EVALUATION OF GREEN GRAM (*Vigna radiata* (L.) R. Wilczek) LINES FOR YMV RESISTANCE USING MOLECULAR MARKERS

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B.Sc. (Ag. Biotech)

MASTER OF SCIENCE IN AGRICULTURE

(MOLECULAR BIOLOGY AND BIOTECHNOLOGY)

2020

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BY

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THESIS SUBMITTED TO PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(MOLECULAR BIOLOGY AND BIOTECHNOLOGY)

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2020.

DECLARATION

I**, MOHD ABDUS SUBHAN SALMAN,** hereby declare that the thesis entitled **"EVALUATION OF GREEN GRAM (*Vigna radiata* (L.) R. Wilczek) LINES FOR YMV RESISTANCE USING MOLECULAR MARKERS"** submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Master Of Science in Agriculture** in the major field of Plant Molecular Biology and Biotechnology, is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

Place: Hyderabad **(MOHD ABDUS SUBHAN SALMAN)**

Date: **I. D. No. RAM/18-104**

CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF GREEN GRAM (*Vigna radiata* (L.) R. Wilczek) LINES FOR YMV RESISTANCE USING MOLECULAR MARKERS"** submitted in partial fulfillment of the requirements for the degree of "Master of Science in Agriculture" of the Professor Jayashankar Telangana State Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Mr. MOHD ABDUS SUBHAN SALMAN** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

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CERTIFICATE

Mr. MOHD ABDUS SUBHAN SALMAN has satisfactorily prosecuted the course of research and that thesis entitled **"EVALUATION OF GREEN GRAM (*Vigna radiata* (L.) R. Wilczek) LINES FOR YMV RESISTANCE USING MOLECULAR MARKERS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Place: **(DR. CH. ANURADHA)**

Date: **Chairperson**

ACKNOWLEDGEMENTS

First and foremost, I am boundlessly thankful to the ***ALMIGHTY*** *for his blessings all through that added to my strength and patience in accomplishing tasks. His selfless love has been of a great support throughout.*

With a deep sense of gratitude, I express my heartfelt thanks to my Chairman ***Dr. Ch. Anuradha,*** *Professor, Department of Plant Molecular Biology and Biotechnology, Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad, for her competent guidance, constant encouragement, meticulous suggestions, constructive criticism, which has had enabled me to complete my research and degree successfully. Her vast and deep knowledge of the subject, sense of dedication throughout the tenure of this investigation will be a part of memory Forever, I will consider myself fortunate to be her disciple.*

I take immense pleasure to express my deep sense of gratitude, ***Dr. V. Sridhar,*** *Assistant Professor, Agricultural Polytechnic, Kampasagar, Nalgonda, a member of my Advisory committee for valuable guidance, incessant inspiration, untiring attention, patience, keen interest and constant motivation in research and personal care with dotting heart throughout the period of my study.*

I take it as a privilege to express my gratitude towards member of advisory committee ***Dr. SNCVL. Pushpavalli,*** *Assistant Professor, Department of Plant Molecular Biology and Biotechnology, Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad, for her motivation and whole hearted cooperation during my research work.*

*I take immense pleasure to express my deep sense of gratitude and indebtedness to****,*** ***Dr. Ch. V. Durga Rani,*** *Director , IBT and* ***Dr. S. Sokka Reddy,*** *Ex-Director, Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad for their help and moral support throughout the course of study and also for their constant encouragement.*

I deem it previllege in expressing my fidelity to Professors ***Dr. Vanisree, Dr. K. Y. N. Yamini, Dr. Balram,*** *Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad for their ready help, support and guidance through the period of my study.*

I am in dearth of words to express my affectionate and heartfelt gratitude for my beloved parents, ***Mr. Md Ghouse*** *and* ***Mrs. Saleha Sultana****, my dear brother* ***Md Abdul Khader Farhan****, for their selfless love, faith and confidence in me. They have been my*

strength throughout and without their moral support I wouldn't have been able to achieve my targets. I thank the almighty for having blessed me with such a beautiful and supportive family.

The instrument of acknowledgement would remain out of tune if I do not express my thanks to my close friends ***Sarfaraz, Yaser Habib, Sadiq*** *and* ***Ganesh*** *whose unconditional love, unbounded affection and moral support were my strongest assets during the period of my research.*

I use this opportunity to sincerely thank my dearest friends ***A. Ravali, Hameeda Afreen, A. Govardhani, P. Navya Padmini, P. Sirisha, Akshay Shendekar, Noor Nihal, Afroz, Hemant, Akash, G. Shridhar, Bidush Ranjan and B. Madhu*** *who have rendered their help during my course works in whose cheerful company I have never felt my work as burden.*

A special word of thanks to my senior's ***Dr. Ravindra kale, Ram Babu, Naresh, Sk Maliha, Anjali,*** *and* ***Nagamani*** *for their support during the entire period of my research. I thank them for their help, support, guidance and inspiration throughout the research period.*

I am thankful to department staff, ***Rekha, Khan, Prabhakar,*** *and other non-teaching staff of the Institute of Biotechnology for their timely assistance and cooperation.*

I am grateful to ***Government of Telangana*** *and* ***Professor Jayashankar Telangana State Agricultural University, Hyderabad*** *for their financial aid for my research work that supported me a lot.*

Finally, I thank all my well-wishers and others who helped me directly or indirectly not placed here, for their kind cooperation and support rendered to me.

Date: *(Mohd Abdus Subhan Salman)*

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percent
&	:	And
/	:	Per
µl	:	Micro litres
µM	:	Micro molar
AFLP	:	Amplified Fragment Length Polymorphism
AFLP-RGA	:	Amplified Fragment Length Polymorphism- Resistant gene analogues
AP-PCR	:	Arbitrarily Primed PCR
AS-PCR	:	Allele Specific PCR
AVRDC	:	Asian Vegetable Research and Development Centre
BC	:	Backcross
bp	:	Base pairs
BSA	:	Bulked Segregant Analysis
C°	:	Centigrade
CAP	:	Cleaved Amplified Polymorphism
cM	:	Centimorgan
cm	:	Centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
CYMV	:	Cowpea Yellow Mosaic Virus
DF	:	Days to 50% Flowering
DNA	:	Deoxy ribonucleic Acid
dNTP	:	Deoxynucleotide Triphosphate
EDTA	:	Ethylene Diamine Tetra Acetic Acid
et al.	:	And other people
g	:	Gram
H^2	:	Heritability
IARI	:	Indian Agricultural Research Institute
ISSR	:	Inter Simple Sequence Repeats
L.ha	:	Lakh hectares
LG	:	Linkage group
LLS	:	Late Leaf Spot
M	:	Molar

M.ha	:	Million hectares
M.t	:	million tonnes
MAS	:	Marker Assisted Selection
Mb	:	Mega bases
mg	:	Milligram
ml	:	Milli liters
mM	:	Micromolar
MYMIV	:	Mungbean Yellow Mosaic India Virus
MYMV	:	Mungbean Yellow Mosaic Virus
ng	:	Nanogram
ng/µl	:	Nanogram per microliter
nm	:	Nano meter
PCR	:	Polymerase Chain Reaction
PH	:	Potential of Hydrogen
PJTSAU	:	Professor Jayashankar Telangana State Agricultural University
PVP	:	Polyvinylpyrrolidone
QTL	:	Quantitative Trait Loci
RAPD	:	Randomly Amplified Polymorphic DNA
RARS	:	Regional Agricultural Research Station
RFLP	:	Restriction Fragment Length Polymorphism
RGA	:	Resistant Gene Analogues
RILs	:	Recombinant Inbred Lines
RPM	:	Revolutions per minute
SB	:	No. of secondary branches
SBR	:	Soyabean Rust
SCAR	:	Sequence Characterized Amplified Region
SD	:	Standard deviation
SDS- PAGE:		Sodium Dodecyl sodium dodecyl sulfate–polyacrylamide gel electrophoresis
Sec	:	Second
SNP	:	Single Nucleotide Polymorphisms
SP	:	Seed per pod
SSR	:	Simple Sequence Repeats
STS	:	Sequence tagged sites
SW	:	Seed weight

TAE	:	Tris Acetate EDTA
Taq	:	*Thermus aquaticus*
TRIS	:	Tris (hydroxyl methyl) amino methane
UV	:	Ultra violet
viz.	:	Vi delicet (namely)
W/V	:	Weight per volume
YMV	:	Yellow Mosaic Virus
$\chi 2$	:	Chi-square

Author	:	**MOHD ABDUS SUBHAN SALMAN**
Title of Thesis	:	**EVALUATION OF GREEN GRAM (*Vigna radiata* (L.) R. Wilczek) LINES FOR YMV RESISTANCE USING MOLECULAR MARKERS**
Degree	:	**MASTER OF SCIENCE IN AGRICULTURE**
Faculty	:	**AGRICULTURE**
Discipline	:	**MOLECULAR BIOLOGY AND BIOTECHNOLOGY**
Major advisor	:	**DR. CH. ANURADHA**
University	:	**PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY**
Year of submission	:	**2020**

ABSTRACT

Green gram (*Vigna radiata* (L.) Wilczek), is one of the important pulse crops mainly grown in developing countries. However, the yield level of the crop is very low due to many biotic and abiotic factors. Among biotic factors, yellow mosaic virus (YMV), which is transmitted by white fly (*Bemisia tabaci*) causes significant yield losses ranging from 10-100% and it leads to severe yield reduction. The biggest challenge in YMD management is the effective utilization of an array of information gained so far, in an integrated manner for the development of genotypes having durable resistance against yellow mosaic virus (YMV) infection. The advancements in the field of biotechnology and molecular biology such as marker assisted selection and genetic transformation can be utilized in developing Yellow mosaic virus (YMV) resistant green gram.

The present investigation was carried out for screening of green gram RILs against Yellow mosaic virus (YMV) and evaluation based on morphological characters and molecular markers. An attempt was made to evaluate microsatellite markers linked to the YMV resistance in a F_6 generation of green gram. The genotypes, MGG 295, susceptible to Yellow Mosaic Virus (YMV) and WGG 42 (Yadadri) resistant to YMV were chosen as parents for development of F_6 population. The studies were carried out at Institute of Biotechnology (IBT), Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad and ARS Madhira, Khammam during 2019-2020 with 128 F_6 RILs to elicit the information on nature and extent of the genetic variability, heritability, genetic advance and molecular evaluation for YMV. For molecular marker

evaluation study of 128 F_6 RILs, SSR primers were employed. Observations were recorded on 13 characters viz., days to initial flowering, days to 50% flowering, days to maturity, number of branches per plant, number of pods per cluster, number of cluster per plant, number of pods per plant, number of seeds per pod, plant height, pod length, seed yield per plant, 100 seed weight and percent of disease incidence under natural incidence of whitefly at hot spot.

Results obtained conveyed that genetic variability was present for all the characters studied indicating that the RILs represented wide variability. The genotypic coefficients of variation for all the characters studied were lesser than the phenotypic coefficients of variation indicating the modifying effects of the environment in association with the characters at genotypic level. High PCV and GCV estimates was noticed for number of pods per plant, seed yield per plant, number of cluster per plant and number of pods per cluster. High heritability along with high genetic advance as percent of mean was observed for number of pods per plant, seed yield per plant, number of cluster per plant, number of pods per cluster, number of branches per plant, number of seeds per pod and plant height indicating the role of additive genes in governing the inheritance of these traits and could be improved through selection. The traits seed yield per plant, number of pods per plant, number of clusters per plant and number of pods per cluster had recorded high PCV, GCV, high heritability along with high genetic advance as percent of mean indicated these traits were less influenced by environment and possess high genetic variability. Hence these RILs would be suitable for green gram breeding programme to develop improved varieties.

The parental DNA was extracted and screened with 185 microsatellite markers to detect the polymorphic markers. Out of these markers, 102 were amplified and 83 markers were not amplified. Of these 102 amplified markers, 15 primers showed polymorphism (14.7%) between the parents, and the rest of the markers were found to be monomorphic. All the 128 F_6 lines were genotyped with the 8 polymorphic markers. CEDG228 was found to be significantly associated with the YMV resistance in green gram. Identification of molecular marker associated with resistance gene in the present study, will increase the efficiency and accuracy in YMV-resistance breeding program and this marker can be used in future for the development of high yielding YMV resistant cultivars in green gram.

Chapter I

INTRODUCTION

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INTRODUCTION

Pulses are the excellent sources of dietary protein. In addition to carbohydrates, lipids, mineral matter and water, pulse grain contains small quantities of vitamins, enzymes, and phytochemicals, which are important for human health. They are also a source of macronutrients and minerals. The pulses also contain plant secondary metabolites that are increasingly being recognized for their potential benefits to human health. A number of bioactive substances including enzyme inhibitors, lectins phytates, oligosaccharides and phenolic compounds, that have a role in human metabolism have been identified. Bioactive compounds present in pulses have both positive and negative impacts on human health.

Besides being a rich source of protein, pulses maintain soil fertility through biological nitrogen fixation in soil thus play a vital role in furthering sustainable agriculture (Kannaiyan *et al*., 1999). Pulses are important food crops that can play a major role in food security and environmental challenges, as well as contribute to healthy diets. The year 2016 was observed as the 'International Year of pulses' by the 68th session of the United Nations General Assembly on December 20, 2013. The 'World Pulses Day' is a designated United Nations global event to recognize the importance of pulses as a global food. Every year, February 10 is celebrated as the world pulses day as nominated by United Nations General Assembly (pulses.org/world-pulses-day-guidelines-2020). Among the pulses, pigeon pea, chickpea, green gram and black gram are the major contributors of the total pulses production.

Green gram (*Vigna radiata* (L.) R. Wilczek var. *radiata*), also known as mung bean or moong, is an important pulse crop in countries of Asia, Africa and latin America, belonging to the papilionoid subfamily, genus Vigna, sub genus *Ceratotrophis* and family *Fabaceae*. Green gram is a self-pollinated diploid grain legume (2n=2x=22) crop with a small genome size of 579 Mb/1C (Arumuganathan *et al*., 1991). This crop play an important role in crop rotation due to their ability to fix atmospheric nitrogen, thus growing green gram helps to improve soil fertility and benefit subsequent crops. It is a widely grown crop due to its early maturity, drought tolerance and ability to fix atmospheric nitrogen.

Green gram is a fast growing, warm season legume and sensitive to frost. It reaches maturity very quickly under tropical and subtropical conditions where optimal temperatures are about 28-30ºC and always above 15ºC. It can therefore be grown in summer and autumn in warm temperate and subtropical region and at altitude below 2000 m in the tropics. Green gram is mostly grown in regions with an average rainfall of 600-1000 mm also it withstands drought well, by curtailing the period of flowering and maturation, but it is susceptible to waterlogging. High humidity at maturity causes damage to seeds leading to seed discoloration or sprouting while still in the field. The crop is utilized in several ways, where seeds, sprouts and young pods are consumed as sources of protein, amino acids, vitamins and minerals, and plant parts are used as fodder and green manure. Green gram protein is easily digested without flatulence. The protein is comparatively rich in lysine, an amino acid that is deficient in cereal grains but cereals are rich in methionine, cystine and cysteine, the sulphur bearing amino acid. Green gram is accentuated due to its nutritional value. Hundred gram of green gram seeds contains energy (234 cal), protein (24.6 %), fat (1.0 %), fiber (2.2 g), carbohydrates (57.5 %), calcium (0.08 g), phosphorus (0.045 g) and iron (5.7 mg), vitamin B (300 mg) and thiamin (0.525 mg) (Srivastava and Ali, 2004). It is an essential source of protein in cereal based diets, in the world over, covering more than six million hectares per year. Whereas, 90 per cent of green gram production in the world is contributed by Asia. India is the largest green gram producer of the world, accounting for about 65 per cent of world's acreage and 54 per cent of its production worldwide (Singh., 2011).

India is the world's largest producer as well as consumer of green gram with an area of about 42.42 lakh hectares production of 20.23 lakh tons and productivity of 477 kg/ha (Indiastat, 2017-18). In Telangana, the area covered under green gram, is about 0.99 lakh hectares with an average production and productivity of 0.6 lakh tons and 652 kg/ha, respectively (Indiastat, 2017-18). However, the standard yield of green gram worldwide is very low (384 kg/ha) and the green gram production has not considerably increased yet. The main cause for the low yield is the susceptibility of the crop to insects, weeds and diseases caused by fungus, virus and bacteria (Anonymous, 2012).

Among the various biotic stresses, the Yellow Mosaic Virus (YMV) disease is considered as the most destructive because of its severity and ability to cause 10-100% yield loss depending on the crop stage at which the plants being infected. (Marimuthu *et al*., 1981). YMV is prevalent in India, Bangladesh, Pakistan and some areas of South East Asia on green gram, soybean (*Glycine max* (L.) Merr.), urd bean (*Vigna mungo* (L.)

Hepper), pigeon pea (*Cajanus cajan* (L.) Millsp.) along with some minor legume crop like horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.), cowpea (*Vigna unguiculata* (L.) Walp.) (John *et al*., 2008).

The YMV Disease in southern Asia is caused by four distinct begomoviruses known collectively as the yellow mosaic viruses (YMVs); mungbean yellow mosaic virus (MYMV), mungbean yellow mosaic India virus (MYMIV), Dolichos yellow mosaic virus (DoYMV) and horsegram yellow mosaic virus (HgYMV) (Qazi *et al*., 2007). Geminivirus (second largest family of plant viruses) causes Yellow mosaic virus (YMV). Begomoviruses are a group of plant viruses containing single-stranded circular DNA encapsidated in geminate particles (Karthikeyan *et al*., 2004). The virus reported from India is not mechanically transmitted but is transmitted by the whitefly vector (*Bemisia tabaci*), to several species in the leguminosae and other families (Nariani, 1960). The symptoms of disease are a few small/minute yellow specks or spots on a few leaves and chlorosis or yellowing of all leaves of the whole plant causing necrosis (complete death). The crop is attacked in an epidemic form and in most of cases, there is yellowing of all leaves and pods, leading to complete yield loss. The disease poses a major crisis to the economic production of green gram in the Asian subcontinent.

Management of YMV is often linked with control of the *Bemisia tabaci* population by spraying insecticides, which is sometimes ineffective because of high population. The chemical management of the vector is expensive as numerous sprays of the insecticides are required to control the whitefly. Spraying often also leads to health hazards and ecological damage. On the contrary, use of virus resistance varieties is the most economical, efficient and environmental friendly approach to alleviate occurrences of YMV in areas where the infection is a major constraint to production.

However, it remains inadequate in developing yellow mosaic virus (YMV) resistant green gram varieties through conventional breeding methods due to rapid detonation of new isolates of YMV and also the complexity of mechanism in regulating its resistance. Identifying the sources of resistance against YMV in green gram is the major hurdle experienced by the scientists. For instance, the variation in the competence of transmission and behaviour of the whiteflies, with respect to host genotypes, vector biotypes and growth conditions etc., pose difficulty in identification of tolerant source. Recognition of resistant lines by the use of conventional method by field screening involves the screening at ‘hot spot’ area. Mostly, the incidence of the disease at the testing site may not be up to the desired level. It is season specific and cannot be created as and

when required by artificial means. Hence, the use of indirect selection methods such as molecular markers linked to YMV resistance gene(s) will be useful in rapid identification of genotypes having these genes without subjecting them to YMV screening. Also, the resistant genotypes can be identified at seedling stage, hence decreasing the time required for production of resistant varieties.

The advancements in the field of biotechnology and molecular biology such as genetic transformation and marker assisted selection could be utilized in developing YMV resistance in green gram (Xu *et al*., 2000). Once the resistance source is identified and their inheritance is known, identification of genomic regions along with molecular markers that are associated with the YMV resistance would increase the selection efficiency in the gene transfer in trait development programme. These techniques are widely accepted as potentially valuable tools for crop improvement in pulses to achieve resistance. The use of DNA marker technology is an ideal strategy to search for DNA markers linked with YMV Resistance since, the selection for resistance genes on phenotype basis is time consuming (Procunier *et al*., 1997). Molecular marker associated with a trait of interest like disease resistance is of great significance. Molecular marker based techniques are reliable and remain unaffected across different growth stages, seasons, locations and agronomic practices.

There are numerous conflicting reports for the inheritance of resistance to YMV claiming both susceptibility and resistance to be dominant. Molecular markers have been used to study the genetic diversity and to tag YMV and MYMIV resistance gene in green gram. The data of linked marker with YMV-resistant gene at protein and DNA level can be applied to marker assisted selection to increase breeding efficiency and also provide the base for gene cloning later.

Diverse molecular markers have been used for the molecular analysis of grain legumes (Gupta and Gopalakrishna, 2008). RAPD marker association with YMV resistance gene in green gram has been identified. The use of ISSR and SCAR markers linked to Yellow mosaic virus has been widely validated. Among several classes of available DNA markers, simple sequence repeat (SSR) or microsatellite marker is one of the most useful genetic marker systems that use polymerase chain reaction (PCR) technique to identify differences in microsatellite repeat units. SSRs are short tandem repeated nucleotide bases distributed throughout the genome. They are widely used because of its codominant, multi - allelic, high polymorphism, reproducibility, abundant informativeness, convenience of assay by PCR distribution throughout the genome,

independent of environments, independent of tissue effects and providing more precise characterization of genotypes and measurement of genetic relationships than other markers (Litt and luty, 1989). Studies revealed that microsatellite markers were found to be associated with YMV resistance which are CEDG293, DMB-SSR008 and DMB-SSR059 (Singh *et al*., 2020), CEDG228 and CEDG044 (Singh *et al*., 2018), MBM 0378 (Lekhi *et al*., 2018) and CEDG 180 (Gupta *et al*., 2013). These Microsatellite markers, which are tightly linked is one of the prerequisites for transfer of genes/QTLs for YMV resistance employing marker-assisted selection to develop a resistant variety for YMV. Marker assisted selection (MAS) has become an essential tool in breeding programs for those traits which are difficult to screen phenotypically. A better understanding of YMV resistance could lead to the identification of genes with different mechanisms that have the potential to complement each other and accordingly, will guide breeders to select appropriate breeding and selection procedures for stable production of green gram over location and year. Markers linked can be used to screen the breeding material and identify YMV resistant and susceptible genotypes of green gram. Molecular markers are now extensively used to track loci and genomic regions for biotic and abiotic resistance in various legume crops and a number of improved varieties have been developed using these markers in recent times (Kumar *et al*., 2011).

As of now, Many QTLs have been identified for YMV resistance in green gram. However, direct utility of QTL linked markers in the MAS programs is limited by lack of co-segregation with the resistance phenotype in the populations. Hence, before using these reported markers directly in MAS programs, it is better to validate them in the segregating populations.

Keeping in view the above perspectives, the present research work was formulated to evaluate green gram lines for YMV resistance using molecular markers with the following objectives:

1. To study the parental polymorphism using SSR markers.
2. Genotyping of the recombinant inbred lines (RIL) population using polymorphic markers.
3. Phenotyping for recombinant inbred line (RIL) population for YMV resistance.

Chapter II

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERARTURE

Green gram also known as mungbean (*Vigna radiata* (L.) Wilczek), is an essential pulse crop in countries of Asia, Africa and latin America, where it is consumed as dry seeds and fresh green pods (Karuppanapandian *et al*., 2006). This crop plays an important role in crop rotation due to their ability to fix atmospheric nitrogen, thus green gram helps to improve soil fertility and benefit subsequent crops. A number of biotic (fungal and viral diseases) and abiotic stresses are the major hurdles for the full realization of the yield potential of the crop. Among the diseases, viral disease mainly Yellow Mosaic Virus (YMV) leads to maximum yield loss. Identification of the resistance lines is essential in the ambit of integrated disease management. Earlier studies indicated that identification of resistant sources is a reliable option for managing YMV disease. Therefore, Molecular markers are reliable tool for screening a large number of germplasm lines and hence, can be used in breeding YMV resistance lines.

2.1 Viruses as a major constraint in pulse production

The annual production of pulses is greatly limited by the Yellow Mosaic disease of legumes caused by Gemini viruses, particularly in tropical and sub-tropical countries (Varma and Malathi, 2003). Plant viral diseases inflict serious economic losses in major crops by reducing yield and compromising quality (Kang *et al*., 2005). Majority of the legume crops such as green gram (*Vigna radiata*), blackgram (*Vigna mungo*), pigean pea (*Cajanas cajan*), soyabean (*Glycine max*), mothbean (*Vigna aconitiolia*) and common bean (*Phaseolus vulgaris*) are affected by YMV (Varma *et al*., 1992). The YMV are included in the genus *Begomovirus*, being transmited by the whitefly (*Bemisia tabaci*) and having bipartite genomes. White fly (*Bemisia tabaci)* delivers these viruses through their proboscis in the phloem cells of the host plant. The genome of bipartite *Begomoviruses* is composed of DNA A, which encodes proteins required for replication, transcription and encapsidation and DNA B, which encodes proteins required for movement functions. Both components are organized into divergent transcription units separated by an intergenic region (IR) that contains sequences conserved between the two DNA components and are referred to as the common region (Girish *et al*., 2005).

In 1940s YMV was first reported in lima bean (*Phaseolus lunatus*) in Western India. Later in 1950, Dolichos (*Lablab purpureus*) showed YMV in Pune. Later, Nariani

(1960s) observed YMV in green gram (*Vigna radiata*) in the experimental fields at Indian Agricultural Research Institute and was subsequently observed throughout India in almost all the legume crops. The loss in yield is more than 60 per cent when infection occurs within 20 days after sowing.

2.2 Symptomology/Epidemiology

The epidemic of YMV caused by MYMV was attributed to their ability to undergo genetic recombination and their transmission by polyphagous pest whitefly (*Bemisia tabaci*), its efficient vector. The MYMV can cause up to 100 per cent yield loss if infection occurs three weeks after planting and loss will be small whenever infection occurs after eight weeks from the day of planting (Karthikeyan *et al*., 2010). Symptoms initially include small yellow patches or spots appear on green lamina of young leaves. Soon, it develops into a characteristic bright yellow mosaic or golden yellow mosaic symptom.

Malathi and John (2008) characterized the epidemiology of the gemini viruses, which infect legumes. Whitefly (*Bemesia tabaci*), which acts as a vector for MYMV is polyphagous with a wide host range. However, it shows strong host preferences and its feeding behaviour is a major factor in deciding the active spread of the virus from one crop species to another. The tropical and subtropical regions of the Indian subcontinent are hot and humid, which favours the growth of *Bemesia tabaci*. The population of whitefly has been reported to be influenced by the climatic factors such as temperature, rainfall and relative humidity. Virus spread is gradual, cumulative and in the control of the prevalent wind depending upon the vector population built up during the course of infection. Yellow mosaic incidence varied from 46-51 percent in spring and 82-86 percent during rainy season in susceptible varieties (Gupta *et al*., 2013).

Based on the percentage of infectivity and the disease incidence to the reproductive parts of the plants, level of resistance rates are followed in different ways. According to the data the plants are classified into six different classes so that it would be easier to separate them category wise from resistant to susceptible lines in the field condition.

2.3 Genetics of resistance to MYMV in crop plants

Studies on the inheritance of MYMV were undertaken by various workers in green gram and black gram. Inheritance of MYMV resistance studies revealed that the resistance is controlled by a single recessive gene (Basavaraja *et al*., 2017; Anusha *et al*., 2014) two recessive genes (Alam *et al*., 2014) two dominant genes (Mahalingam *et al*., 2018) three recessive genes (Mishra and Asthana, 1996), single recessive gene (Reddy, 2012; Saleem *et al*., 1998; Reddy and Singh, 1995; Malik *et al*., 1986; Singh, 1977 and Thakur *et al.*, 1977) complementary recessive genes (Shukla and Pandya, 1985) and a dominant gene (Sandhu *et al*., 1985 and Gupta *et al*., 2005).

Bhanu *et al*. (2019) studied six intervarietal crosses involving two resistant and three susceptible genotypes of green gram with the objectives to determine the mode of inheritance of Mungbean Yellow Mosaic Virus (MYMV) resistance. Artificial inoculation was used for evaluation. The F_2 progeny segregated in the ratio of 9 S: 3 MS:3 MR:1 R suggesting that the resistance was governed by digenic recessive genes (r_m1 and r_m2).

Akbar *et al*. (2018) studied MYMV resistance in terms of number of genes governing it and genetics of related traits. Two contrasting genotypes, Resistant and Susceptible genotypes were crossed to raise six populations for evaluation under protected and unprotected field conditions. Using chi-square test, generation mean and variance analysis, it was discovered that disease resistance was governed by two major genes with additive effects.

Mahalingam *et al*. (2018) screened 10 green gram genotypes for MYMV disease resistance at hotspot. Among them, SML 1815, MH 421 showed resistant reaction whereas, VBN (Gg) 3, VBN (Gg) 2, LGG 460, RMG 10-28 and TM 96-2 showed susceptible reaction. Three crosses were made between resistant and susceptible genotypes. The three F_2 populations were sown along with black gram (CO5) as infector row for evaluation against MYMV resistance. The F_2 population segregated in the ratio of 15R:1S and 13R:3S, suggesting the involvement of two dominant genes in imparting resistance against MYMV disease.

Patel *et al*. (2018) effected hybridization between highly resistant genotype Meha and four susceptible genotypes viz., Pusa Vishal, GJM-1006, GM-4 and GJM-1008 to understand genetics of MYMV resistance in green gram. Evaluation of parents, F_1's, F_2's, F_3's progenies utilizing highly susceptible variety GM-4 as an infector row were

evaluated for their response to MYMV infection. Segregation pattern of resistance and susceptibility in F_2 generation indicated duplicate epistasis (15:1) which was confirmed by non-significant χ^2 test. Ten resistant F_2 plants were selfed to further confirm digenic duplicate epistatic nature of mosaic resistance. The results were also in accordance with the lineage of mosaic resistance in Meha from blackgram.

Basavaraja *et al*. (2017) studied mode of inheritance of resistance to MYMV in intra and inter-specific crosses of green gram. In the field condition, only after 80% of plants showed MYMV incidence and the scoring of the test materials was done by MYMV disease reaction scale. The results showed that in all the three intraspecific F_2 population segregated in 1: 3 (Resistance: Susceptible) ratio indicating single recessive gene whereas in case of all Interspecific crosses between green gram (*Vigna radiata*) and ricebean (*Vigna umbellata*), segregation of F_2 population was in 3(R) : 1(S) ratio which clearly indicated that single dominant gene governed YMV resistance in ricebean.

Thamodhran *et al*. (2016) studied the nature of inheritance of YMV through goodness of fit test and noted it as the duplicate dominant or duplicate recessive in segregating populations of various crosses.

Aski *et al*. (2015) determined mode of inheritance for resistance against MYMIV. Four crosses between resistant and susceptible genotypes were made. The six generations i.e. P_1, P_2, F_1, BC_1, BC_2 and F_2 were grown along with infector row for evaluation against MYMIV resistance. The segregation of resistance response in F_2 populations in ratios of 9 S: 3 MS: 3 MR: 1 R suggested that the resistance was governed by two recessive genes. The results of test crosses/ backcross BC_1 with all susceptible plants and in BC_2 ratio of 1S: 1MS: 1MR: 1R also supported that two recessive genes are involved in imparting resistance against MYMIV.

Alam *et al*. (2014) studied types of gene action governing the resistance to MYMV disease, yield components and effect of MYMV on yield-related traits. P_1, P_2, F_1, F_2, P_1F_1 and P_2F_1 were developed from two crosses involving two susceptible (‘BM1’ and ‘KPS1’) and one resistant (‘BM6’) green gram genotypes. Additive and dominant gene effects were found important in both crosses for all the traits examined. Segregation of the responses in F_2 populations agreed with a ratio of 9 S: 3 MS: 3MR: 1 R, suggesting that the resistance was governed by two recessive genes.

Anusha *et al*. (2014) screened 123 F_2 plants of population under natural screening to assess their resistance or susceptibility against MYMV. This study revealed that single

recessive gene is governing the inheritance of resistance to MYMV. In the F_2 mapping population MYMV resistance segregated in 91 susceptible: 32 resistant i.e. 3:1 ratio suggesting monogenic recessive inheritance.

Vinoth and Jayamani (2014) studied the inheritance of resistance to YMV in F_2, F_3 and RIL population of inter sub-specific cross of blackgram involving resistant cultivar 'VBN (Bg) 4' (*Vigna mungo*) and susceptible accession of *Vigna mungo* var. *silvestris* 22/2, a wild progenitor of blackgram. The results of genetic analysis showed that a single dominant gene controls the YMD resistance in blackgram cultivar 'VBN (Bg) 4'.

Sudha *et al*. (2013) studied the mode of inheritance of resistance to MYMV in inter (TNAU RED × VRM (Gg) 1) and intra (KMG 189 × VBN (Gg) 2) specific crosses of green gram. An infector row technique was used for evaluating parents, F_1, F_2 and F_3 plants for MYMV resistance. Collectively, all the two crosses F_2 and F_3 generations results suggested that a single recessive gene is involved in resistance against the MYMV disease.

Gupta *et al*. (2013) described the inheritance of MYMIV resistance gene in blackgram using F_1, F_2 and $F_{2:3}$ derived from cross DPU 8831 (resistant) × AKU 9904 (susceptible). Single dominant gene was shown to be involved in MYMIV resistance in blackgram genotype DPU 88-31.

Dhole and Reddy (2012) performed six crosses between resistant and susceptible genotypes. Critical evaluation of Parents, F_1 and F_2 plants were done in an infector row technique and F2 populations were classified into four reactions based on severity and distribution of symptoms of MYMV disease. The F_2 segregated in ratios of 9: 3: 3: 1 that showed that the resistance was controlled by two recessive genes.

Kundagrami *et al*. (2009) reported MYMV resistance on blackgram (*Vigna Mungo*) was monogenic recessive nature.

2.4 Genetic variability and parameters for yield components and resistance to MYMV

Genetic variation describes naturally occurring genetic differences among individuals of the same species. This variation permits flexibility and survival of a population in the face of changing environmental circumstances. Consequently, genetic variation is often considered an advantage, as it is a form of preparation for the unexpected. The presence of genetic variability for commercial traits is a key factor for

developing the locally adopted variety with regard to limited and specific traits. Efficient collections of natural variability have not been made and therefore, narrow genetic variability is available in green gram. Genetic variability is very essential as it serves as a basis for crop improvement programme. Johannsen (1909) demonstrated the distinction between genotype and phenotype in the study of heredity. Concept of multiple factor hypothesis might account for the inheritance of many quantitative characters was given by Nilsson-Ehle (1909). Hence, it can be concluded that variability is due to the interaction between genotype and environment.

Genetic variability is prerequisite for commencing an active and profitable breeding programme and it is imperative to study the level of genetic variability available in the existing germplasm. Knowledge on nature and degree of variability present in the population due to genetic and non-genetic cause and also due to interaction between these factors is an important requirement for a planned breeding programme. The study of genetic advance with heritability estimates further enlighten the nature of character, which can be improved through selection.

The assessment of genotypes under different environmental conditions provides information on the relative degree of phenotypic and genotypic variability and the extent of genetic advance that can be made by studying the material under more than one environment has been indicated by Comstock and Robinson (1952), Johnson *et al*. (1955), Neij and Syakudd (1957) and Athwal and Singh (1966).

Heritability is defined as the degree to which individual genetic variation accounts for phenotypic variation seen in a population. Heritability is a statistic used in the fields of breeding and genetics that estimates the degree of variation in a phenotypic trait in a population that is due to genetic variation between individuals in that population. Heritability could increase if either genetic variation increases, causing individuals to exhibit more phenotypic variation or if environmental variance is less. “Broad sense heritability is characterized as the proportion of genotypic variance to the total variance” (Lush, 1940). The perceptible characters are governed by large number of genes and are more influenced by the environment. The phenotype observed is not disseminated entirely to next generation. Therefore, it is necessary to know the distribution of observed variability that is transmitted. Estimating heritability along with genetic gain is usually more useful in predicting the resultant effect from selecting the best individual (Johnson *et al*., 1955). Genetic advance is the difference between the mean genotypic value of the selected lines and the mean genotypic value of the base population.

The Brief review pertaining to the genetic variability, heritability and genetic advance in green gram is provided for the yield and yield related traits are as follows.

Mariyammal *et al*. (2019a) conducted variability studies for eight quantitative traits such as days to first flowering, plant height, number of clusters per plant, number of pods per cluster, total number of pods per plant, number of seeds per pod, 100 seed weight and single plant yield. The variation was recorded to be the highest for plant height followed by number of pods per plant. Hence direct selection based on these traits would be effective. Plant height and single plant yield recorded high heritability coupled with low genetic advance in all these crosses. These findings revealed presence of dominance and epitastatic gene effect and the selection based on these traits may not be rewarding.

Asari *et al*. (2019) studied 44 green gram genotypes to assess the genetic variability parameters, heritability, genetic advance, correlation and path coefficient analysis for yield and yield contributing characters. High heritability along with high genetic advance as percent of mean was observed for plant height, primary branches per plant, clusters per plant, pods per plant and seed yield indicating prevalence of additive gene action. Among the characters studied, days to 50% flowering had high positive direct effect on seed yield per plant while, test weight, clusters per plant, pods per plant and primary branches per plant had low positive direct effect on seed yield per plant. Hence, it can be concluded that more insistence should be given on these characters while selection for high yielding mung bean cultivar.

Malli *et al*. (2018) studied and assessed the magnitude of genetic variability, heritability in broad sense and genetic advance among 40 green gram accessions for growth and grain yield characters. Maximum genotypic and phenotypic variance was recorded for harvest index, primary branches per plant, seed yield per plant, pods per plant. Maximum GCV and PCV were recorded for harvest index, biological yield/plant and seed yield/plant. High heritability was recorded for biological yield/plant, days to maturity, seed/pod, number of branches/plant and seed yield/plant. High heritability coupled with high genetic advance as percent of mean was recorded for different traits.

Mehendi *et al*. (2018) studied 48 green gram genotypes for genetic variability, heritability and genetic advance for yield and 13 yield associated traits. The analysis of variance revealed statistically significant differences ($p<0.05$) indicating the existence of genetic variability among the 48 genotypes for all the traits studied. The characters were

found to be under Additive effect. Consequently, based on the genetic parameter analysis days to 50% flowering, number of effective branches per plant, seed index, seed yield per plant, biological yield per plant and harvest index were suggested more important while formulating a selection strategy for effective improvement of green gram varieties.

Shiv *et al*. (2017) assessed genetic variability, heritability and genetic advance in four F_3 populations for 11 quantitative characters. They took four F_3 generations from crosses viz. Meha x Pusa Vishal, Meha x GJM-1006, Meha x GM-4 and Meha x GJM-1008. Moderate to high GCV and PCV values were observed for pods per plant, cluster per plant, seed yield per plant, straw yield per plant and harvest index. High heritability with high genetic advance was recorded for pods per plant, clusters per plant, seed yield per plant and straw yield per plant. High heritability with high genetic advance for most of the traits was depicted by the F_3 population of crosses viz. Meha x GJM-1006 and Meha x GJM-1008.

Payasi (2015) studied 64 powdery mildew disease tolerant genotypes of green gram, during two seasons in RBD with three replications to compare their performance and existing variability among various yield related traits. Analysis of variance showed significant variation for days to 50% flowering, days to maturity, plant height, number of primary branches per plant, number of clusters per plant, number of pods per cluster, pod length, number of seeds per pod, number of pods per plant, 100 seed weight, hard seed percent, protein content, phenol content and seed yield per plant. High heritability coupled with high genetic advance as percentage of mean was observed for seed yield per plant, number of primary branches, number of clusters per plant and plant height.

Hemavathy *et al*. (2014) investigated and evaluated 13 diverse green gram genotypes for the estimation of genetic variability, heritability, and genetic advance, correlation coefficient for nine quantitative trait and their association level with yield. Association analysis were performed for different traits. Maximum direct effect on seed yield was observed through different traits. They concluded that on the basis of genetic parameters correlation and path analysis, the number of pods per cluster, number of pods per plant, number of seeds per pod and 100 seed weight should be given top most priority while formulating a selection strategy for improvement of yield in green gram.

Raturi *et al*. (2014) studied genetic variability, heritability and genetic advance were conducted during two seasons. The number of pods per plant and seed yield were recorded with significantly higher heritability (>60%), corresponding PCV (>25%) and

GCV (>20%) coupled with more than 30% genetic advance. The number of pods per plant had the maximum direct effect followed by plant height and 1000 seed weight indicating their direct contribution towards seed yield. The study concluded that seed yield and number of pods per plant are greatly influenced by the additive gene effect and greater proposition of variations are heritable for these traits.

Alemu *et al*. (2014) assessed the magnitude of genetic variability, heritability in broad sense and genetic advance in a set of 13 mung bean accessions for growth and grain yield characters. The results showed that number of primary branches, pods per plant, seeds per plant and harvest index had high genotypic and phenotypic coefficients of variation at both locations. The combined results for heritability showed that the high estimates of heritability and genetic advance were scored for seeds per plant and seed yield indicating that these characters were under the control of additive genetic effects.

Nand and Anuradha (2013) observed high PCV and GCV estimates were observed for number of pods per plant, seed yield per plant. High heritability along with high genetic advance as per cent of mean was observed for number of pods per plant, number of seeds per pod, seed yield per plant indicating the role of additive genes in governing the inheritance of these traits and could be improved through selection.

Kamleshwar *et al*. (2013) studied genetic variability, correlation among the yield components and their direct and indirect effects on yield in green gram involving many characters. The trait 100 seed weight exhibited high heritability estimates (narrow sense) coupled with high genetic advance, which shows the importance of additive gene action.

Gadakh *et al*. (2013) studied genetic divergence and clustering pattern of 50 genotypes of green gram for selection of suitable parents that can be utilized in hybridization programme and to study the genetic parameters attributing to yield. Higher genotypic and phenotypic coefficient of variation was observed for harvest index followed by biological yield per plant, 100-seed weight and number of primary branches per plant in mung bean. High heritability coupled with high genetic advance was observed for harvest index, biological yield per plant, and number of pods per plant.

Ullah *et al*. (2011) estimated the heritability and genetic advance in four F_2 populations at two different locations. Combined analysis reports exhibited highly significant difference exist between the genotypes and location for 1000 seed weight, seeds per pod, seed yield and pods per plant.

Abbas *et al*. (2010) studied diverse green gram germplasm (40 genotypes) from India, Pakistan and Thailand using metro glyph analysis. Morphological traits such as plant height, clusters per plant, pods per cluster, 100 seed weight, biological yield and seed yield showed considerable genetic variability.

Rahim *et al*. (2010) studied genotypic and phenotypic variance, coefficient of variance, heritability, genetic advance, correlation and path coefficient for yield and its contributing characters in 26 green gram genotypes. High heritability (broad) along with high genetic advance in percent of mean was observed for plant height, number of pods per plant, number of seeds per pod, 1000 grain weight and grain yield per plant..

Makeen *et al*. (2007) studied 20 diverse green gram genotypes for the estimation of genetic variability, heritability, genetic advance, correlation coefficient and path coefficient analysis for 10 quantitative characters. The genotypes differed significantly for all characters studied. Maximum heritability values were recorded for seed protein content, plant height and test weight. High heritability coupled with high genetic advance was observed in pods per plant, plant height and test weight indicating the importance of additive gene effect for expression of these characters. Character association indicated that pods per plant and plant height had significant positive correlation with seed yield.

Pandey *et al*. (2007) observed high significant variation in seed yield and its attributing characters. High or moderately high genotypic and phenotypic coefficient variations were reported for seed yield, harvest index, plant height, pod number and cluster number. High or moderately high heritability was observed for plant height, seed number, pod length, 100-seed weight and number of clusters. Finally the selection for genotypes with higher harvest index and biological yield, plant height and longer duration could facilitate enhancement of seed yield in green gram.

Raje and Rao *et al*. (2000) studied green gram germplasms in four different environments and recorded high value of GCV for number of primary branches per plant, clusters per plant, pods per plant, seeds per plant and seed yield. Additive gene action controlled 100 seed weight. This showed high heritability at different environments.

2.5 Application of Molecular Markers in Green gram

A molecular marker is a DNA sequence in the genome which can be located and identified. Markers tightly linked to the genes are used in crop improvement employing marker-assisted selection. DNA based markers have shown great promise in expediting plant breeding process. Molecular markers are indispensable for genomic study. Not many genetic markers were developed specifically for green gram. Molecular marker technology has greatly accelerated breeding programs for improvement of various traits including disease resistance and pest resistance in various crops by providing an indirect method of selection. The markers are typically small regions of DNA, often showing sequence polymorphism in different individuals within a species and transmitted by the simple Mendelian laws of inheritance from one generation to the next. These include Allele Specific PCR (AS-PCR) (Sarkar *et al*., 1990), DNA Amplification Fingerprinting (DAF) (Caetano-Anolles *et al*., 1991), Single Sequence Repeats (Hearne *et al*., 1992), Arbitrarily Primed PCR (AP-PCR) (Welsh and Mc Clelland, 1990), Single Nucleotide Polymorphisms (SNP) (Jordan and Humphries, 1994), Sequence Tagged Sites (STS) (Fukuoka *et al*., 1994), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al*., 1995), Simple sequence repeats (SSR) (Anitha, 2008), Resistant gene analogues (RGA) (Chithra, 2008), Random amplified polymorphic DNA - Sequence characterized amplified regions (RAPD-SCAR) (Sudha *et al.,* 2013), Randomly Amplified Polymorphic DNA (RAPD) (Anushya, 2009), Amplified Fragment Length Polymorphism- Resistant gene analogues (AFLP-RGA) (Nawkar, 2009).

When all the members of the population have been scored (genotyped) with a set of molecular markers, the data can be used to make a linkage map (often described as a genetic map). The linkage map describes the linear order of markers within linkage groups. Linkage map is among the most important genetic tools used in contemporary genomic and genetic investigations in crops. Molecular markers have been used to tag the MYMV resistance gene in blackgram, soybean and green gram (Basak *et al*., 2004 and Souframanien and Gopalakrishna, 2006). Randomly amplified polymorphic DNA (RAPD) markers and Intersimple Sequence Repeat (ISSR) markers have been successfully used for the construction of linkage maps in many crop plants including grain legumes (Kalo *et al*., 2000, Lambrides *et al*., 2000 and Cobos *et al*., 2005). Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning.

Microsatellite or Simple Sequence Repeat (SSR) markers, have been developed for green gram (Kumar *et al*., 2002 ; Miyagi *et al*., 2004). The SSR markers have been extensively used in the identification of inheritance pattern, linkage analysis, and QTL mapping in multiple crops. The SSRs are the most widely applied for crop conservation, synteny, and transferability (Marques *et al*. , 2002 ; Gasic *et al*. , 2009).

Genomics tools have become very essential part of crop improvement and genetic resources management programs. Since the last decade, there has been tremendous development in genomics technologies and these tools are helping in identifications of genes/QTLs for all kind of traits, introgression of traits, shortening of breeding cycles, development of new and ideal plant types, development of new variations through utilizing alien species or through mutational approaches, etc.

Earlier to advancement in genome sequencing technologies, several DNA-based molecular markers were also developed (Kaga *et al*., 2000; Barkley *et al*., 2008), and they are still being used particularly which are linked to a trait of interest (Schafleitner *et al*., 2016). These markers were also used in making linkage mapping, and markers were linked to loci governing important traits like seed weight, seed coat colour, resistance for powdery mildew, YMV, bruchids and Cercospora leaf spot (Kim *et al*., 2015). In addition to nutritional qualities, certain traits of green gram like small genome size, short life cycle, self-pollinating, and close genetic relationship with many other legume species makes it a suitable model organism for genetic studies (Kim *et al*., 2015).

2.5.1 Genetic diversity

Extent of genetic diversity present in a population is very vital for any crop improvement programme. Selection of parents based on genetic divergence may form a more sound base for the success of a hybridization programme. Molecular methods have now become an integral part of the studies on genetic diversity as they are more reliable and precise than the methods based on morphological traits. The literature pertaining to molecular marker techniques used to assess the genetic diversity in green gram are presented here.

Pooja *et al*. (2019) identified SNPs suitable for analysis of genetic diversity and established robust SNP marker profile for identification of green gram varieties. A novel set of five SNP regions were identified by analysing a large number of SNP markers developed using whole genome re-sequencing approach for discriminating a set of 34 selected green gram varieties. Polymorphism information content (PIC) and major allele

frequency (MAF) ranged from 0.08 to 0.21 (an average of 0.16) and 0.86 to 0.95 (an average of 0.90), respectively. The results indicated the presence of low genetic distance between the green gram varieties. These novel SNP markers will allow rapid and accurate identification of green gram varieties and will find application in marker assisted green gram breeding programmes.

Nath *et al*. (2019) reported genetic variability within and among plant populations. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix. 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 VI 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.

Wang *et al*. (2018) described the role of genetic resources of one region in widening the genetic background of the local breeding genotypes. They observed the adaptability of green gram accessions collected from the USDA and their genetic diversity was assessed by using SSR markers. High diversity was revealed by the SSR markers with an average of 4.2 alleles per locus and polymorphism information content value of 0.650 per locus.

Sharma *et al*. (2018) studied genetic diversity in green gram using 20 SSR and ISSR primers out of which 10 SSR primers and 7 out of 10 ISSR primers showed amplification. The SSR primers amplified 1 (VR062, VR188, Phi057) to 2(VR0155, VR0222, VR0223, VR0304, VR225, Phi112 and VR040) alleles of 50-600 bp and ISSR primers amplified 1(ISSR GA5), 2(ISSR 842, ISSR 856, ISSR 857, ISSR CA1) and 4(ISSR GA1) alleles of 180-900 bp size. SSR primers and ISSR primers showed 85% and 67.85% polymorphism with highest PIC value marked by VR062 is 0.95 and ISSR 842 is 0.60, respectively. The cluster analysis based on unweighted paired group method of arithmetic means (UPGMA) with 20 SSR and ISSR primers grouped 19 genotypes into four major clusters.

Islam *et al*. (2018) studied kompetitive Allele Specific PCR (KASP) - based single-nucleotide polymorphism (SNP) markers and used them for characterization of 94 cultivated mung bean genotypes from the USDA originating in 27 countries across 10 regions of the world, all being cultivars rather than wild accessions. A total of 42 SNPs from previous sequencing information and were converted to 20 robust KASP assays.

The polymorphism information content of the newly developed markers ranged from zero for monomorphic markers to 0.375 for the most diverse biallelic polymorphic marker (MBkSNP_39) and averaged 0.250 across all loci. An analysis of molecular variance revealed 22% of genetic variation among subpopulations and 78% within subpopulations. The first two axes of region-wide principle coordinate analysis explained 81.26% variation of total variation, indicating the existence of genetic diversity among groups.

Nath *et al*. (2017) studied genetic variability within and among plant populations with ISSR marker. ISSR marker analysis was performed to detect relatedness and diversity among eight parental genotypes. 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 18 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 with total 29 fragments and eight highest scorable bands as well as 100 percent polymorphism.

Vishalakshi *et al*. (2017) investigated the genetic relationships among three varieties and nine accessions of black gram having disease resistance to previously described diseases and susceptibility using RAPD markers. A total of 33 RAPD primers were used for diversity analysis and yielded 206 fragments. Number of amplified fragments ranged from two (OPN-1) to 13 (OPF-1). The highest similarity coefficient was observed between IC-145202 and IC-164118 (0.921), while lowest similarity was between PU-31 and IC-145202 (0.572). The genetic diversity obtained in this study along with disease analysis suggests PU31 as a useful variety for the development of markers linked to MYMV, UCLV, wilt and powdery mildew resistance by marker-assisted backcross breeding and facilitates the production of crosses with multiple disease resistance.

Binyamin *et al*. (2016) screened 127 genotypes against MYMV under the field condition. RAPD analysis showed significant amount of genetic diversity within green gram germplasm. Twenty six primers produced 66% of polymorphism with average 5.36 bands per primer. RAPD analysis revealed an extensive amount of variation.

Singh *et al*. (2016) analysed seven mungbean landraces and cultivars, 4 urdbean landraces and 4 accessions of beachpea (*Vigna marina* (Burm.) Merr) using 24 randomly selected ISSR markers. Seven ISSR markers produced clear amplification profiles. Based on the presence or absence of bands, Jacquard's similarity index was calculated to

construct a dendrogram to show genetic distance between and within the accessions. Similarity index values ranged from 0.67 to 0.92. Matrices derived from ISSR data were used to construct UPGMA dendrograms. The dendrograms derived from ISSR data showed two main groups and four sub groups.

Swati *et al*. (2014) performed ISSR analysis to study genetic diversity in some black gram cultivars. A total number of 10 ISSR primers that produced polymorphic and reproducible fragments were selected to amplify genomic DNA of the urd bean genotypes.

Kalaria *et al*. (2014) identified genetic variation and tightly linked marker associated with MYMV resistance in different green gram lines. Out of 200 RAPD markers, OPG-5, OPJ18 and OPM-20 were the best markers which produced 28, 35, 28 amplicons. respectively Out of 17 ISSR markers used, DE16 was the best marker. The combined dendrogram obtained from these RAPD and ISSR indicated two clearly visible clusters where all resistant lines clearly separated from susceptible lines.

Gupta *et al*. (2013) assessed genetic diversity at molecular level in 29 elite green gram genotypes. A total of 30 green gram SSR markers including 15 genomic SSR markers and 15 EST-SSR markers were used. Each polymorphic SSR marker detected two to three alleles with an average of 2.3 alleles per locus. Polymorphic information content of SSR marker ranged from 0.07 to 0.61 with an average of 0.27. A high discrimination power was revealed by SSR markers and it was observed that a combination of only six SSR markers was sufficient to distinguish 27 out of 29 green gram genotypes. Cluster analysis grouped the green gram genotypes into three clusters which were in accordance with their pedigree and also supported with the results of principle coordinate analysis.

Somta *et al*. (2009) employed 241 SSR markers for the assessment of diversity at the molecular level among 39 parental lines and 5 cultivars derived from breeding lines of green gram. The polymorphic SSR markers detected a total of 175 alleles ranging from 2 to 19 alleles per primer and the PIC value ranging from 0.049 to 0.883. The clustering pattern of entries was in accordance with their origin or pedigree. The five cultivars selected from the breeding lines showed a moderate genetic diversity. Of the 175 alleles detected in 39 parental lines, 69 alleles (39.43 %) were present in the five cultivars. The cultivars were grouped with the Philippine germplasm and most of them were close to one of the parents in their pedigrees.

Lavanya *et al*. (2008) used RAPD profiles to identify the extent of genetic diversity among 54 accessions of green gram. Out of the 40 primers screened, seven primers generated 174 amplification products. Jaccard's similarity coefficients ranged from 0 to 0.48, indicating the presence of wide range of genetic diversity at molecular level.

Karuppanapandian *et al*. (2006) used 20 decamer primers to detect extent of genetic diversity at DNA level among 15 green gram landraces by RAPD analysis. Of 20 primers, 18 showed polymorphism. The primer OPA-03 was found to produce maximum number of polymorphic bands. Jaccard's similarity coefficient ranged from 0.64 to 0.93 and concentrated mostly between 0.76 and 0.93 indicating a narrow genetic base of tested green gram landraces.

2.5.2 Molecular mapping and identification of genes and QTLs associated with important traits in green gram

The strategies of molecular genetic mapping and molecular breeding have made a spectacular impact during the last one and a half decades of the twentieth century. But still they remain "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unravelled.

Singh *et al*. (2020) detected the marker-trait association of a selective diverse panel of 127 mungbean genotypes against MYMIV. A total of 256 genome-wide microsatellite markers were screened on a test panel in which 93 polymorphic markers were used in association studies. Population structure analysis led to formation of six distinct subpopulations. A total of 15 microsatellite markers were detected as associated with MYMIV resistance, among them three microsatellites explained 11–14% phenotypic variation. The specific regions close to CEDG293, DMB-SSR008 and DMB-SSR059 associated with MYMIV resistance were detected and located on linkage group 2, 4 and 9.

Dharajiya *et al*. (2019) performed BSA with a RAPD marker technique in the $F_{2:3}$ individuals of Pusa Ratna (susceptible) × Meha (resistant) cross to identify molecular marker linked to MYMV resistance in mungbean. The RAPD primer, OPP 07 showed the specific band of 895 bp in resistant parent and their bulks, but not in the susceptible parent and their bulks. Co-segregation analysis was performed in resistant and susceptible

$F_{2:3}$ individuals; it confirmed that OPP 07_{895} marker was associated with MYMV resistance in mungbean.

Singh *et al*. (2018) employed 224 molecular markers for the identification of polymorphism between parents. Only 46 markers showed polymorphism between Sonali and V. *radiata* var. *sublobota*. Twenty two polymorphic markers were used to construct a linkage map comprising 11 linkage groups. QTL analysis identified molecular markers linked with MYMIV resistance and agronomic traits viz., no. of pods per plant, no. of seeds per pod and 100- seed weight. The molecular markers linked to the MYMIV and yield attributing traits identified in this study will be useful in marker assisted breeding for development of high yielding mungbean varieties resistant to MYMIV

Kabi *et al*. (2018) screened seven genotypes with PCR based technique employing primer pairs to validate efficiency and reliability of identified marker loci CYR1 and YMV1. Both markers showed consistent polymorphism with respect to disease reaction in seven resistant genotypes. CYR1 was produced an allele size of approximately 90 bp which concluded that seven genotypes have yellow mosaic virus resistant genes and both markers are efficient and ubiquitous for genotyping of YMV reaction.

Lekhi *et al*. (2018) investigated mode of inheritance of MYMV resistance in an interspecific cross of mungbean and identify SSR marker(s) linked to it through BSA. MYMV susceptible *Vigna radiata* genotype SML668 was crossed as female to MYMV resistant *V. mungo* genotype Mash114 to generate F_2 mapping population. The segregation of MYMV in F_2 population fit well in genetic ratio of 3 resistant: 1 susceptible indicating that MYMV resistance from Mash 114 was governed by single dominant gene. The BSA with 67 SSR markers selected from related *Vigna* species and identified 46 markers polymorphic between the parental lines while one SSR marker-MBM 0378 amplified a polymorphic fragment between resistant bulk and susceptible bulk indicating association of this marker with MYMV resistance.

Patel *et al*. (2018) reported identification of markers linked with MYMV resistance gene by investigating F_5 RILs developed from the cross between Meha (resistant) and GM-4 (susceptible). The RILs segregated in 1R:1S ratio in the field screening indicating that the MYMV resistance gene inherited as a monogenic trait. Out of 124 primers used including RAPD, SSR, SCAR and RGA primers, only one primer RGA pair 1F-CG/RGA 1R amplified a single 445 bp band in the genotype Meha (resistant parent) and MYMV resistant bulk which was absent in GM-4 (susceptible parent) and

MYMV susceptible bulk. Same amplicon was detected in individual samples of F_5 RILs constituting the MYMV resistant bulk indicating that this RGA was linked to MYMV resistance.

Kabi *et al.* (2017) developed SCAR marker YMV-1 and tested over 26 genotypes produced amplicon at both 90 bp and/or 200 bp, This primer was revalidated again in next generation which amplified in three genotypes (OBGG-2013-8, OBGG-2013-12, OBGG-2013-39) at 90 bp and in other four genotypes (OBGG2013-21, OBGG-2013-16, OBGG-2013-11, OBGG-2013-20) at 90 bp and 200 bp concluding that these seven genotypes have yellow mosaic virus resistance gene and this marker is useful for genotyping of YMV reaction.

Sai *et al*. (2017) reported molecular markers linked to MYMV resistance and to find the genetic inheritance of MYMV resistance in green gram. A total of 413 germplasm entries were subjected to natural field infection in a MYMV hotspot area (Vamban) and a total of 13 selected resistant lines were subjected to Agrobacterium infection using strains harboring partial genome of two different MYMV isolates, VA221 and VA239. Among the resistant lines, KMG189 showed strain-specific resistance to VA221 and had no symptoms during field trials. Two SCAR markers CM9 and CM815 were developed through BSA, and the linkage analysis proved CM815 SCAR marker to be linked at 5.56 cM with MYMV resistance gene and SCAR CM9 had nil recombination percentage, suggesting it to be very closely linked to the MYMV resistance gene. SCAR marker CM9 was located on chromosome number 3 of green gram suggesting novel locus for virus resistance in green gram.

Rani *et al*. (2017) used RILs derived from JS335 and PI171443 and F_2 population derived from SL525 and NRC101 to study the inheritance of MYMIV resistance and map the gene responsible for MYMIV resistance. BSA was performed using 144 polymorphic SSR markers. MYMIV resistant gene was mapped on chromosome 6 (LG C2) within a 3.5cM genome region between two SSR markers GMAC71 and Satt 322, the size was estimated to be 77.115 kb.

Bui *et al*. (2017) validated mungbean lines V2802, NM92, NM94 and of the wild mungbean line TC1966 (*Vigna radiata* var. sublobata), from F_7 progenies of V2802 x NM94 and F_{12} progenies of TC1966 x NM92 (World Vegetable Center mungbean breeding program) employing putative SNP markers that were identified in populations V2802 x NM94 and TC1966 x NM92 using genotyping by sequencing approach.

Binyamin *et al*. (2015) screened mungbean genotypes consisting of 127 varieties/lines for MYMVD under natural field conditions. The SCAR marker (MYMVR-583) amplified a single band of 583 bp in all 12 genotypes reported to be resistant and moderately resistant, while no amplification was observed in highly susceptible genotypes. Similarly, a second SCAR marker (YMV1) amplified an allele in resistant and moderately resistant genotypes and no fragment was observed in highly susceptible genotypes, indicating that they lack the resistance gene, causing highly susceptible response.

Yadav *et al*. (2015) performed whole-genome resequencing of MYMIV resistant cultivar 'UPSM-534' and susceptible Indian cultivar 'JS335' to identify high-quality SNPs and InDels. A total of 3083987 SNPs (1559556 in UPSM-534 and 1524431 in JS-335) and 562858 InDels (281958 in UPSM-534 and 280900 in JS-335) were identified. Of these, 1514 SNPs were found to be present in 564 candidate disease resistant genes. Among these, 829 non-synonymous and 671 synonymous SNPs were detected in 266 and 286 defence-related genes, respectively. The study generated a large-scale genomic resource such as, SNPs and InDels at a genome- wide scale that will facilitate the dissection of various complex traits through construction of high-density linkage maps and fine mapping.

Kalaria *et al*. (2014) identified genetic variation and tightly linked marker associated with MYMV resistance in different green gram lines. Out of 17 ISSR markers DE-16 proved to be the best marker as it showed highest polymorphism among all. The combined dendrogram obtained from these RAPD and ISSR indicated two clearly visible clusters where all resistant lines clearly separated from susceptible.

Anusha *et al*. (2014) studied marker MB14 present on linkage group 8 which clearly distinguished resistant and susceptible parents, bulks and ten F2 resistant and susceptible plants indicating that this marker could be linked to yellow mosaic virus resistance gene.

Alam *et al*. (2014) employed F_2 and BC_1F_1 populations derived from a cross between susceptible and resistant mungbean genotypes to identify QTLs associated with resistance to MYMIV. Resistance to the virus was evaluated using F_3 and BC_1F_2 populations under field conditions in two locations in Bangladesh. A total of 1165 SSR markers from different legumes were used to detect the polymorphism between the parents, BM1 and BM6. A total of 61 polymorphic markers were used to construct a

linkage map. They identified two major QTLs, qMYMIV2 on linkage group 2 and qMYMIV7 on linkage group 7 conferring resistance in both F_2 and BC_1F_1 populations.

Anusha *et al*. (2014) screened F_2 population with thirteen polymorphic markers to find the markers linked to the resistant gene by bulk segregant analysis. MB14 marker present on linkage group 8 clearly distinguished resistant and susceptible parents, bulks and ten F_2 resistant and susceptible plants indicating that this marker is tightly linked to yellow mosaic virus resistance gene.

Gupta *et al*. (2013) performed molecular tagging of MYMIV resistance gene in blackgram by using 61 SSR markers of which, 31 were found to be polymorphic between the parents. Marker CEDG 180 was found to be linked with resistance gene following the BSA. This marker was mapped in the F_2 mapping population of 168 individuals at a map distance of 12.9 cM.

Sudha *et al*. (2013) identified the molecular markers (SSR, RAPD and SCAR) associated with Mungbean yellow mosaic virus resistance in an inter-specific cross between a mung bean variety, VRM (Gg) 1 x a rice bean variety, TNAU RED. This study revealed that 42 azuki bean markers (39.62%) and four mung bean markers (54.07%) showed parental polymorphism. Among the 42 azuki bean SSR markers surveyed, only 10 markers produced heterozygotic pattern in six F_2 lines viz., 3, 121, 122, 123, 185 and 186. These markers were surveyed in the corresponding F_3 individuals, which skewed towards the mungbean allele.

Kitsanachandee *et al*. (2013) constructed a genetic linkage map using RIL population with SSR markers and identified five QTLs to MYMIV resistance using cross combination of NM10-12-1 (resistant) and KPS2 (susceptible) in Thailand.

Holeyachi *et al*. (2013) identified MYMV resistance marker loci, UBC 499 in mungbean by using BSA.

Kajonphol *et al*. (2012) used the SSR markers to construct a linkage map and identify chromosome regions controlling some agronomic traits in green gram. Twenty QTLs controlling major agronomic characters including days to first flower (FLD), days to first pod maturity (PDDM), days to harvest (PDDH), 100 seed weight (SD100WT), number of seeds per pod (SDNPPD) and pod length (PDL) were located on to the linkage map. Most of the QTLs were located on linkage groups 7 and 5.

Isemura *et al*. (2012) constructed the first genetic linkage map of green gram using 430 SSR and EST-SSR markers from green gram and its related species, where the

number of linkage groups coincided with the haploid chromosome number of green gram. In total, 105 QTLs and genes for 38 domestication related traits were identified, which provides the foundation for the improvement of green gram and related legumes.

Dhole and Reddy (2012) developed SCAR marker linked with a MYMV resistance gene in green gram. Three primers amplified specific polymorphic fragments viz. OPB-07_{600}, OPC-06_{1750} and OPB-12_{820}. The marker OPB-07_{600} was linked (6.8 cM) with a MYMV resistance gene as compared to OPC06_{1750} (22.8 cM) and OPB-12_{820} (25.2 cM). The resistance-specific fragment OPB07_{600} was cloned, sequenced and converted into a SCAR marker and validated in 20 genotypes with different genetic backgrounds.

Kajonphol *et al*. (2012) used SSR markers to construct a linkage map and identify chromosome regions controlling some agronomic traits in green gram with a mapping population comprising 186 F_2 plants. A total of 150 SSR primers were composed into 11 linkage groups, each containing at least five markers. When green gram map was compared with azuki bean (*Vigna angularis*) and blackgram (*Vigna mungo*) linkage maps an extensive genome conservation among the three species was revealed.

Karthikeyan (2010) used Bulk segregant analysis (BSA) and RAPD techniques to analyse the F_2 individuals of cross between susceptible VBN (Gg) 26 and resistant KMG 189 to identify the molecular marker linked to MYMV resistant gene in green gram. Cosegregation analysis was performed in resistant and susceptible F_2 individuals and confirmed that OPBB 05 260 marker was tightly linked to MYMV resistant gene in green gram.

Saxena *et al*. (2009) identified the ISSR marker for resistance to YMV in soybean in the cross JS-335 × UPSM-534. The primer 50 SS was useful to find out the gene resistant to YMV in soybean.

Somta *et al*. (2008) conducted QTL analysis for resistance to *C. chinensis* (L.) and *C. maculatus* (F.) using F_2 (*V. nepalensis* and *V. angularis*) and BC_1F_1 [(*V. nepalensis* and *V. angularis*) and *V. angularis*] populations derived from crosses between the bruchid resistant species *V. nepalensis* and bruchid susceptible species *V. angularis*. Seven QTLs were detected for bruchid resistance; five QTLs for resistance to *C. chinensis* and two QTLs for resistance to *C. maculatus*.

Isemura *et al*. (2007) using SSR marker detected QTLs for seed, pod, stem and leaf-related traits. Few traits, such as pod dehiscence, were controlled by single genes but most traits were controlled by between two to nine QTLs.

Souframanien *et al*. (2006) generated RIL mapping population (F_8) by crossing *Vigna mungo* cv. TU 942 (resistant) with *Vigna mungo* var *silvestris* (susceptible) for screening against MYMV resistant. They employed ISSR marker (ISSR 8111357) linked to the MYMV resistance gene and further developed SCAR marker that co segregated in mapping population of RILs and distinguished the MYMV resistant and susceptible plants in the population.

Selvi *et al*. (2006) identified RAPD marker associated with MYMV resistance in green gram cross ML 267 x CO 4 through Bulked segregant analysis. Total of 149 random decamers were surveyed for identification of polymorphic markers between the DNA bulks of resistant and susceptible F_2 individuals and their parents. Ninety four percent of the primers produced amplification in both parents and bulks. Three primers out of 41 random primers viz. OPT 16, OPS 7 and OPAK 19 produced specific fragments in resistant parents and resistant bulk and were absent in susceptible parents and bulk. OPS 7900 revealed polymorphism in all eight resistant and six susceptible plants, indicating that it was associated with MYMV resistance in ML 267.

Chapter III

MATERIAL AND METHODS

Chapter III

MATERIAL AND METHODS

The present study entitled "Evaluation of green gram (*Vigna radiata* (L.) R. Wilczek) lines for YMV resistance using molecular markers" was conducted during 2019-2020. The material used and methods followed to conduct the present study are described in this chapter.

3.1 LOCATION OF WORK

The present investigation was carried out at the experimental plots of Institute of Biotechnology (IBT), College farm, College of Agriculture, Rajendranagar and Agricultural Research Station (ARS) Farm, Madhira, Khammam. Molecular analysis was carried at Institute of Biotechnology (IBT), PJTSAU, Rajendranagar, Hyderabad.

3.2 EXPERIMENTAL MATERIAL

3.2.1 Plant Material

The genotype, MGG 295 (susceptible to YMV) a high yielding variety released from ARS Madhira (1995) and WGG 42 also known as Yadadri (resistant to YMV) released from PJTSAU, Rajendranagar (2016) were used as parents to develop F_6 (RILs) population. The parental characters are listed in the table 3.1.

Table 3.1. Phenotypic characters of the chosen parents

Character	MGG-295	WGG-42
Pedigree/ Parentage	CO-2 × PIMS-4	Selection from EC-396117 (Exotic collection)
Reaction to YMV	Susceptible	Resistant
Pod size	Medium	long
Pod Colour	Light brown to dark brown	Dark brown to black
Hairiness	Sparse and short	Dense and long
Seed size	Medium	Bold
Duration	65-70 days	55-60 days
100 seed weight (g)	3.7	5.0
Seed yield per plant (g)	4.3	4.0
Year of release	1993	2015
Released by	ARS, Madhira	RARS, Warangal

3.2.2 Equipment's used in the present study is given in Appendix I

3.3 DEVELOPMENT OF RIL POPULATION

RIL population for studying resistance to YMV disease was developed at Institute of Biotechnology (Anuradha *et al*., 2019) from the crosses between the susceptible parent of MGG-295 (female parent) and the resistant variety WGG-42 (male parent) using single seed descent method. The F_5 RILs were advanced to F_6 lines during *Kharif* 2019. Thus, the population comprising of F_6 (RILs) generation was developed. RILs are one of the most extensively used populations in pulses. They offer several advantages over the rest of the mapping populations and hence find usage in both breeding and molecular work. A total of 128 RILs of the F_6 generation along with the parents (namely susceptible parent MGG-295 and the resistant parent WGG-42) were screened for YMV incidence and yield related traits at ARS, Madhira, Khammam during *rabi* 2019-20.

3.4 PHENOTYPING OF F_6 RIL POPULATION

3.4.1 Disease Screening Methodology

The F_6 generation of RILs and parents were sown at ARS Madhira, Khammam (Telangana), the natural hotspot for YMV incidence. Infector rows of the susceptible parent viz., MGG-295 were raised at the experimental plot in order to attract white fly (*Bemisia tabaci*) and enhance infection of YMV under field conditions (Fig 3.1).

The experiment was laid out in a Randomised Completely Block Design (RBD) consisting of 128 RILs of green gram in two replications, sown at ARS, Madhira. Each RIL line was grown in 2 rows of 4 m length with row to row spacing of 30 cm and plant to plant spacing of 10 cm. All the recommended cultural practices viz. fertilizer application and disease management were followed to maintain a good crop. However, the insecticide sprays were not given to encourage the white fly population for the spread of the disease. The crop was regularly monitored for the presence of whitefly and development of YMV disease.

Thirty days after sowing, whitefly population was observed on the plants. The YMV infection and disease incidence was progressed in the next six weeks. The data for the presence or absence of the YMV disease was recorded from initial flowering to harvesting by weekly intervals. Observations were recorded on five randomly selected plants in a row of four meter length in each replication by tagging the plants randomly in each line of a row.

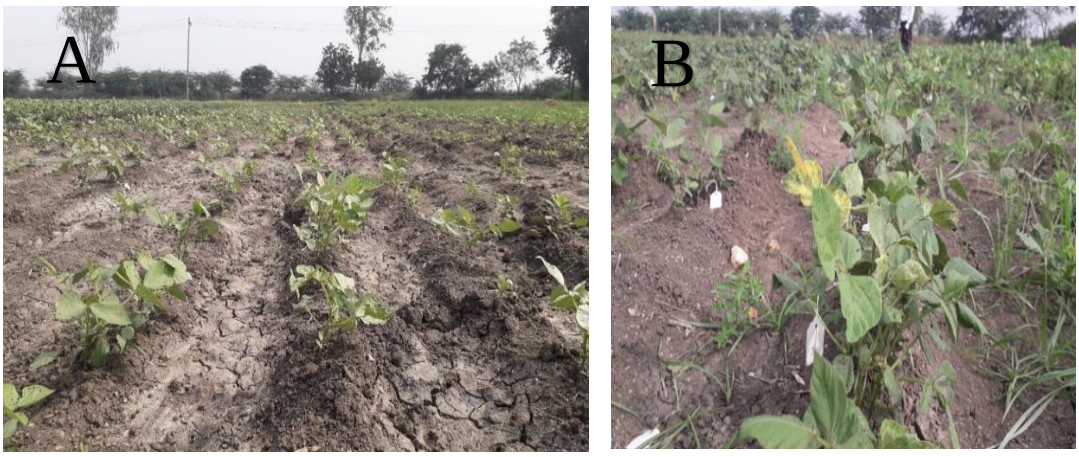

Figure 3.1. Field view of F_6 RILs (A) with YMV infector rows (B) sown during *Rabi*, 2019.

3.4.2 Disease incidence

Incidence of disease was observed at periodic intervals and per cent disease incidence was calculated on the basis of following formulae:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants in a line}}{\text{Total no. of plants in a line}} \times 100$$

In each replication after the first appearance of disease, data was recorded upto 12 weeks after sowing. The genotypes were later grouped into different categories based on the level of resistance from highly resistant to highly susceptible according to Bashir *et al*. (2005).

Table 3.2. Scale used for Yellow Mosaic Virus reaction (Bashir *et al*., 2005)

LEVEL OF RESISTANCE	% INFECTION	INFECTION CATEGORY	REACTION GROUP
0	All plants free of virus symptoms	Highly Resistant	HR
1	1-10% infection	Resistant	RR
2	11-20% infection	Moderately resistant	MR
3	21-30% infection	Moderately Susceptible	MS
4	31-50% infection	Susceptible	S
5	More than 50%	Highly susceptible	HS

3.4.3 Yield and its component agro-morphological traits

Observations were recorded on five randomly selected plants in a row of four meter length in each replication leaving the border plants to avoid border effect in both replications.

1. **Days to initial flowering (days)** : Number of days to first flowering was recorded as days taken from date of sowing to the days when to initial flowers had opened for each line in a plot.
2. **Days to 50% flowering (days)** : Number of days to 50 percent flowering was recorded as days taken from date of sowing to the days when about 50 percent flowers had opened for each line in a plot.
3. **Days to maturity (days)** : Numbers of days taken from the date of sowing to physiological maturity in more than 80 percent of pods in each plot.
4. **Number of branches per plant (no.s)** : The number of main branches of the plant were counted at maturity.
5. **Number of pods per cluster (no.s)** : For this number of effective pods are counted and average of five plants is taken.
6. **Number of clusters per plant (no.s)** : The total number of clusters of pods arising from the same point were counted from every branch of the plant.
7. **Number of pods per plant (no.s)** : The total number of pods present on each plant was counted at maturity.
8. **Number of seeds per pod (no.s)** : Number of seeds from five randomly selected pods from each plant was counted and then average seeds per pod were calculated.
9. **Plant height (cm)** : The height of plant was measured from the base of the plant to the top of the central axis of main shoot of the plant.
10. **Pod length (cm)** : Length of five dry pods from each of the selected plant was recorded and average value for five plants was expressed short (<

8 cm), medium (8 – 10 cm) and long (> 10 cm).

11. 100-seed weight (g) : 100 dry normal seeds from randomly taken plants were weighed and taken as 100-seed weight.

12. Seed yield per plant (g) : Seed yield per plant was measured by threshing and weighed in grams on an electronic balance.

13. Plant growth pattern : It was recorded on fully grown plant and designated as erect, semi erect, spreading or others.

14. Plant habit : It was recorded on 50 % flowering or fully grown plant and designated as determinate and indeterminate.

15. Leaf colour : It was recorded on 50 % flowering and designated as green and dark green.

16. Stem Colour : It was recorded on 50 % flowering stage and designated as Green, Green with purple slashes and purple.

17. Stem pubescence : It was recorded on 50 % flowering stage and designated as absent and present.

18. Pod colour : It was recorded at fully developed pod stage and designed as brown and black.

19. Pod position : It was recorded at fully developed green pod stage and designated as above canopy, Intermediate, and not visible.

20. Pod pubescence : It was recorded on mature green pods and designated as absent and present.

21. Pod curvature of mature pod : It was recorded on fully grown plant and is designed as straight and curved.

22. Seed lusture : It was recorded on mature seeds and is expressed as shiny and dull.

23. Seed shape : It was recorded on mature seeds and is expressed as Oval and Drum shaped.

3.5 MOLECULAR ANALYSIS

3.5.1 DNA isolation

The total genomic DNA was isolated from tender leaves of parents, 128 F_6 (RIL) population at 15-20 DAS (days after sowing), using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method Doyle and Doyle (1987) with steps as follows.

1. Frozen leaf tissues 2 g were ground with 500 µl of CTAB buffer by using mortle and pestle transferred into an eppendorf tubes and were kept in water bath at 65°C.
2. During incubation, the contents were occasionally mixed four to five times by inverting the tubes gently.
3. 500 µl of chloroform: iso-amyl alcohol (24:1) (extraction buffer) was added to the tubes and then mixed thoroughly by gentle inversion.
4. The mixture was then centrifuged for 15 min by keeping in rotator at 12000 rpm (Eppendorf centrifuge, 5804R) at 25^{o}C until clear separation of three layers was attained.
5. The clear upper aqueous phase (400 ul) was then transferred to fresh tubes with the help of micropipette. Care was taken to avoid debris inclusion.
6. To this supernatant, equal volume (500µl) of chloroform: isoamyl alcohol (24:1 v/v) was added and mixed thoroughly by gentle inversion and centrifuged for 10 min by keeping in rotator at 12000 rpm at 25°C until clear separation of three layers was attained.
7. RNase treatment was given by adding 5 µl (10 mg/ml) to each sample tube and incubated at 37°C in water bath for 45 minutes. 3.5 µl of Proteinase K was added and incubated at 37°C for 30 min.
8. To the supernatant, cold isopropanol of about 0.5 to 0.6 volumes (2/3rd of pipette volume) was added. The contents were mixed gently by inversion and kept at 4°C for overnight.
9. Subsequently, the tubes were centrifuged at 12000 rpm for 12 min at 24°C to pellet out DNA. The supernatant was discarded gently and the DNA pellet was washed with 70% ethanol and centrifuged at 13,000 rpm for 4-5 min.
10. This step was repeated twice. The supernatant was removed, the tubes were allowed to air dry completely and the pellet was dissolved in 50 µl $T_{10}E_1$ buffer.
11. DNA was stored at 4°C for further use and for long term stability DNA was stored at -80°C.

3.5.2 Assessment of quality and quantity of DNA

DNA was assessed for its purity and intactness using both agarose gel and NanoDrop spectrophotometer.

3.5.3 Quantification of DNA by 0.8% agarose gel electrophoresis

3.5.3.1 Preparation of 0.8% agarose gel

0.8 g of agarose was placed in conical flask containing 100 ml 1X TAE buffer (Appendix I). The conical flask along with its contents was placed in oven until agarose get melted completely and clear solution was formed. Then the flask was taken out from the oven and allowed the solution to cool until it reached 50-55°C. 3µl of Ethidium Bromide (10 mg/ml^{-1}) was added to 100 ml of agarose gel and mixed it thoroughly. Later this solution was poured slowly into the gel casting tray which was pre-set with 0.5 mm conc without the formation of bubbles. After solidification, the comb was removed gently from the gel and then was placed in gel tank with the casting tray.

3.5.3.2 Electrophoresis of the DNA samples

5 µl of each of dissolved genomic DNA samples was mixed with 3 µl of 6X gel loading dye (40% sucrose and 0.25% bromo phenol blue), were loaded onto 0.8 % agarose-1X TAE gel, which was pre-treated with 3 µl of Ethidium bromide (10 mg/ml) stain, along with lambda (λ) Hind III digest (New England Biolabs, UK). Then, the gel with loaded samples was electrophoresed at 100V at room temperature for about an hour. After that, the gel was visualized in an UV gel documentation system (Biorad Gel Doc XR+ Imaging System) and saved the image for further use. Later, based on the intensity and thickness of genomic DNA compared to λ DNA, the concentration of DNA in individual samples was determined.

3.5.4 Quantification of DNA by NanoDrop

The NanoDrop spectrophotometer model ND1000 was used to assess the quantity and quality of DNA employing following procedure.

- Before initializing the NanoDrop Reader, the pedestal was cleaned with tissue paper to remove dust particles.
- Then for initializing the instrument, 1-2 µl of distilled water was placed on the pedestal and clicked on measure option.
- Then the pedestal was cleaned with tissue paper and placed 1.5 µl of 1X TE buffer on pedestal for blank measurement.

- Finally, the pedestal was cleaned with tissue paper and 1.5 µl of DNA sample was placed and quantity and quality of DNA was measured.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at/or near 280 nm. After quantification DNA samples were diluted to a working concentration of 30 ng/µl.

3.5.5 Amplification of DNA using polymerase chain reaction

DNA was subjected to Polymerase Chain Reaction (PCR) by using microsatellite SSR (Simple Sequence repeats) markers.

PCR tubes of 0.2 ml were taken and 2 µl of DNA (30 ng/µl) was added. PCR reaction was performed in a 10 µl volume of mix containing the components as in table 3.3 and the programme was set as in table 3.4. The steps from 2-4 were repeated for 35 times for amplification of targeted DNA. Annealing temperature of each primer was standardized by doing PCR with the temperature range of 57-60°C.

Table 3.3. PCR components, their concentration and volume used for the reaction.

Component	Concentration	Reaction volume
Taq buffer (10X) with $MgCl_2$	1X	1.0 µl
dNTP mix	2.5 mM	1.0 µl
Taq DNA polymerase	3U/µl	0.2 µl
Forward primer	0.2 µM	0.5 µl
Reverse primer	0.2 µM	0.5µl
Genomic DNA	50 ng/µl	3.0 µl
Sterile distilled water	-	3.8 µl
Total	-	10 µl

The reaction mixture was given a short spin for thorough mixing of the cocktail components and then the PCR tubes with reaction mix were placed in the thermal cycler (AB Veriti, USA and Eppendorf). The PCR products were stored at 4˚C for short periods and at -20˚C for long duration.

Table 3.4. PCR temperature regime

S.NO	STEP	TEMPERATURE	TIME	Cycles
1.	Initial denaturation	95°C	5 minutes	1
2.	Denaturation	94 °C	45 seconds	35 cycles
3.	Annealing	57-60 °C	45 seconds	
4.	Extension	72 °C	1 minute	
5.	Final extension	72 °C	10 minutes	1
6.	4 °C		∞	

3.5.6 Resolving of the PCR products

The PCR product was loaded on to the 3.5% agarose gel (gel was prepared as per the methodology detailed in the section 3.5.3.1.) by mixing with 2 µl of 6X loading dye. A 100 bp ladder was loaded as a reference marker. The gel was run at constant voltage of 100 V for about 2-3 hours, until the ladder got properly resolved. The banding pattern was analysed using gel documentation system (Biorad Gel Doc XR+ Imaging Systems).

3.6 IDENTIFICATION OF POLYMORPHIC MARKERS BETWEEN PARENTS

A total number of 185 SSR primers (selected from Wang *et al*., 2004 and Isemura *et al.*, 2012) were screened among two parents for parental polymorphism study. 15 primers were identified as polymorphic (Table 4.8) between two parents and they were further used in the RIL population. Consistency of the bands was checked by repeating the reaction twice and the reproducible bands were scored in all the samples for each of the primers separately. As the SSR marker is the co-dominant marker bands were present in both resistant and susceptible parents. List of primers and their sequences used in the present study were given in the Supplementary Table 1 (Appendix IV- supplementary table 1).

3.7 GENOTYPING OF F_6 RIL POPULATION

The genotyping of the 128 F_6 (RIL) population and parents was carried out using the polymorphic markers identified between the parents. The allele sizes of the agarose gel resolved PCR products were analysed in comparison to a standard 50 & 100 bp marker and an allele code of 'A' for parent 1 allele (MGG-295), 'B' for parent 2 allele (WGG-42), and 'H' for heterozygote was assigned, to score the entire F_6 RIL population.

3.7.1 Marker – trait association for YMV resistance using Analysis of Variance (ANOVA)

A single factor ANOVA is used to test the null hypothesis that the means of several populations are all equal. The marker data was scored and arranged in format and analysis was performed using Microsoft Excel.

3.8 STATISTICAL ANALYSIS

The data in respect of various characters studied were subjected to the following analysis and was carried out using WINDOSTAT software version 9.2.

1. Chi-Square Analysis
2. Analysis of variance
3. Estimation of Genetic Parameters

3.8.1 Chi-Square Analysis

Chi-square (χ^2) test was applied to fit appropriate genetic ratio for the estimation of number of gene(s) governing resistance. Following equation was used to calculate the chi square values:

$$\chi^2 = \frac{\sum(O - E)^2}{E}$$

Where,

O = Observed frequency

E = Expected frequency

$\sum$= Summation of the data

If the calculated values of χ^2 is significant at 5 per cent level of significance, is said to be poor and one or more observed frequencies are not in accordance with the hypotheses assumed and vice versa. So, it is also known as goodness of fit. The degree of freedom (df) in χ^2 test is (n-1). Where n = number of classes.

3.8.2 Analysis of Variance

Analysis of variance (ANOVA) is a collection of statistical models and their associated estimation procedures used to analyze the differences among group means in a sample. The mean and variances were analyzed based on the formula given by Singh and Chaudhary (1977).

3.8.2.1 Mean

Mean value of each character was determined by dividing the sum of the observed value with the corresponding number of observations.

$$y = \frac{1}{n}\sum_{i=1}^{n} y_i$$

3.8.2.2 Variance

$$Variance = \frac{1}{n-1}[\sum(Y_i - \bar{Y}).^2$$

Where, Yi = Individual value

Y = Population mean

Standard deviation (SD) = $\sqrt{\text{Variance}}$ = $\frac{\sum d^2}{N}$

Where,

d = Deviation of individual value from mean and

N = Number of observations

3.8.3 Estimation of genetic parameters

Genotypic and phenotypic variances and coefficients of variance were computed based on mean and variance calculated by using the data of un replicated treatments. The individual observations made for each trait on F_6 population is used for calculating the phenotypic variance.

Phenotypic variance (σ^2p) = Var F_6.

Where, Var F_6 = variance of F_6 population

3.8.3.1 Genotypic and phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation was computed according to Burton and Devane (1953).

$$\text{Phenotypic coefficient of variability (PCV)} = \frac{\sigma p}{\overline{X}} \times 100$$

$$\text{Genotypic coefficient of variability (GCV)} = \frac{\sigma g}{\overline{X}} \times 100$$

Where,

σg = Genotypic standard deviation,

σp = Phenotypic standard deviation

$\overline{X}$ = General mean of character

GCV and PCV values were categorized as low, moderate and high values as suggested by Sivasubramanian and Menon (1973), as follows

1-10 per cent	: Low
10-20 per cent	: Moderate
20 per cent and above	: High

3.8.3.2 Heritability (h^2)

Heritability in broad sense was computed as the ratio of the total genotypic and phenotypic variance as expressed in percentage as given by Allard (1960).

$$h^2 (bs) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

σ^2g = Genotypic variance

σ^2p = Phenotypic variance

As suggested by Johnson *et al.* (1955) (h^2) estimates were categorized as:

0-30 per cent	: Low
30-60 per cent	: Moderate
60 per cent and above	: High

3.8.4 Genetic advance

Genetic advance was estimated by using the formula given by Johnson *et al*. (1955).

$$GA = h^2 \, k \, \sigma p$$

Where,

h^2 = Heritability in broad sense

k = Selection deferential which is equal to 2.06 at 5 per cent intensity of selection

p = Phenotypic standard deviation

3.8.5 Genetic advance as percent of mean (GAM)

$$GAM = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = General mean of the character

Genetic advance as per cent mean was categorized as low, moderate and high as given by Johnson *et al., (*1955). It is as follows,

0-10 per cent	: Low
10-20 per cent	: Moderate
20 per cent and above	: High

Chapter IV

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

Green gram is one of the important pulse crops of India. The yield level of the crop is often threatened by many biotic and abiotic stresses. Among biotic stresses, yellow mosaic virus (YMV) disseminated by white fly causes significant yield losses ranging from 10-100%. Among the unified disease management approach, development of YMV resistant varieties is the viable option to effectively control the disease. Efforts to develop YMV resistant green gram varieties using conventional methods resulted in very limited success due to its complex nature of inheritance and extraordinary variability in the YMV strains. The molecular marker-assisted selection (MAS) driven varietal development method found promising especially for the traits controlled by oligo genes. As of now, many QTLs governing the YMV resistance have been identified in green gram. However, their direct deployment for development of varieties through MAS requires proper validation in the appropriate donors and the segregating populations. With this background, the present investigation was proposed to evaluate the F_6 RIL population derived from MGG 295 (YMV susceptible parent) and WGG 42 (YMV Resistant parent). The results of the investigation are discussed as follows.

4.1 PHENOTYPING OF PARENTS

The parental genotypes MGG 295 and WGG 42 were evaluated for YMV reaction i.e. susceptible/Resistant as well as yield data was recorded during *Rabi*, 2019 at ARS, Madhira, Khammam. The parent MGG 295 has showed high susceptibility for YMV under field conditions (Fig 4.2), where natural occurrence of disease is more. Whereas the parent WGG 42, did not show any symptoms of YMV. Under heavy infestation of YMV also, the parent MGG 295 exhibited high seed yield of 4.3 g/plant when compared to WGG 42 (4.0 g/plant) (Table 4.2). Hence, molecular based evaluation and validation for incorporation of YMV resistant genes into this variety can further enhance the yield levels.

Figure 4.1. Morphological characters of plant, pod and seed type of parents. Left Panel: The parent MGG295 showing YMV susceptibility; Right Panel: WGG42 exhibiting resistance to YMV under field conditions during *Rabi*, 2019. A,B,C shows susceptible parents characters whereas X,Y,Z shows resistant parent characters.

4.2 DEVELOPMENT OF THE F_6 RIL MAPPING POPULATION

For any trait evaluation study, it is ideal to cross parents which are not only contrasting for the trait of interest, but also which are significantly divergent. In this study, MGG 295 (YMV susceptible) was used as female parent and WGG 42 (YMV resistant) was used as male parent. A mapping population consisting of 128 RILs was developed

from a cross between MGG 295 and WGG 42 by single seed descent method and maintained at IBT, Rajendranagar, Hyderabad and ARS, Madhira (Fig. 4.2).

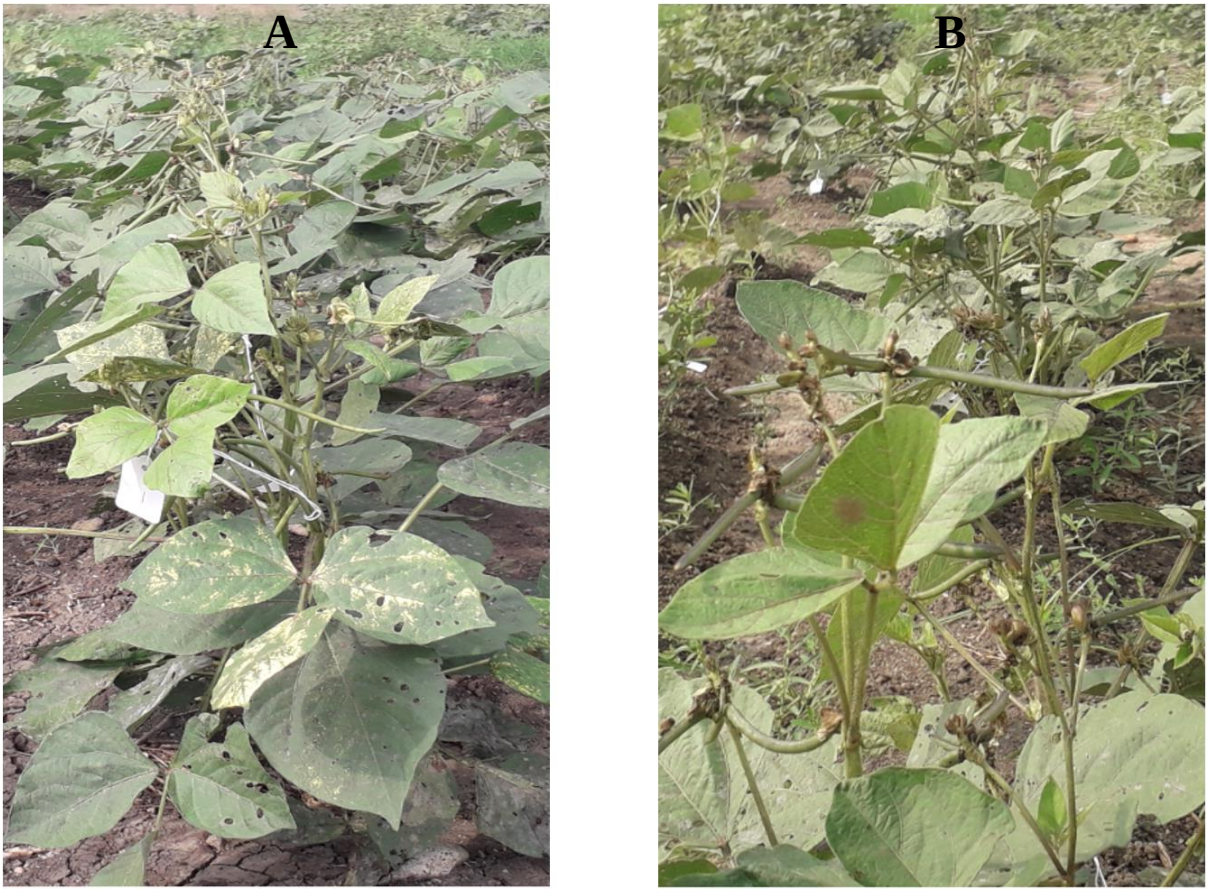

Figure 4.2. Phenotyping of parents [A] MGG 295 showing susceptibility whereas [B] WGG 42 showing resistance in field in field condition.

Figure 4.3. Field experiment site at ARS Madhira.

Figure 4.4. Field screening of F_6 population for YMV incidence during *Rabi*, 2019.

Figure 4.5. Incidence of YMV in F_6 RIL Population.

4.3 PHENOTYPING OF F_6 RIL POPULATION

The phenotyping of a total of 128 F_6 RIL population along with parents for the YMV disease reaction and yield contributing traits was carried out

4.3.1 Screening of F_6 RILs of Green gram for YMV reaction

In any breeding programme, the first and foremost step includes screening of germplasm for trait of interest. Identification and development of resistant variety is considered to be most feasible and durable solution for controlling prevalent diseases, viz., YMV. Hence, screening of genotypes against YMV disease under natural and field conditions will lead the identification of YMV resistant varieties. The difference in the level of resistance shown by RILs based on visual symptoms in response to YMV incidence were studied for 128 RILs during *Rabi*, 2019. Disease rating scale was followed as given by Bashir *et al*. (2005).

Resistant parent WGG-42 selected for crossing showed a disease score of 1 according to the scale (Table: 4.2) and MGG-295 was taken as susceptible parent showed a disease score of 4. The frequency of F_6 RILs showing different scores of resistance/susceptibility to YMV are presented in figure 4.7. The disease incidence symptoms are represented in Figure 4.5. The frequency distribution of YMV is segregated towards normal distribution, with a skewness value of 1.0 and a kurtosis value of 0.45 which indicates normal or mesokurtic curve (values lower than ± 1). With the help of this kurtosis value it can be stated that the population was normally segregated.

The RILs were later grouped into different categories. Based upon the YMY score. The RILs were divided into six categories, Highly resistant (HR), Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), Susceptible (S) and Highly Susceptible (HS). Based on the scale 28 were found highly resistant, 7 were found resistant, 38 were found moderately resistant, 15 were found moderately susceptible, 23 were found susceptible and remaining 17 were found highly susceptible.

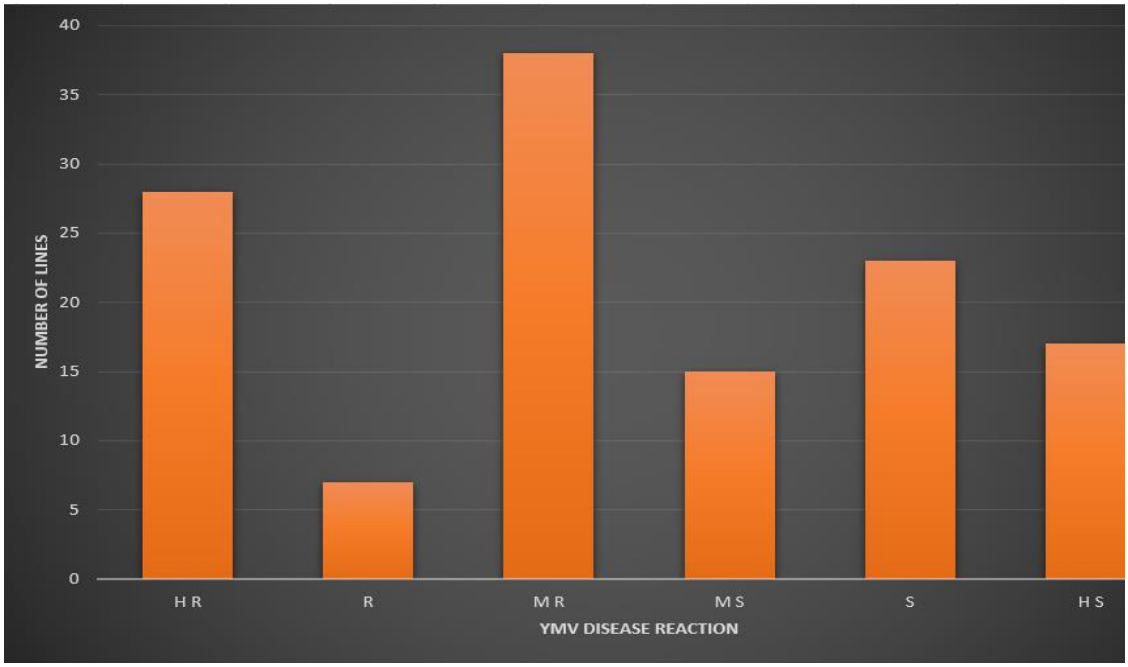

Figure 4.6. Frequency distribution of YMV Incidence recorded from the F_6 RIL population.

Table 4.1. Grouping of 128 F_6 RILs of the cross MGG-295 x WGG-42 based on YMV reaction under field condition.

Scale	Percent plants affected	Infection category	Disease reaction	No of lines	RIL number
0	All plants free of virus symptoms	Highly resistant	**H R**	28	5, 8, 10, 11, 21, 23, 24, 25, 36, 37, 76, 83, 86, 88, 91, 97, 98, 103, 105, 120, 121, 124, 130, 133, 141, 143, 153, 164
1	1-10% infection	Resistant	**R**	7	87, 89, 106, 128, 129, 136, 146
2	11-20% infection	Moderately resistant	**M R**	38	13, 14, 16, 17, 18, 19, 22, 26, 33, 47, 55, 67, 77, 90, 102, 104, 109, 110, 115, 117, 118, 122, 123, 126, 127, 132, 135, 138, 145, 147, 148, 151, 154, 156, 158, 161, 165, 167
3	21-30% infection	Moderately susceptible	**M S**	15	3, 30, 43, 45, 53, 64, 72, 81, 85, 108, 119, 125, 150, 162, 174
4	31-50% infection	Susceptible	**S**	23	2, 4, 20, 29, 31, 32, 38, 42, 63, 65, 66, 75, 78, 79, 101, 107, 116, 131, 134, 159, 162, 166, 170
5	More than 50 %	Highly susceptible	**HS**	17	1, 27, 39, 56, 57, 58, 59, 61, 69, 73, 74, 112, 137, 168, 169, 171, 173

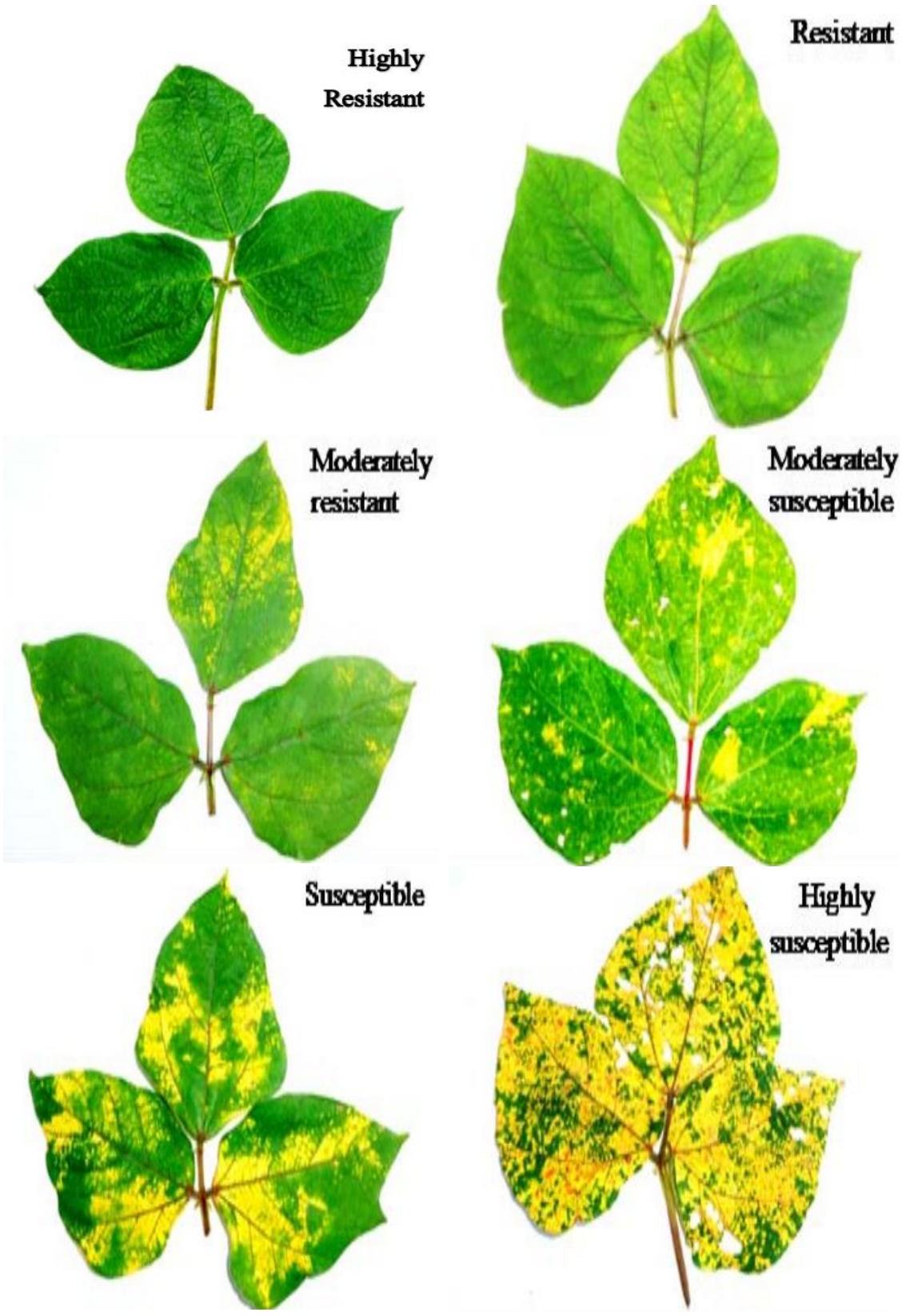

Figure 4.7. Symptoms of different reaction categories against YMV disease as inferred from per cent disease incidence.

4.3.2 Genetics of field resistance to YMV in RIL population

Analysis of the segregation pattern in respect of the disease reaction of the segregating plants in the F_6 RILs of the cross (MGG 295 x WGG 42) corresponded to monogenic inheritance pattern under natural conditions. Chi-square test was performed to check the goodness of fit for ascertaining the number of genes governing the YMV resistance. The RIL's were grouped into two categories i.e. Resistant and susceptible based on the visual expression of the disease. The RIL's divided into two categories, Highly resistant (HR), resistant (R), moderately resistant (MR) plants were included in resistant group and moderately susceptible (MS), susceptible (S) and highly susceptible (HS) were included in susceptible group. The RIL population showed goodness of fit to ratio of 1:1 for disease resistance and susceptible reaction (Table 4.2) revealing monogenic inheritance of YMV resistance. Monogenic inheritance for YMV resistance has also been reported in green gram by previous researchers by Patel *et al*. (2018), Anusha *et al*. (2014) and recessive monogenic resistance in case of black gram was reported by Gupta *et al*. (2013).

Table 4.2. Chi-square test for segregation of disease resistant reaction in F_6 RIL population

RILs (F_6)	Total plants	Disease Resistant Reaction				Ratio R:S	χ 2
		Observed		Expected			
		Resistant	Susceptible	Resistant	Susceptible		
MGG 295 X WGG 42	128	73	55	64	64	1:1	2.53^{NS}

4.4 EVALUATION OF QUANTITATIVE TRAITS IN F_6 SEGREGATING POPULATION.

A total of 128 F_6 RILs along with parents were evaluated for the following morphological traits viz., days to initial flowering, days to 50% per cent flowering, days to maturity, Number of branches per plant, number of pods per cluster, number of cluster per plant, number of pods per plant, number of seeds per pod, plant height, Pod length, Seed yield per plant, 100 seed weight along with YMV score were evaluated.

4.4.1 Analysis of variance

Data recorded on five randomly selected plants in each line in every replication was taken along with parents and were used for statistical analysis. The analysis of variance for thirteen characters studied are presented in Table 4.3. The analysis revealed that the RILs under study exhibited highly significant differences for all the characters under study, both at 0.05 and 0.01 probability levels. Mean performance of the traits studied are given below (Table 4.4).

Mean observation of genotypes for various traits

1. Days to initial flowering (days): The number of days for initial flowering ranged from 36.30 (WGG 42) to 50.30 (RIL 38) with a grand mean value of 44.159. The estimated values of GCV (genotypic coefficient of variation) and PCV (phenotypic coefficient of variation) were low with 4.72 and 7.37.

2. Days to 50% flowering (days): The number of days for 50 per cent flowering ranged from 39.00 (WGG 42) to 53.40 (RIL 38) with a grand mean value of 47.15. The estimates of GCV (4.20) and PCV (7.04) were low.

3. Days to maturity (days): Mean of genotypes for days to maturity ranged from 63.80(RIL 61) to 91.30 (RIL 131) with a grand mean of 73.96. The estimates of GCV(4.29) and PCV (8.94) were low.

4. Number of branches per plant (no.s): The average number of branches ranged from 2.40 (RIL 17) to 5.10 (RIL 116) with a grand mean of 3.49. The values of GCV (14.9) and PCV (15.61) were coupled with moderate values.

5. Number of pods per cluster (no.s): The mean values for number of pods per cluster ranges from 2.20 (RIL 138) to 9.10 (RIL 75) with a grand mean of 4.83. The estimates of GCV(32.53) and PCV (33.07) are coupled with high values.

6. Number of clusters per plant (no.s): The mean values for number of clusters per plant ranges from 2.50 (RIL 150) to 10.20 (RIL 74) with a grand mean of 6.18. The estimates of GCV (28.51) and PCV (28.98) are coupled with high values.

7. Number of pods per plant (no.s): The mean values for number of pods per plant ranges from 5.20 (RIL 98) to 32.40 (RIL 67) with a grand mean of 16.80. The estimates of GCV(46.17) and PCV (46.25) are coupled with high values.

8. Number of seeds per pod (no.s): The number of seeds per pod ranged from 5.50 (RIL 102) to 15.60 (RIL 106) with a grand mean of 10.29. The estimated values of GCV (16.75) and PCV (17.71) were moderate.

9. Plant height (cm): : The mean values for plant height ranges from 13.40 (RIL 116) to 38.22 (RIL 105) with a grand mean of 27.25. The estimates of GCV (12.56) and PCV (13.40) are coupled with moderate values.

10. Pod length (cm): For pod length the values ranged from 6.19 cm (RIL 53) to 8.98 cm (RIL 121) with a grand mean of 7.33 cm. The estimates of GCV (6.53) and PCV (8.64) were low.

11. Seed yield per plant (g): The grand mean value of seed yield per plant was 3.01 and the mean values ranged from 0.83 (RIL 108) to 6.97 (RIL 10) respectively. The values of GCV (38.10) and PCV (38.58) were coupled with high values.

12. 100-seed weight (g): The mean value ranged from 2.57 (RIL 63) to 5.00 (WGG 42) with grand mean of 3.36. The estimated values of GCV (9.53) and PCV (13.77) are low and moderate respectively.

13. Disease incidence (%): The grand mean value of Disease incidence is 24.94 and the mean values ranged from 0.00 (RIL's 5, 8, 10, 11, 21, 23, 24, 25, 36, 37, 76, 83, 86, 88, 91, 97, 98, 103, 105, 120, 121, 124, 130, 133, 141, 143, 153, 164) to 91.66 (RIL 169). The estimated values of GCV (90.50) and PCV (90.82) were coupled with high values.

Table 4.3. Analysis of variance for various traits in Green gram

Source of variation	df	DFF (Days)	DF50 (Days)	DM (Days)	NBP (nos.)	NPC (nos.)	NCP (nos.)	NPP (nos.)	NSP (nos.)	PH (cm)	PL (cm)	SYP (g)	SW (g)	DI (%)
Replication	1	8.952^{NS}	9.855^{NS}	0.418^{NS}	0.002^{NS}	0.071^{NS}	0.056^{NS}	0.380^{NS}	0.279^{NS}	4.181^{NS}	0.359^{NS}	0.001^{NS}	0.028^{NS}	3.815^{NS}
Treatments	129	14.947***	14.972 ***	53.79 **	0.55***	5.03***	6.31***	120.58***	6.300***	25.08***	0.63***	2.67***	0.317***	1023***
Error	129	6.25	7.123	33.651	0.040	0.083	0.103	0.214	0.349	1.629	0.172	0.034	0.112	3.613

***: Significance at 0.01 probability levels; ** & * :Significance at 0.05 and 0.1 probability levels;; NS: Non-significant; DFF: Days to initial flowering, DF50: Days to 50% flowering, DM: Days to maturity, NBP: number of branches per plant, NPC: Number of Pods per cluster, NCP: Number of cluster per plant, NPP: Number of pods per plant, NSP: Number of seeds per pod, PH: plant height, PL: pod length, SYP: Seed yield per plant, SW: 100 Seed weight, DI: Disease incidence

Table 4.4. Mean performance of Green gram RILs in both replication for Thirteen traits under study

S. No	F_6 RIL Lines	DFF (Days)	DF50 (Days)	DM (Days)	NBP (nos.)	NPC (nos.)	NCP (nos.)	NPP (nos.)	NSP (nos.)	PH (cm)	PL (cm)	SYP (g)	SW (g)	DI(%)
1	**1**	48.10	51.10	79.30	3.40	4.30	7.90	29.30	9.50	27.00	7.30	5.78	2.88	82.86
2	**2**	47.10	49.90	79.80	3.40	5.60	4.90	23.50	11.20	29.70	7.10	4.29	3.21	47.73
3	**3**	46.60	49.40	71.30	4.20	4.70	6.10	23.60	10.80	24.80	7.31	4.59	3.25	26.14
4	**4**	41.60	44.40	72.30	3.00	4.10	4.70	8.60	9.30	32.90	7.00	4.01	3.31	47.73
5	**5**	42.50	45.50	85.80	3.50	6.20	6.60	16.00	9.40	28.80	7.14	4.01	3.32	0.00
6	**8**	45.70	48.90	82.30	3.00	3.10	5.30	14.00	10.40	24.60	7.00	3.42	3.13	0.00
7	**10**	42.90	46.40	69.30	3.60	4.70	9.20	27.80	10.00	28.50	7.05	6.97	2.98	0.00
8	**11**	46.70	49.60	83.30	3.80	4.30	6.60	17.30	10.70	26.80	7.00	3.70	2.98	0.00
9	**13**	45.70	48.80	74.30	4.10	5.10	6.30	15.70	10.30	25.60	7.09	3.50	3.12	16.25
10	**14**	43.20	46.40	72.30	4.70	4.80	6.30	19.20	12.60	25.60	6.43	2.38	3.85	16.25
11	**16**	42.70	43.60	80.30	4.70	5.70	7.00	22.10	11.30	25.40	6.70	2.85	3.25	15.56
12	**17**	45.70	49.00	81.80	5.10	5.80	6.80	21.40	12.70	28.20	7.14	2.45	3.75	18.70
13	**18**	44.20	47.40	75.80	3.80	5.20	7.70	27.60	10.00	23.70	7.66	3.42	3.00	19.09
14	**19**	43.70	47.00	82.80	3.40	5.30	4.80	20.60	11.20	26.50	7.06	3.32	2.91	16.67
15	**20**	48.20	51.20	77.30	3.80	4.30	6.30	20.90	10.10	24.30	7.46	2.92	2.84	40.00
16	**21**	46.70	50.00	74.30	3.50	5.30	5.50	23.30	9.40	25.00	6.68	3.08	3.31	0.00
17	**22**	45.20	48.40	75.80	3.30	5.00	7.90	18.60	10.70	29.00	6.90	3.63	3.20	19.09
18	**23**	43.70	46.80	74.30	3.40	6.00	7.90	17.50	10.10	26.70	7.59	2.46	3.50	0.00
19	**24**	44.40	47.70	75.80	3.70	4.70	7.10	24.10	12.00	29.50	7.59	3.28	3.25	0.00
20	**25**	43.40	46.50	71.80	3.90	4.60	8.80	24.80	12.00	29.90	6.56	2.34	3.60	0.00
21	**26**	43.70	46.80	78.30	3.40	5.40	7.20	22.20	10.50	29.30	7.41	2.99	3.35	20.00
22	**27**	46.20	49.30	72.30	3.70	5.50	6.10	29.20	10.20	26.60	7.40	3.99	3.36	58.57
23	**29**	44.40	47.50	64.80	3.80	5.50	6.80	30.30	11.00	27.50	7.48	2.79	3.30	41.67
24	**30**	41.90	45.00	72.30	3.50	5.30	7.50	25.40	11.20	24.90	7.23	1.77	3.14	27.62
25	**31**	42.80	45.90	76.80	3.50	5.70	6.90	26.30	10.70	30.40	7.00	2.59	3.12	36.67
26	**32**	43.50	46.60	79.30	3.60	5.10	9.90	26.30	11.00	31.20	7.11	2.05	3.30	42.86
27	**33**	44.00	47.10	75.80	3.70	6.00	6.20	24.60	9.00	30.50	7.67	2.98	3.78	20.00

28	**36**	46.00	49.10	75.30	3.70	6.10	7.40	25.90	10.30	28.50	6.68	1.44	2.88	0.00
29	**37**	44.50	47.60	76.80	3.70	7.10	8.50	28.10	9.20	33.00	7.10	1.90	2.95	0.00
30	**38**	50.30	53.40	85.30	3.40	6.70	8.10	28.00	10.10	30.70	7.10	2.41	3.00	40.00
31	**39**	45.20	48.30	85.30	4.10	8.20	7.60	29.60	9.10	23.90	6.85	3.45	3.50	58.57
32	**42**	46.70	49.80	84.30	4.10	6.50	7.50	30.30	11.90	29.40	6.55	3.10	2.78	36.67
33	**43**	43.70	46.80	74.30	3.80	5.30	5.60	32.10	12.40	28.30	6.94	1.88	2.85	29.29
34	**45**	44.20	47.30	75.80	4.50	5.20	6.80	30.90	10.90	28.30	6.87	2.41	3.54	23.61
35	**47**	42.70	45.60	72.30	4.60	4.60	7.30	29.50	10.50	29.70	7.35	2.73	2.99	17.14
36	**53**	40.20	43.30	77.30	3.30	6.20	9.00	26.40	10.40	28.90	6.19	2.25	2.97	22.65
37	**55**	45.20	48.30	69.80	3.50	5.10	6.00	26.20	11.50	27.20	6.65	3.69	3.57	15.56
38	**56**	42.70	45.80	71.30	3.80	6.50	6.70	28.00	11.10	31.80	7.35	5.23	2.83	71.67
39	**57**	44.70	47.80	69.80	3.30	7.00	8.00	28.40	11.60	29.80	6.95	3.81	3.17	73.02
40	**58**	41.20	44.30	69.80	4.00	6.70	7.90	27.60	10.60	28.40	7.64	3.23	3.30	84.52
41	**59**	43.20	46.30	66.30	4.10	7.60	6.20	29.90	8.90	29.20	7.11	2.98	3.12	66.07
42	**61**	36.80	39.90	63.80	4.20	7.50	8.90	27.10	10.30	27.50	7.12	3.13	4.02	63.33
43	**63**	44.30	47.40	65.30	4.10	4.50	7.40	27.80	11.50	29.60	7.20	2.57	2.58	36.67
44	**64**	38.30	41.40	65.30	4.10	5.60	8.90	26.60	12.20	28.80	7.40	3.11	3.61	24.87
45	**65**	37.30	40.40	66.30	4.40	5.30	7.00	30.10	12.10	29.20	7.60	3.11	2.88	38.10
46	**66**	40.30	43.40	67.30	3.80	5.40	4.80	30.40	13.70	26.70	7.10	5.36	3.19	37.50
47	**67**	41.80	45.00	68.80	3.40	7.40	5.00	32.40	13.60	30.10	8.05	3.36	2.80	16.25
48	**69**	41.80	44.70	70.80	4.00	7.30	6.90	20.70	9.50	27.40	8.00	2.53	3.75	72.86
49	**72**	44.80	47.80	74.30	3.50	6.60	8.60	11.60	8.80	28.40	7.60	3.66	3.36	23.61
50	**73**	37.80	40.70	70.80	4.40	6.80	7.30	11.00	9.40	27.10	7.70	5.42	3.16	83.33
51	**74**	39.30	42.40	66.80	4.00	8.40	10.20	11.50	9.90	28.20	7.46	5.36	3.15	54.44
52	**75**	43.20	46.10	74.30	4.30	9.10	10.10	11.30	10.20	27.40	7.87	3.98	3.55	33.33
53	**76**	37.00	39.90	64.80	3.70	8.60	7.30	10.90	10.20	27.00	7.07	2.27	3.70	0.00
54	**77**	42.00	44.90	72.80	4.00	7.00	7.90	24.90	10.00	28.10	7.65	4.91	3.35	20.00
55	**78**	42.50	45.40	70.80	3.30	6.50	6.50	20.60	9.30	28.00	6.96	4.99	3.55	38.18
56	**79**	45.00	47.90	66.80	3.10	7.00	6.70	16.80	9.80	26.00	7.11	4.85	2.95	41.43
57	**81**	43.00	45.90	68.80	3.70	7.20	7.80	17.60	9.80	32.10	7.21	4.34	3.50	25.40
58	**83**	43.50	46.40	76.80	3.20	5.30	6.80	14.90	9.60	25.70	7.71	3.69	3.15	0.00
59	**85**	46.50	49.40	77.80	3.10	6.50	6.50	6.60	8.20	24.13	7.02	2.02	3.65	25.40
60	**86**	46.20	49.10	74.80	2.80	5.70	4.40	10.50	10.30	25.41	6.90	2.24	3.40	0.00

61	**87**	45.60	48.50	70.80	3.00	6.50	4.60	8.50	9.40	19.01	6.65	2.17	3.10	7.29
62	**88**	44.10	47.10	76.80	3.60	7.10	9.20	12.00	8.20	25.71	6.32	2.66	3.15	0.00
63	**89**	45.00	48.00	71.80	2.90	7.40	7.60	12.20	9.00	26.89	7.02	2.64	3.49	9.55
64	**90**	44.40	47.40	71.80	2.70	7.20	7.40	8.10	7.30	26.79	6.92	2.44	3.05	14.84
65	**91**	47.40	50.40	81.80	2.60	6.90	5.20	17.10	10.40	16.84	6.87	1.57	3.45	0.00
66	**97**	48.40	51.40	77.80	2.60	6.80	5.10	11.60	7.40	20.78	6.78	1.36	4.00	0.00
67	**98**	45.30	48.30	70.30	3.40	6.20	4.90	5.20	6.20	24.03	7.02	1.91	3.85	0.00
68	**101**	46.30	49.30	70.30	3.90	6.10	5.80	7.80	6.20	28.57	6.94	0.88	3.15	38.89
69	**102**	46.30	49.30	71.80	3.60	6.10	5.50	5.60	5.50	25.51	7.09	1.09	4.35	12.70
70	**103**	45.80	48.80	73.30	3.40	5.90	5.10	9.30	8.70	29.25	6.99	0.97	3.00	0.00
71	**104**	46.80	49.80	74.30	3.60	6.00	3.80	9.10	7.70	28.37	6.94	1.69	3.15	12.70
72	**105**	43.40	46.40	75.80	3.60	4.00	9.30	26.00	9.00	38.22	6.91	2.15	3.55	0.00
73	**106**	42.90	45.90	69.80	4.20	4.20	9.30	24.20	15.60	31.91	7.24	1.75	2.75	9.55
74	**107**	41.30	44.30	70.80	3.80	3.30	7.20	11.10	9.90	32.51	6.83	2.02	3.00	38.89
75	**108**	44.40	47.40	77.30	3.20	3.60	5.30	14.20	11.30	28.57	7.17	0.84	3.30	27.62
76	**109**	41.90	44.90	71.80	4.18	3.20	4.10	10.20	10.60	27.78	7.27	1.71	3.15	13.39
77	**110**	41.40	44.30	69.30	3.30	3.60	4.40	9.30	10.10	26.99	7.48	3.32	2.89	14.29
78	**112**	37.40	40.50	78.80	3.30	3.50	4.00	9.10	10.10	24.82	6.92	3.74	3.83	60.00
79	**115**	49.90	53.10	70.30	2.90	3.70	3.70	10.20	7.40	18.52	6.38	2.15	3.35	15.56
80	**116**	44.40	47.40	70.30	2.40	3.90	3.80	10.10	8.00	13.40	6.70	1.17	3.38	40.00
81	**117**	45.40	48.30	71.30	3.90	4.70	5.50	9.80	12.70	27.28	8.56	2.86	3.00	16.23
82	**118**	47.90	50.90	67.30	3.10	4.10	4.10	12.60	12.00	29.55	8.80	3.44	3.81	13.94
83	**119**	45.40	48.30	79.80	3.00	3.40	4.50	11.00	13.50	30.63	8.88	4.19	4.19	26.14
84	**120**	42.40	45.40	70.30	2.70	3.80	3.40	10.50	8.40	27.78	7.45	2.46	3.37	0.00
85	**121**	43.90	47.00	76.30	4.00	3.50	4.20	11.00	11.90	30.73	8.98	3.15	4.45	0.00
86	**122**	43.40	46.60	71.30	3.20	2.40	4.70	11.80	10.40	27.78	8.85	4.52	3.80	18.33
87	**123**	41.40	44.40	70.80	3.50	2.90	6.80	13.90	9.10	27.97	7.97	5.35	3.15	15.48
88	**124**	44.40	47.40	82.30	3.40	3.70	7.10	11.80	12.10	27.78	8.75	3.47	3.30	0.00
89	**125**	40.90	44.00	68.30	3.20	3.10	5.60	12.80	13.70	27.97	7.40	3.36	4.00	28.64
90	**126**	42.40	45.60	71.30	3.80	4.10	8.60	17.10	10.90	29.16	7.58	4.66	3.13	14.65
91	**127**	42.40	45.40	70.30	3.90	3.20	7.90	18.90	14.00	30.24	7.28	5.23	3.30	8.89
92	**128**	40.40	43.40	70.30	3.80	3.10	8.10	15.60	13.80	28.66	8.98	4.50	3.63	7.74
93	**129**	43.40	46.50	69.30	4.40	3.50	9.00	19.10	13.10	28.76	7.90	4.68	3.46	9.17

94	**130**	43.90	46.80	75.80	4.30	4.90	8.40	12.30	12.60	27.78	8.05	5.02	4.45	0.00
95	**131**	46.40	49.30	91.30	2.80	2.60	4.50	11.80	10.00	31.82	7.00	2.18	3.36	38.10
96	**132**	44.00	46.90	87.80	2.80	2.50	4.40	11.30	11.90	32.51	7.95	3.60	3.79	16.25
97	**133**	45.00	47.70	73.30	2.50	3.80	5.90	16.80	9.70	26.40	7.50	3.33	3.13	0.00
98	**134**	47.00	49.70	77.30	3.60	4.10	4.30	15.90	11.50	30.83	7.55	3.72	3.45	33.33
99	**135**	47.00	49.90	76.30	3.40	3.70	3.60	11.30	11.20	28.66	7.40	2.80	3.40	18.82
100	**136**	45.30	48.20	77.80	3.60	3.30	6.50	14.40	12.90	31.13	7.60	4.73	3.44	10.00
101	**137**	48.00	50.70	77.80	3.50	3.40	7.90	11.40	13.70	27.58	6.90	3.71	3.34	73.48
102	**138**	48.00	50.70	74.80	3.40	2.20	3.40	7.10	9.30	31.72	7.70	2.81	3.80	20.00
103	**141**	47.50	50.20	79.30	3.60	3.00	8.20	11.40	13.40	27.19	7.40	2.63	4.25	0.00
104	**143**	48.00	51.30	79.80	3.60	3.70	6.10	12.20	13.60	29.94	8.05	2.14	4.58	0.00
105	**145**	46.50	49.40	77.30	3.70	4.50	5.30	12.20	10.70	28.57	8.70	1.75	3.15	12.70
106	**146**	45.50	48.20	79.30	3.80	2.50	4.30	8.40	10.10	25.02	7.45	2.40	3.85	6.20
107	**147**	44.00	46.90	72.30	3.10	3.30	8.40	15.20	10.30	29.16	7.15	2.17	3.20	18.33
108	**148**	45.50	48.40	73.30	3.00	3.80	7.00	10.30	10.60	25.51	6.85	2.25	3.00	20.00
109	**150**	47.00	50.10	71.80	3.00	3.90	4.50	10.90	10.80	26.00	7.35	1.97	3.15	29.29
110	**151**	43.50	46.80	71.80	2.70	3.30	2.50	8.30	9.70	23.54	6.65	1.47	3.15	15.56
111	**153**	44.90	46.40	80.30	3.00	4.00	5.50	10.00	9.60	29.55	7.15	3.47	3.45	0.00
112	**154**	39.60	43.20	69.30	2.80	3.50	4.70	22.50	10.20	30.14	7.44	2.54	3.45	16.67
113	**156**	46.90	50.00	82.30	3.00	3.80	4.70	11.40	8.50	23.05	7.35	1.92	3.30	17.14
114	**158**	47.40	50.50	74.80	3.10	3.80	3.90	10.30	10.20	22.85	7.35	2.14	3.25	20.00
115	**159**	46.70	50.40	79.80	3.20	3.00	4.00	13.00	11.50	25.12	7.05	2.12	3.30	38.89
116	**161**	45.40	48.40	79.30	3.10	2.90	3.30	6.10	7.60	24.63	7.60	2.34	3.73	12.70
117	**162**	44.40	47.70	77.30	3.70	2.90	3.60	6.60	7.00	30.14	7.85	2.90	3.40	24.04
118	**163**	39.90	43.10	73.30	2.70	2.90	4.00	8.10	8.10	17.73	7.60	1.71	3.60	36.67
119	**164**	47.40	50.50	68.80	3.50	3.10	4.10	7.80	8.00	23.54	7.30	1.87	3.25	0.00
120	**165**	47.40	50.20	75.30	2.90	2.90	3.40	8.90	8.30	23.74	7.30	2.27	3.15	20.00
121	**166**	43.90	46.80	71.80	2.90	3.10	5.40	7.00	8.10	21.28	6.40	2.45	3.20	40.00
122	**167**	44.40	47.20	76.30	2.70	3.50	4.40	9.70	8.70	22.16	7.90	2.16	2.92	17.14
123	**168**	43.40	46.50	76.30	2.80	3.80	4.30	10.00	8.20	21.47	7.90	2.87	3.10	65.81
124	**169**	45.90	48.70	78.30	3.10	3.30	3.70	9.90	9.40	22.85	7.90	1.81	3.25	91.67
125	**170**	46.40	49.30	77.30	3.20	3.00	3.80	9.80	8.00	23.84	8.30	2.57	3.40	45.00
126	**171**	46.90	49.60	76.30	2.50	2.80	4.70	8.50	9.00	22.06	7.60	2.44	3.15	63.57

127	**173**	42.90	45.90	78.30	2.90	3.50	5.00	9.60	9.30	24.33	7.05	2.12	3.53	80.00
128	**174**	40.40	43.20	77.30	3.20	3.00	4.00	11.10	9.30	21.77	6.95	2.32	3.22	29.29
129	**MGG 295**	45.60	48.50	69.60	3.80	4.00	6.10	19.05	9.80	32.41	8.40	4.30	3.70	45.30
130	**WGG 42**	36.30	39.00	63.80	2.70	2.60	5.20	12.55	9.35	31.62	7.55	4.06	5.00	5.71

DFF: Days to initial flowering, DF50: Days to 50% flowering, DM: Days to maturity, NBP: number of branches per plant, NPC: Number of Pods per cluster, NCP: Number of cluster per plant, NPP: Number of pods per plant, NSP: Number of seeds per pod, PH: plant height, PL: pod length, SYP: Seed yield per plant, SW: 100 Seed weight, DI: Disease incidence

Table 4.5. Mean, Standard deviation, Range, Coefficient of variation and standard error

Characters	Mean	S.D	Range	CV	Standard Error
Days to initial flowering plant	44.159	2.500	36.30-50.30	5.662	1.768
Days to 50% flowering plant	47.154	2.668	39.00-53.40	5.660	1.887
Days to maturity	73.961	5.800	63.80-91.30	7.843	4.101
Number of branches per plant	3.498	0.200	2.40-5.10	5.739	0.141
Number of pods per cluster	4.834	0.287	2.20-9.10	5.945	0.203
Number of cluster per plant	6.182	0.321	2.50-10.20	5.199	0.227
Number of pods per plant	16.802	0.462	5.20-32.40	2.752	0.326
Number of seeds per pod	10.292	0.591	5.50-15.60	5.744	0.418
Plant height (cm)	27.256	1.276	13.40-38.22	4.684	0.902
Pod length (cm)	7.339	0.414	6.19-8.98	5.651	0.293
Seed Yeild per plant (g)	3.016	0.183	0.83-6.97	6.084	0.129
100 Seed weight (g)	3.361	0.334	2.57-5.00	9.937	0.236
Disease incidnece (%)	24.94	1.90	0.00-91.66	7.61	1.34

4.4.2 Seed yield per plant (g)

Seed yield per plant (g) was recorded for each F_6 RILs by taking average of five tagged plants in a row. Mean data of five plants of the F_6 lines is given in the Table 4.3. The seed yield found to have high variation in the F_6 population with a mean and range of 3.01 g/plant and 0.84 – 6.97 g/plant, respectively. The frequency distribution (Fig. 4.8) of yield per plant is segregated towards normal distribution, with a skewness value of 0.67 and a kurtosis value of 0.27 which indicates normal or mesokurtic curve (values lower than ± 1). Based on the analysis it was found that seed yield was normally distributed in F_6 generation of green gram.

Table 4.6. Phenotypic analysis of seed yield per plant (g) in F_6 RIL population.

Seed yield per plant (g)	
Mean	3.36
Median	2.83
Standard deviation	0.183
Kurtosis	0.27
Skewness	0.67
Range	0.83-6.97

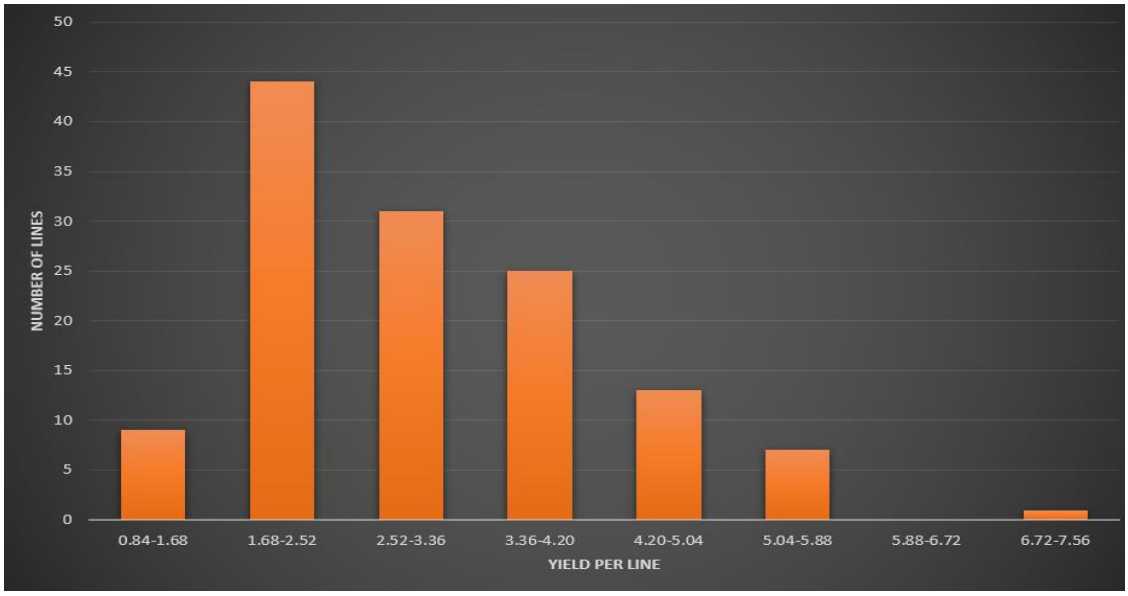

Figure 4.8. Frequency distribution of seed yield recorded from the F_6 RIL population.

4.4.3 Estimation of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), genetic advance, heritability and Genetic advance as percent of mean (GAM) at YMV hotspot area.

In the present study high PCV and GCV estimates were observed for disease incidence (90.82, 90.50), number of pods per plant (46.25, 46.17), seed yield per plant (38.58, 38.10), number of pods per cluster (33.07, 32.53), number of cluster per plant (28.98, 28.51). Lowest PCV and GCV estimates were recorded for Days to 50 % flowering (7.049, 4.201), Days to maturity (8.941, 4.291), days to initial flowering (7.37, 4.72) and Pod length (8.64, 6.53). Lowest GCV estimate is also observed in 100 seed weight (9.537). Moderate values were recorded for the remaining traits. Results are furnished in Table 4.7.

High PCV and GCV estimates for pods per plant, seed yield per plant were reported by Rao *et al.* (2006), Raturi *et al.* (2015), Anand *et al.* (2016), Garg *et al.* (2017) and Parimala *et al.* (2020). High PCV and GCV estimates for pods per plant was observed

by Talukdar *et al.* (2020). Moderate PCV and GCV were found for plant height, number of branches per plant, number of seeds per pod and 100 seed weight. Similar results were also observed by Pandey *et al.* (2007) and Nand and Anuradha (2013) for branches per plant, Rao *et al.* (2006), Makeen *et al.* (2007) and Kumhar and Choudhary (2007) for 100 seed weight. Mehta *et al.* (2019) for number of branches per plant. Lowest PCV and GCV estimates were obtained for days to 50% flowering, pod length and days to maturity. These results are in correspondence with Rao *et al.* (2006), Makeen *et al.* (2007), Kumhar and Choudhary (2007), Nand and Anuradha (2013), Zuge *et al.* (2019) and Asari *et al.* (2019) for days to 50% flowering and days to maturity. Parera *et al.* (2017) for days to maturity. The results obtained for PCV and GCV showed that there is considerable possibility of further improvement and by appropriate selection for these characters and development of high yielding variety can take place. Low values of genotypic and phenotypic coefficient of variation were noted for pod yield, days to maturity and days to 50% flowering which indicated low range of variation for these characters in the genotypes, thus offering little scope for further improvement of these characters through simple selection.

Traits can be selected based on high PCV, GCV, high heritability along with high genetic advance as percent of mean indicating these traits were less influenced by environment and possess high genetic variability.

4.4.4 Genetic advance

A perusal of genetic advance for all the quantitative traits under study ranged from 0.45 percent (hundred seed weight) to 46.34 percent (disease incidence). Moderate genetic advance was expressed by number of pods per plant (15.95) and remaining parameters observed low genetic advance that is plant height (6.60), number of cluster per plant (3.57), number of seed per plant (3.36), number of pods per cluster (3.18), days to maturity (3.13), days to first flowering (2.75), days to 50% flowering (2.43), seed yield per plant (2.33), number of branches per plant (0.97), pod length (0.74) and Hundred seed weight (0.45) (Table 2). Similar results were observed by Nand and Anuradha (2013) and Malli *et al*. (2018) for days to initial flowering, days to 50% flowering, days to full maturity, 100 seed weight and pod length. Raturi *et al*. (2015) for plant height and number of branches per plant.

4.4.5 Heritability, Genetic advance as percent of mean

High heritability was observed in number of pods per plant (99.6), disease incidence (99.30), seed yield per plant (97.5), number of clusters per plant (96.8), number of pods per cluster (96.80), number of seeds per plant (89.50), plant height (87.8), number of branches per plant (86.5). Moderate heritability was observed in pod length (57.2), hundred seed weight (47.9), days to initial flowering (41.07), days to 50% flowering (35.5). Low heritability was observed for days to maturity (23.0). Similar results reported by Natarajan *et al*. (1988). Nand and Anuradha (2013), Hemavathy *et al*. (2015), Garg *et al*. (2017) for high heritability in plant height, seed yield per plant and number of pods per plant. Mahendi *et al*. (2018) for number of pods per cluster, seed yield per plant. Zuge *et al*. (2019) reported high heritability for plant height, number of pods per plant and similarly moderate values were recorded for Pod length. Asari *et al*. (2019) reported high heritability for plant height, number of pods per plant, number of branches per plant, number of pods per cluster, number of cluster per plant, number of pods per plant, number of seeds per pod and seed yield per plant. These results are in correspondence with the findings of Reddy *et al*. (2003) and Makeen *et al*. (2007). For reliable selection high heritability of a character needs to be accompanied by high genetic advance (Johnson *et al*., 1955) because such characters are mostly controlled by additive gene action. Similar results were obtained by the presence of high heritability indicates preponderance of additive gene action in expression of these traits and they can be improved through individual plant selection. Results are furnished in Table 4.7.

The genetic advance as percent of mean was high for the traits i.e. disease incidence (185.78), number of pods per plant (94.94 percent), seed yield per plant (77.51), number of pods per cluster (65.93), number of clusters per plant (57.78), number of seeds per plant (32.65), number of branches per plant (27.82) and plant height (24.25).). Moderate GAM was observed in Hundred seed weight (13.604), pod length (10.18). Lowest GAM is observed for Days to initial flowering (6.22), days to 50% flowering (5.15) and days to maturity (4.24). Similar results were observed by Manivannan *et al*. (1996), Pandiyan *et al*. (2006). Nand and Anuradha (2013) for number of pods per plant, number of seeds per pod, seed yield per plant. Hemavathy *et al*. (2015) for plant height, days to 50% flowering, days to maturity, number of cluster per plant, number of pods per cluster, number of pods per plant, Hundred seed weight and seed yield per plant. Asari *et al*. (2019) for seed yield per plant, number of pods per plant, number of clusters per plant, number of branches per plant and plant height.

High heritability along with high genetic advance as percent of mean was observed for disease incidence, number of pods per plant, seed yield per plant, number of cluster per plant, number of pods per cluster, number of branches per plant, number of seeds per pod and plant height indicating the role of additive genes in governing the inheritance of these traits and could be improved through selection (Table 4.7). Similar results were reported by Baisakh *et al*. (2016) for plant height and pods per plant. Muthuswamy *et al*. (2019) for plant height, number of branches per plant, number of pod per plant, and seed yield per plant. Pavan *et al*. (2019) for pods per plant, seed yield per plant, plant height and number of branches per plant. Asari *et al*. (2019) for plant height, number of branches per plant, number of cluster per plant, number of pods per plant and seed yield per plant. With the help of traits with high heritability coupled with high genetic advance as percent of mean can be selected for breeding programmes in order to develop a high yielding and disease resistant variety.

Table 4.7. Magnitude of Variability and estimates of Heritability and Genetic Advance for various characters of Green gram RILs.

Trait	Coefficient of Variation			H^2(%)	GA	GAM	Mean	CV
	PCV%	GCV%	ECV%					
DFF	7.375	4.722	5.662	41.07	2.751	6.229	44.159	5.662
DF50	7.049	4.201	5.660	35.5	2.432	5.158	47.154	5.660
DM	8.941	4.291	7.843	23	3.138	4.243	73.961	7.843
NBP (nos)	15.617	14.925	5.739	86.5	0.973	27.828	3.498	5.739
NPC (nos)	33.078	32.539	5.945	96.8	3.187	65.939	4.834	5.945
NCP (nos)	28.984	28.514	5.199	96.8	3.572	57.786	6.182	5.199
NPP (nos)	46.254	46.172	2.792	99.6	15.953	94.946	16.802	2.752
NSP (nos)	17.716	16.759	5.744	89.5	3.361	32.659	10.292	5.744
PH (cm)	13.408	12.563	4.684	87.8	6.609	24.250	27.256	4.684
PL (cm)	8.640	6.537	5.651	57.2	0.748	10.187	7.339	5.651
SYP (g)	38.587	38.104	6.084	97.5	2.338	77.513	3.016	6.084
SW (g)	13.773	9.537	9.937	47.9	0.456	13.604	3.361	9.937
DI (%)	90.82	90.50	7.62	99.30	46.34	185.78	24.94	7.62

DFF: Days to initial flowering, DF50: Days to 50% flowering, DM: Days to maturity, NBP: number of branches per plant, NPC: Number of Pods per cluster, NCP: Number of cluster per plant, NPP: Number of pods per plant, NSP: Number of seeds per pod, PH: plant height, PL: pod length, SYP: Seed yield per plant, SW: 100 Seed weight, DI: Disease incidence

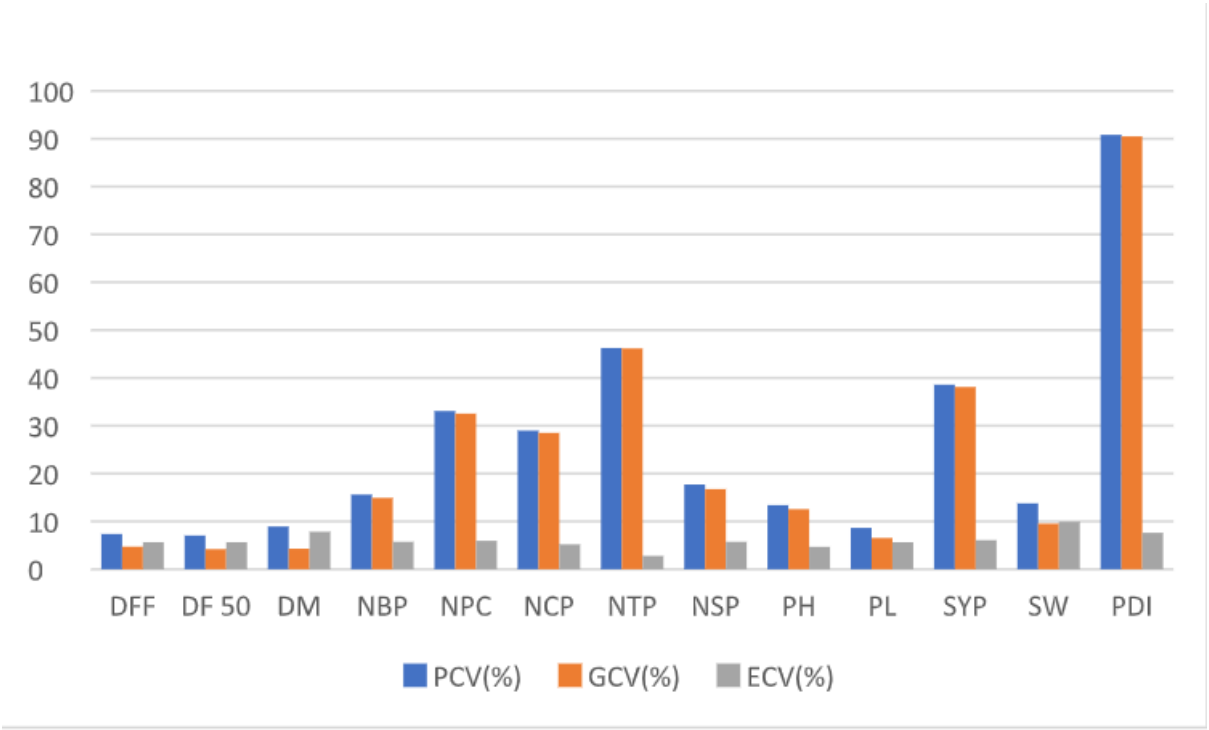

Figure 4.9. Graphical representation of PCV and GCV for yield contributing traits

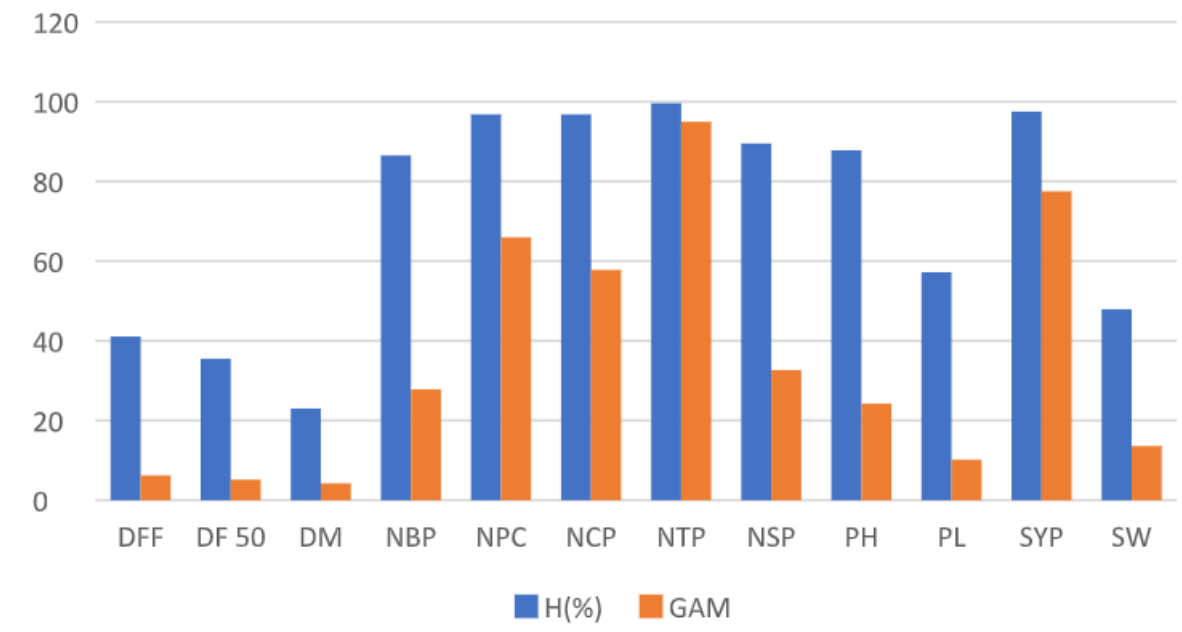

Figure 5.0. Graphical representation of heritability (broad sense) and genetic advance as percent of mean

4.5 EVALUATION OF RIL POPULATION OF GREEN GRAM USING MOLECULAR MARKERS

4.5.1 Parental polymorphism survey using SSR markers

The MGG 295 (YMV susceptible parent) and WGG 42 (YMV Resistant parent) were screened with 185 markers which include microsatellite markers derived from green gram, Azuki bean and genic markers derived from Green gram. Out of these markers, 102 amplified and 83 markers did not amplify. This may be associated to the reason that majority of the markers used in the current study were derived from the Azuki bean genomic sequences. Thus, they may not have the 3' complementarity to anneal properly.

The list of the markers used and their annealing temperatures are given in the Supplementary Table 1 (Appendix IV). Of these 102 amplified markers, 15 markers showed polymorphism with 14.7% between the parents and the rest of the markers were found to be monomorphic. Of 15 markers, 10 markers were derived from Azukibean (CEDG). Representative gel image of parental polymorphism study has been shown in Fig. 5.1. Previously many research groups reported about low polymorphism prevailed in the pulse crops. Low polymorphism was observed by Anjum *et al*. (2010) when examined the population with 387 markers derived from azuki bean and other RGH markers of which 7 markers showed polymorphism which indicated 2% polymorphism. Gupta *et al*. (2013) screened the mapping population with 361 markers among them only 31 markers showed polymorphism indicates 8.6% polymorphism. Alam *et al.* (2014) used 1165 markers from different legumes to detect polymorphism out of these only 61 were found to be polymorphic indicates 5.2% polymorphism. Singh *et al*. (2018) used 224 markers comprising of SSR's, RGA, SCAR, and STS were employed for identification of polymorphism among parents and 45 markers were found to be polymorphic indicating 20.08% polymorphism.

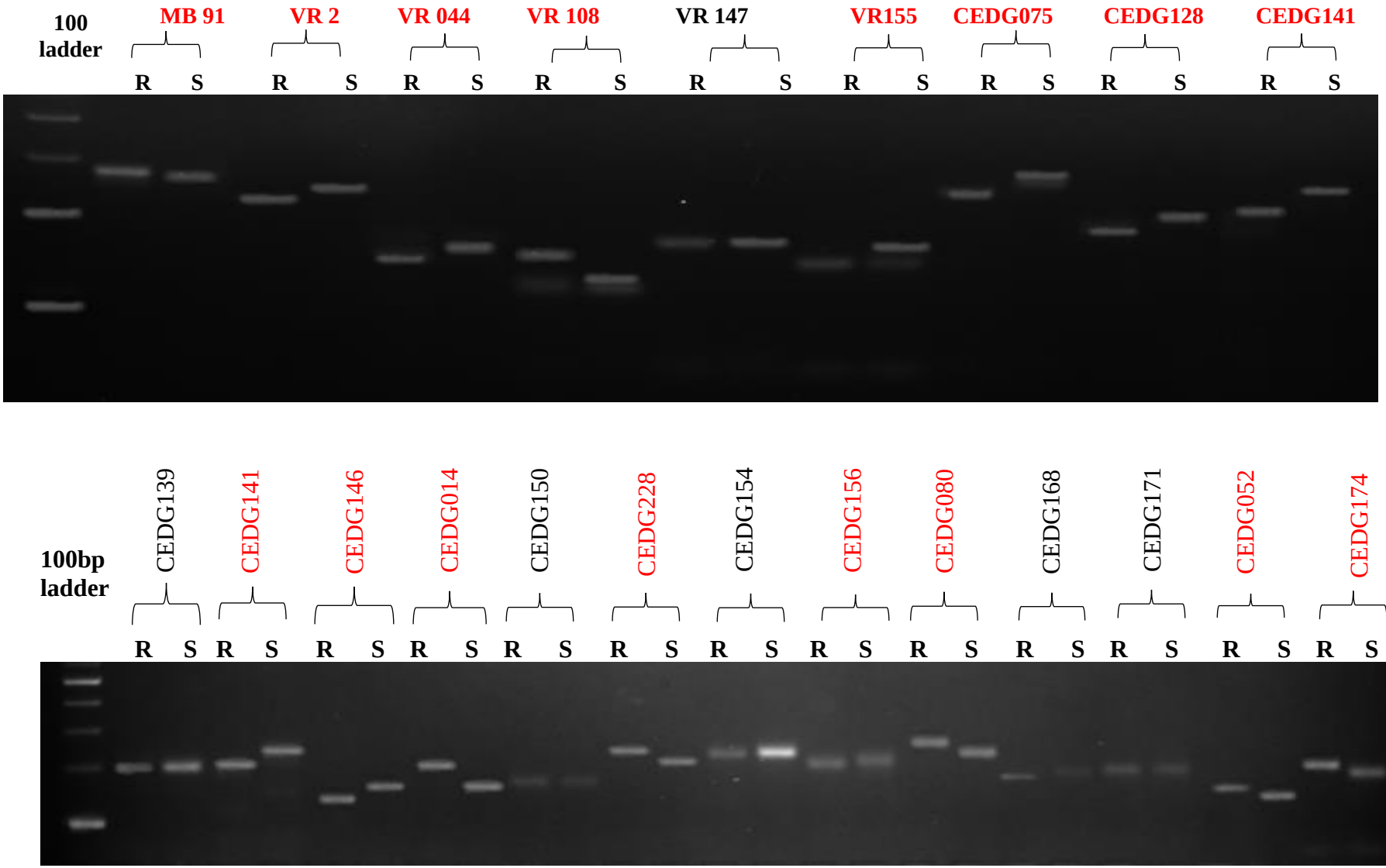

Figure 5.1. Gel image showing parental polymorphism using SSR markers (Highlighted markers are polymorphic)

Table 4.8. List of polymorphic primers identified in the present study

S.no	Primer Name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Ann. Tem. (°C)	Allele Size (bp)	
					MGG 295	WGG 42
1	MB 91	GAGGCCAATCCCATAACTTT	AGCACCACATCAGAGATTCC	58	268.07	275.27
2	VR 2	CGCCCCTCTAGGTTGGTTGG	GGGAAAGACGAAGGGTAGAA	59	237.20	217.30
3	VR044	CCCATGAAGGTATGAGACAACA	GACTGAGAAAGAGAGAGAAGCATTT	58	160.00	150.00
4	VR108	GCTCCAACACTCACTCACAAAC	CAGAAATGCAGGAAAAGAGAGG	59	170.00	160.00
5	VR155	AAGATCACACACAACCAACCC	AATTAGTTCCACAGGCCAGATT	58	130.05	126.06
6	CEDG075	GCGACCTCGAAAATGGTGGTTT	TCACCAACTCACTCGCTCACTG	67.3	267.66	257.60
7	CEDG128	CTGCCAAAGATGGACAACTTGGAC	GCCAACCATCATCACAGTGC	68.4	181.71	169.42
8	CEDG141	CCAGGCATCCATGATGACC	GAAGTTGTTGGTAATGGTTGCCTC	66.4	229.45	187.45
9	CEDG146	GGTGATCGGATTTCAGAG	GGAGAAGAGAATAGAGACG	56.2	150.03	142.08
10	CEDG156	CGCGTATTGGTGACTAGGTATG	CTTAGTGTTGGGTTGGTCGTAAGG	65.3	219.17	210.74
11	CEDG174	GAGGGATCTCCAAAGTTCAACGG	GAAGGCTCCGAAGTTGAAGGTTG	68.6	189.37	194.66
12	CEDG014	GCTTGCATCACCCATGATTC	AAGTGATACGGTCTGGTTCC	58	150.90	200.26
13	CEDG080	CACGTTGGAGGAAGTGACGC	CATCGCCACCACAGAACCA	58	209.98	225.00
14	CEDG052	CAAACCCTTAAGCTAGGGCTG	GGCAAGTGGTGCGCTCCTC	58	150.98	145.02
15	CEDG228	GTCGTTTCCGGAAACTGTTC	GATCCGAACCTCTTTCTGC	58	210.08	230.30

4.6 GENOTYPING OF F6 POPULATION

Genomic DNA was isolated using CTAB method. The quality of extracted DNA was checked by Agarose gel electrophoresis. Using the genomic DNA, which was isolated the 128 F_6 RIL (Figure: 5.2), Screening of RILs were done using fifteen polymorphic SSR markers. Out of these, only eight markers showed clear scorable bands in RIL population. These eight markers are CEDG228 (Fig. 5.3), CEDG 141, VR 2, CEDG014, CEDG146, CEDG 080, MB 91 and CEDG174.

Amplification pattern was analysed in the plants segregating in respect of the response to YMV disease incidence. Screening of all eight scorable markers with RIL population was done in order to distinguished between resistant and susceptible bands.

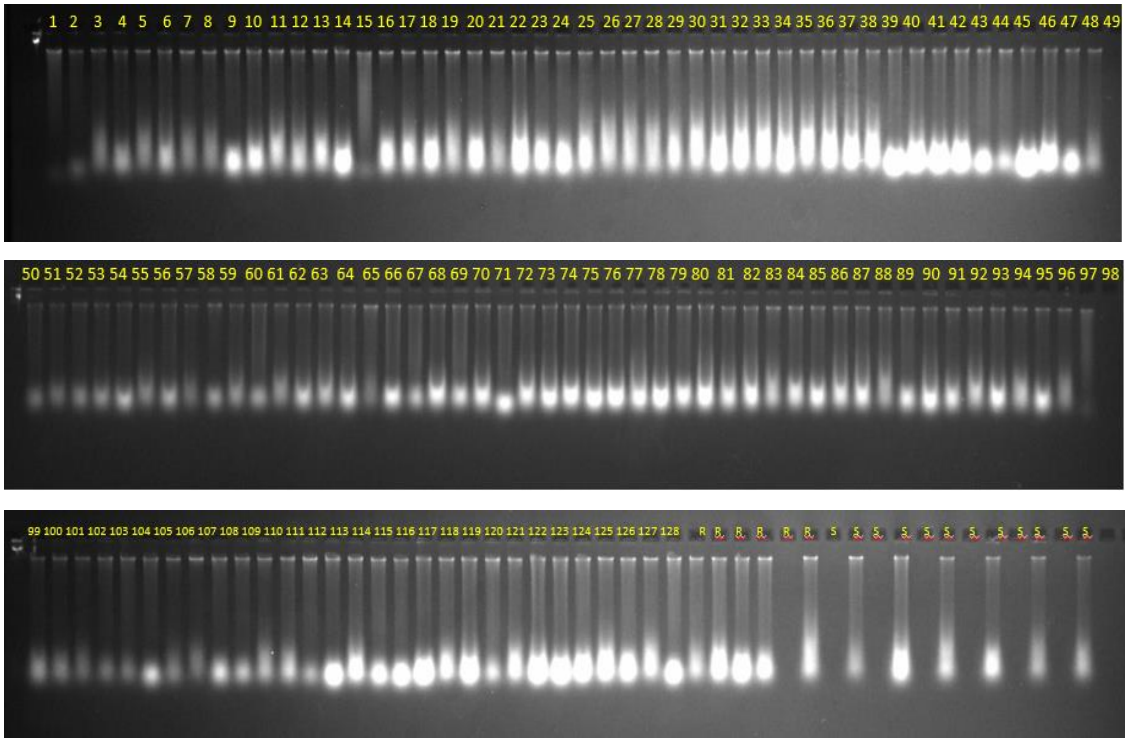

Figure 5.2. Genomic DNA quality checked 0.8% agarose gel and visualized with Ethidium Bromide under Gel Doc (lanes 1-128) and Parents R= WGG 42 & S= MGG295.

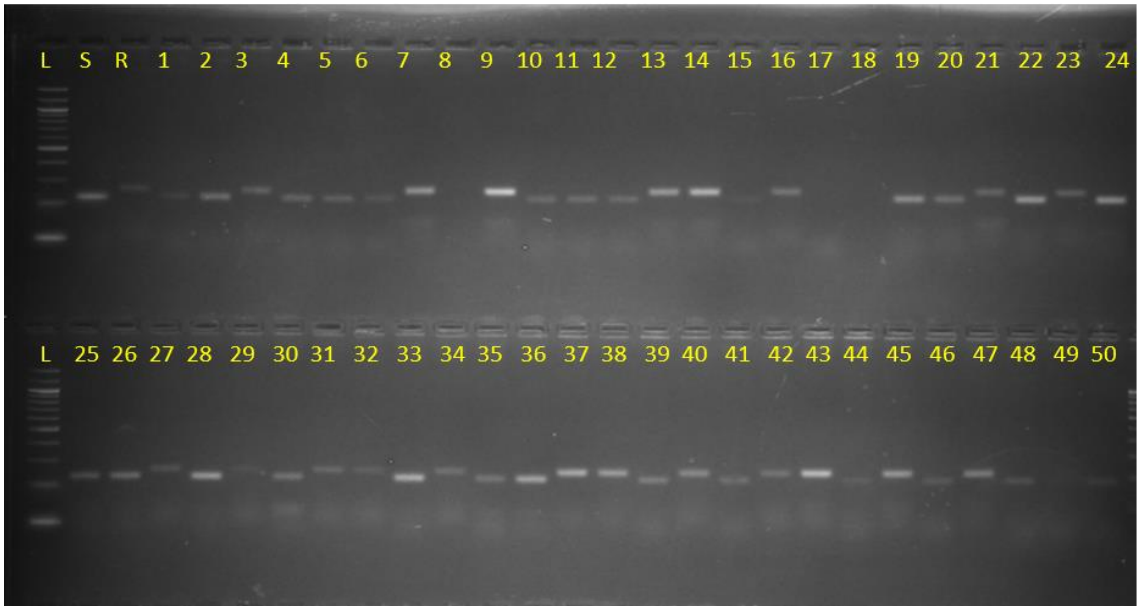

Figure 5.3. A representative gel picture of genotyping of F_6 population with the marker CEDG228: L – 100bp ladder, S – MGG295, R – WGG42

4.6.1 Marker – trait association for YMV resistance

The genotypic data of the eight polymorphic markers was used to evaluate the association of these markers with YMV resistance using Analysis of variance (ANOVA) - single factor by Microsoft excel.

The results of single factor Anova showing probability and *F* value's for all eight markers are given in table 4.9. The co-segregation of CEDG228 with YMV resistance was found to be significant with probability value of 0.03. This implies that CEDG228 is associated with YMV resistance. Further this marker was found to be present on linkage group 9 (Singh *et al*., 2018).

Table 4.9. Anova : single factor for all the markers

S.no	Markers	*F*-value	*P*
1	CEDG228	4.72	0.03*
2	CEDG141	2.77	0.09
3	VR2	0.01	0.90
4	CEDG014	0.03	0.85
5	CEDG146	0.07	0.77
6	CEDG080	0.04	0.83
7	MB91	0.10	0.74
8	CEDG174	1.13	0.28

P-Probability value, *-Significance at 0.05 level.

Similar findings were reported by Singh *et al*. (2018) in which construction of linkage map comprising 11 linkage groups with twenty two polymorphic markers was done. Based on the QTL analysis by single marker analysis, it was reported that CEDG228 was linked with YMV resistance and was found to be present on linkage group 9 at a distance of 62.80. CEDG284, CEDG275 reported by Alam *et al*. 2014 were found to be monomorphic in our study. Gupta *et al*. (2015), Gupta *et al*. (2013) and Isemura *et al*. (2012) reported marker CEDG180 located on LG10 linked with YMV in blackgram. This was found to be monomorphic in our study. CEDG185 was reported in blackgram by Rambabu *et al*. (2018) was found to be monomorphic in our population. This may be due to change in genera and species. These results indicate that gene(s) conferring YMV resistance in green gram and blackgram may be at different loci. Interestingly, it is found in the present investigation that marker CEDG228 is cosegregating with YMV resistance in the parents and population under study.

Hence, this marker has the potential for use in marker-assisted screening and marker assisted breeding for the development of YMV green gram varieties. However, further validation of this marker in a set of resistant and susceptible cultivars and fine mapping would give added strength for the potential of this marker. This would increase the efficiency and accuracy in YMV-resistance breeding program and could be used in future for the development of high yielding YMV resistant cultivars for green gram.

Chapter V

SUMMARY AND CONCLUSION

Chapter V

SUMMARY AND CONCLUSIONS

Mungbean yellow mosaic virus (MYMV) disease is one of the most devastating biotic constraints of mungbean production in India. Development of YMV resistant varieties is the viable option to effectively control the disease among all integrated disease management methods. Characterization of YMV resistance using various advanced molecular and biochemical approaches during plant–virus interactions has unfolded a comprehensive network of pathogen survival, disease severity, and the response of plants to pathogen attack, including mechanisms of YMV resistance in Green gram. The biggest challenge in YMV management is the effective utilization of an array of information gained so far, in an integrated manner for the development of genotypes having durable resistance against yellow mosaic virus (YMV) infection. In this backdrop, the present investigation entitled "Evaluation of Green gram (*Vigna radiata* (L.) R. Wilczek) lines for YMV resistance using Molecular markers" was undertaken at the Institute of Biotechnology (IBT), Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad during 2018-2019. The objectives of the study were to study parental polymorphism in between the resistant WGG 42 and Susceptible MGG 295 and also evaluate molecular markers linked with MYMV resistance in the F_6 generation of RIL population.

The result obtained in the present study are summarised as follows.

- To evaluate the molecular markers linked to yellow mosaic virus resistance, the F_6 RIL population derived by single seed descent method for YMV susceptible MGG 295 and YMV resistant WGG 42 parent's was utilised in the present investigation.
- A total of 128 F_6 RILs along with parents using infector row method were subjected to natural screening against yellow mosaic virus in hotspot using standard disease score scale.
- The field screening of 128 F_6 RILs resulted in the identification of 28 highly resistant, 7 resistant, 38 moderately resistant, 15 moderately susceptible, 23 susceptible and 17 highly susceptible lines.
- Chi-square test for F_6 RIL lines indicate that MYMV resistance in green gram has monogenic inheritance governed by a single gene and gave a goodness of fit to the ratio of 1:1 i.e. 55 susceptible: 73 resistant RIL lines.

- Heritability broad sense (h^2) was recorded high for characters disease incidence, number of branches per plant, number of pods per cluster, number of clusters per plant, number of pods per plant, number of seeds per pod, plant height, seed yield per plant specifying that the above said traits have least influence of environment on their development but selection only based on heritability cannot be done because it is the result of total genetic variance that results due to both additive and non-additive variances. Hence, it is considered with genetic advance for selection.
- High heritability along with high genetic advance as percent of mean was observed for number of pods per plant, seed yield per plant, number of cluster per plant, number of pods per cluster, number of branches per plant, number of seeds per pod and plant height indicating the role of additive genes in governing the inheritance of these traits and could be improved through selection.
- Parental polymorphism was studied using SSR markers. The parents were screened with 185 markers from green gram, black gram and azuki bean to detect the polymorphic markers. Out of these markers, 102 were amplified and 83 markers were not amplified.
- Of these 102 amplified markers, 15 showed polymorphism (14.7%) between the parents and the rest of the markers were found to be monomorphic. These polymorphic markers were used for genotyping of the RILs.
- All the 128 F_6 RILs were genotyped with the 15 polymorphic SSR markers. Out of these 15 polymorphic SSRs, 8 markers showed clear scorable bands. These eight markers were CEDG228, CEDG 141, VR 2, CEDG014, CEDG 146, CEDG 080, MB 91 and CEDG174.
- The RILs 24, 26, 77, 118 and 130 are found to be YMV resistant as well as high yielding.
- SSR marker CEDG228 present on linkage group 9 showed significant association with YMV resistance.

CONCLUSIONS

- The traits viz. seed yield per plant, number of pods per plant, number of clusters per plant and number of pods per cluster had recorded high PCV, GCV, high heritability along with high genetic advance as percent of mean indicated these traits were less influenced by environment and possess high genetic variability.

- High heritability, variability, genetic advance as percent mean in the segregating population can be handled under different selection schemes for improving productivity.
- Sufficient variability exists in the green gram RILs under study which can be used in further breeding programmes, genome mapping studies and QTL analysis.

FUTURE LINE OF WORK

- The RILs developed in the present study can be used for fine mapping, YMV resistance gene and validation of identified markers.
- RILs like 24, 26, 77, 118 and 130 which are as best performing lines can be utilized for development of resistant variety.
- This marker CEDG228 which is identified in the present study can be used to screen large germplasm for Marker assisted breeding for YMV resistance.
- Identification of molecular marker associated with resistance functional gene, will increase the efficiency and accuracy in YMV-resistance breeding program.

LITERATURE CITED

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Abbas, G., Asghar, M. J., Shah, T. M. and Atta, B.M. 2010. Genetic diversity in mungbean (*Vigna radiata* L. Wilczek) germplasm. *Pakistan Journal of Botany*, 42(5): 3485-3495.

Adam-Blondon, A.F., Sevignac, M., Dron, M. and Bannerot, H. 1994. A genetic map of common bean to localize specific resistance genes against anthracnose. *Genome*, *37*(6), pp.915-924.

Akbar, W., Aslam, M., Maqbool, M, A., Ali, M. and Arshad M. 2018. Inheritance pattern of mungbean yellow mosaic disease resistance and gene action for different traits in mungbean (*Vigna radiata* (L.) Wilczek) under protected and unprotected field conditions. *Plant breeding*, 137(5), pp.763-772.

Alam, A.M., Somta, P. and Srinives, P. 2014. Identification and confirmation of quantitative trait loci controlling resistance to mungbean yellow mosaic disease in mungbean [*Vigna radiata* (L.) Wilczek]. *Molecular breeding*, *34*(3), pp.1497-1506.

Alemu, I. D., Petros, Y. and Mebeaselassie, A. 2014. Genetic Variability, Heritability and Genetic Advance in Mungbean (*Vigna radiata* L. Wilczek) Accessions. *Plant Science Today*, 1(2): 94-98.

Allard, R.W. 1960. Principles of plant breeding. New York, *John Wiley and Sons*, pp. 89-98.

Anand, G., Anandhi, K and Paulpandi, V.K. 2016. Genetic variability, correlation and path analysis for yield and yield components in F_6 families of Greengram (*Vigna radiata* (L.) Wilczek) under rainfed condition. *Electron. J. Plant Breed.*, 7:434-437.

Anitha. 2008. Molecular fingerprinting of Vigna sp using morphological and SSR markers. M.Sc Thesis. Tamil Nadu Agriculture University, Coimbatore, India. 45p

Anjum, T., Gupta, S. and Datta, S. 2010. Mapping of Mungbean Yellow Mosaic India Virus (MYMIV) and powdery mildew resistant gene in black gram [*Vigna mungo* (L.) Hepper]. *Electronic Journal of Plant Breeding*. 1(4):1148-1152.

Anonymous. 2012. Selected state wise Area, Production and Productivity of Moong (Kharif and Rabi) in India, Ministry of Agriculture and Farmers Welfare. Govt of India. *Arch Virology* 149: 1643-1652.

Anuradha, Ch. and Sridhar V. 2019. Technical programme of work, IBT, PJTSAU.

Anusha, N. 2014. Identification of molecular markers linked to yellow mosaic virus resistance in greengram (*Vigna radiata* (L.) wilczek) (doctoral dissertation, Acharya NG Ranga Agricultural University, Rajendranagar, hyderabad).

Anusha, N., Anuradha, Ch and Srinivas, AMN. 2014. Genetic parameters for yellow mosaicvirus resistance in green gram *Vigna radiata* (L.). *International Journal of Scientific Research* 3 (9) 23-29.

Anusha, N., Anuradha, C., Srinivas, A.M.N. and Sokka, R.S., 2014. Inheritance of Yellow Mosaic Virus (YMV) in Mungbean [*Vigna radiata* (L.) Wilczek]. *Research Journal Of Biotechnology*, 9(11), pp.60-61.

Anushya. 2009. Marker assisted selection for yellow mosaic virus (MYMV) in mungbean [*Vigna radiata* (l.) wilczek] unpub. M.Sc Thesis. Tamil Nadu Agriculture University, Coimbatore, India. 56p.

Arumuganathan, K. and Earle, E.D. 1991. Nuclear DNA content of some important plant species. *Plant molecular biology reporter*, *9*(3), pp.208-218.

Asari, T., Patel, B.N., Patel, R., Patil, G.B and Solanki, C. 2019. Genetic variability, correlation and path coefficient analysis of yield and yield contributing characters in mungbean [*Vigna radiata* (L.) Wilczek]. *International journal of computer science*, 7(4), pp.383-387.

Aski, M., Dikshit, H.K., Singh, D., Singh, A. and Prapti, P. 2015. Inheritance of resistance to mungbean yellow mosaic India virus (MYMIV) in mungbean (*Vigna radiata*). *Indian Journal of Agricultural Sciences* 85 (9): 1144–7.

Athwal, D.S. and Singh, G. 1966. Variability in Kangani, I. Adaptation and genotypic and phenotypic variability in four environments. Indian Journal of Genetics. 26: 142152.

Baisakh, B., Swain, S.C., Panigrahi, K.K., Das, T.R. and Mohanty, A. 2016. Estimation of Genetic Variability and Character Association in Micro Mutant Lines of

Greengram [*Vigna radiata* (L.) Wilczek] for Yield Attributes and Cold Tolerance. *Legume Genomics and Genetics*, 7.

Barkley, N.A., Wang, M.L., Gillaspie, A.G., Dean, R.E., Pederson, G.A. and Jenkins, T.M. 2008. Discovering and verifying DNA polymorphisms in a mung bean [*V. radiata* (L.) R. Wilczek] collection by Eco TILLING and sequencing. *BMC research notes*, *1*(1), p.28.

Basak, J., Kundagrami, S., Ghose, T.K. and Pal, A. 2004. Development of Yellow Mosaic Virus (YMV) resistance linked DNA marker in *Vigna mungo* from populations segregating for YMV-reaction. *Molecular Breeding*, 14: 375-383.

Basamma, K., Salimath, P.M., Malagouda, P. and Suma, B. 2015. Genetics, molecular dissection and identification of high yielding disease resistant lines to MYMV in blackgram (*Vigna mungo*). *Legume Research-An International Journal*, *38*(6), pp.851-854.

Basavaraja, T., Niranjana, M., Shashi, P. K. and Naik, S. J. 2017. Inheritance of resistance to Mungbean Yellow Mosaic Virus (MYMV) in intra and inter-specific crosses of *Vigna radiata*. *Journal of Food Legumes*, 30 (1), 15–19.

Bashir, M. 2005. Studies on viral diseases of major pulse crops and identification of resistant sources. Technical annual report (April, 2004 to June, 2005) of ALP Project. Crop Sciences Institute, National Agricultural Research Centre, Islamabad. p. 169.

Bhanu, A.N., Singh, M.N. and Srivastava, K. 2019. Genetic analysis of gene-specific resistance to mungbean yellow mosaic virus in mungbean (*Vigna radiata*). *Plant Breeding*, *138*(2), pp.202-206.

Binyamin, R., Khan, M.A., Khan, A.I., Awan, F.S., Khan, N.A. and Akhtar, S. 2016. Genetic diversity of mungbean genotypes in relation to resistance against mungbean yellow mosaic virus. *Pak. J. Bot*, 48(3), pp.1273-1277.

Binyamin, R., Khan, M.A., Khan, N.A. and Khan, A.I. 2015. Application of SCAR markers linked with mungbean yellow mosaic virus disease resistance gene in Pakistan mungbean germplasm. *Genetics and Molecular Research*. 14(1): 2825-2830.

Bui, T.G.T., Hoa, N.T.L., Yen, J. and Schafleitner, R. 2017. PCR-based assays for validation of single nucleotide polymorphism markers in rice and mungbean. *Hereditas*. 154:3.

Burton, G.W. and Devane, E.H. 1953. Estimating the heritability in tall fescue (*Festuca arundinancea*) from replicated clonal material. *Agronomy J.,* 45: 478- 481.

Caetano-Anolles, G., Bassam, B. J. and Gresshoff, P. M. 1991. DNA amplification fingerprinting: a strategy for genome analysis. *Plant Molecular Biology Reporter*, *9*(4), 294-307.

Chaisan, T., Somta, P., Srinives, P., Chanprame, S., Kaveeta, R. and Dumrongkittikule, S. 2013. Development of tetraploid plants from an interspecific hybrid between mungbean (*Vigna radiata*) and rice bean (*Vigna umbellata*). *Journal of Crop Science and Biotechnology*, *16*(1), pp.45-51.

Chithra. 2008. Analysis of resistant gene analogues in mungbean [Vigna radiate (L.) wilczek] and ricebean [*Vigna umbellata* (thunb.) ohwi and ohashi] unpub. M.Sc Thesis. Tamil Nadu Agriculture University, Coimbatore, India. 48 pp.

Cobos, M.J., Fernandez, M.J., Rubio, J., Kharrat, M., Moreno, M.T., Gil, J and Millan, T. 2005. A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli-Desi crosses: location of genes for resistance to fusarium wilt race. *Theoretical and Applied Genetics*. 110: 1347–1353.

Comstock, R.E. and Robinson, H.F. 1952. Genetic parameter, their estimation and significance. *Proceedings of Internation Gross Congress*. 284-291.

Dharajiya, D.T. and Ravindrababu, Y. 2019. Identification of molecular marker associated with mungbean yellow mosaic virus resistance in mungbean [*Vigna radiata* (L.) Wilczek]. *Vegetos*, *32*(4), pp.532-539.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue.

Dhole, V.J. and Reddy, K.S. 2013. Development of a SCAR marker linked with a MYMV resistance gene in mungbean (*Vigna radiata* L. Wilczek). *Plant Breeding*, *132* (1), pp.127-132.

Dhole, V.J. and Reddy, K.S. 2012. Genetic analysis of resistance to mungbean yellow mosaic virus in mungbean (*Vigna radiata*). *Plant breeding*, *131*(3), pp.414-417.

Fukuoka, S., Inoue, T., Miyao, A., Monna, L., Zhong, H.S., Sasaki, T. and Minobe, Y. 1994. Mapping of sequence-tagged sites in rice by single strand conformation polymorphism. DNA Research. 1: 271-277.

Gadakh, S. S., Dethe, A. M. and Kathale, M. N. 2013. Genetic variability, correlations and path analysis studies on yield and its components in mungbean (*Vigna radiata* (L.) Wilczek). *Bioinfolet,* 10(2a), 441-447.

Garg, G.K., Verma, P.K and Kesh, H. 2017. Genetic Variability, Correlation and Path Analysis in Mungbean [*Vigna radiata* (L.) Wilczek]. *International Journal of Current Microbiology and Applied Sciences*, *6*(11), pp. 2166-2173.

Gasic, K., Han, Y., Kertbundit, S., Shulaev, V., Iezzoni, A.F., Stover, E.W., Bell, R.L., Wisniewski, M.E. and Korban, S.S. 2009. Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. *Molecular Breeding*, *23*(3), pp.397-411.

Girish, K. R and Usha, R. 2005. Molecular characterization of two soybean-infecting begomoviruses from India and evidence for recombination among legume-infecting begomoviruses from South-East Asia. *Virus research*, *108*(1-2), pp.167-176.

Gupta, S.K., Souframanien, J. and Reddy, K.S. 2015. Validation of molecular markers linked to yellow mosaic virus disease resistance in diverse genetic background of black gram [*Vigna mungo* (L.) Hepper]. *Electronic Journal of Plant Breeding*, *6*(3), pp.755-763.

Gupta, A.B. and Gupta, R.P. 2013. Epidemiology of yellow mosaic virus and assessment of yield losses in mungbean. *Plant Archives*. Vol. 13 No. 1, pp. 177-180.

Gupta, S.K., Bansal, R., Vaidya, U.J. and Gopalakrishna, T. 2013. Assessment of genetic diversity at molecular level in mungbean (*Vigna radiata* (L.) Wilczek). *Journal of Food Legumes*, *26*, pp.19-24.

Gupta, S., Gupta, D.S., Anjum, T.K., Pratap, A. and Kumar, J. 2013. Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* L. Hepper). *Euphytica*, *193* (1), pp.27-37.

Gupta, S.K. and Gopalakrishna, T. 2008. Molecular markers and their application in grain legumes breeding. *Journal of Food Legumes*. 21: 1-14.

Gupta, S., Kumar, S., Singh, R. A. and Chandra, S. 2005. Identification of a single dominant gene for resistance to Mungbean Yellow Mosaic Virus in blackgram (*Vigna mungo* (L.). Hepper). *Journal of Breeding and Genetics*, 37(2), 85–89.

Hearne, C.M., Ghosh, S. and Todd, J.A. 1992. Microsatellites for linkage analysis of genetic traits. *Trends in Genetics*, 8(8), pp.288-294.

Hemavathy, A. T., Shunmugavalli, N. and Anand, G. 2014. Genetic variability, correlation and path co-efficient studies on yield and its components in mungbean [*Vigna radiata* (L.) Wilezek]. *Legume Research*, 38 (4) 2015: 442-446.

Holeyachi, P. R. and Savithramma, D.L. 2013. Identification of RAPD markers linked to MYMV resistance in Green gram (*Vigna radiata* (L). Wilczek). *Int. J. Life Sci*., 8: 1409-1411.

Isemura, T., Kaga, A., Tabata, S., Somta, P., Srinives, P., Shimizu, T., Jo, U., Vaughan, D.A. and Tomooka, N. 2012. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). *PLoS one*, *7*(8).

Isemura, T., Kaga, A., Konishi, S., Ando, T., Tomooka, N., Han, O.K. and Vaughan, D.A. 2007. Genome dissection of traits related to domestication in azuki bean (*Vigna angularis*) and comparison with other warm-season legumes. *Annals of Botany*, *100* (5), pp.1053-1071.

Islam, A. S. M. and Blair, M. W. 2018. Molecular Characterization of Mung Bean Germplasm from the USDA Core Collection Using Newly Developed KASP-based SNP Markers. *Crop Science*, *58*(4), 1659-1670.

Johannsen, W.L. 1909. *Elements directions Exblich keitelahre*. *Jenal Gustar*, Fisher.

John, P., Sivalingam, P. N., Haq, Q. M. I., Kumar, N., Mishra, A., Briddon, R.W. and Malathi, V.G. 2008. Cowpea golden mosaic disease in Gujarat is caused by a mungbean yellow mosaic India virus isolate with a DNA-B variant. *Archives of virology*, 153(7), p.1359.

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Genotypic and phenotypic correlation in soybean and their implications in selection. *Agronomy Journal*, 47: 477-483.

Jordan, S.A. and Humphries, P. 1994. Single nucleotide polymorphism in exon 2 of the BCP gene on 731-35. *Human molecular genetics*, *3*(10).

Kabi, M., Das, T.R. and Baisakh, B. 2018. Molecular Marker Assisted Selection of Yellow Mosaic Virus Resist Mosaic Virus Resistant Genotypes in Greengram Genotypes In Greengram. *The Bioscan*, 13(2): 581-583.

Kabi, M., Das, T.R., Baisakh, B. and Swain, D., 2017. Resistant gene rnalogous marker assisted selection of yellow mosaic virus resistant genotypes in Greengram (*Vigna radiata*). *Int. J. Curr. Microbiol. App. Sci*, 6(9), pp.3247-3252.

Kaga, A., Ishii, T., Tsukimoto, K., Tokoro, E. and Kamijima, O. 2000. Comparative molecular mapping in Ceratotropis species using an interspecific cross between azuki bean (*Vigna angularis*) and rice bean (*V. umbellata*). *Theoretical and Applied Genetics*, 100 (2), pp. 207-213.

Kajonphol, T., Sangsiri, C., Somta, P., Toojinda, T. and Srinives, P. 2012. SSR map construction and quantitative trait loci (QTL) identification of major agronomic traits in mungbean (*Vigna radiata* (L.) Wilczek). *SABRAO Journal of Breeding & Genetics*, *44*(1).

Kalaria, R.K., Digvijay, C., Mahatma, M.K. and Mahatma, Lalit. 2014. Identification of RAPD and ISSR makers for resistance against Mungbean Yellow Mosaic Virus in mungbean (*Vigna radiata* L.) under south Gujarat agro climatic condition of India. *The Bioscan*, *9*(3), pp.1177-1182.

Kalo, P., Endre, G., Zimanyi, L., Csanadi, G. and Kiss, G.B. 2000. Construction of an improved linkage map of diploid alfalfa (*Medicago sativa*). Theoretical and Applied Genetics. 100: 641–657.

Kamleshwar K., Yogendra, P., Mishra, S. B., Pandey, S. S. and Ravi, K. 2013. Study on genetic variability, correlation and path analysis with grain yield and yield attributing traits in green gram [*Vigna radiata* (L.) Wilczek]. *Indian J. Agric. Sci.*, 8 (4):1551-1555.

Kang, B.C., Yeam, I. and Jahn, M.M. 2005. Genetics of plant virus resistance. *Annual Review of Phytopathology*. 43: 581–621.

Kannaiyan, S. 1999. *Bioresources technology for sustainable agriculture*. Associated Pub. Co, New Delhi, pp 1–29.

Karthikeyan, A. 2010. Studies on Molecular Tagging of YMV Resistance Gene in Mungbean [*Vigna radiata* (L.) Wilczek]. M.Sc Thesis. Tamil Nadu Agricultural University, Coimbatore, India, pp. 58.

Karthikeyan, A.S., Vanitharani, R., Balaji, V., Anuradha, S., Thillaichidambaram, P., Shivaprasad, P.V., Parameswari, C., Balamani, V., Saminathan, M. and Veluthambi, K. 2004. Analysis of an isolate of Mungbean yellow mosaic virus (MYMV) with a highly variable DNA B component. *Archives of virology*, *149* (8), pp.1643-1652.

Karuppanapandian, T., Karuppudurai, T., Sinha, P. B., Haniya, A. H. and Manoharan, K. 2006. Genetic diversity in green gram [*Vigna radiata* (L.)] landraces analyzed by using random amplified polymorphic DNA (RAPD). *African Journal of Biotechnology*. 5: 1214-1219.

Kennedy, J.B. and Neville, A.M. 1976. Basic statistical methods for engineers and scientists. Crowell.

Kim, S.K., Nair, R.M., Lee, J. and Lee, S.H. 2015. Genomic resources in mungbean for future breeding programs. *Frontiers in plant science*, *6*, p.626.

Kitsanachandee, R., Somta, P., Chatchawankanphanich, O., Akhtar, K.P., Shah, T.M., Nair, R.M., Bains, T.S., Sirari, A., Kaur, L. and Srinives, P. 2013. Detection of quantitative trait loci for mungbean yellow mosaic India virus (MYMIV) resistance in mungbean (*Vigna radiata* (L.) Wilczek) in India and Pakistan. *Breeding science*, 63(4), pp. 367-373.

Kumar, J., Choudhary., A. K., Solanki, R. K. and Pratap, A. 2011. Towards marker-assisted selection in pulses: a review. *Plant Breed* 130: 297-313.

Kumar, S.V., Tan, S.G., Quah, S.C. and Yusoff, K. 2002. Isolation and characterisation of seven tetranucleotide microsatellite loci in mungbean, *Vigna radiata*. *Molecular Ecology notes*. 2: 293 - 295.

Kumhar, S.R and Chaudhary, B.R. 2007. Genetic diversity and variability in Mungbean [*Vigna radiata* (L.)Wilczek*]. J. Plant Genet. Resour*, 20(2): 203-208.

Kundagrami, S., Basak, J., Maiti, S., Kundu, A., Das, B., Ghosh, T.K. and Pal, A. 2009. Agronomic, genetic and molecular characterization of MYMIV tolerant mutant lines of *Vigna mungo*. *International Journal of Plant Breeding and Genetics*. 3(1):1- 10.

Lambrides, C.J., Lawn, R.J., Godwin, I.D., Manners, J. and Imrie, B.C. 2000. Two genetic linkage maps of mungbean using RFLP and RAPD markers. Australian Journal of Agricultural Research. 51: 415 - 425.

Lavanya, G.R., Srivastava, J. and Ranade, S.A. 2008. Molecular assessment of genetic diversity in mung bean germplasm. *Journal of Genetics*, *87*(1), p.65.

Lekhi, P., Gill, R.K. and Kaur, S. 2018. Identification of molecular marker linked to mungbean yellow mosaic virus (MYMV) resistance in *Vigna radiata* (L.) Wilczek. *Electronic Journal of Plant Breeding*, *9*(3), pp. 839-845.

Litt, M. and Luty, J. A. 1989. A hyper variable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *The American Journal of Human Genetics*. 44: 397.

Liu, S., M. Banik, K. Yu, S. J. Park, V. Poysa. and Y. Guan. 2007: Marker-assisted election (MAS) in major cereal and legume crop breeding: current progress and future directions. *Int. J. Plant Breed*. 1, 74-88.

Lush, J. L. 1940. Intra-sire correlation and regression of offspring on dam as a method of estimating heritability of characters. *Record of Proceedings. American Society of Animal Production*, pp.293-301.

Mackill, D. J., Nguyen, H. T. and Zhang, J. 1999. Use of molecular markers in plant improvement programs for rainfed low land rice. *Field Crops Res*. 64,177–185.

Mahalingam, A., Satya, V. K., Manivannan, N., Narayanan, S. L. and Sathya, P. 2018. Inheritance of Mungbean Yellow Mosaic Virus Disease Resistance in Greengram [*Vigna radiata* (L.). Wilczek]. *International Journal of Current Microbiology and Applied Sciences*, 7(1), 880-885.

Makeen, K., Abrahim, G., Jan, A. and Singh, A. K. 2007. Genetic Variability and Correlations Studies on Yield and its Components in Mungbean (*Vigna radiata* (L.) Wilezek). *Journal of Agronomy*, 6: 216-218.

Malathi, V.G. and John, P. 2008. Geminiviruses infecting legumes. Characterization, diagnosis and management of plant viruses. *Studium Press*, Houston, pp.97-123.

Malik, L.A., Sarwar, G. and Ali, Y. 1986. Genetic studies in mungbean (*Vigna radiata* L. Wilczek). Inheritance of tolerance to mungbean yellow mosaic virus and some morphological characters. *Pakistan Journal of Botany.* 18:189-198.

Malli, S. R., Lavanya, G. R and Nikhil, B.S.K. 2018. Genetic variability, heritability and genetic advance in mungbean (*Vigna radiata* L. Wilczek) genotypes. *Green Farming* Vol. 9 (2) : 235-238.

Manivannan, N., Ramoorthi, N and Nadarajan. 1996. Genetic variability in green gram. *Madras Agricultural Journal.* 83: 770-771.

Marimuthu, T., Subramanian, C. L. and Mohan, R. 1981. Assessment of yield losses due to yellow mosaic infection in mungbean. *Pulse Crop News Letter*. 1: 104.

Mariyammal, I., Pandiyan, M., Vanniarajan, C., Kennedy, J.S. and Senthil, N. 2019a. Genetic variability in segregating generations of greengram (*Vigna radiata* L. Wilczek) for quantitative traits. *Electronic Journal of Plant Breeding*, *10*(1), pp.293-296.

Mariyammal, I., Seram, D., Samyuktha, S.M., Karthikeyan, A., Dhasarathan, M., Murukarthick, J., Kennedy, J.S., Malarvizhi, D., Yang, T.J., Pandiyan, M. and Senthil, N. 2019b. QTL mapping in *Vigna radiata*× *Vigna umbellata* population uncovers major genomic regions associated with bruchid resistance. *Molecular Breeding*, *39*(7), p.110.

Marques, C., Brondani, R., Grattapaglia, D. and Sederoff, R. 2002. Conservation and synteny of SSR loci and QTLs for vegetative propagation in four Eucalyptus species. *Theoretical and Applied Genetics* 105, 474 – 478.

Mehandi, S., Mishra, S.P., Tripathi, R.C and Singh, I. P. 2018. Genetic variability, heritability and genetic advance for yield and its related traits in mungbean [*Vigna radiata* (L.) Wilczek] genotypes. *International Journal of Current Microbiology and Applied Sciences*, pp. 3818 - 3824.

Mehta, C. M. 2019. Estimation of variability through genetic parameters and identification of superior pure lines for yield attributing traits in green gram [*Vigna radiata* (L.)]. *Technology*.

Ministry of agriculture and farmers welfare. Government of India. Indiastat. 2017-18.

Mishra, S. P. and Asthana, A. N. 1996. Inheritance of yellow mosaic virus resistance in mungbean (*Vigna radiata* (L.) Wilczek). Recent Advances in Mungbean Research. *Indian Society of Pulses Research, IIPR*, Kanpur, 214–219.

Miyagi, M., Humphry, M., Ma, Z.Y., Lambrides, C.J., Bateson, M and Liu, C.J. 2004. Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). *Theoretical and Applied Genetics*. 110: 151- 156.

Muthuswamy, A., Jamunarani, M. and Ramakrishnan, P. 2019. Genetic Variability, Character Association and Path Analysis Studies in Green Gram (*Vigna radiata* (L.) Wilczek). *Int. J. Curr. Microbiol. App. Sci*, 8(04), pp.1136-1146.

Nand, M.J. and Anuradha, C. 2013. Genetic variability, correlation and path analysis for yield and yield components in mungbean (*Vigna radiata* L. Wilczec) J. *Res. ANGRAU* 41: 31- 39.

Nariani, I. K. 1960. Yellow mosaic of mungbean (*Phaseolous aureus* L.). *Indian Phytopathology*. 13:24-29.

Natarajan, C., Thiyagarajan, K and Rathnaswamy, R. 1988. Association and genetic diversity studies in green gram (*Vigna radiata* (L.) Wilczek). *Madras agric. J*. 75(7-8): 238-245.

Nath, A., Maloo, S.R. and Meena, B.L. 2019. Genetic confirmation of mungbean genotypes (*Vigna radiata* (L.) Wilczek) using molecular markers. *Journal of Food Legumes*, *32*(1), pp.1-8.

Nath, A., Maloo, S.R., Meena, B.L., Devi, A.G. and Tak, S. 2017. Assessment of Genetic Diversity Using ISSR Markers in Green Gram [*Vigna radiata* (L.) Wilczek]. *Int. J. Curr. Microbiol. App. Sci*, *6*(5), pp.1150-1158.

Nawkar. 2009. Identification of sequence polymorphism of resistant gene analogues (RGAs) in Vigna species. M.Sc Thesis. Tamil Nadu Agricultural University, Coimbatore, India. 60p.

Neij, S. and Syakudd, K. 1957. Genetic parameters and environments II: Heritability and genetic correlations in rice plants. *Japan Journal of Genetics*. 32: 235-241.

Nilsson-Ehle, H. 1909. *Kreuzungsuntersuchungen an hafer und weizen* (Vol. 5, No. 2). H. Ohlssons buchdruckerei.

Pandey, M.K., Srivastava, N. and Kole, C. R. 2007. Selection Strategy For Augementation Of Seed Yield in Mungbean (*Vigna radiata* L. Wilczek). *Legume Research*, 30 (4) : 243 – 249.

Pandiyan, M., Subbalakshmi, B and Jebaraj, S. 2006. Genetic variability in greengram. *International Journal of Plant Sciences*. 2006. 1(1): 72-75.

Parent, J.G. and Page, D. 1998. Identification of raspberry cultivars by sequence characterized amplified region DNA analysis. *HortScience*, *33*(1), pp.140-142.

Parimala, N.K., Harinikumar, K.M., Savitramma, D.L., Sritama, K and Shailja, C. 2020. Genetic variability and correlation studies on yield and yield related attributes in mungbean [*Vigna radiata* (L.) Wilczek]. *Mysore Journal of Agricultural Sciences*, 54(1), pp.15-19.

Patel, P., Modha, K., Kapadia, C., Vadodariya, G. and Patel, R. 2018. Validation of DNA Markers Linked to MYMV Resistance in Mungbean (*Vigna radiata* (L). R. Wilczek), *Int. J. Pure App. Biosci.* 6(4): 340-346.

Pavan K, Reddy, P. and Mehta, C. M. 2019. Estimation of variability through genetic parameters and identification of superior pure lines for yield attributing traits in green gram [*Vigna radiata* (L.)]. *Journal of Pharmacognosy and Phytochemistry*.3: 55-61.

Payasi, D. K. 2015. Genetic Variability Analysis for Seed Yield and its Components in Mungbean (*Vigna radiata* L. Wilczek). *International Journal of Plant Breeding and Genetics* 9 (3): 177-188.

Perera, U.I.P., Chandika, K.K.J and Ratnasekera, D. 2017. Genetic variation, character association and evaluation of mungbean genotypes for agronomic and yield components. *Journal of the National Science Foundation of Sri Lanka*, 45(4), pp.347-353.

Pooja, B., Ashok, C., Bhavana, T., Sanjay, K., Ratna, K. and Bhat, K.V. 2019. Utility of single nucleotide polymorphism markers for DNA fingerprinting and genetic diversity analysis in mungbean. *Annals of Agri Bio Research*, *24* (1), pp.7-10.

Procunier, J.D., Gray, M.A., Howes, N.K., Knox, R.E. and Bernier, A.M. 1997. DNA markers linked to a T10 loose smut resistance gene in wheat (*Triticum aestivum* L.). *Genome*, *40* (2), pp.176-179.

Qazi, J., Ilyas, M., Mansoor, S. and Briddon, R. 2007. Legume yellow mosaic viruses: genetically isolated begomoviruses. *Molecular plant pathology*, 8(4): 343–348

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

Raje, R.S. and Rao S.K. 2000. Genetic parameters of variation for yield and its components in mungbean (*Vigna radiata* L. Wilczek) over environments. *Legume Research- An International Journal*, 23(4): 211-216.

Rambabu, E., Anuradha, Ch., Sridhar, V. and Sokka, Reddy. S. 2018. Identification of Molecular Markers Linked to Yellow Mosaic Virus Resistance in Blackgram (*Vigna mungo* (L.) Hepper). *Int.J.Curr.Microbiol.App.Sci* 7(2): 3810-3817.

Rani, A., Kumar, V., Gill, B.S., Rathi, P., Shukla, S., Singh, R.K. and Hussain, S.M. 2017. Linkage mapping of mungbean yellow mosaic India virus (MYMIV) resistance gene in soybean. *Breeding Science*. 67: 95-100.

Rao, C.M., Rao, Y.K. and Reddy, M. 2006. Genetic variability and path analysis in Mungbean. *Legume Res.*, 29 (3): 216-218.

Raturi, A., Singh, S. K., Sharma, V. and Pathak, R. 2014. Genetic variability, heritability, genetic advance and path analysis in mungbean [*Vigna radiata* (L.) Wilczek. *Legume Research*, 38 (2) 2015 : 157-163.

Raturi, A., Singh, S.K., Sharma, V. and Pathak, R. 2015. Genetic variability, heritability, genetic advance and path analysis in mungbean [*Vigna radiata* (L.) Wilczek]. *Legume Research-An International Journal*, *38*(2), pp.157-163.

Reddy, K.R. and Singh, D.P. 1995. Inheritance of resistance to mungbean yellow mosaic virus. *The Madras Agricultural Journal*. 88(2):199201.

Reddy, K.S. 2012. A new mutant for yellow mosaic virus resistance in mungbean (*Vigna radiata* L. Wilczek) variety SML-668 by recurrent Gamma-ray irradiation. Induced Plant Mutation in the Genomics Era. *Food and Agriculture Organization of the United Nation, Rome,* pp. 361-362. 361-362.

Reddy, V.L.N., Reddisekhar, M., Reddy, K.R and Reddy, K.H. 2003. Genetic variability for yield and its components in mungbean, [*Vigna radiata* (L.) Wilczek]. *Legume Research.* 26(4):300-302.1852.

Reddy, K.R., and Singh DP. 1995. Inheritance of resistance to mungbean yellow mosaic virus. *Madras Agric. J*., 88: 199–201.

Sai, C.B., Nagarajan, P., Raveendran, M., Rabindran, R., Bapu, K.J.R. and Senthil N. 2017. Understanding the inheritance of Green gram yellow mosaic virus (MYMV) resistance in Green gram (*Vigna radiata* L. Wilczek). *Molecular Breeding*. 37:63.

Saleem, M., Haris, W.A. and Malik, I.A. 1998. Inheritance of yellow mosaic virus resistance in mungbean. *Pakistan Journal of Phytopathology.* 10: 30-32.

Sandhu, T.S., Brar, J.S., Sandhu, S.S. and Verma, M.M. 1985. Inheritance of resistance to mungbean yellow mosaic virus in green gram. *Journal of Research Punjab Agricultural University*. 22 (1):607-611.

Sarkar, G., Cassady, J., Bottema, C. D. and Sommer, S. S. 1990. Characterization of polymerase chain reaction amplification of specific alleles. *Analytical biochemistry*, *186*(1), 64-68.

Saxena, P., Kamendra, S., Usha, B. and Khanna, V.K. 2009. Identification of ISSR marker for the resistance to yellow mosaic virus in soybean [*Glycine max*. (L). Merrill]. *Pantnagar Journal of Research*. Vol. 7 No. 2 pp. 166-170.

Saxena, R.R., Singh, P.K and Saxena, R. R. 2007. Correlation and path analysis in Mungbean cultivars (*Vigna radiata* (L.) Wilczek). *J. Interacademicia*. 11(2):143-148.

Schafleitner, R., Huang, S.M., Chu, S.H., Yen, J.Y., Lin, C.Y., Yan, M.R., Krishnan, B., Liu, M.S., Lo, H.F., Chen, C.Y. and Long-fang, O.C. 2016. Identification of single nucleotide polymorphism markers associated with resistance to bruchids (*Callosobruchus* spp.) in wild mungbean (*Vigna radiata* var. sublobata) and cultivated V. radiata through genotyping by sequencing and quantitative trait locus analysis. *BMC Plant Biology*, *16*(1), p.159.

Selvi, R., Muthiah, A.R., Manivannan, N., Raveendran, T.S., Manickam, A. and Samiyappan, R. 2006. Tagging of RAPD marker for MYMV resistance in mungbean (*Vigna radiata* (L.) Wilczek). *Asian J Plant Sci*, *5*(2), pp.277-280.

Sharma, S. and Sirohi, A. 2018. Estimation of genetic diversity of Mungbean (*Vigna radiata* L.) Cultivar Using SSR and ISSR Marker Analysis. *Journal of Pharmacognosy and Phytochemistry*; SP1: 207-211.

Shiv, A., Ramtekey, V., Vadodariya, G.D., Modha, K.G. and Patel, R.K. 2017. Genetic variability, heritability and genetic advance in F_3 progenies of mungbean [Vigna radiata (L.) Wilczek]. *International Journal of Current Microbiology and Applied Sciences*, 6(12): 3086-3094.

Shukla, G.P. and Pandya, B.P. 1985. Resistance to yellow mosaic in green gram. *SABRAO Journal of Genetic and Plant Breeding*. 17: 165.

Singh, A. K., Roy, S.D., Kumar, R.R., Biswas, U., Swain, S., Singh, V., Velmurgan, A., Gautam, R.K., Sujatha, T. and Ahmed, S.Z. 2016. ISSR Markers Analysis of Genetic Relationship Between Underutilized Beachpea [*Vigna marina* (Burm.) Merr.], Mungbean & Urdbean landraces of Bay Islands. *Vegetos*, 29, pp.14-21.

Singh, B. B. 2011. Project coordinators report. All India Coordinated Research Project on MULLaRP. Annual Group Meet ; Kanpur: ICAR, Indian Institute of Pulses Research.

Singh, C.M., Pratap, A. and Gupta, S. 2020.Association mapping for *Green gram yellow mosaic India virus* resistance in Green gram (*Vigna radiata* L. Wilczek). *3 Biotech* 10, 33.

Singh, N., Mallick, J., Sagolsem, D., Mandal, N. and Bhattacharyya, S. 2018. Mapping of molecular markers linked with MYMIV and yield attributing traits in mungbean. *Indian J. Genet*, 78(1), pp.118-126.

Singh, R.K. and Chaudhary, B.D. 1977. Biometric methods in quantitative genetics analysis. *Kalyani Publishers*, pp. 304.

Sivasubramanian, S. and Menon, M. 1973. Heterosis and inbreeding depression in rice. *Madras Agric. J*, *60*(7), pp.1139-1140.

Somta, P., Kaga, A., Tomooka, N., Isemura, T., Vaughan, D.A. and Srinives, P. 2008. Mapping of quantitative trait loci for a new source of resistance to bruchids in the wild species Vigna nepalensis Tateishi & Maxted (*Vigna Ceratotropis*). *Theoretical and Applied Genetics*, *117*(4), pp.621-628.

Somta, P., Sommanas, W. and Srinives, P. 2009. Molecular diversity assessment of AVRDC–The World Vegetable Center elite-parental mungbeans. *Breeding Science*, *59*(2), pp.149-157.

Souframanien, J. and Gopalakrishna, T. 2006. ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [*Vigna mungo* (L.) Hepper]. *Plant breeding*, *125* (6), pp.619-622.

Srivastava, R.P. and Ali, M. 2004. Nutritional quality of common pulses. *Bulletin* IIPR/2004/07, *IIPR Publication, Kanpur, India.*

Sudha, M., Anusuya, P., Mahadev, N.G., Karthikeyan, A., Nagarajan, P., Raveendran, M., Senthil, N., Pandiyan, M., Angappan, K. and Balasubramanian, P. 2013. Molecular studies on mungbean (*Vigna radiata* (L.) Wilczek) and ricebean (*Vigna umbellata* (Thunb.)) interspecific hybridisation for Mungbean yellow mosaic virus resistance and development of species-specific SCAR marker for ricebean. *Archives of Phytopathology and Plant Protection*, 46(5), pp.503-517.

Sudha, M., Karthikeyan, A., Anusuya, P., Ganesh, N. M., Pandiyan, M., Senthil, N., Raveendran, N., Nagarajan, P. and Angappan, K. 2013. Inheritance of resistance to mungbean yellow mosaic virus (MYMV) in inter and Intra specific crosses of mungbean (*Vigna radiata* L. Wilczek). *American Journal of Plant Sciences*. 4: 1924-1927.

Swati, D., Das, S.S. and Ghosh, P., 2014. Assessment of molecular genetic diversity in some green gram cultivars as revealed by ISSR analysis. *Advances in Applied Science Research*, *5*(2), pp.93-97.

Talukdar, N., Borah, H.K. and Sarma, R.N. 2020. Genetic Variability of Traits Related to Synchronous Maturity in Greengram [*Vigna radiata* (L.) Wilczek]. *Int. J. Curr. Microbiol. App.* Sci, 9(1), pp.1120-1133.

Thakur, R.P., Patel, P.N. and. Verma, J.P. 1977.Genetical relationships between reactions to bacterial leaf spot, yellow mosaic and cercospora leaf spot diseases in mungbean (*Vigna radiata*). Euphytica 26:765-774.

Thamodhran, G., Geetha, S and Ramalingam, A. 2016. Genetic study in URD bean (*Vigna mungo* (L.) Hepper) for inheritance of mungbean yellow mosaic virus resistance. *International Journal of Agriculture, Environment and Biotechnology*, *9* (1), pp.33-37.

Ullah, H., Khalil, I.H., Badshah, H., Shahwar, D. and Lightfoot, D.A. 2011. Location effect on heritability estimates of yield traits in mungbean derived from F2 populations. *African Journal of Biotechnology*, Vol.10 (83): 19309-19317.

Varma, A. and Malathi, V.G. 2003. Emerging geminivirus problems: A serious threat to crop production *Annals of Applied Biology*, *142*(2), pp.145-164.

Varma, A., Dhar, A.K. and Mandal, B. 1992. Proceedings of mungbean yellow mosaic disease. Asian Vegetable Research and Developmental Centre. 8-27.

Vinoth, R. and Jayamani, P. 2014. Genetic inheritance of resistance to yellow mosaic disease in inter sub-specific cross of blackgram (*Vigna mungo* (L.) Hepper). *Journal of Food Legumes* 27(1): 9-12

Vishalakshi, B., Umakanth, B., Shanbhag, A.P., Ghatak, A., Sathyanarayanan, N., Madhav, M.S., Krishna, G.G. and Yadla, H. 2017. RAPD assisted selection of black gram (*Vigna mungo* L. Hepper) towards the development of multiple disease resistant germplasm. *3 Biotech*, *7*(1), p.1.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van De Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J. and Kuiper, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*. 23: 4407-4414.

Wang, L., Bai, P., Yuan, X., Chen, H., Wang, S., Chen, X. and Cheng, X. 2018. Genetic diversity assessment of a set of introduced mungbean accessions (*Vigna radiata* L.). *The Crop Journal* 6: 207-213.

Wang, X.W., Kaga, A., Tomooka, N. and Vaughan, D.A. 2004. The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. *Theoretical and Applied Genetics*, *109* (2), pp.352-360.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research*, *18*(24), pp.7213-7218.

Windostat. 2011. Windostat Services, Hyderabad, Telangana, India. www.windostat.org.

World pulses day. 2020. (https://pulses.org/world-pulses-day-guidelines-2020)

Xu, R.Q., Tomooka, N., Vaughan, D.A. and Doi, K. 2000. The *Vigna angularis* complex: genetic variation and relationships revealed by RAPD analysis and their implications for in-situ conservation and domestication. *Genetic Resources and Crop Evolution*. 46: 136 -145.

Yadav, C.B., Bhareti, P., Muthamilarasan, M., Mukherjee, M., Khan, Y., Rathi, P. and Prasad, M. 2015. Genome-wide SNP identification and characterization in two

soybean cultivars with contrasting mungbean yellow mosaic India virus disease resistance traits. *PloS one*, 10(4), p.e0123897.

Zuge Sopan, S and Sao Abhinav, T.N.A. 2019. Genetic variability of yield and yield related traits in mungbean (*Vigna radiata* L. Wilczek) genotypes. *Agric Res J*, 56(1), pp.163-165.

APPENDICES

APPENDIX I

EQUIPMENTS USED

- Agarose gel electrophoresis system (Bio-rad)
- Autoclave
- DNA thermal cycler (Eppendorf master cycler gradient and Peltier thermal cycler)
- Freezer of -20ºC and -80ºC (Sanyo biomedical freezer)
- Gel documentation system (Bio-rad)
- Ice maker (Sanyo)
- Magnetic stirrer (Genei)
- Microwave oven (LG)
- Microcentrifuge (Eppendorf)
- Pipetteman (Thermo scientific)
- pH meter (Thermo orion)
- U.V absorbance spectrophotometer (Thermo electronic corporation)
- Nanodrop (Thermo scientific)
- U.V Transilluminator (Vilber Lourmat)
- Vaccum dryer (Thermo electron corporation)
- Vortex mixer (Genei)
- Water bath (Cintex)

APPENDIX II

LIST OF CHEMICALS

- Agarose (Sigma)
- 6X loading dye (Genei)
- Chloroform (Qualigens)
- dNTPs (Deoxy nucleotide triphosphates) (Biogene)
- EDTA (Ethylene Diamino Tetra Acetic acid) (Himedia)
- Ethidium bromide (Sigma)
- Ethyl alcohol (Hayman)
- Isoamyl alcohol (Qualigens)
- Isopropanol (Qualigens)
- NaCl (Sodium chloride) (Qualigens)
- NaOH (Sodiun hydroxide) (Qualigens)
- Phenol (Bangalore Genei)
- Poly vinyl pyrrolidone
- Taq polymerase (Invitrogen)
- Trizma base (Sigma)
- 100 bp ladder (NEB)
- MgCl2 buffer (Jonaki)
- Primers (Sigma)

APPENDIX III

BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

2 % (w/v) CTAB (Nalgene) - 10g

100 Mm Tris HCl, pH 8.0 - 100 ml of 0.5 M Tris HCl (pH 8.0)

20 mM EDTA, pH 8.0 - 20 ml of 0.5 M EDTA (pH 8.0)

1.4 M NaCl - 140 ml of 5 M NaCl

PVP (Sigma) - 200 mg

All the above ingredients except CTAB were added in respective quantities and final volume was made up to 500ml with double distilled water, the solution was autoclaved. The solution was allowed to attain room temperature and 10g of CTAB was dissolved by intense stirring, stored at room temperature.

EDTA (0.5M) 200ml

Weigh 37.22g of EDTA, dissolve in 120ml of distilled water by adding 4g of NaoH pellets. Stir the solution by adding another 25ml of water and allow EDTA to dissolve completely. Then check the pH and try to adjust to 8 by adding 2N NaoH drop by drop. Then make the volume to 200ml

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Equal parts of equilibrated phenol and Chloroform: Isoamyl alcohol (24:1) were mixed and stored at 4^{o}C.

50X TAE Buffer (pH 8.0)

400 mM Tris base

200 mM Glacial acetic acid

10 mM EDTA

Dissolve in appropriate amount of sterile water.

Tris-HCl (1 M)

12.1g of tris base is dissolved in 50 ml if distilled water, then check the pH using litmus paper. If pH is more than 8 then add few drops of HCL and then adjust pH to 8 then make up the volume to 100ml.

5M NaCl (1000ml)

Weigh 292.5g of NaCl and add it to 600ml of distilled water. Keep it on a magnetic stirrer until the salt is completely dissolved and then make up the volume to 1000 ml with distilled water.

APPENDIX IV

Supplementary Table 1. List of primers used in the present study for parental polymorphism.

S.No	Primer name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Annealing Temperature (oC)
1	VR073	GGTAGTTCATTTCGGCCACTT	GGTAGTTCATTTCGGCCACTT	59
2	VR102	CATGTGAGCTACCCTTTCAACA	CAAGGACTGCTATATCCAAGGC	58
3	VR216	TTCCCTGTGTCCTTATATGTCC	GAGGATAGTGAATTTTGAAGGC	58
4	VR303	AGACGAAGAAGAAAACGCAGAC	CCTCACACACAACACAACAGAA	59
5	VR304	GAAGCGAAGAAGCCATAGAAAA	CCTCACACACAACACAACAGAA	58
6	VR323	ATATCAGCCATTGTTGCTTTCC	TTCCCAGTTCAGACAACCAAGT	59
7	VR326	GATGGCTCTGCATTGAAACC	GATCTTCCCAACTTTCCCTCTC	58
8	VR357	GCCCGATGTCCTAGCTTTTAG	CCTCAAAACAATCAGAACTCTCG	59
9	VR361	CTTGGACTTCGTCTCTGCG	CAAAACAACCAACGCCATTAC	59
10	MB 7	CTTGCTTGCGAGGATGAC	TCCAGTGCAGCAGATTGA	57
11	MB 13	GCAGCAACAACAGCAACA	GCAGGTTTTGTGGCTCAG	57
12	MB 14	TGGAATTTGGAAGGAAGGA	GATGCAGGTGTTTGGGAG	57
13	MB 17	ACCTGCAAGTTGGCAAGA	TATGTGCACGCATGGAAG	57
14	MB 77	GGAGAGGAAGGAACAGGG	GGCAGAGCATAAACATGGC	57
15	MB 87	TCCCTTGTGGGAGATCCT	CTTTGCCACACTCCTTGC	57
16	MB 91	GAGGCCAATCCCATAACTTT	AGCACCACATCAGAGATTCC	58
17	VR 1	AGCCCTTCGTGCTAGGAAAT	CCCTACCGGTTGGTTGGT	59
18	VR 2	CGCCCCTCTAGGTTGGTTGG	GGGAAAGACGAAGGGTAGAA	59
19	VR 3	GCCCCCCTTAGGTTGGTTGG	CCTTGTATTTGGATTCACAAGA	56
20	VR 4	TGGTTGGTTGGTTCACAAGA	CACGGGTTCTGTCTCCAATA	58
21	VR 5	TCACAAAGGGAGGGAAGAGA	CCCCAGGTTTGGTTGGTTGGA	59
22	VR 9	TGACGGAGAGAGAGAGAGAGAG	TGCTTCCTTTTGTCTGAGTTAGAA	57
23	VR006	CCTTCTATCTCATGTTCACCGTC	TGGAATAGGGACAAAATGGACT	59
24	VR029	GAAAGAAGCCAAACAAAACAGG	TGGCAGAGAAGGTAAATAAGGG	59
25	VR040	TGACAACATGGGAAGAAGAAGA	ACACCAACACAAAAGCAAACAC	58

26	VR044	CCCATGAAGGTATGAGACAACA	GACTGAGAAAGAGAGAGAAGCATTT	59
27	VR062	CGAAGACGAAATCTGAAGACAA	TTACTTCTCCCAGCACTCCAAT	58
28	VR070	TGATTGTTGGAGAGTGCTCATT	CAATGTAGTTGATCCATCCGAA	59
29	VR078	CATGTGGCAACGCAGAAG	TCAACTTATTCCTCCTTTCTCTCAC	58
30	VR084	GAGCCACTTTGCCATATTTCT	ATTCTCCATTGTTCTCGTTCTC	57
31	VR086	GAGATCCTCCTACGGATTTGC	TTTCCTTCTCCAATTCTTGCTC	59
32	VR095	GAAATGGGAGTTCAAAGAGGAA	TGGAGAAGTCTGGAAGAGAACC	58
33	VR099	ATACTTCGATCCGACCACTAGG	CAAAGACAGGAGGAGAACAAGG	58
34	VR108	GCTCCAACACTCACTCACAAAC	CAGAAATGCAGGAAAAGAGAGG	59
35	VR111	TGCATCTTTATTGAGTTCCGTG	GTTTTGGGGTGAATGTTGGATA	59
36	VR133	GAAGTGGCGGAAGATTGATAAG	GGTAGATGGAAGGTAGAGGAATGA	59
37	VR135	GCCCAGATTTGTTCATCCTAGA	ACTGTTTTGAGTGGGGAAAAGA	59
38	VR140	GGTGTTGTTGTTGAGGAATGAA	AACATTGAGGACCCACATATCC	59
39	VR147	CCATGTGTGTGAATGTGAGTGA	CCTTTGATTTTGTGGGATGTGT	58
40	VR148	CCGTTGTTGTTGCTGTTGTG	GAGCTTGCTAACCCTCTCCAAT	59
41	VR153	AATTGTGAAGCAACAGAAAGCC	AGAAATAGGCAGGCAGTTTTCA	59
42	VR155	AAGATCACACACAACCAACCC	AATTAGTTCCACAGGCCAGATT	58
43	VR163	AGGAGAAATTGTTGTTGTTCGG	GTGTTGATTGTTAGGGAGGGAG	59
44	VR169	GGAAGATAGCGGAGATGAAGAG	CACCATACACCATAACATTCCTG	58
45	VR188	ATACAAGGGCAGGTGTAGCATC	CAGAAAACTTCATCCCCAGCTA	59
46	VR198	AAGAAGAATGCGAGAAAGAAGC	GTCCTAGAAGTTAGGGTTTGTGATT	58
47	VR200	TGGGAAATAAAGAAAGCGTAGG	CTCTTCTCCTTTGCCTCTACAAA	58
48	VR212	AAACCAAAACGTAAGATCAGGG	ATAGAAAGAAGTTGGCGCAGAA	58
49	VR222	TCTCTTCTCTCTTCTCTCTTCTTCTTC	TTGTGTCTGAGGCTATGTTGGT	57
50	VR223	GCGTGATCGAGGCAGACTAT	GTGGGTAGCTCGGTAATAGCAC	59
51	CEDG 073	CCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC	65.4
52	CEDG074	CGAGTGAATGGAAGGGAGTC	ATTCTTACAGCACGGACCAC	63.5
53	CEDG075	GCGACCTCGAAAATGGTGGTTT	TCACCAACTCACTCGCTCACTG	67.3
54	CEDG076	GGTGGTTTACTTACTGGCATTT	GGTCTATTCCACCATCTATCAA	58.5
55	CEDG077	ATCCCGTGACCCTTCTTCCT	GCTCAAGCGAAAACCCAGCA	65.1
56	CEDG086	GAGTTTACAACAGATGGGGCTAA	AGGTCTTGATTGACTTTCTGGGT	63.2
57	CEDG91	CTGGTGGAACAAAGCAAAAGAGT	TGCGTCTTGGTGCAAAGAAGAAA	65.4
58	CEDG92	TCTTTTGGTTGTAGCAGGATGAAC	TACAAGTGATATGCAACGGTTAGG	64.2

59	CEDG93	AAAACCCATGTAAAAGTTCA	CAATCCATTCCCTTCTTAAT	56.7
60	CEDG96	TTACGAAACTGTGGCCTTCAT	TGAACAAAGATGACTTCGGTG	62.3
61	CEDG97	GTAAGCCGCATCCATAATTCCA	TGCGAAAGAGCCGTTAGTAGAA	64.4
62	CEDG98	AAAGGAGTAGAAGGTGCATA	ACAAAATTGGTTGACTCACC	57.6
63	CEDG99	TGGGTGAGCATGGATGTGGA	GGTTCAAGGTGGAAGGCAGA	68.4
64	CEDG107	GAAGTTGACCTTCAATGGAGAAAA	TTGTAGCGTAAAATTAATCCACGC	64.1
65	CEDG111	TGGAAGTTTCCAAGAGGGTTTTC	TCTCACCACCTTTTACCTTCTCA	64.4
66	CEDG113	ATGGCGTTGGAGTTATAGACG	GTTTGACCCTACTGCGTTGTT	62.6
67	CEDG115	GGCTCATTGTACCACTGGATAT	A T G C C T C C T T T C A G G T G A T T G T	62.3
68	CEDG116	TTGTATCGAAACGACGACGCAGAT	AACATCAACTCCAGTCTCACCAAA	65.7
69	CEDG117	GTACACTTCCACTAATCCAAAATT	T G G T A C C T T C C T T A T C T G A A A T T A	58.7
70	CEDG118	AACCCAACCAACCCTTGTGGTAAG	G C T G G A A T C A T A A T A C C G C C T T G T	68.6
71	CEDG121	CTTTCAAAATAATGTTGAGGCATA	C A A T A C A T A A A T A A C C T T T T C T G C	58.4
72	CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC	63.2
73	CEDG128	CTGCCAAAGATGGACAACTTGGAC	GCCAACCATCATCACAGTGC	68.4
74	CEDG130	GTTGCGTGGATGATCCTACTTCAC	C T A A C A C A A G G A G A T A G T G C C A C	66.3
75	CEDG132	GGGTGTAATCCGTCAGAGGC	C T T C C C C C T C T T C C G T T C T C	65.2
76	CEDG136	GTTCCAAGTCTCCAATCCGTAC	C A C T T C A C T A G A A C T G G T T C A G	63.4
77	CEDG139	CAAACTTCCGATCGAAAGCGCTTG	G T T T C T C C T C A A T C T C A A G C T C C G	70.2
78	CEDG141	CCAGGCATCCATGATGACC	G A A G T T G T T G G T A A T G G T T G C C T C	66.4
79	CEDG146	GGTGATCGGATTTCAGAG	G G A G A A G A G A A T A G A G A C G	56.2
80	CEDG147	CTCCGTCGAAGAATTGGTTGAC	G C A A A A A T G T G G C G T T T G G T T G C	68.4
81	CEDG150	GAAGGGAATGAAAATGAAACCC	G T T C A A T C C C A T T C A G T C T C C	64.6
82	CEDG153	CAAGTATCTACCAACACAACTG	CAATCGAGAATCTTGCATAAG	57.8
83	CEDG154	GTCCTTGTTTTCCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG	66.4
84	CEDG156	CGCGTATTGGTGACTAGGTATG	CTTAGTGTTGGGTTGGTCGTAAGG	65.3
85	CEDG166	GGTACAACATTCTTCTATTTG	GGCTTATGAGTTTATCTTATC	51.5
86	CEDG168	CTGCTTGGTGTTGAAGCTTC	CATTCTACATTCCAGACCTGC	62.4
87	CEDG171	CTTGAGAACCAACTCGAACTTC	GGGAAATCGAAGAGGGACAG	61.1
88	CEDG172	GCTGACGTAGGTGACAACC	CGGCTTGTGCTTCATTGTCTG	65.2
89	CEDG174	GAGGGATCTCCAAAGTTCAACGG	GAAGGCTCCGAAGTTGAAGGTTG	68.6
90	CEDG176	GGTAACACGGGTTCAGATGCC	CAAGGTGGAGGACAAGATCGG	67.2
91	CEDG180	GGTATGGAGCAAAACAATC	GTGCGTGAAGTTGTCTTATC	57.4

92	CEDG184	GAGTAGCCAAAGAACTTTGTAG	CTTCTGATTCTTCACGACCC	60.1
93	CEDG185	CACGAACCGGTTACAGAGCG	CATCGCATTCCCTTCGCTGC	60
94	CEDG186	GGATGGGAGTAAGAAG	GCATGGCATGATGACTTG	55.6
95	CEDG191	CAATAAGCAATCTGTGGAGAG	CTGCAGGAAACTTGGAATTGC	52.3
96	CEDG198	CAAGGAAGATGGAGAGAATC	CCTTCTAAGAACAGTGACATG	56.1
97	CEDG199	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG	68.4
98	CEDG200	GAACCCACTTCTGAAGTTC	GAACAACCTCTGCAGTAG	54.2
99	CEDG201	GAGTGTGCATATGTGAGAG	CAAGTCTAATCTCTGACTCC	53.8
100	CEDG202	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	57.2
101	VR225	CAGCAACAGAACTACAATCCCA	CGGCAATCCTCCTATATTCATT	56
102	VR226	GCTTCTCTTTCTTGCATTCATC	GACTAGGCGCTGGGAAAA	57
103	VR238	ATTCTCTGCCTGCCATTTT	ACGATTGTGTTTGTTGATGC	58
104	VR244	GCTCTAAAACACGAAAGGGGT	TCATGGTGGAAGAAAAGCAA	58
105	VR248	ATTCGGTTCCAGTGCTAAGAAG	AGCAGAAGTGCTTATCCCAGAG	58
106	VR256	GCTGTGGTGTATTTACCTTGGG	ATCCTCCGGTCATTATCTTGTG	58
107	VR257	AGGAAGATGAGGGGAAAGTGA	TATTCTATACCTGCCACCCCAC	59
108	VR274	ATTCGGGTAAAGTTCTGCATCT	AATGTTCACACACGTCATAGCA	59
109	VR293	GTGGCTCACAAGGTAGTGCTAA	GAGAGAAACAACCAACCAAAGG	59
110	CEDC036	GAAAAAGTAATCAAAGCTGGG	CTTTACTAACTCCAACTCCTAACTC	58
111	CEDG268	CATCTCCCTGAAACTTGTG	GCTATCAATCGAGTGCAG	56.7
112	CEDG022	AGGAATGTGAGATTTG	AATCGCTTCAAGGTCAAGCC	62.3
113	CEDG133	GCATACATAATGTGGTGAGATG	GTCTCGTGCCTTTCACAC	64.4
114	CEDG225	GAGGAAGTGTTGCAGCACC	GTAGACTCTGCAGAGGGATG	57.6
115	CEDG284	GGTGCTAACGTTGGAAACTGAG	CACTCCATTCTGAGGATCAATCC	68.4
116	CEDG014	GCTTGCATCACCCATGATTC	AAGTGATACGGTCTGGTTCC	64.1
117	CEDG245	GATAGAGCTTAAACCCTC	CTTTTGATGACAAATGCCC	64.4
118	CEDG112	GCAATATTCGCATTATTCATTCA	GTGTTTCAAAGCACTATACTTAA	62.6
119	CEDG011	GTCCGACTTTATGTGTGGAG	TTTCTAGTTCCAGCCCCGAC	62.3
120	CEDG104	TATGGCCCGAGCAAACCTTG	CCGTTCGGTCTTCGGTTGAA	65.7
121	CEDG275	CACACTTCAAGGAACCTCAAG	GTAGGCAACCTCCATTGAAC	58.7
122	CEDG271	GCACTAAAGTTAGACGTGGTTC	CACTCCCACTGCCAAACAAGG	68.6
123	CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACCC	58.4
124	CEDG016	TTAGTTCACTCCGCTTGGTC	CACGTCATCCTCTGTTAGAC	63.2

125	CEDG228	GTCGTTTCCGGAAACTGTTC	GATCCGAACCTCTTTCTGC	68.4
126	CEDG173	GATAAGAGATGCATCACTC	CTTCTCTTCCATCACATCTG	66.3
127	CEDG143	GATGAACTCGTCTCGCTCATCG	CTGGACGCGTCTACTCAGAC	65.2
128	CEDG231	GAGGCTACGAGAAGAGAGTG	CGCCAACCATCATCACAGTG	63.4
129	CEDG267	GTGCTTGCATATGCAATGTCC	GGCATCTAAGAAATCCATGTCG	70.2
130	CEDG256	CCTTCACTATGTCCACATCC	GTTGTTGGTCGGTTCAGAG	66.4
131	CEDG254	CGATGTCTCTTGCTTCAAGG	GTGAAGGACTAGCCAAGTTTG	56.2
132	CEDG065	GGAATTTTGAGAACGGATTTGC	CCACCGACCACGGCCTTC	68.4
133	CEDG165	GCTCTGTCAGTTCCCACTAC	GGTCCTGAACCCAGATGAAC	64.6
134	CEDG055	CAAACACTTTTGTAACTCCC	GCTTCTAACCTTGATCCTTC	57.8
135	CEDG125	TGGAATATACTGTTTAATAGAG	AGATTAATTTGATCACTCATTC	66.4
136	CEDG251	ATATCTCAAAACCCTTCCTG	CCTCAATAACAATGATACGAC	65.3
137	CEDG257	GACTACTCTCAAGACCAAAG	GATGGTTGTAGATAACACTCC	51.5
138	CEDG265	GTAAAACAAACACACAGGAC	GCTCTCAACGAGAATGAAC	62.4
139	CEDG270	GTGCGTCACTAGTCCATTGC	GCAGAAGATTGAATCCTGGACC	61.1
140	CEDG286	CGAGCAGAACACTGATCATG	CCTCTTAGAGGTCATTGCTC	65.2
141	CEDG080	CACGTTGGAGGAAGTGACGC	CATCGCCACCACAGAACCA	68.6
142	CEDG134	CTCCGTGTTGAAACAATGACG	GGTCTTTCTGATCTACGAACTTG	67.2
143	CEDG243	GACAACTCATCCATTCTTGAG	CCTATGGATAGTGATACAGC	57.4
144	CEDG273	GTTTAGCTTCTTCTGCTG	CCAAACTGTCAATATCTGC	60.1
145	CEDG042	CACAGTGGTTTGGGCAACAG	TCAGAGGTTCCCATTTCCCG	60
146	CEDG295	CAAAGGTTAGATCCAACATCG	GGTTAGTCATCAACAACTCC	55.6
147	CEDG010	TGGGCTACCAACTTTTCCTC	TGAGCGACATCTTCAACACG	52.3
148	CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	56.1
149	CEDC027	ACTGGATGAGGGTTTAGTGCG	CTGTCTTGTCTTGTGGGTTCGTTC	68.4
150	CEDC036	GAAAAAGTAATCAAAGCTGGG	CTTTACTAACTCCAACTCCTAACTC	64.1
151	CEDG103	CACCGCTGTCCATTGAAGTATTA	TCTTAGAGTGCCCTGTGAGATTG	64.4
152	CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	62.6
153	CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	62.3
154	CEDG232	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG	65.7
155	CEDG253	CACTTCCATGATGACTCACC	CACCCTTCTTTATCCTCTTCG	58.7
156	CEDG294	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC	68.6
157	CEDG007	GAAGTTGACACTCATCCACC	GTGCAGCCACTACATGAATG	58.4

158	CEDG019	TATAGAGAGGCGAGAAAGGG	AGGGAAACTCAGAACACGTG	63.2
159	CEDG023	GCTCTCCATGAATGGAGTTG	TCATTCATTCACCCCTCC	68.4
160	CEDG025	TAGTCAACCGTTACTATGCC	CGAGAAAAATGAATCTCCCC	66.3
161	CEDG052	CAAACCCTTAAGCTAGGGCTG	GGCAAGTGGTGCGCTCCTC	65.2
162	CEDG057	GGGTGATCTTCCCCAAAATCG	GACAAATTCTCTGATGCCATTCC	63.4
163	CEDG069	GGTGGAGTCGTAGTGGTTCGGTG	CGGACCACCTTTACCGGACC	70.2
164	CEDG082	CACTCAAATAGGATTTGGTTGC	ACAATGTTGCATATCCCTTTCC	66.4
165	CEDG095	TAAATAGGCACCTCCTCAGCGTC	TACCTGGAGTAGTAGGGCTTGCA	56.2
166	CEDG102	GCCAAGGTGAACGGTGGTG	GAGCGAGAATGGCGGAAGG	68.4
167	CEDG105	AAACGGAACGGGCTGGAGG	TGTGACGTGGCGAGGGATG	64.6
168	CEDG106	GCCTTTGACTCTGACCCTCTT	AGCCTCCTCAGGGATGACTTT	57.8
169	CEDG109	TATGCCATGTCAAGTCACCATTA	CCTAACTCCAAAGAATCCCATCA	66.4
170	CEDG126	GGCATGAATGATTTTGACATGG	GTTCAATACGTGATTTTTGACATC	65.3
171	CEDG129	GCTTAGCAAGGTGAGAGCTTTC	GAACAGCTTCCCATGGTTGC	51.5
172	CEDG137	GGTAATCATTTCCAAACAAG	GTGAAGAATTGATTAGGTTG	62.4
173	CEDG138	CATTCTGATGAAAAGATCAAGG	CAATGTAACAGACTCACTGG	68.4
174	CEDG170	CTTCTAAGGTTGGAACAGCTTC	GGAAGCTTAAGAAGGTGAGAGC	64.1
175	CEDG193	GTGTGAAACATGTAGCACGGTG	GGTTCTCTCTCTCCCTCTC	64.4
176	CEDG196	GGTGTAGGTTATGGCTGAAACC	CCCACGTTCTTCTATACTCTTCC	62.6
177	CEDG209	CAGTGAAATCGTTAGGAGTTCAG	CTTCATACTCCTTGGCTATTCTG	62.3
178	CEDG211	GAGTGTGCATATGTGAGAG	CAAGTCTAATCTCTGACTCC	65.7
179	CEDG217	CGACGCAGCTGAATCAAATTCACG	CAAGCAACGGAGTAGAATGGAACG	58.7
180	CEDG218	CTTGCACCACAGTCTCCTTG	CCAATTGGAGGGTTGGTGTC	68.6
181	CEDG220	GGTATTGAAGTCACATGGTCC	GGTTGTTATCTTTGTGCACTCC	58.4
182	CEDG221	CACCCATCACATTTCCTC	CACTACACACACACGTAG	63.2
183	CEDG222	CCATGCACATGCCTGAGGAC	GAGGAGAACTGGGTGACAGC	68.4
184	CEDG223	GCAGAGCAGAGATTTGAG	GGTATAGTCCCTTAAGTGTC	66.3
185	CEDG224	GGTATGTGAAGTGAATGGCTG	CTCACTCTCACTCCAGTAC	65.2