-1, pod length, number of grains pod-1, number of grains plant-1, grain yield plant-1and 100-grain weight. Grain yield plant-1 revealed highly significant phenotypic correlation with pods plant-1 (0.65), grains plant-1 (0.61), and with 100-grain weight. Grain yield plant-1 showed significant genotypic correlation with pods plant-1 (0.71) and with days to pods formation (0.70). Based on present studies Ramzan and AEM-96 can be used for further breeding study regarding yield improvement.

2.6 Path coefficient analysis in greengram

Labanya and Toms (2009) studied path analysis among yield attributing characters in l32 greengram genotypes. Number of primary branches per plant, clusters/plant, pods/cluster, pods/plant, seeds/pod and l00 seed weight showed positive direct effect on seed yield/plant. Plant height, days to maturity and 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 l00 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.

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

Jena (2010) partitioned the correlation into direct and indirect effect by path analysis. Pods/plant had highest direct positive effect on yield followed by seeds/pod and pod length.

Tabasum *et al.* (2010) in a study of greengram reported clusters per plant, pods per plant, total plant weight and harvest index having positive significant genotypic and phenotypic correlations with seed yield. Positive direct effects were exerted through secondary branches, pods per plant, pod length, 100 seed weight, totalplant weight and harvest index while primary branches, plant height, clusters per plant and pods percluster had negative direct effects.

An experiment on 56 greengram genotypes was conducted by Khajudparn and Tantasawat (2011) and they found clusters per plant showing the highest positive direct effect on seed yield followed by 100 seed weight, seeds per pod, TDM and pods per plant. However, the effect of 100 seed weight was substantially minimized by the negative indirect effects of clusters per plant, pods per plant and seeds per pod.

Mondal *et al.* (2011) conducted field experiments to investigate the path coefficient analyses in 45 mungbean genotypes. Path coefficient analysis further revealed that number of flowers, pods and 100-seed weight constituted central important sinks which exerted direct positive influence on seed yield. The results indicated that pod yield could be increased by increased raceme and flower production, while seed yield could be increased by increasing pod production.

Thirty five divergent genotypes of greengram [*Vigna radiata*. (L.) Wilczek] was evaluated by Reddy *et al.* (2011) for yield and yield attributes. Path co-efficient analysis revealed that days to flowering, days to maturity, number of pods/plant, seed protein, shoot dry matter/plant and 100-seed weight had positive direct effects on seed yield/plant. Hence, selection on these traits could be improving seed yield in greengram

2.7 Genetic diversity in greengram

Muhammad *et al.* (2007) analysed 40 mungbean génotypes for l4 quantitative traits. All the traits were analyzed using multivariate analysis technique (cluster and principal component analyses). The genotypes were categorized in to 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.

Thirty four diverse mungbean (*Vigna radiata* L. Wilczek) accessions for eight agronomic were assed by Rahim *et al.* (2008). Among eight quantitative traits, number of seeds per pod contributed maximum towards divergence. Considering the yield, growth duration and number of seeds per pod genotypes of cluster I and IV, and cluster II and IV exhibit high heterosis for yield as well as earliness, and yield as well as large number of seeds per pod, respectively.

Genetic divergence of 26 mungbean genotypes for yield and yield contributing characters was studied by Rahim *et al.* (2010).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.

Chattopadhyay *et al.* (2011) took an investigation aimed to assess morphological and molecular diversity of seventy eight mungbean germplasm of Indian sub-continent. The estimated genetic distances based on combined binary data of molecular markers and qualitative characters along with standardized data of quantitative characters were substantially supported by the linearity evaluation with morphological features, localization of gene pool and the nature and parentage. ‘Kopargaon’, a variety from Maharastra, which was the highest yielder with highest number of pods/plant, was found most diverse from ‘TRCM-5-1’, a breeding line of Tripura possessing highest seed weight based on both D^2 and combined analysis using morphological and molecular data. This method of the comprehensive estimation of genetic distances could possibly pave the path for the required modification in the process of diversity analysis and varietal description of mungbean.

Genetic divergence was estimated among thirty greengram genotypes using Mahalanobis's D^2 statistic by Gokulakrishnan *et al.* (2012). Total of six clusters were formed. Cluster II contained the highest number of thirteen genotypes followed by cluster I with eight genotypes and clusters V and VI contained one genotype each. The pattern of distribution of genotypes from different geographical locations into six clusters was random, demonstrating that geographical isolation may not be the only factor causing genetic diversity. The highest intra-cluster distance was observed for cluster V (17.30) and the lowest was observed for cluster I (9.52). While the highest inter-cluster distance was observed between cluster I and V (39.22). Cluster V recorded the highest mean for seed yield per plant, number of pods per plant, number of branches per plant, number of seeds per pod and 100 seed weight. Therefore, it was concluded that more emphasis should be given on cluster V for selecting genotypes as parents for crossing with the genotypes of cluster I which may produce new recombinants with desired traits.

Abna *et al.* (2012) evaluated genetic diversity of 20 genotypes of mungbean. Eight morphological characters including plant height, number of fruiting branches per plant, number of pods per plant, number of pod clusters per plant, pod length, number of seeds per pod, 1000-seed weight and total seed yield per plant were measured. A total of four groups were defined through cluster analysis and distinct genetic variations were observed among these groups. Using cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) method, all genotypes were grouped into three main groups and one minor group. Cluster I consisted of nine genotypes, cluster II of seven, cluster III of one and cluster IV of three genotypes. Principal component analysis was done to evaluate diversity and morphological traits which had more effects on diversity and three components explained near 79% of total variation among genotypes. By plot of first two components score for genotypes confirmed the result of cluster analysis.

2.8 Mechanism of cold resistance

Tropical and subtropical plants exhibit marked physiological and biochemical days functions commonly referred to as chilling injury when they are exposed to temperature below 10ºC - 12ºC (Graham and Patterson, 1982; Guy, 1990). These dysfunctions include alteration in membrane structure and lipid composition (Lyons and Raison, 1979) metabolic modifications; Trevanion *et al.*, 1995), changes in protein content (Marmiroli *et al.*, 1986); and enzyme activities (Kumar and Tripathy, l998), 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 (Hughes and Dunn, l996; 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 4ºC for 2 days and found induced irreversible chilling injury. The major cation in the leakage from tissues of unacclimated seedlings was K^+ the loss of which was 7 – 10 folds greater than that of Ca^{2+} or Mg^{2+}. Acclimation of seedlings at l0ºC protected them from the injuries caused by 4ºC treatment. Acclimation of seedling at 10ºC 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 28ºC-root/28ºC shoot, control seedlings. Though in the 28ºC root/4º C-shoot treatment did not suffer noticeable injury, but seedlings in the 4ºC-root/4ºC 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 28ºC root/4ºC shoot seedlings were similar to those of controlled seedlings.

Mung bean (*Vigna radiata* L. cv. TN5, a chilling-sensitive cultivar) was employed to evaluate the importance of glutathione in hydrogen peroxide (H_2O_2)-induced chilling tolerance by Yu *et al* (2003). Seeds germinated at 25°C for 7 d were subjected to differenlt periods of chilling treatment, prior to analysis of the glutathione contents of their leaves. In a comparison of acclimation temperatures from 2-12°C, it was found that an 8°C acclimation for 36 h induced a 5.7-fold increase, the highest glutathione level among the temperatures tested. Seedlings acclimated at 8°C for 36 h showed 97% survival after a 36-h, 4°C chilling stress, compared with 33% survival of non-acclimated plants. Pretreatment with 200 mM H_2O_2 for 12 h before a 36-h, 4°C chilling treatment increased glutathione levels by 30% and reduced electrolyte leakage to 43%, relative to the untreated control. Treated seedlings also showed a survival rate of 71% after the same chilling treatment. Application of 1 mMbuthioninesulfoximine, a specific inhibitor of glutathione synthesis, reversed the protection against chilling stress provided to seedlings either by acclimation at 8°C for 36 h or H_2O_2 pretreatment. The role of glutathione in chilling acclimation or H_2O_2-pretreatment-induced chilling tolerance is thus confirmed.

Fang *et al.* (2002) found application of a 8°C, 36 h-chilling in dark resulted in a 2-fold increase in the glutathione (GSH) levels in 7-d-old mung bean seedlings, compared to 25°C unchilled plants. However, this chilling-induced GSH accumulation was prohibited by white-light irradiation under the same chilling treatment. The inhibitory effect of light on the GSH accumulation was enhanced with the increasing of light intensity and reached the maximal level at 100 µmol m^{-2} s^{-1}. Whereas this light-dependent inhibitory effect promptly declined when the white-light intensity was more than 100 µmol m^{-2} s^{-1}.Noteworthily, electrolyte leakage analysis showed that the light-reduced GSH accumulation had no negative impact on the cold tolerance of mung bean seedlings. A combination of cold treatment (8°C, 36 h) and 100 µmol m^{-2} s^{-1} red (640-660 nm) or blue light irradiation (420-460 nm) further indicated that red light was the main contributor for GSH inhibition in mung bean seedlings. Taken together, these results strongly suggest that in addition to growth and development, red/far-red light receptor phytochrome is also involved in the development of cold tolerance in plants.

Survival rates of greengram seedlings chilled at 4°C for 36 hour was studied by Yu-Chin-Wen *et al.* (2003). 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

Chen (2007), used mung bean (*Phaseolus radiatus* Linn.) and garden pea (*Pisum sativum* Linn.), which were stressed 4 days under a low temperature of 10^0C, as materials to study the cold tolerance of plant with different resistance. On the 2nd and 3rd day under 10 degrees C stress, both the malondialdehyde (MDA) content and the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities increased significantly in hypocotylar cells of mung bean, so did SOD activity in garden pea, but other physiological indexes in garden pea were not different from the non-treatment groups). In hypocotylar cells of mung bean, SOD activity always maintain at the highest level in a period of time,and so does POD activity. Ultrastructural results after stress indicated as follows: (1) Plastids in hypocotylar cells of mung bean accumulated much starch (Plate I-6), whereas the form of plastids in hypocotylar cells of garden pea changed maskedly to become dumb-bell-shaped, round or irregular, with the last one being the most common form); (2) In both mung bean and garden pea, central vacuole was divided into small vacuoles and the number of mitochondria increased and became aggregated. Judging from the activities of protective enzymes and ultrastructures, 10^0C low temperature caused non-lethal, temporary injuries to hypocotyls ultrastructures in mung bean, but no visible injury at all and even improved its cold tolerance to a certain degree in garden pea.

2.9 Seed storage protein pattern

Plant seeds accumulate massive storage proteins in discrete vesicles termed protein bodies as a source of amino acids for use during germination and postgerminative growth of seedings. Extensive research has been carried out of the molecular level in the past two decades on seed storage proteins. Osborne (1924) pioneered the systematic study of plant proteins by categorizing them on the basis of their solubility in a series of solvents, as follows: first, water solubilizes the albumins; next, extraction in dilute salt solutions solvates the globulins; subsequent extraction inalcohol-water mixtures yields the prolamins; and finally, treatment with dilute acids or alkali releases the glutelins. The globulins are further divided into two subgroups according to their sedimentation coefficients: 7S vicilin-type and 11S legumin-type.

In dicotyledonous plants, seed storage proteins have been classified as either albumin or globulin types. Globulins are the major storage proteins in most dicot seeds, especially legumes, although a low-molecular-mass sulfur-rich albumin (one of the 2S albumins, named for their sedimentation coefficient) also is present in these and other species. The globulins account for up to 70% of the total seed nitrogen (Croy and Gatehouse, 1995), and in the legumes they consist of two major protein families, the 7S vicillin/ convicilin and the 11S legumin groups, which are differentiated by molecular mass. Both groups are holoproteins composed of regularly assembled subunits (Bewley and Black, 1994). They differ in their digestibility and amino acid content, and therefore their relative abundance affects the nutritional value of the seed (Perrot, 1995).

Mungbean seeds contain about 20-25% protein. It is considered to be a major source of proteins as a source of the 10 amino acids that are essential in the diet of non-ruminant animals, including human. Mungbean supplies a substantial part of the protein consumed by the poor, especially in developing countries. It also serves as an excellent source of easily-digestible protein and particularly nutritious when combined with cereals because, while the cereals compensate for mungbean's low levels of sulfur amino acid, the beans compensate the shortage of lysine in cereal. Most developing countries of the world today face the problem of inadequate protein intake by a large section of their population. However, utilization of legume protein is below their potential partly due to the deficiency of some essential amino acids in their proteins and also due to the presence of some antinutritional factors associated with their protein (Kavas and Nehir, 1992). Sulfur containing amino acids like cysteine and methionine are the most nutritionally limiting amino acids in mungbean protein. Unlike other leguminous seed crops, there is a dearth of information on the seed storage protein profiles and protein subunits of mungbean. Improvement of the nutritional quality of mungbean by increasing its protein content will contribute to the improvement of the nutritional quality of the diet for people. Breeding and molecular genetic methods are new tools for improving nutritional quality of mungbean.

The investigation on the protein components of seed storage proteins in mungbeans by studying different in banding patterns and subunit molecular weight determination by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) will help to develop initial information of profiles for seed storage protein improvement and to facilitate the mungbean breeding program of Odisha.

Ghafoor *et al.*(2002) used thirty seven diverse genotypes of Vigna mungo and three of V. radiata resembling to V. mungo for seed characters. Seed proteins were analyzed through slab type SDS-PAGE. Based on SDS-PAGE, specific bands were suggested to be used for identifying Vigna radiata from mixed germplasm with Vigna mungo. The SDS-PAGE proved to be a powerful tool for differentiating Vigna radiata and Vigna mungo; whereas a low level inter-specific genetic diversity was observed and no clear differentiation was observed both for agronomic characteristics and for geographical origin.

Ghafoor *et al.* (2005) evaluated one hundred and five genotypes of blackgram from diverse origin for agronomic traits for two years and analyzed seed proteins using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) through vertical slab type unit. Screening analysis for markers to quantitative traits revealed its significance in determining quantitative trait loci (QTL) in blackgram through SDS-PAGE markers. The factors affecting quantitative traits may occur as individual genes or gene clusters scattered throughout the genome, therefore, quantitative traits were expected differently at several loci. Variation in seven quantitative traits out of ten was significantly associated with 9 protein sub-units, however, the actual number of QTLs might be fewer because several of these traits were correlated. Variation at protein peptides in the vicinity of QTL in blackgram may be an indication of genetic variation potentially available to breeding programmes. Expansion of genetic base for blackgram breeding might be accomplished by systematic use of germplasm that differ from common banding pattern and known to be associated with variation in quantitative traits.

Biochemical analysis of seed storage polypeptides of three Vigna species through SDS-PAGE was studied by Borah *et al.* (2007) and revealed difference in banding pattern. There was variation both in the number and MW of the polypeptides. The major components of all the three species were in the MW range of 66-32kD. It was evident that gel electrophoresis of proteins could be a useful tool in species identification.

Rajesh *et al.* (2008) reportd late embryogenesis abundant (LEA) proteins to protect against water stress deficit in plants. An over expression system for mungbean late embryogenesis abundant protein, *emv2* was constructed in a pET29a vector, designated pET-*emv2* which is responsible for higher expression under the transcriptional /translational control of T7/lac promoter incorporated in the *Escherichia coli* BL21 (DE3).Induction protocol was optimized for pET recombinants harboring the target gene. Overexpressed EMV2 protein was purified to homogeneity and the protein profile monitored by SDS-PAGE.

Seed storage protein profiles of 8 kabuli (white seeded) chickpea mutants/genotypes was analysed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by Hameed et al, (2009) . Total soluble proteins were resolved on 10% SDS polyacrylamide gels. Low variability in tested kabuli germplasm was observed. On the bases of seed protein banding patterns, 5 genotypes could be identified clearly and biochemical fingerprints of these varieties are reported. Dendrogram based on electrophoretic data clustered the genotypes in three groups. Three genotypes viz., Pb-1, CM-94/99 and PKV Kabuli 2 showed 100% homology therefore could not be separated on the bases of seed storage proteins. One exotic genotype ILC-195 and two mutants CM-2000 and CM-98/99 were comparatively divergent from other genotypes. Identified protein markers i.e., KSSP-100, KSSP-93 and KSSP-64 can be used for identification of CM-98/99, ILC-195 and CM-2000 respectively. Moreover CM-315/99 and Noor-91 can also be identified by combination of identified protein markers. In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins can economically be used to assess genetic variation and relation in germplasm. The specific bands of seed storage protein profiles may be used as markers for identification of the mutants/ genotypes.

Tripathy *et al.* (2010) employed a method based in protein gel electrophoresis in order to improve the analysis of genetic relationships among populations of *Vigna* to estimate genetic divergence among 34 improved varieties, five local land races, one wild progenitor of mungbean (*Vigna sublobata*) and a land race of urdbean. In total, 20 polypeptide bands were resolved for seed storage proteins (albumin and globulin) by SDS-PAGE. The electrophoregrams revealed 11 and nine polypeptide bands for albumin and globulin respectively that have exhibited an array of polymorphism both in quality and quantity of bands. Mutants of mungbean differed in polypeptide banding pattern as compared to their parents indicating the mutation of genes in multigene families for seed storage protein expression. Cluster analysis revealed high genetic diversity. The results obtained support the idea that seed storage protein analysis can be successfully applied to phylogenetic analysis of *Vigna genotypes.*

Genetic diversity of greengram based on SDS-PAGE of albumin seed storage protein was under taken by Tripathy *et al.* (2010a). 11 polypeptide bonds were observed for albumin and 41 greengram genotypes were distributed in to to six clusters.

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CHAPTER-III

Materials and Methods

MATERIALS AND METHODS

The present investigation on "Genetic architecture of yield and cold tolerance in mutant lines of greengram [*vigna radiata* (L.) Wilczek]" was taken up in the Department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar. The field experiment was conducted at the EB-II Section during *rabi* season of 2011-12 and laboratory work was taken up in S.K.Sinha Molecular Breeding Laboratory of the department. The materials consisted of 30 cultures/varieties of greengram (*Vigna radiata (*L.) Wilczek). Twenty two of these were induced mutants (now in M7 generation) of Sujata and OBGG-52, 02 were parents and other 06 were standard genotypes including 04 locals. The induced genetic variants were developed at OUAT. The mutants were developed through selection for higher yield than the parental material by applying selection pressure from M_2 - M_6. Finally, the selected cultures were tried in yield trial in present investigation. The source of the materials and their pedigree are given in Table 1.

3.1 Field plot technique

The field experiment was laid out in a randomized block design (RBD) with 3 replications with 30 entries. The crop was shown on 25.09 2011. Each entry was represented by 3 rows of 3 m length. The intra and inter rows distances were 10 cm and 30 cm respectively. Fertilizers were applied at the rate of 20:40:20 kg of $N:P_2O_5:K_2O$ with 300 cft. of farm yard manure per hectare. All the FYM, phosphatic, potasic and half of the nitrogenous fertilizers were applied as basal dose and rest half of the nitrogenous fertilizers were applied at 21 days after sowing. Hoeing and hand weeding were done at the time of top dressing. No plant protection measure was taken as there was no incidence of diseases and insect pest. The crop was harvested during first fortnight of December, 2011.

Table 1 List of cultures/varieties with their pedigree and source of origin

Sl. No.	Cultures	Pedigree	Source
1	SN1-2	T_7 - NG 0.005 %, PS - 6 hrs. D - 6 hrs.	OUAT
2	SE3-2	T_6 - EMS 0.6%, PS - 6 hrs. D - 6 hrs.	OUAT
3	SE1-2	T_4 - EMS 0.2 %, PS - 6 hrs. D - 6 hrs.	OUAT
4	SG1-1	T_1 - Gamma-rays 20 Kr	OUAT
5	SN3-3	T_9 - NG 0.015 %, PS - 6 hrs. D - 6 hrs.	OUAT
6	SG3-3	T_3 - Gamma-rays 60 kR	OUAT
7	SE2-3	T_5 EMS 0.4 %, PS - 6 hrs. D - 6 hrs.	OUAT
8	SM1-3	T_{10} - MH 0.01 %, PS - 6 hrs. D - 6 hrs.	OUAT
9	SE2-2	T_5 - EMS 0.4 %, PS - 6 hrs. D - 6 hrs.	OUAT
10	SG1-2	T_1 - Gamma-rays 20 kR	OUAT
11	ON3-2	T_9 - NG 0.015 %, PS - 6 hrs. D - 6 hrs.	OUAT
12	ON3-3	T_9 - NG 0.015 %, PS - 6 hrs. D - 6 hrs.	OUAT
13	OM1-3	T_{10} - MH 0.01 %, PS - 6 hrs. D - 6 hrs.	OUAT
14	ON3-1	T_9 - NG 0.015 %, PS - 6 hrs. D - 6 hrs.	OUAT
15	OE1-2	T_4 - EMS 0.2 %, PS - 6 hrs. D - 6 hrs.	OUAT
16	OE2-3	T_5 - EMS 0.4 %, PS - 6 hrs. D - 6 hrs.	OUAT
17	OG3-3	T_3 - Gamma-rays 60 kR	OUAT
18	OG1-1	T_1 - Gamma-rays 20 kR	OUAT
19	OG3-2	T_3 - Gamma-rays 60 kR	OUAT
20	OG1N2-3	T_{14} - Gamma-rays 40 kR + NG (0.010 %), PS - 6 hrs. D-6 hrs.	OUAT
21	OM2-3	T_{11} - MH 0.02 %, PS - 6 hrs. D - 6 hrs.	OUAT
22	OG1M2-3	T_{15} - Gamma-rays 40 kR + MH (0.02 %), PS - 6 hrs. D-6 hrs.	OUAT
	PARENTS		
23	Sujata	L24-2 x Pusa Baisakhi	Odisha
24	OBGG-52	A mutant of K-851 (EMS@ 0.375%)	OUAT
	STANDARD VARIETIES		
25	TARM-1	RUM 5 x TPM 1	BARC
26	PDM54	Selection from germplasm collected from Kundawa Bahraich (UP)	IIPR
	LOCAL		
27	Sonpur-2	A local genotype collected from Balangir	OUAT
28	Jhainmung(B-2)	A local genotype collected from Bargarh	OUAT
29	Kendrapara(A)	A local genotype collected from Kendrapara	OUAT
30	Kopargaon	A selection from local genotypes of Kopergaon	MR

3.2 Sampling and recording of observations

Twelve quantitative characters related to growth, vigor and productivity including seed yield were recorded. Out of the 12 quantitative traits, days to 50% flowering was recorded on the plot basis and for other characters the 10 randomly selected competitive plants per plot in each replication were recorded. The characters observed were as follows:

3.2.1 Days to flowering- Number of days from sowing to flowering was recorded on plot basis, when about 50% of the plants of the plants in the plot started blooming.

3.2.2 Plant height- Plant height was recorded at the time of maturity. The plant height was measured to the nearest centimetre (cm) from the base of the plant *i.e.* ground level to the tip of main shoot.

3.2.3 Clusters per plant- Number of fruit bearing bunches or cluster in the 10 random plants per plot in each replication were recorded.

3.2.4 Pods per plant- Number of seed bearing pods were recorded at maturity of those randomly chosen plant.

3.2.5 Pod length-Ten random pods of the sample plant were taken for their length measurement in centimeter.

3.2.6 Seeds per pod- Number of seeds per pod in each random plant were calculated by dividing total number of seeds from the plant by total number of pods of the plant .

3.2.7 100-seed weight- After recording the yield of individual randomly chosen plant, it was divided by no of seeds of that plant and was multiplied by 100 to get 100-seed weight (g). Seed weight of each randomly selected plant was recorded in gram.

Response to cold exposure 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 to study their viability. Observations were taken on number of plant wilted in each genotype every day continuously and day to survival under cold stress was recorded. Analysis of seed storage protein pattern was done using protein gel electrophoresis.

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

3.3 Estimation of parameters of variability

The observations recorded on the 12 component characters were recorded on the 30 greengram genotypes in each of three replications. The replication wise mean values for the individual characters of different treatments were subjected to analysis of variance using RBD design. Character wise range, mean, coefficient of variations and the significance of treatments were worked out.

3.3.1 Analysis of variance (ANOVA) and test of significance

Analysis of variance (ANOVA) for each character was carried out with plot means for partitioning of the total variance into components, ascribable to replication, genotypes and error. The test of significance of difference between replications and among varieties for any character was done by 'F' test. The test of significance of difference between means of any two lines was tested by 't' test and critical difference (CD at 5%).

The analysis of variance was done on the basis of the following model.

$$Y_{ij} = M + g_i + r_j + e_{ij}$$

Where,

Y_{ij} = phenotypic observation of the i^{th} genotype in j^{th} replication

M = general mean

g_i = effect of the i^{th} genotype

r_j = effect of the j^{th} replication

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

The structure of analysis of variance is as follows:

Table 2 ANOVA for R.B.D with expectations of mean sum of squares (EMS)

Sources of variation	Degrees of freedom (d.f.)	Sum of squares (SS)	Mean sum of squares (MSS)	F-values	Expectations of mean sum of squares (EMS)
Replication	(r-1)	$(1/g_j)\ \sum y_j^2$-CF	MS_r	MS_r/MS_e	$\sigma^2_e+g\sigma^2_r$
Genotype	(g-1)	$(1/r_j)\ \sum y_i^2$-CF	MS_g	MS_g/MS_e	$\sigma^2_e+r\sigma^2_g$
Error	(r-1) (g-1)	By subtraction	MS_e		σ^2_e
Total	(rg-1)	$\sum y_{ij}^2$-CF			

Where,

r	=	number of replications
g	=	number of genotypes
MS_r	=	mean square due to replications
MS_g	=	mean square due to genotypes
MS_e	=	mean square due to error
σ^2_e	=	environmental variance
σ^2_g	=	genotypic variance
σ^2_p	=	phenotypic variance

σ^2_e = MS_e

σ^2_g = $\dfrac{MS_g - MS_e}{r}$

σ^2_p = $\sigma^2_g + \sigma^2_e$

3.3.2 Estimation of variance components

The phenotypic, genotypic and environmental variance components for different characters were estimated from E.M.S. in the ANOVA according to Al-Jibouri *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.

3.3.3 Estimation of mean, range, standard error and critical differences

The different parameters of variability like mean, range, standard error of mean (SEm), standard error of difference (SEd) and critical difference (CD) were estimated as follows:

Mean values of each character was averaged out over replications. The lowest and the highest values for each character were taken as range.

The significance of difference between means of any two genotypes was ascertained by using critical difference (CD), calculated as follows:

Standard error of difference (SEd) = $\sqrt{\frac{2\sigma_e^2}{r}}$

Critical difference (CD) = $\sqrt{\frac{2\sigma_e^2}{r}}$ x 't' at error d.f (5% level of significance)

Where,

r = number of replications

σ^2_e = error mean sum of squares

3.3.4 Estimation of genotypic, phenotypic and environmental coefficients of variation

The phenotypic, genotypic and environmental variance components for different characters were estimated from ANOVA using the expectations of mean square following Al-Jibouri *et al.* (1958).

Variability for different characters was estimated by the formula suggested by Burton (1952). The phenotypic, genotypic and environmental coefficients of variation for different characters were estimated as follows:

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

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

ECV (Environmental coefficient of variation in per cent) = $\frac{\sigma_e}{\bar{x}} \times 100$

Where σ_p and σ_g are phenotypic and genotypic standard deviations respectively and $\bar{X}$ is general mean for the character.

3.3.5 Heritability

Heritability (in broad sense) coefficients of different characters were estimated using the component of variance already defined previous section.

Heritability in broad sense was calculated as follows:

Heritability (h^2) = h^2_{bs} = $\frac{\sigma^2_g}{\sigma^2_p}$

Where,

σ^2_g = genotypic variance

σ^2_p= phenotypic variance

and h^2 (in %) = $(\sigma^2_g / \sigma^2_p) \times 100$

3.3.6 Estimation of genetic advance

The expected genetic advance (GA) or gains from selection among varieties for different characters was calculated using the following formula:

$$G.A. = K \times h \times \sigma_g \text{ (or } = K \times h^2 \times \sigma_p)$$

Where,

K = Standardized selection deferential which takes the values of 2.06 at 5% selection intensity.

h = Square root of heritability coefficient

σ_g = Genotypic standard deviation for the character

σ_p = phenotypic standard deviation

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

3.5 Correlation studies

The phenotypic, genotypic and environmental correlations were estimated to examine the pattern of association between the component characters.

3.5.1 Estimation of covariance components

The analyses of covariance (ANCOVA) between all possible pairs of ten characters were carried out on mean values. Phenotypic, genotypic and environmental components of covariance were estimated from the ANCOVA using the expectations of mean products (which are analogous to those of the mean squares in ANOVA) in the same way as the variance components.

Table 3 Analysis of covariance (ANCOVA) with expectations of mean sum of products (MSP)

Source	d.f	MSP	Expectations of MSP
Replication	(r-1)	MP_r	$\sigma_e\,(xy) + g\,\sigma_r\,(xy)$
Genotype	(g-1)	MP_g	$\sigma_e\,(xy) + r\,\sigma_g\,(xy)$
Error	(r-1) (g-1)	MP_e	$\sigma_e\,(xy)$

The components of covariance between two characters x and y were estimated according to Al-Jibouri *et al.* (1958) as follows:

Phenotypic covariance between x and y $= \sigma_p\,(xy) = \sigma_g\,(xy) + \sigma_e(xy)/r$

Environmental covariance between x and y $= \sigma_e\,(xy) = MP_e$

Genotypic covariance $= \sigma_g\,(xy) = \dfrac{MP_g(xy) - MP_e(xy)}{r}$

Where,

MP_g and MP_e are mean sum of products of genotype and error respectively and r is the number of replications

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.

3.5.2 Estimation of genotypic and phenotypic correlation

The variance and co-variance analysis were made for the characters based on the 30 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.

Genotypic correlation $(r_g) = \dfrac{\sigma_g(xy)}{\sigma_g(x)\sigma_g(y)}$

Where, $\sigma_{g\,(xy)}$ 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.

$$\text{Phenotypic correlation } (r_p) = \frac{\sigma_p(xy)}{\sigma_p(x)\sigma_p(y)}$$

$$\text{Environmental correlation } (r_e) = \frac{\sigma_e(xy)}{\sigma_e(x)\sigma_e(y)}$$

Where,

σ_p (xy) = phenotypic covariance between x and y

σ_p (x) and σ_p (y) = phenotypic standard deviations of the characters x and y respectively

σ_g(xy) = genotypic covariance between x and y

σ_g (x) and σ_g (y) = genotypic standard deviations of the characters x and y respectively

σ_e (xy) = environmental covariance between x and y

σ_e (x) and σ_e(y) = environmental standard deviations of the characters x and y respectively

Standard errors of the correlation coefficients were calculated using the following formula.

$$\text{SE } (r_p) = \sqrt{\frac{1 - r_p^2}{(g-2)}}$$

$$\text{SE } (r_g) = \sqrt{\frac{1 - r_g^2}{(g-2)}}$$

$$\text{SE } (r_e) = \sqrt{\frac{1 - r_e^2}{(r-1)(g-1)-1}}$$

Significance of correlation co-efficient was tested by ‘t’-test with (n – 2) degrees of freedom for r_g and r_p, and at {(r-1) (g-1)-1} degrees of freedom for r_e. by the formula.

Where,

g is the number of genotypes and r is the number of replications\

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

Where,

‘r’ is the correlation co-efficient and ‘n’ us the number of genotypes.

3.5.3 Path coefficient analysis

The method of analysis by path coefficients requires a cause-and-effect relationship among correlated variables. Path coefficients are standardized partial regression coefficients which individually provide a measure of the direct effect of a causal factor on the effect variable. They permit partitioning of the correlations between a causal factor and the effect variable into components of direct and indirect effects and thus, give a better picture of the associations of the 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, I92I; Dewey and Lu, I959).

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

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

....

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

Where,

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

The solutions for path coefficients, direct and indirect effects of the causal factors were estimated as the values of the individual terms of the above equation in RHS.

3.6 Multivariate analysis of diversity among genotypes

Multivariate analysis of divergence among 30 greengram genotypes based on the 12 productivity traits was done in two methods.

(i) D^2 analysis of genetic divergence

(ii) Canonical analysis

(i) D^2 analysis of genetic divergence

Genetic divergence with regard to twelve characters was estimated by Mahalanobis' D^2-statistic following Rao (1952). D^2 (Genetic divergence) between any 2 cultures/varieties is given by the formula:

$$D^2_p = \sum_{i=1}^{p} \sum_{j=1}^{p} W^{ij} d_i d_j$$

Where, W^{ij} is the inverse of the common dispersion matrix W_{ij} and d_i and d_j are the difference in the means of the two populations for ith and jth characters.

As computation by this formula is laborious, the character means were transformed into sets of uncorrelated variables. The transformation was done by pivotal condensation of the common dispersion matrix following Rao (1952). After this transformation, the formula for genetic divergence becomes:

$$D^2_p = \sum_{j=1}^{p} d_{2_i}$$

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

All possible $D^2{}_s$ among the 30 cultures/varieties were computed, the relative contribution of individual characters to divergence was assessed by (a) ranking of components $D^2{}_s$ as well as by (b) percentage contribution to total D^2 over all combinations.

(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 the ranks of each character are summed over all the 435 D^2 combination to get the rank total and then rank average is estimated.

(b) **Average D^2:** Average contribution of each character to all the 435 D^2 combinations is worked out.

3.6.1 Grouping of cultures/ genotypes into different clusters

1. Tocher's method

Usually a cluster is defined as a group of population/cultures such that any two populations belonging to the same cluster should, on an average, show a smaller D^2 then those belonging to two different clusters. A simple device suggested by Tocher (Rao, 1952) for construction of cluster is to start with two most closely related populations (having the smallest D^2) and then find a third one which has smaller average D^2 for 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 the cluster. Similarly, construction of 2nd, 3rd and other clusters are formed till all the population are included in one or the other cluster.

2. Canonical root method

This method is an extension of multiple regression analysis and is concerned with the study of association or interdependence of two sets of variables. This method can be used as a forecast model and also for clustering purpose. The canonical analysis (Rao, 1952) involves calculation of canonical vectors or canonical roots and the two first canonical root values (Z^1 and Z^2) of each population/culture are taken for two dimensional presentations of the cultures in graph. This can be supplemented to grouping by Tocher's method.

Seed storage proteins

3.7 Techniques in seed storage protein, molecular weight determination by SDS-PAGE

Estimation of molecular weight of a polypeptide is central importance to the characterization of proteins and is finding increasing use in molecular biology. Molecular weight determination of polypeptide using SDS-polyacrylamide gel electrophoresis (PAGE) was first introduced, empirically, by Shapiro *et al.* (1967). It was confirmed and extended by Weber and Osborn (1969) and Dunker and Rueckert (1969). These authors showed that the relative mobilities of the polypeptides were related to their molecular weights. Explanation for this behavior of polypeptides in SDS-PAGE was provided by Pitt-Rivers and Impiombato (1968) and Reynolds and Tanford (1970). They found that, under appropriate condition, all reduced polypeptides bind the same amount of SDS on a weight basis (1.4 g SDS/g polypeptide).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE); SDS is sodium dodecyl sulfate (sodium lauryl sulfate). The hydrophobic tail of dodecyl sulfate interacts strongly with polypeptide chains. The number of SDS molecules bound by a polypeptide is proportional to the length (number of amino acid residues) of the polypeptide. Each dodecyl sulfate contributes two negative charges. Collectively, these charges overwhelm any intrinsic charge that the protein might have. SDS is also a detergent that disrupts protein folding. SDS-PAGE is usually run in the presence of sulfhydryl-reducing agents such as β–mercaptoethanol so that any disulfide links between polypeptide chains are broken (Garrett and Grisham, 1995).

The electrophoretic mobility of protein upon SDS-PAGE is in inversely proportional to the logarithm of the protein's molecular weight. SDS-PAGE is often used to determine the molecular weight of a protein.

3.7.1 Materials

General Solutions

- Acrylamide stock solution: 30% Acrylamide, 0.8% (30.8% T 2.6% C) Bis (N, N' Methylenebisacrylamide)
- 1.5M tris buffer PH- 8.8 with 0.2 gm SDS
- 10% Ammoniumpersulfate (APS)
- Coomassie Brilliant Blue staining solution: 0.025% Coomassie Brilliant Blue R-250, 50% methanol 10% acetic acid
- Destaining solution I: 40% methanol, 7% acetic acid
- Destaining solution II: 5% methanol, 7% acetic acid
- Electrophoresis buffer: 0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3
- 2X Protein sample loading buffer: 0.5 M TrisCl, 20% SDS, 100% glycerol, 100% 2-mercaptoethanol, 1% Bromphenol Blue, pH 6.8
- 4X Resolving gel buffer: 1.5 M TrisCl, pH 8.8
- 20% SDS (Sodium Dodecyl Sulfate) stock solution
- 4X Stacking gel buffer: 0.5 M TrisCl, pH 6.8
- TEMED (N, N, N', N'- Tetramethylethylenediamine)

General Equipment

- Analytical and Precision Balances
- Centrifuge
- Gel Electrophoresis
- Hot Plate Stirrer
- Magnetic stirrer
- Mixer, Vortex
- Mortar
- pH Meter
- Spectrophotometer
- Rocker

Plant Materials

The experimental materials comprised of 30 genotypes including 22 mutants, two parents, two improved varieties and four local land races of Odisha. Albumin and globulin seed storage proteins were extracted with pre-chilled distilled water and 0.5M NaCl respectively, denatured with an equal volume of cracking buffer (0.125M Tris HCL pH 6.8, 20% SDS, 100% glycerol, 100% 2-mercaptoethanol, 1% bromophenol blue)at 65 degree C in hot water bath. Seed proteins were analyzed through verticalslab gel (12,5% polyacrilamide gel) SDS-PAGE of 60mA (2.5mA per lane for two gels run each time) for four hours. Reproducibility was confirmed by minimum of two repeats of each runoff gel electrophoresis under similar electrophoretic conditions. After electrophoresis, gels were stained with 0.125%w/v coomassie brilliant blue R 250, 50% v/v methanol, 10% v/v glacial acetic acid for four hours with intermittent shaking followed by distaining overnight in 50% methanol and 10% acetic acid; and finally several washing with5% methanol and 7% acetic acid. The molecular weights of the dissociated polypeptides were determined by using molecular weight marker with known molecular weights

3.7.2 Protein banding pattern analysis

Seed storage protein subunit profile was analyzed by visible protein bands from which intensity of the bands were classified into groups of protein banding patterns. Protein bands were put in matrix comparison plot of score 0 and 1 for missing and appearing of protein bands, and the data were analyzed for distance similarity using the NTSYSpc 2.01e program software (Adams and Rohlf, 2000).

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CHAPTER-IV

Results and Discussion

RESULTS AND DISCUSSION

The present investigation on "Genetic Architecture of yield and cold tolerance in mutant lines of greengram [*Vigna radiata* (L.) Wilczek]" was undertaken for development of superior mutant lines with improvement in yield, yield attributing traits, seed protein and tolerance to cold. Seed samples of two greengram varieties, *viz.*, Sujata and OBGG-52 with distinct morphological features were treated with varying doses/concentrations of one physical mutagen (Gamma rays), three chemical mutagens (EMS, NG and MH) and combinations of physical and chemical mutagens (Table 4). Observations on biometrical characters were recorded in M_7 generation. The experimental data were analyzed with broad objectives to evaluate the genotypes for their yield and other characters performances *per se* including cold sensitivity; to study the nature and extent of variation in these characters and examine the scope of selection of the traits; to evaluate these lines for components of productivity and asses genetic divergence among them by multivariate analysis; to examine the genetic changes induced by different mutagenic treatments by analyzing the clustering pattern of mutant clusters; to examine the association of different characters by correlation studies; and to analyze the seed protein profile by SDS-PAGE.

Thirty genotypes of greengram including 22 mutant lines, two parents and two standard varieties along with four land races were evaluated in M_7 generation. The trial was conducted in RBD with three replications. Observations on eight quantitative traits, *viz.,* days to flowering, plant height, clusters per plant, pods per plant, pod length, seeds per pod, 100-seed weight and seed yield/plant were recorded on 10 randomly chosen plants where as response to cold and seed protein were studied in laboratory. Analysis of variance for the twelve traits showed significant differences among the genotypes under study (Table 5).

Table 4 **Details of mutagenic treatments**

Tr. No.	Treatment* symbol	Mutagen	Dose/ concentration	Duration of pre-soaking in distilled water	Duration of treatment with mutagenic solution
1	T1G1	Gamma-rays	20 kR	-	-
2	T2G2	Gamma-rays	40 kR	-	-
3	T3G3	Gamma-rays	60 kR	-	-
4	T4E1	EMS	0.2 %	6 hours	6 hours
5	T5E2	EMS	0.4 %	6 hours	6 hours
6	T6E3	EMS	0.6 %	6 hours	6 hours
7	T7N1	NG	0.005 %	6 hours	6 hours
8	T8N2	NG	0.010 %	6 hours	6 hours
9	T9N3	NG	0.015 %	6 hours	6 hours
10	T10M1	MH	0.01 %	6 hours	6 hours
11	T11M2	MH	0.02 %	6 hours	6 hours
12	T12M3	MH	0.03 %	6 hours	6 hours
13	T13G1E2	Gamma-rays 40 kR + EMS (0.4 %)		6 hours	6 hours
14	T14G1N2	Gamma-rays 40 kR + NG (0.010 %)		6 hours	6 hours
15	T15G1M2	Gamma-rays 40 kR + MH (0.02 %)		6 hours	6 hours
16	T16C	Control (distilled water)		6 hours	-

Table 5. Analysis of variance for twelve characters in greengram

Sl. No	Character	Source	d.f	S.S.	M.S.	"F"
1	Days to 50% flowering	Replication	2	12.163	6.081	2.313
		Genotype	29	249.396	8.600	3.271**
		Error	58	152.504	2.629	
2	Plant height	Replication	2	3.287	1.644	0.289
		Genotype	29	2016.818	69.545	12.222**
		Error	58	330.043	5.690	
3	Cluster per plant	Replication	2	0.861	0.430	2.197
		Genotype	29	20.869	0.720	3.674**
		Error	58	11.359	0.196	
4	Pods per plant	Replication	2	2.138	1.069	1.183
		Genotype	29	278.605	9.607	10.628**
		Error	58	52.428	0.904	
5	Pod length	Replication	2	0.801	0.401	3.006
		Genotype	21	21.795	0.752	5.638**
		Error	58	7.731	0.133	
6	Seeds per pod	Replication	2	3.297	1.648	3.221*
		Genotype	29	89.879	3.099	6.056**
		Error	58	29.684	0.512	
7	Survival of 10 days old seedling at 10°C (d)	Replication	2	3.822	1.911	0.976
		Genotype	29	2217.822	76.477	39.077**
		Error	58	113.511	1.957	
8	Survival of 20 days old seedling at 10°C (d)	Replication	2	6.756	3.378	1.325
		Genotype	29	2847.656	98.195	38.505**
		Error	58	147.911	2.550	
9	Survival of 30 days old seedling at 10°C (d)	Replication	2	1.623	0.812	0.334
		Genotype	29	5002.457	172.499	70.935**
		Error	58	141.043	2.432	
10	Survival of 40 days old seedling at 10°C (d)	Replication	2	14.156	7.078	3.280*
		Genotype	29	3572.889	123.203	57.085**
		Error	58	125.177	2.158	
11	100-seed weight	Replication	2	0.072	0.036	1.078
		Genotype	29	17.708	0.611	18.273**
		Error	58	1.938	0.033	
12	Yield per plant	Replication	2	0.626	0.313	2.806
		Genotype	29	19.907	0.686	6.159**
		Error	58	6.465	0.111	

* Significant at 5% level $\quad$ ** Significant at 1% level

4.1 Variation of quantitative traits in the mutant cultures/genotypes

The estimates of means for different characters of the mutant cultures of both varieties Sujata (V1) and OBGG-52(V2) and other genotypes are presented in table 6.

4.1.1 Days to 50% flowering

The range of variation in days to 50% flowering among all the genotypes was 36.00 (Jhainmung) to 44.33(Sonepur-2) days as against 40 days in both the parent Sujata and OBGG-52 (Table 7). Range of variation in 10 mutant cultures of Sujata was 39 (SG3-3) to 44 days (SE1-2) as against 40 days (OG1M2-3) to 43 days (ON3-1) in parent OBGG-52. In both the cases, none of the mutant cultures flowered significantly earlier than the parent variety. However, seven cultures in Sujata and two cultures in OBGG-52 flowered significantly late than their respective parent. The late and early genotypes can be used in improvement programme as per situation demand.

4.1.2 Plant height

The range of variation for plant height in 30 greengram genotypes was 14.567cm (TARM-1) to 33.033 cm (SE2-2) Mutant cultures of Sujata and OBGG-52 showed a range of variation of 16.833 cm (SE1-2) to 33.033 cm (SE2-2) and 17.333 cm (ON3-3) to 30.667cm (OG1M2-3), respectively for plant height as against 25.333 cm and 26.967cm in the respective parent variety. In Sujata two (SE2-2, SG1-2) mutant cultures were significantly taller and five (SE1-2, SN1-2, SE3-2, SG1-1, SE2-3) were significantly shorter in height than the parent. In OBGG-52, non mutant cultures were significantly taller and six (ON3-3, OM1-3, OG1-1, OM2-3, OG3-3, ON3-2) were significantly shorter in height than the parent. The taller and the shorter mutant culture will be utilized in different cropping system as per the need of the farming community.

4.1.3 Clusters per plant

Range of clusters per plant was 2.467 (SE2-2) to 4.067 (SG1-2) among the experimental material. The range of variation in clusters/plant among the mutant cultures of Sujata and OBGG-52 was 2.467 (SE2-2) to 4.067 (SSG1-2) and 2.533 (OM2-3) to 3.933 (OE2-3) respectively, as against 3.933 and 2.800 in respective parents. In case of Sujata none of the mutant cultures showed significantly higher clusters per plant where as three (OE2-3, OG1-1, ON3-3) mutant cultures of OBGG-52 were with significantly higher clusters/plant. Culture with significantly higher no. of clusters per plant may be utilized by the breeder for future improvement of the greengram crop.

4.1.4 Pods per plant

Range of Pods per plant found to be 4.733 (SG1-1) to 10.533 (OE2-3) in the study materials. Mutant cultures derived from the variety Sujata showed wide variation of 4.733 (SG1-1) to 8.533 (SE2-2) pods per plant while in parent it was 8.600 and none of the mutant cultures showed significant increase in pods per plant. In case of OBGG-52, the range of variation among mutant cultures for pods per plant was 4.933 (OM2-3) to 10.533 (OE2-3) as against 8.333 in the parent and only one mutant culture exhibited significant increase in pods per plant than the parent. Higher no of pods per plant may be good combination in the plant architecture for achieving higher productivity.

4.1.5 Pod length

The experimental genotypes exhibited a range of variation of 4.233cm (OM1-3) to 7.200cm (Kopargaon) for Pod length. Variation among the mutant cultures of Sujata and OBGG-52 had a range of 4.900 cm (SG1-1) to 5.733 cm (SG3-3) and 4.233 cm (OM1-3) to 5.900cm (OE2-3), respectively, as against 5.267cm of both the parents. Only one mutant cultures of OBGG-52 showed significant increase in pod length than its parent variety.

4.1.6 Seeds per pod

The range of Seeds per pod was 4.200 (OM1-3) to 8.333 (Kopargaon) in the 30 genotypes under investigation. The mutant cultures of these two varieties differed significantly in seeds/pod. The range of variations in seeds/pod in mutant cultures of Sujata and OBGG-52 were 4.667 (SE3-2) to 7.967 (SE2-2) and 4.200 (OM1-3) to 8.233 (OG3-2) respectively, as against 6.533 and 8.067 in respective parents. Numbers of mutant cultures showing significantly higher seeds/pod than respective parents were 01 (SE2-2) in case of Sujata and none in case of OBGG-52.

4.1.7 100-seed weight

The variation in 100-seed weight was significant with range of 1.937(Jhainmung (B-2) to 3.783g (OM1-3). It was 2.740g (SE1-2) to 3.660g (SE2-2) and 3.150g (OG1N2-3) to 3.783g (OM1-3) in the mutant cultures of Sujata and OBGG-52, respectively, as against 3.083g and 3.397 g in the respective parent varieties. In Sujata three mutant cultures recorded significantly higher 100-seed weight than its parent while number of mutant culture showing significantly higher 100-seed weight than parent was two (OM1-3,ON3-3) in case of OBGG-52.

4.1.8 Yield per plant

The mutant cultures developed from both varieties differed significantly for seed yield/plant. The range of variation in yield/plant among the cultures of Sujata and OBGG-52 was 1.610g (SE1-2) to 3.170g (SE2-2) and 1.960g (OM1-3) to 3.590g (OE2-3), respectively, as against 2.730g and 2.440g in the respective parent. One (SE2-2) of the mutant cultures of Sujata and three (OM1-3, ON3-1, ON3-3) mutant cultures of OBGG-52 recorded significantly higher seed yield per plant than their parent varieties.

Table 6 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)	Seeds/ pod	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)	100-seed weight (g)	Yield (g/pl.)
1	SN1-2	43.000	19.333	2.933	7.467	5.533	7.200	15.000	17.000	5.000	19.333	3.160	2.380
2	SE3-2	44.000	20.333	2.533	5.867	5.267	4.667	8.000	15.000	10.000	12.000	3.073	2.030
3	SE1-2	44.000	16.833	2.533	5.000	5.067	7.133	16.000	23.000	12.000	27.000	2.740	1.610
4	SG1-1	43.000	20.967	3.000	4.733	4.900	6.467	16.000	23.000	29.000	29.000	3.307	2.090
5	SN3-3	40.000	21.567	2.667	5.933	5.667	6.967	12.000	21.000	22.000	26.000	3.147	2.390
6	SG3-3	39.000	22.067	3.000	6.667	5.733	6.600	22.667	12.000	19.000	9.000	3.483	2.400
7	SE2-3	44.000	20.933	2.533	6.267	5.533	6.467	3.000	10.000	19.000	7.000	3.257	2.340
8	SM1-3	43.000	23.367	3.600	6.667	5.567	6.633	14.000	17.000	15.000	16.000	3.613	2.520
9	SE2-2	43.000	33.033	2.467	8.533	5.633	7.967	14.000	18.000	17.000	14.000	3.660	3.170
10	SG1-2	42.000	33.000	4.067	7.133	5.167	6.100	12.000	18.000	21.000	21.000	3.367	2.890
11	ON3-2	42.000	22.067	3.267	6.600	5.667	6.033	1.000	17.000	5.000	23.000	3.440	2.530
12	ON3-3	42.000	17.333	3.533	6.000	4.800	5.400	12.000	11.000	15.333	25.000	3.727	2.643
13	OM1-3	42.000	17.633	2.867	5.800	4.233	4.200	8.000	13.000	6.000	10.000	3.783	1.960
14	ON3-1	43.000	24.133	3.467	9.600	5.567	7.167	13.000	14.000	24.000	21.000	3.563	3.350
15	OE1-2	42.000	27.133	2.567	9.333	5.200	6.767	13.000	22.667	39.000	17.000	3.283	2.917

Sl. No.	Genotypes	Days to 50% flowering	Plant height (cm)	Cluster/ plant	Pods /plant	Pod length (cm)	Seeds/ pod	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)	100-seed weight (g)	Yield (g/pl.)
16	OE2-3	42.000	27.867	3.933	10.533	5.900	7.600	3.000	15.000	20.000	31.000	3.540	3.590
17	OG3-3	43.000	21.533	2.800	5.167	5.233	5.467	13.000	23.000	18.000	15.000	3.370	2.170
18	OG1-1	40.000	19.833	3.600	6.400	5.467	6.400	14.000	35.000	9.000	26.333	3.247	2.500
19	OG3-2	42.000	25.167	3.400	8.600	5.667	8.233	15.000	17.000	8.000	10.000	3.243	3.037
20	OGN2-3	41.000	28.300	3.067	8.600	5.333	8.200	10.000	22.000	19.000	16.000	3.150	3.200
21	OM2-3	40.000	20.633	2.533	4.933	5.767	7.900	10.000	8.000	13.000	19.000	3.617	2.530
22	OGM2-3	40.000	30.667	3.133	5.800	5.333	7.233	7.000	12.000	3.000	18.000	3.673	2.840
23	Sujata	40.000	25.333	3.933	8.600	5.267	6.533	4.000	21.000	17.000	17.000	3.083	2.730
24	OBGG-52	40.000	26.967	2.800	8.333	5.267	8.067	3.000	15.000	22.000	22.000	3.397	2.440
25	TARM-1	44.000	14.567	3.133	5.933	5.533	7.367	10.000	16.000	18.000	23.000	2.993	2.640
26	PDM54	42.000	29.300	3.133	9.467	6.100	7.467	10.000	13.000	15.000	25.000	2.777	3.010
27	Sonepur-2	44.333	25.800	2.533	9.400	5.667	6.300	1.000	11.000	15.000	11.000	2.350	2.580
28	Jhainmung(B-2)	36.000	20.733	3.400	9.667	6.167	7.300	8.000	13.000	15.000	12.000	1.937	2.440
29	Kendrapara(A)	44.000	17.933	2.600	5.467	5.400	6.300	12.667	11.000	8.000	21.000	2.217	1.990
30	Kopargaon	44.000	26.933	3.867	10.467	7.200	8.333	11.000	9.000	14.000	14.000	2.707	2.920
	Mean	42.278	23.388	3.097	7.299	5.495	6.816	10.378	16.456	15.744	18.556	3.197	2.561
	CD (5%)	2.661	3.914	0.726	1.560	0.598	1.174	2.296	2.621	2.559	1.989	0.298	0.546

4.1.9 Response to low temperature

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 20ºC [Graham and Patterson (1983)) 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 or prevent productive development. Each plant species has an optimum range of temperature for its 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 and the help of mutation breeding can also be availed.

Present genotypes when exposed to low temperature (10° C) at 10 days seedling stage (Table 7) the maximum days of survival (22.667 days) was observed in case of SG3-3 followed by SE1-2 and SG1-2 (16.000 days). They all are mutants of Sujata. The minimum day of survival of seedling was observed in case of ON3-2, Sonepur-2 (1.000 days) followed by SE2-3, OE2-3 and OBGG-52 (3.000 days) a parent in present investigation indicating their susceptibleness to the cold temperature at 10 days seedling state among all the genotypes considered together. Standard varieties survived up to 10.00 days (TARM1 and PDM-54), whereas OBGG-52 was most susceptible to the cold temperature at early stage at seedling. The genotype having tolerance to low temperature at early stage of their growth may be used in the interior district of Odisha where the crop was shown towards the end of winter season.

Table 7. Reaction of different days old seedling of greengram to cold

Sl. No.	Genotypes	Survival duration (days)				Mean (survival days)	Yield (g/plant)
		10 days old seedling stage	20 days old seedling stage	30 days old seedling stage	40 days old seedling stage		
1	SN1-2	15.000	17.000	5.000	19.333	14.083	2.380
2	SE3-2	8.000	15.000	10.000	12.000	11.250	2.030
3	SE1-2	16.000	23.000	12.000	27.000	19.500	1.610
4	SG1-1	16.000	23.000	29.000	29.000	24.250	2.090
5	SN3-3	12.000	21.000	22.000	26.000	20.250	2.390
6	SG3-3	22.667	12.000	19.000	9.000	15.666	2.400
7	SE2-3	3.000	10.000	19.000	7.000	9.750	2.340
8	SM1-3	14.000	17.000	15.000	16.000	15.500	2.520
9	SE2-2	14.000	18.000	17.000	14.000	15.750	3.170
10	SG1-2	12.000	18.000	21.000	21.000	18.000	2.890
11	ON3-2	1.000	17.000	5.000	23.000	11.500	2.530
12	ON3-3	12.000	11.000	15.333	25.000	15.833	2.643
13	OM1-3	8.000	13.000	6.000	10.000	9.250	1.960
14	ON3-1	13.000	14.000	24.000	21.000	18.000	3.350
15	OE1-2	13.000	22.667	39.000	17.000	22.916	2.917
16	OE2-3	3.000	15.000	20.000	31.000	17.250	3.590
17	OG3-3	13.000	23.000	18.000	15.000	17.250	2.170
18	OG1-1	14.000	35.000	9.000	26.333	21.083	2.500
19	OG3-2	15.000	17.000	8.000	10.000	12.500	3.037
20	OGN2-3	10.000	22.000	19.000	16.000	16.750	3.200
21	OM2-3	10.000	8.000	13.000	19.000	12.500	2.530
22	OGM2-3	7.000	12.000	3.000	18.000	10.000	2.840
23	Sujata	4.000	21.000	17.000	17.000	14.750	2.730
24	OBGG-52	3.000	15.000	22.000	22.000	15.500	2.440
25	TARM-1	10.000	16.000	18.000	23.000	16.750	1.640
26	PDM54	10.000	13.000	15.000	25.000	15.750	3.010
27	Sonepur-2	1.000	11.000	15.000	11.000	9.500	2.580
28	Jhainmung(B-2)	8.000	13.000	15.000	12.000	12.000	2.440
29	Kendrapara(A)	12.667	11.000	8.000	21.000	13.166	1.990
30	Kopargaon	11.000	9.000	14.000	14.000	12.000	2.920

When 20 days old seedlings of the cultures/genotypes and standard varieties were exposed to artificial low temperature at 10°C maximum (35 days) of survival was noticed incase of mutant OG1-1 followed by SG1-1 and SE1-2 (23 days). Local Jhainmung survived for 13 days as against 35 days in case of OG1-1. OM2-3was the most susceptible variety (08 days) at 20 days of seedling growth exposed to 10°C followed by Kopargaon (09 days).

Exposure of 30 days old seedlings of the mutant cultures, local genotypes and released varieties to 10°C resulted in maximum tolerance of the cold stress by OE1-2 for 39.00 days followed by SG1-1 (29daya) and ON3-1(24 days). OGM2-3 was very susceptible to cold (3 days survival) followed by SN1-2, ON3-2 (05 days) and OM1-3 (06 days). The mean survival days of 15.74. Genotypes statistically superior than the general mean were more in number in case of Sujata mutant than OBGG 52 mutant. This age of the seedling coincide with the flowering of the crop resulting maximum yield loss. Genotype having higher tolerance to cold at this stage will be very much favorable for harvesting good crop.

When 40 days old seedlings of greengram were exposed to artificial cold condition at 10°C, the maximum survival was noticed in OE2-3 (31 days), followed by genotype SG1-1 (29 days) and SE1-2 (27 days), where as the most susceptible genotype was the SE2-3 (07 days) followed by SG3-3 (09 days) and OM1-3 (10days). The parental genotypes survived for 17 days (Sujata) and 22days (OBGG-52). The superior mutant identified in this study will be a source of cold tolerance in future breeding programme.

Considering the average of all the stages of seedling expose to low temperature (Table 7), it was observed that most tolerant genotype was SG1-1 (24.250 days of survival) followed by OE1-2 (22 days of survival) and OG1-1 (21 days of survival). Al these were the mutant cultures including the most susceptible genotype OM1-3 having 9.25 days of survival. The genotypes which were found to be tolerance or susceptible to cold may be adapted to the appropriate ecological situations of Odisha.

The genotypes having maximum per plant yield (3.590g to 2.917g) and moderately tolerant to cold (12.50 days to 22.916 days survival) were OE2-\3, OGN2-3, OG3-2 and OE1-2. 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 mid-late stage of their vegetative growth when exposed to the low temperature. This may be due to more concentration of glutathione levels and reduce electrolyte leakage in the tolerant genotypes (Yu *et al.,* 2002), deposit of malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Chen, 2007) along with action of multiple low-temperature (Lin and Yun, 2002) regulatory pathway.

Levitt (1939), Hughes and Dumn (1999), Chang *et al.* (2000), Yu *et al.* (2003), Chen (2007), Jena (2010), Pradhan (2011) also reported variation of cold tolerance in different stages of greengram seedling exposed to cold for different duration.

4.2 Variability, heritability and genetic advance of characters

The mutant cultures of these two varieties showed wide range of variation for all the 12 quantitative characters studied. The genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance for these nine characters are presented in the Table 8.

The range of the variation of the characters that the highest limit of variation was about 20-40% higher than the lower limit for days to flowering and Clusters per plant 40-60% for plant height, pods per plant, pod length, seeds per pod and 100-seed weight and yield per plant and for other characters it about 70-100%. These indicated that the strains possess tremendous potential differences for the characters. Variability in greengram for different traits have been studied earlier by many workers Idress *et al.*(2006), Tabasum *et al.*(2010), Rahim *et al.*(2010), Tabasum *et al.*(2010), Abna *et al.*(2012), Roy and Chowdhury *et al.*(2012),

4.2.1 Coefficients of variability

The extent of variability among the mutant cultures, parents, standards and locals genotypes with respect to different quantitative characters was estimated in terms of PCV and GCV. The PCV and GCV ranged from 4.00 to 48. 65 and 3.34 to 48.03 respectively in days to 50% flowering and survival of 10 days old seed ling at 10^0C.

Both the PCV and GCV estimates were high (more than 10%) for Plant height, Clusters per plant, Seeds per pod, 100-seed weight, Yield per plant in the mutant cultures/genotypes of the experimental material.

The PCV and GCV estimates revealed precise trend and their relative magnitude would indicate that the coefficient of variation were high for survival of 10, 30, 40 days old seedling at 10ºC, pods per plant. GCV for character were smaller than that of PCV, indicating some influence of environment on them These variability estimates for the traits under study in mutant lines of both varieties were in close agreement with earlier findings in greengram Momin and Misra,2004; Kumar *et al.*,2008, Rahim *et al.*(2010), Roychowdhury *et al.*(2012).

4.2.2 Heritability

The heritability (broad sense) estimates for different characters of the mutant cultures, parents, standards and locals ranged from 69.43% (for days to 50% flowering) to 98.59% (for survival of 30 days old seedling at 10ºC). The heritability estimates were high (more than 90%) for 100-seed weight, plant high and pods per plant. The heritability estimates were low (less than 70%) for days to 50% flowering.

4.2.3 Genetic advance

The expected genetic advance (GA) for different characters of the mutant cultures, parents, standards and locals was estimated at 5% selection intensity and was expressed as percentage of population mean. The estimated GA (% of mean) under selection for characters varied from 5.73% for days to 50% flowering to 45.75% for pods per plant. The expected genetic advance was high (more than 30%) for plant height, pods per plant, and yield/plant in the population in addition to their response to cold at different growth stage.

High heritability and high GA was observed for plant height, pods per plant, survival of 10 days old seedling at 10ºC, survival of 20 days old seedling at 10ºC, survival of 30 days old seedling at 10ºC and survival of 40 days old seedling at 10ºC indicating additive gene action where selection will be effective. Low heritability and low GA was found for character days to 50% flowering indicating the character to be highly influenced by environmental effect and selection will not be effective.

The heritability estimates for different characters in greengram were in broad agreement with reports of Momin and Misra (2004), Idress *et al.* (2006), Babu *et al.* (2007), Tabasum et al.(2010), Rahim *et al* .(2010), Reddy *et al* (2011), Makeen et al.(2011) and Roychowdhury *et al.*(2012).

Table 8. Estimation of different parameters of variability and genetic advance under section (at 5%) for twelve characters

Sl. No	Characters	Range	Mean	PCV (%)	GCV (%)	h² (%)	GA (5%)	GA (% of mean)
1	Days to 50% flowering	36.000-44.333	42.278	4.00	3.34	69.43	2.421	5.73
2	Plant height (cm)	14.567-33.033	23.388	20.59	19.73	91.82	9.107	38.94
3	Clusters per plant	2.467-4.067	3.097	15.82	13.49	72.78	0.734	23.71
4	Pods per plant	4.733-10.533	7.299	24.52	23.34	90.59	3.340	45.75
5	Pod length (cm)	4.233-7.200	5.494	9.11	8.26	82.26	0.848	15.44
6	Seeds per pod	4.200-8.333	6.816	14.91	13.63	83.49	1.748	25.65
7	Survival of 10 days old seedling at 10°C (d)	1.000-22.667	10.378	48.65	48.03	97.44	10.135	97.66
8	Survival of 20 days old seedling at 20°C (d)	8.000-35.000	16.456	34.77	34.31	97.40	11.480	69.76
9	Survival of 30 days old seedling at 30°C (d)	3.000-39.000	15.744	48.16	47.82	98.59	15.400	97.82
10	Survival of 40 days old seedling at 40°C (d)	7.000-31.000	18.556	34.54	34.23	98.25	12.970	69.90
11	100-seed weight (g)	1.937-3.783	3.197	14.11	13.72	94.53	0.879	27.48
12	Yield per plant (g)	1.610-3.590	2.561	18.68	17.09	83.76	0.827	32.23

GA = Genetic advance as percentage of population mean

4.2.4 Productive mutants in relation to mutagenic treatments

The mutant cultures of Sujata, OBGG 52 were derived through different mutagenic treatments using gamma rays, EMS, NG, and MH. The superiority of performance of each mutant culture was judged by analysis of variance of means of each character. The cultures, statistically at par with highest mean for each character and their source of origin are presented in Table 9. The mutagenic treatments responsible for positive change of a character were analyzed Table 10.

Table 9 Mutant cultures with superior characters performances and their origin in greengram

Character	Superior Mutant culture at per with highest Mean	Origin from Mutagenic Treatment
Yield/ plant	SE 2-2, O N 3-1,OE2-3, OG N2, OGN2-3	T_5, T_7,T_5,T_{14}
100 seed weight	SM1-3, SE2-2, ON3-3, OM1-3, ON3-1, OE2-3, OM2-3, OGN2-3	T_{10},T_5, T_9, T_{10}, T_9,T_5,T_{11},T_{15}
Survival of 40 days old seedlings at 10^0 c	OE2-3	T_5
Survival of 30 days old seedlings at 10^0c	OE1-2	T_4
Survival of 20 days old seedlings at 10^0c	SG3-3	T_1
Survival of 10 days old seedlings at 10^0c	SG3-3	T_3
Seeds/pod	SN1-2, SE2-2, ON3-1, OE2-3, OG3-2, OGN2-3, OM2-3, OGM2-3	T_7, T_5, T_9,T_5,T_3, T_{14}, T_{11},T_{15}
Pod length	-	-
Pods/plant	ON3-1, OE1-2, OE2-3	T_9, T_4,T_5
Clusters/plant	SM1-3, SG1-2, ON3-3, ON3-1, OE2-3, OG1-1, OG3-2	T_{10}, T_1, T_9, T_9,T_5,T_1,T_3
Plant height	-	-
Days to 50% flowering.	-	-

Examination of the data revealed that T5E2 was able to change six out off twelve characters in the positive direction; T9N3 was able to change four out off twelve characters in the positive direction; T1G1 and T3G3 were able to change three out of twelve characters in the positive direction; and

T4E1, T7N1, T10M1,T11M2 T14G1N2 and T15G1M2 changed two out of twelve characters in the positive direction; while T2G2 ,T6E3,T8N2, T12M3, T13G1E2, and T16C changed none of the twelve character in positive direction.

Table 10 **Potentiality of mutagenic treatments in inducing positive change in character(s)**

Mutagenic treatment	Parent	Treatment No.	Characters changed in positive direction	
			Number of character	characters
GR 20kR	Sujata, OBGG-52	T_1	3	Survival of 20 days old seedlings at 10^0c, plant height, cluster/plant, plant height,
GR 40kR	-	T_2	NILL	-
GR 60kR	OBGG-52	T_3	3	Survival of 10 days old seedlings at 10^0c, seed/pod, cluster/plant
EMS 0.2 %	OBGG-52	T_4	2	Survival of 30 days old seedlings at 10^0c, pods/plant
EMS 0.4%	Sujata, OBGG-52	T_5	6	Yield/plant, 100-seed weight, Survival of 40 days old seedlings at 10^0 c, seed/pod, pods/plant, cluster/plant
EMS 0.6%	-	T_6	NILL	-
NG0.005%	Sujata, OBGG-52	T_7	2	Yield/plant, seeds/pod
NG 0.010%	-	T_8	NILL	-
NG 0.015%	Sujata, OBGG-52	T_9	4	100-seed weight, seeds/pod, pods/plant, cluster/plant
MH 0.01%	Sujata, OBGG-52	T_{10}	2	100-seed weight, cluster/plant
MH 0.02%	OBGG-52	T_{11}	2	100-seed weight, seed/pod
MH 0.03%	-	T_{12}	NILL	-
GR 40Kr + EMS 0.4%	-	T_{13}	NILL	-
GR 40kR + NG 0.010%	OBGG-52	T_{14}	2	Yield/plant, seed/pod
GR 40kR + MH 0.02%	OBGG-52	T_{15}	2	100-seedweight, seed/pod
CONTROL (distilled water)	-	T_{16}	NILL	-

In one extreme, the positive change in six out of twelve characters has been brought about by T5E2 (EMS@0.4%). This is expected because the potentialities of EMS in inducing point mutation have been proved by many workers, [Das and Misra, (2005); Das *et al.* (2006); Singh and Singh, (2007); Singh *et al.* (2008); Balai and Krishna, (2009); and Kozgar *et al.* (2010), Thilagavathi and Mullainathan (2011)]. This may be due to the facts that EMS induces C- to T- changes resulting in C/G to T/A substitution , transition from GC to AT, less transversion and frame-shift mutations and with rare chromosomal breaks. This was followed by T9N3 (NG@ 0.015%) which bring about positive changes in four out of twelve characters. It may be due to the fact that NG is an alkylating agent containing methyl group. Methyl group of NG reacts with purine bases and produces more of methylated guanine than methylated adenine. T1G1 (Gamma ray 20kR) and T3G3 (Gamma rays 60kR) changed 3 characters in positive direction than other treatments. Tah and Saxena (2009) and Kumar *et al.* (2009) and Thilagavathi and Mullainathan (2011), have reports the effect of this physical agents earlier. MH is an effective chromosome breaker and acts preferentially on heterochromatin region. Since heterochromatin is the site of majority of the polygene (Mather's hypothesis) and MH reacts on the heterochromatin, it can be effectively utilized for induction of micromutation in quantitative traits (Ghose and Chatterjee (1989). Induction of mutations by MH treatments were reported by Reddy and Smith (1984), Ghose and Chatterjee (1989), Misra (1990), Senapati (2007) and Das (2011).

4.3 CHARACTER ASSOCIATION

4.3.1 Phenotypic and genotypic correlation

The phenotypic correlation (r_p) estimates among the 12 characters are presented in Table 11. The r_p estimates ranged from 0.739 between pods per plant and yield per plant to maturity to -0.409 between pod length and 100-seed weight. Of these 66 r_p estimates, 12 were significant. The genotypic correlation (r_g) estimates (Table 12.) ranged from 0.860 between plant height and yield to -0.666 between days to flowering and 100 seed-weights. Fifteen of 66 r_g estimates were found to be significant. In general the r_p and r_g estimates followed almost similar trend for all the characters under study. The magnitude of r_g was greater than r_p in almost all cases indicating higher genotypic correlation in case of positive association and lower in case of negative association would reinforce selections based on phenotype.

4.3.1 Correlation of yield and with other characters

Of the association of character components plant height, cluster per plant, and pods per plant, pod length and seeds per pod showed highly significant positive correlation with yield per plant both at phenotypic and genotypic level. Similar high positive correlation was reported by many workers [Rahim *et al.* (2010) for pods per plant, number of seeds per pod. Reddy *et al.* (2011) for plant height, pods per plant, seeds per pod, 100 seed weight. Khajudparn and Tantasawat (2011) for pods per plant, clusters per plant, seeds per pod. Makeen *et al.* (2011) for pods per plant, plant height]. Hundred seed weight, survival of 30 days old seedling at 10^0C exhibited moderate positive correlation with yield at both phenotypic and genotypic level. Similar result was reported earlier by Jena (2010) and Pradhan (2011).

4.3.2 Correlation among the component traits

Thirty one out of 55 phenotypic correlation among the yield components were positive out of which six were significant where as only one phenotypic correlation was negatively significant. In general the phenotypic and genotypic correlation estimates followed almost similar trend. The characters seeds per pod, pods per plant, pod length showed high positive correlation among themselves both at genotypic and phenotypic level which is in close agreement with the report of Rahim *et al.* (2010), Makeen *et al*, (2011), Begum *et al* , (2012). Plant height showed moderate but significant positive association with seeds per pod, and pods per plant. Clusters per plant also had moderate positive association with pods per plant where as pods per plant also have moderate non- significant positive correlation with survival of 30 days old seedling at 10ºC. Pod length had a moderate but negative correlation with survival of seedling at different stage of their growth at 10ºC and moderate but positive correlation with all other character. Seeds per pod showed highly significant positive correlation with pods per plant and pod length but low non-significant positive correlation with survival of 30 and 40 days old seedling at 10ºC. Survival of seedling at their different growth stage at 10ºC almost had a negative and negligible correlation with all other yield traits. Correlation of 100-seed weight was significantly negative correlated with days to 50% flowering and pod length where as in all other cases it was negligible. Correlation study in greengram for different biometrical traits have been reported by earlier scientist Reddy D. *et al.* (2011), Makeen *et al*, (2011) and Begum *et al.* (2012).

Thus the significant association of plant height, pod length, pods per plant and seeds per pod with yield and among themselves indicated that selection of genotype based on these characters will be effective for yield improvement.

4.3.3 Path analysis of yield components

In order to study the cause and effect relationship between component traits on seed yield, path coefficient analysis was used. Through this, the correlation of the component traits with yield were partitioned into direct and indirect effect that would reflect on the nature of these associations and the relative importance of the component traits in determining the yield. The phenotypic correlation coefficients were used for carrying out path coefficient analysis in 30 genotypes (Table 13) and the analyzed had high R^2 value of 85.360% and low residual effect of 0.383.

Table 11 Phenotypic Correlation (r_p) between all pairs of twelve characters

Character	Plant height	Cluster/plant	Pods/plant	Pod length	Seeds/pod	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)	100-seed weight	Yield/plant
Days to 50% flowering	-0.249	-0.126	0.129	0.128	-0.113	-0.096	-0.215	-0.038	-0.185	-0.573**	-0.259
Plant height		0.254	0.580**	0.279	0.414	-0.165	-0.025	0.239	-0.085	0.168	0.778**
Cluste per plant			0.391*	0.253	0.110	-0.047	0.088	-0.038	0.236	0.106	0.454**
Pods per plant				0.562**	0.491**	-0.264	-0.151	0.256	-0.133	-0.247	0.739**
Pod length					0.617**	-0.057	-0.297	-0.043	-0.095	-0.409	0.375*
Seeds per pod						0.069	-0.070	0.150	0.131	-0.142	0.467**
Survival of 10 days old seedling at 10°C							0.282	0.137	0.037	0.113	-0.136
Survival of 20 days old seedling at 10°C								0.208	0.365*	0.088	-0.077
Survival of 30 days old seedling at 10°C									0.157	0.076	0.221
Survival of 40 days old seedling at 10°C										0.086	0.021
100-seed weight											0.255

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

Table 12. Genotypic Correlation(r_g) between all pairs of twelve characters

Character	Plant height	Cluster/plant	Pods/plant	Pod length	Seeds/pod	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)	100-seed weight	Yield/plant
Days to 50% flowering	-0.291	-0.180	0.158	0.267	-0.106	-0.106	-0.274	-0.030	-0.241	-0.666**	0.323
Plant height		0.278	0.641**	0.339	0.474**	0.178	-0.020	0.252	-0.081	0.177	0.860**
Cluste per plant			0.469**	0.352	0.210	-0.086	0.112	-0.045	0.283	0.123	0.521**
Pods per plant				0.652**	0.568**	-0.288	-0.162	0.264	-0.145	-0.273	0.857**
Pod length					0.704**	-0.264	-0.341	-0.046	-0.104	-0.463**	0.447*
Seeds per pod						0.075	-0.086	0.168	0.147	-0.133	0.574**
Survival of 10 days old seedling at 10°C							0.827**	0.143	0.037	0.114	-0.158
Survival of 20 days old seedling at 10°C								0.212	0.371*	0.091	-0.088
Survival of 30 days old seedling at 10°C									0.159	0.076	0.239
Survival of 40 days old seedling at 10°C										0.088	0.032
100-seed weight											0.292

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

Table 13 Direct (diagonal) and indirect effect of component traits on their phenotypic correlation with seed yield in greengram

Character	Days to 50 % flowering	Plant height	Cluster/ plant	Pods/ plant	Pod length	Seeds/ pod	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	100-seed weight	Yield/ plant
Days to 50 % flowering	**-0.0480**	-0.0912	-0.0064	0.0767	0.0108	-0.0009	-0.0061	0.0107	0.0022	-0.0207	-0.1862	-0.2590
Plant height	0.0119	**0.3663**	0.0129	0.3449	0.0165	0.0033	-0.0104	0.0012	-0.0138	-0.0095	0.0546	0.7780
Cluster per plant	0.0060	0.0930	**0.0508**	0.2325	0.0150	0.0009	-0.0030	-0.0044	0.0022	0.0264	0.0344	0.4540
Pods per plant	-0.0062	0.2124	0.0199	**0.5947**	0.0333	0.0040	-0.0167	0.0075	-0.0148	-0.0149	-0.0803	0.7390
Pod length	-0.0087	0.1022	0.0129	0.3342	**0.0593**	0.0050	-0.0036	0.0148	0.0025	-0.0106	-0.1329	0.3750
Seeds per pod	0.0054	0.1516	0.0056	0.2920	0.0366	**0.0081**	0.0044	0.0035	-0.0087	0.0147	-0.0461	0.4674
Survival of 10 days old seedling at 10°C	0.0046	-0.0604	-0.0024	-0.1570	-0.0034	0.0006	**0.0632**	-0.0141	-0.0079	0.0041	0.0367	-0.1360
Survival of 20 days old seedling at 10°C	0.0103	-0.0092	0.0045	-0.0898	-0.0176	-0.0006	0.0178	**-0.0499**	-0.0120	0.0409	0.0286	-0.0770
Survival of 30 days old seedling at 10°C	0.0018	0.0875	-0.0019	0.1522	-0.0026	0.0018	0.0087	-0.0104	**-0.0579**	0.0176	0.0247	0.2210
Survival of 40 days old seedling at 10°C	0.0089	-0.0311	0.0120	-0.0791	-0.0056	0.0011	0.0023	-0.0182	-0.0091	**0.0020**	0.0279	0.0210
100-seed weight	0.0275	0.0615	0.0054	-0.1469	-0.0243	-0.0011	0.0071	-0.0044	-0.0044	0.0096	**0.3249**	0.2550

R^2=85.360 Residual effect=0.383

Considering the direct effect of the component traits on seed yield in the genotypes it was observed that pod per plant had the highest direct effect (0.595) followed by plant height (0.3663) and 100-seed weight (0.3249). The direct effect of seeds per pod, survival of 40 days old seedling at 10ºc on yield were positive but of very low magnitude (less than 0.05) whereas clusters per plant, pod length, survival of 10 days old seedling at 10ºC showed positive and moderate (>0.05 to < 0.30) direct effect. Direct effect of days to 50% flowering and survival of 30 days old seedling at 10ºC was negative.

In present investigation, path analysis revealed that pods per plant, plant height, 100-seed weight had the highest positive direct effect on seed yield, whereas direct effects of other traits on yield were very low to moderate having positive magnitude except days to 50% flowering and survival of 20 and 30 days old seedling at 10ºC.This indicated that selection for higher yield on the basis pods/plant, plant height and 100-seed weight be most effective. This is in broad conformity with path analysis studies in greengram as reported by Mishra and Pradhan(2006), Tabasum *et al.* (2010), Reddy *et al.* (2011), Mondal *et al.* (2011), and Khajudparn and Tantasawat (2011).

4.4 Multivariate analysis of genetic divergence

4.4.1 Multivariate test of diversity

Simultaneous variation in all the 12 characters of 30 green gram genotypes were tested for assessing the nature of genetic divergence among them following Mahlanobis' D^2-statistics and canonical analysis. The data recorded were subjected to analysis of variance. The aggregate effect of all 12 character tested by Wilk's criterion indicated highly significant difference among the genotypes (χ^2 for 348 d.f.). It is therefore, worthwhile to classify the population on the basis of characters study.

4.4.2 Genetic divergence among the strains

D^2 values for the 435 pair combinations among 30 strains ranged from 41.244 between SM1-3 and SE2-2 to 735.765 between ON3-2 and OE1-2 indicating that, while some strains were quite close to each other genetically, others are quite diverse. The genetic closeness between SM1-3 and SE2-2 was apparently due to their similarity to days to flowering, plant height, clusters per plant, pods per plant ,pod length, seeds per pod ,100-seed weight, yield per plant, Response to 10°C temperature at 10 days, 20 days, 30 days and 40 days seedling stage. On the other hand, the higher distance between ON3-2 and OE1-2 could be attributed to the wide difference in all the characters except days to 50% flowering, pod length and seeds per pod. Other divergent and close combinations of the genotypes have been presented in Table 14. The D^2 values of the Sujata mutants (Table 15) for all the 45 paired combinations among 10 entries ranged from 41.244 between SM1-3 and SE2-2 to 464.139 between SG1-1 and SE2-3 while genetic divergence (D^2) among 66 paired of OBGG-52 varied from 42.488 between ON3-3 and OM2-3 to 710.406 between OE1-2 and OGM2-3. (Table 16)

Table 14. Highly divergent and close combinations of greengram genotypes

Divergent combination (D^2 more than 550)	Close combination (D^2 less than 50)
ON3-2 and OE1-2	SM1-3 and SE2-2
OE1-2 and OG1M2-3	ON3-3 and OM2-3
SG3-3 and OE2-3	SE3-2 and OM1-3
SG1-1 and OM1-3	SE2-2 and OG1N2-3
	SN3-3 and SG1-2
	SG1-2 and OG1N2-3

The highest D^2 value was observed between ON3-2 and OE1-2 which were the mutant of same parent OBGG 52 but with different mutagenic treatments (T9N3 i.e. NG 0.015 % and T4E1 i.e. EMS 0.2%). In the next order of sequence same genotype OE1-2 exhibited greater diversity with OG1M2-3. Usually, the higher genetic diversity between two genotypes is the indication of good combiners for crossability and might ultimately yield appreciable recombinants. On the other hand the lowest diversity between SM1-3 and SE2-2 was an indicative of the reverse situation.

The magnitude of D^2 values indicated considerable genetic diversity among the strains despite the fact that they constitute an elite group, all selected for high yield. At the same time, the results were in consistent with the diverse origin of the strains, majority of which originated from different mutagenic treatments. Similar results were observed by Mishra and Pradhan (2006), Momin *et al.* (2006) and Das (2011).

4.4.3 Relative contribution of the characters

The relative contribution of different characters to total divergence could be accessed through comparison of total D^2's for individual characters over the 435 pair combinations. Rank totals over the paired combinations were also use as an additional criterion for assessing the relative contribution of the character. Considering the first criterion (i.e. D^2 over all combinations), survival of 30 days old seedling at 10°C, made the greatest contribution (26.968%) to divergence followed survival of 40 days old seedling at 10°C (20.400%), survival of 10 days old seedling at 10°C (15.655%), survival of 20 days old seedling at 10°C (13.859%), 100-seed weight (6.120%), plant height (3.975%), pods per plant (3.682%), seeds per pod (2.396%) and yield per plant (2.348%). Rank totals brought out same pattern of relative contribution

Table 15 D^2 values for mutant lines of parent Sujata

	SE3-2	SE1-2	SG1-1	SN3-3	SG3-3	SE2-3	SM1-3	SE2-2	SG1-2	Sujata
SN1-2	75.500	98.480	367.031	172.279	209.181	229.297	64.679	126.648	166.160	154.432
SE3-2		182.024	401.280	206.935	193.190	83.199	69.454	136.419	171.660	113.795
SE1-2			145.818	64.178	267.559	370.235	123.030	206.290	134.882	203.521
SG1-1				61.176	320.593	464.139	220.441	268.689	128.630	327.078
SN3-3					254.455	288.388	102.491	147.488	49.240	137.545
SG3-3						221.651	109.763	139.942	227.533	360.214
SE2-3							118.235	152.093	214.238	154.125
SM1-3								41.244	63.156	107.754
SE2-2									72.911	145.246
SG1-2										93.677

Table 16 D^2 values for mutant lines of parent OBGG 52

	ON3-3	OM1-3	ON3-1	OE1-2	OE2-3	OG3-3	OG1-1	OG3-2	OG1N2-3	OM2-3	OG1M2-3	OBGG 52
ON3-2	181.342	183.959	394.420	375.765	181.771	295.060	245.466	227.838	207.680	168.665	55.629	155.557
ON3-3		191.035	82.655	391.267	185.315	161.388	288.706	193.278	153.408	42.488	154.455	188.245
OM1-3			311.522	646.034	497.640	174.841	370.725	107.376	220.016	149.593	129.228	284.560
ON3-1				170.411	163.793	148.374	360.558	182.288	78.289	102.743	261.410	83.086
OE1-2					481.966	220.474	549.303	476.735	214.452	444.797	710.406	281.805
OE2-3						400.556	387.098	396.368	205.982	223.977	240.925	77.434
OG3-3							177.270	136.881	73.074	182.318	270.440	189.059
OG1-1								295.717	195.041	373.608	309.495	314.786
OG3-2									96.177	128.750	126.439	229.116
OG1N2-3										145.431	182.878	72.640
OM2-3											97.777	114.752
OG1M2-3												173.513

of all twelve characters judged by the first criterion. The maximum contribution to divergence was by survival of 40, 30, 20 and 10 days old seedling at 10^{o}C followed by 100 seed weight, plant height, pods per plant and seed yield (Table 17.) which was in close agreement with the observations of Jena (2010) and Pradhan (2011).

Table 17. Relative contribution of different characters to genetic divergence among thirty culture/varieties

Sl. No.	Character	Average D^2	Average D^2 (%)	Rank total	Rank average
1	Days to 50% flowering	2.180	1.078 (12)	3854	11.358 (1)
2	Plant height (cm)	8.040	3.975 (6)	2882	8.493 (7)
3	Cluster per plant	2.338	1.156 (11)	3723	10.972 (2)
4	Pods per plant	7.447	3.682 (7)	2953	8.703 (6)
5	Pod length	4.780	2.364 (9)	3651	10.760 (3)
6	Seeds per pod	4.846	2.396 (8)	3296	9.714 (5)
7	Survival of 10 days old seedling at 10°C	31.662	15.655 (3)	2031	5.985 (10)
8	Survival of 20 days old seedling at 20°C	28.030	13.859 (4)	2114	6.230 (9)
9	Survival of 30 days old seedling at 30°C	54.543	26.968 (1)	1772	5.222 (11)
10	Survival of 40 days old seedling at 40°C	41.259	20.400 (2)	1702	5.016 (12)
11	100-seed weight (g)	12.377	6.120 (5)	2616	7.709 (8)
12	Yield per plant (g)	4.750	2.348 (10)	3336	9.832 (4)
13	Total	202.252	100	33930	100

4.4.4 Group constellations:

Thirty strains were grouped on the basis of genetic affinity / diversity as measured by D^2 into nine clusters or group consisting of one to fourteen strains (Table 18). Group 1 was the largest cluster consisting of 14 strains, 12 of which were mutant cultures of different parents but with different mutagenic treatments. The varieties and the mutant cultures were from diverse origin but clustered in one group. Group II included four strains which three were local varieties except one mutant SE2-3. Group III consistent of two mutant cultures. They were of from same parent OBGG 52 but again of different mutagenic treatments. Group IV included only three strains consisting of one parent and one of its mutant along with one standard variety. Group V contain one mutant and one local. Whereas Group VI includes only two mutants of different parent. Other three groups have single genotype each of different parents and different mutagenic treatment. These observations showed that diverse type of genetic changes were induced by different mutagens and selection towards higher yield made them separate from the parental material.

Table 18: Composition of genetic cluster using D^2 value

Cluster	No. of genotypes	Name of genotype
I	14	SN1-2, SE3-2, SN3-3, SM1-3, SE2-2, SG1-2, ON3-3, ON3-1, OG3-3, OG3-2, OG1N2-3, OM2-3, Sujata, TARM-1
II	4	SE2-3, Sonepur-2, Jhainmung(B-2), Kopargaon local
III	2	ON3-2, OG1M2-3
IV	3	OE2-3, OBGG-52, PDM-54
V	2	SE1-2, Kendrapara local-A
VI	2	SG1-1, OE1-2
VII	1	SG3-3
VIII	1	OM1-3
IX	1	OG1-1

Similar D^2 analysis and grouping of greengram genotypes into genetic clusters have been reported by Muhhamad *et al.* (2007), Das *et al.* (2010), Rahim *et al.* (2010), Das (2011), Gokulakrishnan *et al.* (2012) and Abna *et al.* (2012).

4.4.5 Inter and intra-cluster divergence and character means

The inter cluster average D^2's ranged from 149.844 to 634.792 (Table 19.) and average genetic distances (D's) ranged from 12.241 to 25.195 (Table 20. and Fig 1). Clusters I and V were least distant from each other indicating their closeness genetically. Maximum inter cluster distance was observed between III and VI. Thus, clusters III and VI were most diversed genetically. The intra cluster average D^2 for the clusters containing two or more strains ranged from 55.625 to 143.421 and the average intra-cluster distance (D's) ranged from 7.458 to 11.976 (Table 19 and Table 20). Cluster VI (2 strains) having highest average D^2, was genetically most heterogeneous group followed by cluster I (14 strains), cluster V (2 Strains) and cluster II (4 strains), Cluster IV (3 Strains) and cluster III (2 strains). The rest clusters had one strains each.

The means for 12 traits of nine different clusters are given in Table 21. Yield per plant, cluster per plant, pods per plant, seeds per pod were highest for cluster IV. The survival of 20, 40 days old seedling was highest in group in IX where as Group VIII was highest with survival of 10 days old seedling at 10°C along with lowest mean for days to flowering. Maximum pod length was observed in case of cluster II whereas maximum 100-seed weight was noticed in cluster VIII. All the other clusters were intermediate in their mean character components.

Table 19 Intra and inter cluster average D^2 values among clusters

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	**112.125**	168.808	196.510	170.562	149.844	283.041	202.120	192.083	252.150
II		**87.848**	226.202	219.146	243.212	444.911	259.633	194.903	463.580
III			**55.625**	175.678	228.419	634.792	483.562	156.592	277.489
IV				**84.049**	235.033	337.906	438.623	365.840	326.745
V					**94.592**	351.186	251.483	239.406	202.297
VI						**143.421**	312.167	606.786	430.717
VII							**000**	264.298	499.863
VIII								**000**	370.722
IX									**000**

Table 20 **Intra and inter cluster average distance (D) = √ D^2 values among clusters**

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	**10.588**	12.992	14.018	13.060	12.241	16.824	14.217	13.859	15.879
1		**9.373**	15.040	14.803	15.595	21.092	16.113	13.961	21.531
III			**7.458**	13.254	15.113	25.195	21.990	12.513	16.658
IV				**9.168**	15.331	18.382	20.943	19.127	18.076
V					**9.726**	18.740	15.858	15.473	14.223
VI						**11.976**	17.668	24.663	20.754
VII							**000**	16.257	22.358
VIII								**000**	19.254
IX									**000**

Table 21: Character means for different genetic clusters

Character / Cluster	Days to 50% flowering	Plant height	Cluster/ plant	Pods/ plant	Pod length	Seeds/ pod	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	100-seed weight	Yield/plant
I	42.142	23.402	3.152	7.073	5.428	6.842	11.571	17.000	15.880	18.166	3.340	2.620
II	42.083	23.599	3.083	8.950	6.141	7.100	5.750	10.750	15.750	11.000	2.562	2.570
III	41.000	26.367	3.200	6.200	5.500	6.633	4.000	14.500	4.000	20.500	3.556	2.685
IV	41.333	28.044	3.288	9.444	5.755	7.711	5.333	14.333	19.000	26.000	3.238	3.013
V	44.000	17.383	2.566	5.233	5.233	6.716	14.333	17.000	10.000	24.000	2.478	1.800
VI	42.500	24.050	2.783	7.033	5.050	6.617	14.500	22.833	34.000	23.000	3.295	2.503
VII	39.000	22.067	3.000	6.667	5.733	6.600	22.667	12.000	19.000	9.000	3.483	2.400
VIII	42.000	17.633	2.867	5.800	4.233	4.200	8.000	13.000	6.000	10.000	3.783	1.960
IX	40.000	19.833	3.600	6.400	5.467	6.400	14.000	35.000	9.000	26.333	3.247	2.500

Plant breeders often use varieties or genotypes 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 IV x VII, IV x IX and IV x VI are expected to produce more transgressive, yield segregants in later generation. But these crosses are to be tested to get the final conclusion.

4.4.6 Canonical analysis

Canonical analysis is a multivariate analysis which is an extension of multiple regression analysis. The canonical analysis (Rao, l952) involved estimation of canonical vectors or canonical roots and the first t5wo 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 30 greengram genotypes based on twelve characters was done and the contribution of first two canonical roots Z_1 and Z_2 were 32% and 22%, 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. 2). Depending on the closeness of points representing genotypes in the scatter diagram, the 30 greengram genotypes were grouped into seven clusters (Table 22).

Table 22 Composition of genetic cluster using Canonical analysis

Cluster	No. of genotypes	Name of genotype
I	16	SN1-2, SE1-2, SN3-3, SM1-3, SE2-2, SG1-2, ON3-3, ON3-1, OG3-3, OG1N2-3, OM2-3, Sujata, OBGG-52, TARM-1, PDM54, Kendrapara (A)
II	6	SE3-2, SE2-3, OG3-2, Sonpur-2, Jhainmung(B-2), Kopargaon
III	2	ON3-2, OG1M2-3
IV	2	OE2-3, OG1-1
V	2	SG1-1, OE1-2
VI	1	OM1-3
VII	1	SG3-3

Cluster I represented by 16 genotypes occupied the central position in the scattered diagram while cluster II occupied the position near to cluster I which includes six genotyppes including three mutant culture and three local genotypes. Cluster III, IV and V also each having two genotype but almost at equidistance from cluster I which is at the center. Cluster V composed of two genotypes SG1-1 and OE1-2 which were highly cold tolerant and with satisfactory yield. Cluster VI and VII were having one genotype each and were located near to each other. Cluster VII was away from cluster I in comparison to cluster VI. The genotype of cluster VI, OM1-3 having highest cold sensitivity and very low yielding ability. The genotype of cluster VII, SG3-3 was a cold tolerant genotype with satisfactory yield level Clustering pattern on the basis of D^2 Tocher's method and canonical analysis were almost similar with few deviations. Cluster I of canonical method acquired two genotypes PDM 54, OBGG-52 from cluster IX of Tocher's method and donated two genotypes (SE3-2, OG3-2) to cluster II of Canonical method.

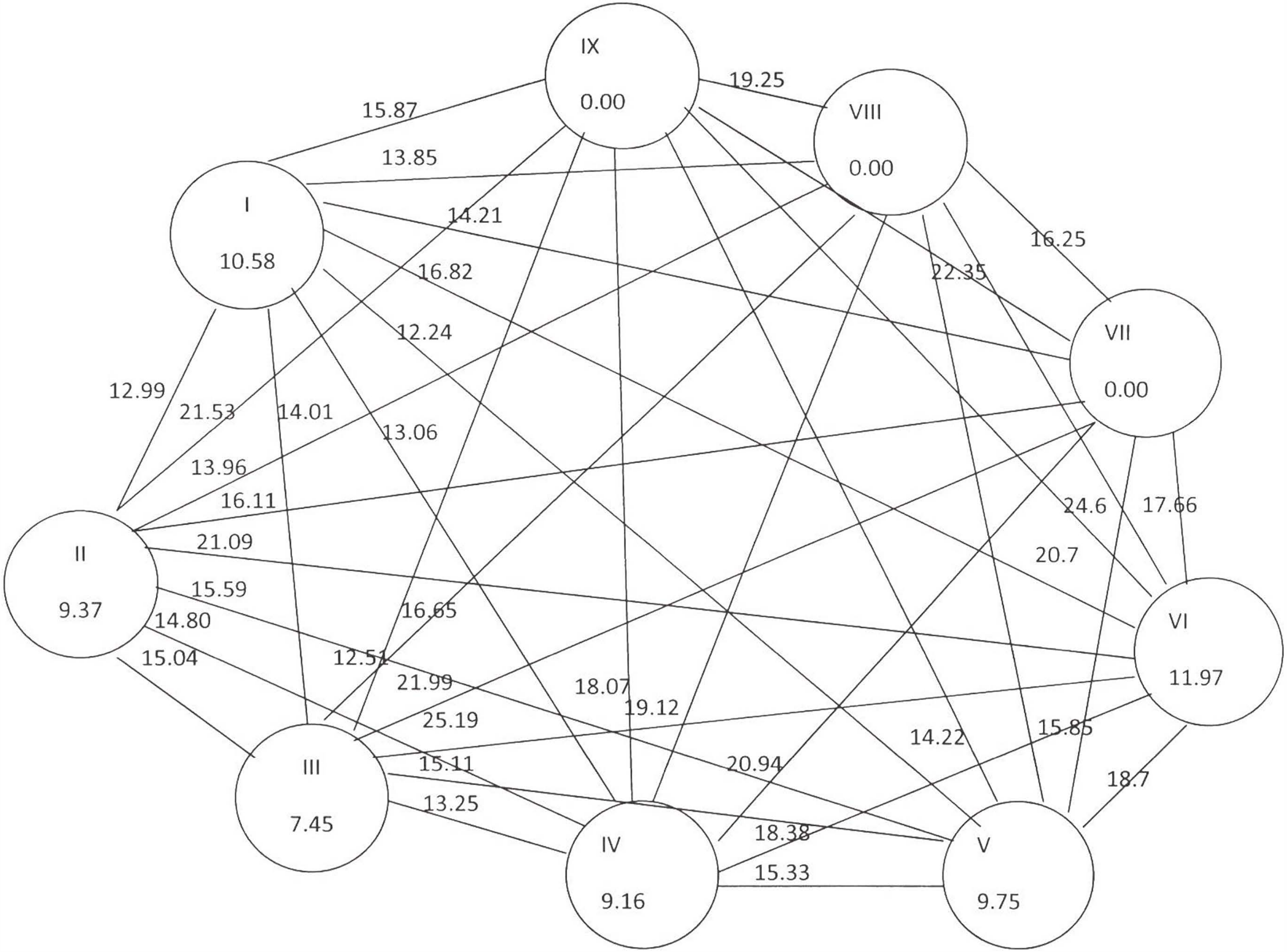

Fig. 1. Relative deposition of the cultures showing average distance (D) between and within them

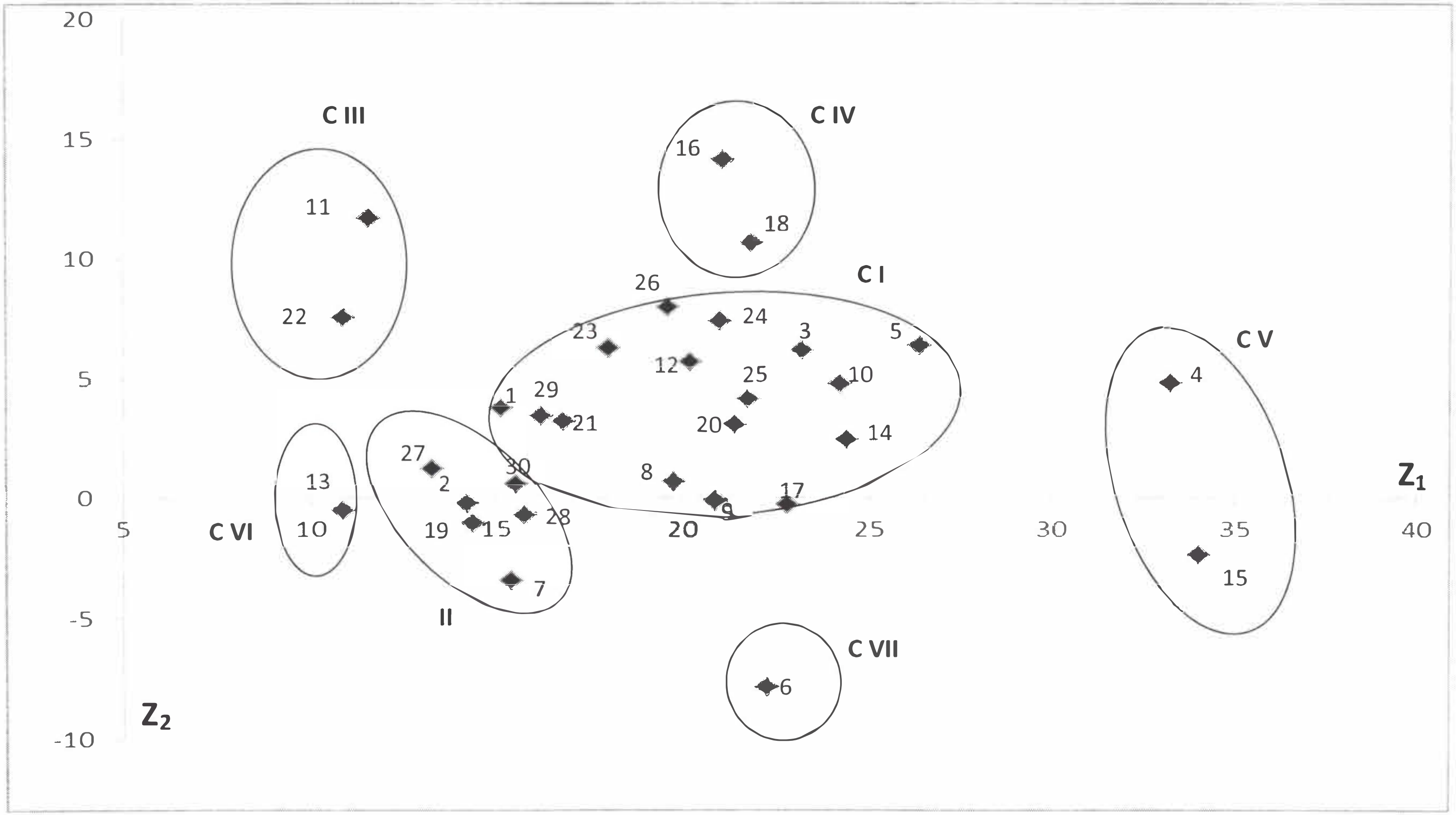

Fig. 2. Group constellations of 30 cultures or varieties (Z_1 –Z_2) Graph

4.5 SDS- PAGE

SDS-PAGE of proteins generates different profiles composed of several polypeptide subunits which migrate in the gel according to their molecular weights. The number of such subunits indicates the no. of multigene families involved (De, 1990). Mutation in these gene families or their regulatory regions leads to deletion of some or all the genes or production of new alleles which form the basis of variation in the polypeptide banding patterns involving presence or absence of bands. In addition, intensity of polymorphism could provide information in shutting of some members of the multigene family that could produce fewer copies in the genotype exhibiting faint bands. Electrophoregrams of a fairly large number of genotypes may also reveal distinct allelism for gene families comprising two molecular variants of a polypeptide subunit under monogenic control. Such polymorphic polypeptides with varying molecular weight are considered as polypeptide markers. Electrophoretic patterns of storage seed proten could be used for verification of varietal identity (Hussain *et al.* 1986).

Seeds storage protein contains various protein fractions including globumin, albumin, glutenin, prolimine, arceline and lectin which defer in their solubility. Since albumin is water soluble and globulin is salt (Sodium Chloride) soluble, polypeptide binding pattern has been generated for these protein fractions singly or in combination. The polymorphism of polypeptides markers are in vogue used for characterization and categorization of genotypes in addition to its use in hybrid selection, marker assisted selection, elucidation of genetic control of protein expression, linkage of polypeptide bands, stability of the polypeptide binding pattern, genome homology, centre of genetic diversity and evolutionary pathway.

Electrophoresis result for albumin and globulin (accounting 85- 90% of seed storage, protein in greengram) protein fractions reveled twelve and eight polypeptide bands with different molecular weights (Fig. 3). The albumin globulin profile included bands AL1 TO AL12 with molecular weights 98.7, 92.4, 83.1, 72.1, 66.3, 60.1, 52.1, 50.9, 40.8, 31.7, 28.3, 22.4 kDa. Globulin profile is a bit smaller which included bands GL1 to GL8 with molecular weights 84.4, 74.6, 62.5, 51.2, 42.7, 34.9, 30.3and 26.7kDa respectively.

Polymorphism of polypeptide bands were revealed in terms of presence (+)/absence (-) of bands as well as thickness of band at appropriate molecular weight positions in the electrophoregrams. The thickness of bands was given due important to reveal degree of quantitative variations in the polypeptides. Five distinct major bands of albumin (AL2-92.4,AL3- 83.1, AL6-60.1,AL7- 52.1,AL8- 50.9,) and three major band globulin (GL2:76.4, GL4:51.6, GL5:42.7kDa) were observed in the electrophoregrams. The present sets of genotypes did not reveal any polymorphism for polypeptide band AL2, AL7, AL8 ,AL9, AL10 and AL12 (92.4kd,52.1kDa50.9kDa,40.8kDa and 31. 7kDa, 22.4 kD respectively) and GL-2, GL-4, GL-5, GL-7 (74.6, 51.2, 42.7, 30.3kDa) as these major bands were present in all accessions. However, thickness of these bands varied in the genotypes. The polypeptide band AL3 was medium thick but sharp in all genotypes except genotype 5 to 10 and 20 and 22 indicating absence of gene responsible for production of that polypeptide in them which can be used as marker for those particular genotype. Similarly GL1 and GL3 which were faint and thin in nature were absent in few genotypes and can be used as molecular marker for genotypes OG3-2, SN1-2 and SE3-2 respectively.

Considering polypeptide banding pattern pulled over albumin and globulin, most of the mutant had similar protein band type with their parents but differ in intensity of globulin bands and albumin banding patterns. Further most of the mutant cultures were of same protein type for albumin but differ in globulin polypeptide banding pattern and vice versa. The potential power of electrophoresis technique for determining genetic variation in crop germplasm has been demonstrated earlier for seed storage protein and isozymes (Ferguson and Grabe (1986), Morphy *et al.* (1990) and Tripathy *et al.* (2010).

Genotype SN1-2 and SE3-2 reveal absence of globulin bands GL3 of 62.5 kDa, but it was present in all other accession. Similarly, globulin band GL1 of 84.4kDa was absent in all the genotypes except in the accession number OG3-2. Absence/presence of these bands could serve as molecular markers for identification of accessions. Absence of few polypeptide bands have been observed in SN1-2, SE3-2, SM1-3, SE2-3. This could be due to fact that mutant could have acquired new protein component in the course of mutagenic treatments.

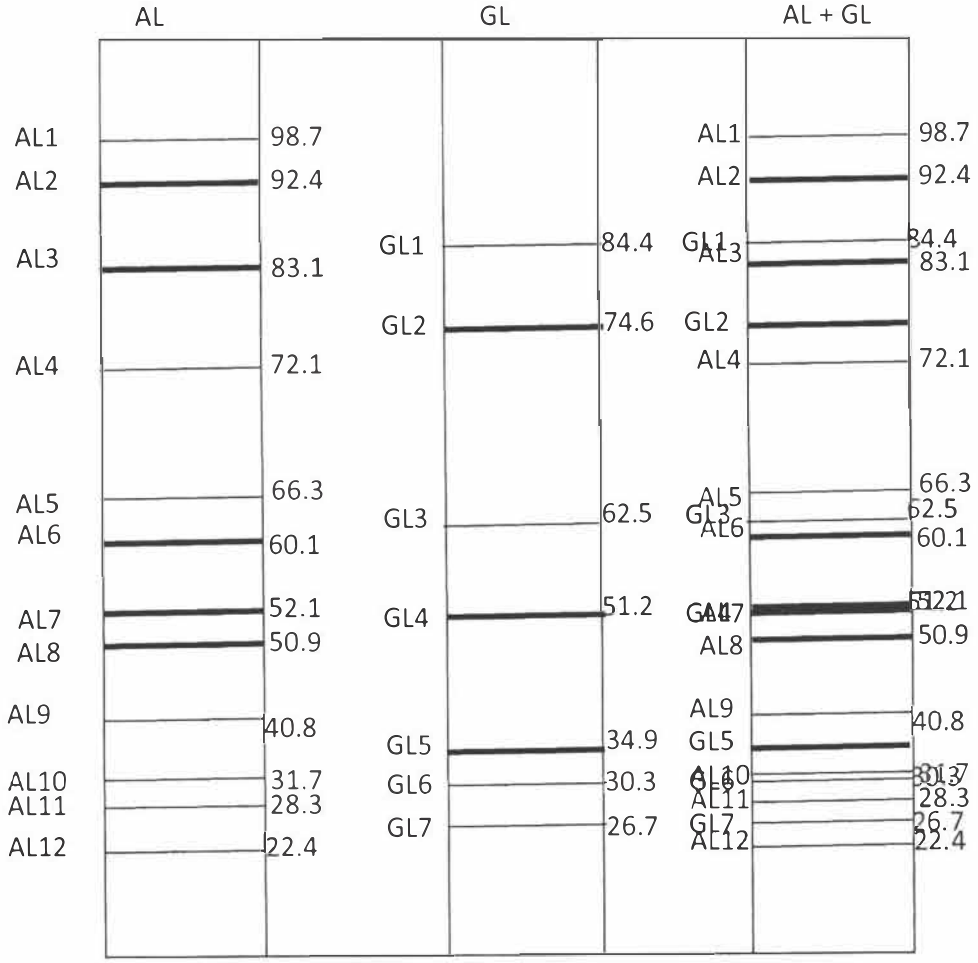

Fig. 3. **Seed storage protein fraction profile (AL: Albumin, GL : globulin and AL + GL : combined albumin and globulin pattern**

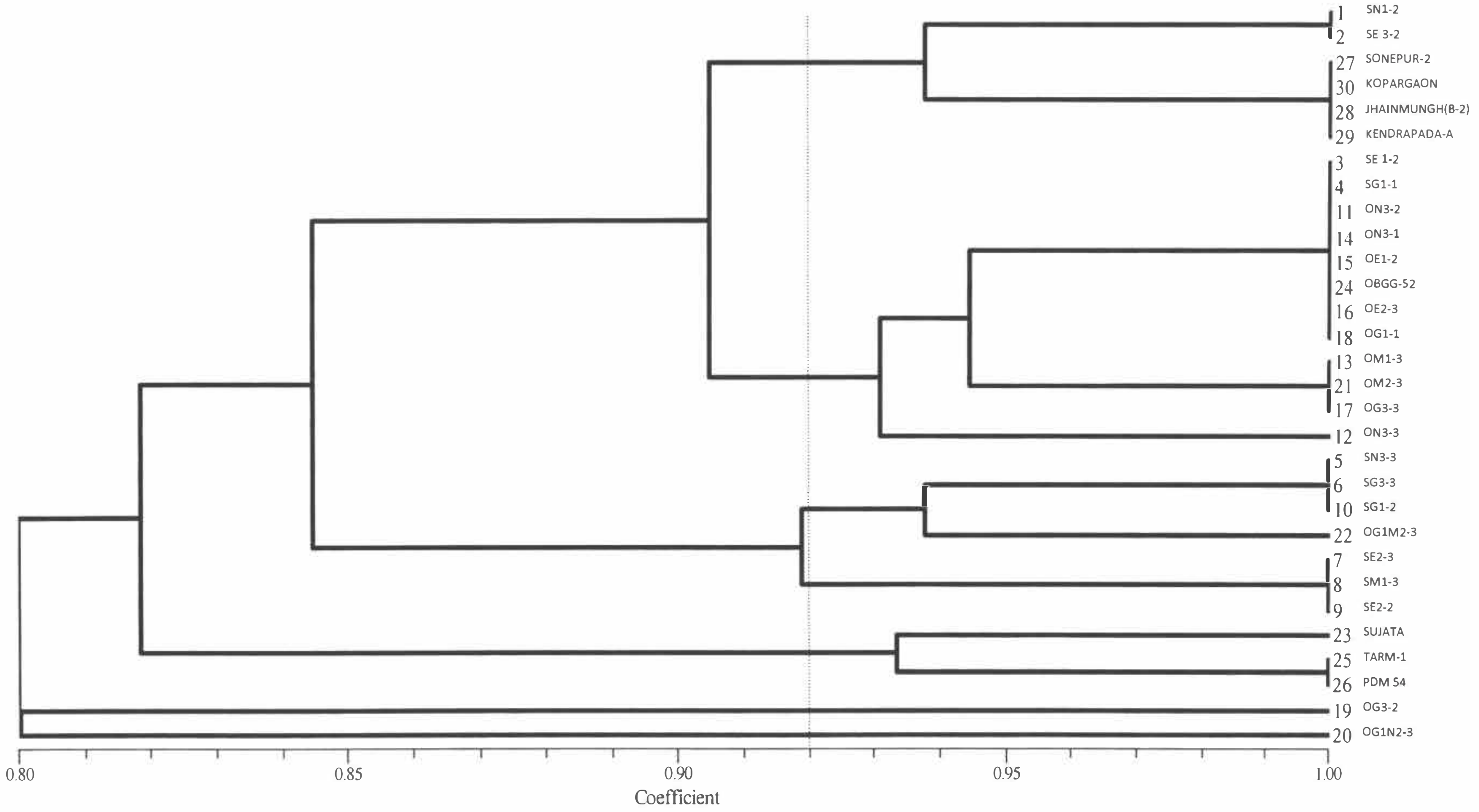

Fig. 4. Dendrogram showing genetic diversity of genotypes based on combined albumin and globulin polypeptide banding pattern

large genetic diversity and genotype specific protein type among the test materials. Ghallab *et al.* (2007) delineated the genetic relationship among a set of greengram genotypes from a dendogram which showed three different genetic clusters. Electrophoretic assessment of 581 local strains of greengram collected from different regions of Asia revealed eight protein types on the basis of combination of four albumin and two globulin bands Tomooka *et al* (1992). However; Roy (2003) observed very restricted level of polymorphism among some Indian cultivar and wild accessions of greengram genotypes.

In present case the genotypes were grouped into 12 clusters at SI=1 in which all the local genotypes were in one group (cluster-II), where as two parents grouped them in to two separate clusters. Parent OBGG-52 grouped itself along with some of its mutants and Sujata's where as OBGG-52 was alone. The parents of the mutant cultures form separate cluster from their mutants indicating occurrence of mutation in them.

In this pursuit few genotypes (OG3-2, OG1N2-3) were clubbed in a separate cluster at a low phenon level. Besides each of the genotypes (SN1-2, SE3-2, OE2-3, ON3-1, and SE2-2) had also maintained their genotypic identity. All the local genotypes being placed in a separate small cluster at moderate phenon level, exhibited appreciable genetic difference in polypeptide banding pattern with other genotypes. However, more polymorphic bands needed to be scored to reveal its distinctiveness at low phenon level. Thus, all the above genotypes considered to be quite divergent from rest of the genotypes and could serve as valuable breeding materials for improvement in protein quality and quantity *per se.*

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The genotypes OG3-2 was with highest number of polypeptide bands and hence may be considered as superior in seed protein quality provided the polypeptide revealed are not associated with anti-nutritional activity. The genotypes ON3-3, OM1-3, OG3-3, and OM2-3 were observed with second highest number of polypeptide bands. These genotypes differed in protein type and may serve as excellent breeding materials for future crop improvement in protein quality and quantity *per se*.

Polypeptide banding pattern of mutants derived from OBGG 52 and Sujata differed from their parents indicating mutation of genes in multigene families for seed storage protein expression.

Genetic variation in germplasm has an important role in identification of desirable varieties. Polymorphism in electrophoretic banding pattern of seed storage protein is associated with the genetic background of the protein and thus could be used to certify the genetic makeup. Further, the genetic variation study in the present set of materials could serve as a source of genetic diversity. The genotype having absence/presence of few bands can be combined through the hybridization and would lead to the possibilities of isolation of qualitatively superior segregants showing protein profile with presence of all the 20 polypeptide bands. A choice of few other genotypes combination can also be made basing on complementary banding pattern for further genetic improvement of protein quality. Besides, the protein fingerprinting develop in the present investigation would serve as a molecular tool for identification and characterization of promising genotypes.

The present set of greengram genotypes were distributed over 7divergent cluster at comparatively low phenon (SI=0.92) level (fig-4). At finer level (SI= 1.00) the genetic variation was virtually dissociated into single variety in few cases to cluster of few varieties in some other. This envisaged

CHAPTER-V

Summary & Conclusion

SUMMARY AND CONCLUSION

The present investigation on "Genetic architecture of yield and cold tolerance in mutant lines of 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 seed storage protein banding pattern.

Thirty greengram genotypes consisting of twenty two mutant cultures, two parents, two standard varieties and four land races cultivated in Odisha were evaluated in RBD during *rabi*, 2011-12 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 and SDS-PAGE were studied at S. K. Sinha Molecular Breeding Laboratory of Plant Breeding and Genetics Department.

The genotypes showed wide variation in all 12 traits and the difference were highly significant. The range of variation in yield/plant among the cultures of Sujata and OBGG-52 was 1.610g (SE1-2) to 3.170g (SE2-2) and 1.960g (OM1-3) to 3.590g (OE2-3), respectively, as against 2.730g and 2.440g in the respective parent. High yielders were OE2-3, ON3-1, OG1N2-3, SE2-2. PCV and GCV estimates were high for reaction to cold at 10,30,40 days old seedling expose to 10°C. Heritability and genetics advance of the traits ranged from 69.43 to 98.59 and 0.734 to 15.400, 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 seeds per pod 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 -0.409 to 0.739 and -0.666 to 0.860, respectively. Character association among component traits showed two sets strongly interrelated characters *viz*., pods/plant, plant height, seeds/pod, and pod length and reaction to cold at 10 days old seedling.

The characters plant height, pods/plant, cluster per plant, and seeds/pod showed positive correlation with yield while, reaction to cold at 10, 20 and 30 days old seedling stage 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 plant height, and 100- seed weight.. Positive correlation of most traits with yield was mostly influenced by in direct positive effect *via* pods/plant and plant height.

Among the different mutagenic treatment, T5E2 was found to be most effective as it was able to change six out of twelve characters in the positive direction followed by T9N3 which change four characters in the positive direction.

Multivariate analysis of divergence among the 30 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

D^2 values for the 435 pair combinations among 30 strains ranged from 41.244 between SM1-3 and SE2-2 to 735.765 between ON3-2 and OE1-2 indicating that, while some strains were quite close to each other genetically, others are quite .The characters survival of 30 days old seedling at 10°C (d), survival of 40 days old seedling at 10°C (d) and survival of 10 days old seedling at 10°C (d) contributed maximum to divergence, while days to 50% flowering , clusters/plant, yield per plant, , pod length, seeds/pod contributed least to divergence.

Using the Tocher's method, the genotypes were grouped into 09 clusters. 14 genotypes including 1 standard variety, 12 mutant cultures and one of the two parents were grouped in a single cluster (Cluster-I) while cluster II was with one mutant and three local land races cultivated in Odisha. Other clusters were with 1 to 3 genotypes. The mutant cultures were distributed among all the clusters indicating a diverse type of genetic changes being induced by different mutagenic treatments. The clustering of different varieties from different sources with some mutant cultures, and conversely, the distribution of mutant cultures originating from same treatment over different clusters indicated no definite relationship or parallelism between the geographic origin of the strains and the genetic diversity among them as measured by D^2.

Considering inter-cluster average D^2 value, cluster mean for different character including yield and character complementation in productivity traits, crosses between cluster IV x VII, IV x IX and IV x VI 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 32% and 22 % respectively of the divergence. Clustering on basis of canonical analysis was similar to that on basis of D^2, with very few exchanges.

SDS-PAGE of proteins generates different profiles composed of several polypeptide subunits which migrate in the gel according to their molecular weights. Electrophoresis result for albumin and globulin protein fractions reveled twelve and eight polypeptide bands with different molecular weights. The albumin globulin profile included bands AL1 TO AL12 with molecular weights 98.7, 92.4, 83.1, 72.1, 66.3, 60.1, 52.1, 50.9, 40.8, 31.7, 28.3, 22.4 kDa. Globulin profile is a bit smaller which included bands GL1 to GL8 with molecular weights 84.4, 74.6, 62.5, 51.2, 42.7, 34.9, 30.3and 26.7kDa respectively. Five distinct major bands of albumin (AL2-92.4,AL3-83.1, AL6-60.1,AL7- 52.1,AL8- 50.9,) and three major band globulin (GL2:76.4, GL4:51.6, GL5:42.7kDa) were observed in the electrophoregrams. However, thickness of these bands varied in the genotypes. The polypeptide band AL3 was medium thick but sharp in all genotypes except genotype SN3-3 , SG3-3, SE2-3, SM1-3, SE2-2, SG1-2, OGN2-3 and OGM2-3 indicating absence of gene responsible for production of that polypeptide in them which can be used as marker for those particular genotype. Similarly GL1 and GL3 which were faint and thin in nature were absent in few genotypes and can be used as molecular marker for genotypes OG3-2, SN1-2 and SE3-2 respectively. The genotypes OG3-2 was with highest number of polypeptide bands and hence may be considered as superior in seed protein quality provided the polypeptide revealed are not associated with anti-nutritional activity. The present set of greengram genotypes were distributed over seven divergent cluster at comparatively low phenon (SI=0.92) level. In present case the genotypes were grouped into 12 clusters at SI=1 in which all the local genotypes were in one group (cluster-II), where as two parents grouped them in to two separate clusters. Parent OBGG-52 grouped itself along with some of its mutants and Sujata's where as OBGG-52 was alone. The parents of the mutant cultures form separate cluster from their mutants indicating occurrence of mutation in them. In this pursuit few genotypes (OG3-2, OG1N2-3) were clubbed in a separate cluster at a low phenon level. Besides each of the genotypes (SN1-2, SE3-2, OE2-3, ON3-1, SE2-2) had also maintained their genotypic identity. All the above genotypes considered to be quite divergent from rest of the genotypes and could serve as valuable breeding materials for improvement in protein quality and quantity *per se.*

CONCLUSION

The study of variability parameters among 30 greengram genotypes in 12 traits including yield has provided valuable information regarding the genetic variability, heritability and genetic advance for the characters and efficacy of different mutagenic treatment. Genetic parameters of traits, correlation among traits and path analysis revealed that selection for pods/plant, plant height; 100-seed weight would be effective in isolation of high yielding genotypes.

Multivariate analysis of divergence by two methods D^2 and Canonical analysis grouped the 30 genotypes into nine clusters indicating presence of divergence. Clustering by Canonicals analysis was similar to D^2 analysis. The crosses cluster between cluster IV x VII, IV x IX and IV x VI and are expected to produce transgressive segregants for yield.

SDS-PAGE produced albumin and globulin profile included bands AL1 TO AL12 with molecular weights 98.7, 92.4, 83.1, 72.1, 66.3, 60.1, 52.1, 50.9, 40.8, 31.7, 28.3, 22.4 kDa where as globulin profile was a bit smaller which included bands GL1 to GL8 with molecular weights 84.4, 74.6, 62.5, 51.2, 42.7, 34.9, 30.3and 26.7kDa respectively.. The polypeptide band AL3 was medium thick but sharp in all genotypes except genotype SN3-3 , SG3-3, SE2-3, SM1-3, SE2-2, SG1-2, OGN2-3 and OGM2-3 indicating absence of gene responsible for production of that polypeptide in them which can be used as marker for those particular genotype. The present set of greengram genotypes were distributed over seven cluster at comparatively low phenon (SI=0.92) level. At finer level (SI=1.00) the genetic variation was virtually dissociated into single variety in few cases to cluster of few varieties in some other leading to lusters but with 12 sub clusters.

Several amplification products or band were present or absent in only one or two to three of the greengram genotypes. Such rare present or absent of a band in any genotype would help in DNA fingerprinting and molecular characterization of genotypes or varieties. Genotypes (OG3-2, OG1N2-3) were clubbed in a separate cluster at a low phenon level. Besides each of the genotypes (SN1-2, SE3-2, OE2-3, ON3-1, and SE2-2) had also maintained their genotypic identity. Thus, all the above genotypes considered to be quite divergent from rest of the genotypes and could serve as valuable breeding materials for improvement in protein quality and quantity *per se*.

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