COMBINING ABILITY ANALYSIS FOR GRAIN YIELD IN GREEN GRAM

[*Vigna radiata* (L.) Wilczek **]**

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ANAMIKA NATH

THESIS

Doctor of Philosophy in Agriculture

(Plant Breeding & Genetics)

2016

DEPARTMENT OF PLANT BREEDING AND GENETICS
RAJASTHAN COLLEGE OF AGRICULTURE
MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
UDAIPUR-313001 (RAJASTHAN)

CERTIFICATE –I

Dated 30 / 12 /2015

This is to certify that **Miss ANAMIKA NATH** has successfully completed the Comprehensive/Preliminary Examination held on 02 /07 /2014 as required under the regulation for degree of **Doctor of Philosophy in Agriculture.**

(Dr. S.P. Sharma)
Professor & Head
Department of Plant Breeding & Genetics
Rajasthan College of Agriculture
Udaipur (Raj.)

Maharana Pratap University of Agriculture and Technology

Rajasthan college of Agriculture, Udaipur

CERTIFICATE –II

Dated: 30/ 12 /2015

This is to certify that the thesis entitled **"Combining ability analysis for grain yield in green gram [***Vigna radiata* (L.) Wilczek **]"** submitted for the degree of **Doctor of Philosophy in Agriculture** in the subject of **Plant Breeding and Genetics,** embodies bonafide research work carried out by **Miss Anamika Nath** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of this thesis was also approved by the advisory committee on 23 / 12 /2015.

(Dr. S.P.Sharma)
Prof. & Head
Deptt. of Plant Breeding & Genetics

(Dr. S.R. Maloo)
Major Advisor

(Dr. S.R. Maloo)
Dean
Rajasthan College of Agriculture
Udaipur

Maharana Pratap University of Agriculture and Technology

Rajasthan college of Agriculture, Udaipur

CERTIFICATE –III

Dated: - / - /2016

This is to certify that the thesis entitled **"Combining ability analysis for grain yield in green gram [*Vigna radiata* (L.) Wilczek]"** submitted by **Miss. Anamika Nath** to the Maharana Pratap University of Agriculture and Technology, Udaipur in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** in the subject of **Plant Breeding and Genetics** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on her thesis has been found satisfactory; we therefore, recommended that the thesis be approved.

(Dr. S.R. Maloo)
Major Advisor

(Dr. S.P. Sharma)
Advisor

(Dr. A. Joshi)
Advisor

(Dr. K.B. Shukla)
Advisor

(Dr. Anila Doshi)
Dean
Rajasthan College of Agriculture
Udaipur

(Dr. S.L. Mundra)
DRI Nominee

External Examiner

Approved by

Dr. R. A. Kaushik
Director of
Resident Instructions
Maharana Pratap University of Agriculture and Technology, Udaipur (Raj.)

Maharana Pratap University of Agriculture and Technology

Rajasthan college of Agriculture, Udaipur

CERTIFICATE –IV

Date: - / - /2016

This is to certify that **Miss Anamika Nath** student of Doctor of Philosophy in Agriculture, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur has made all the corrections/modifications in the thesis entitled **"Combining ability analysis for grain yield in green gram [***Vigna radiata* (L.) Wilczek **]" ,** which were suggested by the external examiner and the advisory committee in the oral examination held on / /2016. The final copies of the thesis duly bound and corrected were submitted on / /2016, are enclosed herewith for approval.

(Dr. S.R. Maloo)
Major Advisor

(Dr. R .B. Dubey)
Prof. & Head
Department of Plant Breeding and Genetics

(Dr. Anila Doshi)
Dean
Rajasthan College of Agriculture,
MPUAT, Udaipur

Combining Ability Analysis for Grain Yield in Green Gram [*Vigna radiata* (L.) Wilczek]

ABSTRACT

Miss Anamika Nath* **Ph.D. Research Scholar**

Dr. S. R. Maloo** **Major Advisor**

The present investigation entitled, "Combining ability analysis for grain yield in Green gram [*Vigna radiata* (L.) Wilczek]" was undertaken by crossing eight diverse genotypes namely viz. IPM 99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 in diallel fashion (excluding reciprocals). Molecular analysis of these parental genotypes was also carried out using RAPD and ISSR markers. The experimental material comprising 8 parents along with 28 F_1s was evaluated during *kharif* season, 2014 in randomized block design with three replications at Instructional Farm of Rajasthan College of Agriculture, Udaipur, India.

The analysis of variance for experimental design was performed for sixteen characters. It revealed significant differences for all the characters indicating presence of adequate amount of variation among the genotypes. Further partitioning of mean squares due to parents were significant for all the characters except clusters per plant and pod length, while F_1s were significant for all the characters, revealed that adequate amount of variation was present for parents and F_1s . However, mean squares due to Parent v/s hybrid component were significant for plant height, pods per cluster, pods per plant, 100-seed weight, seed protein content and seed methionine content, which depicted presence of heterosis for these characters. The valus presented in appendices v indicate that the variation was very limited for primary branches, secondary branches, pods/ clusterseeds per pod and 100 seed weight. This is the reason that the ss due to hybrids vs parents was found to be non significant.

Mean performance of parents and their hybrids reveled that parent BM-4 followed by IPM 99-125 appeared to be the best parent for seed yield and most of the yield contributing charactes while IPM 02-03 was superior for seed protein content, Similarly, cross BM-4 x PDM-139 exhibited high mean performance for seed yield per plant and RMG-10435 x RMG-1045 for seed protein content.

Crosses viz. BM-4 x PDM-139 and RMG-1035 x RMG-1045 exhibited heterosis for grain yield and its components. For seed protein content and seed methionine content maximum significant positive heterosis as well as heterobeltiosis was recorded in cross RMG-1035 x RMG-1045 and BM-4 x PDM-139, respectively.

* Ph.D. Research Scholar, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur.

** Dean, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, (Raj.), India.

Combining ability analysis was performed by employing method 2, Model I of Griffing (1956), it revealed that the mean squares due to GCA were significant for all the sixteen attributes. Higher magnitude of GCA effects than SCA effects were observed for twelve characters indicating predominance of additive gene effects. Higher magnitude of SCA effects than GCA effects were observed for characters pods per plant, pod length, seed protein content and seed methionine content indicating preponderance of non-additive gene action for these characters.

While critically analyzing the over all performance of eight parents studied, BM-4 appeared to be the most promising followed by PDM-139 for seed yield and its components, whereas RMG-1035 depicted its superiority for seed protein content and ML-131 showed promising for for seed methionine content. This might be due to the accumulation of favourable genes in these *elite* lines. Therefore these genotypes could be utilized in breeding programmes.

Crosses BM-4 x PDM-139 and RMG-1035 x RMG-1045 exhibited heterosis and high significant SCA effects for seed yield, out of these RMG-1035 x RMG-1045 depicted high heterosis and significant SCA effects for seed protein while BM-4 x PDM-139 showed superior for high heterosis and significant SCA effects for methionine content.

Twenty five RAPD and ISSR markers were used for the present investigation. 17 RAPD and 18 ISSR primers were amplified and produce total 391 and 563 amplified fragments, respectively. Out of 104 scorable RAPD bands, 91 were polymorphic that showed 88 per cent polymorphism and out of 109 scorable ISSR bands, 88 bands were polymorphic and showed 81 per cent polymorphism. OPP-10 and ISSR-01 proved to be best RAPD and ISSR primer, respectively. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix. The similarity coefficient values lay between 0.46-0.68. The cluster tree analysis showed that the eight genotypes could be divided into 4 clusters.

Therefore, the cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance, GCA effects, heterosis with significant SCA effects for seed yield and its components. Further molecular analysis through combined RAPD and ISSR markers revealed its parental genetic diversity having 53 per cent dissimilarity. The parent BM-4 was grouped in cluster IV while PDM-139 in

cluster II A, thereby confirming that there was concurrence between the results obtained by molecular (RAPD and ISSR) and morphological markers along with their origen and known pedigree. Therefore this cross can be gainfully utilized.

On the basis of present investigations suggestions regarding breeding methodology for improvement in seed yield, seed protein and seed methionine content of green gram have been given.

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1. INTRODUCTION

Pulses constitute an important ingredient of the vegetarian as well as non-vegetarin diet in the Indian sub-continent and play a significant role in Indian farming because of their value in providing quality food to teeming million and restoring soil fertility through biological nitrogen fixation. Pulses offer one of the viable options for diversification of contemporary agriculture and management of natural resources. India is the largest producer and consumer of pulses in the world accounting 33 per cent of the area and 25 per cent of the global out-put.

Green gram [*Vigna radiata* (L.) Wilczek) is the most important legume (Pulse) crop in India after chickpea and pigeonpea. It belongs to family Leguminaceae, subfamily Papillionaceae, genus *Vigna*, species *radiata* and its chromosome number is 2n = 2 x = 22. According to Vavilov (1926) and Zukoveskij (1962), green gram has originated from Indian Sub continent. Green gram is extensively grown in India under varying soil types and climatic conditions. It is fairly well adapted to sandy loam soils and dry conditions. It improves soil fertility by fixing atmospheric nitrogen. It is drought tolerant crop and suitable for dry land farming and predominantly used as intercrop with other crops.

India is the primary green gram producer and contributes to about 75 per cent of the world pulses production. It contributes to about 14% of total pulses cultivation area and 7% of total pulses production in India. Maharashtra, Rajasthan, Madhya Pradesh, Bihar, Punjab and Andhra Pradesh are the leading producers of green gram. In India, pulses are grown in nearly 25.4 million hectare area with production status of nearly 19.66 million tonnes at an average productivity level of 770 kg/ha (Economic Survey 2013-2014). Among various pulse crops grown in India, green gram is grown on an area of 2.75 million hectares with a production of 1.19 million tonnes and productivity of 436 kg/ha (Economic Survey 2013). In Rajasthan, it was grown on an area of 965.6 thousand ha with a production of 453.6 thousand tonnes and productivity of 470 kg/ha (Govt. of Rajasthan, 2015-16).

The country has experienced progressive decline in per capita availability of pulses per day from 70.3 g in 1956 to 41.9 g in 2013 (Agril. Statistics at a glance,2014). This decline is mainly attributed to the steady marginalization of their

cultivation in the wake of the green revolution, meager productivity advances and burgeoning population. Several reasons have been suggested for low productivity of this crop, which include inherent low potential of yield, damages caused by several diseases like yellow mosaic virus, anthracnose, little leaf and pest, abiotic stress factors and poor management. It generally felt that there is an urgent need to break the bottleneck for increasing productivity of this crop.

Among pulses, green gram has important place as it contains more digestible proteins. The seeds are easily digestible and form excellent source of food for infants in the absence of milk. It does not produce heaviness or flatulence. It is rich source of proteins and calories in the Asian diet. It is rich in vitamin B and is regarded as a remedy for beriberi. When it is allowed to sprout, ascorbic acid (vitamin C) is synthesized. The amount of riboflavin and thiamine are also increased. It contains 25.0 per cent proteins with all essential amino acids, which is almost three times more than that of cereals (Saini *et al.,* 2010). It is consumed in the form of split pulses as well as whole pulse, which is an essential supplement of cereal based diet. The biological value improves greatly, when wheat or rice is combined with green gram, because of the complementary relationship of the essential amino acids, it is particularly rich in Leucine, Phenylalanine, Lysine, Valine, Isoleucine etc.

Green gram is small herbaceous annual plant with a slight tendency to twining in the upper branches. Being a short duration (60-65 days) crop with wide adaptability green gram grown all over the world as a sole crop and as an inter crop or mixed crop with cereals. Besides being a rich source of protein, green gram enriches soil fertility through atmospheric nitrogen fixation with the help of rhizobium bacteria in nodules and humus thus, plays a crucial role in furthering sustainable agriculture.

For any successful breeding programme to improve grain yield and component characters, it is essential to know precisely the genetic architecture of these characters under prevailing conditions. For appropriate breeding strategy knowledge of nature of inheritance is essential. To understand the nature of inheritance of yield and its components, genetic parameters like heterosis and combining ability are necessary. Hence, estimation of heterosis along with general combining ability and specific combining ability may be very helpful in selection of parents and identification of desirable crosses.

Serious attention is required to develop high yielding varieties of green gram using various crop breeding techniques. The diallel cross analysis technique (Griffing, 1956) could be used as one of the approaches to identify superior parents and crosses. Further knowledge on genetic architecture and mode of inheritance of different characters related to yield and quality is necessary for the effective implementation of selection programme aimed at improvement of genotypes. The combining ability determined through diallel analysis is useful to assess the nicking ability of the parents and at the same time it elucidates the nature and magnitude of different types of gene actions involved.

Exploration of heterosis on commercial scale in major field crops has increased both production and productivity per unit area of land. Heterosis is directly relevant to development of hybrids in cross pollinated crops, but it is also important in self pollinated crops because it also provide potential of crosses. Green gram being a highly self pollinated crop, utility of heterosis *per se* may not be of much use but cross combinations showing excellent heterosis involving parents with high general combining ability can be used in developing high yielding pure lines. The study of heterosis will provide the basic information regarding the breeding methodology to be employed for the varietal improvement. It also helps in rejecting large number of crosses in first generation itself and selecting only those with high potential. In short the study of heterosis helps the plant breeder in eliminating the less productive crosses in early generations.

Superiority of parents depends on their ability to combine well and also on the potentiality to produce transgressive segregants. In this context, combining ability analysis is useful in isolating superior genotypes and in identifying gene action involved in the inheritance of characters of economic importance. Yield is the most important character for improvement of a crop and it has a complex inheritance governed by large number of genes and greatly affected by environmental factors.

Currently, the genetic diversity of plants has been assessed more efficiently after the introduction of the methods that reveal polymorphism directly at the DNA levels. Ever since thermostable DNA polymerase was introduced, the use of PCR (Mullis *et al.* 1986) in research has increased tremendously. Genotypic selection at the DNA level can be exploited in marker assisted selection to identify desirable genotypes. DNA based markers find widespread application in plant science and plant

production. Morphological markers with their complex and undeciphered genetic control were used for the individual identification and diversity studies, they may be affected by environmental effects and cultivation practices, (Khan *et al*., 2000). The present study was conducted with an objective of selecting suitable parents for future use in improvement of green gram. Incorporation of the molecular approaches along with the conventional techniques is genetically more informative.

Keeping in view the above facts, the work was undertaken to increase the breeding efficiency, parental genetic distance based on morphological and molecular markers (RAPD and ISSR) has been used as a potential tool to predict hybrid vigour in green gram. Besides this, nature and magnitude of different gene effects were determined by mating 8 parents in diallel fashion. To develop suitable genotype of green gram and capitalizing high yield, the present investigation entitled "Combining ability analysis for grain yield in green gram [*Vigna radiata* (L.) Wilczek]" was attempted to analysis following objectives:

a. To estimate the magnitude of heterosis for grain yield and its component characters.
b. To estimate the general and specific combining ability for grain yield.
c. To elucidate the gene action and to identify suitable parents and crosses based on morphological and molecular marker

On the basis of above parameters appropriate future breeding methodology has been suggested in green gram.

2. REVIEW OF LITERATURE

The long term objective of most plant breeding programmes is to increase yield with a concomitant improvement in quality. Development of commercial hybrids in green gram may be one of the way to increase yield and grain protein content.

One of the most important factors in determining feasibility of hybrid is the nature and amount of heterosis and its exploitation. The presence of heterosis indicates the ability of diverse parents to combine in a hybrid combination. Hence in addition to the understanding of heterosis in green gram, it is also necessary to find out gene actions involved in the expression of quantitative and quality traits of economic importance. This, in turn, helps in determining the most appropriate breeding methodology to be adopted to achieve maximum genetic improvement in our breeding programmes. Combining ability analysis is frequently used for testing the performance of lines in hybrid combinations and also to know the nature of gene actions involved in the inheritance of various characters. Further identification of parental lines in cross combinations at molecular level is very important for plant breeding in authentication and genetic resource management. The literature on green gram pertaining to studies on heterosis, general combining ability effects of parents, specific combining ability effects of crosses, nature of gene action involved in the inheritance of various characters and molecular marker profiling is reviewed and presented in this chapter under the following heads:

2.1. MAGNITUDE OF HETEROSIS:

Discovery of heterosis is found to be one of the most important breakthrough in the field of plant breeding. The phenomenon of heterosis has been proved to be the most important genetic tool in boosting the yield of self as well as cross-pollinated crops. Heterosis or hybrid vigour represents the superiority of F_1's over the parents and was first reported in plants by Koelreuter (1766). Shull (1914) termed this phenomenon as heterosis and the manifestation effects as "hybrid vigour". Fonseca and Patterson (1968) coined term 'heterobeltiosis' to describe the superiority of heterozygote over better parent having practical utility. Meredith and Bridge (1972) used the term useful heterosis which refers to the superiority of F_1 over the standard

commercial variety. Heterosis is being worked out extensively in maize and other cross pollinated crops like pearl millet, tomato, onion (Adam and Shanks, 1959, Lonnquist and Gardner, 1961). Green gram being a highly self-pollinated crop, scope for exploitation of hybrid vigour depends on the direction and magnitude of heterosis, biological feasibility of crop and nature of gene action. It has been recorded that heterosis in green gram was observed by several authors for many characters including yield and its components. However, relevant and salient findings are reported in tabular form (Table 2.1).

Table 2.1: Important findings related to heterosis in green gram

Reference	Year	Important findings
Reddy *et al.*	1992	The cross combinations J-45 x 11/395 (70.85%) and J-45 x J-1 (63.64 %) had exhibited high and positive heterosis over better parent for seed yield/ plant.
Ghafoor *et al.*	1993	The grain yield had positive association with non significant harvest index. The hybrids NH 13-1 x NCM 7 had high harvest index along with high grain yield/ plant and exhibited high degree of heterosis for harvest index and grain yield.
Patil *et al.*	1996	Magnitude of heterosis was significantly high for the characters like pods/ plant and seed yield / plant. Significant negative heterosis was found for days to flower, days to maturity and weight of 100-seeds.
Reddy	1998	Highest range of heterosis over mid-parent and better parent (-5.56 to 125.68 % and -9.85 to 111.89 %, respectively) was observed in pods/plant followed by grain yield/plant (-16.27 to 94.50 % and -18.28 to 63.80 %).
Aher *et al.*	1998	The heterosis for protein content was -4.74 to 6.29 % over mid-parent while it was -5.92 to 5.09 per cent over better parent.
Joseph & Santoshkumar	2000	All the crosses exhibited significant relative heterosis for pods/plant, seeds/ pod and seed yield, while heterobeltiosis for test weight and seed yield and standard heterosis for plant height

		and seed yield.
Aher *et al.*	2000	Heterosis for grain yield/ plant over mid-parent, better parent and standard variety was 52.50, 42.23 and 53.65 % respectively. Maximum range of heterosis was obtained for grain yield/plant (-9.33 to 42.23 %).
Khattak *et al.*	2002	The cross combination having ML-5 as one parents produce segregates with yield potential through more number of pod clusters on main stem. Hybrid VC 3902A × ML-5, produced high heterotic effects for pod clusters on main stem.
Loganathan *et al.*	2001	Highest range of heterosis over mid-parent and better parent was observed for pods/plant while seed yield showed highest heterosis over mid-parent and better parent (64.57 per cent and 61.24 per cent, respectively).
Khattak *et al.*	2002	The extent of heterosis varied bi-directionally according to crosses and characters, with the maximum for seed yield/plant and harvest index in hybrids VC 1560D x ML-5 and 6601 x VC 1560D, respectively. The hybrids involving ML-5, VC 1560D and VC 3902A as one of the parents gave superior yield components than others.
Chen Xin *et al.*	2003	Identified superior F_1s were CM5 x K7, CM4 x K7, CM4 x KPS1 and CM3 x K7 while K7 was the most promising parental line for future hybrid production, and its F_1s gave significant heterobeltiosis in most crosses.
Patil *et al.*	2003	Heterosis for seed yield ranged from 6.54 to 70.75 and -6.86 to 51.91 % over mid parent and better parent, respectively. Out of 20 crosses, 17 over mid parents and 11 over better parents showed significant heterosis.
Reddy *et al.*	2003	Heterosis of 110.77 % and 77.54 % over the mid and better parent, was recorded for grain yield/plant.
Sawale *et al.*	2003	Significant heterosis over mid and better parent was present for pods/ plant, pods/ cluster, 100 seed weight and seed yield/ plant. The cross

		AKM-9505 x PM-9651 had the highest heterosis over mid and better parent.
Anbumalarmathi *et al*.	2004	Hybrids ML 682 x Vamban 2 and VGG 77 x Vamban 1 recorded significant standard heterosis for all the traits except no of branches/ plant. Three hybrids could be exploited for recombination breeding and four cross combinations can be utilized for heterosis breeding.
Kumar *et al*.	2007	The magnitude of heterosis was positive for days to maturity in most of the hybrids. The hybrids VGMGg-1 x 02-SM-51 expressed the highest positive mid-parent and better parent heterosis for seed yield/plant.
Barad *et al.*	2008	Genotype HUM 1 was found as good general combiner for six characters including seed yield. None of the testers found good general combiner for all the characters. Crosses HUM10 x Kopergaon, Pusa 9331 x K 851 and SML395 x Gujarat Mung 3 showed significant positive standard heterosis for seed yield/plant.
Dethe & Patil	2008	The highest heterosis to the extent of 26.1 % over mid parent and 22.8 % over better parent for seed yield/plant was observed in the cross BM 4 x TARM 18, which exhibited high heterosis for one or more yield contributing traits.
Intwala *et al.*	2009	The pods/plant was the most heterotic component followed by seed yield/ plant. Highest heterosis for seed yield/plant was shown by the cross $VRMG_g$-1 x SM-52 followed by crosses GM-4 x Pusa bold-1, GM-4 x Pusa 9632 and GM-3 x Pusa 9632.
Patel *et al.*	2009	The highest heterobeltiosis (62.50 %) was recorded for the cross PDM-87 x K851 while PDM-143 x GM-3 showed the highest standard heterosis (36.90 %).The highest standard heterosis for early flowering was noted by the crosses PDM-143 x PDM-87 followed by PDM-143 x PDM-11

Saravanan *et al.*	2009	The highest mid parent heterosis (103.48 %) and better parent heterosis (53.56 %) were recorded for seed yield/plant. Crosses L_1 x T_3, L_2 x T_3, L_8 x T_3, L_3 x T_3, and L_6 x T_3 showed high significant positive heterosis for seed yield/ plant.
Dhuppe *et al.*	2010	Crosses Jal-781 x AKM-9504 (87.96 %) and Jal-781 x PM-9002 exhibited significant positive heterosis over standard check BM-4 for grain yield while Kopergaon x ML-131 exhibited significant positive heterosis for 100-seed weight.
Zubair *et al.*	2010	High level of hybrid vigour was observed for plant height, pods/ plant and grain yield / plant. Cross NM 51 x VC 3902 showed strong heterotic effects for pods/plant, grains/ pod and grain yield/plant.
Reddy *et al.*	2011	The cross PUSA 9672 x WGG 2 and MGG 341 x LGG 407 shows the maximum better parent heterosis of 149.71 % and the mid parent heterosis of 159.69% respectively, for seed yield/plant.
Jayamani &Sathya	2011	The crosses 'VBN(Gg)3' x 'SML 1023' & 'VBN(Gg)3' x 'EC 396117' showed superior mean, *sca* effects & standard heterosis for seeds/ pod, 100–seed weight, clusters/plant and seed yield/plant.
Sujatha *et al.*	2011	Moderate to high heterosis was observed for seed yield and its 11 component traits. Highest better parent heterosis for seed yield/plot was reflected by the cross 'Chinamung' × 'VC-1' and 'Chinamung' × 'BPMR-1' (77.42%).The highest harvest index was shown by the cross 'Pusa Baisaki' × 'TARM-1' (34.12%).
Ramakant & Srivastava	2012	Positive and significant standard heterosis for seed yield/plant was registered for all the six crosses. Most heterotic crosses for yield were cross were identified for *zaid* and *kharif* season.
Srivastava & Singh	2013	The highest heterosis to the extent of 80.76% over standard variety and 72.39% over better parent for

		seed yield/plant was observed in the cross Narendra Mung-1 x PS-16 which also exhibited heterosis for yield and yield components.
Patil *et al*.	2014	High heterotic effects were observed for days to flowering, days to maturity, branches/plant, clusters/plant, pods/plant, pod length, seeds/ pod, seed yield/plant and 100-seed weight.
Ghaffar *et al.*	2015	High heterosis, heritability and genetic advance were observed for pod plant-1 which could be utilized for identification of best segregates from crosses K0031-10 × K0052-10 and K0019-10 × K0031-10.
Yadav *et al.*	2015	Out of 21 F1 hybrids,crosses SML382 x WGG37 and PUSA9871 x WGG37 exhibited highly significant positive standard heterosis for clusters/plant, pods/cluster, pods/plant, seeds/pod, 100-seed weight and seed yield/plant.
Das *et al.*	2015	The hybrid between IC 333153 and PDM 84143 showed high heterosis towards desired direction for all the traits with high yielding protein rich lines with low level of trypsin inhibitor.

2.2 COMBINING ABILITY AND NATURE OF GENE ACTION:

The concept of combining ability was enunciated in maize by Sprague and Tatum (1942). They defined the term GCA as an average performance of a line involved in crosses, while SCA as those instances where certain cross combinations do relatively better or worse than would be expected on the basis of average performance of parental lines involved. It has also been revealed that GCA is primarily due to additive gene effects and additive x additive interaction variance, while SCA is the consequence of non-additive genetic effects arising from dominance and epistatic deviations. It is also being realized that high yielding lines may not necessarily be able to transmit their superiority to their hybrids (Allard, 1960). Hence an estimate of GCA and SCA effects may be more reliable test rather than their *per se* performance.

Choice of the parents is one of the important practical problems encountered by plant breeders, which plays important role in hybridization and produce superior off springs. The most useful technique that helps plant breeders in this direction is the

analysis of combining ability. Combining ability not only helps in developing improved hybrids and high yielding varieties but also aids to identify the best combiner in breeding procedure, thus it is an unavoidable phenomenon for the plant breeders. In a crop improvement programme, much of the success rests upon isolation of valuable gene combination as determined in the form of lines with high combining ability. Several workers have estimated general and specific combining ability variances in green gram and the findings having relevance to the present study are reviewed in tabular form (Table 2.2).

Table 2.2: Important findings related to combining ability in green gram

Reference	Year	Important findings
Malhotra *et al*.	1980	Suggested that pedigree system would be more efficient method of obtaining lines with high pod and cluster numbers. Simultaneously, diallel selective mating among the good general combiners can be followed for release of greater genetic variability.
Ahuja	1980	Non additive gene effects were important for all traits except 100-seed weight and harvest index for which additive gene effects were more important. Significant specific combining ability for height and pod number and seed yield/plant while for general combining ability for all traits.
Basaeeruddin & Nagur	1981	The variance due to specific combining ability (σ^2 sca) was much higher than the variance due to general combining ability (σ^2 gca) indicating the operation of non-additive gene action for expression of grain yield.
Wilson *et al*.	1985	The existence of both additive and non-additive gene action for days to flowering, days to maturity, plant height, pods/plant, pod length, seeds/pod, seed yield/plant, 100-seed weight and protein content. However, additive gene action was predominant in the expression of all the characters except protein content.
Singh and Paroda	1985	Parents T44 and K8S1 had significant positive estimates of gca. effects for the nodulation traits and would be useful as parent in hyridization programmes. Some cross combinations showed significant heterosis for the three

		traits.
Thimmappa *et al.*	1989	The variance due to gca effects was more prominent for branches /plant and 100-grain weight, while sca variance was prominent for plant height, days to flower, clusters/plant, pods/cluster, pods/plant, seeds /pod and grain yield.
Natarajan *et al.*	1990	Both additive and non-additive gene action were important for plant height, clusters/plant, pods/plants, seeds/pod and seed yield. The additive gene action was predominant for plant height and seed yield.
Pathak *et al.*	1990	Mungbean parent ML 5 and Sabarmati were a good general combiners for seed yield, pods/plant, clusters/plant and tallness etc. The crosses showing high sca effects for yield also had significant and positive effects for two important yield components.
Reddy *et al.*	1992	The additive gene action was predominant for days to flowering, maturity, plant height, seeds/ pod and 100-seed weight whereas non-additive gene action was predominant for seed yield/ plant.
Saxena & Sharma	1992	Additive and non-additive variances were significant for branches/plant, pods /plant, seeds /pod and seed yield/plant. However an additive component was higher than that of non-additive component.
Tiwari *et al.*	1993	The gca variance found higher than sca variance for branches/plant and test weight, while sca variance was higher than gca variance for days to maturity, plant height, pods/plant, seed yield/plant and protein content.
Holkar	1994	Both gca and sca variances were significant for all the characters except branches/plant and pod length. The sca was predominantly higher in all the characters except days to flowering, 100-seed weight and seed yield.
Ram	1997	The dominance gene action was involved in the expression of yield, pods/plant, plant height, pod length and custers/plant in mungbean. Additive x additive and dominance x dominance played important role in the expression of plant height, branches/plant, clusters/plant,

		pods/plant and grain yield/plant.
Aher *et al*.	1998	Recorded that out of 28 F_1's only 4 F_1's exhibited positive significant sca effects.
Dasgupta *et al.*	1998	Additive gene effects were predominant for seed yield/plant, pods/cluster and harvest index. B1 and B105 were good general combiners for seed yield/plant. PS16 was a good general combiner for protein content. The three best specific combinations for seed yield/plant and other traits involved one parent with good GCA effects.
Kute *et al*.	1999	Additive gene effect was predominantly higher for all the characters except seed yield/plant.
Aher *et al*.	1999	Days to 50 % flowering, days to maturity, plant height, primary branches/ plant, seeds/ pod, 100-seed weight and protein content were under the control of additive gene actions. On the other hand, pods/ plant and seed yield /plant were governed by non-additive gene actions.
Joseph & Santhoshkumar	2000	The specific combining ability (sca) effects were found to be higher in magnitude than the general combining ability (gca) effects for all the six characters. The ratio of gca to sca variance was less than unity for all the characters, indicating the presence of the non-additive gene action for all the characters studied.
Aher *et al.*	2001	The variance due to both gca and sca were highly significant for all the characters except primary branches/plant, indicating the importance of both additive and non-additive gene effects. However, the additive gene effects showed pre-dominance for all the characters studied.
Gawande and Patil.	2002	Variances due to gca and sca were significant for all the characters studied. The good general combiners consisted of Kopargaon and TARM 18 for days to 50% flowering and maturity; PIP 3-85-2 for plant height, pods/ plant, 100-seed weight, and grain yield /plant; and AKM 8802 for pods/ plant, and grain yield /plant.

Manivannan	2002	All the characters *viz*., plant height, branches/plant, pods/plant and seed yield /plant displayed predominance of gca than that of sca variance in mungbean.
Kute & Deshmukh	2002	Predominance of additive effects for days to flowering, pods/cluster, pods/plant and 100-seed weight in was recorded most of the crosses. The dominance effect was also noticed in most of the crosses for clusters/plant, pods/plant and seed yield/plant.
Singh & Dikshit	2003	The estimates of sca variance were higher than gca variance for all the traits indicating the importance of non-additive gene effects in the expression of the traits. IPM 99-124 x IPM 99-125 was the best combination, because of its high and desirable sca effect for seed yield/plant.
Anbumalrmathi *et al.*	2004	The ratio of gca : sca indicated the preponderance of non additive type of gene action for all the traits. The parents VGG 77, MI 267, Vamban 2 and KM 2 were the best general combiners for most of the characters.
Kumar *et al.*	2005	The magnitude of sca variances for all ten characters indicated the influence of non-additive gene action.
Aziz *et al*.	2005	Analysis of variance revealed highly significant differences for female and male parents and their interaction in F1 and F2 generations.Estimation of genetic components of variation revealed the significance of both additive and non-additive components in both generations.
Singh & Singh	2005	General and specific combining ability mean squares were significant for 2 nitrogen fixation traits. Parents K-851, PS-7 and PIMS-2 showed significant positive gca effects and would be useful as parents in breeding programmes.
Gopi *et al.*	2006	The mean squares due to LXT were significant for all the characters indicating greater variability among the parents. The interaction effects between lines and testers were significant for all characters except branches/ plant. The variances due to sca were of greater magnitude than gca indicating the majority of non-additive gene action.
Gupta *et al.*	2006	The parent VC 3760-88 was the best general combiner for

		grain yield/plant, pods/plant, pods/ cluster and clusters/ plant. SML 668 x VC 3760-88 had the highest sca values for grain yield/ plant, clusters /plant, pods /cluster, pods/plant and seeds /pod along with their high *per se* performance in mungbean.
Ajmal *et al*.	2007	The additive genetic effects appeared to be important for pod length and 100-seed weight and non-additive effects were pronounced for pods/plant, seeds/pod, and grain yield/plant. Directional dominance was observed for pods/ plant, seeds/pod, and grain yield/plant.
Singh *et al*.	2007	The presence of additive, dominance and epistatic gene effects in almost all the crosses indicated importance of both additive and non-additive gene actions for the expression of the characters namely, days to 50 % flowering, days to maturity, plant height, primary branches, pods/ plant, pod length, seeds/ pod and yield/plant.
Vasline *et al*.	2007	The sca variances were greater in magnitude than the gca indicating the preponderance of non-additive gene action for all the yield attributing characters. The parents LGG 450, LGG 410, ADT 3 and KM 2 were identified as good general combiners based on gca effect. The hybrids LGG 450/ADT 3, VRMGG Local/KM 2 and LGG 410/ADT 3 exhibited high sca effects for all the economic traits.
Barad *et al.*	2008	The additive gene action was involved in controlling days to flowering, days to maturity and seed yield /plant. While other characters *viz.,* plant height, clusters /plant, pods /plant, pod length, seeds/pod, 100-seed weight and protein content were under the control of non-additive gene action.
Dethe *et al.*	2008	The mean squares due to gca and sca were significant for all the characters thereby revealed preponderance of additive gene effects for days to 50% flowering, days to maturity and pods/plant. Vaibhav and BM 4 proved to be good general combiners with good *per se* performance for most of the characters.
Intwala *et al.*	2009	Both the general and specific combining ability were significant for seed yield and its related traits. GM-4, $VRMG_g$-1,Pusa bold-1, and Pusa 9632 were good general combiner for seed yield and its related traits. Crosses $VRMG_g$-1 x SM-7 and GM-4 x Pusa bold-2 were the best on the basis of *per se* performance, sca effect, and

		heterobeltiosis.
Vasline & Suguna	2009	The sca variances were greater in magnitude than the gca indicating the preponderance of non-additive gene action for all the yield attributing characters. The parents, LGG 450, VRMGg Local, and ADT 3 were identified as good general combiners based on gca effect.
Rout *et al.*	2009	The parents LGG 460, Pant M 4, and OGG 12 were judged as good general combiners for seed yield. The cross combinations OGG 12/LGG 460, LGG 460/Pant M 4 and TARM 1/OUM 11 5 were identified as potential on the basis of their mean performance and specific combining ability in mungbean.
Patel *et al.*	2009	Parents GM-4 and GM-9918 were good general combiners for seed yield and yield attributing traits. The highest sca effect for seed yield was observed in cross PDM-11 x GM-4 followed by PDM-143 x GM9918. The crosses PDM-143 x GM-9918 and PDM-87 x GM-4 were superior for MYMV incidence.
Rehman *et al.*	2010	Eight diverse parents were selected for a diallel set of crosses to study the mode of inheritance. ANOVA showed the significance of additive and dominance effects for all the traits in both generations. Significance of D, H1 and H2 also confirmed the contribution of both additive and dominance effects in controlling the inheritance of traits.
Kumar *et al.*	2010	Parent EC 30072 was a good general combiner for grain yield/plant, whereas VBN 1 and PMB 27 were a good general combiners for 100-seed weight. Cross combinations *viz.*, VRMGG Local/VBN 1, VRMGG 1/EC 30072, VRMGG 1/PMB 27, M 123/LGG 450 and VBN 1/PMB 27 recorded high specific combining ability.
Patil *et al.*	2011	The ratio of gca to that of sca indicated preponderance of non-additive type of gene action for most of the characters except for plant height and pods/plant. The good general combiners for seed yield /plant were GM-4, Pusa vishal and K-851. The best specific cross combiners were Pusa vishal x SML-668, GM-4 x GM-3,GM-2k3 x GM-4 showed the highest sca effect for seed yield/plant.
Kujur & Lavanya	2011	The variance due to female were significant for characters such as plant height, primary branches, cluster/ plant, pods/plant, seeds/ pod and 100-seed weight. Variance due to males were significant for all characters.
Jayamani & Sathya	2011	High magnitude of sca variance than gca variance was recorded for all characters indicating role of non additive gene action. The parents,'Co 6', 'Barimung 7' and

		'Binamung 7' were identified as good general combiners for yield and yield contributing characters.
Alam *et al*.	2012	Studied mixing ability and inter-genotypic competition from 7x7 mechanical diallel mixtures excluding reciprocals of mungbean, the biblend BD-6901+BD-6922 was found as the best biblend mixture for different characters. Averaged biblends were 1.35% higher yielding than the mean yield of their uniblend components.
Narasimhulu *et al.*	2014	MGG-351, WGG-42, LGG-460 and TM-96-2 were considered as superior parents as they recorded high *per se* performance with positive significant effects for seed yield /plant and other yield contributing traits. Cross combinations viz., MGG-351 x PM-115, MGG-295 x PM-110 and WGG-42 x PM-110 were good specific combinations for seed yield/ plant and other desirable traits.
Katiyar & Kumar	2015	Out of thirty hybrids, seven cross combinations for plant height, six for branches per plant, six for pods/plant, 4 for grains/pod, 12 for 100-grain weight and 10 for grain yield/ plant exhibited significant sca effects.

2.3 MOLECULAR MARKERS:

Morphological and biochemical markers used for discriminating cultivars / varieties are not adequate as they are subject to environmental influences, whereas the molecular markers especially DNA based, have proven better. The latter may or may not correlate with phenotypic expression of a genomic trait. Varietal profiling methods that directly utilize DNA have been found to potentially address all the limitations associated with morphological and biochemical data. They offer numerous advantages over conventional, phenotype-based characters as they are stable and detectable in all situations regardless of growth, differentiation, development or defense status of the cell. Additionally, they are not confounded by environmental, pleotropic and epistatic effects. The DNA markers become the marker of choice for the study of crop genetic diversity, especially those based on DNA sequence variations which are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection studies. Application of molecular markers to plant breeding has established the need for information on variation in DNA sequence even

in those crops in which little classical genetic and cytogenetic information is available.

Randomly Amplified Polymorphic DNA (RAPD)

Among the DNA markers, development of RAPD-PCR based DNA finger printing has proved to be versatile (Gherardi *et al.*, 1998). RAPD markers have been used for the identification and assessing the genetic diversity among cultivars of several crops like green gram (Saini *et al.,* 2010), *Medicago sativa* L. (Mohammadzadeh *et al.*, 2011), *Oryza sativa* L. (Easmin *et al.,* 2008) and black gram (Srivastava *et al.,* 2011) etc. Moreover, RAPD-derived genetic information helps to compare each germplasm and to choose competent parents for hybridization. Among the various molecular markers, PCR based RAPD markers have become popular since their application does not need any prior information about the target sequences on the genome and is simple and fast.

Lakhanpaul *et al.* (2000) studied 32 Indian cultivars of green gram to randomly amplified polymorphic DNA (RAPD) analysis using 21 decamer primers. A total of 267 amplification products were formed at an average of 12.71 per primer with an overall polymorphism of 64 per cent. The extent of polymorphism was moderate to low. Jaccard's similarity coefficient values ranged from 0.65 to 0.92. Mainly three clusters revealing greater homology between cultivars.

Ranade and Gopalakrishna (2001) used 75 random RAPD primers to differentiate between 12 black gram varieties. Consistent results were obtained with about 40 primers. Use of these 40 primers resulted in the amplification of 74 polymorphic bands. Putative variety specific markers were amplified in four cases. The black gram varieties were discriminated by analysing the amplification patterns generated by four primers**.**

Samarajeewa *et al.* (2002) characterized 13 germplasm collection of *Vigna* species using RAPD markers. Total ten RAPD primers were used, out of which five were found to produce clear banding pattern. A total of 134 bands were produced, out of which 104 were found to be polymorphic. SAS program (version 8.0) was used to construct dendrogram which divided 13 accessions into two clusters comprising of six accessions in one and the other included rest of the accessions.

Afzal *et al.* (2004) studied 21 green gram cultivars were subjected to RAPD analysis using 34 decamer primers. All the primers produced polymorphic amplification products with some extent of variation. Green gram cultivars exhibited 75.0 per cent polymorphism. Jaccard's similarity coefficient ranged from 0.54 to 0.85 and concentrated mostly between 0.61 to 0.74. This indicated a rather narrow genetic base of tested cultivars. Genetic similarity obtained in this study may be used for selecting parents for breeding purposes. Clustering of cultivars into four groups showed reasonable variability that may be exploited for yield improvement.

Chattopadhyay *et al.* (2005) employed markers like RAPD and ISSR to assess the genetic diversity among selected germplasm comprising varieties, landraces and wild accessions. Though polymorphism among the varieties was moderate, it was high (83%) when the whole set of germplasm was considered. One green gram variety, PS-16 with determinate growth habit and a wild accession, Sub-14 was found most diverse as revealed from the lowest Jaccard's similarity coefficient value (0.34).

Diouf *et al.* (2005) used RAPD and SSR techniques to assess genetic diversity among cowpea genotypes. A total of 61 RAPD primers were used and twelve showed polymorphism. Fifteen of the 30 microsatellite primer pairs were polymorphic, detecting one to nine alleles per locus in cowpea landraces.

Karuppanapandian *et al.* (2006) studied the extent of genetic diversity at DNA level by RAPD analysis using 20 decamer primers which generates 200 bands with an average of 10 per primer and exhibited 83.0 per cent polymorphism. Jaccard's similarity coefficient ranged from 0.64 to 0.93 and concentrated mostly between 0.76 to 0.93. This indicated a rather narrow genetic base of tested green gram landraces. Clustering of green gram landraces into two groups showed reasonable variability that may be exploited for selecting parents for breeding purposes. .

Lakshanpaul *et al.* (2006) studied genetic variability among 32 Indian green gram cultivars using RAPD markers. Total 21 decamer primers were used that produced 267 bands with an average of 12.71 per primer. Polymorphism percentage was 64. Jaccard's similarity coefficient values ranged from 0.65 to 0.92. The cluster analysis divided all the cultivars into three clusters revealing greater homology between cultivars, as the genotypes were released from the same source and showing commonness with their pedigree.

Lavanya *et al*. (2008) used 54 accessions of green gram for RAPD profiling to identify the extent of diversity. The RAPD profiles were analysed for Jaccard's similarity coefficients that was found to be in the range from 0 to 0.48, indicating the presence of wide range of genetic diversity at molecular level. The dendrogram resolved all the accessions into two major clusters, I (with 11 accessions) and II (with 43 accessions).The distribution of the accessions in different clusters and sub clusters appears to be related to their performance in field conditions for 10 morphological traits.

Chattopadhyay *et al*.(2008) studied 80 accessions of green gram having diverse origin. One green gram genotype (B1) was differentiated from wild and cultivated accessions belonging to twelve other indigenous *Vigna* species and subspecies by employing one each of microsatellite, ISSR and RAPD primers. The highly informative primers could be utilized in generating useful molecular descriptors for fingerprinting green gram genotypes.

Arulbalachandran *et al.* (2009) evaluated genetic variation through RAPD markers in four black gram mutants (high seed protein, tall, bushy and dwarf mutants) along with parent cultivar (control) by taking 20 random primers which had generated 202 fragments that scored 58 polymorphic DNA bands. The average DNA bands were 10.1 per locus that ranged from 1 to 9. The average polymorphic rates were 38.37 per cent. Primer OPK–06 and OPK-11 revealed 62.5 per cent DNA polymorphism. Jaccard's similarity coefficient ranged between 0.621 to 0.785.

Abd El-Hady *et al.* (2010) evaluated genetic variations of seven *Vigna* species using RAPD and ISSR markers. Total five random primers generated 64 fragments, of which 31 were polymorphic with an average of 12.8 bands per primer. The amplified products varied in size from 2556 to 255 bp; eleven selected ISSR primers produced 128 bands across seven genotypes of which 89 were polymorphic with an average of 11.64 per primer. The size of amplified bands ranged from 2838 to 264 bp. The results indicated that both the marker systems RAPD and ISSR, individually or combined can be effectively used in determination of genetic relationship among the *Vigna* species.

Saini *et al.* (2010) studied RAPD primers for differentiate a set of 39 green gram genotypes. The primers S-1 and S-2 were more efficient with iso-frequency

distribution of most of their banding patterns and a combination of any one of the primers with S-1 or S-2 could identify all the genotypes.

Ullah *et al.* (2010) were assessed genetic diversity among 15 green gram genotypes of Pakistan through Random Amplified Polymorphic DNA (RAPD) analysis. Total 30 random decamer primers were used and showed 91.6 per cent polymorphism. Maximum similarity was observed between NM-51, NM-54 and NM-98 (0.86). The analysis revealed that the inter-varietal genetic relationship of several genotypes is related to their centre of origin.

Sheikh *et al.* (2011) done RAPD analyses of genomic DNA, which was carried out using 80 RAPD primers in ten different genotypes each of A and R lines of pigeonpea.76 of the 80 RAPD primers produced clear and unambiguous banding pattern among A and R lines. Out of these, 72 were polymorphic and 4 were monomorphic. A total of 702 bands were amplified, out of which 544 were polymorphic. This evinced on an average 7.55 polymorphic bands per primer; though the average numbers of amplified bands per primer were 8.77.

Binyamin *et al.* (2011) screened forty black gram varieties/lines against the virus under field conditions, but none of the lines appeared to be highly resistant. RAPD analyses revealed an extensive amount of variation, which could be used for cultivar identification. Genetic differentiation among black gram genotypes was similar to the field screening data.

Srivastava *et al.* (2011) investigated the genetic diversity and relationships among eight black gram (*V. mungo*) varieties from diverse geographic locations using RAPD markers. Forty decamer primers could generate a total of 346 RAPD fragments, of which 338 or 97.68 percent were polymorphic. The similarity coefficient was maximum between PLU-289 and PLU-456(0.76) that indicated less divergence between them. Lower similarity was observed between LBG-20 and PLU-289 (0.4337) indicating more divergence.

Undal *et al.* (2011) reported genetic diversity and their phylogenetic status among the wild and cultivated species of green gram using 36 random decamer primers. Twenty eight out of 36 primers amplified across all species scoring 537 amplicons of which 498 were polymorphic and 39 were monomorphic. Average percentage of polymorphism among the seven species was 92.82%. Out of 28, two

primers were reported to generate a maximum of 95 percent polymorphism. The dendrogram obtained categorized the seven species in to two distinct clusters.

Ghafoor *et al.,* (2012) studied thirty seven black gram genotypes using RAPD markers. Among 53 primers 36 produced polymorphic fragments in black gram. The RAPD markers were found useful for studying genetic diversity but clustering did not exhibited indication for agronomic performance, whereas quantitative traits contributed more towards agronomic performance. Cluster revealed that only a portion of genetic diversity has been exploited for black gram improvement that should broaden involving diverse parents from various clusters.

Pandiyan *et al.* (2012) measured genetic diversity among 36 accessions of cultivated and wild *Vigna* species using 12 quantitative morphological cultivars. The 36 *Vigna* accessions formed 8 clusters at 16.77 dissimilarity level. The accessions namely *V. dalzelliana, V. unguiculata, V. trilobata, V. mungo* var. *mungo* and *V. trinervia* var. *bourneae* were clustered in the separate groups. Hence the variations were found to occur at species level.

Sony *et al.* (2012) studied genetic diversity of 13 green gram cultivars using RAPD markers analysis. 20 arbitrary decamer oligonucleotide primers used, out of that 10 produced total 379 different bands with an average of 37.9 bands per primer. Banding pattern showed 100 per cent polymorphism. Dendrogram based on Nei's genetic distance using UPGMA segregated these cultivars into two major clusters. The highest genetic distance (1.0852) was found between cultivars BARI Mung-2 and Mung-6.

Panigrahi *et al.* (2014) in their experiment molecular markers have been used to study the genetic diversity in green gram cultivars. RAPD marker association with YMV resistance gene in green gram has been identified for validation and confirmation of several marker bands like VMYR 1. CEDG 180 and several RGA markers were taken into consideration to identify the exact marker which is linked to YMV resistance.

Bhuyan *et al.* (2014) analyzed the genetic diversity and relationship in 7 exotic and 3 advance green gram germplasm using 3 RAPD primers (OPA01, OPB06 and OPB07). On an average, 6 amplified products per primer were formed and per cent polymorphism was found to be 78.33. The similarity coefficient values was highest

(0.93) between genotypes AVRDC-3 and AVRDC-4, indicating less divergence amongst those genotypes and was least (0.39) between AVRDC-5 and AVRDC-6, indicating more divergence.

ISSR MARKERS

ISSR offers several advantages and this technique is already used in many crop plants. ISSR markers have been successfully utilized for assessing the genetic diversity in the genus *Vigna* by various scientists.

Ajibade *et al.* (2000) investigated the utility of inter simple sequence repeat (ISSR) DNA polymorphisms to distinguish taxa within the genus *Vigna*. Total 19 primers were used out of which 15 primers showed effective polymorphisms for distinguishing taxa at the species level. In contrast, ISSR analysis was not able to clearly differentiate subgeneric divisions within *Vigna*. They attribute this loss of resolution at the subgeneric level to the high rate of evolution of the sequences examined.

Chattopadhyay *et al.* (2005) reported RAPD and ISSR were employed to assess the genetic diversity among selected germplasm varieties, landraces and accessions. Through polymorphism among the varieties was moderate, it was high (83%) when the whole set of germplasm was considered. One green gram variety, PS-16 with determinate growth habit and a wild accession, Sub-14 was found most diverse as revealed from the Jaccard's similarity coefficient value The efficiency of ISSR markers over RAPD markers was well visualized from higher frequency polymorphic bands and polymorphic information content values.

Souframanien and Gopalakrishna (2006) employed inter simple sequence repeat (ISSR) marker technique to identify markers linked to the MYMV resistance gene. Of the 100 primers screened, 54 showed amplification of which 36 exhibited polymorphism .The ISSR8111357 marker was sequenced and sequence characterized amplified region (SCAR) primers were designed (YMV1-F and YMV1-R) to amplify the marker.

Reddy *et al*. (2008) analysed genetic diversity present in green gram, 30 genotypes were analysed at the DNA level by simple sequence repeat (SSR) and intersimple sequence repeat (ISSR) markers. A total of 19 SSR and 35 ISSR primers were used, of which 10 and 20 primers, respectively, showed amplification. The 20

ISSR primers, which showed amplification, produced 116 bands of which 72 were polymorphic (62%). The number of amplified bands ranged from 2 to 12 and the size ranged from 200 to 2700 bp.

Kanimozhi *et al.* (2009) studied genetic diversity among 23 black gram genotypes involving 16 released varieties, six pre release cultivars and one wild species *V. mungo var. silvestris* using twelve ISSR primers. The level of polymorphism was 82.05 per cent. The resulting dendrogram distributed the 23 black gram genotypes into five main clusters. The results of PCA were comparable to that of grouping based on UPGMA in that the 23 genotypes could be grouped into four clusters.

Tantasawat *et al.* (2010) determined genetic diversity and relatedness in 17 green gram (*V. radiata* (L.) Wilczek) and 5 black gram (*V. mungo* (L) Hepper) genotypes by means of ISSR analysis and morphological characters. In total, 341 fragments were amplified for the two *Vigna* species by using 18 ISSR primers. The analysis revealed 90.6 per cent polymorphism UPGMA analysis (based on ISSR) exhibited 2 major clusters; cluster I containing all green gram genotypes and cluster II containing all black gram genotypes.

Elham *et al*. (2010) evaluated genetic diversity of seven *Vigna* species using RAPD and ISSR markers. They selected 11 ISSR primers that produced 128, bands out of which 89 were polymorphic with an average of 11.64 bands per primer. The size of amplified bands ranged from 264 to 2838 bp. The similarity coefficient values ranged from 0.118-0.822 (ISSR) and 0.115-0.899 (RAPD and ISSR). The results indicated that both marker systems RAPD and ISSR, individually or combined can be effectively used in determination of genetic relationship among the *Vigna* species.

Pardhe and Satpute (2011) studied phylogenetic relationships of ten *Vigna* genotype by using inter simple sequence repeats (ISSR) markers. Average polymorphism was 54.05 per cent and average of 6.66 polymorphic fragments per primer. ISSR based dendrogram classified ten genotypes into 3 clusters (I, II and III) with 6, 3 and 1 genotypes respectively.

Datta and Lal (2011) had used 20 ISSR and 22 SSR markers to differentiate 2 major pulse crops (*Cicer arietinum* L. and *Cajanus cajan* L.) possessing different important traits. The average number of amplicon generated was 1.3 (SSR) and 8.1

(ISSR) that showed a polymorphism of 95% (ISSR) and 93% (SSR). UPGMA cluster analysis of eight genotypes using both the markers showed clear demarcation between the two genera falling in two separate clusters with their respective resistant and susceptible genotypes.

Bhareti *et al.* (2012) studied DNA polymorphism of black gram cultivars using 10 ISSR markers. Amplification pattern yielded 71 bands, of which 65 were polymorphic with an average of 6.5 polymorphic bands per primer. Cluster analysis grouped the genotypes into two main clusters, I and II comprised of 22 and 11 of that genotypes, respectively. The Jaccard's similarity coefficient values between different *Vigna* genotypes ranged from 0.47 to 1.00.

Khajudparn *et al*. (2012) utilized inter-simple sequence repeat (ISSR) markers for identifying putative F_1 hybrids from six cross combinations whose morphological characteristics were very similar to those of their respective female parents and could not be visually discriminated from the self-pollinated progeny. Based on 10 ISSR primers, polymorphisms were found between female and male parents of all six cross combinations. F_1 hybrids of all six cross combinations could be differentiated from the self-pollinated progeny of their female parents by using only either ISSR 841 or 857 primers, together with the ISSR 835 primer.

Liu *et al*. (2012) examined genetic relationship among five species of *Pinellia* endemic in China using inter-simple sequence repeat (ISSR) marker. Twelve ISSR primers showed 88.58 percent polymorphism, whereas 38 SRAP primer combinations showed 78.37 percent polymorphism. Cluster analysis showed that ISSR, SRAP and ISSR + SRAP dendrograms of the five species of *Pinellia* generally exhibited similar clustering patterns.

Singh *et al*. (2013) obtained a total of 108 bands using 30 green gram genotypes through 22 ISSR markers of which 68 were polymorphic. ISSR primers gave 58.3 per cent polymorphism and the PIC value ranged from 0.09 to 0.71 with an average of 0.46. The UPGMA distributed the 30 genotypes into five main clusters; ISSR markers were found useful in the assessment of green gram diversity and the selection of core collection to enhance the efficiency of germplasm management for use in green gram breeding and conservation.

Bainade *et al.* (2014) undertaken an experiment to identify ISSR markers associated with powdery mildew resistance in green gram (*Vigna radiata* (L.) Wilczek) cross between Kopargaon (susceptible) and BPMR-48 (resistant). Among the 75 ISSR markers, 54 were amplified in DNA extracted from green gram. Four markers were polymorphic for the two parents (Kopargaon and BPMR-48) whereas, only one marker was found polymorphic between the parents as well as resistant and susceptible population.

Das *et al*. (2014) assessed the phylogenetic relationships among the 5 green gram varieties based on DNA data. A total number of 10 ISSR primers that produced polymorphic and reproducible fragments were selected to amplify genomic DNA of the green gram genotypes. The value of Jacaard's similarity coefficient ranged from 0.724 to 0.793 and these five green gram cultivars were grouped into 3 clusters at 72.4 per cent similarity. Overall a moderate degree of genetic diversity among the green gram cultivars was recorded.

Singh *et al*. (2014) analysed the extent of genetic diversity among 35 *Vigna* genotypes using SSR, ISSR and RAPD markers. SSR (21), ISSR (17) and RAPD (25) markers produced a total of 319 bands, of which 284 exhibited polymorphism. Higher marker indices were obtained for ISSR markers, which also proved to be the most efficient marker system in terms of average heterozygosity values. The pooled allelic diversity data grouped 35 genotypes into 4 major clusters with most of the genotypes reflecting relationship according to the distribution.

Sao *et al.* (2015) analyzed five lines of four mungbean genotypes for polymorphism using a total of five Inter Simple Sequence Repeat (ISSR) and two Simple Sequence Repeat (SSR) markers. Amplification of genomic DNA of most popular four Indian mungbean genotypes with these ISSR and SSR primers yielded 162 fragments that could be scored, of which 56 were polymorphic, with an average of 8.0 polymorphic fragments per primer. The results indicated that both of the markers ISSR and SSR individually can be effectively used in determination of genetic relationship among Vigna species. It could be concluded that, the information of genetic similarities and diversity among Vigna genotypes can easily be identified using molecular techniques

3. MATERIALS AND METHODS

The present investigation entitled, **"Combining ability analysis for grain yield in green gram [*Vigna radiata* (L.) Wilczek]"** was conducted at Instructional Farm of Rajasthan College of Agriculture, Udaipur, India. Udaipur is situated at an elevation of 579.50 meters above mean sea level on latitude of 24^{o}-$35^{'}$ N and longitude of 70^{o}-$42^{'}$ E. The experiment was conducted on clay-loam soil under irrigated conditions. The meteorological data on maximum and minimum temperatures, relative humidity and rainfall for the crop period are given in Appendix I.

3.1 EXPERIMENTAL MATERIALS:

Eight diverse green gram genotypes namely IPM-99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 were selected as parents for crossing programme on the basis of their origin, pedigree maturity, adaptability, diversity and yield potential characters. Pedigree and source of parents are given in Table 3.1. The experimental material for the present investigation comprised of 36 entries including 8 parents and their 28 F_1 crosses. Cross success percentage was less in

open field at normal environmental condition, therefore crosses were attempted at green house during *spring*, 2013-14 in diallel fashion (excluding reciprocals). Greengram pods showing protrudence of stigma in emasculated bud (Fig: 3.1). Further the evaluation of F_1s and their parents were done during *kharif*, 2014 at RCA college farm.

Table 3.1: Name, pedigree and source of the parents used

Parent	Pedigree	Source
IPM 99-125	PM 3 x APM 36	IIPR, Kanpur
BM 4	MUTANT of T44	ARS, Badnapur
ML 131	ML 1 x ML 23	ARS, Durgapura
IPM 02-03	IPM 99-125 x Pusa bold 2	IIPR, Kanpur
PDM 139	ML 20/19 x ML 5	IIPR, Kanpur
RMG 1035	RMG 492 x ML 818	ARS, Durgapura
RMG 344	MOONG SEL.1 x J 45	ARS, Durgapura
RMG-1045	RMG-62 x KM 2170	ARS, Durgapura

3.2 EXPERIMENTAL DESIGN AND CROP HUSBANDRY:

Final experimental trial comprising 8 parents along with 28 F_1s was evaluated during *kharif,* 2014 in randomized block design with three replications. Parents and F_1s were grown in two rows. Sowing was done by dibbling the seeds at a distance of 10 cm in the rows of 2 m length with row to row spacing of 30 cm. Non-experimental rows were planted around the layout to eliminate border effects. A basal dose of 20 kg N and 40 kg P_2O_5 ha-1 was applied at sowing time.Five irrigations were given during the entire crop period. Recommended crop production and protection practices were followed for raising a good crop.

The observations on following traits were recorded on ten randomly selected competitive plants in parents and F_1s per treatment per replication. A brief description of the procedure adopted for recording of the observations for various traits as under:

(A) Morphological traits :

1. **Days to 50% flowering:**

   The numbers of days from the date of sowing to the date of flowers appearance on 50 per cent plants in a plot were recorded as days to 50% flowering for each genotype in each replication.

2. **Days to maturity:**

   The period from date of sowing to the date of eighty per cent pods matured on plants were recorded and expressed as days to maturity.

3. **Plant height (cm):**

   The height of plant was measured in centimeters (cm) from the ground level to the tip of the main stem at the time of eighty per cent maturity.

4. **Number of primary branches per plant :**

   Branches arising from main stem were considered as primary branches, which were counted and recorded at the time of harvest.

5. **Number of secondary branches per plant:**

   Pod bearing branches arising from secondary branches on plants were counted and recorded at the time of harvest.

6. Number of clusters per plant:

The peduncles which possessed one or more pods were considered as clusters per plant and were counted at maturity and averaged was analysed.

7. Number of pods per cluster:

This was estimated by dividing the total number of pods per cluster.

8. Number of pods per plant:

Total numbers of dry fully matured pods on each plant were harvested and average was counted.

9. Pod length (cm):

From the harvested pods of each plant five pods were selected at random and their length was measured in centimeters and the average was computed.

10. Number of seeds per pod :

For this, the pod's were selected which was used for measuring the length. Seeds of the same five pods were counted and average number of seeds per pod was derived and recorded.

11. 100-seed weight (g):

One hundred seeds were randomly collected from the produce of the selected five plants. The weight of 100 clean and undamaged seeds was taken by using single pan electronic balance and recorded in grams (g).

12. Seed yield per plant (g) :

The dry pods of each selected plant were threshed and cleaned. The seed yield of individual plant was weighted in grams (g).

13. Biological yield per plant (g) :

The weight of randomly selected plants from each plant per entry was taken after sun drying for constant weight in grams and averaged as biomass yield per plant.

14. Harvest index (per cent):

Harvest index is the ratio of economic yield (grain) to biological yield expressed in percentage and was calculated as per Donald (1962).

$$\text{Harvest index (\%)} = \frac{\text{Seed yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100$$

(B) Quality traits :

15. Protein content (per cent):

Bulk seeds of five plants of each entry from each replication were taken for the purpose of biochemical analysis. These seeds were crushed and the flour was used for nitrogen estimation by standard micro-Kjeldahl's method. Value for total nitrogen content were converted to crude protein per cent by multiplying with factor 6.25. The details of the method used are given in Appendix II.

16. Methionine content (per cent):

The methionine content was estimated by Colorimetric method as prescribed by Horn *et al.* (1946). Three seed samples per treatment per replication were analysed to obtain methionine content as given in Appendix-III

(C) Molecular characterization: Molecular profiling by RAPD and ISSR banding pattern was performed in section 3.3.4

3.3 STATISTICAL ANALYSIS

3.3.1 Analysis of Variance:

The analysis of variance for randomized block design was carried out for the data recorded on each character as per standard procedure given by Panse and Sukhatme (1985). The skeleton of ANOVA is as under:

Table 3.2: Analysis of variance and expectations of mean squares for Parents and hybrids

Source	d.f.	SS	MSS	EMS
Replication	(r-1)	S_r	M_1	$V_e + gV_r$
Genotype	(g-1)	S_g	M_2	$V_e + bV_g$
Parents	(p-1)	S_p	M_3	$V_e + bV_p$
F_1's	(F_1-1)	S_{F1}	M_4	$V_e + bV_{F1}$
Parents v/s Hybrid	1	S_P vs. S_{F1}	M_3 vs. M_4	-
Error	(r-1) (g-1)	S_e	M_6	V_e

Where, r = number of replications; g = number of genotypes,

p = number of parents; F_1 = number of hybrids

Test of Significance:

The F ratio was calculated for each source of variation by dividing M_6 (Error Mean Square) to their respective mean squares.

$$F = \frac{M_i}{M_6}$$

Where

M_i = Mean Square due to i^{th} source.

Critical Difference:

$$CD = \sqrt{\frac{2M_6}{r}} \times t_{[(r-1)(g-1)]} \text{ at 5 \% or 1 \% level of significan ce}$$

Coefficient of Variation:

$$CV = \frac{\sqrt{M_6}}{\overline{X}} \times 100$$

Where

$$\overline{X} = \frac{\sum_{i=1}^{g} \sum_{j=1}^{r} X_{ij}}{\overline{X}}$$

X_{ij} = Value of i^{th} genotype in j^{th} replication.

r and g = Number of replications and genotypes.

3.3.2 Estimation of Heterosis:

Per cent increase or decrease of F_1 over the mid parent referred as relative or average heterosis, while heterobeltiosis (Fonseca and Patterson, 1968) denotes the increase or decrease of F_1 over the better parent and economic heterosis defined as the increase or decrease of F_1 over the best parent. Following formulae have been used in estimation of heterosis:

Heterosis:

$$\frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \text{ X } 100$$

It's significance was tested by student 't' test as follows:

$t_{[(t-1)(r-1)]} = (\overline{F_1} - \overline{MP}) / SE_{(\overline{F_1} - \overline{MP})}$

$SE_{(\overline{F_1} - \overline{MP})} = \sqrt{3MSe/2r}$

Heterobeltiosis:

$$\frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

It's significance was tested by student 't' test as follows:

$t_{[(t-1)(r-1)]} = [\overline{F_1} - \overline{BP}] / SE_{(\overline{F_1} - \overline{BP})}$

$SE_{(\overline{F_1} - \overline{BP})} = \sqrt{(2MSe/2r)}$

Economic heterosis

$$\frac{\overline{F_1} - \overline{SC}}{\overline{SC}} X\ 100$$

It's significance was tested by student 't' test as follows:

$t_{[(t-1)(r-1)]} = [\overline{F_1} - \overline{SC}] / SE_{(\overline{F_1} - SC)}$

$SE_{(\overline{F_1} - \overline{SC})} = \sqrt{(2MSe/2r)}$

Where$_C$

$\overline{F_1}$ = Mean value of cross,

$\overline{MP}$ = Mean of two parents of corresponding F_1 i.e. $(\overline{P^1} + \overline{P^2})/2$,

$\overline{P_1}$ = Mean value of first parent,

$\overline{P_2}$ = Mean of second parent,

$\overline{BP}$ = Mean value of better parent,

~~SC~~ = Standard check,

r = Number of replications,

MSe = Error mean square,

t = Table value of 't' at error d.f. corresponding to 5% or 1% level of significance.

Negative direction was considered for days to 50% flowering and days to maturity. Whereas, positive direction was considered for rest of the characters.

3.3.3 Combing ability analysis:

Analysis of variance:

The combining ability analysis was carried out according to method 2 (Parents and one set of F_1s without reciprocals) Model-I (fixed effects) of Griffing (1956). In

this model, experimental material was regarded as population about which inference was to be drawn and combining ability effects of parents could be compared when parents themselves are used as tester to identify good combiner. It was assumed that variety and block effects were constant but error (environment and other uncontrollable components) was variable and normally and independently distributed with mean zero and variance. Following statistical model for combining ability was followed.

$$Xij = \mu + g_i + g_j + s_{ij} + 1/r \sum_K e_{ijk}$$

Where

μ = Population means

g_i = General combining ability (gca) effects of 1st parent.

g_j = General combining ability effects of J^{th} parent.

s_{ij} = Specific combing ability (SCA) effect of ij^{th} cross/ hybrid.

e_{ijk} = Environmental component pertaining to ijk^{th} observation.

i and j = Female & male parents responsible for producing ij^{th} cross/hybrid.

r = Number of replications.

Table 3.3: Analysis of variance for combining ability Model I

Source of Variation	**d.f.**	**SS**	**MSS**	**Expected M.S.**
GCA	*P*-1	Sg	Mg	$\sigma^2 e + (P+2) \frac{1}{(P-1)} \sum gi^2$
SCA	$\frac{P(P-1)}{2}$	Ss	Ms	$\sigma^2 e + \frac{2}{P(P-1)} \sum\sum S_{ij}^2$
Error	(r-1)(*P*-1)	Se	Me	$\sigma^2 e$

Method 2 (Griffing, 1956)

Where

$$Sg = \frac{1}{(P+2)}\left[\sum_i (x_i + x_{ij})^2 - \frac{4}{P} X^2..\right]$$

$$Ss = \sum_i \sum_j X^2{}_{ij} - \frac{1}{P+2}\sum_i (X_i + X_{ij}) + \frac{2X^2..}{(P+1)(P+2)}$$

Where

Se = MSe/r, and

$$X = \text{Mean over replications i.e.} = \left[\sum_{j=1}^{r} \frac{X_{ij}}{r}\right]$$

P =Number of parents.

The variance ratio (F) was used as a test of significance of gca and sca mean squares.

Combining ability effects:

General and specific combining ability effects were calculated as follows:

1. General combining ability (gca) effects of i^{th} parent (g_i):

$$g_i = \frac{1}{P+2}(X_i + X_{ij} - \frac{2}{P} X..)$$

2. Specific combining ability (sca) effects of ij^{th} cross (s_{ij}):

$$S_{ij} = X_{ij} - \frac{1}{P+2}(X_{i.} + X_{ij} + X_{j.} + X_{jj}) + \frac{2}{(P+1)(P+2)} X..$$

The restrictions imposed to this model are:-

$\sum_i g_i = 0$ and $\sum_j s_{ij} + s_{ij} = o$ (for each i)

Estimation of variance of these effects and difference was as under:-

$$SE\ gi = \sqrt{(P-1)M^l e / P(P+2)}$$

$$SE\ sij = \sqrt{(P+2)\ X\ M^l e / P(P+1)(P+2)}$$

$$SE\ gi - gj = \sqrt{2M^l e/(P+2)}$$

$$SE\ sij - sik = \sqrt{2(P+1)M^l e/(P+2)}$$

$$SE\ sij - ski = \sqrt{2\ X\ M^l e/(P+2)}$$

Significance was tested by using‘t’ test.

't' test for gca $t = \frac{g_i}{SE(g_i)}$ and

't' test for sca $t = \frac{s_{ij} - 0}{SE(s_{ij})}$

The't' value thus obtained was tested against the table 't' value at 5per cent and 1 per cent level of significance at error degree of freedom.

For testing significance of difference between two effects, the critical difference was divided by respective standard error of difference and compared with table value of 't' at error degree of freedom

3.3.4 Molecular analysis using RAPD and ISSR markers

It was done exclusively for the parental material only. DNA extracted from different green gram cultivars were compared using RAPD and ISSR methodology. DNA was extracted from young leaves (3–4 weeks old) using CTAB method. DNA was amplified by using decamer random oligonucleotide primer in a DNA thermo cycler (Biometra). The amplified samples were separated on agarose gel electrophoresis (1.2%). The bands were scored for their presence or absence. The details of the technique of DNA isolation, RAPD and ISSR are as given below:

Plant material:

Eight diverse green gram parents viz., IPM-99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 were studied for genetic diversity using RAPD and ISSR at Department of Molecular Biology and Biotechnology, RCA, Udaipur by fulfilling the terms and conditions as per the centre guidelines. Following biochemical steps were followed in genetic diversity analysis.

Chemicals and glasswares

All chemicals used in DNA isolation and PCR were of analytical grade. These were purchased from the Himedia Laboratory, SRL and Bangalore Genei Pvt. Ltd., India and provided by Department of Molecular Biology and Biotechnology.

Collection of leaves for DNA extraction

Healthy and tender leaves (3-4 weeks old) were selected for DNA isolation.

DNA isolation:

The leaves were harvested after 21 days and DNA was isolated with following

protocol (Doyle and Doyle, 1987):

1. 5g of leaf tissue material was ground well using liquid nitrogen in a pestle and mortal. The contents were transferred to the pre heated capped polypropylene tubes containing 2X CTAB extraction buffer and incubated for one hour at 60 ^{0}C with occasional mixing by gentle swirling in water bath.
2. Tubes removed from water bath and added equal volume of chloroform: isoamylalcohol in the ratio of 24: 1(v/v). Mixed the content gently by inverting the tubes to form emulsion.
3. Centrifuged the tubes at 5000 rpm for 10 min. Three phases appeared and the aqueous phase was taken and transferred to another fresh tube with cut tips and DNA was precipitated by the addition of 75 percent of isopropanol.
4. DNA – CTAB complex was precipitated as fibrous network, lifted by cut tips and transferred to another tube and washed with 70 percent alcohol. The pellet was collected by centrifugation at 15000 rpm for 15 min.
5 The pellet was rewashed by keeping it with 70 percent alcohol for 20 min followed by centrifugation. The pellet so formed dried by inverting the tube on paper towel and kept it overnight for drying.
6. The pellet was re-dissolved in 1000 µl of $T_{10}E_1$ buffer by keeping overnight at -20 ^{0}C without agitation.

Purification of DNA:

1. 0.5 ml of crude DNA preparation was incubated with 2.5µl of RNase (10mg/ml.) at 37^0 C for 1 hr.
2. 400µl of chloroform: isoamylalcohol in the ratio of 24:1(v/v) mixed thoroughly for 15 minutes to form an emulsion.
3. Centrifuged the tubes for 15 min at 15000 rpm and supernatant was collected in another tube avoiding the whitish layer of interphase.
4. The DNA was precipitated by addition of double quantity of absolute alcohol.
5. The solution was centrifuged at 5000 rpm for 10 minutes. The pellet settled down and washed with 70 percent alcohol and dried overnight.
6. The DNA was dissolved in 200 µl of $T_{10}E_1$ buffer.

Analysis of DNA:

(a) Quantification of DNA:

Concentration of DNA was estimated by measuring the optical density at 260 nm and 280 nm by using a UV spectrophotometer (also with gel

electrophoresis with standard of λ Hind III bacteriophage DNA) in following steps:

1 2 µl of DNA diluted to 1000 µl of $T_{10}E_1$ in a cuvette mixed properly and recorded the optical density (OD) at both 260 and 280 nm.

2 The concentration of dsDNA was calculated by using formula-

1 O.D.=50µg/ml

$$\text{DNA concentration } (\mu g/\mu l) = \frac{\text{O.D.}(260) \text{ x } 50 \text{ x dilution factor}}{1000}$$

3 The quality of DNA was judged from the ratio of OD value recorded at 260 and 280 nm.

(b) Gel analysis:

Electrophoresis was carried out in solid matrix, the agarose gel containing EtBr.DNA was visualized under trans-illuminated UV light.

(c) Casting of gel:

1. Prepared 0.8 per cent agarose gel in 1X TAE buffer the mixture was heated for few second till the agarose dissolved and then cooled.
2. To the cooled solution 5µl EtBr per 100 ml solution was added and mixed well.
3. The solution was poured into the gel caster having desired comb and allow to set when the gel was set the comb was removed and the gel was kept in the gel unit.

(d) Preparation of sample:

10µl DNA solution mixed with 2 µl 6X loading spined for few seconds in order to mix well.

(e) Electrophoresis:

Filled the gel tray with 1X TAE buffer and 10µl DNA sample was loaded with micropipette. The gel was run at 50 volt till the bromophenol blue dye traveled 2/3rd of the length of the gel, removed the gel and visualized under UV light and Photographs were taken with the help of GEL DOCUMENTATION system.

RAPD analysis:

(a) Dilution of DNA for PCR:

The DNA was diluted to final concentration of 10.0 ng/µl in $T_{10}E_1$ buffer (10 mM Tris HCL, 1 mM EDTA, pH 8.0).

(b) List of primers:

A set of 30 decanucleotide RAPD primers were used for PCR amplification. The sequences of these primers were selected from literature and were purchased from Bangalore Genei Pvt. Ltd. The details of operon code sequence of the primer and G:C contents are given below: (Table 3.4)

Table 3.5: PCR reaction mixture content

Components	**Final concentration**	**Single tube/20 (µl)**
DNA template	50ng	2.00 µl
Master Mixture		
(i) dNTP MIX	200µM	1.6 µl
(ii) Taq polymerase	1 U	0.33µl
(iii) Reaction buffer (10x)	1X	2.00 µl
(iv) Primer	0.5 µM	2.00 µl
(vi) dd H_2O		12.07µl

Table 3.6: PCR reaction cycle

Cycle	**Denaturation**		**Annealing**		**Extension**	
First cycle	94 ^{0}C	4 min	-	-	-	-
45 Cycle	94 ^{0}C	1 min	37 ^{0}C	1 min	72 ^{0}C	2 min
Last cycle	-	-	-	-	72 ^{0}C	10min

*Annealing temperature same for primer to primer

PCR amplification: PCR amplifications were performed in total volume of 25 µl.

(c) Preparation of solution for PCR amplification:

A master mix DNA was prepared for 10 tubes to reduce pipeting error. The master mix was then distributed to each PCR tube (20µl each) and then 2µl of different DNA template was added to each tube. The mixture was gently mixed and spined for 30 seconds at 4000 rpm. The PCR amplification was achieved in a gradient thermocycler programmed. (Table 3.5 and 3.6)

(d) Agarose gel electrophoresis**:**

Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.5 per cent agarose gel. The gel was prepared in 1X TAE buffer containing [(0.50 µg/ml) of ethidium bromide] the samples and loading dye were mixed in 1:1 ratio and loaded with micropipette. Electrophoresis was carried out at 100 volt for 3 hrs.

Data analysis:

Photographs from ethidium bromide containing gel were used to score the data manually and independently for RAPD analysis. Presence of amplified product were scored as 1 and its absence as 0 for all genotypes and primer combinations. These data matrices were then entered into NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System Programme) developed by Rohlf (1993).

ISSR analysis :

For the ISSR reactions, 25 primer pairs were used. The DNA content in 20 µl of the reaction mixture was 50 ng. The sequences of these primers were purchased from Bangalore Genei Pvt. Ltd. The details of operon code sequence of the primer and G:C contents are given on table 3.7.

The reaction contained 10X reaction buffer, 200 µM each of dNTPs ("Bangalore Genei"), 0.5 µM of each primer and 1 unit of Taq DNA polymerase (Bangalore Genei).

Table 3.8: PCR reaction cycle

Cycle	**Denaturation**		**Annealing**		**Extension**	
First cycle	94°C	5 min	-	-	-	-
2-35 Cycle	94°C	1 min	Tm (Pr)	45 sec	72 °C	2 min
Last cycle	-	-	-	-	72°C	10min

Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.2% agarose gel. The gel was prepared in 1X TAE buffer (Sambrook *et. al.,* 1989) containing 0.5 µg/ml of ethidium bromides. The samples and loading dye were mixed in 1:1 ratio and loaded with micropipette. Electrophoresis was carried out at 100 V for 3 hr in 1X TAE electrophoresis buffer. Gel was viewed under UV transilluminator and photographed by gel documentation system (Bio Rad Gel DOC).

Scoring the RAPD and ISSR Products:

In order to score and preserve banding patterns, photographs of the gel were taken by a Gel Documentation System, under UV transilluminator. RAPD and ISSR bands were designated on the basis of their molecular size ranging between 100-1000 bp. DNA ladder loaded simultaneously, with the PCR product. Molecular size of PCR products were estimated by referencing to the DNA ladder. The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored, but a major band corresponding to faint bands was considered for scoring.

Statistical Molecular Analysis for Similarity Coefficient:

The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix (qualitative data matrix). The pair-wise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient (Jaccard, 1908). The equation for calculating Jaccard's similarity coefficients 'F' between two samples A and B is:

$f = n_{xy} / (n_1 - n_z)$

n_{xy} = Number of bands common to sample A and sample B.

n_1 = Total number of bands present in all samples.

n_z = Number of bands not present in sample A or B but found in other samples.

Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method (Sneath and Sokal, 1973). The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genotypes using computer program NTSYSpc version 2.02 (Rohlf, 1993).

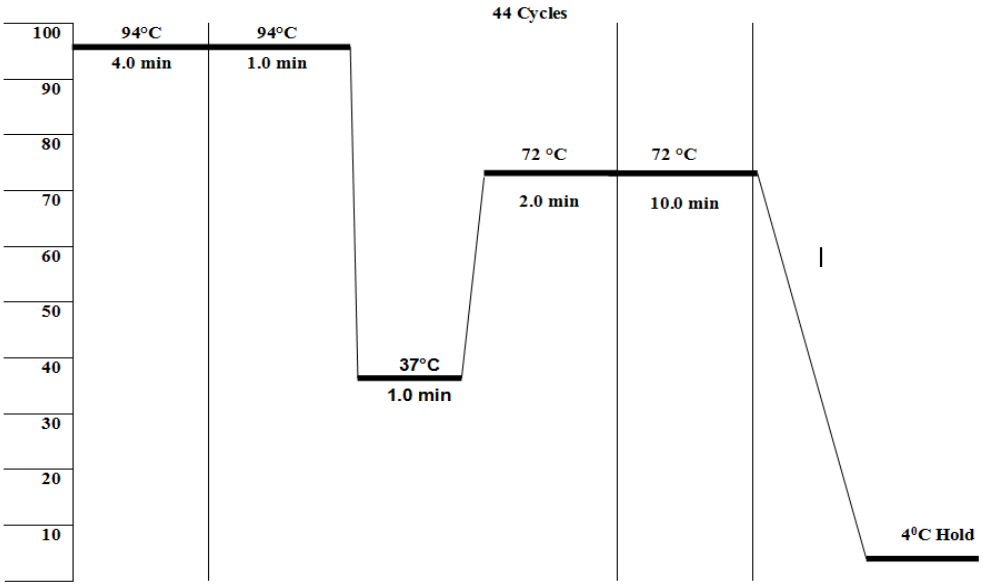

Figure 3.2: Protocol used for RAPD primers for PCR amplification

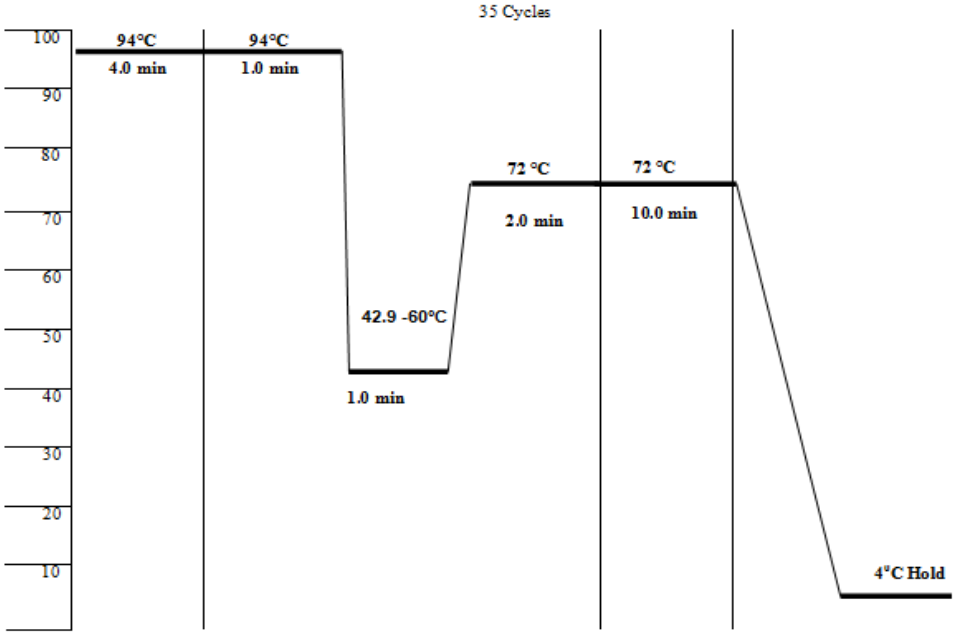

Figure 3.3: Protocol used for ISSR primers for PCR amplification

4. RESULTS AND DISCUSSION

Green gram is the third important pulse crop in India after chickpea and pigeon pea and also an outstanding source of easily palatable protein (24% protein). The consumption of green gram in India has increased over the years and demand will continue to grow faster than that of any crop. Since there is no scope for further expansion in the area under cultivation, the productivity enhancement through genetic means is the only option. Genetic restructuring of plant type under changing environmental/climatic situations and exploitation of heterosis may serve as potential alternatives for stabilizing the productivity level. Choice of the best parents is prerequisite in any crop breeding programmes, so evaluation of parents for their transmission potential for yield and yield components will pave a way for better selection.

In the light of above facts, efforts were made to investigate the combining ability and heterosis for different quantitative and quality characters. Genetic diversity analysis using RAPD and ISSR was carried out for parental lines. The material consisted of a diallel set (Griffing's, 1956 method 2, Model I) having total 36 entries including 8 diverse parents and their 28 F_1s (excluding reciprocals). The experiment was laid out at the Instructional Farm of Rajasthan College of Agriculture, Udaipur during *kharif*, 2013-14. The results obtained are presented under following heads:

1. (a) Analysis of variance for experimental design, and

   (b) Mean performance of parents and their F_1 progenies
2. Estimation of heterosis
3. Combining ability analysis
4. Molecular analysis using RAPD and ISSR markers

1. (a) ANALYSIS OF VARIANCE FOR EXPERIMENTAL DESIGN:

The analysis of variance for experimental design was performed for sixteen quantitative and quality characters (Table – 4.1). It revealed significant differences for all the characters indicating presence of adequate genetic variation among the genotypes.

Further partitioning of mean squares due to parents were significant for all the characters except clusters per plant and pod length, while F_1s were significant for all the characters, revealed that adequate amount of variation was present for parents and F_1s. However, mean squares due to Parent v/s hybrid component were significant for plant height, pods per cluster, pods per plant, 100-seed weight, seed protein content and seed methionine content, which depicted presence of high heterosis for these characters.

The valus presented in appendices v indicate that the variation was very limited for primary branches, secondary branches, pods/ clusterseeds per pod and 100 seed weight. This is the reason that the ss due to hybrids vs parents was found to be non significant.

(b) MEAN PERFORMANCE OF PARENTS AND THEIR HYBRIDS:

The mean values, grand mean (GM), standard error of mean (SEm), critical difference (CD) and coefficient of variation (CV) of parents and their crosses for all the characters are presented in Table 4.2 and Appendix –V, VI, VII and VIII . A perusal of mean values of parents and hybrids indicated high *per se* values of hybrids in comparison to parents for most of the characters.

The general, parental and hybrids means for days to 50% flowering and days to maturity were estimated as 39.71, 39.84, 39.67 days and 76.19, 77.10, 75.92 days, respectively. Mean values for days to flowering and days to maturity varied from 33.16 to 43.53 days and 66.96 to 79.37 days respectively. The study of mean values of hybrids in comparison with parental mean values indicated that emergence of 50% flower and days of maturity were earlier in the hybrids than the parents. The parent IPM 02-03 (33.16 d) was earliest in flowering followed by RMG-1045 (37.16 d), ML-131 and BM-4. Similarly parent RMG-1045 (67.63 d) attended minimum days for maturity followed by IPM 02-03 (74.47d) and BM-4. The hybrid IPM 02-03 x RMG-1045 (35.38) flowered early followed by hybrids IPM 99-125x RMG-1045 and BM-4 x IPM 02-03.Where as hybrid ML-131x RMG-344 (66.96 d) emerged out as the earliest followed by IPM 99-125x RMG-1045, BM-4xML-131 and BM-4x RMG-1045.

Therefore, it could be concluded that parent RMG-1045 and hybrid IPM 99-125x RMG-1045 appeared to be the best parent and best hybrid for days to 50%

flowering and days to maturity in terms of earliness, respectively (Table 4.2 and Appendix V).

General mean estimated for plant height was 51.00 cm. The mean values recorded for parents and hybrids were 46.41 and 53.73 cm, respectively. On an average, F_1 hybrids were found to record greater plant height than parents. Plant height varied from 34.18 to 66.72 cm in hybrids in the experimental genotypes (Table 4.2 and Appendix V). Among the parents ML-131 (54.00) and cross IPM-02-03 x RMG-1045 (66.72) followed by ML-131 x RMG-1035 **(**65.34) in F_1 hybrids, recorded highest plant height in comparison to their respective mean values. Dryland agricultural condition was chosen for this research work, hence taller plant height is more preferable than dwarf.

The general, parental and hybrid means were depicted as 2.80, 2.77 and 2.80 for primary branches per plant; 7.05, 7.01 and 7.06 for secondary branches per plant and 7.13, 7.12 and 7.13 for clusters per plant, respectively. The mean values recorded for parents and hybrids depicted that the hybrids had the higher primary branches, secondary branches and clusters per plant than parents. In general, parent BM-4, IPM 99-125 and ML-131 exhibited high mean performance for primary branches, secondary branches and clusters per plant. On the overall basis the cross IPM 99-125 x BM-4 was found more promising for the above three characters (Table 4.2, Appendix V and VI).

The general mean for pods per cluster, pod length and pods per plant were estimated as 3.39, 6.74 and 18.14, respectively. Pods per cluster varied from 2.83 to 3.97; pod length 4.92 cm to 9.00 cm and pods per plant 9.29 to 33.18. On overall basis parent PDM-139 followed by ML-131 had good pod length with higher no of pods per cluster and pods per plant. Hybrid BM-4 x RMG-344(3.96), ML-131x RMG-1035(3.96) followed by BM-4 x RMG-1035 for pods per cluster; BM-4 x IPM 02-03(33.18) followed by ML-131 x PDM-139 (29.26) for pods per plant and BM-4 x IPM 02-03(9.00 cm) followed by BM-4 x ML-131(8.44 cm) for pod length showing superior performance than others (Table 4.2 and Appendix VI). Therefore, it could be concluded that parent PDM-139 found to be the best parent for pods per cluster and pod length, while hybrid BM-4 x IPM 02-03 appeared to be the best hybrid for good pod length with maximum no of pods per plant.

100- Seed weight and seeds per plant are among the important traits that influence green gram yield. In this study seeds per pod varied from 8.6 to 13.32 and 100-seed weight from 3.20 g to 6.13 g. As evident from Table 4.2, general mean for seeds per pod recorded as 10.76 and for 100-seed weight 3.94 g. On overall basis, parent RMG-1045 followed by ML-131 was pointed out superior in parents for seeds per pod and parent IPM 02-03 followed by RMG-1045 for 100-seed weight. In general, cross BM-4 x PDM-139 (13.32) followed by BM-4 x RMG-344 for seeds per pod and IPM 99-125 x BM-4(6.13 g) followed by IPM 99-125 x ML-131 for 100-seed weight exhibited high mean performance, respectively (Appendix VI and VII).

In relation to these yield components, seed yield per plant varied from 7.57 g to 12.83 g in parents and 9.34 g to 13.33 g in hybrids. The general, parental and hybrids means were observed as 11.41 g, 11.32 g and 11.44 g, respectively. In general, hybrids had the highest grain yield per plant than parents. Among the parents highest mean values were recorded for BM-4(12.83 g) and IPM 99-125(12.56 g) followed by PDM-139 and ML-131(Appendix VII), whereas among the crosses, best seed yield surpassing parental yield was recorded for BM-4 x PDM-139 (13.33 g) and BM-4 x ML-131 (13.15) followed by ML-131 x PDM-139, IPM 99-125 x BM-4, IPM 9-125 x ML-131, IPM 99-125 x PDM-139 and RMG-344 x RMG-1045 (Table 4.2).

Population mean exhibited for biological yield per plant was 40.54g. The mean performance of parents and hybrids were 40.48 and 40.56g, respectively. In general, biological yield per plant range from 32.7 to 52.15 g (Table 4.2 and Appendix VII). The cross combination IPM 99-125 x IPM 02-03(49.35 g) followed by BM-4 x RMG-344(45.14 g) showed highest biological yield per plant in F_1s and among parents ML-131 (52.15 g) followed by RMG-344. Examination of data revealed that hybrids had more photosynthetic efficiency compared to parents.

Proportion of seed yield to total dry biomass or assimilate partitioning is given by harvest index. Harvest index depicted general, parental and hybrid mean values were 28.53, 28.44 and 28.56 per cent, respectively. In general, it revealed that F_1s showed higher mean values as compared to parents. IPM 99-125 (38.44%) among parents and the cross combination IPM 99-125 x ML-131(36.89 %) depicted the highest harvest index per cent age followed by RMG-344 x RMG-1045(36.30%), IPM 99-125 x PDM-139 and BM-4 x PDM-139 all in F_1s (Table 4.2 and Appendix VII).

Quality traits:

Seed protein and seed methionine content varied from 19.47 to 24.19 per cent and 0.98 to 1.29 per cent respectively, while general mean was observed as 21.71 per cent for seed protein and 1.12 per cent for seed methionine. Among parents, maximum protein content were recorded in IPM 02-03 (23.51%) followed by IPM 99-125(23.33%) and PDM-139, while maximum methionine has been found by RMG-344(1.26%) followed by IPM 99-125 and RMG-1035(1.24%). However, high estimates were recorded in cross, RMG-1035 x RMG-1045(24.19%) followed by IPM 99-125 x RMG-1045(23.88%) for protein content and BM-4 x PDM-139(1.29%) followed by PDM-139 x RMG-344(1.26%) for methionine content. Therefore, IPM 99-125 and cross IPM 99-125x RMG-1045 exhibited high mean performance for these two characters (Table 4.2 and Appendix VIII).

In the present study higher mean values of the hybrids over parents revealed superiority of F_1 than parents, which was also reported in green gram by Joseph and Santoshkumar (2000), Loganathan *et al.*(2001), Khattak *et al.* (2002), Sawale *et al.* (2003), Anbumalarmathi *et al.* (2004), Kumar *et al.* (2007), Barad *et al.* (2008), Patel *et al.* (2009), Saravanan *et al.* (2009), Zubair *et al.* (2010), Sujatha *et al.* (2011), Srivastava & Singh (2013), Patil *et al.* (2014) and Yadav *et al.* (2015).

Therefore, it could be concluded that parent BM-4 appeared to be the best parent for seed yield per plant, primary branches per plant, secondary branches per plant, clusters per plant, pods per cluster and harvest index (Table 4.3). Besides this, parent IPM 02-03 was also spotted as superior for earliness, 100-seed weight and highest protein content, while IPM 99-125 exhibited high harvest index, seed yield per plant, plant height, secondary branches, protein and methionine content. Similarly parent ML-131 appeared the best for plant height, pods per plant, biological yield per plant and seeds per pod. Further RMG-1045 was superior for seeds per pod, 100-seed weight and days to maturity.

An examination of mean values of crosses indicated that, cross IPM 99-125 x RMG-1045 took least days for earliness, IPM 02-03 x RMG-1045 had maximum plant height, while RMG-1035 x RMG-1045 was superior for seed protein content. With respect to seed yield per plant and methionine content cross BM-4 x PDM-139 were found to be the best these crosses had also the high magnitude of *per se* performance for other yield attributes (Table 4.3).

2. ESTIMATION OF HETEROSIS

The commercial exploitation of heterosis in crop plant is regarded as one of the major breakthrough in the field of plant breeding. Use of heterosis on commercial scale in major field crops has increased both production and productivity per unit area of land. Green gram being a highly self pollinated crop, utility of heterosis *per se* may not be of much use, so the production of economically viable hybrid in green gram still remains a challenge to breeders, but cross combinations showing good heterosis involving parents with high general combining ability can be used in developing high yielding pure lines. The study of heterosis will provide the basic information regarding the breeding methodology to be employed for the varietal improvement. It also helps in rejecting large number of crosses in first generation itself and selecting only those with high potential. In short the study of heterosis in green gram helps the plant breeder in eliminating the less productive crosses in early generations. The aim of heterosis study in green gram is to spot out cross combination which might generate desired segregants in the succeeding generations.

The scope of exploitation of hybrid vigour depends on directions and magnitude of heterosis and type of gene action involved. Heterosis is measured as per cent increase or decrease over mid parent (relative heterosis), over better parent (heterobeltiosis) and over best parent (economic heterosis). The manifestation of heterosis, heterobeltiosis and economic heterosis are presented as under: (Table 4.4, 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10).

Perusal of heterosis values of the twenty eight crosses showed that significant negative heterobeltiosis and economic heterosis for days to 50% flowering was observed in three crosses and thirteen crosses, respectively. Heterobeltiosis, i.e. improvement over the early flowering parent of cross, was observed in three crosses, IPM 99-125 x RMG 1045 (-14.11 %), BM-4 x RMG-1035, and RMG-1035 x RMG 1045. Significant negative economic heterosis for this trait was ranged from – 20.02 % to -2.60 % and the highest estimates of economic heterosis was recorded in the hybrid IPM 02-03 x RMG 1045 (-20.02%) followed by IPM 99-125x RMG-1045 (-18.44%) and BM-4 x IPM 02-03. However, crosses IPM 99-125xRMG-1045, BM-4 x RMG-1035 and RMG-1035 x RMG-1045 depicted significant heterobeltiosis and economic heterosis for early flowering (Table 4.4).

The estimates of relative heterosis, heterobeltiosis and economic heterosis for early maturity were negatively significant in four, five and nine crosses, respectively. Maximum significant economic heteros for early maturity was exhibited by the cross ML-131 x RMG-344 followed by cross IPM 99-125 x RMG-1045, BM-4 x ML-131 and BM-4 x RMG-1035 (Table 4.4).

The cross combinations viz. IPM 99-125 x ML-131,IPM 99-125 x RMG-1045, BM-4 X RMG-1045, BM-4 x ML-131 and ML-131 x RMG-1035 appeared most promising for *per se* performance, significant average heterosis, heterobeltiosis and economic heterosis in desired direction for days to 50% flowering and days to maturity. Patil *et al.* (1996), Reddy (1998), Aher *et al.* (2000), Loganathan *et al.* (2001), Reddy *et al.* (2003), Anbumalarmathi *et al.* (2004), Saravanan *et al.* (2009) and Reddy *et al.* (2011) also reported significant heterosis for days to 50% flowering and days to maturity in green gram.

Table 4.4 revealed seventeen, fifteen and seven cross combinations, which depicted significant heterosis, heterobeltiosis and economic heterotis (respectively) in desired (positive) direction for plant height. For this trait relative heterosis, heterobeltiosis and economic heterosis ranged from -13.49% - 59.90%, 8.01% - 52.07% and 2.16% - 23.56 %. Heterosis values revealed cross IPM 02-03 x RMG-1045 followed by cross BM-4 x RMG-1045, IPM 02-03 x RMG-1035, IPM 02-03 x RMG-344, ML-131 x RMG-1045 ML-131 x RMG-1035 and ML-131 x IPM 02-03 showed maximum significant positive heterosis, as well as heterobeltiosis and economic heterosis for plant height.

It has been found that, crosses IPM 02-03 x RMG-1045, ML-131 x RMG-1045 and ML-131 x PDM-139 has good *per se* performance with significant heterosis, heterobeltiosis and economic heterosis for plant height, days to earliness and days to maturity. The present findings were in close association with results reported by Joseph and Santoshkumar (2000), Reddy *et al.* (2003), Kumar *et al.* (2007) and Saravanan *et al.* (2009).

As evident from table 4.5, heterosis, heterobeltiosis and economic heterosis for primary branches per plant was significant in five, nine and twelve crosses, respectively, out of which two crosses, viz. cross IPM 02-03 x RMG-1045 (30.89%) and ML-131 x RMG-1045 (25.24%) depicted positive significant heterosis for this trait. RMG-1035 x RMG-1045 exhibited the highest estimates of significant average

heterosis, however, this cross also highlighted positive heterobeltiosis for secondary branches per plant. Patil *et al.* (1996), Anbumalarmathi *et al.* (2004), Kumar *et al.* (2007), Intwala *et al.* (2009), Patel *et al.* (2009), Saravanan *et al.* (2009) and Dhuppe *et al.* (2010) also reported similar results in green gram for primary branches per plant and secondary branches per plant.

Table 4.5 revealed that for clusters per plant, significant heterosis and heterobeltiosis were recorded in two crosses, while economic heterosis for six crosses. None of the cross combination showed significant heterosis in desired diraction.

Heterosis for pods per cluster range from -25.72% (IPM 99-125 x PDM-139) to 17.23% (BM-4 x RMG-344) and was significant in thirteen crosses, four of these crosses depicted positive heterosis for this trait. Maximum significant positive heterosis was recorded in cross BM-4 x RMG-344 followed by cross IPM 02-03 x RMG-344, RMG-344 x RMG-1045 and IPM 99-125 x RMG-344. Cross IPM-02-03 x RMG-344 and BM-4 x RMG-344 showed significant heterosis as well as heterobeltiosis and turned out to be the best as indicated in table 4.6.

Further a perusal of table 4.6 indicated that relative heterosis for pods per plant ranged from -61.31% to 183.24 %. It was found significant in twenty crosses, out of which only three significant positive heterosis was recorded The highest estimates of significant positive heterobeltiosis and relative heterosis for pods per plant was noticed by cross BM-4 x IPM 02-03 and IPM 02-03 x RMG-1045. The results reported by Patil *et al.* (1996), Reddy (1998), Aher *et al.* (1999), Aher *et al.* (2000), Joseph and Santoshkumar (2000), Loganathan *et al.* (2001), Reddy *et al.* (2003), Sawale *et al.* (2003), Kumar *et al.* (2007), Dethe and Patil (2008), Saravanan *et al.* (2009), Dhuppe *et al.* (2010) and Reddy *et al.* (2011) were in close association with the present findings for pods per cluster and pods per plant.

The relative heterosis and heterobeltiosis for pod length ranged from -26.50 to 46.05% and -31.26 to 25.67% respectively. It was positively significant in four crosses for heterosis and two crosses for heterobeltiosis. Cross BM-4 x IPM 02-03 followed by BM-4 x ML-131 recorded maximum level of significant positive heterosis and heterobeltiosis. While cross BM-4 x IPM 02-03 (25.67%) also depicted positive significant economic heterosis and turned out to be the best hybrid for this trait (Table 4.6). The present findings were in close association with the results

reported by Reddy (1998), Reddy *et al*. (2003), Intwala *et al*. (2009) and Vasline *et al*. (2009) were also reported heterosis for pod length in green gram.

Average heterosis for seeds per pod was found to be significant in eleven crosses, out of which six crosses depicted positive heterosis with the range in between-14.93 % - 30.58 %. Maximum significant positive heterosis was recorded in cross BM-4 x RMG-344 followed by cross IPM 99-125 x RMG-344, BM-4 x PDM-139 and cross BM-4 x RMG-1035. While, significant heterobeltiosis for seeds per pod was observed in thirteen crosses, and the highest estimates of positive heterobeltiosis was exhibited by the hybrid BM-4 x PDM-139, which also showed the desired economic heterosis for this trait (Table 4.7). The results were in agreement with the findings of Reddy (1998), Aher *et al*. (2000), Joseph and Santoshkumar (2000), Reddy *et al.* (2003), Kumar *et al*. (2007), Intwala *et al.* (2009), Saravanan *et al.* (2009), Vasline *et al.* (2009), and Dhuppe *et al*. (2010).

Relative heterosis for 100-seed weight ranged from -25.00 % (IPM 99-125 x IPM 02-03) to 41.28% (IPM 99-125 x BM-4) as showed in table 4.7. High magnitude of significant relative, better parent and economic heterosis was recorded in cross IPM 99-125 x BM-4 followed by cross IPM 99-125 x ML-131. Five, nine and eleven crosses were significant for heterosis, heterobeltiosis and economic heterosis respectively. These results are in parallel with the findings of Patil *et al.* (1996), Aher *et al*. (2000), Reddy *et al*. (2003), Sawale *et al*. (2003), Dethe and Patil (2008), Intwala *et al.* (2009), and Patel *et al.* (2009).

For seed yield per plant, significant heterosis was recorded in the cross BM-4 x PDM-139 (5.17) and RMG-1035 x RMG-1045 (Table 4.7). Heterosis for biological yield per plant ranged from -17.50% to 35.36 %. Maximum significant positive heterosis was recorded in cross IPM 99-125 x IPM 02-03 and IPM 99-125 x RMG-1035, while this two cross also recorded significant hetrobeltiosis for this trait. For harvest index, relative heterosis and heterobeltiosis ranged from -31.22% to 37.14% and -40% to 32.39%, respectively. Cross RMG-344 x RMG-1045, PDM-139 x RMG-1035, IPM 99-125 x ML-131 and BM-4 x PDM-139 showed significant positive heterosis for the trait (Table 4.8). While, the highest estimate of significant heterosis as well as heterobeltiosis was exhibited by the hybrid RMG-344 x RMG-1045 and turned out to be the best as indicated in table 4.8. Similar to the present findings Intwala *et al*. (2009), Khattak *et al*. (2002), Sujatha *et al.*(2011) and Yadav *et al.*(2015) also reported heterosis for seed yield per plant, biological yield and harvest index in green gram.

Quality characters:

Table 4.9 reveled relative heterosis and heterobeltiosis for protein content ranged from – 14.80% to 10.33% and 0.46 % to 8.70% respectively,Significant heterosis was observed in seventeen crosses and of these eight showed significant positive heterosis, while for heterobeltiosis five crosses showed significant in desired direction. Maximum significant positive heterosis as well as heterobeltiosis was recorded in cross RMG-1035 x RMG-1045 followed by cross ML-131x RMG-1035, ML-131 x RMG-344, RMG-1035 x RMG-344 and cross BM-4 x RMG-1035. Similar results were reported by Aher *et al*. (1998) and Patel *et al*. (2009).

Relative heterosis for methionine content ranged from -19.74% (IPM 99-125 x PDM-139) to 13.32% (BM-4 x PDM-139). It was significant in fourteen crosses, of which only two crosses depicted positive heterosis. Maximum significant positive heterosis as well as heterobeltiosis for this trait was recorded in cross BM-4 x PDM-139 and turned out to be the best as indicated in table 4.9. Similar results were reported by Singh S. (2000).

Therefore, the results revealed significant positive as well as negative heterosis and heterobeltiosis in many crosses for different characters studied (Table 4.10). The high values for heterotic effects indicated that the parents used for the study appeared to be genetically diverse. Considerable high heterosis in certain cross combination and low in other revealed that nature of gene action varied with the genetic architecture of the parents which might help in identifying superior cross combination, (Sharma, 2001) .

A perusal of Table 4.11 revealed best heterotic cross for seed yield and yield contributing traits involving genetically as well as geographically diverse parents thereby, confirming the established facts as enunciated by Falconer (1981). It has been found that cross BM-4 x PDM-139 and RMG-1035 x RMG-1045 showed significant heterosis for seed yield with most of the yield contributing characters. It is well established that there could be no separate gene system for yield *per se* as yield was an end product of the multiplicative interaction between its various components. Thus heterosis for grain yield could be determined by finding the effect of heterosis for individual yield components. Based on heterotic studies, the best direct yield contributing character was seeds per pod, pods per plant, plant height and harvest

index. Importance of these characters was also emphasized by Khattak *et al.* (2002), Intwala *et al.* (2009), Sujatha *et al.* (2011) and Yadav *et al.* (2015) in green gram.

Similarily, for quality traits maximum significant positive heterotic response for seed protein and seed methionine content was recorded in four crosses and two crosses respectively, as indicated in Table 4.12. Most of these crosses exhibited less superiority for seed yield which might be due to the negative correlations of seed yield and grain protein content. However, cross RMG-1035x RMG-1045 followed by ML-131 x RMG-344 showed significant heterosis for seed protein content and while cross BM-4 x PDM-139 followed by BM-4 x ML-131 exhibited significant seed methionine content as well as heterosis for other traits .

Table 4.11: Best heterotic crosses for seed yield and its component traits in green gram

Superior heterotic crosses	**Significant heterosis over mid parent (MP) and better parent (BP) for seed yield and its component traits**
RMG 1035 X RMG 1045	Days to 50% flowering, seed yield per plant, plant height, secondary branches per plant, biological yield and protein content
BM-4 X PDM 139	Seeds per pod, pods per plant, methionine and seed yield per plant
IPM 02-03 X RMG-1045	Days to 50% flowering, maturity, plant height, primary branches per plant, and pods per plant
IPM 99-125 X ML 131	100 seed weight, harvest index, days to 50% flowering and maturity
RMG 344 X RMG -1045	Plant height, pods per cluster, pods per plant, pod length and harvest index
ML131 X PDM 139	Pod length. Plant height, days to 50% flowering and maturity
ML 131 X RMG-1045	Days to 50% flowering, maturity, plant height, primary branches per plant, and pod length
M1L31 X RMG 344	Days to 50% flowering , maturity and plant height

Table 4.12: Best heterotic hybrids for quality traits (seed protein and seed methionine content) and for other traits in green gram

Superior heterotic hybrids for seed protein and seed methionine content over mid and better parent	**Heterosis for other traits**
Crosses for seed protein content	
IPM 99-125 X RMG-1045	Days to 50% flowering and maturity
BM 4 X RMG-1045	Plant height, days to 50% flowering and maturity
RMG1035 X RMG-1045	Days to 50% flowering, plant height, secondary branches per plant, biological yield per plant and seed yield per plant
ML 131 X RMG 344	Plant height, days to 50% flowering and maturity
Crosses for seed methionine content	
BM 4 X ML 131	Pod length, days to flowering and maturity
BM 4 X PDM 139	Seeds per pod and methionine content

The cross combinations viz. BM-4 x PDM 139 appeared as the most promising for *per se* performance and average heterosis for seed yield and also for seed methionine content, while cross RMG-1035 x RMG-1045 was pointed out to be superior for Seed protein content, which indicated a possibility of producing superior progenies combining seed protein or seed methionine content with seed yield. These crosses could be extensively used in breeding programme to develop superior segregants and better pure lines could be derived in further breeding programmes.

3. COMBINING ABILITY ANALYSIS

The concept of combining ability analysis has significant practical implication in plant breeding as it allows the prediction of the relative efficiency of parents based on early generation performance besides enabling to study the comparative performance of lines in hybrid combinations. General and specific combining ability estimates help ascertain the breeding potential of genotypes. While the former reveals about the ability of various genotypes, when used as parents, to reliably transmit their genetic potential to hybrid progeny, the latter highlights the unexpected favourable or unfavourable genic interactions in various genotypes. Without genetic direction, plant

breeders lack the rational basis to guide him in the choice of parents, in the manipulation of progenies and isolation of superior parents.

Combining ability analysis provides clues to the usefulness of individuals to be employed as the parents in the hybridization programme as well as simultaneously to screen the hybrids. General combining ability effects and additive x additive gene action are theoretically fixable. On the other hand, specific combining ability attributed to non additive gene action may be due to dominance or epistasis or both and is not fixable. Thus based on such information breeder can spot superior donor parents having high GCA effect and *per se* performance and also identifying promising crosses for use in improvement work. In self-pollinated crop like green gram where pure line breeding is a thumb rule, crosses with high mean and SCA effects are more likely to show transgressive segregation and lead to development of superior pure lines.

In the light of above facts, a diallel set comprising 8 parents, their 28 F_1s using method 2, Model I (Griffing, 1956) was evaluated for estimation of combining ability effects for different yield contributing characters and grain protein and methionine content .

Analysis of variance:

The analysis of variance for combining ability of each character is presented in Table-4.13. In the present investigation the mean squares due to GCA were significant for all the sixteen attributes. Whereas mean squares due to SCA were significant for all the characters except for days to 50% flowering, clusters per plant and seed yield per plant. Non-significant of SCA for these three characters indicated that characters were under the control of fixable portion of genetic variance. Higher magnitude of GCA effects than SCA effects were observed for days to 50% flowering, maturity, plant height , primary branches per plant, secondary branches per plant, clusters per plant, pods per cluster, seeds per pod,100 seeds weight, seed yield per plant, biological yield per plant, and harvest index indicating predominance of these traits by additive gene effects. Higher magnitude of SCA effects than GCA effects were observed for characters pods per plant, pod length, protein content and methionine content**.** Similar to the present findings, Malhotra *et al.* (1980), Thimmappa *et al.* (1989), Saxena and Sharma (1992), Reddy *et al.* (1992), Dasgupta *et al.* (1998), Kute *et al.* (1999), Aher *et al.* (1999), Kute and Deshmukh (2002), Singh *et al.* (2007),

Rehman *et al*. (2010) Patil *et al.* (2011) recorded importance of additive gene effects for one or other aforesaid characters in green gram.

Table 4.13: Analysis of variance showing mean squares for sixteen characters in Green gram

S. No	Characters	GCA [7]		SCA [28]		Error [70]
1	**Days to 50 % flowering**	16.69	**	3.66		2.61
2	**Days to maturity**	24.72	**	9.93	**	4.86
3	**Plant height**	136.44	**	67.75	**	7.41
4	**Primary branches / plant**	0.80	**	0.09	**	0.04
5	**Secondary branches / plant**	1.82	**	0.24	*	0.14
6	**Clusters / plant**	1.42	**	0.21		0.18
7	**Pods / cluster**	0.06	*	0.13	**	0.02
8	**Pod length**	0.49	*	0.93	**	0.20
9	**Pods / plant**	30.69	**	47.18	**	1.67
10	**Seeds / pod**	1.42	**	1.02	**	0.13
11	**100 Seed weight**	0.70	**	0.39	**	0.05
12	**Seed yield / plant**	6.52	**	0.27		0.23
13	**Biological yield**	33.93	**	13.76	**	6.03
14	**Harvest index**	30.30	**	11.03	**	3.17
Quality traits						
15	**Seed protein content**	0.55	**	1.78	**	0.08
16	**Seed methionine content**	0.01	**	0.01	**	0.01

* Significant at 5 per cent level and **Significant at 1 per cent level

General and specific combining ability effects:

The result pertaining to general combining ability effects of the parents (g_i) and specific combining ability effects of the crosses (S_{ij}) were estimated separately and are presented in (Table 4.14, 4.15, 4.16, 4.17, 4.18 and 4.19). Variable results were observed with regard to GCA and SCA effects for different characters.

For days to 50% flowering and maturity the GCA and SCA effects of the parents and hybrids in negative direction were considered to be desirable as the

earliness is preferred over the late varieties. GCA effects computed for days to 50% flowering revealed that the two parent's viz. IPM-02-03 and RMG-1045 exhibited negative significant GCA effects, indicating as good combiners for days to 50% flowering. The *per se* performance of these two parents were also in accordance with their GCA effects. Out of twenty eight crosses, maximum negative significant SCA effects was displayed by the cross combination IPM 99-125 x RMG-1045 (-3.46) followed by cross ML-131 x RMG-344 (-1.73) (Table 4.14 and 4.16).

Only one parent RMG-1045 exhibited negative significant GCA effect for days to maturity. However, four crosses exhibited significant negative SCA effects. The highest significant negative SCA effects were exhibited by cross ML-131 x RMG -344(-8.27) followed by cross IPM 99-125 x RMG-1045(-6.73) and BM-4 x RMG-1035 (Table 4.14 and 4.16).

For plant height four parents exhibited positive significant GCA effect in the as evident in Table 4.17. The result revealed that parent IPM 02-03(3.92) followed by ML-131 (3.20) and RMG-1045 turned out to be the best combiner for plant height (Table 4.14 and 4.16). Out of nine significant crosses, six crosses exhibited significant positive SCA effects. The highest significant positive SCA effects was exhibited by cross BM-4 x RMG-1045 (13.43) followed by cross ML-131 x RMG-1035 and IPM 02-03 x RMG-10455.

Wilson *et al.* (1985), Reddy *et al*. (1992), Kute *et al.* (1999), Aher *et al* (2001), Gawande *et al.* (2002), Kute and Deshmukh (2002), Manivannan *et al.* (2002), Barad *et al.* (2008) and Kumar *et al*. (2010) also identified superior parents and cross combinations on the basis of GCA and SCA effects for days to 50% flowering, days to maturity and plant height in green gram.

Four parent displayed positive significant GCA effect for primary branches per plant and Parent ML-131 followed by RMG-344 exhibited the highest significant positive GCA effects. Out of twenty eight crosses, only three crosses showed positive significant SCA. The highest significant positive SCA effects was exhibited by IPM 99-125 x BM-4 followed by cross IPM 02-03 x RMG-1045 and RMG-1035 x RMG-344 in F_1s. For secondary branches per plant significant positive GCA effects exhibited by one parent BM-4. Out of four significant crosses, only one cross RMG-1035 x RMG-1045 showed positive significant specific combining ability effects. Thimmappa *et al.* (1989), Saxena and Sharma (1992), Tiwari *et al*. (1993), Aher

et al. (1999), Gawande *et al.* (2002), Manivannan (2002), Singh *et al.* (2007), and Barad *et al*. (2008) also identified good general combiners for these two traits in green gram.

GCA effects computed for clusters per plant revealed that parent BM-4 and ML-131 depicted significant positive effects, indicating as good general combiner for this trait (Table 4.15 and 4.16). Estimates of specific combining ability effects were negatively significant for all the crosses as also reported by Aher *et al*. (2001) and Gupta *et al*. (2006)

For pods per cluster only parent RMG-1035 recorded highly significant positive GCA effects. Estimates of specific combining ability effects showed that out of fourteen significant crosses, six crosses showed positive significant specific combining ability effects. However, cross BM-4 x RMG-344 followed by cross ML-131 x RMG-1035 and IPM 02-03 x PDM-139 depicted the highest significant positive SCA effects recorded (Table 4.15 and 4.16). Kute and Deshmukh (2002) and Gupta *et al*. (2006) also recorded the similar results.

ML-131(0.34) exhibited significant positive GCA effects for pod length thus could be regarded as good general combiner for this trait. SCA effects revealed that the crosses BM-4 x IPM 02-03, BM-4 x ML-131, RMG-344 x RMG-1045 and IPM 99-125 x RMG-1035 were positively significant. Out of twenty eight, 13 crosses were significant and six crosses exhibited significant positive SCA effects for this trait (Table 4.15 and 4.16).

Three parents ML-131, RMG-1035 and PDM -139 revealed significant positive GCA effects for pods per plant. Estimates of SCA effects revealed that out of twenty-one significant crosses, five crosses exhibited positive significant SCA effects. The cross BM-4 x IPM 02-03 followed by IPM 02-03 x RMG-1045 , RMG-344 x RMG-1045 and ML-131 x PDM-139 depicted higher significant positive SCA effects for pods per plant (Table 4.17 and 4.19). Manivannan (2002), Kute and Deshmukh (2002), Dethe *et al*. (2008) and Kumar *et al*. (2010) also recorded high SCA effects in their material .

Parents BM-4 and IPM 99-125 displayed significant positive GCA effects for seeds per pod hence appeared as the best combiners. Five crosses depicted significant positive SCA effects for the trait (Table 4.17 and 4.19). The hybrid BM-4 x PDM-139

depicted the highest SCA effect followed by cross BM-4 x RMG-344, IPM 99-125 x RMG-344 and BM-4 x RMG-1035 for the trait. Reddy *et al.* (1992), Aher *et al.* (2001), Aher *et al.* (1999), Anbumalarmathi *et al.* (2004) and Kumar *et al.* (2010) also identified superior parents and cross combinations on the basis of GCA and SCA effects for seeds per pod in green gram.

For 100-seed weight three parents showed significant positive GCA effects in green gram. viz., IPM 99-125 exhibited highest significant positive GCA effect for this trait followed by BM-4 and IPM 02-03. Estimates of SCA effects revealed that out of ten significant crosses, three crosses in exhibited positive significant SCA effects (Table 4.17 and 4.19). The cross IPM 99-125 x BM-4(1.63) depicted the highest significant positive SCA effects in for 100-seed weight followed by cross IPM 99-125 x ML-131(1.54). Similar result has been found by Thimmappa *et al.* (1989), Tiwari *et al.* (1993), Kute and Deshmukh (2002), Gupta *et al.* (2006) and Ajmal *et al.* (2007).

A perusal of Table 4.17 and 4.19 indicated that four parents revealed significant positive GCA effects for seed yield per plant. These were BM-4(0.74), PDM -139, ML-131 and IPM 99-125. Estimates of SCA effects revealed that three crosses exhibited positive significant SCA effects. The cross RMG-344 x RMG-1045, followed by RMG-1035 x RMG-1045 and BM-4 x PDM-139 depicted maximum positive SCA effects for seed yield per plant. Manivannan (2002), Barad *et al.* (2008) Intwala *et al.* (2009) and Jayamani and Sathy (2011) also identified superior general and specific combiners for grain yield in their green gram material.

GCA effects estimated for biological yield per plant revealed that only one parent ML-131 depicted positive significant GCA effects. Out of twenty eight crosses, only three crosses showed significant SCA effects and IPM 99-125 x IPM 02-03 exhibited highest SCA effects and was considered as the best specific combiner.

A perusal of Table 4.18 and 4.19 indicated that IPM 99-125 and PDM-139 exhibited significant positive GCA effects, hence appeared as the best combiners for harvest index. Four crosses viz., RMG-344 x RMG-1045 the depicted highest SCA effects followed by IPM 99-125 x ML-131(6.01) , PDM-139 x RMG-1035, and RMG-1035 x RMG-1045 displayed significant positive SCA effects.

Quality traits:

Parents RMG-1035 and IPM 99-125 displayed significant positive GCA effects in F_1s for seed protein content hence appeared as the best combiners. Out of twenty significant crosses, ten crosses depicted significant positive SCA effects for the trait. The hybrid ML-131 x RMG-344 displayed the highest SCA effect for protein content followed by RMG-1035 x RMG-1045, IPM 99-125 x RMG-1045 and ML-131 x RMG-1035. Aher *et al.* (1999) and Dasgupta *et al.* (1998) also identified superior general and specific combiners for seed protein content in green gram.

Parents ML-131 and RMG-1045 showed significant positive general combining ability effects for seed methionine content. (Table 4.18 and Table 4.19).While, out of sixteen significant crosses, seven crosses depicted significant positive SCA effects for the trait. The hybrid BM-4 x PDM-139 showed the highest seed methionine content followed by cross PDM-139 x RMG-344, IPM 99-125 x ML-131 and IPM 99-125 x RMG-1045.

Therefore, an attempt has been made to identify atleast superior parents/best combiners and crosses/specific combiners for different characters on the basis of various parameters viz. *per se* performance, GCA effects and SCA effects and heterotic response over mid and better parent over F_1 in green gram (Summary Table 4.22).

Since SCA effects were related to GCA effects of their parents, performance of crosses on the basis of GCA was more efficient than that of SCA. Therefore, more stress should be laid on GCA effects rather than SCA effects. In the present study, an overall appraisal of GCA effects revealed that IPM 99-125 and BM-4 were good combiners for the majority of characters. High GCA effects are related to additive gene effects or additive x additive interaction effects which represented the fixable genetic component of variation. Hence BM-4 could be efficiently used for exploiting seed yield, whereas, IPM 99-125 appeared as most promising for seed protein content and ML-131 and RMG-1045 appeared as best parents for seed methionine content.

In contrast to GCA effects, SCA effects represent dominant and epistatic component of variation which are non-fixable and do not contribute tangibly to the improvement of self pollinated crops, except where commercial exploitation of heterosis is feasible. In the present study, estimates of SCA effects indicated that no cross combination was consistently good for all the characters studied. However, Significant SCA effects in desired direction for grain yield and its contributing traits

were recorded in hybrids BM-4 x PDM-139 and RMG-1035 x RMG-1045 involving two and atleast one of the parent with high GCA effects and good *per se* performance for seed yield and its important components .

Whereas, for seed protein content cross RMG-1035 x RMG-1045 appeared promising and parent RMG-1035 as best general combiner with high mean values. Similar to grain yield cross BM-4 x PDM-139 also appeared promising for seed methionine content and parent ML-131 as good combiner with high mean values. Thus these crosses could produce desirable transgressive segregates giving rise to new populations (Table 4.21).

Performance of crosses was also compared on the basis of SCA effects and their heterotic response over mid and better parent. High SCA effects denote high heterotic response. In general, there was less degree of association between these two for the characters studied, though ranking occupied by them was some what different.

For grain yield the crosses BM-4 x PDM-139 identified as promising on the basis of its high *per se* performance, average heterosis with high significant SCA effects. This cross involved parents of high x high general combining ability capable of producing progenies with high transgressive effects. The manifestation of heterosis and SCA effects for seeds per pod and pods per plant seemed to be responsible for increased seed yield in these crosses (Table 4.20).

On the other hand for seed protein content cross RMG-1035 x RMG-1045 possessed highly significant SCA effects, high average heterosis and heterobeltiosis response and medium *per se* performance in F_1. This crosses involved RMG-1035 with high GCA effects with high *per se* performance for seed protein content as well as significant SCA effects for seed yield per plant, secondary branches per plant and harvest index

An attempt has also been made in the present study to identify few crosses with those parents who possessed high GCA and *per se* performance with insignificant heterosis and SCA effects to exploit additive gene effects in advance generations. The crosses IPM 99-125 x BM-4, IPM 99-125 x ML-131, BM-4 x ML-131and ML-131 x PDM-139 exhibited insignificant SCA effects and heterosis, while the crosses involved both the parents with high GCA effects and high *per se* performance for seed yield per plant. The parents of these crosses were good general

combiners for these traits. Similarily, the crosses IPM 99-125 x RMG-1035 for seed protein content and cross ML-131 x RMG-1045 for seed methionine displayed insignificant SCA effects but these crosses having both the parents with high GCA effects. These parents having additive type of gene action which is fixable component and might be gainfully exploited in future breeding programmes.

4. MOLECULAR ANALYSIS USING RAPD AND ISSR MARKERS

Morphological markers with their complex and undeciphered genetic control were used for the individual identification and diversity studies; they may be affected by environmental effects and cultivation practices. In contrast to the morphological markers, molecular markers, are now available (Khan *et al*., 2000) in plant system involves improvement in the efficiency of conventional plant breeding by carrying out indirect selection through QTL, RAPD and ISSR techniques that provide a new alternative for cultivar identification (Gunter *et al.,* 1996. Lashermes *et al.,* 1996, Bouchired, 1997 and Colombo *et al.,* 2000). Ever since thermostable DNA polymerase was introduced in 1988 (Saiki, *et al*., 1988), the use of PCR (Mullis *et al*., 1986, Mullis and Faloona, 1987) in research has increased tremendously.

Molecular characterization is helpful in understanding the phylogenetic relationship between plant species and to reveal the genetic diversity within a given taxonomic group. Evaluation of genetic diversity would promote the efficient use of genetic variations (Paterson *et al.,* 1991), effective conservation and purity of the genotype to be determined as well as utilization of germplasm in crop improvement (Samarajeewa *et al.,* 2002). Apart from morphological and biochemical markers, molecular markers have been found to be useful and superior in analyzing the genetic differences in plant populations at the DNA level (Yoon *et al.,* 2000). The molecular markers are a powerful tool and they yield significant information on genetic variation thereby enhancing their scope (Das *et al.,* 2014). Data from even duplicate accessions can be distinguished using molecular markers.

Among several efficient methods for revealing genetic variability within and among plant populations, some of the most widely applied methods are RAPD (Wolff 1993; Wachira *et al.,* 1995; Brummer *et al*., 1995; Swoboda and Bhalla, 1997) and ISSR (Guo *et al.*, 2006; Joshi and Dhawan 2007; Heikal *et al*., 2008). Further, RAPD and ISSR markers are commonly used because they are quick, simple and environment non-sensitive enabling genetic diversity analysis in several types of plant

material like natural populations, population in breeding programmes and germplasm collections (Williams *et al.,* 1990). To achieve breakthrough in the yield and quality characters of *V. radiata* L., genetic divergence analysis has been attempted so that the highly diverse genotypes could be selected for molecular breeding programmes.

The present investigation was carried out to analyse relatedness and diversity among eight parents viz. IPM 99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045. Purified and isolated DNA was subjected to PCR based markers (RAPD and ISSR) for assessment of genetic diversity. The results obtained are discussed here:

Table 4.23: Quality and quantity of total genomic DNA of *V. radiata* L. isolated and purified by CTAB method

Genotypes	Parents' Name	Concentration (ng/ µl)	Ratio 260/280
P1	IPM 99-125	1420	1.81
P2	BM-4	968	1.77
P3	ML-131	1250	1.79
P4	IPM 02-03	1518	1.89
P5	PDM-139	1251	1.80
P6	RMG-1035	1012	1.81
P7	RMG-344	757	1.74
P8	RMG-1045	998	1.82

Total genomic DNA was isolated with CTAB method Doyle and Doyle (1987). The podwered plant tissues extracted with extraction buffer containing chelating agent (EDTA) which helped to inactivate nucleases released from the plant cells which could cause serious degradation of the genomic DNA. Major contaminants in crude DNA preparation are RNA, proteins and polysaccharides. The RNA was removed by treating with RNase. Extraction with phenol–chloroform mixture was employed for eliminating most of the proteins. The quality of DNA was determined by calculating the ratio between A_{260} and A_{280} which ranged from 1.74-1.89. Quality of DNA was also supported by appearance of single, compact, sharp band that was not sheared on 0.8% agarose gel electrophoresis corresponded to the high molecular weight DNA compared with standard λ Hind III DNA marker.

The amount of DNA isolated from various genotypes of *V. radiata* L. ranged from 757 to 1518 ng/µl (Table 4.23). The genotype IPM 02-03 yielded the highest amount of DNA (1518 ng/µl). Whereas the lowest amount of DNA (757 ng/µl) was obtained from genotype RMG-344. The ratio of absorbance (A260/A280) ranged from 1.70 to 1.89 revealing that the DNA obtained was free from contaminants like polysaccharides, protein and RNA. The quality of DNA as also checked by gel electrophoresis revealed a single discrete band in all genotypes (Plate-5) showing that genomic DNA was intact and had high molecular weight, free from any mechanical or enzymatic degradation, free from RNA contamination and was of high quality.

4.1 MOLECULAR MARKER (RAPD and ISSR) ANALYSIS

RAPD has been used extensively for classification of varieties, identification of cultivars and diversity estimation in various crops such as green gram (Karuppanapandian *et al.,* 2006). Similarly, ISSR markers are useful in detecting polymorphism among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome (Reddy *et al.*, 2002). The RAPD and ISSR techniques are more informative for estimating the extent of genetic diversity and relationships between green gram varieties. So far, very little attention has been given to varietal improvement of legumes (Sultana *et al.*, 2006; Nisar *et al.,* 2006). The present study aimed to analyze the extent of genetic diversity, using a total of 25 RAPD and 25 ISSR primers, respectively, to generate DNA fingerprints of eight parents of *V. radiata* L. with a view to detect polymorphism and access to information on diversity among these genotypes.

Optimization of PCR Conditions for RAPD and ISSR Analysis

PCR amplification conditions such as concentration of template DNA, primers, concentration of $MgCl_2$, *Taq* DNA polymerase and annealing temperature were optimized for RAPD and ISSR primers. Reproducible and clear banding patterns were obtained in a reaction mixture of 20 µl containing 50 ng of template DNA, 2 µl of 10 X *Taq* DNA polymerase buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.30 µM of primer and 1 U of *Taq* DNA polymerase, at an annealing temperature of 37°C (RAPD) and 42.9°-67° C (ISSR) for PCR amplification. Similarily, optimization of the concentration of template DNA, $MgCl_2$, *Taq* polymerase and of primers were

found similar to findings reported by Khamassi *et al.* (2011). An annealing temperature of 37°C (RAPD) and 41.3°-67.2° C (ISSR) were found optimum for PCR amplification.

4.1.1 DNA Polymorphism in *V. radiata* L.

RAPD

The RAPD technique has been applied to assess molecular polymorphism in chickpea (Sonnante and Beckman, 1997), *V. ungularis* (Yee *et al.,* 1999), green gram (Lakshanpaul *et al.,* 2000) and black gram (Souframanien and Gopalakrishna, 2004). However, in this study, twentyfive RAPD primers having 60% or more GC content were used for the present investigation. Out of 25 primers only 17 were amplified. A total of 104 amplified bands were obtained of which 91 were polymorphic and 13 monomorphic that showed 88 % polymorphism. The total number of amplified bands varied between 5 and 8. The average number of bands per primer was found to be 6.12 and average numbers of polymorphic bands per primer were 5.35. The polymorphism amongst all genotypes of *V. radiata* L. was 88% and the overall size of PCR amplified products ranged between 100 bp to 2500 bp. The per cent polymorphism ranged from as low as 60 % (OPA-15 and OPB-06) to as high as 100 % (OPA-09, OPA-10, OPA-08, OPB-03, OPB-07, OPE-03, and OPA-16).

The DNA amplification and polymorphism generated among various genotypes of green gram using RAPD primers are presented in Table 4.24. The representative photographs of the DNA banding pattern of individual plant DNA samples from genotypes of *V. radiata* L. after amplification with RAPD primers are depicted in Plate-1 to Plate-5.

Electrophoresis pattern of RAPD profile was studied on 1.2 per cent agarose gel. Only those fragments which consistently amplified were considered for analysis. Each RAPD band was assumed to represent a single locus and data were scored as presence of bands (1) and its absence as (0). Results are illustrated in Appendix IX.

Similar to present finding, Datta *et al.* (2012) studied a total of sixty RAPD primers that were used to assess the diversity of Indian green gram cultivars. The total number of polymorphic bands amplified was 224 (90%). The size of products ranged from 250 bp to 2600 bp. Undal *et al.* (2011) obtained high level of polymorphism of

92.82 per cent among green gram genotypes. Earlier, Saini *et al*. (2010) used RAPD molecular markers to analyze genetic diversity between 39 green gram genotypes.

Table 4.24: Polymorphism information of RAPD primers analyzed

Sl No	Primer	Total No of bands (a)	Total no. of polymorphic bands (b)	Polymorphism % (b/a X 100)	Range of band size
1	OPA-02	7	6	86	200-1000
2	OPA-05	6	5	83	300-2000
3	OPA-07	7	6	86	300-1000
4	OPA-08	7	7	100	400-2000
5	OPF-19	6	4	67	200-1500
6	OPP-03	5	4	80	300-1500
7	OPB-06	5	3	60	100-900
8	OPA-10	6	6	100	200-1000
9	OPP-10	8	8	100	200-1500
10	OPA-11	6	5	83	400-1500
11	OPA-14	NA	NA	NA	-
12	OPA-15	5	3	60	400-1000
13	OPC-01	NA	NA	NA	-
14	OPB-03	6	6	100	100-1500
15	OPA-09	7	7	100	200-2500
16	OPB-07	6	6	100	300-1000
17	OPC-05	NA	NA	NA	-
18	OPE-03	5	5	100	400-1500
19	OPA-16	6	6	100	400-2000
20	OPC-06	NA	NA	NA	-
21	OPB-02	6	4	67	400-2000
22	OPB-04	NA	NA	NA	-
23	OPB-05	NA	NA	NA	-
24	OPB-08	NA	NA	NA	-
25	OPB-10	NA	NA	NA	-
Total		**104**	**91**	**88**	-
Average		**6.12**	**5.35**		

NA- Not amplified

Thirty primers were used resulting in the amplification of 411 fragments, of which 328(92.9%) were polymorphic. Reddy *et al.* (2008) reported that accessions

with most distinct DNA profiles are likely to contain the greatest number of novel alleles.

Table 4.25: Details of the RAPD primers used for amplification of genomic DNA of green gram

Total number of primers	25
Number of primers which showed amplification	17
Number of primer which showed polymorphism	17
Total number of monomorphic bands	13
Total number of polymorphic bands	91
Total number of bands	104
Total number of amplicon produced	391

ISSR

Twenty five ISSR primers were used for the present investigation out of which eighteen primers showed amplification in all genotypes. A total of 109 amplified bands were obtained from the 18 primers, out of which 88 were polymorphic. The total number of amplified bands varied between 5 and 8 (Table 4.26). The average number of bands per primer was found to be 6.22 and average numbers of polymorphic bands per primer were 4.89.

The polymorphism percentage ranged from 43 % (UBC-845) to 100% for five primers (ISSR-01, UBC-817, UBC-818, UBC-820 and UBC-854) used. Average polymorphism across all the genotypes of *V. radiata* L. was found to be 79%. Overall size of PCR amplified products ranged between 100 bp to 2000 bp. The PCR amplification using ISSR primers gave rise to reproducible amplification products. The number of potential ISSR markers depends on the variety and frequency of microsatellites, which tends to change with species (Despeiger *et al.,* 1995).

Electrophoresis pattern of ISSR profile was studied on 1.2 per cent agarose gel. Only those fragments which consistently amplified were considered for analysis.

Each ISSR band was assumed to represent a single locus and data were scored as presence of bands (1) and its absence as (0). Results are illustrated in Appendex –X.

The DNA amplification and polymorphism generated among various genotypes of green gram using ISSR primers are presented in Table 4.26.

The representative photographs of the DNA banding pattern of individual plant DNA samples from genotypes of *V. radiata* L. after amplification with ISSR primers are depicted in Plate-5 to Plate-10.

Similar results were shown by Das *et al.* (2014) who used ten primers that amplified a total number of 353 bands under 93 loci across five genotypes of green gram with an average of 9.3 loci / primer, exhibiting an overall polymorphism of 52.69%. Singh *et al*. (2011) also studied ISSR markers that were used to study DNA polymorphism in elite green gram genotypes. They found that percentage polymorphism had ranged from 25% to 85%. Tantasawat *et al.* (2010) measured genetic diversity and relatedness in 17 green gram and 5 blackgram genotypes by ISSR analysis. The 18 ISSR primers had produced 341 scorable fragments of which 309 fragments were found to be polymorphic (90.6%).

Table 4.27: Details of the ISSR primers used for amplification of genomic DNA of green gram

Total number of primers	25
Number of primers which showed amplification	18
Number of primer which showed polymorphism	18
Total number of monomorphic bands	21
Total number of polymorphic bands	88
Total number of bands	109
Total number of amplicon produced	563

4.1.2 Genetic relationship and Cluster Tree Analysis

The data obtained by using RAPD and ISSR primers (Appendix-IX and X) were further used to construct similarity matrix of eight *V. radiata* L. genotypes using 'Simqual' sub-programme of software NTSYS-pc. Dendrograms were constructed using similarity matrix values as determined from RAPD and ISSR data for *V. radiata* L. genotypes using unweighted pair group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc software.

Similarity Matrix

(A) Similarity matrix based on RAPD Markers

Based on RAPD similarity matrix data, the value of similarity coefficient ranged from 0.34 to 0.57 (Table 4.28). The average similarity across the eight parents was found out to be 0.46 showing that genotype were moderately diverse from each other. Maximum similarity value of 0.57 was observed between genotypes PDM-139 and RMG-1035; PDM-139 and RMG-1045 followed by PDM-139 x RMG-344 and RMG-1035 x RMG-344 with a similarity coefficient value of 0.54. Likewise, minimum similarity value of 0.34 was observed between genotypes IPM 99-125 and RMG-1045.

The findings confirm with that of Saini *et al.* (2010). They determined similarity coefficient among green gram genotypes to range from 40.8 per cent (ML 131 and UPM 98) to 90.3 per cent (MH 98-1 and ML 682). Lavanya *et al*. (2008) found similar results in other green gram varieties.

(B) Similarity matrices based on ISSR Markers

Based on ISSR similarity matrix data, the value of similarity coefficient ranged from 0.43 to 0.80 (Table 4.29). The average similarity across the eight parents was found out to be 0.62 showing that genotype were diverse from each other. Maximum similarity value of 0.80 was observed between genotypes RMG-1035 and RMG-1045 followed by RMG-1035 and RMG-344 with a similarity coefficient value of 0.79. Likewise, minimum similarity value of 0.43 was observed between genotypes BM-4 and IPM 02-03.

Similar findings were reported by Das *et al.* (2014) in green gram cultivars. The value of Jacaard's similarity coefficient ranged from 0.566 to 0.793. Singh *et al.*

(2011) also recorded similar results in 30 green gram genotypes through Dice analysis, the similarity coefficient ranging from 0.65 to 0.85 with an average of 0.69.

(C) Similarity Matrix Values Based on Combined RAPD and ISSR Markers

Perusal of the combined RAPD and ISSR similarity matrix data revealed that the values for different genotypes ranged from 0.42-0.68 (Table 4.30). The average similarity value across the genotypes was found out to be 0.55, indicating that there is sufficient genetic diversity among the genotypes. The genotypes that exhibited the highest similarity matrix values (0.68) are RMG-1035 and RMG-344; RMG-1035 and RMG-1045. However, BM-4 and IPM 02-03 were found to be genetically diverse with a minimum similarity value of 0.42.

4.1.3. Cluster Tree Analysis

The average linkages between *V. radiata* L. genotypes were used for constructing a tree depicting the phylogenetic relationship among eight *V. radiata* L. genotypes.

(A) RAPD Marker Based Cluster Tree Analysis

The Jaccard'ssimilarity coefficient based on UPGMA displayed in the range of 0.34 to 0.57. The RAPD cluster tree analysis of eight *V. radiata* L. genotypes showed that they could be divided into 2 major clusters viz., cluster I and cluster II at a similarity coefficient of 0.40 (Fig. 4.1). The dendrogram clearly indicated that cluster I included two genotypes IPM 99-125 and IPM 02-03 at 0.46 similarity coefficient. The cluster II was larger than cluster I, included six genotypes, viz. BM-4, ML-131, PDM-139, RMG-1035, RMG-1045 and RMG-344. Cluster II was devided intwo two sub-clusters, cluster II-A has only one genotype BM-4, where as cluster II-B has two sub-clusters, II B1 included genotype ML-131 and second sub cluster II B2 included four genotypes PDM-139, RMG-1035, RMG-1045 and RMG-344. II B2' has two sub-clusters, sub-cluster II B2'a included PDM-139 and RMG-1035 with 0.57 similarity coefficient, Looking to the morphological similarity both are having hard seed with approx. similar maturity time and yield potential and RMG-1045 included sub-cluster II $B2^1$ b. While, the genotype RMG-344 was grouped single in separate cluster (II B2").

Saini *et al*. (2010) reported almost similar results among 39 genotypes of green gram using 30 RAPD primers. Jaccard similarity coefficient ranged from 40.8 to 90.3%. The genotypes have been clustered into 11 clusters, three main groups and minor groups based on UPGMA. Similar results have also been shown by Datta *et al.* (2012), their dendrogram revealed genetic similarity among the twenty four varieties of green gram which ranged from 0.45 to 0.78.Similar findings have been reported by Lavanya *et al*. (2008).

(B) ISSR Marker Based Cluster Tree Analysis

The ISSR data based derivation of similarity matrix shown in Table 4.28 reveal the similarity values lay between 0.43-0.80. The dendrogram clearly indicated four major clusters (Fig. 4.2). Cluster I included two genotypes IPM 99-125 and ML-131 are similar to eachother at a similarity coefficient of 0.64. Cluster II is the main one that included four genotypes *viz.,* PDM-139, RMG-1035, RMG-1045 and RMG-344. It could be divided into two, sub-clusters IIA which joined with sub cluster IIB at similarity coefficient of 0.62. Subcluster IIA included only one genotype viz. PDM-139. Sub cluster IIB included three genotypes and also devided as IIB 1 and IIB 2. IIB 1 has two genotypes RMG-1035 and RMG-1045 are similar to eachother at a similarity coefficient of 0.80, these two genotypes showed the maximum similarity coefficient. Subcluster BII 2 included only one genotype viz. RMG-344.

Cluster III and cluster IV included only one genotype IPM 02-03 and BM-4 respectively. Cluster II joined with cluster IV at similarity coefficient of 0.49.

Similar results have been reported by Singh *et al.* (2013). The UPGMA distributed the 30 genotypes into five main clusters; clusters with Dice's analysis indicated similarity coefficient values ranging from 0.65 to 0.8. One genotype namely, ML 818 forms an out-group by not falling in any cluster. The variety PDM-139 used as standard check was grouped separately. The genetic variation amongst advanced lines of diverse crosses could be useful for selecting parents for crossing so as to yield populations required for breeding for yield and related agronomic traits.

(C) RAPD and ISSR Markers Based Combined Cluster Tree Analysis

The RAPD and ISSR data were evaluated to obtain a combined similarity matrix (Table 4.30). The similarity coefficient values lay between 0.46-0.68. The RAPD and ISSR cluster tree analysis showed that the eight genotypes could be divided into 4 clusters (Fig. 4.3). Cluster I included two genotypes *viz.*, IPM 99-125 and ML-131 that were similar to each other at a coefficient of 0.56. Cluster II included two sub clusters,sub cluster II A included genotype PDM-139 and II B devided intwo two sub clusters, genotypes RMG-1035 and RMG-344 included in sub clusters IIB 1 at similarity coefficient 0.68, while sub clusters IIB 2 has only one genotype RMG-1045. Cluster III and cluster IV included with each other at similarity coefficient 0.46

Singh *et al* (2014) compared three marker systems ISSR, RAPD and SSR to characterize the genetic diversity in 35 *Vigna* accessions. The similarity coefficient values ranged between 0.36 to 0.92. Based on their pooled allelic diversity data, the 35 genotypes could be grouped into four major clusters. Similar results have also been observed by Dikshit *et al.* (2009).

Breeding Methodology Suggested:

The present study has provided some meaningful information regarding nature and magnitude of gene effects for various characters Green gram.

Seed yield and its most of the important components showed importance of both additive and non-additive gene effects with preponderance of additive gene effects. However, the grain protein and seed methionine content was under influence of both additive and non additive gene effects. Higher magnitude of GCA effects than SCA effects were observed for days to 50% flowering, days to maturity, plant height , primary branches per plant, secondary branches per plant, clusters per plant, pods per cluster, seeds per pod,100 seeds weight, seed yield per plant, biological yield per plant ,and harvest index indicating predominance of these traits by additive gene effects. Higher magnitude of SCA effects than GCA effects were observed for characters pods per plant, pod length, protein content and methionine content.

While critically analyzing the over all performance of eight parents studied, BM-4 appeared to be the most promising followed by PDM-139 for seed yield and its

components, whereas RMG-1035 depicted its superiority for seed protein content and ML-131 showed promising for for seed methionine content. This might be due to the accumulation of favourable genes in these *elite* lines. Therefore these genotypes could be utilized in breeding programmes. On the basis of high heterosis and significant SCA effects, crosse BM-4 x PDM-139 involving both good combiners were identified for grain yield and its components. While BM-4 x PDM-139 displayed superiority for seed methionine content and RMG-1035 x RMG-1045 superior for seed protein content.

Since greengram is predominantly self-pollinated crop under such situations, the improvement in these crosses under study may be expected through standard selection procedures, which may first exploit the additive gene effects. Simultaneously, care should be taken that non-additive effects were nor dissipated, rather concentrated. It is therefore, suggested to develop pure lines by progeny selection for early improvement. Biparental mating will mop-up additive and additive x additive genetic variances. It will also allow breaking-up of unfavorable linkage groups present in repulsion phase. This will ensure full utilization of both types of gene effects additive and non-additive and ultimately lead to the fixation of the characters at the desired level.

Therefore, the cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance, GCA effects, heterosis with significant SCA effects in both for seed yield and its components. Further molecular analysis through RAPD and ISSR markers profiling revealed its parental genetic diversity having 53 per cent dissimilarity (47% similarity). In the dendrogram generated using combined RAPD and ISSR UPGMA cluster analysis based on Jaccard,s similarity coefficient, the parent BM-4 was grouped in cluster IV while PDM-139 in cluster II A thereby confirming that there was concurrence between the results obtained by molecular (RAPD and ISSR) and morphological markers along with their known pedigree. Therefore this cross can be gainfully utilized.

5. SUMMARY

The present investigation was carried out to elicit information on the the heterosis, combining ability and nature of gene action to identify desirable parents and crosses.An assessment of genetic diversity of parents using RAPD and ISSR markers in greengram [*Vigna radiata* (L.) Wilczek] was also performed.The experimental material comprised eight varieties viz. IPM 99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 which were selected on the basis of their origin, pedigree, maturity, adaptability, diversity and yield potential characters.

A 8 x 8 diallel set was prepared by crossing these varieties in all possible combinations (excluding reciprocals). Parents and their F_1 were planted during *kharif*, 2014 in randomized block design with three replications at Instructional Farm of Rajasthan College of Agriculture, Udaipur. Parents and F_1s were grown in two rows with two metre row length. Row to row and plant to plant distances were maintained as 30 cm and 10 cm, respectively. Non-experimental rows were planted all around the experimental plot to eliminate border effects. All the recommended cultural practices were adopted to raise the timely sown good crop.

The observations were recorded on ten randomly selected competitive plants in parents and F_1s for sixteen characters viz. days to 50% flowering, days to maturity, plant height, primary branches per plant, secondary branches per plant, clusters per plant, pods per cluster, pod length, pods per plant, seeds per pod, 100-seed weight, seed yield per plant, biological yield, harvest index and two quality traits viz. seed protein content and seed methionine content.

Following results were obtained after subjecting the data to analysis of variance, estimation of heterosis over mid and better parent, combining ability analysis by method 2, Model I of Griffing (1956). Molecular analysis of parents was also carried out using RAPD and ISSR molecular markers.

Salient features of the findings are summarized here as under:

1. The analysis of variance for experimental design was performed for sixteen characters. It revealed significant differences for all the characters indicating presence of adequate amount of variation among the genotypes, parents and

F_1s. However, mean squares due to Parent v/s hybrid component were significant for plant height, pods per cluster, pods per plant, 100-seed weight, seed protein content and seed methionine content, which depicted presence of heterosis for these characters.

2. Mean performance of parents and their hybrids reveled that parent BM-4 appeared to be the best parent for seed yield and most of the yield contributing charactes followed by IPM 99-125 and ML-131, while IPM 02-03 was superior for seed protein content. Similarly, cross BM-4 x PDM-139 exhibited high mean performance for seed yield per plant, while cross RMG-1035 x RMG-1045 exhibited superior *per se* performance for quality traits.

3. Heterosis over mid parent, for seed yield ranged from -19.85(RMG-344 x RMG-1045) to 9.93(IPM 02-03 x RMG-1035) per cent. Significant positive heterosis was depicted by BM-4 x PDM-139 and RMG-1035 x RMG-1045 and turned out to be the promising hybrids for seed yield and its components.Manifestation of heterosis for seed yield was mainly through pods per plant, pods per cluster, seeds per pod, 100-seed weight and harvest index.

4. Significant relative heterosis and heterobeltiosis for seed protein content was observed in eight and five crosses respectively. Maximum significant positive heterosis as well as heterobeltiosis was recorded in cross RMG-1035 x RMG-1045 followed by cross ML-131x RMG-1035, ML-131 x RMG-344 and cross BM-4 x RMG-1035.

5. Significant positive relative heterosis and heterobeltiosis for seed methionine content were also recorded. Maximum significant positive heterosis as well as heterobeltiosis was recorded for BM-4 x PDM-139 and turned out to be the best.

6. In the present investigation the mean squares due to GCA were significant for all the sixteen attributes. Whereas mean squares due to SCA were significant for all the characters except for days to 50% flowering, clusters per plant and seed yield per plant, indicating control of these three characters under fixable portion of genetic variance. Higher magnitude of GCA effects than SCA effects were observed for days to 50% flowering, days to maturity, plant height , primary branches per plant, secondary branches per plant, clusters per

plant, pods per cluster, seeds per pod,100 seeds weight, seed yield per plant, biological yield per plant and harvest index indicating predominance of these traits by additive gene effects. Higher magnitude of SCA effects than GCA effects were observed for characters pods per plant, pod length, protein content and methionine content indicating preponderance of non-additive gene action for these characters.

7. While critically analyzing the over all performance of eight parents studied, BM-4 appeared to be the most promising followed by PDM-139 for seed yield and its components, whereas RMG-1035 depicted its superiority for seed protein content and ML-131 showed promising for for seed methionine content. This might be due to the accumulation of favourable genes in these *elite* lines. Therefore these genotypes could be utilized in breeding programmes.

8. GCA effects for grain yield were associated with GCA effects of its component characters. In general, *per se* performance of parents was related to their GCA effects.

9. The crosses BM-4 x PDM-139 and RMG-1035 x RMG-1045 exhibited high heterosis and significant SCA effects for seed yield. Out of these RMG-1035 x RMG-1045 depicted high heterosis, heterobeltiosis and significant SCA effects for protein content and BM-4 x PDM-139 showed superior for high heterosis, heterobeltiosis and significant SCA effects for seed methionine content.

10. In general, SCA effects were related to the GCA effects of their parents. There was also some degree of association between SCA effects and heterotic response.

11. RAPD and ISSR marker analysis was performed to detect relatedness and diversity among eight parental genotypes.

    (a) Twenty five RAPD primers having 60% or more GC content were used for the present investigation. Out of 25 primers only 17 were amplified and produce total 391 amplified fragments (amplicon) ranged between 100 bp to 2500 bp. Out of 104 scorable bands, 91 were polymorphic that showed 88 per cent polymorphism. The average number of bands per

primer was found to be 6.12 and average numbers of polymorphic bands per primer were 5.35. OPP-10 proved to be best primer in our investigation with total 52 fragments and eight highest scorable bands as well as 100 per cent polymorphism.

(b) Out of 109 scorable bands, 88 bands were polymorphic and the level of polymorphism was 81 per cent.Twenty five ISSR primers were used, out of which eighteen primers showed amplification in all genotypes. The average number of bands per primer was found to be 6.22 and average numbers of polymorphic bands per primer were 4.89. ISSR-01 proved to be best primer in our investigation with total 29 fragments and eight highest scorable bands as well as 100 per cent polymorphism.

12. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix. The similarity coefficient values lay between 0.46-0.68. The RAPD and ISSR cluster tree analysis showed that the eight genotypes could be divided into 4 clusters. The genotype BM-4 was grouped in separate IV cluster. However, PDM-139 was grouped on cluster IIA. In the light of RAPD and ISSR study the parents of the cross BM-4 x PDM-139 were also noticed for their genetic diversity, having 53% dissimilarity and grouped into the separate clusters.

13. Therefore, the cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance, GCA effects, heterosis with significant SCA effects in both for seed yield and its components. Further molecular analysis through RAPD and ISSR markers revealed its parental genetic diversity having 53 per cent dissimilarity. The parent BM-4 was grouped in cluster IV while PDM-139 in cluster II A, thereby confirming that there was concurrence between the results obtained by molecular (RAPD and ISSR) markers and morphological variation along with their centre of origin and known pedigree. Therefore this cross can be gainfully utilized.

14. On the basis of present investigations suggestions regarding breeding methodology for improvement in seed yield and grain protein content of greengram have been given.

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Combining Ability Analysis for Grain Yield in Green Gram [*Vigna radiata* (L.) Wilczek]

ABSTRACT

Miss Anamika Nath* **Dr. S. R. Maloo****

Ph.D. Research Scholar **Major Advisor**

The present investigation entitled, "Combining ability analysis for grain yield in Green gram [*Vigna radiata* (L.) Wilczek]" was undertaken by crossing eight diverse genotypes namely viz. IPM 99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 in diallel fashion (excluding reciprocals). Molecular analysis of these parental genotypes was also carried out using RAPD and ISSR markers. The experimental material comprising 8 parents along with 28 F_1s was evaluated during *kharif* season, 2014 in randomized block design with three replications at Instructional Farm of Rajasthan College of Agriculture, Udaipur, India.

The analysis of variance for experimental design was performed for sixteen characters. It revealed significant differences for all the characters indicating presence of adequate amount of variation among the genotypes. Further partitioning of mean squares due to parents were significant for all the characters except clusters per plant and pod length, while F_1s were significant for all the characters, revealed that adequate amount of variation was present for parents and F_1s . However, mean squares due to Parent v/s hybrid component were significant for plant height, pods per cluster, pods per plant, 100-seed weight, seed protein content and seed methionine content, which depicted presence of heterosis for these characters. The valus presented in appendices v indicate that the variation was very limited for primary branches, secondary branches, pods/ clusterseeds per pod and 100 seed weight. This is the reason that the ss due to hybrids vs parents was found to be non significant.

Mean performance of parents and their hybrids reveled that parent BM-4 followed by IPM 99-125 appeared to be the best parent for seed yield and most of the yield contributing charactes while IPM 02-03 was superior for seed protein content, Similarly, cross BM-4 x PDM-139 exhibited high mean performance for seed yield per plant and RMG-10435 x RMG-1045 for seed protein content.

Crosses viz. BM-4 x PDM-139 and RMG-1035 x RMG-1045 exhibited heterosis for grain yield and its components. For seed protein content and seed methionine content maximum significant positive heterosis as well as heterobeltiosis was recorded in cross RMG-1035 x RMG-1045 and BM-4 x PDM-139, respectively.

* Ph.D. Research Scholar, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur.

** Dean, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, (Raj.), India.

Combining ability analysis was performed by employing method 2, Model I of Griffing (1956), it revealed that the mean squares due to GCA were significant for all

the sixteen attributes. Higher magnitude of GCA effects than SCA effects were observed for twelve characters indicating predominance of additive gene effects. Higher magnitude of SCA effects than GCA effects were observed for characters pods per plant, pod length, seed protein content and seed methionine content indicating preponderance of non-additive gene action for these characters.

While critically analyzing the over all performance of eight parents studied, BM-4 appeared to be the most promising followed by PDM-139 for seed yield and its components, whereas RMG-1035 depicted its superiority for seed protein content and ML-131 showed promising for for seed methionine content. This might be due to the accumulation of favourable genes in these *elite* lines. Therefore these genotypes could be utilized in breeding programmes.

Crosses BM-4 x PDM-139 and RMG-1035 x RMG-1045 exhibited heterosis and high significant SCA effects for seed yield, out of these RMG-1035 x RMG-1045 depicted high heterosis and significant SCA effects for seed protein while BM-4 x PDM-139 showed superior for high heterosis and significant SCA effects for methionine content.

Twenty five RAPD and ISSR markers were used for the present investigation. 17 RAPD and 18 ISSR primers were amplified and produce total 391 and 563 amplified fragments, respectively. Out of 104 scorable RAPD bands, 91 were polymorphic that showed 88 per cent polymorphism and out of 109 scorable ISSR bands, 88 bands were polymorphic and showed 81 per cent polymorphism. OPP-10 and ISSR-01 proved to be best RAPD and ISSR primer, respectively. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix. The similarity coefficient values lay between 0.46-0.68. The cluster tree analysis showed that the eight genotypes could be divided into 4 clusters.

Therefore, the cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance, GCA effects, heterosis with significant SCA effects for seed yield and its components. Further molecular analysis through combined RAPD and ISSR markers revealed its parental genetic diversity having 53 per cent dissimilarity. The parent BM-4 was grouped in cluster IV while PDM-139 in cluster II A, thereby confirming that there was concurrence between the results obtained by molecular (RAPD and ISSR) and morphological markers along with their origen and known pedigree. Therefore this cross can be gainfully utilized.

On the basis of present investigations suggestions regarding breeding methodology for improvement in seed yield, seed protein and seed methionine content of green gram have been given.

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iSr`d vkSj muds ladj esa BM-4 cht mit vkSj mit ds y{k.kksa ds ;ksxnku ds fy, lcls T;knk iSr`d iznf'kZr gqvk tcfd IPM 02-03 cht izksVhu vo;o ds fy, csgrj Fkk tcfd blh rjg ladj RMG-10435 ×

RMG-1045 cht izksVhu vo;o ds fy, csgrj izn'kZu n'kkZ;k x;k tcfd ladj BM-4 × PDM-139 la;a= izfr cht mit ds fy, mPp ek/; izn'kZu fd;kA

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*LukRdkRrj 'kk/k Nk=] ikni çtuu ,o vkuokf'kdh foHkkx] jkt LFkku Ñf"k egkfo|ky;] mn;ijA

**vkpk; ,o foHkkxk/;{k] ikni çtuu ,o vkuokf'kdh foHkkx] jkt LFkku Ñf"k egkfo|ky;] mn;ijA

BM-4 × PDM-139 vkSj RMG-1035 × RMG-1045 mit vkSj mlds ?kVdksa ds fy, ladj vkst ds fy, izn'kZu fd;kA

cht izksVhu vo;o vkSj cht esfFkvksfuu] vo;o vf/kdre egRoiw.kZ ldkjkRed ladj vkst ds #i esa vPNh rjg ls ntZ dh xbZA vfrladj vkst Øe'k% RMG-1045 × PMG-1045 vkSj BM-4 × PDM-139 esa ntZ dh xbZA

fof'k"V la;kstu {kerk izHkko ls lkekU; la;kstu {kerk vf/kd egRoiw.kZ ik;k x;kA iq"ikoLFkk esa yxs fnu] ifjiDork] ikS/ks dh Å¡pkbZ] izkjfEHkd Vguh izfr ikS/kk] f}rh; Vguh izfr ikS/kk] dyLVj izfr Qyh] Qyh izfr cht] 100 cnkuksa ds Hkkj] nkuk mit izfr ikS/kk] tSfod mit izfr ikS/kk vkSj Qly lwpdkad] ;ksT; tho izHkko dh izcyrk dk ladsr gSA

lkekU; la;kstu {kerk izHkko ls fof'k"V la;kstu {kerk izHkko vf/kd ik;k x;k bu y{k.kksa ds fy, Qyh ij ikS/kk] Qyh dh yackbZ] cht izksVhu vo;o vkSj cht esfFkeksfuu vo;o vkSj ;ksT; y{k.kksa ds fy, ik;k x;kA

APPENDIX - II

Estimation of crude protein by micro-kjeldahl's method using Nessler's reagent

Principle

Nessler's reagent is an alkaline aqueous solution of potassium mercuric iodide ($Kl.Hgl_2$). It reacts with NH_3 (or NH_4 – salts) to give reddish-brown colour or precipitate. In the presence of sodium silicate the coloured precipitate are rapidly and completely removed from the solution leaving behind a clear, non-turbid coloured solution. The colour developed remains stable upto 15^{th} at room temperature ($20\text{-}40^{o}$ C) and its intensity is proportional to the initial concentration of NH_3 nitrogen. It is, therefore, possible to calorimetrically assay the concentration of NH_3 nitrogen by Nesslerization. The colour can be read at 440-650 nm but sensitivity is more at shorter wavelength.

Procedure

A. Digestion

1. Grind the seed material, weigh 0.1 g of sample and put in a dried Kjeldahl's flask.
2. Add 2 ml of concentrated H_2SO_4 (Analar) and digest on heater for 1.30 h (a short funnel may be used as a reflux).
3. To this add 0.5 ml of H_2O_2 (30%) with alternate heating and cooling till the colour disappears. Heat further until H_2O_2 fumes escapes.
4. Transfer the contents of Kjeldahl's flask to 100 ml volumetric flask and make volume.

B. Colour Development

5. Take 5 ml aliquot in 50 ml volumetric flask, add 2 ml and 1 ml of 10 % solution of NaOH and sodium silicate, respectively. Add 106 ml of Nessler's reagent and finally make volume with distilled water. Allow 10 min for full colour to develop.

C. Standard curve

Dissolve 0.1185 g of ammonium sulphate in distilled water and make the volume of one litre. Pipette out 0, 1, 2, 3, 4, 5, 6, 7 and 10 ml of this solution in 50

ml of volumetric flask. Colour is developed by procedure given above. Draw a graph of 'ppm nitrogen' versus optical density.

D. Estimation of Crude protein

Determine the N-content of samples of using the standard curve. The crude protein is calculated by multiplying the N-content with a conservation factor 6.25 for green gram seed.

APPENDIX - III

Steps involved in the estimation of methionin content by Horn *et al.* (1946) method.

1. One of the defatted sample was taken in a 250 ml conical flask, added 25 ml of 2N HCL and mixed well.
2. The solution was autoclaved at 15 PSI for one hour.
3. The colour of the hydrolysate was get rid off by adding a pinch of activated charcol and boiling it.
4. It was than filtered and the charcol was washed with hot water.
5. The washing was collected.
6. The colour free hydrolysate was neutralised with 10 N NaOH and the pH was brought to 6.5.
7. The volume of the hydrolysate was made up to 50 ml.
8. For colour development, 2 ml of acid hydrolysate was added to 3 ml of distilled water in a test tube.
9. Then, 1 ml of 5 N NaOH and 0.1 ml of 10 % sodium nitroprusside solution was added.
10. The mixture was shaken for 5 min.
11. 2 ml of 3 % glycine was added followed by 2 ml of orthophosphoric acid (85%) slowly with constant shaking.
12. A reddish colour was developed.
13. The intensity which was read at 530 mm against blank prepared by separately by omitting sodium nitroprusside.

CALCULATION:

A standard curve for methionin from 200-1000 ug versus optical density was plotted and the amount of methionin was calculated from it using the formula.

(Methionin content from the graph X 4) mg per 100 mg of protein.

APPENDIX- IV

List of reagents used for experimental analysis:

1.	**CTAB Extraction Buffer** Chemicals Stock Working Tris HCl 500mM 100mM EDTA 100mM 20mM NaCl 5 M 1.4 M CTAB - 2% β- merceptoethanol - 2.5µl **Autoclaved**
2.	**TE Buffer: (100ml)** Tris Buffer 10mM EDTA 1mM Set the pH at 8.0.
3.	**70 per cent Alcohol** To 70 ml of absolute alcohol add 30 ml of distilled water**.**
4.	**Chloroform: Isoamylalcohol (24: 1)** To make 50 ml add 48 ml of chloroform and add 2 ml of Isoamyl alcohol.
5.	**Electrophoresis Buffer TAE (50 X)** Tris base 242 gm Glacial acetic acid 57.1 ml EDTA 0.5 M Make up the volume 1000 ml (pH-8.0) and Autoclaved.
6.	**ß – mercaptoethanol (BME)** It is obtained as 14.4 M solution. Store in a dark bottle at 4^0 C.

7.	**RNase that is free from DNase** Dissolved pancreatic RNase (RNase A) at a concentration of 10 mg/ml in 0.01 M sodium acetate (pH – 5.2). Heated to 100^0C for 15 minutes. Allowed to cool slowly at Room Temperature (RT). Adjusted pH by adding 0.1 volume of 1 M. Tris. HCl. (pH – 7.4) Dispensed in aliquots and stored at – 20^0 C.
8.	**Ethidium Bromide (10 mg/ml)** Added 1 gm of Ethidium Bromide to 100 ml of H_2 O. Stirred on magnetic stirrer for several hours to ensure that the lye has dissolved. Wrap the container in aluminum foil or transfer the solution in dark bottle and store at room temperature.
9.	**1.) Agarose 0.8 per cent** 0.8 gms dissolved in 90ml of distilled water with 10 ml of 1 x TAE. **2.) Agarose 1 per cent** 1 gms dissolve in 90ml of distilled water with 10 ml of 1 x TAE .
10.	**Gel loading dye** 0.25 gms of Bromophenol Blue 0.25 gms of Xylene cyanol 50 per cent glycerol Make 100 ml final volume with 50 per cent glycerol
11.	**Assay buffer for Taq DNA polymerase (10 x)** 100mM TrisCl (pH – 9.0) 15 mM $MgCl_2$ 500mM KCl 0.1 Per cent Gelatin

APPENDIX – V

Mean values for Days to flowering, Days to maturity, Plant height, Primary branches / plant and Secondary branches / plant

S. No	Genotype and crosses	Days to flowering	Days to maturity	Plant height	Primary branches / plant	Secondary branches / plant
1	IPM 99-125	42.01	81.34	52.28	3.08	7.60
2	BM-4	39.85	76.82	46.39	3.37	7.70
3	ML-131	39.20	79.25	54.00	3.08	7.53
4	IPM-02-03	33.16	74.48	43.87	2.71	7.02
5	PDM-139	41.72	77.92	43.39	3.21	6.90
6	RMG-1035	44.24	81.38	44.83	2.00	5.92
7	RMG-344	41.45	78.06	46.92	2.91	7.27
8	RMG-1045	37.16	67.63	39.58	1.83	6.11
	Hybrids					
9	IPM- 99-125 × BM-4	43.09	78.38	34.18	3.68	8.03
10	IPM-99-125 x ML-131	38.71	74.31	34.80	3.17	7.39
11	IPM 99-125 x IPM 02-03	41.32	79.11	50.09	2.81	6.98
12	IPM 99-125 x PDM-139	41.66	76.87	43.37	3.15	7.58
13	IPM-99-125x RMG-1035	42.17	78.23	44.47	1.98	5.48
14	IPM-99-125 x RMG-344	43.53	77.51	46.67	3.07	7.35
15	IPM-99-125 x RMG-1045	36.08	67.39	45.81	2.32	7.45
16	BM-4 x ML-131	36.90	70.64	48.43	2.78	7.94
17	BM-4 x IPM 02-03	36.23	75.99	53.88	2.35	7.62
18	BM-4 x PDM-139	42.10	78.83	38.83	2.69	7.99
19	BM-4 x RMG-1035	38.26	73.99	44.00	2.24	6.32
20	BM-4 x RMG-344	36.85	76.53	52.34	3.33	7.85
21	BM-4 x PRMG-1045	37.05	72.60	63.91	2.55	7.07
22	ML-131 x IPM 02-03	39.19	78.65	63.06	3.25	7.26
23	ML-131 x PDM-139	37.65	73.58	61.25	3.50	7.86
24	ML-131 x RMG-1035	41.57	79.37	65.34	2.50	5.88
25	ML-131 x RMG-344	37.83	66.96	58.32	3.17	7.38
26	ML-131 x PRMG-1045	39.27	74.45	61.73	3.08	6.85
27	IPM 02-03 x PDM-139	41.06	76.14	59.21	3.22	7.38
28	IPM 02-03 x RMG-1035	40.80	79.36	63.84	2.14	5.08
29	IPM 02-03 x RMG-344	40.55	75.84	63.55	3.10	6.95
30	IPM 02-03 x PRMG-1045	35.38	74.02	66.72	2.97	6.90
31	PDM-139 x RMG-1035	41.71	79.23	53.23	2.21	6.37
32	PDM-139 x RMG-344	41.59	76.43	56.23	3.09	7.74
33	PDM-139 x PRMG-1045	40.48	77.46	56.88	2.57	6.31
34	RMG-1035 x RMG-344	39.90	79.00	55.16	2.85	6.05
35	RMG-1035 x RMG-1045	39.25	78.05	57.90	1.86	7.24
36	RMG-344 x RMG-1045	41.09	76.96	61.17	2.85	7.49
	GM	39.71	76.19	51.00	2.80	7.05
	SE (m)	2.33	3.19	2.72	0.29	0.55
	CD (5 %)	4.65	6.36	7.68	0.58	1.09
	CD (1%)	6.18	8.45	10.20	0.78	1.45
	CV (%)	7.04	5.01	9.25	12.54	9.28

APPENDIX - VI

Mean values for Clusters / plant, Pods / cluster, Pod length, Pods / plant and Seeds / pod.

S. No	Genotype and crosses	Clusters / plant	Pods / cluster	Pod length	Pods / Plant	Seeds / Pod
1	IPM 99-125	7.45	3.65	6.42	23.56	10.77
2	BM-4	7.77	3.82	6.37	12.46	10.80
3	ML-131	7.55	3.75	6.61	33.04	11.09
4	IPM-02-03	7.11	3.42	5.96	10.96	10.59
5	PDM-139	7.06	3.97	7.11	28.21	10.78
6	RMG-1035	6.53	3.69	7.04	31.10	9.88
7	RMG-344	7.07	2.93	7.17	23.08	8.60
8	RMG-1045	6.39	3.82	6.73	16.55	12.20
	Hybrids					
9	IPM- 99-125 × BM-4	8.03	3.68	5.97	16.54	10.72
10	IPM-99-125 x ML-131	7.48	3.09	5.59	14.06	10.18
11	IPM 99-125 x IPM 02-03	7.29	3.30	6.59	16.43	11.40
12	IPM 99-125 x PDM-139	7.87	2.83	6.29	11.67	11.43
13	IPM-99-125x RMG-1035	5.51	3.92	7.13	13.73	12.24
14	IPM-99-125 x RMG-344	7.40	3.66	6.88	11.77	12.13
15	IPM-99-125 x RMG-1045	7.18	3.60	5.42	9.29	11.79
16	BM-4 x ML-131	8.04	3.31	8.44	16.47	11.50
17	BM-4 x IPM 02-03	7.11	3.10	9.00	33.18	11.10
18	BM-4 x PDM-139	7.83	3.49	6.89	22.06	13.32
19	BM-4 x RMG-1035	7.33	3.95	4.92	16.79	12.45
20	BM-4 x RMG-344	7.89	3.96	5.97	15.54	12.67
21	BM-4 x PRMG-1045	7.12	3.11	6.40	13.16	9.85
22	ML-131 x IPM 02-03	7.45	3.40	7.86	17.76	10.24
23	ML-131 x PDM-139	7.84	3.01	8.23	29.26	11.12
24	ML-131 x RMG-1035	6.65	3.96	6.40	18.12	10.42
25	ML-131 x RMG-344	7.45	3.03	6.44	10.86	10.25
26	ML-131 x PRMG-1045	6.81	3.50	7.87	20.51	10.25
27	IPM 02-03 x PDM-139	7.40	3.73	5.34	12.15	9.29
28	IPM 02-03 x RMG-1035	6.09	3.03	7.15	21.78	10.54
29	IPM 02-03 x RMG-344	7.03	3.65	7.17	16.40	9.69
30	IPM 02-03 x PRMG-1045	6.61	3.32	5.81	28.65	10.66
31	PDM-139 x RMG-1035	6.50	3.57	7.63	20.39	10.35
32	PDM-139 x RMG-344	8.09	3.68	6.88	15.14	9.23
33	PDM-139 x PRMG-1045	5.60	3.00	7.10	14.68	9.77
34	RMG-1035 x RMG-344	6.33	3.32	6.03	13.61	10.61
35	RMG-1035 x RMG-1045	6.11	3.67	5.87	16.50	9.67
36	RMG-344 x RMG-1045	7.57	3.79	8.07	24.30	9.73
	GM	7.13	3.39	3.58	18.14	10.76
	SE (m)	0.62	0.15	0.27	1.29	0.52
	CD (5 %)	1.24	0.42	0.55	3.64	1.04
	CD (1%)	1.64	0.55	0.73	4.84	1.39
	CV (%)	10.41	7.54	9.16	12.33	5.83

APPENDIX - VII

Mean values for 100 seed weight, Seed yield / plant, Biological yield / plant and Harvest index

S. No	Genotype and crosses	100 seed weight	Seed yield / plant	Biological yield / plant	Harvest index
1	IPM 99-125	4.36	12.56	32.70	38.44
2	BM-4	4.31	12.83	39.98	32.08
3	ML-131	4.42	12.31	52.15	23.64
4	IPM-02-03	5.08	11.10	40.21	27.92
5	PDM-139	4.44	12.53	42.32	29.69
6	RMG-1035	3.41	7.57	32.81	22.82
7	RMG-344	4.35	11.52	46.25	25.52
8	RMG-1045	4.68	10.17	37.45	27.42
	Hybrids				
9	IPM- 99-125 × BM-4	6.13	13.10	41.71	31.48
10	IPM-99-125 x ML-131	5.87	12.88	35.00	36.89
11	IPM 99-125 x IPM 02-03	3.54	11.06	49.35	22.82
12	IPM 99-125 x PDM-139	3.54	12.62	39.13	32.43
13	IPM-99-125x RMG-1035	3.65	9.70	42.22	23.08
14	IPM-99-125 x RMG-344	4.12	11.67	39.74	29.13
15	IPM-99-125 x RMG-1045	3.51	10.65	35.72	29.89
16	BM-4 x ML-131	3.56	13.15	43.71	30.18
17	BM-4 x IPM 02-03	4.47	11.46	39.60	29.06
18	BM-4 x PDM-139	4.35	13.33	42.00	31.77
19	BM-4 x RMG-1035	3.20	10.15	41.26	24.96
20	BM-4 x RMG-344	4.53	11.83	45.14	26.20
21	BM-4 x PRMG-1045	3.62	11.43	40.94	27.91
22	ML-131 x IPM 02-03	3.79	12.02	42.02	28.75
23	ML-131 x PDM-139	3.88	13.11	44.03	30.10
24	ML-131 x RMG-1035	3.27	10.74	40.12	26.75
25	ML-131 x RMG-344	3.91	11.60	41.88	28.14
26	ML-131 x PRMG-1045	3.67	11.11	42.52	26.34
27	IPM 02-03 x PDM-139	4.37	11.77	40.71	29.04
28	IPM 02-03 x RMG-1035	3.70	10.26	42.91	23.89
29	IPM 02-03 x RMG-344	4.13	10.89	39.85	27.41
30	IPM 02-03 x PRMG-1045	4.17	10.50	42.63	24.76
31	PDM-139 x RMG-1035	3.42	10.96	35.20	31.30
32	PDM-139 x RMG-344	3.71	11.71	41.75	28.10
33	PDM-139 x PRMG-1045	4.48	10.91	36.05	30.48
34	RMG-1035 x RMG-344	3.27	9.34	40.61	23.00
35	RMG-1035 x RMG-1045	3.22	10.29	36.29	29.48
36	RMG-344 x RMG-1045	4.48	12.03	33.54	36.30
	GM	3.94	11.41	40.54	28.53
	SE (m)	0.23	0.70	3.55	2.58
	CD (5 %)	0.66	1.39	7.09	5.14
	CD (1%)	0.87	1.84	9.41	6.82
	CV (%)	10.26	7.30	10.49	10.81

APPENDIX – VIII

Mean values for Seed protein content and Seed methionin content

S. No	Genotype and crosses	Seed protein content	Seed methionin content
1	IPM 99-125	23.33	1.24
2	BM-4	22.06	1.08
3	ML-131	22.38	1.17
4	IPM-02-03	23.51	1.2
5	PDM-139	23.27	1.19
6	RMG-1035	21.6	1.24
7	RMG-344	22.36	1.26
8	RMG-1045	22.26	1.11
	Hybrids		
9	IPM- 99-125 × BM-4	23.440	1.17
10	IPM-99-125 x ML-131	19.470	1.24
11	IPM 99-125 x IPM 02-03	22.980	1.09
12	IPM 99-125 x PDM-139	20.300	0.98
13	IPM-99-125x RMG-1035	22.560	1.14
14	IPM-99-125 x RMG-344	23.460	1.03
15	IPM-99-125 x RMG-1045	23.880	1.24
16	BM-4 x ML-131	21.170	1.19
17	BM-4 x IPM 02-03	22.740	1.11
18	BM-4 x PDM-139	22.K2210	1.29
19	BM-4 x RMG-1035	23.090	0.98
20	BM-4 x RMG-344	19.960	1.08
21	BM-4 x PRMG-1045	22.940	1.11
22	ML-131 x IPM 02-03	20.800	1.24
23	ML-131 x PDM-139	22.480	1.11
24	ML-131 x RMG-1035	23.620	1.19
25	ML-131 x RMG-344	23.840	1.11
26	ML-131 x PRMG-1045	21.240	1.19
27	IPM 02-03 x PDM-139	22.740	1.07
28	IPM 02-03 x RMG-1035	22.290	1.23
29	IPM 02-03 x RMG-344	23.180	1.05
30	IPM 02-03 x PRMG-1045	19.700	1.21
31	PDM-139 x RMG-1035	22.970	1.03
32	PDM-139 x RMG-344	20.680	1.26
33	PDM-139 x PRMG-1045	22.220	1.21
34	RMG-1035 x RMG-344	23.410	1.16
35	RMG-1035 x RMG-1045	24.190	1.19
36	RMG-344 x RMG-1045	21.350	1.21
	GM	21.710	1.12
	SE (m)	0.290	0.03
	CD (5 %)	0.810	0.08
	CD (1%)	1.080	0.1
	CV (%)	2.300	4.21

APPENDIX – IX

Pattern of amplified product appeared on agarose gel [Green gram genotype (P1 to P8) with different RAPD primer]

OPA-02

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	0	1	0	0	0	1	0	0
2	900	0	0	1	0	1	0	0	0
3	800	1	1	1	1	1	1	1	1
4	600	0	0	1	0	1	0	0	0
5	500	0	0	0	1	0	1	0	1
6	400	1	1	1	0	1	0	0	0
7	200	0	0	1	0	1	0	0	0
Total		2	3	5	2	5	3	1	2

OPA-05

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	1	0	1	1	1	1	1	1
2	1500	1	0	1	0	1	1	0	1
3	1000	1	0	0	0	1	1	0	1
4	600	1	1	1	1	1	1	1	1
5	400	1	1	1	0	1	1	1	1
6	300	1	0	1	0	1	1	1	1
Total		6	2	5	2	6	6	4	6

OPA-07

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	0	1	0	0	1	1	0	1
2	900	0	0	0	1	1	1	0	0
3	800	1	0	0	1	0	0	1	0
4	700	0	0	0	0	0	1	0	0
5	600	1	1	1	1	1	1	1	1
6	400	0	0	0	1	0	0	0	0
7	300	0	0	0	1	0	0	0	0
Total		2	2	1	5	3	4	2	2

OPP-03

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	1	0	1	1	1	1	1	1
2	1000	0	0	1	0	0	1	0	0
3	600	1	1	1	1	1	1	1	1
4	400	0	0	0	0	1	1	0	1
5	300	0	1	0	0	0	1	1	0
Total		2	2	3	2	3	5	3	3

OPB-06

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	900	1	1	1	1	1	1	1	1
2	700	1	0	1	1	0	0	1	0
3	400	1	1	1	1	1	1	1	1
4	300	0	1	1	0	0	1	1	0
5	100	0	0	1	0	0	0	1	0
Total		3	3	5	3	2	3	5	2

OPA-10

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	0	0	0	0	1	0	1	0
2	800	0	1	0	1	1	1	1	1
3	700	1	0	1	0	0	0	0	0
4	400	0	1	1	0	1	1	1	1
5	300	0	1	1	0	1	1	1	1
6	200	1	0	0	0	0	0	0	0
Total		2	3	3	1	4	3	4	3

OPP-10

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	0	0	0	0	1	0	0
2	1000	1	1	1	1	1	0	1	0
3	700	1	0	0	0	1	0	1	0
4	600	0	0	0	0	0	0	1	0
5	500	0	0	0	0	0	0	0	1
6	400	0	1	1	1	1	1	0	0
7	300	0	0	0	0	0	0	0	1
8	200	1	1	0	0	1	0	0	1
Total		3	3	2	2	4	2	3	3

OPA-11

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	1	0	0	0	0	0	0	0
2	900	0	1	0	1	0	1	1	1
3	700	1	0	0	0	0	0	0	0
4	600	0	1	1	1	1	1	1	1
5	500	0	0	0	0	0	1	1	1
6	400	1	0	1	1	0	0	0	0
Total		3	2	2	3	1	3	3	3

OPA-09

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2500	0	1	0	0	1	1	1	0
2	1500	0	0	1	0	0	0	0	0
3	1000	1	1	0	0	1	1	1	1
4	600	0	0	0	0	0	0	0	1
5	500	1	0	1	1	0	1	0	0
6	300	0	0	0	1	0	0	0	0
7	200	0	0	0	1	0	0	0	0
Total		2	2	2	3	2	3	2	2

OPA-08

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	0	1	0	0	0	0	0	0
2	1500	0	1	0	0	0	0	0	0
3	1000	1	1	0	1	1	1	1	1
4	800	0	0	0	0	1	1	1	1
5	700	1	1	1	0	0	0	0	0
6	600	0	1	0	1	1	0	1	1
7	400	1	0	1	0	0	1	1	0
Total		3	5	2	2	3	3	4	3

OPB-07

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	0	1	1	1	1	1	0
2	800	0	0	0	0	0	0	0	1
3	700	0	1	0	0	1	1	0	0
4	600	1	1	1	1	1	1	1	0
5	400	1	0	0	0	0	0	1	0
6	300	0	0	0	0	0	0	0	1
Total		3	2	2	2	3	3	3	2

OPE-03

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	1	1	1	0	1	1	0	1
2	1000	0	0	1	0	1	1	1	0
3	600	0	0	1	1	1	1	1	1
4	500	1	1	0	0	0	0	0	0
5	400	0	0	0	0	0	0	1	0
Total		2	2	3	1	3	3	3	2

OPA-16

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	0	0	0	0	1	1	1	1
2	1000	1	1	0	0	0	0	0	0
3	900	0	0	0	1	1	0	0	0
4	800	0	0	0	0	1	0	1	1
5	600	1	1	1	1	0	1	0	0
6	400	1	0	0	0	0	0	0	1
Total		3	2	1	2	3	2	2	3

OPB-02

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	0	0	0	0	0	1	1	0
2	1000	1	1	1	1	1	1	1	1
3	800	0	0	1	0	0	1	1	0
4	700	1	0	0	0	0	0	0	0
5	600	1	1	1	1	1	1	1	1
6	400	0	0	0	0	0	0	0	1
Total		3	2	3	2	2	4	4	3

OPB-06

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	900	1	1	1	1	1	1	1	1
2	700	1	0	1	1	0	0	1	0
3	400	1	1	1	1	1	1	1	1
4	300	0	1	1	0	0	1	1	0
5	100	0	0	1	0	0	0	1	0
Total		3	3	5	3	2	3	5	2

OPF-19

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	1	0	1	0	0	1	1
2	1000	0	0	1	0	1	1	1	1
3	700	1	1	1	1	1	1	1	1
4	500	1	1	1	1	1	1	1	1
5	300	1	0	0	1	0	0	0	0
6	200	1	0	0	1	0	0	0	0
Total		4	3	3	5	3	3	4	4

OPA-15

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	1	1	1	1	1	0	1
2	800	0	0	1	0	0	0	0	0
3	600	1	1	1	1	1	1	1	1
4	500	0	1	0	0	1	0	0	0
5	400	1	1	1	1	1	1	1	1
Total		3	4	4	3	4	3	2	3

OPB-03

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	1	1	1	1	0	1	1
2	500	0	0	1	1	0	1	0	1
3	400	1	0	0	1	0	0	0	0
4	300	0	0	0	0	0	0	0	1
5	200	1	1	0	1	0	1	0	0
6	100	0	0	0	0	0	1	0	0
Total		2	2	2	4	1	3	1	3

APPENDIX – X

Pattern of amplified product appeared on agarose gel [Green gram genotypes (P1 to P8) with different primer]

ISSR-01

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	0	1	0	0	0	0	0
2	1000	1	0	0	0	0	0	0	0
3	800	0	0	0	0	1	1	1	1
4	600	1	0	1	1	0	0	0	0
5	400	0	0	1	1	1	1	1	1
6	300	0	1	0	1	0	0	0	0
7	200	0	1	0	1	1	1	1	1
8	100	1	0	1	0	1	1	1	1
Total		3	2	4	4	4	4	4	4

ISSR-02

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	1	1	0	0	1	1	1	1
2	1500	1	1	1	1	1	1	1	1
3	1000	1	1	0	0	1	1	1	1
4	500	1	1	1	0	1	1	1	1
5	400	1	1	0	0	1	1	1	1
6	300	1	1	1	1	1	1	1	1
7	200	1	1	1	1	1	1	1	1
Total		7	7	4	3	7	7	7	7

ISSR-04

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	700	1	0	1	0	1	0	0	0
2	400	1	1	1	1	1	1	1	1
3	300	1	0	0	1	0	1	1	1
4	200	1	1	0	1	0	0	1	1
5	100	1	1	1	1	1	1	1	1
Total		5	3	3	4	3	3	4	4

ISSR-05

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	0	0	0	0	0	0	1	0
2	1000	0	0	0	0	0	0	1	0
3	700	1	0	1	0	1	1	1	1
4	600	0	0	1	1	1	1	1	1
5	400	1	1	1	1	1	1	1	1
6	300	1	1	1	1	0	1	1	1
7	200	1	0	1	1	0	1	1	1
Total		4	2	5	4	3	5	7	5

UBC—854

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	1	0	0	1	0	0	0	1
2	800	1	0	1	1	1	1	1	0
3	700	0	0	0	0	0	0	1	0
4	400	1	1	1	1	0	1	1	1
5	300	0	0	0	0	0	0	0	1
6	200	1	0	1	1	1	0	1	0
Total		4	1	3	4	2	2	4	3

ISSR-07

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	0	1	0	1	1	1	1
2	1000	1	1	1	1	1	1	1	1
3	900	0	0	1	1	1	0	1	0
4	600	0	0	1	0	1	1	1	1
5	500	0	0	1	0	1	1	1	1
6	400	0	1	1	0	0	1	1	1
7	300	0	1	1	0	0	1	1	0
8	100	1	1	1	1	1	1	1	1
Total		2	4	8	3	6	7	8	6

ISSR-08

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	1	1	0	1	1	1	1
2	900	1	1	1	0	1	1	1	1
3	800	1	0	1	1	1	0	1	0
4	500	1	0	1	0	1	1	1	0
5	400	1	1	1	0	1	1	1	1
6	300	1	1	1	0	0	1	1	1
7	200	1	1	0	0	0	0	0	0
8	100	1	1	1	1	1	1	1	1
Total		8	6	7	2	6	6	7	5

ISSR-09

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	700	1	0	1	0	0	1	1	1
2	500	1	0	1	0	0	1	1	1
3	300	1	1	1	1	1	1	1	1
4	200	1	1	1	1	1	1	1	1
5	100	1	1	1	1	0	0	0	0
Total		5	3	5	3	2	4	4	4

UBC-810

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	1	1	1	1	1	1	1
2	900	1	1	1	1	1	1	1	1
3	700	1	0	0	0	1	1	1	1
4	600	1	1	1	1	1	1	1	1
5	500	0	0	0	0	1	1	1	1
6	400	1	0	1	1	1	1	1	1
7	300	1	0	0	0	1	1	1	1
Total		6	3	4	4	7	7	7	7

UBC-811

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	1	1	1	1	1	1	1
2	900	0	0	0	1	1	0	0	0
3	700	0	1	1	1	1	0	1	1
4	600	0	0	0	0	0	1	1	0
5	500	1	0	0	1	1	0	1	0
6	400	0	0	0	1	1	1	1	0
7	300	0	0	0	1	1	0	0	0
Total		2	2	2	6	6	3	5	2

UBC-817

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	600	0	0	1	0	0	1	0	0
2	500	0	1	1	1	1	1	1	0
3	400	1	0	0	0	0	0	0	0
4	300	0	1	0	0	0	0	1	1
5	200	1	1	1	0	1	1	1	1
Total		2	3	3	1	2	3	3	2

UBC-818

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	0	0	0	0	0	0	1	0
2	900	1	0	1	0	0	1	1	1
3	600	1	0	1	1	1	1	1	1
4	500	1	1	1	0	0	1	1	1
5	300	0	1	1	1	1	1	1	1
6	200	0	1	0	1	0	0	0	0
Total		3	3	4	3	2	4	5	4

UBC-820

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	700	1	0	0	1	0	1	0	1
2	500	1	1	1	0	0	0	0	0
3	400	0	0	0	1	1	1	1	1
4	200	1	1	1	0	0	0	0	0
5	100	0	0	0	1	1	1	0	1
Total		3	2	2	3	2	3	1	3

UBC-822

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1500	0	0	0	0	1	1	0	1
2	1000	0	1	0	0	1	1	1	1
3	500	1	0	1	1	0	0	0	0
4	400	0	0	0	0	1	1	1	1
5	300	1	0	1	1	0	0	0	0
6	200	1	1	1	1	1	1	1	1
7	100	1	1	1	1	1	1	1	1
Total		4	3	4	4	5	5	4	5

UBC-836

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	900	1	0	1	0	0	1	1	1
2	800	1	0	1	1	1	1	1	1
3	500	1	1	0	0	1	0	0	1
4	400	1	1	1	1	1	1	1	1
5	300	1	0	1	0	0	0	1	1
Total		5	2	4	2	3	3	4	5

UBC-845

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	600	1	0	1	0	1	0	1	0
2	500	1	1	1	1	1	1	1	1
3	300	1	0	0	0	0	0	0	1
4	200	1	1	0	1	0	1	1	1
Total		4	2	2	2	2	2	3	3

UBC-848

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	1000	1	0	1	1	0	1	1	0
2	800	1	0	1	1	0	1	1	1
3	500	1	1	1	1	0	1	1	1
4	400	0	0	1	1	0	1	0	1
5	300	1	1	1	1	1	1	1	1
Total		4	2	5	5	1	5	4	4

UBC-878

Sl No	bp	P1	P2	P3	P4	P5	P6	P7	P8
1	2000	0	0	1	1	0	1	1	1
2	1500	1	0	1	1	1	1	1	1
3	800	0	1	1	1	1	0	1	1
4	500	1	1	1	1	1	1	1	1
Total		2	2	4	4	3	3	4	4