Studies on yield and stress tolerance in greengram

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(Plant Breeding and Genetics)

By

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CERTIFICATE - I

This is to certify that the thesis entitled **"Studies on yield and stress tolerance in greengram"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT BREEDING AND GENETICS)** of the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of *bona fide* research work carried out by **PUSAPATI NARMADA VARMA** under my guidance and supervision. No part of the thesis has been submitted for the award of any other degree or diploma.

It is further certified that the assistance and help availed by her from various sources during the course of investigation has been duly acknowledged.

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Advisory committee

CERTIFICATE-II

This is to certify that the thesis entitled **"Studies on yield and stress tolerance in greengram"** submitted by **PUSAPATI NARMADA VARMA** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT BREEDING AND GENETICS)** has been approved by the students' advisory committee and external examiner.

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Place:

ABSTRACT

Fifty six genotypes of greengram were evaluated in RBD for yield and component traits during 2014-15. Next season, eleven selected genotypes of the previous year entries based on their yield performance were evaluated for cold and drought analysis in S.K. Sinha Molecular Breeding Laboratory of Department of Plant Breeding and Genetics. Molecular work was taken up for these eleven genotypes regarding YMV resistance. The genotypes showed wide and highly significant variation in all these traits. Seed yield of the genotype varied from 1.8 to 6.1 g/plant. High yielding genotypes were IPM-02-03, IPM-02-14 and Sujata X LGG-460. PCV and GCV estimates were high for primary branches per plant. Plant height, pods per plant, days to 50% flowering, and maturity had high heritability with high genetic advance which indicated additive gene effect. The characters plant height, clusters per plant, pods/ plant, pod length, and 100 seed weight showed positive correlation with yield. Pods /plant had highest direct positive effect on yield followed by 100 seed weight. Divergence analysis was done by Mahalnobis D^2 statitics and clustering of genotype was done by Tocher's method and the 56 genotypes were grouped into 7 clusters. 100 seed weight contributed maximum to divergence. Crosses between cluster V and VI, II and VI, VI and VII are expected to produce more transgressive segregants in the later generation. From cold study, it was noticed that the cold tolerant genotype Sujata X LGG-460 is a moderate yielder but IPM-02-03 is a high yielder having medium tolerance to cold. So crosses between genotype having high yielding ability and cold tolerance are expected to produce superior genotypes combining both the characters. In drought analysis genotypes PDM-139, IPM-99-125 and Bhawanipatna showed high tolerance index values indicating their tolerance to drought. In molecular analysis, it was observed that two primers (RGA and YMV1) linked to YMV resistance produced band in 6 genotypes. YMV1 produced amplicon at 130 bp whereas RGA produced amplicon at both 90 bp and/ or 180bp. The genotypes OBGG-52 X Pant-M-4, Sujata X LGG-460, IPM-02-14, IPM-02-03, IPM-99-125 and KPS-1 were found to possess resistance against YMV.

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INTRODUCTION

Pulses are important component of human diet as a source of protein. On an average, pulses contain 20-25% of protein in dry seeds, which is about 2.5-3.0 times that of cereals. When supplemented with cereals, they provide a perfect mix of essential amino acids with high biological value. So they have a special role in meeting the protein requirement of particularly vegetarian population and poor masses. Besides, pulse crops function as mini nitrogen factories due to their ability to fix atmospheric nitrogen in symbiotic association with *Rhizobium* bacteria, thereby improving the physical, chemical and biological properties of the soil. Thus these crops are excellent crops in cropping system for sustainable agriculture and nutritional security. The commonly grown pulses in India are Chickpea, Pigeonpea, Greengram, Blackgram, Cowpea, Fieldpea, Lentil, Moth bean, and French bean.

Greengram

Vigna radiata (L.) Wilczek, commonly known as greengram, belongs to family Fabaceae. Greengram recently moved from the genus *Phaseolus* to *Vigna* due to closeness in floral and seed morphology with the genus *Vigna*. This crop belongs to the subgenus Ceratotropis in the genus *Vigna*. It is a self pollinating diploid grain legume (2n=22) with a genome size of 560 Mb (Arumuganathan and Earle 1991).

According to Vavilov (1926) greengram is a native of India and central Asia. It is grown in these areas since pre-historic period. De Candolle (1986) believes that mungbean has originated in India.

Greengram is one of the important pulse crops in Asia particularly India and South-East Asia. It is widely cultivated throughout Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia, and south China. India is the largest producer of greengram in the world and accounts for 65% area (second after China) and 54% production (Pratap *et al*, 2012). Most of the production in India is traded and consumed locally, whereas Thailand is the world's largest exporter of greengram.

In India greengram is the third major pulse crop followed by chickpea and pigeonpea. It occupies 3.55 million hectares of area with a production of 1.5 million tons. In India, major greengram producing states are Andhra Pradesh, Odisha,

Maharastra, Madhya Pradesh, Rajasthan, Bihar and Tamil Nadu. In Odisha, greengram ranks first in terms of both area and production amongst the pulse crops. In Odisha, greengram is cultivated in an area of 833.11 thousand ha with a production of 396.93 thousand ton and productivity of 476 kg/ha (OAS, 2013-14) and being cultivated in Ganjam, Kalahandi, Bolangir, Bargarh, Nayagarh, Cuttack, Nuapada.

Though greengram can be grown during pre-*rabi*, *rabi* and summer in Odisha, the majority of areas are in rice fallows of coastal districts after the harvest of kharif rice under residual moisture condition. In the interior districts, it is generally cultivated during kharif and pre-*rabi*. However, now the crop has been rapidly penetrating into several non-traditional areas, e.g, during *rabi* season in interior districts and during spring/ summer season under assured irrigated condition.

Though the estimated pulse requirement in Odisha by 2020 is focused to be 49.4 lakh ton (Nayak, 2009), the present productivity is very low to achieve the target. The low productivity may be due to sowing on marginal and sub-marginal land under residual moisture in rice fallows, lack of high yielding genotypes, negligence in plant protection measures, occurrence of cold stress and terminal drought during *rabi*, high temperature stress during spring/ summer and due to prevalence of Yellow Mosaic virus (YMV) disease. All these factors either independently or jointly result in the poor productivity of this crop.

Research on Greengram

Research on greengram was started in 1925 at Pusa. But systematic and well organized research for development of high yielding, disease/ insect-pest resistant varieties and production technology was started with the establishment of All India Coordinated Pulse Improvement Programme (AICPIP) in 1967 which was later on bifurcated into three groups later in i.e. AICRP on Chickpea, AICRP on MULLaRP (Mungbean, Urdbean, Lentil, Lathyrus, Rajmash and Pea), AICRP on Pigeonpea, Under the aegis of AICRP, more than 100 varieties of greengram have been released so far cultivation in different agro-ecological regions and seasons. Despite the systematic and continuous breeding efforts through conventional breeding method, substantial genetic gain in production and productivity of these two crops could not be achieved.

Thus the present investigation in greengram with 56 genotypes with fifteen selections from crosses, seven selections from induced mutants, seven selections from local cultivars developed at OUAT along with 27 breeding lines was taken up with the following objectives:

1) To evaluate for yield and yield attributing traits.

2) To study the nature and extent of variability for different traits.

3) To study correlation among different traits and direct and indirect effects of component traits on seed yield.

4) To study genetic divergence among these genotypes by multivariate analysis.

5) To study the reaction to drought stress in these genotypes

6) To study the reaction to cold stress in these genotypes

7) To study the reaction to Mungbean yellow vein mosaic virus (MYVMV) in these genotypes.

The results of this study will help in selecting genotypes having high yielding ability, resistance to biotic stress like YMV, and abiotic stress like low temperatures and drought which may be further utilized in hybridization programme for developing a suitable genotype with all the desirable traits.

REVIEW OF LITERATURE

Planning effective breeding methods to bring about improvement in any crop largely depends upon the nature and magnitude of genetic component of variation for yield and its component traits. The choice of best parent that possess high heritability and genetic advance for various traits (Khan *et al.* 2005) in any crop breeding programmes can be achieved by conventional methods involving hybridization or by induction of mutation for the success of breeding programme. Evaluation of parents for their transmission potential for yield and its component will pave the way for better selection. As all available parents with high order of performance may not be able to transmit their superior traits into their progenies, therefore, selection of desirable parent is increasingly used now a day in crop improvement. Knowledge of genetic variability on different yield parameters is also an important criterion for yield enhancement. Adequate information on origin, distribution, cultivation system, cytology, cytogenetic and genomic relationship, crossability with wild relatives, available range of variability in morphological and economic traits, genetics of important traits, reaction to biotic and abiotic stress, nutritional qualities etc. are very important factors for a breeder.

2.1 Genetic variability, heritability and genetic advance in quantitative traits of green gram

Suresh *et al.* (2010) reported high heritability (broad) along with high genetic advance (as % of mean) for plant height, number of pods per plant, number of seeds per pod, 100 seed weight and single plant yield indicating that these characters would be amenable for phenotypic selection.

Rahim *et al.* (20 l0) evaluated 26 greengram genotypes for genetic variability study and significant variations were observed among the genotypes for all characters. High heritability (broad) along with high genetic advance (% of mean) was observed for plant height, number of pods per plant, seeds per pod, test weight and grain yield per plant indicating these characters would be best for phenotypic selection.

Reddy *et al.* (2011) evaluated 35 divergent genotypes of greengram for yield which showed significant difference among characters studied. High genetic advance coupled with high heritability was observed for plant height, number of pods/plant,

shoot dry matter/plant and seed yield/plant, indicating thereby the preponderance of additive gene action.

Zaid *et al*. (2012) tested the greengram genotypes for genetic variability .Maximum plant height was observed for genotype NFM5-63-19 cm; maximum number of pods plant^{-1} was recorded for genotype NFM5-63-19, while genotypes NFM-12-8 and NFM-6-5 were found with a maximum pod length. Similarly the maximum number of seed pod^{-1}, biological yield and grain yield was observed in genotype NFM-6-5, NFM-12-6 and NM- 98 respectively. The high heritability was recorded for pod length (99%) and plant height (70%), while pods plant^{-1} (29%) and seed pod^{-1} (17%) had low heritability.

Gadakh *et al.* (2013) estimated the genetic variability, heritability and genetic advance for 15 quantitative characters in 50 diverse genotypes of green gram. High heritability coupled with high genetic advance was observed in biological yield per plant, harvest index indicating the impact of additive gene affecting expression of these characters.

Narasimhulu *et al*. (2013) evaluated 40 greengram genotypes and observed high GCV and PCV for number of branches, pods/plant, biological yield and harvest index. Genetic advance(% of mean) was high in case of 100 seed weight and harvest index. High heritability coupled with genetic advance was observed for plant height, pods per plant, harvest index, biological yield, and seed yield indicating their control by additive gene action.

Singh *et al*. (2013) observed a significant difference among 36 genotypes studied for almost all traits. Heritability is found to be affected by environment. Traits like plant height, primary branches, secondary branches, pod mass, seed mass, biological yield and harvest index showed low environmental influence comprising of high heritability and selection response.

Kumar *et al. (*2013) studied the genetic variability in greengram and observed that only 100 seed weight exhibited high heritability estimates (narrow sense) coupled with high genetic advance, indicating the preponderance of additive gene action. Selection based on this trait will be rewarding. Phenotypic coefficient of variation was slightly higher than the genotypic coefficient of variation.

Garje *et al*. (2014) revealed highly significant difference for all characters under study indicating the presence of sufficient amount of variability in 40 genotypes of green gram for different quantitative characters. The highest GCV and PCV were observed for seed yield per plant, number of pod per plant, secondary branches per plant, clusters per plant and pod length respectively. High estimates of genetic advance were observed for plant height and number of pod per plant.

Raturi *et al*. (2014) reported that 44 mung bean genotypes exhibited significant to highly significant differences with respect to all the morphological and biochemical characters studied. Five genotypes were recorded with > 3% fat, 16 with > 3% fibre, four with > 5% ash, six with > 29% protein and eight with > 65% carbohydrate contents. Fat and fibre contents were recorded with significantly higher heritability (> 90%) with corresponding PCV and GCV (> 15%) coupled with > 35% genetic advance, which validates that these characters are greatly influenced not only by the additive gene effect but also by greater proportion of heritable variation.

Sahu *et al.* (2014) studied on homogeneity and homozygosity of F_5 and F_6 populations of 35 greengram genotypes to assess genotypic variability and character association considering nineteen nitrogen fixing and yield attributing parameters. Straw protein content (0.306*), nitrogen fixation (0.342*), plant height (0.474*), pod per plant (0.388*), 100 seed weight (0.442*) were found to be major yield factors. Variability coefficients exhibited approximate similar pattern for both generations indicating stabilization of parameters with generation advance.

Degefa *et al*. (2014) found high PCV over GCV indicating a higher environmental influence for number of primary branches, pods per plant, seeds per pod and harvest index while studying the genetic variability, heritability (broad sense) and genetic advance among 13 greengram accessions. High estimates of heritability and genetic advance were scored for seeds per plant and seed yield indicating that these characters were under the control of additive genetic effects. High genetic advance (% of mean) coupled with high heritability was observed for number of primary branches, number of seeds per plant.

Hemavathy *et al*. (2014) evaluated thirteen greengram genotypes for nine quantitative traits, all of which showed a significant difference for all the characters

under study. Higher GCV and PCV was observed for seed yield, pods per plant and clusters per plant.

Katiyar *et al*. (2015) estimated higher heritability for all the traits except branches per plant and seeds per pod while studying 45 advanced lines of greengram. High heritability coupled with high genetic advance was found in case of seed yield, days to flowering and plant height indicating minimal environmental influence.

2.2 Correlation Study in Green Gram

Tabasum *et al.* (2010) reported that clusters per plant, pods per plant, total plant weight and harvest index having positive significant genotypic and phenotypic correlations with seed yield.

Jena *et al*. (2010) while studying correlation in local green gram, found pods/plant, pod length and seeds/pod had positive correlation with yield.

Rahim *et al*. (2010) reported that the pods per plant, panicle length and seeds per pod were positively correlated with grain yield 26 in mung bean genotypes

Reddy *et al*.(2011) revealed 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, seed protein and shoot dry matter/plant.

Khajudparn and Tantasawat (2011) identified the direct and indirect effects on seed yield in 56 mung bean accessions. Seed yield was significantly and positively correlated with pods per plant, clusters per plant, total dry matter, seeds per pod, seeds per plant, biomass and branches/plant and negatively correlated with days to maturity.

Mondal *et al.* (2011) investigated that leaf area index (LAI) was the most important source that determined total dry mass (TDM) yield, and reproductive characters like number of racemes, flowers and pods plant were the most important sinks that determined seed yield. Contrarily, reproductive efficiency (R.E) did not show significant relationship with pod number and seed yield, indicating that selection of high yield based on RE may be misleading.

Makeen *et al*. (2011) reported that pods per plant and plant height have significant positive correlation with seed yield in greengram.

Begum *et al.* (2012) reported that grain yield plant^{-1}had highly significant phenotypic correlation with pods/ plant (0.65), grains/ plant (0.61) and 100-grain

weight and significant genotypic correlation with pods plant^{-1} (0.71) and days to pods formation (0.70).

Zaid *et al*. (2012) tested the greengram genotypes for correlation among different yield contributing traits i.e., plant height, pods plant^{-1}, pod length, seed pod^{-1}, biological yield, and grain yield. Based on genotypic correlation analysis characters like plant height, pods plant^{-1} , pod length and on phenotypic basis grain yield and seed pod^{-1} could be the best criteria in any breeding program for increasing yield in mungbean genotypes under various agro-climatic conditions.

Suresh *et al.* (2013) concluded that the number of pods per plant was positively and highly significantly correlated with single plant yield.

Kumar *et al. (*2013) found that number of secondary branches per plant, number of bunches per plant, number of pods per plant, number of grains per pod, pod length and 100 seed weight had significant positive correlation along with their high positive direct effect with grain yield, suggesting these parameters as prime traits during selection to have the higher potential of yield.

Gadakh *et al.*(2013) reported that harvest index and 100 seed weight had significant positive correlation with seed yield.

Thippani *et al.* (2013) reported that the seed per plant showed positive and significant relation with plant height, no. of pods per cluster and no. of seeds per pod both at genotypic and phenotypic level.

Aqsa *et al.* (2013) found that primary and secondary branches per plant, pod length and 100-seed weight exhibited negative and non-significant genotypic and phenotypic correlations with seed yield. Clusters per plant, pods per plant, total plant weight and harvest index showed positive significant genotypic and phenotypic correlations with seed yield.

Lalinial *et al. (*2014) reported a strong correlation between 100-seed weight, number of pods per plant, number of seeds per pod and pod weight with seed yield.

Garje *et al.* (2014) found that the seed yield per plant was significantly and positively correlated with number of primary and secondary branches per plant, number of cluster per plant, number of pod per plant and number of seed per pod.

Mishra *et al*. (2014) found that the number of pods/plant, pod length and seeds/pod exhibited significant positive correlation with green gram yield. However, the association of seed yield with days to 50% flowering, maturity duration, plant height and dry biomass was found to be significantly negative.

Javed *et al. (*2014) found that the phenotypic correlation of seed yield per plant with germination % and seeds per pod was positive and highly significant while pods per plant was negatively correlated. Higher heritability and significance correlation indicated that germination %, 100-seed weight and seeds per pod may be used to select higher yielding greengram genotypes.

Degefa *et al.* (2014) showed that seed yield per plot was significantly associated with harvest index, seed yield per plant and biomass. However, number of secondary branches and number of pods per plant were also significantly associated with seed yield per plot.

Sahu *et al*. (2014) while studied on F_5 and F_6 populations of thirty five mung bean genotypes found that nitrogen fixation had positive and significant correlation with primary branches, seed per pod, yield per plant and straw protein in both the generations.

Pathak *et al*. (2015) reported highly significant and positive association of all the quantitative characters with seed yield except with that of days to 50% flowering.

Muralidhara *et al*. (2015) found that number of pods per plant, pod yield per plant and threshing percentage had shown positive and significant correlation along with their high positive direct effect with seed yield, suggesting that these parameters may be considered as prime traits during the course of selection to have the higher potential of yield in green gram

2.3. Path coefficient analysis in greengram

Rahim *et al.* (2010) reported that the number of pods per plant and number of seeds per pod are important characters for yield through path analysis.

Tabasum *et al.* (2010) reported that Positive direct effects were exerted through secondary branches, pods per plant, pod length, 100 seed weight, total plant weight and harvest index while primary branches, plant height, clusters per plant and pods per cluster had negative direct effects.

Khajudparn and Tantasawat (2011) found that clusters per plant showing the highest positive direct effect on seed yield followed by 100 seed weight, seeds per pod 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) investigated the path coefficient analyses in 45 mung bean genotypes and found that number of flowers, pods and 100-seed weight constituted central important sinks which exerted direct positive influence on seed yield.

Reddy *et al.* (2011) 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 green gram.

Mishra *et al. (*2012) reported that pods per plant had the highest direct effect followed by branches per plant, 100-seed weight, seeds per pod and pod length on seed yield per plant.

Aqsa *et al.* (2013) found that positive direct effects were exerted through secondary branches, pods per plant, pod length, 100 seed weight, total plant weight and harvest index while primary branches, plant height, clusters per plant and pods per cluster had negative direct effects.

Suresh *et al.* (2013) opined that the number of pods per plant followed by seeds per pod was the important characters for increasing yield in mung bean.

Thippani *et al.*(2013) reported that the maximum direct positive effects exerted by no. of pods per cluster, no. of seeds per pod, pod length, plant height and 100 seed weight towards seed yield per plant based on correlation and path analysis.

Lalinial *et al. (*2014) found that the maximum direct and positive effects were related to number of seeds per pod, number of pods per plant, 100-seed weight and pod weight. Performing factor analysis through principal component analysis, three factors in total 82.03 (2009), 81.64 (2010) and 78.20 (combined dataset) percent of the changes were justified. These factors were named: yield and yield component traits, phenological and physiological parameters, respectively. Yield can be increased by improving traits of the first factor. Also, selection of cultivars with shorter

phonological traits (factor2) and higher content of physiological traits (factor3) can help to avoid dry period**.**

Sahu *et al.* (2014) opined that maximum direct positive effects towards yield were exerted by nitrogen fixing per plant (1.111), seeds per pod (0.423), pods per cluster (0.837), 100-seed weight (0.561) and plant height (0.257).

Garje *et al.* (2014) revealed that pods per plant had maximum direct effect on seed yield followed by cluster per plant and number of secondary branches per plant.

Pathak *et al*. (2015) found that pods per plant had the maximum direct effect towards seed yield followed by plant height and 100-seed weight.

2.4 Genetic diversity in green gram

Rahim *et al.* (2010) reported that 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 among all the characters in 3 clusters. Crosses involving cluster I and III may exhibit high heterosis for yield as well as earliness.

Das *et al*. (2010) explained that the genetic divergence and clustering pattern for selection of suitable parents that can be utilized in hybridization programme to study the genetic parameters attributing to yield in 6 characters and grouped the 23 strains into 8 clusters by using D^2 statistics. Cluster V has the highest inter cluster distance and can be used for hybridization programme. Among the six characters the no. of seeds per pod contributed maximum amount towards divergence.

Chattopadhyay *et al.* (2011) assessed the morphological and molecular diversity of 78 greengram germplasm. '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.

Abna *et al.* (2012) evaluated genetic diversity of 20 greengram genotypes and 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. 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.

Mishra *et al.* (2012) assessed the genetic divergence among the mutants for 9 quantitative traits in M_4 generation. 25 mutants selected from a gamma irradiated population of green gram variety Sujata were grouped in 8 clusters based on D^2 values. All the clusters (cluster II to VIII) were divergent from the cluster I, the largest group that included the parent variety Sujata. D^2 analysis of genetic divergence showed that branches per plant followed by pod length, yield per plant and plant height were the major contributors to divergence among mutant cultures of Sujata.

Gokulakrishnan *et al*. (2012) estimated genetic divergence among 30 green gram genotypes using Mahalanobis's D^2 statistic and 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. 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 indicating 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.

Garje *et al*. (2013) studied the nature and magnitude of genetic divergence using D^2 Statistics in 40 green gram genotypes for 10 important quantitative traits and grouped them into 13 clusters. Cluster III was largest with ten genotypes followed by cluster II with eight genotypes. Three characters viz. seed yield per plant (g), no. of pod per plant, and pod length (cm) contributed maximum in manifestation of genetic diversity.

Gadakh *et al.* (2013) examined the genetic divergence and clustering pattern of 50 genotypes of greengram. The crosses of genotypes from cluster I, i.e**.** Kopergaon, Vaibhav, BM-4 and BM-2005-1 with those of genotypes BM-2003-2, PM-203- 18, AKM-9907 and AKM-08-01 belonging to cluster III and RVSM-11, PM-201-19, ML-1354, AKM-0603 belonging to cluster II has the highest inter-cluster distance and might produce high level of segregating population in regards to yield as well as earliness. Among the thirteen characters the protein content contributed maximum amount towards divergence.

Divyaramakrishnan *et al*. (2014) determined the extent of variability existing among 374 mung bean genotypes through Principal Component Analysis (PCA), cluster analysis. 4 principal components (PC) had eighteen values more than unity and accounted for 65.76% of the total variance among 12 characters. Amongst first four PCs, PC1 was accounted high proportion of total variance. 374 accessions were classified into 8 clusters through hierarchical cluster analysis method. Cluster I had maximum number of genotypes under study. Based on the cluster analysis results it was recommended that crosses could be made between the genotypes of Cluster VI and VIII, Cluster V and VIII, Cluster III and VIII and Cluster V and VIII.

Mehandi *et al.* (2015), performed multivariate analysis in green gram using twenty-one genotypes and grouped the genotypes into ten and five clusters based on the extent of genetic divergence following Tochers and non-hierarchical Euclidian clustering methods. Based on the maximum diversity obtained in Tochers method genotype KM 10-1064 of cluster V and genotypes KM 10-1046, KM 10-1059 and KM 10-1070 of cluster VI were found suitable for improving the plant structure, whereas concerning high diversity along with high trait contribution towards total divergence, the clusters KM 10-1064 of cluster V and KM 10-1042 of cluster VIII were found to be appropriate for hybridization. The genotype KM 10-1068, which represents the mono genotypic cluster in case of both the clustering methods signifies that it could be the most diverse from other genotypes and it would be the suitable candidate for hybridization with genotypes present in other clusters to tailor the agriculturally important traits and ultimately, to enhance the seed yield in green gram

2.5. Green gram and drought

Water deficit can change the growth pattern of the grain which inhibits the cell elongation and enlargement. This decreases the productivity of mungbean and disturbs normal turgor pressure. It may lose the cell turgidity. The effects of drought stress can seen by measuring root and shoot ratio which increases during stress conditions. As well as index area of leaf is decreases whereas increased cell thickness, lignifications and cutinization amounts are noticed. This grain is reported to more susceptible to drought than many other grain legumes (Pandey *et al*. 1984). Therefore, this affects the productivity of a crop, mainly during spring and summer climate.

Effect of polyethylene glycol : Osmotic solutions of different concentration are used to impose water stress reproducibly under *in vitro* conditions (Pandey and Agarwal, 1998). Polyethylene glycol molecules with a MW ≥ 6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Lu and Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita *et al*., 1979). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low molecular osmotica, which infiltrate the cell wall with solutes (Veslues *et al*, 1998)

Dutta *et al*. (2008) screened 15 mungbean genotypes for drought tolerance under laboratory condition using PEG 6000. The genotypes showed reduction in all the growth parameters under water stress except root dry weight in some of the genotypes. Screening of genotypes based on single morphological parameter appears to be limiting due to inconsistency in growth responses of different seedling parts are more affected than other parts of the same seedling in response to drought. However, tolerance index which is the ratio of dry weight of seedling under stress to dry weight of seedling under control is a more stable character and can be considered as a useful tool to screen drought tolerant genotypes.

Aslam *et al*. (2013) screened 17mungbean genotypes for drought tolerance at seedling stage to find out best selection criterion against drought conditions. Shoot length (SL), root length (RL), root shoot ratio (R/S), stem diameter (S.D), shoot weight (SW), emergence percentage (E %) and energy of emergence (EE)were studied. Line graph, biplot graph and principle component analysis were used for evaluation of seedlings performance at different moisture levels i.e. 80% (T1), 50% (T2) and 30% (T3) of the field capacity(FC).Genotypes AUM51, AUM-2002, M-2006, AUM-28, NM-58 and AUM-25 performed better under all the three studied environments.. Parameters corresponding early growth and development proved as best selection standard for low moisture stress tolerance.

Purbajanti *et al.* (2013) studied the performance of drought stress for legumes using PEG-6000 on ten kinds of legume like Calopo (*Calopogonium mucunoides*), Crotalaria (*Crotalaria juncea*),Centro (*Centrosema pubescens*), Puero (*Pueraria phaseoloides*), Leucaena (*Leucaena leucocephala*), Gliricideae (*Gliricideae*

maculate), Sesbania (*Sesbania glandifora*), green grams (*Vigna udulata*), kidney beans (*Vigna radiata*), and Soybeans(*Glycine max*). Parameter like germination percentage, root length, shoot length, shoot weight, root weight and shoot/root ratio were studied. Kidney beans have highest seedling percentage of shoot length, root length and shoot weight. Gliricideae have lowest seedling percentage of shoot length, root length, shoot weight and root weight. Kind of legumes have significance difference of shoot length affect by PEG-6000. The increasing concentration of PEG 6000 given declining percentage germination, shoot length, root length, shoot weight and root weight. Highest shoot/root ratio in legume was kidney beans and the lowest was Gliricideae. Calopo, Centro and Gliricideae were very sensitive to drought stress.

Swapna *et al*. (2014) studied the influence of water stress on germination and seedling growth of two greengram genotypes ML-267 and TM 96-2 by subjecting the cultivars to water stress using PEG-6000. Decreased osmotic potential caused a reduction in germination percentage. Shoot and root length reduced significantly with rise in PEG.

2.6 Green gram and cold

Abiotic stress like cold causes considerable losses in productivity of many crops. Low temperature is one of the most crucial signals affecting plant growth and even leading to death as low temperature induces the over-production of reactive oxygen species (ROS) like superoxide radical (O_2 •−), H_2O_2, and hydroxyl radical (HO^-) in plant cells (Hung *et al*., 2005). ROS are highly reactive to membrane lipids, protein, and DNA and are believed to be one of the major contributing factors to chilling injuries and to cause rapid cellular damage. When plants are exposed to low temperature, electron-transport chains tend to form O_2 •−, which dismutase to form H_2O_2. In the chloroplast, low temperature limits the dark reactions, thus limiting the supply of $NADP^+$ and favouring reduction of O_2 by photo-system II. Therefore, exposure to low temperature in combination with high light intensity leads to more serious damage in plants (Allen and Ort, 2001).

Chang *et al.* (2000) exposed green gram seedling to 4ºC for 2 days and found induced irreversible chilling injury. Acclimation of seedling at l0ºC for 2 to 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 28º C root/4º C-shoot treatment did not suffer noticeable injury, but seedlings in the 4º C-root/4º C shoot treatment did.

Yu Chin-Wen *et al*. (2003) studied survival rates of greengram seedlings chilled at 4ºC for 36 hour from pre-treatment with 200 mMH_2O_2 increased survival rate of seedling from 30 to 70 % and suggested 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.

Hung *et al*. (2005) said that an oxidative burst caused by biotic or abiotic stress leads to a disturbance in the cellular redox balance and is highly toxic to cells. Recently, H_2O_2, in addition to being a toxicant, has been regarded as a signaling molecule and a regulator of the expression of some genes in cells. These include genes encoding antioxidants, cell rescue/defense proteins, and signaling proteins such as kinase, phosphatase, and transcription factors.

Chen X W *et al.* (2005) subjected greengram to stress for 4 days under a low temperature of 10ºC and reported that on the 2nd and 3rd day under 10 degree C stress, both the malondialdehyde (MDA) content and the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities increased significantly in hypocotylar cells, SOD activity always maintain at the highest level in a period of time and so does POD activity. He finally concluded that at 10º C low temperature caused non-lethal, temporary injuries to hypocotyls ultrastructure in mung bean, but no visible injury at all.

Hung *et al*. (2007) studied greengram seedlings of the cultivar Tainan No. 5 (a chilling-sensitive cultivar) by pre-treating with multiple sprays of 200 mM H_2O_2 which showed a tolerance to chilling at 4ºC for 36 h, measured by electrolyte leakage, that was greater than that induced by a single treatment and similar to that induced by cold-acclimation at 10ºC for 48 h.. Chilling tolerance induced by H_2O_2 depended on accumulation of glutathione (GSH), which could be significantly reversed by pre treatment with buthionine-sulf-oximine (BSO).

Roy *et al.* (2009) postulated that low temperature in growing season may reduce germination, retards vegetative growth by inducing metabolic imbalance and or can delay/prevent productive development. Each plant species has an optimum temperature for their growth and development. It varies among genotypes within species and the specific temperature also depend on growth stage and development of particular genotype

Satya *et al*. (2011) reported that chilling stress is one of the major abiotic stresses for greengram during germination stage. During germination starch and protein reserves in greengram cotyledons are degraded, which is hampered by chilling stress. They investigated the effect of chilling stress on mobilization of cotyledon reserves, production of phenolics and activation of antioxidant enzymes and showed that tolerant genotypes were more capable of channeling the cotyledon reserves for seedling growth, resulting in higher seedling vigor. During the recovery period, the tolerant genotype exhibited higher phenolics production. The significant increase in peroxidase activity during stress induction and recovery period suggests that peroxidase activity may be used as a biochemical marker for tolerance to chilling stress during germination of mung bean.

Baisakh *et al*. (2013) evaluated 21 land races of greengram for twelve characters. Maximum variability was recorded with respect to total days of survival of 10, 30 and 40 days old seedling exposed to 10^{o}C and yield / plant. Land races Nayagarh local, Jharsuguda local, Bhawanipatna localB; Kapurgaon local, Keonjhar local, Kalahandi local-A; Ratila local, Khadabhanga local, Bhawanipatna local-B; Kalahandi local-2B and Bhawanipatna local-B were found to be good donors for cold tolerance at all the stages of seedling exposure to cold.

Swain *et al*. (2014) studied the response of 30 entries to cold exposure under controlled conditions and observed that the 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). All of them were the mutant cultivars including the most susceptible genotype OM1-3 having 9.25 days of survival. These genotypes which are found to be tolerant or susceptible to cold may be adapted to the ecological situations of Odisha. The genotypes having maximum per plant yield (3.590 g to 2.917 g) 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.

2.7. Green gram and YMV

In greengram, the major devastating viral disease caused by Mung bean Yellow Mosaic Virus (MYMV) is Yellow Mosaic Virus (YMV) transmitted through white fly, *Bemicia tabaci* which leads to severe yield reduction and it necessitates developing MYMV resistant lines for improved crop yield. MYMIV belonging to the

genus begomovirus causes the YMV in a number of economically important edible grain legumes including greengram, blackgram and soybean. Some varieties may posseses genes for resistance to YMV. This resistance genotype may be screened by molecular analysis and later this resistance genotype can be included in hybridization programme for transfer of resistance gene to the high yielding genotype but sensitive to YMV.

Conventional breeding methods have been employed in the past to solve some of the problems at a slow pace. The use of molecular marker technology can help accelerating greengram improvement process through the marker assisted selection (MAS) technique. Among the markers micro satellites offers several advantages, viz. they are highly reproducible, highly polymorphic, PCR based and readily portable within a species. The breeding and selecting process for developing a new high yielding variety with resistance to disease is an arduous process that may take up 8 – l0 years. Multi environment testing will always be required to confirm that the identified phenotype have desired agronomic characters combined with resistance. Hence, tools for determining the genotypes of the experimental lines increase the efficiency of the selection process.

Using suitable DNA markers for selection will help in identifying the right genotype that are resistant. Development of markers to identify YMV resistance in greengram and deploying them through marker-aided selection in breeding programme would fasten the process of developing resistant lines.

Simple sequence repeats (SSRs) consisting of randomly repeated units of short nucleotide motifs that are of 1-6 bp long, di-tri and tetra nucleotide [e.g, (CA) n, (AAT) n and (GATA) n respectively] repeats are the most common and are widely distributed throughout the genome of plants and animals (James and Lagoda, 1996).

Basak *et al.* (2004) developed YMV-tolerant lines from a single YMV-tolerant plant within a large population of the susceptible cultivar T-9, by crossing with T-9, and F_1, F_2 and F_3. The different generations were phenotyped for YMV-reaction by forced inoculation using viruliferous white flies. Of 24 pairs of resistance gene analog RGA primers screened, only one pair, RGA 1F-CG/RGA 1R, was found to be polymorphic among the parents. Selected F_2 individuals and F_3 families were genotyped with the polymorphic RGA primer pair and the polymorphism was found

to be linked with YMV-reaction. This primer pair amplified a 445bp DNA fragment only from homozygous tolerant and the heterozygous lines. The 445bp marker band was sequenced and named 'VMYR1'.

Souframanien *et al*. (2006) developed recombinant inbred line (RIL) mapping population (F_8) by crossing *Vigna mungo* (cv. TU 94-2) with *Vigna mungo* var. *silvestris* and screened for mungbean yellow mosaic virus (MYMV) resistance. The $ISSR811_{1357}$ marker was sequenced and sequence characterized amplified region (SCAR) primers were designed (YMV1-F and YMV1-R) to amplify the marker. Screening for the SCAR marker in the RIL population distinguished the MYMV resistant and susceptible plants, agreeing well with the phenotypic data. The $ISSR811_{1357}$ marker was validated using diverse blackgram genotypes differing in their MYMV reaction. The marker will be useful for the development of MYMV-resistant genotypes in blackgram.

Maiti *et al*. (2011) took up study to develop molecular markers linked to MYMIV-resistance to facilitate genotyping of urd bean and mung bean germplasms for MYMIV-reaction. Two MYMIV-resistance marker loci, YR4 and CYR1, were identified and of these two CYR1 is completely linked with MYMIV-resistant germplasms and co-segregating with MYMIV-resistant F_2, F_3 progenies of urdbean. This study demonstrated that these two markers could be efficiently employed together in a multiplex- PCR-reaction for genotyping both *V. mungo* and *V. radiate* germplasms from field grown plants and also directly from the seed stock.

Prasanthi *et al*. (2013) screened 45 lines along with PU-31 and PU-19 resistant checks for YMV under field conditions with artificial inoculation and identified 19 lines having 1 score with no disease symptoms. PCR reactions using SCAR marker for screening the disease reaction with genomic DNA of these lines resulted in identification of 19 resistant sources with specific amplification for resistance to YMV at 532bp with SCAR 20F/20R developed from OPQ1 RARD primer linked to YMV disease. Considering the YMV reaction and resistance linked SCAR marker, it is possible to identify the new resistance sources in a short time and they can be utilized in breeding programme or for direct release.

Sowmini *et al*. (2014) found four markers which were closely associated with YMD resistance *viz*., VMYR1, YR4, CYR1 and SCARISSR 811 were validated using

14 genotypes of black gram. Disease screening was performed at two locations viz., Coimbatore and Vamban and disease reaction of the genotypes was observed to be similar in both the locations. The marker VMYR1 was monomorphic and not linked with YMD resistance genes. The other three markers viz., YR4, CYR1 and SCARISSR 811 behaved as a dominant marker that produced respective allele in the susceptible genotypes and absent in resistant genotypes with few deviation. Thus, these three markers were found to be partially linked with YMD resistance genes and it could be used in the MAS only for the genotypes whose marker data coincides with the disease reaction. The present study suggested that identification makers associated with YMD resistance genes and specific to south Indian viral species (MYMV) is highly important to develop YMD resistant varieties in greengram.

Singh *et al*. (2014) Inheritance of MYMIV tolerance was determined in two sets of recombinant inbred lines (RILs) of mungbean. The two sets comprising 143 and 79 RILs each derived from the cross between PM5 x Sub2 and B1 X Sub2, respectively were considered for the study. Sub2 was a Sublobata derived lines whereas, B1 was a popular small seeded cultivar of west Bengal, susceptible against MYMIV. It was observed that one or two major genes with a few modifiers played a significant role in resistance mechanism against MYMIV in the lower Gangetic alluvial zone. Resistance alleles for Sub2 and PM5 are probably allelic as observed from frequency distribution pattern. A set of 177 SSRs were employed for identification of polymorphism between parents. Only 37 SSRs showed polymorphism between Sublobata and B1 or PM5. But only eight SSRs were polymorphic between two high yielding cultivars. Markers linked with MYMIV tolerance like RGA, SCAR and others from earlier studies were also considered and found that only one RGA derived marker showed polymorphism between resistance and susceptible parents.

Narasimhan *et al*. (2016) found that revelation of only 2 markers being associated genetically with MYMV in mungbean, but not in urdbean identified in this study would be helpful in marker assisted breeding. Putative markers identified in this study viz., RGH-1TG and MtB99 could be further validated using mapping populations. The study also indicated that there is high potential for the transfer of RGHs from closely related species, circumventing the laborious cloning and screening procedures involved in characterizing RGHs for mungbean and urdbean.

MATERIALS AND METHODS

The present study on "Studies on yield and stress tolerance in greengram" was undertaken to evaluate the performance of some greengram genotypes for yield and stress tolerance (MYMV, low temperature and drought). The field experiment was conducted at the EB-II Section in the department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar during Rabi season of 2014-15 and 2015-16 and laboratory work was taken up in S.K. Sinha Molecular Breeding Laboratory of the department. The first year materials consisted of 56 genotypes (Table-1) of green gram [*Vigna radiata* (L.) Wilczek] including selections from local varieties (7), selections from crosses (15), selections from mutants (7) and selections from breeding lines (27).

3.1 Field experiment

The field experiment was conducted in a randomized block design (RBD) in 3 replications with 56 entries in first year of experiment. The trail was sown on 20.10.2014 and irrigated on the same day. Each genotype was represented by five rows with a spacing of 30cm X 10 cm. Fertilizers were applied @ 20:40:20 kg of $N:P_2O_5:K_2O$ with 300 cft. of farm yard manure (FYM) 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. The crop was harvested during January, 2015.

The materials for second year study comprised of eleven genotypes (Table-2) which were selected from 56 genotypes. Out of eleven genotypes three genotypes from seven local land races and eight genotypes from rest 49 genotypes were selected.

3.2 Sampling and recording of observations

Observations on ten quantitative traits were recorded. Out of the 10 quantitative traits, days to 50% flowering and maturity were recorded on the plot basis and for the rest of eight characters, the observations were recorded on ten randomly selected competitive plants per plot in each replication and average was calculated. The characters observed were as follows:

Table 1: List of 56 greengram genotypes with their code and source

Sl. No	Code	Name of Genotype	Source
1	OPBGG-2016-1	Makarjholalocal	Local variety of odisha
2	OPBGG-2016-2	Kalahandi	Local variety of odisha
3	OPBGG-2016-3	Kendrapada	Local variety of odisha
4	OPBGG-2016-4	Ambagaon	Local variety of odisha
5	OPBGG-2016-5	Bhawanipatna	Local variety of odisha
6	OPBGG-2016-6	Keonjhar A	Local variety of odisha
7	OPBGG-2016-7	Jharsuguda	Local variety of odisha
8	OPBGG-2016-8	TARM-1 x LGG-460	Crossed material from OUAT
9	OPBGG-2016-9	OBGG-52 X Pant-M-4	Crossed material from OUAT
10	OPBGG-2016-10	Sujata x TARM-1	Crossed material from OUAT
11	OPBGG-2016-11	Sujata x LGG-460	Crossed material from OUAT
12	OPBGG-2016-12	OBGG-52 X Kendrapada Local	Crossed material from OUAT
13	OPBGG-2016-13	TARM-1 X OUM-11-5	Crossed material from OUAT
14	OPBGG-2016-14	TARM-1X OUM-11-5	Crossed material from OUAT
15	OPBGG-2016-15	OUM-11-5 x Kendrapada-A	Crossed material from OUAT
16	OPBGG-2016-16	LGG 460 X OUM-11-5	Crossed material from OUAT
17	OPBGG-2016-17	OGG-12	Crossed material from OUAT
18	OPBGG-2016-18	OGG-12	Crossed material from OUAT
19	OPBGG-2016-19	OBGG- 177	Crossed material from OUAT
20	OPBGG-2016-20	Sujata x TARM-1	Crossed material from OUAT
21	OPBGG-2016-21	OUM-11-5	Crossed material from OUAT
22	OPBGG-2016-22	V2-22	Mutant of OPBGG-52 (NG 0.01%)
23	OPBGG-2016-23	V2-18	Mutant of OPBGG-52 (EMS 0.6%)
24	OPBGG-2016-24	V1-9	Mutant of Sujata (Gamma- rays 600 kr)
25	OPBGG-2016-25	V1-2	Mutant of Sujata (Gamma- rays 200 kr)
26	OPBGG-2016-26	V2 -11	Mutant of OPBGG-52 (EMS 0.2%)
27	OPBGG-2016-27	V1-19	Mutant of Sujata (NG 0.005%)
28	OPBGG-2016-28	V2-20	Mutant of OPBGG-52 (NG 0.005%)
29	OPBGG-2016-29	OUM-62	Crossed material from OUAT
30	OPBGG-2016-30	T-32-2-3	BARC Trombay
31	OPBGG-2016-31	T-43-1-3	BARC Trombay
32	OPBGG-2016-32	IPM-02-14	IIPR, Kanpur
33	OPBGG-2016-33	EC-693358	AVRDC, Hyderabad
34	OPBGG-2016-34	IPM-02-03	IIPR, Kanpur
35	OPBGG-2016-35	IPM-99-125	IIPR, Kanpur
36	OPBGG-2016-36	ML-1666	Ludiana
37	OPBGG-2016-37	PAU-911	Ludiana
38	OPBGG-2016-38	VC-6173	AVRDC, Hyderabad
39	OPBGG-2016-39	VC-6368	AVRDC, Hyderabad
40	OPBGG-2016-40	KPS-1	AVRDC, Hyderabad
41	OPBGG-2016-41	IPM-02-17	IIPR, Kanpur
42	OPBGG-2016-42	KPS-2	AVRDC, Hyderabad
43	OPBGG-2016-43	EC-693369	AVRDC, Hyderabad
44	OPBGG-2016-44	ML-818	Ludiana
45	OPBGG-2016-45	NM-94	Ludiana
46	OPBGG-2016-46	ML-1299	Ludiana
47	OPBGG-2016-47	EC-693368	AVRDC, Hyderabad
48	OPBGG-2016-48	PDM-139	GBP, Pantnagar
49	OPBGG-2016-49	NM-92	AVRDC, Hyderabad
50	OPBGG-2016-50	EC-693363	AVRDC, Hyderabad
51	OPBGG-2016-51	EC-693367	AVRDC, Hyderabad
52	OPBGG-2016-52	VC- 693370	AVRDC, Hyderabad
53	OPBGG-2016-53	VC-6372	AVRDC, Hyderabad
54	OPBGG-2016-54	EC-693356	AVRDC, Hyderabad
55	OPBGG-2016-55	EC-693376	AVRDC, Hyderabad
56	OPBGG-2016-56	LGG-460	LAM, ANGRAU

3.2.1 Days to 50% flowering- Number of days from sowing to the day on which about 50% of the plants in the plot started blooming.

3.2.2 Days to maturity - Number of days from the date of sowing to the day when about 75% of the plants in the plot bearded mature pod.

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

3.2.4 Primary branches per plant- Number of Primary branches in the 10 randomly selected plants per plot in each replication were recorded.

3.2.5 Clusters per plant- Number of pod bearing bunches or cluster in the 10 randomly selected plants per plot in each replication were recorded.

3.2.6 Pods per plant- Number of seed bearing pods were recorded at maturity of the randomly chosen plants in each replication.

3.2.7 Pod length-Ten random pods of the sample plants were observed for their length and the length measurement was expressed in centimeter.

3.2.8 Seeds per pod- Number of seeds per pod in each of the randomly selected plants were calculated by dividing total number of seeds from the plant by total number of pods of the plant.

3.2.9 100-seed weight (g)- Weight of 100 seed drawn from random sun dried samples from each genotype in each replication was recorded in gram.

3.2.10 Yield per plant (g) - Weight of all the seeds obtained from the 10 sample plants was recorded and averaged.

Table 2: List of 11 genotypes with their respective code

Sl. No	Name of Genotype
1	OPBGG-2016-1
2	OPBGG-2016-5
3	OPBGG-2016-7
4	OPBGG-2016-9
5	OPBGG-2016-11
6	OPBGG-2016-32
7	OPBGG-2016-34
8	OPBGG-2016-35
9	OPBGG-2016-40
10	OPBGG-2016-48
11	OPBGG-2016-49

3.3 Statistical analysis

The replication wise mean values of individual characters of different treatments were subjected to statistical analysis (Gomez and Gomez, 1983). Observations of all the characters related to seed yield were analyzed for variability and other genetic parameters like character association, path analysis and genetic divergence studies.

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 among replications and varieties for any character was done by 'F' test. The test of significance of difference among 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

Table 3: 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)	Expectations of mean sum of squares (EMS)	F- values
Replication	(r-1)	$(1/g_j) \sum y_j^2$-CF	MS_r	$\sigma^2 e+g\sigma^2 r$	MS_r/MS_e
Genotype	(g-1)	$(1/r_j) \sum y_i^2$-CF	MS_g	$\sigma^2 e+r\sigma^2 g$	MS_g/MS_e
Error	(r-1) (g-1)	By subtraction	MS_e	$\sigma^2 e$	
Total	(rg-1)	$\sum^2 y_{ij}$-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 = $\frac{MS_g - MS_e}{r}$

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 :(σ_e^2) = MS_e

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

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

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 and 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 suggested by Hanson *et al*. (1956).

Heritability in broad sense was calculated as follows:

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

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

Where,

σ^2_g = genotypic variance

σ^2_p= phenotypic variance

3.3.6 Estimation of genetic advance

The expected genetic advance (GA) or gain from selection among varieties for different characters was calculated using the following Johnson *et al* (1953):

$$GA = K \times h \times \sigma_g (= K \times h^2 \times \sigma_p)$$

Where,

K = Standardized selection deferential which is 2.06 at 5% selection intensity.

h = Square root of heritability coefficient in broad sense

h^2 = heritability coefficient in broad sense

σ_g = Genotypic standard deviation

σ_p = phenotypic standard deviation

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

3.3.7 Correlation studies

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

3.3.7.1 Estimation of phenotypic and genotypic correlation

Utilizing the various components of variance, the genotypic and phenotypic correlations were computed according to Al-Jibouri *et al*. (1958) as per the following formula.

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

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

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.

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

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

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

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

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

Where,

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

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

3.3.8 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 9 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, l92l; Dewey and Lu, l959).

$$P_{1.10} + r_{1.2}\, p_{2.10} + r_{1.3}\, p_{3.10} + \ldots\ldots\ldots\ldots + r_{1.10}\, p_{9.10} = r_{1.10}$$

$$r_{2.1}\, p_{1.10} + p_{2.10} + r_{2.3}\, p_{3.10} + \ldots\ldots\ldots\ldots + r_{2.10}\, p_{9.10} = r_{2.10}$$

……

$$r_{10.1}\, P_{1.10} + r_{9.2}\, P_{2.10} + r_{9.3}\, P_{3.10} + \ldots\ldots + P_{9.10} = r_{9.10}$$

Where,

r_{ij} is the correlation co-efficient between i^{th} and j^{th} character and $p_{i,\ 10}$ is the path co efficient (direct effect) of i^{th} character on yield (10^{th} 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 LHS.

The residual effect (P_R) was calculated as follows:

$$1 = P^2_{RPY} + P_{IPY}.\, r_{IPY}$$

3.3.9. Genetic divergence

Genetic divergence with regard to ten characters was estimated by Mahalanobis' D^2-statistic following Rao (1952). D^2 (Genetic divergence) between any 2 genotypes was estimated using the formula:

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

Where,

W_{ij} is the inverse of the common dispersion matrix W_{ij} and d_i and d_j are the difference in the means of the two populations for i^{th} and j^{th} 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:

$$D_p^2 = \sum_{i=1}^{p} d_i^2 .$$

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 56 genotypes 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.

3.3.9 Grouping of genotypes into different clusters

3.3.9.1. Tocher's method

Usually a cluster is defined as a group of genotypes such that any two genotypes 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 2^{nd}, 3^{rd} and other clusters are formed till all the genotypes are included in one or the other cluster.

3.4.0 Drought analysis

The experiment on drought tolerance was laid out in CRD (completely randomized design) with two factors, comprising eleven greengram genotypes using four concentrations of PEG (6000 MW), with (0% control, 2.5%, 5.0%, 7.5%) in Hoagland Solution. 15 seeds of each greengram genotype were placed on Petri dish lined with moistened filter paper to provide appropriate moisture stress during seed germination. Following drought stress, observations on shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were recorded at 10 days after treatment. Data were analyzed for ANOVA with genotypes and PEG concentration as first and second factor respectively to assess the varietal sensitivity to different water stress levels.

3.4.1 Formulation of Hoagland Solution:

The Hoagland solution is a hydroponic nutrient solution that was developed by Hoagland and Arnon in 1938. It is one of the most popular solution compositions for growing plants. The solution described by Hoagland and Arnon in 1950 has been modified several times, mainly to add iron chelates, the original concentrations for each element are shown below in table-4**.**

Table 4: Formulation of Hoagland Solution

Component	**Stock solution**	**mL stock solution/ 1L**
macronutrients		
$Ca(NO_3).4H_20$	1M	3ml
$Mg(SO_4)2.6H_2O$	1M	2ml
KNO_3	1M	2ml
KH_2PO_4	1M	2ml
Micronutrients		
$H3BO_3$	2.5g	1ml
$MnCl_2$	1.5g	
$ZnCl_2$	0.1g	
$CuCl_2$	0.05g	
$HMoO_3$	0.05g	
Iron source		
$FeCl_2$	5.0g	1ml
Tartaric acid	5.0g	

3.4.2 Observations recorded

Shoot length (cm): Shoot lengths were measured for randomly selected ten normal seedlings and then mean length was calculated.

Root length (cm): Root lengths were also measured for same ten normal randomly selected seedlings and then mean length was calculated.

Shoot Fresh weight (g): Fresh shoot weight of above mentioned randomly selected ten normal seedlings was recorded on precision balance in grams.

Root Fresh weight (g): Fresh root weight of above mentioned randomly selected ten normal seedlings was recorded on precision balance in grams.

Shoot dry weight (g): The selected seedlings were oven dried and the shoot dry weights were recorded on precision balance in grams.

Root dry weight (g): The selected seedlings were oven dried and the root dry weights were recorded on precision balance in grams.

Tolerance index (TI): It is the ratio of dry weight of seedlings in stress (g) to the dry weight of seedlings in control (g).

3.5 Cold analysis

Response to cold exposure was studied under controlled condition in the S.K. Sinha Molecular breeding laboratory where the genotypes were exposed to 15^0C temperature for 10 days, 20 days stage of growth to study their viability. In field, three sets of 11 genotypes were sown in plastic glass with three replications. Ten days after sowing the first set was taken into plant growth chamber (Fig.1 and 2). Observations of number of genotypes that wilted everyday and days to survival under 15^0C plant growth chamber was recorded. After recording the observations in incubated condition, the genotypes were exposed to field condition and their survival in field condition was recorded every day. Then total survival day (plant growth chamber and field) was calculated for screening of genotype tolerance to cold. Second set was taken into plant growth chamber 20 days after sowing respectively and observations were taken in the similar way to first set and then field exposure was done. In field condition, all the three set of treatments were compared with control and observations were taken every day for survival.

FIG 1. Plant growth chamber for cold tolerance study

FIG 2. Exposure of seedlings to cold stress in plant growth chamber

3.6. Molecular analysis

3.6.1 Plant materials

The eleven greengram genotypes selected from 56 genotypes were taken for molecular analysis. Seedlings were grown in pots and fresh and young leaf samples were collected from 5- 20 days old seedlings for the isolation of genomic DNA.

3.6.2 Isolation of genomic DNA

Genomic DNA was isolated from tender young leaves of 15 days old which were harvested freshly before sunrise and washed thoroughly with cold autoclaved, distilled water and then blotted to dry. About two grams of young leaves were excised from the upper tip portion and DNA was extracted on the same day of collection. Total genomic DNA from the leaves was isolated by using standard CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, l990). Two grams of fresh leaves of each variety were taken and ground to fine powder with liquid nitrogen in a mortar and pestle. The solution was then transferred to a sterile 50 ml centrifuge tube, containing preheated l0 ml of DEB (DNA extraction buffer-l00 mM Tris-HCL pH 8.3, 20 mM EDTA (pH 8.0), 2 % CTAB, 5 M NaCl, 0.2 % mercaptoethanol) which was preheated at 65ºC in water bath. Each sample was mixed thoroughly and was incubated in water bath at 65ºC for 1 hour by occasional gentle shaking. After cooling, equal volume of 24:l chloroform: isoamyl alcohol (v/v) was added and mixed gently. The centrifugation was carried out at l0,000 rpm for 20 min at 25ºC. The upper supernatant was carefully transferred into another centrifuge tube. Then same volume of pre-chilled isopropanol was added to precipitate the DNA. The DNA was precipitated into another micro-centrifuge tube and washed with 70 % ethanol twice and kept for air-drying at room temperature. The air dried crude DNA was then dissolved in sufficient amount of $T_{10}E_1$ (Tris l0 mM-1 mM EDTA, pH 8.0) buffer. After DNA isolation, the quality of DNA was checked by gel electrophoresis in 0.8% gel. In the well, 4µl of bromophenol blue and 4µl of DNA sample were loaded and run at 70v for 20-25 min. After that, gel was checked in Gel documentation System. The band which appeared near the well was identified as DNA and the band which appeared distant to the well was identified as RNA and these corresponding sample required purification.

3.6.3 Purification of DNA

The dissolved DNA is the crude DNA and requires further purification. The RNA was removed by giving RNase treatment. Quantity of RNase was added to DNA depending on the quantity of RNA present in the sample. Quantity of RNase treated varies from 3 µl - 30 µl. and it is known after gel run. The solution was incubated in water bath at 37ºC for 1 hour. After 1 hour it was removed from the water bath and equal volume of phenol about 500 µl was added and mixed gently for about 10 min. The solution was then centrifuged in l0,000 rpm for 10 minutes with a medium speed centrifuge and upper aqueous phase was pipetted out to another centrifuge tube. After that equal volume of chloroform-isoamylalcohol (24:1) was added to sample and shaken for 10 min as phenol treatment and centrifugation was also done for 10 min at 10,000 rpm. For further purification, the DNA solution was washed with phenol:chloroform: isoamylalcohol (25:24:l). The upper aqueous phase was separated after centrifugation as per the procedure described earlier and mixed with l/10th volume of 3M sodium acetate (pH 4.8). DNA was precipitated by adding equal volume of chilled isopropanol and pelleted by spinning at 10, 000 rpm for 10 min. The pellet was washed twice with 70 % ethanol carefully and dried under air. The dried DNA was dissolved in minimum amount of $T_{10}E_1$ buffer (pH 8.0).

3.6.4 Test for quantity of the purified DNA:

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

3.6.5 PCR (Polymerase chain reaction) analysis

For PCR 2 selected primers (MERCK SPECIALITIES PRIVATE LIMITED) like SCAR (sequence characterized amplified region) and RGA (Resistance gene analog) were used for PCR amplification. Each amplification reaction mixture of 25 µl contain 2µl primer (each forward and reverse),0.5µl dNTP, 0.5µl of 3 unit Taq DNA polymerase, 2.5µl Taq buffer, 17.5µl PCR water and 2µl of DNA sample. Amplification condition were 1 cycle at 94ºC for 5 min., 45 cycles at 94ºC for 1 min, 60^0C for 1 min., and 72^0C for 2 min. followed by 1 cycle of 12 min. at 72^0C. Amplification products were electrophoresed on 1.5% agarose gel.

Table 5 : List of primers used in PCR

Primers	Base sequences	% GC
RGA22F2	GGGTGGTTTGGGTAAGACCAC	57.1
RGA24R2	TTCGCGGTGTGTGAAAAGTCT	47.6
YMV1-F	GAGAGAGAGAGAGAGACAAAG	47.6
YMV1-R	GAGAGAGAGAGAGAGACAGGA	52.4

3.6.6 Agarose Gel Electrophoresis

The amplicons were separated in l.5% agarose gel. Three grams of agarose was added to 200ml 1XTBE buffer boiled for complete melting of agarose, then cooled to 50ºC. Ethidium bromide (EtBr) (2µl/50ml of gel solution) was added to the gel and casted on the gel-casting tray. 33 well comb was used for formation of quality gels. After complete gelling, the gel was transferred to the submarine gel tank containing lX TBE buffer. Prior to loading the samples the comb was removed. In the submerged gel 25µl of the PCR samples were loaded in each well along with a single well loaded with standard DNA ladder (l00 bp-3kb DNA ladder). The electrophoresis was performed in a constant voltage at 80v for 3 hours. The run was stopped when bromophenol blue dye has travelled 2/3 length of the gel. The gels were placed on the Gel doc system (UVITECH, Cambridge, UK) and was photographed under U.V. light and was used for scoring the bands. The sizes of the amplicons were determined by comparing them with that of the ladder.

RESULTS

During the course of research on the project entitled “Studies on yield and stress tolerance in greengram”, observation on seed yield per plant and its component characters were recorded. The mean values of the traits studied were statistically analyzed to study the variability, heritability and genetic gain for selection and their association with yield as well as among themselves. The direct and indirect effect of these traits on seed yield, the nature and extent of genetic divergence among 56 genotypes were also studied. The salient findings as revealed from the investigation are given below:

4. A.1 Analysis of variance

The variance (mean square values) between genotypes for 10 characters such as days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, clusters per plant, pods per plant, pod length, seeds per pod, 100 seed weight and yield per plant are presented in the Table 6.The data revealed the existence of significant difference among the genotypes for the characters studied. All the characters are significant at 1% level.

4. A.2 Mean performance and co-efficient of variation

The mean performance, standard error of variance (S.E.) and critical difference values are presented in Table 7.

4. A.2.1 Days to 50% flowering

A significant variability ranging from 31.33 days to 38.6 days was noticed with respect to days to 50% flowering. Among the 56 genotypes, OPBGG-2016-13 and OPBGG-2016-21 are the early flowering genotypes (31.33) whereas OPBGG-2016-4 and OPBGG-2016-19 was found to be late flowering genotypes (38.66).

4. A.2.2 Days to maturity

Among the 56 genotypes, genotypes OPBGG-2016-13, OPBGG-2016-26, OPBGG-2016-43 and OPBGG-46 were found to be the earliest maturing genotype (61.00 days) and the highest late maturity (70.00 days) was observed in the genotype OPBGG-2016-11.

4. A.2.3 Plant height (cm)

A significant moderate variability ranging from 30.66 cm to 53.00 cm was noticed with respect to plant height. The plant height was maximum for genotype OPBGG-2016-10 and the minimum height was observed in OPBGG-2016-43.

4. A.2.4 Primary branches per plant

OPBGG-2016-39 and OPBGG-2016-45 had the lowest number of primary branches per plant (0.00) and the highest no. of primary branches (2.73) was recorded in OPBGG-2016-14.

4. A.2.5 Clusters per plant

A medium range of variation was observed in case of number of clusters per plant among the genotypes. The highest value (6.66) was recorded in genotype OPBGG-2016-47. The genotype which showed the lowest clusters per plant (2.00) was OPBGG-2016-55.

4. A.2.6 Pods per plant

Wide range of variability was found in case of pods per plant. The highest value (19.66) was found in case of OPBGG-2016-11. The lowest value (9.00) for pods per plant was recorded for OPBGG-2016-19.

4. A.2.7 Pod length (cm)

The highest value (10.00) for pod length was recorded in case of genotype OPBGG-2016- 54 while the lowest value (5.00) was recorded in genotypes OPBGG-2016-7, OPBGG-2016-8 and OPBGG-2016-11.

4. A.2.8 Seeds per pod

A moderate range of variation was observed in case of seeds per pod. Maximum value (12.66) was recorded in genotype OPBGG-2016- 56. However lowest value (9.00) for seeds per pod was found in case of genotype OPBGG-2016-8.

4. A.2.9 100 Seed weight (gm.)

A wide variability ranging from 2.13 to 4.68 was recorded for this trait in the 56 genotypes. OPBGG-2016-43 exhibited the maximum 100 seed weight (4.68) whereas the lowest value (2.13) was recorded for OPBGG-2016-52.

4. A.2.10 Yield per plant (gm.)

A significant amount of variability was recorded for this trait. Maximum value (6.16) for this trait was found in OPBGG-2016-34. Minimum value (1.82) for yield per plant was recorded in OPBGG-2016-19.

Table 6: Analysis of variance for ten characters in greengram

Sl. No.	Character	Source	d.f	S.S	M.S	F value
1	Days to 50% flowering	Replication	2	5.57	2.78	10.08**
		Genotype	55	565.95	10.29	37.20**
		Error	110	30.42	0.27	
2	Days to maturity	Replication	2	1.17	0.58	2.24
		Genotype	55	582.25	10.58	40.40**
		Error	110	28.82	0.26	
3	Plant height	Replication	2	36.99	18.49	1.94
		Genotype	55	4773.32	86.78	9.10**
		Error	110	1048.33	9.53	
4	Clusters Per plant	Replication	2	1.79	0.89	2.27
		Genotype	55	120.51	2.19	5.53**
		Error	110	43.53	0.39	
5	Primary branches Per Plant	Replication	2	0.11	0.05	0.53
		Genotype	55	76.21	1.38	13.08**
		Error	110	11.64	0.10	
6	Pods per plant	Replication	2	0.14	0.07	0.04
		Genotype	55	1095.31	19.91	13.10**
		Error	110	167.18	1.51	
7	Pod length	Replication	2	0.51	0.25	0.72
		Genotype	55	181.51	3.30	9.35**
		Error	110	38.82	0.35	
8	Seeds per pod	Replication	2	0.58	0.29	0.64
		Genotype	55	107.61	1.95	4.35**
		Error	110	49.41	0.44	
9	100 seed weight	Replication	2	0.00	0.00	0.06
		Genotype	55	59.53	1.08	42.95**
		Error	110	2.77	0.02	
10	Yield per plant	Replication	2	0.06	0.03	0.12
		Genotype	55	130.54	2.37	9.74**
		Error	110	26.79	0.24	

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

Table 7: Mean performance of the 56 green gram genotypes for ten characters

Sl. No	Genotypes	Days to 50% flowering	Days to maturity	Plant height	Clusters/ plant	Primary branches/ plant	Pods/ plant	Pod length	Seeds / pod	100seed weight	Yield /plant
1	OBPGG-2016-1	34.66	64.66	42.00	4.00	1.00	15.33	6.00	10.00	3.29	3.56
2	OPBGG-2016-2	35.66	66.00	35.66	3.00	1.60	11.33	6.00	10.66	2.81	2.55
3	OPBGG-2016-3	36.67	65.66	41.00	4.00	1.33	11.00	5.66	10.66	2.86	3.21
4	OPBGG-2016-4	38.66	68.66	31.66	3.00	0.93	10.00	6.00	10.33	2.41	2.63
5	OPBGG-2016-5	34.66	64.66	40.00	4.00	1.60	13.00	6.00	10.66	2.84	3.65
6	OPBGG-2016-6	35.66	65.66	38.66	4.66	1.73	12.66	6.00	11.66	2.58	2.88
7	OPBGG-2016-7	35.33	66.33	31.00	3.66	1.00	14.00	5.00	10.66	3.58	3.94
8	OPBGG-2016-8	35.00	63.66	41.00	4.00	2.00	13.33	5.00	9.00	3.69	3.43
9	OPBGG-2016-9	36.00	62.66	40.66	4.66	2.00	16.00	6.00	10.00	3.19	5.03
10	OPBGG-2016-10	35.33	64.00	53.00	5.33	1.46	15.66	6.66	10.66	3.46	4.36
11	OPBGG-2016-11	38.00	70.00	47.00	6.00	1.00	19.66	5.00	9.66	3.71	5.21
12	OPBGG-2016-12	33.66	62.00	47.00	3.33	1.00	12.00	6.00	11.66	3.71	3.68
13	OPBGG-2016-13	31.33	61.00	44.00	4.66	1.00	15.00	6.66	11.00	3.83	4.67
14	OPBGG-2016-14	38.00	65.00	44.33	4.66	2.73	15.66	7.00	12.00	2.41	3.76
15	OPBGG-2016-15	33.66	62.33	40.66	5.66	2.53	16.00	6.00	10.00	3.23	3.79
16	OPBGG-2016-16	37.00	65.00	38.66	5.00	2.00	14.00	6.66	10.66	3.75	4.17
17	OPBGG-2016-17	36.66	65.00	47.00	4.33	1.00	12.33	6.00	10.66	3.34	3.10
18	OPBGG-2016-18	35.66	65.00	48.00	3.66	1.00	14.00	7.00	11.66	3.04	3.56
19	OPBGG-2016-19	38.66	65.00	34.00	3.00	1.73	9.00	6.00	10.00	2.53	1.82
20	OPBGG-2016-20	32.33	61.33	45.66	5.00	1.00	17.33	6.00	11.00	3.33	4.56
21	OPBGG-2016-21	31.33	61.33	38.00	4.66	0.86	14.33	6.00	10.66	3.32	3.94
22	OPBGG-2016-22	35.00	65.00	47.00	5.66	1.00	15.66	6.00	9.66	3.02	4.13
23	OPBGG-2016-23	34.00	62.33	45.00	5.00	1.00	14.33	6.00	11.66	3.10	3.86
24	OPBGG-2016-24	35.66	65.00	44.66	6.00	2.00	18.66	5.66	10.00	3.39	4.48
25	OPBGG-2016-25	36.66	64.00	38.00	4.66	2.00	17.66	5.33	10.33	2.99	4.37
26	OPBGG-2016-26	32.66	61.00	39.00	4.66	1.00	15.00	6.00	11.00	3.22	3.96
27	OPBGG-2016-27	33.66	62.66	49.00	4.33	1.73	15.00	6.00	11.33	3.16	4.32
28	OPBGG-2016-28	37.66	64.66	51.66	5.00	2.00	13.66	5.66	10.66	3.16	3.40
29	OPBGG-2016-29	34.66	62.33	49.00	3.66	2.66	14.00	7.00	11.66	2.94	3.42
30	OPBGG-2016-30	35.33	63.00	44.00	4.66	2.00	17.00	6.00	11.00	3.17	4.33

Sl. No	Genotypes	Days to 50% flowering	Days to maturity	Plant height	Clusters/ plant	Primary branches/ plant	Pods/ plant	Pod length	Seeds / pod	100seed weight	Yield /plant
31	OPBGG-2016-31	33.66	62.00	39.00	5.00	2.00	18.00	6.66	11.00	2.84	4.43
32	OPBGG-2016-32	33.66	62.33	39.00	5.00	1.00	17.33	7.00	10.33	4.22	5.65
33	OPBGG-2016-33	33.00	62.33	42.00	3.66	1.66	9.66	9.00	11.66	4.42	4.19
34	OPBGG-2016-34	34.00	65.00	36.66	5.00	1.06	18.66	6.66	11.00	4.00	6.16
35	OPBGG-2016-35	35.66	62.33	38.66	4.00	0.13	15.33	6.66	12.00	3.65	5.07
36	OPBGG-2016-36	36.00	64.66	41.66	4.66	2.00	16.66	6.00	10.00	3.36	4.34
37	OPBGG-2016-37	34.00	64.00	38.00	4.66	1.60	14.33	7.00	12.33	3.12	3.96
38	OPBGG-2016-38	35.66	63.33	48.00	4.66	0.06	13.66	7.66	12.00	3.71	4.35
39	OPBGG-2016-39	32.66	62.00	36.00	3.66	0.00	14.00	7.00	11.66	3.64	4.34
40	OPBGG-2016-40	33.66	62.00	45.00	4.66	0.86	16.66	7.00	12.00	3.38	5.06
41	OPBGG-2016-41	35.00	63.66	49.66	3.66	1.60	11.66	7.00	12.00	4.67	4.70
42	OPBGG-2016-42	32.66	62.00	43.66	4.00	0.06	12.00	8.66	12.00	3.66	3.58
43	OPBGG-2016-43	32.00	61.00	30.66	4.00	0.06	12.66	9.33	11.00	4.68	4.35
44	OPBGG-2016-44	34.00	62.66	49.00	4.66	1.60	13.33	7.66	11.33	4.28	4.74
45	OPBGG-2016-45	32.66	62.33	35.66	3.66	0.00	10.00	7.66	10.66	4.46	3.65
46	OPBGG-2016-46	32.00	61.00	42.00	4.66	0.66	15.33	6.66	11.00	3.36	4.58
47	OPBGG-2016-47	35.00	64.00	46.00	6.66	2.53	12.00	7.00	11.33	3.64	3.83
48	OPBGG-2016-48	31.66	61.33	39.66	5.00	1.00	19.00	6.66	11.66	3.16	5.00
49	OPBGG-2016-49	31.66	61.33	42.66	4.66	2.06	16.67	8.00	12.33	3.24	5.15
50	OPBGG-2016-50	35.66	64.33	38.66	3.00	1.00	11.00	6.00	10.66	2.25	1.94
51	OPBGG-2016-51	35.66	64.00	45.00	5.33	1.60	16.33	6.66	12.33	2.32	3.43
52	OPBGG-2016-52	36.00	63.66	35.66	3.66	1.00	12.66	6.66	12.00	2.13	2.55
53	OPBGG-2016-53	36.66	66.33	45.66	4.00	1.46	10.66	9.00	11.66	3.08	2.80
54	OPBGG-2016-54	33.66	63.33	34.00	4.00	1.60	17.00	10.0	11.00	2.50	3.63
55	OPBGG-2016-55	35.66	65.00	32.00	2.00	2.00	13.66	6.66	10.33	2.32	2.62
56	OPBGG-2016-56	35.66	63.33	38.66	3.66	1.00	11.00	7.00	12.66	2.81	2.61
	MEAN	34.76	63.64	41.58	4.38	1.35	14.32	6.63	11.04	3.29	3.94
	SE	0.429	0.418	2.521	0.514	0.266	1.007	0.485	0.547	0.130	0.403
	CD	0.851	0.828	4.995	1.018	0.527	1.995	0.961	1.085	0.257	0.799
	PV	3.614	3.703	35.283	0.994	0.533	7.652	1.335	0.952	0.378	0.954
	GV	3.338	3.441	25.752	0.598	0.427	6.132	0.982	0.502	0.352	0.710

4. A.3 Genetic Variability

4. A.3.1 Co-efficient of variance (C.V.)

The co-efficient of variation with respect to different characters are presented in Table 8 which ranged from 0.65 to 19.28. The highest variation (19.28) was recorded for primary branches per plant followed by clusters per plant (11.6). The lowest variation was recorded in days to maturity (0.65) followed by days to 50% flowering (1.23). Therefore, basing on the C.V. value, the characters can be grouped into three classes such as

i. Low variability (C.V = 5% or less)

ii. Moderate variability (C.V. = 5-10%)

iii. High variability (C.V. =>10%)

The traits like primary branches per plant, clusters per plant and pods per plant showed high variability. On the contrary, the traits like plant height, pod length, seeds per pod, 100 seed weight and yield per plant showed moderate variability. The traits like days to 50% flowering, days to maturity and exhibited low variability.

4. A.3.2 Estimation of genetic parameters

The estimates of genetic parameters such as genotypic variance and phenotypic variance are presented in table 7 and their respective co-efficient of variation, broad sense heritability and their genetic gain for selection are presented in table 8.

The genotypic variance ranged from 2.91 for days to maturity to 48.38 for primary branches per plant. The phenotypic variance ranged from 3.02 for days to maturity to 54.05 for primary branches per plant. In general, all the traits exhibited parallel values between those two variances showing lower value in the former than later.

The perusal of data in table 8 revealed that the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits studied. The PCV was highest (54.05) for primary branches per plant while the traits like days to maturity (3.02) and days to 50% flowering (5.46) exhibited the

lowest value of PCV. However, the traits like pods per plant (19.31) and 100 seed weight (18.68) exhibited medium value.

More or less similar trend was observed in the estimates of GCV for all the traits with primary branches per plant having the highest value (48.38) followed by test weight (18.04) while the days to maturity exhibited the lowest value (2.91) followed by days to 50% flowering(5.25). However moderate values of GCV were obtained for traits like clusters (17.63), pods per plant (17.29) and plant height (12.20).

Table 8: Genetic parameters of 10 characters in 56 greengram genotypes

Character	Mean	Range	CV (%)	GCV (%)	PCV (%)	h^2 (%)	GA	GA (% of mean)
Days to 50% flowering	34.76	31.33-38.66	1.23	5.25	5.46	92.00	3.61	10.40
Days to maturity	63.64	61.00-70.00	0.65	2.91	3.02	92.00	3.67	5.78
Plant height(cm)	41.58	30.66-53.00	6.06	12.20	14.28	72.00	8.92	21.47
Cluster per plant	4.38	2.00-6.66	11.60	17.63	22.72	60.00	1.23	28.18
Primary branches/plan t	1.35	0.00-2.73	19.20	48.38	54.05	80.00	1.20	89.21
Pods per plant	14.32	9.00-19.66	11.20	17.29	19.31	80.00	4.56	31.88
Pod length	6.63	5.00-10.00	7.31	14.93	17.41	73.00	1.74	26.38
seeds per pod	11.04	9.00-12.66	5.10	6.41	8.83	52.00	1.05	9.60
100 seed weight	3.29	2.13-4.68	3.90	18.04	18.68	93.00	1.18	35.91
Yield per plant	3.94	1.82-6.16	10.20	21.37	24.77	74.00	1.49	37.99

4. A.3.3 Heritability

Heritability denotes the proportion of phenotypic variance that is due to genotype, i.e., heritable. Heritability (broad sense) estimates as presented in table 8 ranged from the lowest for seeds per pod (52.00%) to highest for 100 seed weight (93.00) followed by flowering and maturity (92.00%), pod per plant (80.00%) and branches (80.00%), yield per plant (74.00%), plant height (72.00), clusters (60.00).

4. A.3.4 Genetic advance

The genetic advance varied from lowest (1.05) for seeds per pod followed by 100 seed weight (1.18), primary branches (1.20), clusters per plant (1.23), yield per plant (1.49), pod length (1.74), days to 50 % flowering (3.61) followed by days to maturity(3.67), pods per plant (4.56), to highest (8.92) for plant height..

4. A.4 Character association

Estimates of Phenotypic and Genotypic correlation co-efficient of all pairs of 10 characters related to yield are presented in table 9.

4. A.4.1 Phenotypic correlation

The phenotypic correlation (r_p) among the 10 traits in the 56 genotypes ranged from the lowest (-0.397) between days to 50% flowering and 100 seed weight to the highest (0.772) between days to 50% flowering and maturity

Yield per plant was positively and significantly associated with traits like pods per plant (0.699), 100 seed weight (0.577), clusters/ plant (0.490), at 1% level of significance. Plant height (0.206), seeds per pod (0.121), pod length (0.079) showed positive correlation with yield. But yield was negatively correlated with branches (-0.052), maturity (-0.300), and days to 50 % flowering (-0.380).

Days to 50% flowering was positively and significantly correlated with days to maturity (0.772), at 1% level of significance, primary branches (0.292) at 5% level of significance. It was positively correlated with plant height (0.023). But it was negatively correlated with 100 seed weight (-0.397), yield per plant (-0.380), pod length (-0.38), seed per pod (-0.199), pods/ plant (-0.180) and cluster/ plant (-0.080).

Days to maturity was positively correlated with primary branches per plant (0.176). It was negatively correlated with clusters per plant (-0.047), plant height (-0.061), pods per plant (-0.116), seeds per pod (-0.288), yield per plant (-0.300), 100 seed weight (-0.303) and pod length (-0.305).

Plant height was positively and significantly correlated with clusters/ plant (0.373) at 1% level of significance and positively correlated with yield per plant (0.206), seeds per pod (0.200), 100 seed weight (0.161), primary branches per plant (0.158), and pods per plant (0.139). It was negatively correlated with pod length (-0.014).

Cluster per plant was positively and significantly correlated with pods per plant (0.571) and yield per plant (0.490) at 1% level of significance. It was

positively correlated with primary branches (0.180) and 100 seed weight (0.177). It was negatively correlated with seeds per pod (-0.084) and pod length (-0.134).

Primary branches per plant was positively correlated with pods per plant (0.166) and negatively correlated with yield per plant (-0.052), seeds per pod (-0.123), pod length (-0.128) and 100 seed weight (-0.294).

Pods per plant were positively and significantly correlated with yield per plant (0.699) at 1% level of significance. It was positively correlated with 100 seed weight (0.010) and negatively correlated with seeds per pod (-0.093) and pod length (-0.158).

Pod length was positively and significantly correlated with seeds per pod (0.432) at 1% level of significance and positively with 100 seed weight (0.235) and yield per plant (0.079).

Seeds per pod were positively correlated with yield per plant (0.121) and negatively correlated with 100 seed weight (-0.017). 100 seed weight was positively and significantly correlated with yield per plant (0.577) at 1% level of significance.

4. A.4.2 Genotypic correlation

The genotypic correlation (r_g) among the 10 traits in the 56 genotypes ranged from the lowest (-0.467) between days to 50% flowering and 100 seed weight to the highest (0.838) between days to 50% flowering and days to maturity.

Yield per plant was positively and significantly correlated with pods per plant (0.706), 100 seed weight (0.639) and clusters per plant (0.613) at 1% level of significance and positively correlated with plant height (0.248), pod length (0.068) It was negatively correlated with seeds per pod (-0.038), primary branches per plant (-0.152) days to maturity (-0.367), and days to 50% flowering (-0.467)

Days to 50% flowering was positively and significantly correlated with days to maturity (0.838) at 1% level of significance and branches (0.356) at 5% level of significance and positively correlated with plant height (0.064), It was negatively correlated with clusters per plant (-0.128), pods per plant (-0.221) seeds per pod (-0.282), pod length (-0.382), 100 seed weight (-0.433) and yield per plant (-0.467).

Days to 50% maturity was positively correlated with primary branches per plant (0.204), and negatively correlated with plant height (-0.059), clusters per plant (-0.086), pods per plant (-0.147), 100 seed weight (-0.318**)** pod length (-0.361), yield per plant (-0.367) and seeds per pod (-0.386).

Plant height was positively and significantly correlated with clusters per plant (0.509) at 1% level of significance. It was positively correlated with yield per plant (0.248) and primary branches (0.232), 100 seed weight (0.191), pods per plant (0.172), and seeds per pod (0.141). It was negatively correlated with pod length (-0.096).

Number of clusters per plant was positively and significantly correlated with pods per plant (0.656), and yield per plant (0.613) at 1% level of significance. It was positively correlated with primary branches (0.249), 100 seed weight (0.221) and negatively correlated with seeds per pod (-0.103) and pod length (-0.154).

Primary branches per plant was positively correlated with pods per plant (0.148) and negatively correlated with yield per plant (-0.152), seeds per pod (-0.218), pod length (-0.248) and 100 seed weight (-0.346).

Pods per plant was positively and significantly correlated with yield per plant (0.706) at 1 % level of significance and positively correlated with 100 seed weight (0.004). It was negatively correlated with seeds per pod (-0.189) and pod length (-0.223).

Pod length was positively and significantly correlated with seeds per pod (0.562) at 1% level of significance and 100 seed weight (0.271) at 5% level of significance and positively correlated with yield per plant (0.068). Seeds per pod were negatively correlated with seed weight (-0.030) and yield per plant (-0.038). Seed weight was positively and significantly correlated with yield per plant (0.639) at 1% level of significance.

Table 9: Phenotypic correlation (r_p) and genotypic correlation (r_g) among the 10 characters in 56 greengram genotypes

Character		Days to maturity	Plant height	Clusters/ plant	Primary branches/ Plant	Pods/plant	Pod length	Seeds / pod	100 seed weight	Yield/ plant
Days to 50% flowering	rp	0.772**	0.023	-0.080	0.292*	-0.180	-0.318	-0.199	-0.397	-0.380
	rg	0.838**	0.064	-0.128	0.356*	-0.221	-0.382	-0.282	-0.433	-0.467
Days to maturity	rp		-0.061	-0.047	0.176	-0.116	-0.305	-0.288	-0.303	-0.300
	rg		-0.059	-0.086	0.204	-0.147	-0.361	-0.386	-0.318	-0.367
Plant height(cm)	rp			0.373**	0.158	0.139	-0.014	0.200	0.161	0.206
	rg			0.509**	0.232	0.172	-0.096	0.141	0.191	0.248
Clusters/ plant	rp				0.180	0.571**	-0.133	-0.085	0.177	0.490**
	rg				0.249	0.656**	-0.154	-0.103	0.221	0.613**
Primarybranches/ Plant	rp					0.166	-0.128	-0.123	-0.294	-0.052
	rg					0.148	-0.248	-0.218	-0.346	-0.152
Pods/plant	rp						-0.158	-0.093	0.010	0.699**
	rg						-0.223	-0.189	0.004	0.706**
Pod length	rp							0.432**	0.235	0.079
	rg							0.562**	0.271*	0.068
Seeds/ pod	rp								-0.017	0.121
	rg								-0.030	-0.038
100 seed weight	rp									0.577**
	rg									0.639**

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

4. A.5 Path co-efficient analysis

The correlation of seed yield with other characters was further analysed to assess the cause and effect relationship between the component traits and yield by path co-efficient analysis. Path co-efficient analysis was carried out for 10 quantitative characters in greengram. The correlation of seed yield per plant with other characters was partitioned into components of direct and indirect effects that would reflect on the nature of these associations and the relative importance of the components in determining the seed yield. Indirect effect of each character indicates their role in affecting correlation of other characters with yield.

The phenotypic correlation co-efficient of seed yield with the 9 component traits were partitioned into direct and indirect effects of component traits on yield by path co-efficient analysis The phenotypic correlation co-efficient analysis and the results are presented in table 10.

The present path co-efficient analysis showed low residual effect of 0.28518 indicating that most of the major yield components were included in this study.

Pods per plant had the highest direct positive effect (0.708) on yield .The characters 100 seed weight (0.643) and seeds per pod (0.124) had the moderate positive direct effect on yield. Days to flowering (0.075), Clusters per plant (0.047), showed negligible direct effect on seed yield. Pod length (-0.019), primary branches (-0.024), plant height (-0.043) and days to maturity (-0.073), showed the negative direct effect on seed yield.

Highest positive indirect effect was contributed by pods per plant (0.464) and 100 seed weight (0.142) via clusters per plant followed by pod length (0.174) via test weight and100 seed weight (0.122) and pods per plant (0.121) via plant height respectively on seed yield.

Negative indirect effect was contributed by days to 50% flowering on seed yield via plant height (-0.002), and clusters per plant (-0.006) followed by clusters per plant via 50% flowering (-0.009) and primary branches (-0.006). Also the negative indirect effect of seeds per pod on seed yield per plant was counteracted by clusters per plant (-0.004), plant height (-0.006).

Table 10: Direct (diagonal and bold) and indirect effects of 9 component traits on seed yield in 56 greengram genotypes

Character	Days to 50% flowering	Days to maturity	Plant height (cm)	Clusters/ plant	Primary branches /plant	pods/plant	Pod length	Seeds/ pod	100 seed weight
Days to 50% flowering	**0.075**	-0.061	-0.002	-0.006	-0.008	-0.156	0.007	-0.035	-0.278
Days to maturity	0.062	**-0.073**	0.002	-0.004	-0.005	-0.103	0.007	-0.048	-0.204
Plant height(cm)	0.004	0.004	**-0.043**	0.024	-0.005	0.121	0.001	0.017	0.122
Clusters/plant	-0.009	0.006	-0.022	**0.047**	-0.006	0.464	0.003	-0.012	0.142
Primary branches/plant	0.026	-0.014	-0.010	0.011	**-0.024**	0.104	0.004	-0.027	-0.222
Pods /plant	-0.016	0.010	-0.007	0.030	-0.003	**0.708**	0.004	- 0.023	0.002
Pod length	-0.028	0.026	0.004	-0.007	0.006	-0.158	**-0.019**	0.070	0.174
Seeds / pod	-0.021	0.028	-0.006	-0.004	0.005	-0.133	-0.010	**0.124**	-0.019
100 seed weight	-0.032	0.023	-0.008	0.010	0.008	0.002	-0.005	-0.003	**0.643**

Residual effect = 0.28518

4. A.6 Clustering pattern

56 genotypes were grouped into 7 different genetic clusters on the basis of genetic affinity or diversity by Tocher's method. Cluster IV consists of 24 genotypes while Cluster II consist of 11 genotypes, Cluster VI with 6 genotypes, Cluster I and VII consist of 5 genotypes each, cluster III consist of 3 genotypes and Cluster V consists of 2 genotypes. The names of the genotypes are presented in Table 11.

Table 11: Clustering of 56 greengram genotypes using Tocher's method

Cluster	No. of genotypes	Name of Genotype
I	5	OPBGG-2016-1, OPBGG-2016-2, OPBGG-2016-3, OPBGG-2016-21 OPBGG-2016-46.
II	11	OPBGG-2016-4, OPBGG-2016-5, OPBGG-2016-6, OPBGG-2016-7 OPBGG-2016-8, OPBGG-2016-9, OPBGG-2016-10, OPBGG-2016-11, OPBGG-2016-12, OPBGG-2016-24, OPBGG-2016-36.
III	3	OPBGG-2016-13, OPBGG-2016-20, OPBGG-2016-26.
IV	24	OPBGG-2016-14, OPBGG-2016-15, OPBGG-2016-16, OPBGG-2016-17, OPBGG-2016-18, OPBGG-2016- 19, OPBGG-2016-22, OPBGG-2016-23, OPBGG-2016-25, OPBGG-2016-27, OPBGG-2016-28, OPBGG-2016-29, OPBGG-2016-30, OPBGG-2016-31, OPBGG-2016-32, OPBGG-2016-33, OPBGG-2016-34, OPBGG-2016-35, OPBGG-2016-37, OPBGG-2016-38, OPBGG-2016-39, OPBGG-2016-40, OPBGG-2016-41, OPBGG-2016-42.
V	2	OPBGG-2016-50 OPBGG-2016-52
VI	6	OPBGG-2016-43, OPBGG-2016- 44, OPBGG-2016-45, OPBGG-2016-47, OPBGG-2016-48, OPBGG-2016-49.
VII	5	OPBGG-2016-51, OPBGG-2016-53, OPBGG-2016- 54, OPBGG-2016-55, OPBGG-2016-56.

4. A.6.1 Intra and inter cluster distances.

From the average intra and inter cluster distance presented in table 14 and their corresponding D^2 values is presented in table13, It was evident that Cluster V had the minimum intra-cluster distance (D =3.79) whereas maximum intra-cluster distance (D=12.18) was observed in Cluster VI.

The average inter-cluster distance revealed that the most divergent clusters were Cluster V and VI (D =15.77), followed by cluster II and VI (D=15.30) and cluster VI and VII (D =15.04).

4. A.6.2 Relative contribution of characters to divergence.

The relative contributions of 10 quantitative characters to genetic divergence among the 56 genotype of greengram were assessed by rank average of individual character. The character contributing to maximum divergence needs greater emphasis for deciding on the cluster for the purpose of selection in hybridization programme. The number of times, each of the component character appeared first in rank and its respective percent of contribution towards genetic divergence was analyzed.

Among the yield contributing characters, the maximum contribution towards divergence was made by 100 seed weight (25.8%), followed by yield per plant (25.6%), Rest of the characters exhibiting divergence in order were days to maturity (17.5%), 50% flowering (10.7), pods per plant (7.0%), primary branches (5.0%), plant height (2.4%), pod length (2.4%), clusters (1.7%) and seeds per pod (1.6%).

Table 12: Relative contribution of 10 characters to genetic divergence

Character	No. of first rank	% Contribution
Days to 50% flowering	166	10.7792
Days to maturity	270	17.5325
Plant height(cm)	37	2.4026
Clusters/ plant	27	1.7532
Primary branches/ Plant	77	5.0000
Pods/plant	108	7.0130
Pod length	37	2.4026
Seeds/ pod	25	1.6234
100 seed weight	398	25.8442
Yield per plant	395	25.6494
Total	1540	100

4. A.6.3 Characteristic features of 10 quantitative characters of 7 green gram clusters

Maximum value for days to 50% flowering was observed in cluster-V (35.83) and minimum value in cluster-III (32.11) as presented in table14. Maximum value for days to maturity was observed in cluster-II (65.21) while minimum value was observed in cluster-III (61.11). Maximum value of plant height was observed in cluster-IV (43.00) and lowest value in cluster-V (37.16). Highest value of primary

branches per plant was observed in cluster-VII (1.53) and lowest value in cluster-III and cluster-V (1.00). For clusters per plant highest value was observed in cluster-III and cluster-VI (4.77) and lowest value in cluster-V (3.33). Cluster-III showed highest value for pods per plant (15.77) among all other clusters and lowest value was observed in cluster-V (11.83) for pods per plant. For pod length highest value was observed in cluster-VII (7.86) and lowest value was observed in cluster-II (5.75). In case of seeds per pod highest value was observed in cluster-VII (11.60) and lowest value was observed in cluster-II (10.39). Cluster -VI showed highest value for 100 seed weight (3.91) among all other cluster and lowest value (2.19) was observed in cluster-V. For character yield per plant highest value was observed in case of cluster-VI (4.45) and lowest value was observed in cluster-V (2.24).

Table 13: Average intra and inter cluster D^2 values for 7 clusters in 56 greengram genotypes

Cluster	I	II	III	IV	V	VI	VII
I	133.937	135.044	116.532	116.384	112.274	166.715	126.946
II		113.662	205.685	125.218	124.924	234.232	132.856
III			19.142	129.162	187.209	95.373	189.748
IV				111.565	125.514	166.177	127.967
V					14.361	248.734	57.329
VI						148.342	226.408
VII							91.954

Table 14: Average intra and inter cluster distance values for 7 clusters in 56 greengram genotypes

Cluster	I	II	III	IV	V	VI	VII
I	11.573	11.621	10.795	10.788	10.596	12.912	11.267
II		10.661	14.342	11.190	11.177	15.305	11.526
III			4.375	11.365	13.682	9.766	13.775
IV				10.562	11.203	12.891	11.312
V					3.790	15.771	7.572
VI						12.180	15.047
VII							9.589

Table 15: Cluster wise mean values of 10 quantitative characters in 56 genotypes of green gram.

Character / Cluster	Days to 50% flowering	Days to maturity	Plant height	Clusters/ plant	Primary branches/ plant	Pods/ plant	Pod length	Seeds/ pod	100 seed weight	Yield/ plant
I	34.067	63.733	39.733	4.067	1.093	13.467	6.067	10.600	3.132	3.571
II	35.818	65.212	41.485	4.485	1.521	14.697	5.758	10.394	3.271	3.971
III	32.111	61.111	42.889	4.778	1.000	15.778	6.222	11.000	3.466	4.403
IV	35.014	63.431	43.000	4.472	1.394	14.569	6.708	11.222	3.385	4.151
V	35.833	64.000	37.167	3.333	1.000	11.833	6.333	11.333	2.191	2.247
VI	32.833	62.111	40.611	4.778	1.211	13.944	7.722	11.389	3.917	4.459
VII	35.467	64.400	39.067	3.800	1.533	13.733	7.867	11.600	2.611	3.022

Top 11 genotypes were selected from 56 genotypes based on their yield performance and further investigation (response to drought, cold and molecular analysis) was carried out during the year 2015-16. These 11 genotypes were also the mixture of selection from local genotype (3), selections from crosses (2) and breeding lines (6).

4.B. Response to Drought

4. B.1 Analysis of variance

The variance (mean square values) among 11 selected genotypes for 6 characters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight is presented in table 16. The data revealed the existence of significant difference among the genotypes for the characters under study. The characters are significant at 1 % level.

Table16: ANOVA for seedling characters under artificial drought stress induced by PEG.

Source	d.f	M.S.S of Characters					
		Shoot length	Root length	Fresh shoot weight	Fresh Root weight	Dry shoot weight	Dry Root weight
A	10	224.45**	40.62**	25039.04**	126.35**	612.69**	13.77**
B	3	583.38**	89.41**	104994.30**	780.47**	1322.80**	20.89**
A X B	30	28.97**	4.50**	9547.30**	100.59**	310.13**	0.94
Error	88	1.43	1.26	672.70	68.64	132.83	1.96

A-Genotypes * Significant at 5 % level, ** Significant at 1% level
B- PEG concentration

4. B.2 Mean performance of genotypes for different seedling characters

The mean performance of genotypes for various seedling characters under drought stress induced by PEG and under control conditions were presented in tables 17-22 and fig: 3.

4. B.2.1 Shoot length (cm)

A significant variability ranging from 4.32 to 18.55cm (table-17) was noticed under overall effect of PEG. The shoot length was minimum for OPBGG-2016-1 (4.32)

and maximum for OPBGG-2016-35 (18.55). At 2.5% conc, shoot length was minimum (6.2) for OPBGG-2016-1and maximum (19.00) for OPBGG-2016-40. At 5.0% conc, a minimum value (2.66) was recorded in OPBGG-2016-7 and a maximum (18.20) in OPBGG-2016-35. At 7.5% conc, OPBGG-2016-7 showed minimum (1.26) and maximum (17.93) by OPBGG-2016-48. Under control conditions OPBGG-2016-7 showed the lowest (12.20) and OPBGG-2016-32 showed highest shoot length (20.4).

4. B.2.2 Root length (cm)

Among 11 genotypes, OPBGG-2016-48 (7.88) showed the maximum root length, (table-18) while the minimum was seen in OPBGG-2016-7 (1.34) when overall effect of PEG was considered. At 2.5% conc, OPBGG-2016-9 showed a minimum root length (2.60) and the maximum (7.90) by OPBGG-2016-48. At 5.0% conc, OPBGG-2016-7 showed minimum root length (0.66) and OPBGG-2016-48 showed a maximum value (7.73). At 7.5% conc, OPBGG-2016-9 showed minimum value (0.43) and OPBGG-2016-48 showed a maximum value (8.03). Under control conditions OPBGG-2016-11 (9.83) showed highest root length while the lowest (3.06) being OPBGG-2016-7.

4. B.2.3 Fresh shoot weight (gm)

A wide range of variability ranging from 16.48gm to 161.70gm was noticed with respect to fresh shoot weight (table-19). The shoot fresh weight was maximum for genotype OPBGG-2016-7 (161.70) and the minimum was observed in OPBGG-2016-11 (16.48gm). At 2.5% conc, OPBGG-2016-1 showed a minimum shoot weight (71.26) and the maximum (365.93) by OPBGG-2016-7. At 5.0% conc, OPBGG-2016-11 showed minimum shoot weight (3.16) and OPBGG-2016-35 showed a maximum value (122.4). At 7.5% conc, OPBGG-2016-11 showed minimum value (1.60) and OPBGG-2016-35 showed a maximum value (132.13). Under control conditions OPBGG-2016-7 (392.3) showed the highest weight and OPBGG-2016-9 (116.6) showed lowest weight.

4. B.2.4 Fresh root weight (gm)

A medium range of variation was observed in case of fresh root weight (table-20). The highest value was recorded in case of OPBGG-2016-5 (13.96) and the lowest in OPBGG-2016-11 (3.84gm) under overall effect of PEG. At 2.5% conc, OPBGG-2016-11

showed a minimum root weight (5.44) and the maximum (17.33) by OPBGG-2016-5. At 5.0% conc, OPBGG-2016-34 showed minimum root weight (1.24) and OPBGG-2016-5 showed a maximum value (11.60). At 7.5% conc, OPBGG-2016-49 showed minimum value (0.18) and OPBGG-2016-5 showed a maximum value (12.96). OPBGG-2016-7showed the lowest weight (9.66) and the highest being OPBGG-2016-34 (44.76) under control conditions.

4. B.2.5 Dry shoot weight (gm)

The highest value (21.55) was found in case of OPBGG-2016-7 while the lowest value was found for OPBGG-2016-11 (2.54) under overall PEG effect (table-21). At 2.5% conc, OPBGG-2016-11 showed a minimum shoot weight (6.89) and the maximum (48.76) by OPBGG-2016-7. At 5.0% conc, OPBGG-2016-11 showed minimum shoot weight (0.48) and OPBGG-2016-35 showed a maximum value (16.28). At 7.5% conc, OPBGG-2016-32 showed minimum value (0.22) and OPBGG-2016-35 showed a maximum value (16.06). Under controlled conditions OPBGG-2016-7 (52.30) showed maximium shoot dry weight and OPBGG-2016-5 (13.80) showed minimum shoot dry weight.

4. B.2.6 Root dry weight (gm)

The highest value for root dry weight was recorded in case of OPBGG-2016-5 (4.63gm) while the lowest value was recorded in OPBGG-2016-34(0.80 gm) under overall effect of PEG (table-22). At 2.5% conc, OPBGG-2016-7 showed a minimum root dry weight (1.36) and the maximum (5.75) by OPBGG-2016-5. At 5.0% conc, OPBGG-2016-34 showed minimum root dry weight (0.23) and OPBGG-2016-5 showed a maximum value (3.86). At 7.5% conc, OPBGG-2016-34 showed minimum value (0.13) and OPBGG-2016-5 showed a maximum value (4.29). Under control conditions OPBGG-2016-5 (5.53gm) showed the maximum root dry weight and OPBGG-2016-7 (1.53) showed the minimum value.

Table17: Mean performance of genotypes over different concentrations of PEG for shoot length (cm.).

Genotypes	control	Concentration of P.E.G			Overall effect of PEG
		2.5%	5.0%	7.5%	
OPBGG-2016-1	14.40	6.20	4.30	2.46	4.32
OPBGG-2016-5	17.86	14.80	16.73	16.70	16.07
OPBGG-2016-7	12.20	11.90	2.66	1.26	5.27
OPBGG-2016-9	13.60	12.00	4.20	1.30	5.83
OPBGG-2016-11	18.40	13.80	4.56	2.43	6.93
OPBGG-2016-32	20.46	14.93	12.73	6.13	11.26
OPBGG-2016-34	20.20	16.80	15.56	9.83	14.06
OPBGG-2016-35	19.03	18.53	18.20	18.93	18.55
OPBGG-2016-40	19.83	19.00	10.23	5.66	11.63
OPBGG-2016-48	18.10	17.83	17.76	17.93	17.84
OPBGG-2016-49	18.03	15.90	10.66	5.10	10.53

Table18: Mean performance of genotypes over different concentrations of PEG for root length (cm.).

Genotypes	**control**	**Concentration of P.E.G**			**Overall effect of PEG**
		2.5%	**5.0%**	**7.5%**	
OPBGG-2016-1	8.00	3.40	2.13	0.73	2.08
OPBGG-2016-5	5.86	5.23	4.93	4.73	4.93
OPBGG-2016-7	3.06	2.83	0.66	0.53	1.34
OPBGG-2016-9	3.56	2.60	1.96	0.43	1.66
OPBGG-2016-11	9.83	4.60	3.36	1.70	3.22
OPBGG-2016-32	4.90	3.46	2.36	1.16	2.33
OPBGG-2016-34	7.50	6.86	5.30	2.60	4.92
OPBGG-2016-35	5.80	5.10	4.60	5.60	5.10
OPBGG-2016-40	8.73	6.66	5.06	3.40	5.04
OPBGG-2016-48	8.40	7.90	7.73	8.03	7.88
OPBGG-2016-49	6.13	5.06	2.26	0.90	2.74

Table 19: Mean performance of genotypes over different concentrations of PEG for shoot fresh weight (gm.).

Genotypes	**control**	**Concentration of P.E.G**			**Overall effect of PEG**
		2.5%	**5.0%**	**7.5%**	
OPBGG-2016-1	207.96	71.26	53.50	3.13	42.63
OPBGG-2016-5	130.00	93.66	112.67	98.67	101.60
OPBGG-2016-7	392.33	365.93	63.33	56.00	161.70
OPBGG-2016-9	116.63	75.16	6.00	1.86	27.60
OPBGG-2016-11	154.33	44.70	3.16	1.60	16.48
OPBGG-2016-32	222.00	145.03	106.33	2.43	84.57
OPBGG-2016-34	143.92	109.24	95.86	74.20	93.06
OPBGG-2016-35	131.98	124.36	122.43	132.13	126.30
OPBGG-2016-40	195.53	185.06	101.96	54.43	113.81
OPBGG-2016-48	125.48	122.87	121.92	121.67	122.15
OPBGG-2016-49	160.43	140.72	95.10	44.25	93.35

Table 20: Mean performance of genotypes over different concentrations of PEG for root fresh weight (gm).

Genotypes	Control	Concentration of P.E.G			Overall effect of PEG
		2.5%	5.0%	7.5%	
OPBGG-2016-1	16.03	13.10	3.36	1.36	5.94
OPBGG-2016-5	16.66	17.33	11.60	12.96	13.96
OPBGG-2016-7	9.66	8.63	4.53	3.60	5.58
OPBGG-2016-9	10.73	8.83	5.46	0.58	4.92
OPBGG-2016-11	11.72	5.44	4.03	2.10	3.84
OPBGG-2016-32	11.23	8.50	6.46	3.03	5.99
OPBGG-2016-34	44.79	13.10	1.24	0.63	5.01
OPBGG-2016-35	12.97	11.44	10.64	12.29	11.46
OPBGG-2016-40	14.51	10.64	8.60	5.71	8.31
OPBGG-2016-48	10.64	10.00	9.80	10.18	9.99
OPBGG-2016-49	12.95	10.69	4.33	0.18	5.07

Table 21: Mean performance of genotypes over different concentrations of PEG for shoot dry weight (gm).

Genotypes	Control	Concentration of P.E.G			Overall effect of PEG
		2.5%	5.0%	7.5%	
OPBGG-2016-1	22.21	7.56	5.72	0.33	4.53
OPBGG-2016-5	13.80	9.56	11.49	12.33	11.12
OPBGG-2016-7	52.30	48.76	8.44	7.45	21.55
OPBGG-2016-9	15.93	10.32	0.81	0.28	3.80
OPBGG-2016-11	23.70	6.89	0.48	0.24	2.54
OPBGG-2016-32	21.16	13.54	9.86	0.22	7.87
OPBGG-2016-34	15.46	11.74	10.28	7.97	9.99
OPBGG-2016-35	16.04	15.16	16.28	16.06	15.83
OPBGG-2016-40	22.69	21.48	11.85	6.36	13.23
OPBGG-2016-48	14.75	14.45	14.34	14.26	14.35
OPBGG-2016-49	19.53	17.14	11.59	5.33	11.35

Table 22: Mean performance of genotypes over different concentrations of PEG for root dry weight (gm.).

Genotypes	control	Concentration of P.E.G			Overall effect of PEG
		2.5%	5.0%	7.5%	
OPBGG-2016-1	3.56	2.93	0.74	0.30	1.31
OPBGG-2016-5	5.53	5.75	3.86	4.29	4.63
OPBGG-2016-7	1.53	1.36	0.71	0.57	0.86
OPBGG-2016-9	2.81	2.58	1.41	0.14	1.34
OPBGG-2016-11	3.09	1.47	2.03	1.24	1.58
OPBGG-2016-32	2.49	1.88	1.43	0.67	1.31
OPBGG-2016-34	2.97	2.04	0.23	0.13	0.80
OPBGG-2016-35	2.69	2.37	2.34	2.33	2.34
OPBGG-2016-40	3.37	2.46	2.01	1.32	1.91
OPBGG-2016-48	3.22	3.03	2.96	3.08	2.96
OPBGG-2016-49	2.51	2.03	0.85	0.43	1.92

Table 23 – Tolerance index of root dry weight and Over-all mean values of genotypes for different seedling characters under drought stress induced by PEG.

Genotypes	Shoot length	Root length	Fresh shoot weight	Fresh Root weight	Dry shoot weight	Dry Root weight	Tolerance index of root dry weight
OPBGG-2016-1	4.32	2.088	42.63	5.94	4.53	1.31	0.36
OPBGG-2016-5	16.07	4.933	101.6	13.96	11.12	4.63	0.84
OPBGG-2016-7	5.27	1.343	161.7	5.58	21.55	0.86	0.56
OPBGG-2016-9	5.83	1.664	27.6	4.92	3.80	1.34	0.47
OPBGG-2016-11	6.93	3.222	16.48	3.84	2.54	1.58	0.54
OPBGG-2016-32	11.26	2.333	84.57	5.99	7.87	1.31	0.51
OPBGG-2016-34	14.06	4.92	93.06	5.01	9.99	0.80	0.26
OPBGG-2016-35	18.55	5.10	126.30	11.46	15.83	2.34	0.86
OPBGG-2016-40	11.63	5.04	113.81	8.31	13.23	1.91	0.57
OPBGG-2016-48	17.84	7.88	122.15	9.99	14.35	2.96	0.92
OPBGG-2016-49	10.53	2.74	93.35	5.07	11.35	1.92	0.76

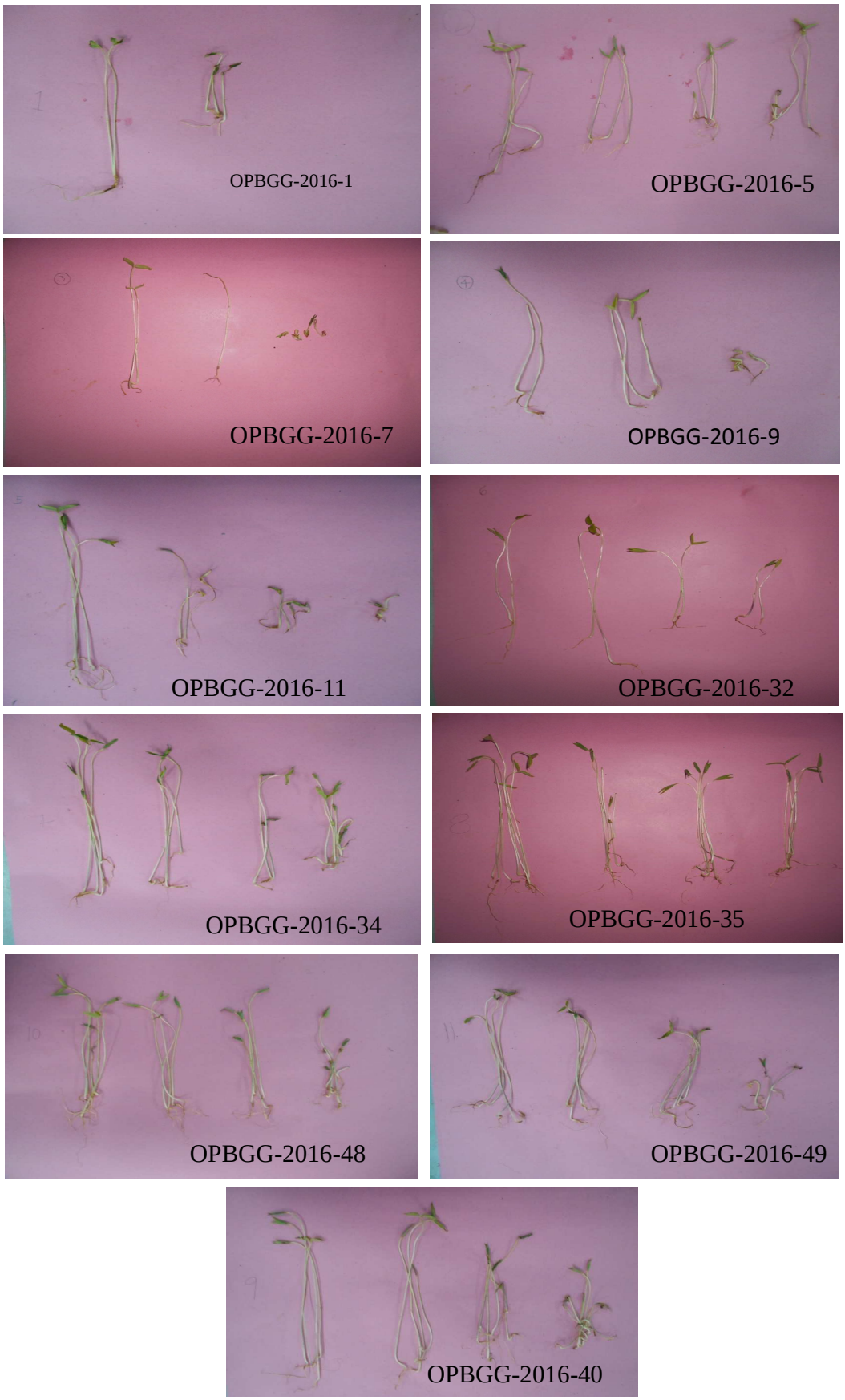
Table 24: Mean values of genotypes for different seedling characters under normal condition.

Genotypes	Shoot length	Root length	Fresh shoot weight	Fresh Root weight	Dry shoot weight	Dry Root weight
OPBGG-2016-1	14.40	8.00	207.96	16.03	22.21	3.56
OPBGG-2016-5	17.86	5.86	130.00	16.66	13.80	5.53
OPBGG-2016-7	12.20	3.06	392.33	9.66	52.30	1.53
OPBGG-2016-9	13.60	3.57	116.63	10.73	15.93	2.81
OPBGG-2016-11	18.40	9.83	154.33	11.72	23.70	3.09
OPBGG-2016-32	20.46	4.90	222.00	11.23	21.16	2.49
OPBGG-2016-34	20.20	7.50	143.92	44.79	15.46	2.97
OPBGG-2016-35	19.03	5.80	131.98	12.97	16.04	2.69
OPBGG-2016-40	19.83	8.73	195.53	14.51	22.69	3.37
OPBGG-2016-48	18.10	8.40	125.48	10.64	14.75	3.22
OPBGG-2016-49	18.03	6.13	160.43	12.95	19.53	2.51

4. B.3 Overall mean values and tolerance index

On comparision of mean values of the seedling characters under control with that of the PEG treatement shown in tables-24 and 23, the genotypes varied significantly in tolerance indices (TI) under water stress conditions. Maximum value of tolerance index was found in case of OPBGG-2016-48(0.92) followed by OPBGG-2016-35(0.86) and OPBGG-2016-5(0.84) where as OPBGG-2016-34 (0.26) showed minimum value.

Fig. 3 Growth response of greengram seedlings under different concentrations of PEG (control , 2.5 %, 5 % and 7.5 %



4. B.4 Character association

Estimates of phenotypic correlation co-efficient of all pairs of 6 seedling characters related to drought stress are presented in table 25-26.

4. B.4.1 Phenotypic correlation

The phenotypic correlation (r_p) among the 6 traits in the 11 genotypes under drought stress induced by PEG ranged from 0.165 between dry shoot weight and dry root weight to 0.874 between fresh root weight and dry root weight (table-25).

Root dry weight was positively and significantly correlated with root fresh weight (0.874) at 1% level of significance and with shoot length (0.622) at 5% level of significance. It was positively correlated with root length (0.572), fresh shoot weight (0.191) and dry shoot weight (0.165).

Shoot length was positively and significantly correlated with root length (0.855), fresh root weight (0.749), at 1% level of significance and with root dry weight (0.622) at 5% level of significance. It was positively correlated with shoot dry weight (0.524) and shoot fresh weight (0.486).

Root length was positively and significantly correlated with root fresh weight (0.631) at 5 % level of significance. It was positively correlated with root dry weight (0.572), shoot fresh weight (0.350), and shoot dry weight (0.312).

Shoot fresh weight was positively and significantly correlated with shoot dry weight (0.845) at 1% level of significance. It was positively correlated with root fresh weight (0.470) and root dry weight (0.191).

Root fresh weight was positively and significantly correlated with root dry weight (0.875) at 1% level of significance. It was positively correlated with dry shoot weight (0.510).

Shoot dry weight was positively correlated with root dry weight (0.165). Under controlled conditions the phenotypic correlation among 6 seedling characters in the 11 genotypes ranged from -0.217 between shoot fresh weight and root fresh weight to 0.958 between shoot fresh weight and shoot dry weight at 1% level of significance (table-26).

Dry root weight was positively correlated with root length (0.354), shoot length (0.236), fresh root weight (0.145). It was negatively correlated with fresh shoot weight (-0.537) and dry shoot weight (-0.578).

Shoot length showed positive correlation with root length (0.509), fresh root weight (0.363), and dry root weight (0.236). It showed negative correlation with fresh shoot weight (-0.481), dry shoot weight (-0.572)

Root length showed positive correlation with dry root weight (0.354) and fresh root weight (0.227). It showed negative correlation with dry shoot weight (-0.374) and fresh shoot weight (-0.402)

Shoot fresh weight showed positive and significant correlation with dry shoot weight (0.958) at 1 % level of significance. It showed negative correlation with fresh root weight (-0.217) and dry root weight (-0.537).

Root fresh weight was positively correlated with root dry weight (0.145). It was negatively correlated with dry shoot weight (-0.276).

Dry shoot weight was negatively correlated with root dry weight (-0.578).

Table 25- Inter-relationship of different seedling characters under drought stress induced by PEG.

Characters	Root length	Fresh shoot weight	Fresh Root weight	Dry shoot weight	Dry Root weight
Shoot length	0.855**	0.486	0.749**	0.524	0.622*
Root length		0.350	0.631*	0.312	0.572
Fresh shoot weight			0.470	0.845**	0.191
Fresh Root weight				0.510	0.874**
Dry shoot weight					0.165

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

Table 26 - Inter-relationship of different seedling characters under normal condition.

Characters	Root length	Fresh shoot weight	Fresh Root weight	Dry shoot weight	Dry Root weight
Shoot length	0.509	-0.481	0.363	-0.572	0.236
Root length		-0.402	0.227	-0.374	0.354
Fresh shoot weight			-0.217	0.958**	-0.537
Fresh Root weight				-0.276	0.145
Dry shoot weight					-0.578

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

4. C Response to cold

Eleven genotypes selected from 56 genotypes based on their yield performance were subjected to cold response by exposing these genotypes to artificial low temperature in laboratory by growing at 15^0c incubator for 10 days and then transferring the plans to field for evaluation in field condition.

Table 27: Reaction of different days old seedling of green gram to cold (15ºC)

Sl No.	Genotype	10 days old seedling			20 days old seedling			Yield (gm /plant)
		Survival in incubator (in days)	Survival in field (in days)	Total survival (in days)	Survival in incubator (in days)	Survival in field(in days)	Total survival (in days)	
1	OPBGG-2016-1	10	14	24	10	8	18	3.85
2	OPBGG-2016-5	10	13	23	10	5	15	3.65
3	OPBGG-2016-7	10	12	22	10	1	11	3.94
4	OPBGG-2016-9	10	15	25	10	8	18	5.03
5	OPBGG-2016-11	10	18	28	10	10	20	5.21
6	OPBGG-2016-32	10	16	26	10	11	21	5.65
7	OPBGG-2016-34	10	15	25	10	12	22	6.16
8	OPBGG-2016-35	10	11	21	10	9	19	5.07
9	OPBGG-2016-40	10	15	25	10	10	20	5.06
10	OPBGG-2016-48	10	16	26	10	11	21	5.00
11	OPBGG-2016-49	10	12	22	10	9	19	5.15

The above listed 11 genotypes (table-27) when exposed to low temperature, at 10 days seedling stage, the maximum days of survival was observed in case of OPBGG-2016-11 (28 days) followed by OPBGG-2016-32 and 48 (26 days), OPBGG-2016-9 (25 days), OPBGG-2016-1 (24 days), OPBGG-2016-5 (23 days), OPBGG-2016-7 and OPBGG-2016-49 (22 days), OPBGG-2016-IPM-99-35 (21 days).

When 20 days old seedling were exposed to cold, the maximum days of survival was found in case of OPBGG-2016-34 (22 days), followed by OPBGG-2016-32and 48 (21 days), OPBGG-2016-11 and 40 (20 days), OPBGG-2016-35 and 49 (19 days), OPBGG-2016-1 and 4 (18 days), OPBGG-2016-5 (15days), OPBGG-2016-7 (11 days). Considering both the yield and tolerance to cold at the 2 stages of growth of the crop, following observations were recorded:

It was observed that the genotype which showed maximum survival to cold at both the stages(10 days and 20 days) i.e. OPBGG-2016-32 is a high yielder (5.65 g/plant).

The genotype OPBGG-2016-34 (6.16g/plant) whose yield was highest among all genotypes is highly tolerant to cold at 20 days old seedling stage but showed medium tolerance at 10 days old seedling stage whereas the genotype OPBGG-2013-7 (3.94g/plant) was though a high yielder among the local varieties but very sensitive to cold at 20 days old seedling stage. Some genotypes were average yielders but showed high tolerance to cold like OPBGG-2013-11 (5.21 g/plant), OPBGG-2016-48 (5.00 g/plant), and OPBGG-2016-40 (5.06g /plant).

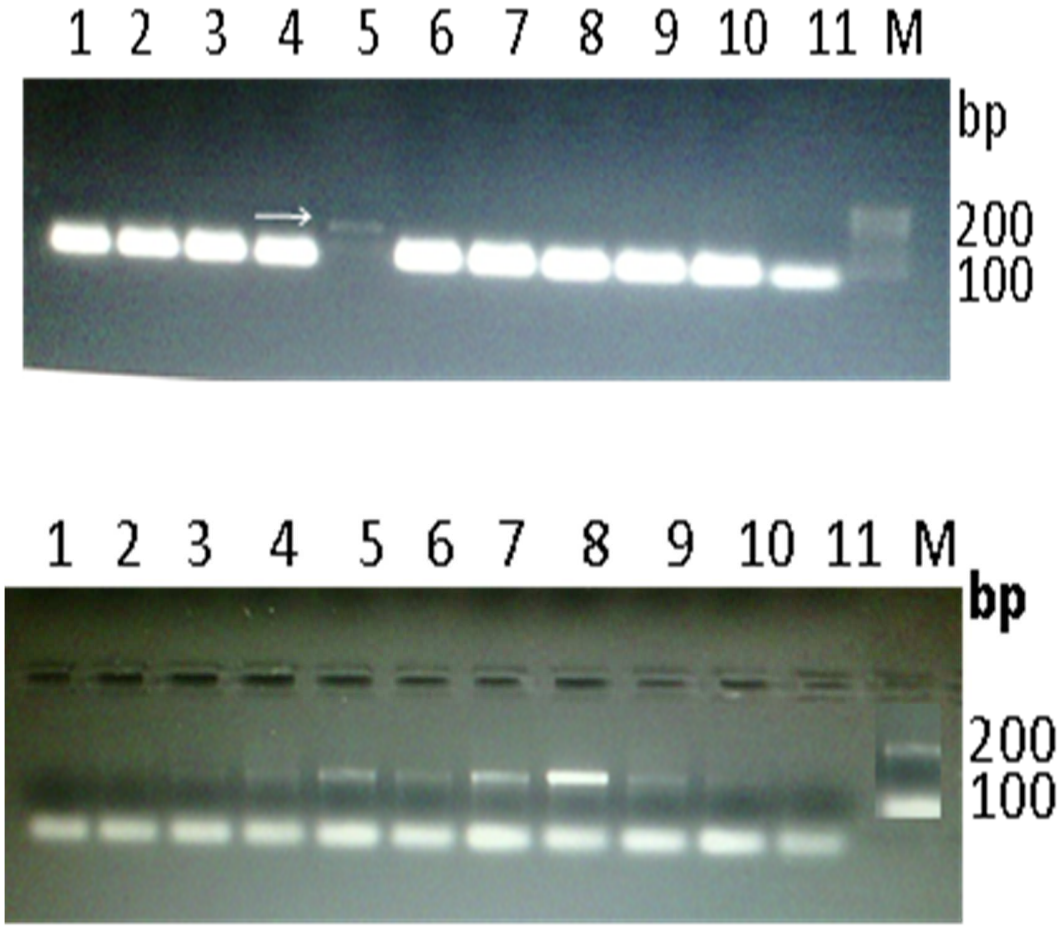
OPBGG-2016-34 (6.16g/ plant) was high yielder but showed moderate sensitivity to cold at 10 days old seedling stage. The local genotypes OPBGG-2016-1 (3.85g/plant) and OPBGG-2016-7(3.65g/plant) though high yielders are highly sensitive to cold at 20 days seedling stage.

4. D Molecular Markers Linked with Yellow Mosaic Disease Resistance in Greengram (*Vigna radiats* (L.) Wilczek).

Screening for YMV in greengram is difficult owing to practical difficulties in creation of artificial epiphytotic conditions and dependency on several factors such as vector population and climatic conditions. The aim of the present investigation is to validate markers associated with YMV resistance in greengram. Eleven genotypes were selected for molecular marker study with respect to MYMV reactions of *V. radiata* were screened with PCR-based technique employing primer pairs to validate efficiency and reliability of identified marker loci (CYR 1 and YMV1).

Both CYR 1 and YMV1 showed consistent polymorphism with respect to disease reaction (fig. 4). CYR1 showed amplifications in six genotypes while YMV showed amplification in a single genotype. The genotypes were OPBGG-2016-9, OPBGG-2016-11, OPBGG-2016-32, OPBGG-2016-34, OPBGG-2016-35, OPBGG-2016-40, which showed band in 4,5,6,7,8 and 9 well of the lane respectively for CYR 1 marker and a single genotype OPBGG-2016-11 showed band in 5^{th} well of the lane in case of YMV1 marker. In figure white arrow indicates the resistance genotype.

RGA22F2/RGA24R2 (CYR1) markers produced an allele size of approximately 180 bp in contrast to that of findings obtained by Maiti *et al*. (2010) i.e.1236bp. In case of YMV1 all the genotypes showed bands at 90bp excepting genotype OPBGG-2016-11 which revealed a spurious band at around 130bp.



1. OPBGG-2016-1	6. OPBGG-2016-32
2. OPBGG-2016-5	7. OPBGG-2016-34
3. OPBGG-2016-7	8. OPBGG-2016-35
4. OPBGG-2016-9	9. OPBGG-2016-40
5. OPBGG-2016- 11	10. OPBGG-2016-48
	11. OPBGG-2016-49

Fig. 4. PCR with YMV1 and CYR 1 primers for 11 genotypes of greengram.

DISCUSSION

Greengram [*Vigna radiata* (L.) Wilczek,] is an important and well known leguminous crop grown in India. It is popular because of its nutritional quality and its suitability for multiple cropping systems. However, the productivity of greengram is low. The constraints limiting yield are losses due to biotic and abiotic stresses. Severe yield losses have been reported due to the incidence of YMV, powdery mildew, cercospora leaf spot and bacterial leaf spot. Of these, YMV assume the greatest significance generally causing yield reduction to the extent of 20-40% and 10-100% respectively (Reddy *et al*., 2004 and Verma *et al*, 1993) and among abiotic stresses, cold stress and drought stress are the major stresses affecting the crop productivity.

A systematic and scientifically based approach is necessary to develop high yielding genotypes having resistant to these stresses. Generally, the carefully selected donor parent possessing high degree of resistance to a given biotic stress is used to create variability which is used by the breeder to make effective selections combining both resistance and productivity. Therefore, screening of the available diverse collections is the first step in such programme. Further diversity analysis on these collections would enable the breeder to plan and develop crosses keeping in view the diversity and complementarity of desired traits coming from two parents like resistance and productivity traits.

The research entitled “Studies on yield and stress tolerance in greengram” was initiated in the department of Genetics and Plant Breeding at OUAT, Bhubaneswar taking into considerations all the above facts and studies were planned and conducted to achieve the set of objectives.

5.1 Genetic variability, heritability and genetic advance in quantitative traits of greengram

Wide genetic variability among genotypes was observed for ten yield attributing traits including yield/plant. Genotypic difference among these genotypes was found to be statistically significant at 1 % level of significance (Table 6) for all the characters suggesting the presence of substantial variability in the material under investigation which will be much beneficial for the selection of breeding material. Similar wide range of variations for different traits in greengram with different set of

collection has been reported by Das *et al*. (2010), Degefa *et. al*. (2014), Reddy *et al.* (2014), Garje *et al.* (2014), Pathak *et al*. (2015). The range of variation in different traits in the present study is wide because of their diverse origin and geographical adaptation.

All the genotypes under the study displayed considerable amount of differences in their mean performance with respect to all the characters studied. An assessment of heritable and non-heritable components in the total variability is indispensable in adopting suitable breeding procedure. The heritable portion of the overall observed variation can be ascertained by studying the components of variation such as coefficients of genotypic and phenotypic variability, heritability and predicted genetic advance. Presence of narrow gap between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for all the characters under study suggested that expression of these traits have low environmental influence (Table 8). Although range can provide a preliminary idea about the variability but coefficient of variation is reliable as it is independent of unit of measurement. The extent of variability as measured by PCV and GCV also gives information regarding the relative amount of variation in different populations. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) showed wide variation for most of the characters under study. The difference between PCV and GCV values reflect the influence of environmental effect on character. The high, medium and low PCV and GCV indicate the potentiality with which the characters expression occurs.

From the experimental results, it was concluded that PCV was higher than GCV for all the character under study which indicate that there was some environmental influence on these traits. The influence of environment should be taken care for precise selection of material for the improvement of the crop. This type of findings were earlier reported by Garje *et al*. (2013), Gadakh *et al.* (2013), Kumar *et al. (*2013), Degefa *et al*. (2014). PCV and GCV were higher for primary branches per plant, yield per plant, clusters per plant, pods per plant and 100 seed weight. It is in close agreement with Narasimhulu *et al*. (2013), Garje *et al.* (2014), Degefa *et al*. (2014). It was observed that branches per plant exhibited maximum difference between PCV and GCV which indicate the higher environmental influence on this character. While selecting this character, much care should be taken up.

Heritability estimates reveals the heritable portion of variability present in different characters. The knowledge of heritability enables the plant breeder to decide the course of selection procedure to be followed under a given situation. However, heritability values coupled with genetic advance would be more reliable (Johnson *et al*., 1955) and useful in formulating selection procedure. In the present study, heritability estimates in broad sense and genetic advance as percent of mean was estimated. Heritability is the proportion of observed differences on a trait among individuals of population that is due to genetic differences. Factors including genetics, environment and random chance can all contribute to the variation between individuals in their observable characteristics (in their "phenotypes"). Genetic advance as percentage of mean along with heritability provides clear picture regarding the effectiveness of selection for improving the plant characters. Estimation of heritability along with genetic gain is usually more useful in predicting the resultant effect from selecting the best individual.

Moderate heritability (75-90%) was found for characters like primary branches per plant and pods per plant. High heritability was observed for 100 seed weight followed by flowering and maturity. Similar observations have been reported earlier by Das *et al*.(2010), Rahim *et al*. (2010), Reddy *et al*. (2011), Garje *et al*.(2013), Gadakh *et al*.(2013), Suresh *et al*.(2013), Singh *et al*.(2013), Degefa *et al*.(2013) and Anusha *et al*.(2014).

In the present study, genetic advance was high for plant height, pods/plant, days to maturity and days to 50% flowering as earlier reported by Reddy *et al.* (2011). Considering heritability and genetic advance jointly, moderate to high heritability coupled with high genetic advance was observed for the characters pods per plant, days to maturity and days to 50% flowering which is in close agreement with Rahim *et al*. (2010), Reddy *et al.* (2011), Gadakh *et al.* (2013), Anusha *et. al*. (2014), Garje *et al*. (2013), Pathak *et al*. (2015). This indicates there was lesser influence of environment in expression of these characters suggesting the role of additive gene effect and possibilities of achieving high genetic progress through selection.

5.2. Correlation Study in Greengram

Quality and quantity seldom go together and all the efforts of plant breeders are aimed at bringing these together. Hence, a knowledge of inter relationships

existing among various characters is necessary when selection for simultaneous improvement of these traits is to be most effective. If two-favourable characters are associated, selection for one character will automatically be good enough for the other. Grafius (1959) reported that there may not be genes for yield as such, but operate only through its components. So correlation analysis provides the information on nature and magnitude of the association of different components characters with seed yield, which is regarded as highly complex trait in which the breeder is ultimately interested. So it is a matter of great importance to the plant breeders to find out as to which of the characters are correlated with yield and also how they are associated among themselves, if negative association between characters is due to pleiotropic effects it would be very difficult to obtain the desired combinations while if linkage is involved, special breeding programmes are needed to break these linkage blocks. Knowledge of the correlations that exist between important characters may be helpful in the choice of good genotypes for any crop improvement programme.

Considering r_p and r_g of the component traits with yield from the results, it was observed that yield per plant was significantly and positively correlated with pods per plant, 100 seed weight clusters per plant, plant height, pod length and seeds per pod both phenotypically and genotypically except for seed per pod which showed negative correlation genotypically. Earlier similar findings have been reported by Suresh *et al.* (2013), Kumar *et al. (*2013), Garje *et al*. (2014) and Mishra *et al*. (2014). Yield is negatively correlated with days to 50% flowering, days to maturity and primary branches which was earlier reported by Mishra *et al. (*2014).

5.3. Path Coefficient Analysis in Greengram

Path analysis is the standardized partial regression coefficient, which splits the correlation coefficient into the measures of direct and indirect effects of a set of independent variables on the dependent variable. If the correlation between yield and character is due to the direct effects of character, it reflects true relationship between them, selection can be practiced for such a character in order to improve yield, however if correlation is due to indirect effect of the character through another component trait, the breeder has to select for the latter trait through which indirect effect is exerted. The result of negative direct effect indicated that these characters had low association and selection based on these characters would not be effective.

It was observed that pods per plant had highest effect on yield which was earlier reported by Suresh *et al.* (2013), Mishra *et al*. (2014), Garje *et al*. (2014) and Sahu *et al.* (2014). From the experimental findings, it was observed that 100 seed weight, seeds per pod, had direct positive effect on yield which has been confirmed earlier by Thippani *et al.* (2013), and Lalinial *et al. (*2014). Highest positive indirect effect was contributed by pods per plant and 100 seed weight *via* clusters per plant.

5.4. Genetic diversity in greengram

The divergence within the cluster indicates the divergence among the genotypes in the same cluster. On the other hand, inter cluster divergence suggests the distance (divergence) between the genotypes of different clusters. Inter and intra cluster D^2 values were worked out from divergence analysis. Critical assessment of clusters showed that clusters were heterogeneous within themselves and between each other based on major character relation.

From the experimental findings, it was observed that maximum to moderate contribution to D^2 by seed weight, followed by yield per plant, days to maturity, days to 50% flowering, and pods per plant which was in agreement with Mishra *et al*. (2012).

On the basis of D^2 values using Tocher's method, the 56 greengram genotypes were grouped into 7 genetic clusters (Table 11). Cluster IV consists of 24 genotypes while Cluster II consist of 11 genotypes, Cluster VI with 6 genotypes, Cluster I and VII consist of 5 genotypes each cluster III consist of 3 genotypes, and Cluster V consists of 2 genotypes each indicated that wide diversity in the experimental materials existed for majority of the characters. Distance between all pairs of genotypes was calculated using squared Euclidean distance method and the genotypes were clustered basing on Tocher's method. Similar findings have also been reported by Rahim *et al*. (2010), Das *et al*. (2010), Chattopadhyay *et al.* (2011), Abna *et al.* (2012), Mishra *et al.* (2012), Gokulakrishnan *et al*. (2012), Garje *et al*. (2013), Gadakh *et al.* (2013). It was observed from the results that cluster IV consisted of maximum number of genotypes having different sources of origin. Also materials of same source have been grouped in different clusters indicating no parallelism between sources of origin and clustering.

Plant breeders often use varieties/genotype possessing high genetic divergence in 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.

Considering inter-cluster D^2 value and cluster mean for different characters including yield and character complementation in yield traits in 56 genotypes, of present study, crosses between Cluster V and VI, Cluster II and VI, VI and VII are expected to produce more transgressive segregants in the later generation.

5.5. Greengram and drought:

Greengram is an important pulse crop grown throughout the country and it thrives well under drought prone condition. However, there is a great variability for drought tolerance among greengram genotypes under drought condition. Among different growth processes, early seedling growth stages are considered critical for raising a successful agricultural crop, since they indirectly determine the crop density and consequently yield. Due to inconsistency of the genotypes in length and fresh or dry weight under moisture stress, it becomes cumbersome to screen the genotypes based on these traits. However, tolerance indices might be useful to screen the genotypes as dry weight of a plant at particular age is universally considered as a more stable character than other morphological parameters.

In the present investigation attempts have been made to establish suitable method for screening drought tolerant genotypes based on tolerance index. The genotypes varied significantly in tolerance indices (TI) under water stress conditions (Table-23). Maximum value of tolerance index was found in OPBGG-2016-48 followed by OPBGG-2016-35 and OPBGG-2016-5 where as OPBGG-2016-34 showed minimum value and rest genotypes are either moderately tolerant or susceptible. Similar findings have also been reported by Dutta *et al*. (2008). Root dry weight was positively and significantly correlated with root fresh weight and shoot length under treated conditions. Under control conditions root dry weight was positively correlated with root length, shoot length, fresh root weight.

5.6. Greengram and cold

Greengram is cultivated in Odisha mostly in *rabi* season. The crop grown in interior districts suffer from winter if grown in rice fallow cropping pattern. The genotypes with little winter hardiness will be beneficial to the agro climatic zone having *rabi* temperature around 15^0C

The genotypes of OPBGG-2016-11, OPBGG-2016-32, OPBGG-2016-48, OPBGG-2016-9, OPBGG-2016-1 are found to be cold tolerant in early stages of their growth as they could sustain $15C^0$ temperature in incubator for 10 days and when they were exposed to field, the genotype OPBGG-2016-11showed maximum tolerance to cold in field condition. The genotypes which were susceptible to cold may be due to lipid peroxidation, membrane leakage and increase in hydrogen peroxide level, while activities of catalase, peroxidase and ascorbate peroxidase were decreased as reported by Saleh (2007). The tolerance to cold may be due to higher autolysis of protein bodies and depletion of starch reserves supported better seedling growth as reported by Satya *et al.* (2011). These findings need further conformation at molecular level.

The genotypes OPBGG-2016-34, OPBGG-2016-32, OPBGG-2016- 48, OPBGG-2016-11, OPBGG-2016-40, showed maximum tolerance to artificial cold at their later stages of growth (20 days old seedling) as it was observed when the seedling survived in artificial cold condition in incubator. When these genotypes were exposed to field, the maximum survival day was found in case of OPBGG-2016-11. So it was concluded that the genotype OPBGG-2016-11 is the most cold tolerant genotype. The genotype will be very useful to eco- geographical area where winter is little late. The tolerance to cold at later stage of growth (20 days old) may be due to H_2O_2 dependent accumulation of glutathione (GSH) as reported by Hung *et al.* (2007).

The genotypes having cold tolerance at different stages of their growth will be more beneficial to Odisha farming community where greengram crop is grown during *rabi* season in rice fallow cropping system.

5.7 Greengram and YMV

Among the biotic stress YMV is considered to be the prominent one. Genotypes having high yielding ability were exposed to natural field condition of

occurrence of YMV. There was no appearance of YMV in the field in the year under study which may be an escape. However, the genotypes were examined for having resistance to YMV on the basis of molecular marker like YMV1 (SCAR marker) and CYR1 (RGA marker). The primer CYR1 amplified in genotypes like OPBGG-2016-9, OPBGG-2016-11, OPBGG-2016-32, OPBGG-2016-34, OPBGG-2016-35 and OPBGG-2016-40 produced band approximately of 180bp indicating the presence of resistance gene in their genotypic make-up. Same primers were used for identification of YMV resistance nature in greengram and blackgram by Maiti *et al* (2010) but they found amplification at 1236bp. Residual heterozygosity is still maintained as far as YMV resistance locus is concerned in these segregating pedigree lines (OPBGG-2016-9, OPBGG-2016-11) and other breeding lines(OPBGG-2016-32, OPBGG-2016-34, OPBGG-2016-35, OPBGG-2016-40). These above materials need to be further selfed and purified for YVMV resistance using RGA marker for YVMV tolerance

YMV1 primer amplified in one genotype (OPBGG-2016-11) at 130bp. Souframanien *et al*. (2006) used this marker for identification of YMV resistance in blackgram. However the results need further confirmation by phenotyping in field under forced inoculation procedure. Other markers such as VMYR1 have also been used by Sowmini *et al*. (2014) for the identification of YMV genotype of greengram.

SUMMARY AND CONCLUSION

The present investigation on “Studies on yield and stress tolerance in greengram” was undertaken to study the variability parameters of the productivity traits, association among the traits and for the grouping of genotypes into different clusters basing on the productivity traits through multivariate analysis.

Fifty six greengram genotypes consisting of local varieties (7), selection from mutants (7), selection of entries from crosses (15) and selection from breeding lines (27) were evaluated in RBD during rabi 2014-15 and observations on days to 50% flowering, days to maturity, plant height, branches per plant, clusters per plant, pods / plant, pod length, seeds/pod, 100 seed weight and yield / plant were recorded from the field trial at EB-II section of the department. In the next season, eleven genotypes were selected from the 56 genotypes based on their performance and further evaluation (cold, drought and YMV) was done. Studies on the response to cold and drought stress were carried out at S.K. Sinha Molecular Breeding Laboratory of this department. Molecular work was done in these eleven genotypes for identifying YMV resistant entries.

Genetic variability is the prime objective for crop improvement fraternity. Higher the amount of variation for a character greater will be the scope of its improvement through selection.The genotypes showed wide variation in all traits under study and the difference were highly significant. Seed yield of the genotypes varied from 1.8 to 6.1g/plant. High seed yielders were OPBGG-2016-34 (IPM-02-03), OPBGG-2016-32(IPM-02-14), OPBGG-2016-11(Sujata X LGG-460), OPBGG-2016-49 (NM-92).

PCV and GCV estimates were high for primary branches per plant. Heritability of the traits ranged from 60.00 to 93.00. GA ranged from 1.05 to 8.92 for the productivity traits. Estimation of heritability along with genetic gain is usually more useful in predicting the resultant effect for selecting the best individual. Primary branches, pods per plant, days to maturity and days to 50% flowering had moderate to high heritability accompanied with high genetic advance indicating additive gene

effect. Characters like 100-seed weight and yield per plant with high to moderate heritability but low genetic advance indicated non additive gene effects.

The phenotypic (r_p) and genotypic (r_g) correlation among traits ranged from (-0.397 to 0.772) and (-0.467 to 0.838), respectively. Character association among component traits showed strongly interrelated characters *viz.*, pods/plant, pod length, seeds/pod and 100-seed weight.

All the characters like plant height, clusters per plant, pods/ plant, pod length 100 seed weight and seeds/pod showed positive phenotypic and genotypic correlation except for seed per pod which showed negative genotypic correlation with yield while days to 50% flowering and days to maturity was negatively correlated with yield.

Phenotypic correlation of seed yield with component traits was partitioned into direct and indirect effects by path analysis. Pods/plant had the highest direct positive effect on yield followed by 100 seed weight and seeds/pod. Highest positive indirect effect was contributed by pods per plant and 100 seed weight *via* clusters per plant followed by pod length (0.174) *via* test weight and100 seed weight (0.122) and pods per plant (0.121) *via* plant height. Correlation of most traits with yield was mostly influenced by indirect positive effect *via* pods /plant and pod length.

Multivariate analysis of divergence among the 56 greengram genotypes based on the 10 and 9 productivity traits were done in the following two methods,

1) D^2 analysis of genetic divergence

2) Tocher's method

Genetic divergence (D^2) estimates among 56 genotypes ranged from 19.14 to 248.73 (inter cluster). It was observed that the character seed weight followed by yield per plant contributed maximum to divergence, while plant height, pod length, seeds per pod, clusters per plant contributed least to divergence.

Using the Tocher's method, the 56 genotypes were grouped into 7 clusters. Though the genotypes were of diverse origin, these observations indicated no relationship between genetic diversity and geographical diversity. Considering inter-cluster D^2 value and cluster mean for different character including yield and character

complementation in productivity traits in 56 genotypes crosses between Cluster V and VI, Cluster II and VI, VI and VII are expected to produce more transgressive segregants in the later generation.

From drought analysis the genotypes varied significantly in tolerance indices (TI) under water stress conditions. Maximum value of tolerance index was found in OPBGG-2016-48 (PDM-139), followed by OPBGG-2016-35 (IPM-99-125), and OPBGG-2016-5 (Bhawanipatna) indicating these genotypes to be drought tolerant where as OPBGG-2016-34 (IPM-02-03) showed minimum tolerance index which may be considered as susceptible to drought.

From cold analysis among 11 genotypes, it was found that the most cold-resistant genotype OPBGG-2016-11 (Sujata X LGG-460) was a moderate yielder (5.21 g/plant) but the genotype OBGG-2013-34 (IPM-02-03) was a high yielder(6.16) having medium tolerance to cold. So crosses between genotypes which were high yielders and the genotype with tolerance to cold were expected to produce superior genotype combining both the characters.

DNA isolation was made from eleven greengram genotypes using young seedling leaves and the molecular analysis was carried out for these genotypes using two primers YMV1 (SCAR) and CYR1 (RGA). The gel electrophoresis figure showed that six genotypes namely OPBGG-2016-9 (OBGG-52 X Pant-M-4), OPBGG-2016-11 (Sujata X LGG-460), OPBGG-2016-32 (IPM-02-14), OPBGG-2016-34 (IPM-02-03), OPBGG-2016-35 (IPM-99-125), OPBGG-2016-40 (KPS-1), were resistant to YMV. YMV1 primer amplified in one genotype OPBGG-2016-11(Sujata X LGG-460) at both 90bp and 130bp but CYR1 produced amplicon approximately of 180bp. However this needs further investigation both at molecular and field condition.

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