Identifying Donors for Gall midge Resistance from Traditional Rice Varieties by Functional Markers

by

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(2014-11-122)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM- 695 522
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2016

DECLARATION

I, hereby declare that this thesis entitled **"Identifying Donors for Gall midge Resistance from Traditional Rice Varieties by Functional Markers"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to....

Parents

Teachers

God

Nation

ACKNOWLEDGMENT

THANK YOU is a miraculous eight letter word that can make someone's heart go over whelmed. Right from birth to grave we are indebted to others in one way or other, but rarely get a chance to acknowledge. Let me take this opportunity to present my token of love and respect to those who directly or indirectly became a reason for my smile and peace.

I do love surprises and it was such a wonderful surprise that I got ***Dr. V. G. Jayalekshmy*** *as my major advisor. I extend my sincere gratitude for providing excellent guidance, care and an excellent atmosphere for doing my research. Above all she was a guide, mentor, criticizer and a mother. It was a beautiful surprise she gave me with a proposal comprising with* ***Rice-*** *the first crop I learned and* ***Gall midge*** *–the first pest I was taught. In fact, I could not have imagined having any better advisor. Thank you mam for believing in me.*

I express my deepest gratitude and veneration to the esteemed members of my Advisory committee, ***Dr. Leena Kumary S,*** *Professor and Head, Division of Genetics and Plant Breeding, RRS, Moncompu for her peerless advice, valuable suggestions and meticulous guidance throughout the course of my investigation. I have no words to appreciate your passion to your profession. Above all, you have been a great source of inspiration to me.*

A special bunch of thanks is presented to ***Dr. Shanas S,*** *Assistant Professor, Division of Agricultural Entomology, RRS, Moncompu, for his patience listening, constructive criticism, constant encouragement and frequent updates on entomological wonders which helped me to pursue research with total commitment. Words will not be sufficient to express my gratitude towards you sir.*

I am grateful to ***Dr. P. Manju,*** *Professor and Head, Department of Plant Breeding and Genetics, for her ceaseless kind help and guidance, constructive criticism, timely advice and for facilitating me to complete the thesis in an orderly manner.*

I owe special debt of thanks to ***Dr. P. Maya Devi,*** *Professor (Rtd.) Department of Plant Breeding and Genetics, for her keen interest, for valuable suggestions, timely advice and personal care given throughout my research work.*

I would also like to express my heartfelt thanks to faculty members ***Dr. S. Radha Devi, Dr. D. Wilson, Dr. Sunny K. Oommen*** *(Rtd. Professors),* ***Dr. Arya K, Dr. Mareen Abraham, Dr. Lekha Rani, Mrs. Seeja*** *and* ***Dr. Beena Thomas*** *Department of Plant*

Breeding and Genetics, for their constant encouragement and valuable suggestions rendered throughout my research work.

I do acknowledge the guidance and hope relayed on me by ***Dr. H. C. Lohitaswa,*** *(Prof and Head)* ***Dr. P. Mahadevu*** *(Professor) Dept. Plant Breeding and Genetics and* ***Mr. Maruthi J. B*** *(PhD scholar), University of Agricultural Sciences, Bangalore.*

I am immensely indebted and owe my due regard to ***Mr. Ramaling Hundekar*** *(Ph D. Scholar) and* ***Ms. Anju Krishnan*** *(Research Assistant, RRS Moncompu) for their guidance, valuable suggestions, persistent encouragement and support beyond words that have made me capable of completing my research work.*

I express my sincere thanks to all the nonteaching ***staff members****, Department of Plant Breeding and Genetics and Rice Research Station, Moncompu, for the timely help they offered in completion of my research work.*

Like all, my parents do believe that their kid has got some kind of superpowers, keeping up their belief is my ever ending challenge. I feel very fortunate and lucky to have the ceaseless encouragement, Support, blessing and love from ***my family-Achan, Amma, Mummy and Aswani****.*

I may fall short of words if I tend to acknowledge the helps rendered to me by ***Bharath K, Ambareesh Reddy, Lakshmi Krishna, Athul V, Joel Joy, Aju Aniyan, Geetha B V, Henry Nickolas and Sindya*** *you all have been the torch bearers in the darkest path of life. Also, Inexplicable is my sense of affection to* ***Keerthishree M, Vidya K M, Ishrath P K, Neethu Chandra C, Rajib Das, Anju Padmanabhan, Reshma Gopi, Jeena George, Nikitha J, Saranya V S, Satish Kumar, Harikrishnan P J, Shalini K R, Siddesh N K, Sini, Ramya, Sumina, Liji, Prinita, Akhila*** *for being there when I needed them the most.*

Hey, my woes and foes, it's your grims on my gladness made me to fight against all odds. I do owe a thank you ;).

Finally I owe a High-five to ***lord almighty*** *for the quick replies to my prayers. It is your benevolence that helped me in accomplishment of this thesis. You were amazing buddy. Tight hugs.*

Everyone is not mentioned...but no one is forgotten.... ***THANK YOU***

ASHA A NAIR

CONTENTS

Sl. No.	CHAPTER	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	3
3	MATERIALS AND METHODS	18
4	RESULTS	26
5	DISCUSSION	36
6	SUMMARY	48
7	REFERENCES	51
	ABSTRACT	63

LIST OF TABLES

Table No.	Title	Between pages
1	Reported gall midge biotypes and their region of occurrence.	10-11
2	List of varieties used for the study.	24
3	Sequence, Annealing temperature and other details of the selected primers.	25
4	Allele size of resistant gene sources reported for the primers under study	25
5	0-9 Scoring scale of Standard Evaluation System in Rice (SES)	25
6	Quality and Quantity of DNA of rice varieties used in the study	28-29
7	Presence of various genes in selected varieties	30
8	Selected varieties and gall infestation percentage observed at 30 DAT & 50 DAT.	32-33
9	Gall infestation scoring scale and reaction showed by the traditional varieties under study.	34
10	Classification of the varieties based on the overall reaction to rice gall midge attack	35
11	Varieties with one gene of resistance for gall midge.	44
12	Varieties with two genes of resistance for gall midge	45
13	Varieties with three genes of resistance for gall midge	45
14	Varieties with four genes of resistance for gall midge	46

LIST OF FIGURES

Fig. No.	Title	Between pages
1	Field layout for screening for gall midge resistance in rice	21-23
2	Map position of SSR marker RM444 on rice chromosome flanking gene *Gm1*	36-38
3	Map position showing the location of candidate gene LRR flanking the gall midge resistance gene *Gm4*	39-41
4	Map positions of gall midge resistance genes *Gm1, Gm2, Gm4* and *Gm8* on respective chromosomes with reported markers and their physical locations	40-49
5	Percentage gall infestation at 30 DAT and 50 DAT	43-44
6	Comparison of gall infestation per cent in varieties with single gene *Gm1, Gm2, Gm4* and *Gm8.*	43-44
7	Trend of per cent gall infestation at 30 and 50 DAT	43-44
8	Treatment comparison of varieties under field screening	45-46
9	Comparison of gall infestation per cent in varieties with two genes for resistance	45-46

LIST OF PLATES

Plate No.	Title	Between pages
1a & b	Amplification pattern of *Gm1* linked SSR marker RM444 in 16 varieties and two check varieties.	26-27
	Amplification pattern of *Gm1* linked SSR marker RM444 in 16 varieties and two check varieties	26-27
2a & b	Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PF10	29-30
	Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PF10	29-30
3a & b	Amplification pattern of genomic DNA of 12 varieties and two check varieties using the primer LRR del.	29-30
	Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer LRR del.	29-30
4a & b	Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PRP	29-30
	Amplification pattern of genomic DNA of 12 varieties and two check varieties using the primer PRP	29-30
5,6	General view of Nursery and experimentation field view	31-30
7	General view of silver shoot or gall	31-30
8	Life cycle of gall midge observed from the plot	31-30
9	Intensity of gall midge attack from filed.	35-36

LIST OF ABBREVIATIONS

A	Absorption
AICRP	All India Coordinated Rice Improvement Programme
et al.	And others
Avr	Avirulence gene
Bp	Base Pairs
cM	Centimorgan
CD	Critical Difference
DAT	Days after Transplantation
^{0}C	Degree Celsius
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
Fig.	Figure
F:	Forward
Gm	Gall midge
GMB	Gall midge biotype
G	Gram
Ha	Hectare
Hrs	Hours
HR	Hypersensitive Reaction
Kb	Kilo base

MAS	Marker Assisted Selection
µM	Micro molar
µl	Microlitre
mM	Mill molar
Ml	Millilitre
Mm	Millimetre
viz.	Namely
Ng	Nanogram
O.	Orseolia
PCR	Polymerase Chain Reaction
RBD	Randomized Block design
R	Resistance gene
R:	Reverse
Rpm	Rotations per Minute
SCAR	Sequenc Characterized Amplified Regions
SSR	Simple Sequence Repeats
SDS	Sodium Dodecyl Sulphate
Taq	*Thermus aquaticus*
TE	Tris EDTA buffer
UV	Ultra Violet
V	Volt

Introduction

1. INTRODUCTION

Rice (*Oryza sativa*) is botanically a caryoptic seed of a moncot grass. It is a cereal of global importance with more than half the world population relying on it as a major source of food energy. The green lush rice fields cover about 164 million hectares of world area spanning 114 countries, producing nearly 750 million tonnes annually (FAO, 2014). It meets the calorific requirement of millions of people which accounts to nearly 20 per cent of the world's dietary energy supply. More than 90 per cent of the world's rice is produced and consumed in Asia, where it forms an integral part of culture and tradition. It is a crop which can be grown in wide range of climatic arrays ranging from wettest lowlands to driest deserts, even below sea levels. The slogan "Rice is life" is most appropriate for India as this crop plays a vital role in our national food security and is a means of livelihood for millions of rural household (Yadav, 2007).

Right from plot to plate, rice crop is exposed to various biotic as well as abiotic stresses. Of the various biotic stresses, blast and bacterial blight, brown plant hopper, yellow stem borer, gall midge etc. are considered as the major causes of severe yield loss in rice. Rice gall midge (*Orseolia oryzae)*, is an endemic dipteran pest of rice, causes an annual loss of about US$80 million (Krishnaiah and Varma, 2011). The larvae of this pest feeds on fertile tillers converting them into 'onion shoot' like galls rendering the tiller sterile and causes yield loss (Hidaka, 1974). Damage is most prominent at vegetative stage of crop, during the wet season.

To reduce this biotic stress, especially in the rice cultivating coastal states of India including Kerala, Andhra Pradesh, Karnataka, Manipur, Chattisgrah etc. deployment of resistant cultivars is considered to be an effective approach. The emergence of more virulent new insect biotypes have always lead to breaking down of the available host plant resistance. This calls for new breeding strategies. Kerala is a state known to harbor rich diversity of rice cultivars, especially the traditional land

races. Exploitation of this diversity to trace the gene donors against different biotypes of gall midge is highly essential for attaining sustenance in rice cultivation of Kerala.

So far 11 gall midge resistance genes have been reported and several traditional landraces were traced out as gene donors for many of these genes.

A clear understanding of allelic relationship of the gall midge and host resistance helps in identifying the different sources of resistance. Identifying different sources of resistance offer us dual advantage of pyramiding the genes for resistance as well as developing varieties with wider adaptability. Crop improvement programs are not complete without the screening and selection from the traditional germplasm sources.

Use of molecular markers in identifying and tracking genes of interest is a revolutionary leap in crop improvement programmes. Molecular markers are nucleotide sequences used to highlight the genes of interest. Apart from their use in marker assisted breeding program, these molecular markers offer a great opportunity in understanding the relationship between resistance genes and the origin and mechanism of resistance (Hartl *et al.*, 1995). Molecular markers do support classical breeding in crop plants by saving time and labour. Marker assisted selection offer great opportunity for improved efficiency and effectiveness in selecting plant genotypes with desired combinations of traits (Singh, 2012).

With this background, this project is proposed to explore the rice germplasm of Kerala with the help of functional markers, with the following specific objectives,

- Identifying the traditional rice varieties with gall midge resistance genes (*Gm1, Gm2, Gm4 and Gm8*) using associated functional markers.
- Field scoring of the identified varieties under pest stress conditions.

Review of Literature

2. REVIEW OF LITERTAURE

The literature survey conducted to collect the reports of related studies pertaining to the project investigation on "Identifying donors of gall midge resistance from traditional rice varieties by functional markers" are presented in this chapter. The relevant information are reviewed here under specific sub headings.

2.1 RICE-AS A GLOBAL STAPLE

Rice is one among the three globally important cereals, on which human population largely subsists. More than half of the world population rely on rice as their energy source, thus ranking it as an important plant on planet earth (Shimamoto, 1995 and Goff, 1999).

It is the staple food crop grown extensively all over the world which provides 21 per cent of global human per capita energy and 15 per cent per capita protein and hence, occupy a pivotal position in the food security system. Rice is grown in 114 countries across the world on an area about 164 million hectares with annual production of over 750 million tonnes (FAO, 2014).

In Indian agricultural scenario, rice is the staple food for more than 60 per cent of the population. It provides about 29.40 per cent of total calories capita^{-1} day^{-1} in Asian countries (FAO, 2006). To meet the demand of increasing population and to maintain the self-sufficiency, the present production level needs to be increased up to 114 million tonnes by 2035 (Singh *et al.*, 2015).

In India it occupies an area of 38.35 mha with 103.61 mt production and 2.3 t/ha of productivity (Anon., 2016). However rice production is affected by various biotic and abiotic stresses, of which insect pest alone cause about 25 per cent losses accounting to Rs.240, 138 million (Dhaliwal *et al.*, 2007).

Rice landraces are known to possess high genetic diversity on specific traits such as disease resistance, environmental tolerance and nutritional quality which are often used in crop improvement (Prakash *et al.*, 2005).

Kerala is known for such rich genetic diversity of the traditional rice varieties grown in various seasons and in different agro-climatic conditions. (Kumari, 2010).

2.2 BIOECOLOGY OF THE PEST-RICE GALL MIDGE *Orseolia oryzae.*

Gall midge was first reported as an unidentified pest from rice in India by Riley in 1881. Later it was called as *Cecidomyia oryzae* Wood-Mason (Cotes, 1889). It was Felt (1921) who renamed it as *Pachydiplosis oryzae* and then to *Orseolia oryzae* (Gagne, 1973).

There are two main species of rice gall midge identified as Asian rice gall midge, *Orseolia oryzae* (Wood mason) and African rice gall midge, *Orseolia oryzivora.* They belong to family Cecidomyiidae under order Diptera. Adult flies of rice gall midge are mosquito like, pink (female) to brownish (male) bodied, and live only for a few days (Sardesai *et al.*, 2001; Bentur *et al.*, 2003; Harris *et al.*, 2003).

2.2.1 Occurrence of the pest

Gall midge started grabbing the attention of scientific world from the last forty years due to their extensive damageability to rice crop. Among the various insect pests, it is ranked as the third major pest in India based on its relative importance.

In Asia, the pest is known to occur in countries like Bangladesh, Pakistan and Srilanka (Singhe, 1969) in Burma and Cambodia (Grist and Lever, 1969), in China (Yen *et al.*, 1941), in India (Cotes, 1889; Reddy, 1967) and in Indonesia, Laos, Nepal, Thailand and Vietnam (Ladell, 1933), Philippines etc.

The pest is predominately seen in Indian states like Andhra Pradesh, Assam, Bihar, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Orissa, and

Tamil Nadu and in certain parts of Uttar Pradesh and West Bengal. Certain regions *viz.*, the coastal and northern Telangana regions of Andhra Pradesh, Ranchi area in Bihar, Kuttanadu region of Kerala, Chattisgarh region of Madhya Pradesh, Vidarbha region of Maharashtra, coastal and Sambalpur areas of Orissa, and Madurai region of Tamil Nadu are considered as "hot spots" based on its intensity and regularity of occurrence (Rao *et al.*, 2004).

2.2.2 Economic importance of the pest

The Asian rice gall midge, *Orseolia oryzae* (Wood Mason) is regarded as a serious pest of rice (*Oryza sativa* L.). It is estimated that Asian gall midge can cause an annual loss of US$500 million (Herdt, 1991).

Widawsky and O'Toole (1996) reported that in eastern and southern India, gall midge caused an annual yield loss of 477,000 t of rice worth US$80 million.

Mishra and Sarawgi (1997) also reported about 60 per cent yield loss in rice from Chhattisgarh by gall fly. An annual loss of about US $80 million is accounted from gall midge attack (Rao *et al.*, 2004).

Devika *et al.* (2004) reported a yield loss of about 90 per cent, worth US$1.8 million, from gall midge infestation from Kuttanad rice tract of Kerala.

2.2.3 Significance of gall midge damage in rice.

The major insect pests that can cause severe economic losses in rice of Asian countries are stem borer, plant hoppers, leaf folder, gall midge, rice hispa and rice bugs. Among these, gall midge alone causes about 28-35 per cent yield loss (Herdt, 1991).

Lima *et al.* (2007) reported that around 28-35 per cent annual crop loss have been caused by gall midge from India. Gall midge trouble gets compounded due to the emergence of new biotypes.

The rice gall midge has a major impact on the fitness of a rice plant. Early attack of the plant results in stunting and profuse tillering, but few tillers produce panicles. In India, systematic screening for resistant rice genotypes was initiated in 1948 (Sardesai *et al.*, 2001).

Sardesai *et al.* (2002) opined that the yield loss by gall midge mainly depends upon the severity of attack, which often can cause about cent percent loss in extreme cases. For every unit percent increase in gall infestation, yield losses can range from .502 per cent to 2.5 per cent increase.

Rawat *et al.* (2013) reported Asian rice gall midge, *Orseolia oryzae* as third most destructive insect pest in rice.

2.2.4 Life cycle of gall midge

Plant feeders of Cecidomyiidae are generally noted for certain biological peculiarities (Bentur *et al.*, 2003). They generally produce unisexual progenies, Female lays eggs which hatch out as either only daughters or only sons (Sardesai *et al.*, 2002). Secondly, the third instar larva shows a prolonged diapause. Even it can extend upto 12 years as in case of wheat midge (Barenes *et al.*, 1956). Thirdly, they all produce galls in the plants.

Life cycle of gall midge consists of egg, larvae, pupa and adults. The life cycle from oviposition to adult emergence takes about two to three weeks (Joshi and Venugopal, 1984).

Adult flies are mosquito like, exhibiting sexual dimorphism. They emerge during early hours and mate before dark. Females generally remain less active during the subsequent day and start active oviposition after sunset. Unmated females emit strong sex pheromones to attract males for mating (Sain and Kalode, 1988).

Females mate only once and lay about 100-400 eggs in several clusters. Eggs are sigar shaped pinkish coloured ones which are generally layed on rice leaf or leaf

blade or on hairs on stem, either singly or in groups (Sain and Kalode, 1988). Eggs hatch on 3rd or 4th day and hatched maggot crawl towards the space between leaf sheaths to reach up to apical meristem, stimulating the formation of gall (silver shoot) in place of normal tillers. The larvae feeds actively for about two weeks, and moults twice before pupating.

Pupal period lasts for about 2-8 days (Rajamani *et al.*, 2004). With cessation of maggot feeding, pupa wriggles up to the tip of the gall, drills an exit hole and partly pushes out of the gall. Adult emerges through eclosion of the puparium (Fig. 1). Adult male lives for about 1-3 days whereas, female lives for 1-5 days (Kumar *et al.*, 2009).

Bentur *et al.* (2004) reported that, the adult emergence is soon marked by copulation and female mate only once while male flies mate for many number of times. Five to seven overlapping life cycles of gall midges have been reported from a year of seasons.

2.2.5 Symptoms of damage.

Mated female midge selects the host plant for oviposition. Female lays about 100-400 eggs either on leaf sheath or on the hairs of ligules in a group of 2-6 eggs. Eggs take about 4 days to hatch.

Hatched maggot crawls down between the leaf sheaths until it reaches the growing point of the apical or lateral buds, feeds and stimulates the formation of a gall, which when fully formed consists of a long ivory-white tube terminated with a solid plug of white tissue, called as the "silver shoot" (Harris and Gagne, 1982).

Upon infestation, the gall midge converts the leaf sheaths into sterile onion shoot like structures commonly called as silver shoots. Virulent maggot lives inside the gall and completes its life cycle. During this course, the attack site serves as sink for photosynthetic assimilates and thereby nourishes the insect maggot at the cost of plant growth (Harris *et al.*, 2003).

2.3 HOST PLANT RESISTANCE IN GALL MIDGE MANAGEMENT.

Flor (1971) suggested a gene-for-gene hypothesis to explain disease resistance in plants. According to this hypothesis, the product of a pathogen avirulence (*avr*) gene, or a factor produced by it, is recognized by a corresponding plant disease resistance (R) gene product and thereby triggers the resistance response.

Host plant resistance is the sum total of adaptations which are evolved by plants to safeguard themselves from the impact of herbivores. Painter (1951) defined HPR as relative amount of heritable qualities that influence the ultimate degree of damage done by the insect.

Host plant resistance can be considered as a logical methodology for tackling the yield losses (Heinrich and Pathak, 1981). However, biotype emergence can easily overcome the host-plant resistance from the existing populations (Kalode and Bentur, 1989).

In pest endemic areas, where, resistant cultivars are not available, farmers do depend on chemicals like Carbofuran, Phorate, Cartap, Isazophos, Fipronil, etc. for effective control of gall midge (Bentur *et al.*, 2003). Spray formulations are found to be less effective as the internally feeding maggot escape from the chemical. Also, the use of insecticides are not proven as either completely effective or environmentally safe making, the development of gall midge resistant varieties as the only effective and safest way of controlling gall midge infestation (Sardesai *et al.*, 2001).

More than about 45 gall midge resistant cultivars have been released for cultivation in India as reported by Rao *et al.* (2004). But frequent emergence of virulent biotypes creates a challenging platform for crop improvement programmes (Pasalu *et al.*, 2004). This invokes the need for understanding insect-host plant interaction for better use of available resistant sources.

Several morphological features of the plants *viz,* pigmentations and compactness of leaf sheath, hairiness of leaves etc., were found associated with gall midge resistance as reported by Israel *et al.* (1963); Joshi and Venugopal (1984).

Sastry and Rao (1975) and Sain (1988) observed that no specific mechanical barriers were offered by any plants to gall midge maggots, preventing them from reaching the apical meristem. Similarly, no ovipositional preferences were noted between resistant and susceptible cultivars as reported by Hidaka 1974; Sain and Kalode 1985.

It was in the year 1996 that, Bentur and Kalode reported presence of hypersensitive reaction in rice against gall midge *O. oryzae*.

When the gall midge attacks a rice plant, two different types of interactions are observed. In a susceptible variety, the insect is allowed to establish followed by gall formations. This reaction is said to be compatible. Whereas, in a resistant variety, plant produces a hypersensitive response (HR) wherein the host cells around the larva undergo apoptotic cell death, resulting gradually in the death of larvae. Some other resistant varieties, though restrict the larval establishment, do not show any HR reactions. Sardesai *et al.* (2001) commented that it might be due to some kind of defense chemicals produced inside the plant system. Hence the reaction offered by plants could be categorized as HR+ or HR- type.

Whether the reaction should be HR+ or HR−, depend upon the resistance gene the cultivar carries. It was observed that varieties with the *Gm2* gene show HR+ response, while those with *Gm1* and *Gm 8* show HR− response. Results of crosses between varieties carrying *Gm2* with those carrying *Gm1* showed that HR+ response is epistatic to HR− response (Bentur and Amudhan, 1996).

Himabindu *et al.* (2010) reported the existence of a specific gene to gene interaction between the insect biotypes and rice cultivars. So far eleven R gene loci

have been characterized from different rice varieties and nine biotypes of pest has been reported (Yasala *et al.*, 2012).

Gm1 and *Gm8* are known to be confer HR- type resistance, whereas all the other reported genes confer HR + type resistance (Himabindu *et al.*, 2010). Singh (2012) cited that, cultivar W1263, though did not exhibit HR+ reaction, offered resistance via antibiosis.

2.4 BIOTYPES

The major problem confronted by breeders and entomologists are the emergence of biotypes. Biotypes are morphologically indistinguishable and capable of interbreeding but differ in their reaction to rice genotypes (Grover and Prasad, 1980).

First report on gall midge biotype was made by Khan and Murthy (1955) from Nizamabad (India). Gall midge menace is added up by the emergence of resistance-breaking biotypes which contribute to the spread of this pest to new regions causing more than 85 per cent of crop damage (Rao and Kittur 1989; Krishnaiah *et al.*, 2000).

Kuttandu region of Kerala also marked the frequent outbreak of gall midge since 1990, coinciding with the emergence of biotype 5 as reported by Nair and Devi (1994). Currently a total of 14 biotypes of the Asian rice gall midge are reported in literature (Verma, 2006).

Table 1. Reported gall midge biotypes and their region of occurrence.

Biotypes	Endemic region of occurrence in India	References
GMB 1	Raipur (Chattisgrah), Warangal (Andhra Pradesh) and Sambhalpur (Orissa).	Bentur *et al.*, 1987
GMB 2	Cuttack (Orissa), Mangalore (Karnataka), Sakoli (Maharashtra) and Goa	Siddiq *et al.*, 1991
GMB 3	Ranchi (Bihar) and Wangbal (Manipur)	Kalode and Bentur 1989

Table 1. Reported gall midge biotypes and their region of occurrence (Contd.)

Biotypes	Endemic region of occurrence in India	Refernces
GMB 4	Srikakulam ,Vijayanagram ,Vishakapatanam(A.P.) Bhandra (Maharashtra)	Kalode and Bentur, 1988
GMB 5	Moncompu (Kerla)	Nair and Devi, 1994
GMB 6	Manipur	Bentur and Amudhan, 1996

Kumar (2006) opined that, there is an increasing need for identifying new genes and gene combination to develop resistant varieties with diverse genetic backgrounds. This would help in effectively countering the biotype variation in gall midge populations.

2.5 MOLECULAR MARKERS IN STRESS BREEDING

Identification and development of molecular markers which are tightly linked to the gene of interest has improved the efficiency of conventional plant breeding (Hittalmani *et al.*, 2000). Sardesai *et al.* (2002) opined that the molecular markers, tightly linked to the gene of interest enables the breeders to screen the varieties year round without depending on the annual occurrence of insects.

As opined by Biradar *et al.* (2004) molecular markers support conventional breeding by saving time and labour in breeding programmes. And among the several molecular markers available, the SSR markers have found to be more advantageous over other markers (Fjellstrom *et al.*, 2006) as they are more reliable, multi-allelic, chromosome specific, co-dominant and highly informative (Sundaram, 2007). Thus, use of marker assisted selection (MAS) protocol for the selection of resistant phenotypes have proved to be a boon for the plant breeders.

Rapid advancements in molecular biology provides DNA based markers which are more useful and reliable for linkage map construction, identification of gene specific genotypes, assessment of genetic variability of breeding lines etc. (Kumar, 2006).

Apart from this, Candidate Gene markers are also used for identifying and tracing important plant traits like yield and resistance to biotic or abiotic stresses. Candidate genes (CG) are sequenced genes of known biological action involved in the development or physiology with the manifestation of the trait. They may be structural genes or genes in regulatory or biochemical pathways, which affect trait expression. One CG hypothesis states, "The significant proportion of the quantitative trait loci (QTL) affecting trait variation are in fact CGs associated with that trait" (Rothschild and Soller, 1997).

To date, nearly about 11 major genes conferring resistance to gall midge have been identified (Kumar, 2006; Himabindu *et al.*, 2009).

There are available linked molecular markers available for 8 of the 11 gall midge resistance genes (*Gm1*, *Gm2*, *Gm4*, *Gm5*, *Gm6*, *Gm7*, *Gm8*, and *Gm11*), seven of which (except *Gm5*) have been mapped onto the rice genome (Himabindu *et al.*, 2010).

Ribaut *et al.* (2010) suggested molecular markers are one of the important tools with many potent applications for crop improvement.

Himabindu *et al.* (2010) opined that identification of simple sequence repeat (SSR) which are tightly linked to resistant genes can be employed for gene pyramiding for attaining durable resistance.

Yasala *et al.* (2012) identified several candidate genes that involved in rice-gall midge interaction. They reported that genes coding for no apical meristem protein, F-box family protein were common near region of *Gm2, 3, 6, 7* and *Gm4*, which was

responsible for conferring an HR+ type of resistance to gall midge attack. They also deduced out that, SET domain protein was common for *Gm1* and *Gm8* regions, which rendered an HR- type of reaction in plants with *Gm1* and *Gm8* gene.

2.6 GENES CONFERRING RESISTANCE TO GALL MIDGE UNDER STUDY

The inheritance studies arrived at a conclusion that, gall midge resistance in rice, in most of the cases, is a monogenic character controlled by a single dominant gene (Tomar and Prasad, 1992).

Investigations made by Chaudhary *et al.* (1985) to study the relationships of the resistance genes in different varieties revealed the presence of a single dominant gene for resistance. The gene was designated as *Gm1* and few other varieties had another dominant gene non-allelic to *Gm1* and was designated as *Gm2*. Thus, the different gall midge resistance genes provide resistance against different sets of biotypes of the insect (Bentur and Amudhan, 1996).

Latest studies confirmed the presence of nine non-allelic resistant genes, conferring gall midge resistance. *Gm1* and *Gm2* genes reported from 'Samridhi' and 'Surekha' were the first R genes to be identified (Chaudhary *et al.*, 1986). Further studies on gall midge resistance reported several other genes from different sources *viz*, *gm 3* from RP2068-18-3-5 by Kumar *et al.*(1998), *Gm4* from 'Abhaya' (Shrivastava *et al.*,1993), *Gm5* from ARC 5984 (Kumar *et al.*, 1998), *Gm6* from Chinese cultivar 'Duokang1'(Zhang *et al.*,2000), *Gm7* from RP2333-156-8 (Kumar *et al.*, 1998), *Gm8* from 'Jhitpiti' (Kumar *et al.*, 2001), *Gm9* from 'Madhuri line 9' (Shrivastava *et al.*, 2003). All these genes, except for *gm3*, are dominant resistance genes.

Identifying new sources of resistance can help in accelerating gene-pyramiding efforts (Mohan *et al.*, 1997b; Bentur *et al.*, 2003). Hittalmani *et al.* (2000) reported that even the gall midge resistance genes like, *Gm2, Gm4* and *Gm7* in rice, can also be used for marker assisted pyramiding for imparting blast resistance in rice.

2.6.1 *Gm1* Gene

Shastry (*et al*). 1975 reported the presence of a resistance gene *Gm1* in W1263, hybridized from the native rice land race '*Eswarakora*'. This was later confirmed by the works of Chaudhary *et al*., (1986). They reported that W1263 is resistant to many biotypes of gall midge and that the resistance is controlled by a single dominant gene. Similar reports were also presented by Kalode *et al*. (1993) stating that resistance in W1263 has been derived from *Eswarakora,* as W1263 is a cross between *Eswarakora* and MTU15. Also, Reddy *et al*. (1997) confirmed the presence of the dominant gene *Gm1* in the genome of W1263 by genetic analysis studies.

Bentur *et al*. (2003) reported that this gene is effective against biotypes GMB 1, GMB 3, GMB 5 and GMB 6, thus demonstrating its multiple and broad range of resistance. This gene was observed to deploy long lasting resistance in varieties like *Kavya, Samridhi* and *Mahamaya.*

Biradur *et al*. (2004), in their study, tagged and mapped the dominant gene *Gm1* from the cultivar W1263, on chromosome number 9, using SSR markers. They also reported that an SSR marker RM 444 produced alleles at 320bp with resistant genotypes, while susceptible genotype (TN1) produced allele at 183bp. They also showed that the markers RM316, RM444 and RM219, located on chromosome 9, exhibited co-segregation with the trait phenotype with linkage distances of 8, 4.9 and 5.9 cM, respectively, from *Gm1*. Apart, selection efficiency of these markers were also estimated as 92.40, 94.50 and 95.80 per cent respectively for the markers RM 316, RM 219 and RM444.

Sundaram (2007) have mapped *Gm1* gene on chromosome number 9 at a location of 0.18 Mb between two SSR markers RM23941 and RM23956.

Himabindu *et al.* (2010) from their study on allelism test using flanking SSR markers for gall midge resistance genes in rice reported TN 1 as showing susceptible reaction to six biotypes under investigation. Also they used RM 444 and RM 219 as flanking markers for *Gm1* gene detection and W1263 produced bands at 320bp with RM 444, while the susceptible cultivar TN1 got amplified as clear band at 183 bp.

In a study aimed in testing the efficiency of flanking markers which are closely linked to *Gm1* for MAS, markers RM444, 126GIR, RM219 and P33 flanking the gall midge resistance gene *Gm1* showed 92.45, 88.88, 92.59 and 81.48 per cent efficiency respectively proving the reliability of RM 444 marker (Singh, 2012).

2.6.2 *Gm2* Gene

The first gall midge resistance gene to be mapped is the *Gm2* gene that confers resistance to biotypes 1, 2 and 5 of gall midge (Mohan *et al.*, 1994). The results revealed that *Gm2* segregated closely with four RFLP markers that map to chromosome number 4 of rice.

Pasalu and Rajamani (1996) also presented a report showing the presence of *Gm2* in a rice cultivar *'Phalguna'*, which showed a dominant resistance against biotypes GMB 1, GMB 2 and GMB 5.

Nair *et al.* (1995) identified PCR based marker linked to *Gm2* gene by converting RAPD markers into more reliable sequence characterized amplified regions (SCARs). They showed that *Phalguna* cultivar produced amplicons at 600bp with PF10 marker and the susceptible entry made its amplicon at 1700bp.

Sardesai *et al.* (2001) cited *Phalguna* as a variety showing resistance to the gall midge biotypes 1, 2 and 5, while it is susceptible towards biotypes 3 and 4. While, Lakshmi *et al.* (2006) compiled a report stating that *Phalguna* contain *Gm*2 gene capable of offering resistance against gall midge biotypes GMB 1, 2 and 5.

This gene was later mapped on chromosome number 4 at 0.66 Mb (between SSR markers RM17473 and RM17503) by Sundaram (2007).

2.6.3 *Gm4* Gene

Shrivastava *et al.* (1993) reported an introgressed rice cultivar Abhaya with gall midge resistance. They also reported Abahya and R296-260 as donor source for *Gm4* gene. The *Gm4* gene was tagged and mapped using RAPD (Nair *et al.*, 1996) and RFLP (Mohan *et al.*, 1997a) markers on to chromosome 8 of rice.

Pasalu and Rajamani, (1996) reported that *Gm4* gene showed resistance against the biotypes GMB1, GMB2, GMB3 and GMB 4. A similar report was presented by Lakshmi *et al.* (2006) stating that *Gm4* gene in Abhaya provides HR+ type of induced resistance against five of the seven gall midge biotypes GMB1, GMB2, GMB3, GMB4 and GMB 4M.

Himabindu *et al.* (2009) mapped the gene on chromosome 8 within 0.33 Mb region flanked by the SSR markers RM22551 and RM22562.

Later, Divya *et al.* (2015) identified LRR gene as the candidate gene for *Gm4* based on physical location, structural diversity, co-segregation and functional validation. Also revealed LRR-del as a functional marker which can be used for detecting *Gm4* as this marker produces amplified fragments at 620bp in TN1 (susceptible check) and 350bp in Abhaya (resistant check).

Yasala *et al.* (2012) implicated the presence of WRKY family proteins in regions of *Gm4* and *Gm11* as well as the presence of *RPM1* (a disease resistant protein).

2.6.4 *Gm8* Gene

Gm8 gene was mapped to chromosome 8 with SSR markers RM 22685 and RM 22709 at 400kbp region by Sama *et al.* (2012), reported that LRR del primer can

be effectively used as a candidate gene to confirm the presence of *Gm8* at base pairing of R allele observed at 350bp.

Bentur *et al.* (2011) reported that HR- type resistance conferred by *Gm8* can be effectively used against biotypes GMB1, GMB2, GMB3, GMB4 and GMB4M as well.

Sama *et al.* (2012) reported rice cultivar Aganni can be used as a check for identifying gall midge resistance against GMB1, GMB 2, GMB3, GMB 4 and GMB4M as it possess *Gm8* gene in its genome.

Divya *et al.* (2016) cited that the indica rice cultivar Aganni, possess gall midge resistance gene *Gm8*, has a wide range of resistance across gall midge biotypes, 1,2,3 and 4.

Sama *et al.* (2012) mapped *Gm8* gene on chromosome 8. Further, *in-silico* analysis were made by Yasala *et al.*(2012) revealing several functional gene loci and later attempts narrowed down the search with a candidate gene coding for Proline Rich Protein in the genomic region of *Gm8*, validated by Divya *et al.* (2015). This marker-PRP was used for identification of several genotypes with *Gm8* gene.

A gene encoding pentatricopeptide was observed to be present along the regions of *Gm2, 3, 6, 7, 4* and *8.* And is found responsible for regulation of extra chromosomal RNAs (Saha *et al.*, 2007). Zhou *et al.* (2010) while studying the maize gene mediated response in rice, against bacterial streak pathogen, *Xanthomonas oryzae pv. Oryzicola* revealed the activity of pentatricopeptide genes in rendering resistance. Yasala *et al.* (2012) reported the presence of genes encoding pentaricopeptide in genomic vicinity of *Gm4, gm3* and *Gm8*, which was also found responsible of offering resistance to gall midge. This findings points out the functional significance of employing these genes in disabling the genes coding for susceptibility towards various pest and disease complexes in rice.

Materials and Methods

3. MATERIALS AND METHODS

The current study on identifying gall midge resistance genotypes from traditional rice varieties using functional markers was carried out in the Dept. of Plant breeding and Genetics, College of Agriculture, Vellayani and at the Rice Research Station, Moncompu.

The first experiment of molecular screening of 26 traditional and two popular rice varieties of Kerala for four reported genes *viz*, *Gm1, Gm2, Gm4* and *Gm8* was done in molecular lab, Dept. of Plant Breeding and Genetics. List of the varieties used in the study are given in Table 2.

Second experiment of field screening was laid out at the Rice Research Station, Moncompu, the endemic area reported for gall midge in Kerala. This field screening was carried out with 20 traditional varieties obtained from first experiment along with the corresponding resistant and susceptible checks.

3.1 MOLECULAR MARKER ANALYSIS USING FUNCTIONAL MARKERS

3.1.1 Isolation of genomic DNA

- Fresh tender leaf sample weighing about 1gm was taken in well sterilized mortar and was crushed using liquid nitrogen.
- Crushed powder so obtained was transferred into an eppendorf tube (2ml) and 1ml extraction buffer was added into it. [formulation of extraction buffer 1.00 g SDS (1 per cent), 1.576 g Tris HCl (100 mM), 0.584 g Sodium chloride (100 mM), 0.372 g EDTA (10 mM), volume made upto 100 ml with distilled water].
- Tubes were then homogenized in water bath maintained at 60 °C for 30 minutes with frequent shaking of tubes at intervals.
- The mixture was then centrifuged at 10000 rpm at 4 °C for 10 minutes.

- The aqueous phase was collected, into which 400 µl of phenol chloroform (25:24) was added and mixture was again centrifuged at 10000 rpm at 4 °C for 10 minutes.
- Supernatant so obtained was added with 200 µl of chloroform iso-amyl alcohol (24:1) and centrifuged for 10 minutes at 10000 rpm at 4 °C.
- 200 µl of chloroform iso-amyl alcohol (24:1) was added to the aqueous phase collected and was again centrifuged at 10000 rpm at 4 °C for 5 minutes.
- The supernatant was collected and 60 µl of 3 M sodium acetate and 600 µl ice-cold isopropanol were added and kept overnight at – 20 °C for precipitation.
- After about 16 hours, the solution was centrifuged for 10 minutes at 12000 rpm and the supernatant was discarded without dislodging the pellet.
- The precipitate was then washed twice using 70 per cent ethanol and dried
- Dried precipitate was then dissolved in 100 µl 0.1x TE buffer [tris buffer 0.12 g (10 mM), EDTA 0.037 g (1 mM)] and stored at -20 °C.

3.1.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit as per the following procedure.

- Agarose (4 per cent) was prepared by weighing 6gm agarose and melted in 1x TAE buffer
- After cooling the solution to 42-45 °C, ethidium bromide was added at the rate of 2 µl per 100 ml.
- The solution was then poured on to a preset, sealed gel casting tray with comb fixed in position, to a height of 3 mm – 5 mm.
- The gel was allowed to solidify for 15-20 minutes and comb was removed after solidification.
- Then the tray was submerged in the electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to a height of 1 mm.

- DNA samples, mixed with loading dye [glycerol 30 per cent + bromophenol blue] in 5:1 ratio, were fed into wells preceded by 100kb ladder (5 µl) and corresponding check DNA sample near the negative terminal.
- After attaching cathode and anode of the electrophoresis unit to the power supply, a constant voltage of 60 V was used for the run.
- Power supply was turned off when the loading dye moved about $3/4^{th}$ of the gel.
- The gel was documented in the documentation system.

3.1.3 Quantification of DNA

Spectrophotometer reading was relied on to assess the quality and quantity of DNA samples used for the study. 5 µl of sample DNA dissolved in 0.1x TE was added to 3 ml of distilled water and absorbance at 260 and 280 nm was read against distilled water as blank, using UV spectrophotometer. The concentration of DNA in sample was calculated using formula

$$\text{Amount of DNA (µg/ml)} = \frac{A_{260} \times 50 \times \text{Dilution factor}}{1000}$$

Where, A_{260} = absorption at 260 nm.

Ratio of absorbance values at 260 nm and 280 nm gives the quality of DNA. A ratio of 1.8 - 2.0 indicates best quality DNA.

3.1.4 Primers

Reported gene specific SSR and SCAR markers were used for PCR amplification. Primers, their sequence and annealing temperatures are given in Table 3.

3.1.4.1 PCR Aliquot

DNA amplification was done using the protocol of Cordeiro *et al.* (2002).

The reaction mixture was made to 25 µl containing 3 µl genomic DNA, 2.5 µl $MgCl_2$, 2.5µl Taq buffer B, 1 µl total dNTPs, 0.3 unit of Taq DNA polymerase and 1unit of each of forward and reverse primer and made up using 13.7 µl sterile distilled water.

3.1.4.2 PCR - Cycle

Amplification was done in programmable thermo cycler (Bio-Rad) that was programmed as follows:

Generally an initial denaturation at 94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute, at annealing temperature for 1 minute and 74 °C for 2 minutes; followed by a final elongation at 72 °C for 5 minutes and a 4 °C hold. But there were different programmed timings followed for specific primers. The details are given in Table 4.

Amplified products were separated by agarose gel electrophoresis using 4 per cent gel as described earlier and photographed using gel documentation system.

3.2 FIELD SCREENING

Field screening experiment was conducted with 26 traditional varieties selected from experiment 1, along with specific resistant check varieties and one susceptible check in an area of 4 cents during *Kharif* season, 2015 at Rice Research Station, Moncompu.

Nurseries were prepared and 20 days old seedlings were pulled out for transplanting to the main field.

3.2.1 Design of Experiment

Randomized Block Design was used for the field level screening of the varieties. 20 varieties were taken and they were arranged in lines, with each line

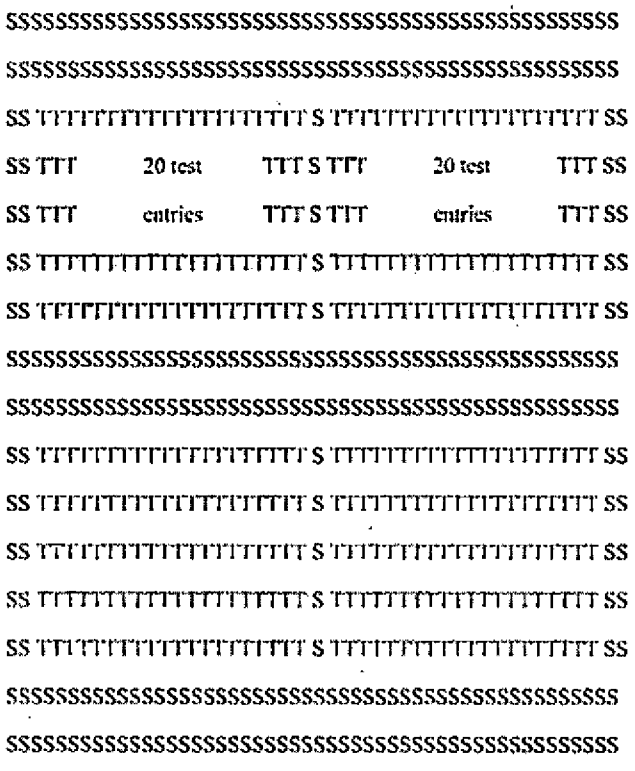


Figure 1. Field layout for screening for gall midge resistance in rice (Bentur *et al.*, 2003)

containing 20 hills in 3 replications. Layout was carried out as per DRR specification used for screening of rice germplasm against *Orseolia oryzae* (Bentur *et al.*, 2003).

Susceptible variety was sown as border rows on all sides of the bed after every 20 test entries, to spread the inoculums. Test material was sown in rows perpendicular to the border rows (Plate.2). Periodical observations on total plant tillers and number of infected tillers at 30 and 50 DAT were taken and scored as per Standard Evaluation System (SES) (IRRI, 2002) (Table 5).

3.3 DATA ANALYSIS

The data generated from the different experiments were subjected to analysis of variance (ANOVA). Mean comparisons were also made to figure out the treatments which were on par with each other.

Table 2. List of the varieties used for the study.

Treatment No.	Varieties	Place of collection
1	Aruvakkari	RARS, Patambi
2	Arrikkirai	RARS, Pattambi
3	Aryan	RARS, Patambi
4	Athikkiraya	RARS, Patambi
5	Bolgatti Kayama	RARS, Patambi
6	Chenellthondi	RRS, Moncompu
7	Chenkayma	RRS, Moncompu
8	Chennellu	RRS, Moncompu
9	Chettivirippu	RRS, Moncompu
10	Chitteni	RRS, Moncompu
11	Chuvanna IR8	RRS, Moncompu
12	Ittikandappan	RRS, Moncompu
13	Kaathikannan	RRS, Moncompu
14	Kalluruli	RARS, Patambi
15	Mattatriveni	RRS, Moncompu
16	Njavara Kunnathoor	RRS, Moncompu
17	Palthondi	RRS, Moncompu
18	Paluveliyan	RRS, Moncompu
19	Karimundakan	RRS, Moncompu
20	Parambuvattan	RARS, Patambi
21	Thavalakkannan	RARS, Patambi
22	Veluthittaryan	RARS, Patambi
23	Thekken Cheera	RARS, Patambi
24	Urulan Kayama	RRS, Moncompu
25	Vellari	RRS, Moncompu
26	Velutha Vattan	RRS, Moncompu
27	Nooranvella	RRS, Moncompu
28	Kirwana	RRS, Moncompu
29	Abhaya (Check)	AICRP Trial, RRS, Moncompu
30	Aganni (Check)	AICRP Trial, RRS, Moncompu
31	Phalguna (Check)	AICRP Trial, RRS, Moncompu
32	W 1263 (Check)	AICRP Trial, RRS, Moncompu
33	TN1 (Check)	AICRP Trial, RRS, Moncompu

RARS- Regional Agricultural Research Station
RRS- Rice Research Station
AICRP- All India Coordinated Rice Improvement Programme

Table 3. Sequence, Annealing temperature and other details of the selected primers.

GENES	PRIMERS	TYPE OF MARKER	SEQUENCE	ANNEALING TEMPERATURE
Gm1	RM444	SSR	F-5' GCTCCACCTGCTTAAGCATC 3' R- 5'GCTCCACCTGCTTAAGCATC 3'	62°C
Gm2	PF10	SCAR	F-5' GGAAGCTTGGCTTATAGTAACTAG3' R-5'GAAGCTTGGAAATGCAAGATCTT 3'	57°C
Gm4	LRR-del	Candidate gene marker	F-5' GTGGATCGAGAGAAGACAAG3' R- 5' CTTGAGGACGATATTCAAGC 3'	60°C
Gm8	PRP	Candidate gene marker	F-5' TCATGTTGTGCAGATCAACC3' R- 5'AGCCATATGAAAACCACCAA 3'	57°C

Table.4. Allele size of resistant gene sources reported for the primers under study

GENES	PRIMERS	ALLELE SIZE FOR R SOURCE	RESISTANCE GENE SOURCE
Gm1	RM444	320 bp	W1263
Gm2	PF10	600 bp	Phalguna
Gm4	LRR del	350 bp	Abhaya
Gm8	PRP	300 bp	Aganni

Table.5.Standard Evaluation System in Rice (SES) Scoring scale, (IRRI, 2002)

Scale	Percentage	Scale of resistance
0	No damage	Highly resistant (HR)
1	<1 %	Resistant (R)
3	1-5%	Moderately Resistant (MR)
5	6-10%	Moderately Susceptible (MS)
7	11-25%	Susceptible (S)
9	>25%	Highly Susceptible (HS)

Results

4. RESULTS

Twenty six traditional rice varieties of Kerala were screened for the presence of four genes (*Gm1, Gm2, Gm4* and *Gm8*) conferring resistance to gall midge using linked molecular markers. The varieties with resistance genes were screened under pest stress condition at Rice Research Station, Moncompu. The results obtained is presented in this chapter.

4.1 MOLECULAR MARKER ANALYSIS

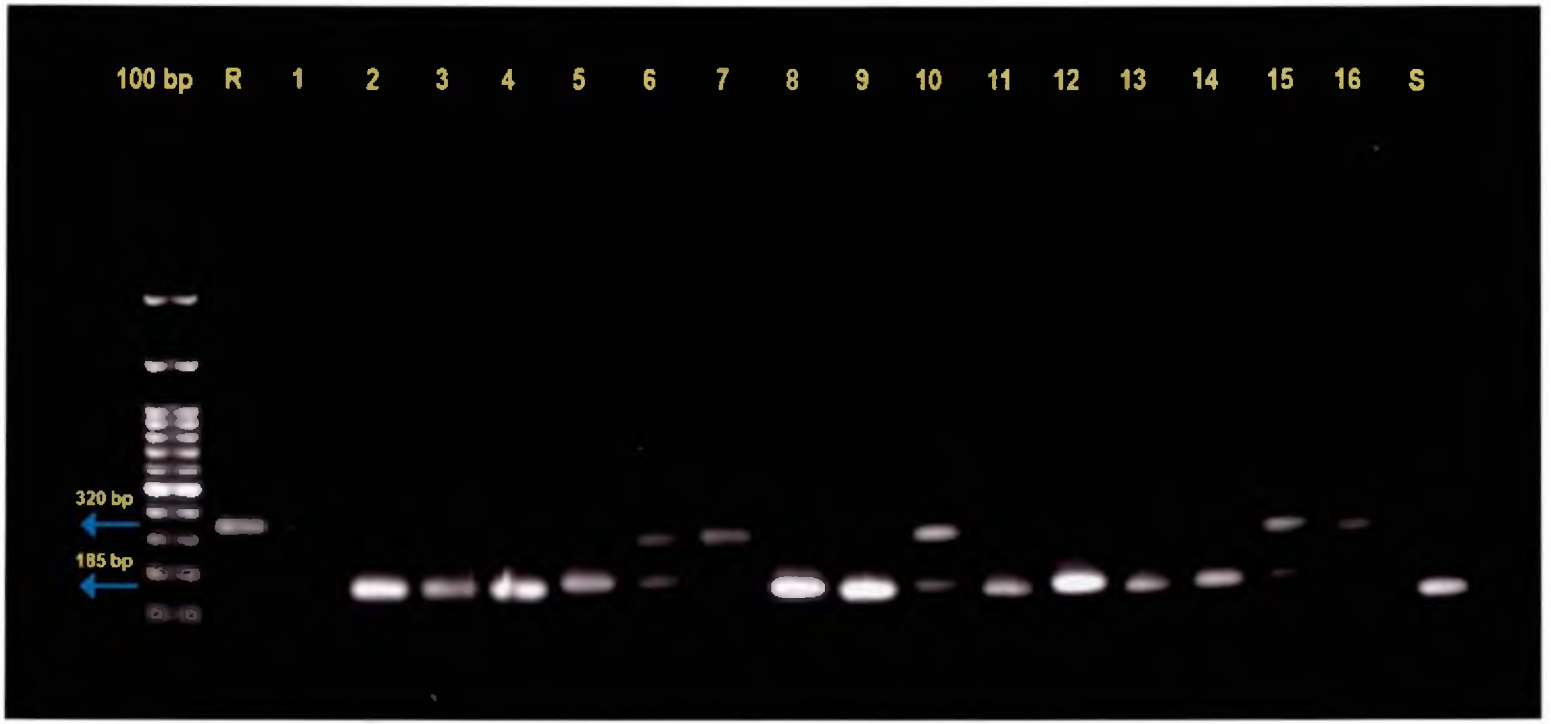
Molecular marker analysis of the varieties were done using the markers tightly linked to the genes under study namely , *Gm1, Gm2, Gm4* and *Gm8*. Good quality genomic DNA was isolated from 26 traditional and 2 popular varieties were checked for quality by using 0.8 per cent agarose gel electrophoresis and by spectrophotometer reading. The quality of DNA obtained ranged from 1.22 to 2.80 (Table 6).

4.1.1 Screening for the Presence of Gene *Gm1*

Presence of *Gm1* gene in the accessions under study was confirmed using SSR marker, RM444 linked to the gene (Plates 1a and 1b). Resistant genotype W1263 was used as check, which produced an allele amplified at 320bp. Susceptible samples produced allele at 185bp.

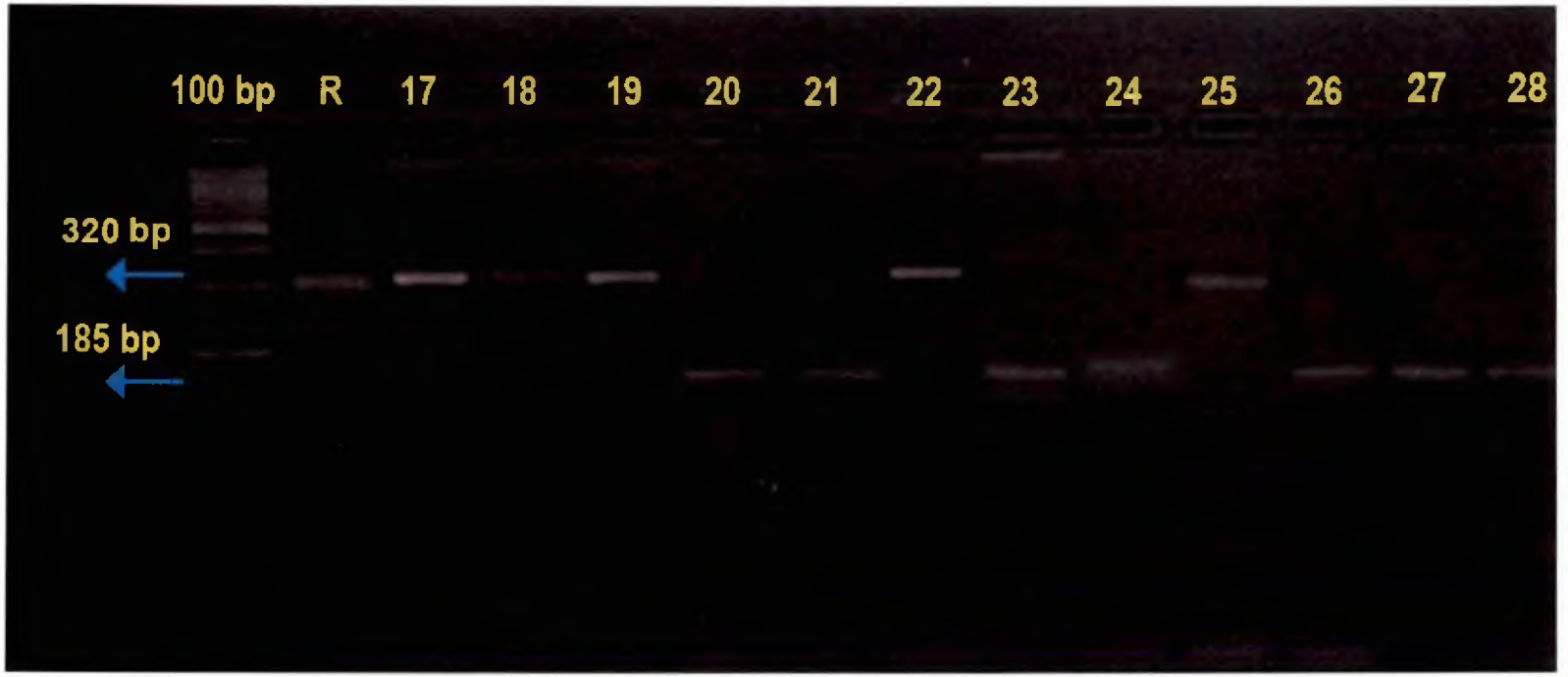
Eleven genotypes produced allele at 320bp matching with that of resistant check. Among them, six varieties viz, Arrikkirai, Vellari, Kalluruli, Chettivirippu, Urulan Kayama and Veluthittaryan, produced heterozygous loci and Parambuvattan, Aruvakkari, Thavalakkannan and Thekken Cheera and Ittikandappan produced allele at 320bp confirming the presence of gene.

Plate No. 1a. Amplification pattern *Gm1* linked SSR marker RM444 in 16 varieties and two check varieties (R- W1263, S- TN1).



1. **Arrikkirai** 2. Aryan 3. Athikkiraya 4. Bolgatti Kayama 5. Chuvanna IR 8 **6. Vellari** **7. Kalluruli** 8. Velutha Vattan 9. Mattatriveni **10. Chettivirippu** 11. Chitteni 12.Kaathikannan 13. Palthondi 14. Paluveliyan **15. Urulan Kayama** **16. Veluthittaryan**

Plate No.1b Amplification pattern of *Gm1* linked SSR marker RM444 in 16 varieties and two check varieties (R- W1263).



17. Thavalakkannan 18. Thekken Cheera 19. Aruvakkari 20. Chennellu 21. Chennelltondi **22. Ittikandappan** 23. Njavara Kunnathoor 24. Chenkayama **25. Parambuvattan** 26. Karimundakan 27.Nooranvella 28.Kirwana

4.1.2 Screening for the Presence of Gene *Gm2*

The variety *Phalguna* was used as the resistant check with SCAR marker PF10 for identification of resistance gene. Seven varieties *viz*, Aryan, Bolgatti Kayama, Kaathikannan, Chuvanna IR8, Parambuvattan, Nooranvella and Kirwana produced allele at 600bp as in the profile of *Phalguna*. Rest of the samples produced allele at 1700kb which is in concurrence with the susceptible check (Plates 2a and 2b).

4.1.3 Screening for the Presence of Gene *Gm4*

Candidate gene primer LRR del was used for screening for the presence of *Gm4*. Among the samples screened, 10 genotypes showed alleles at 350 bp similar to resistant check Abhaya. Rest 16 samples confirmed their susceptibility by producing alleles at 600bp. Vellari, Kalluruli, Velutha Vattan, Chettivripu, Kaatikannan, Paluveliyan, Thavalakkannan, Njavara Kunnathoor, Parambuvattan and Thekken Cheera produced allele at 350bp which confirmed the presence of *Gm4* gene (Plates 3a and 3b).

4.1.4 Screening for the Presence of Gene *Gm8*

SSR primer PRP was used for identifying *Gm8* gene from the accessions under study. Aganni was used as resistant check which showed allele at 300bp with PRP primer. Arrikirai, Aryan, Vellari, Kalluruli, Velutha Vattan, Chettivirippu, Kaathikannan, Paluveliyan, Chenkayama, Veluthittaryan, Karimundakan and Thekken Cheera showed the presence of gene *Gm8* which was confirmed by the presence of allele at 300bp (Plates 4a and 4b).

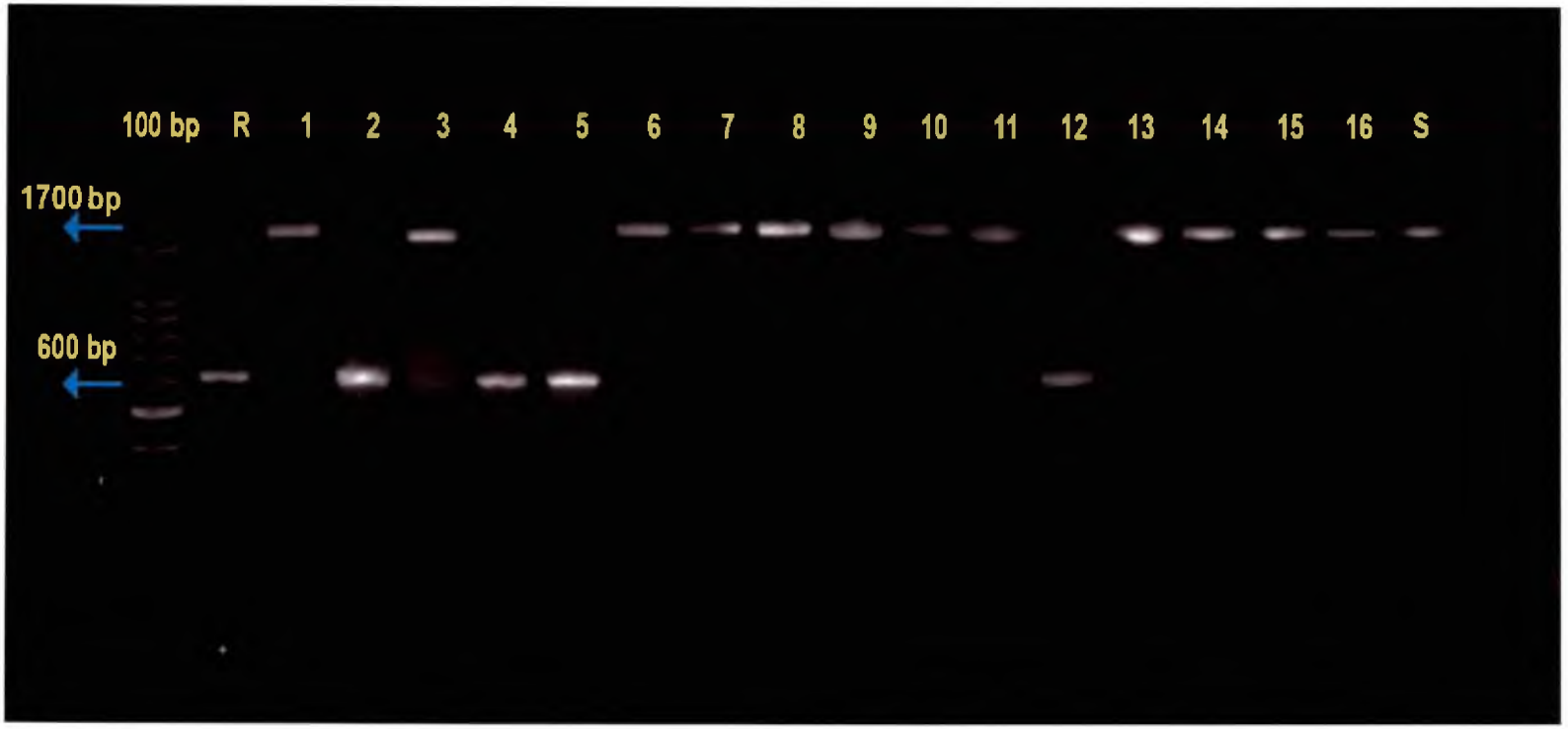
Table 6. Quality and Quantity of DNA of rice varieties used in the study

Sl. No.	Varieties	A 260 nm	A 280 nm	A260/A280	DNA Yield (ng/µl)
1	Abhaya (Check *Gm4*)	0.012	0.007	1.71	360
2	Aganni (Check *Gm8*)	0.028	0.015	1.87	840
3	Aruvakkari	0.006	0.004	1.50	180
4	Arrikkirai	0.004	0.002	2.00	120
5	Aryan	0.011	0.009	1.22	330
6	Athikiraya	0.008	0.005	1.60	240
7	Bolgatti Kayama	0.014	0.007	2.00	420
8	Chenelthondi	0.011	0.006	1.83	330
9	Chenkayma	0.027	0.015	1.80	810
10	Chennellu	0.019	0.012	1.58	570
11	Chettivirippu	0.010	0.006	1.67	300
12	Chitteni	0.015	0.007	2.14	450
13	Chuvanna IR8	0.013	0.008	1.63	390
14	Ittikandappan	0.011	0.006	1.83	330
15	Kaathikannan	0.004	0.002	2.00	120
16	Kalluruli	0.016	0.010	1.60	480
17	Karimundakan	0.010	0.007	1.43	300
18	Kirwana	0.009	0.006	1.50	270
19	Mattatriveni	0.024	0.015	1.60	720
20	Nooranvella	0.026	0.014	1.86	780
21	Njavara Kunnathoor	0.011	0.009	1.22	330
22	Palthondi	0.014	0.005	2.80	420
23	Paluveliyan	0.010	0.006	1.67	300
24	Parambuvattan	0.004	0.003	1.33	120
25	Phalguna (Check *Gm2*)	0.003	0.002	1.50	90
26	Thavalakkanan	0.019	0.012	1.58	570
27	Thekken Cheera	0.006	0.003	2.00	180
28	TN1(Susceptible check)	0.017	0.009	1.89	510

Table 6. Quality and Quantity of DNA of 30 traditional rice varieties used in the study (Contd.)

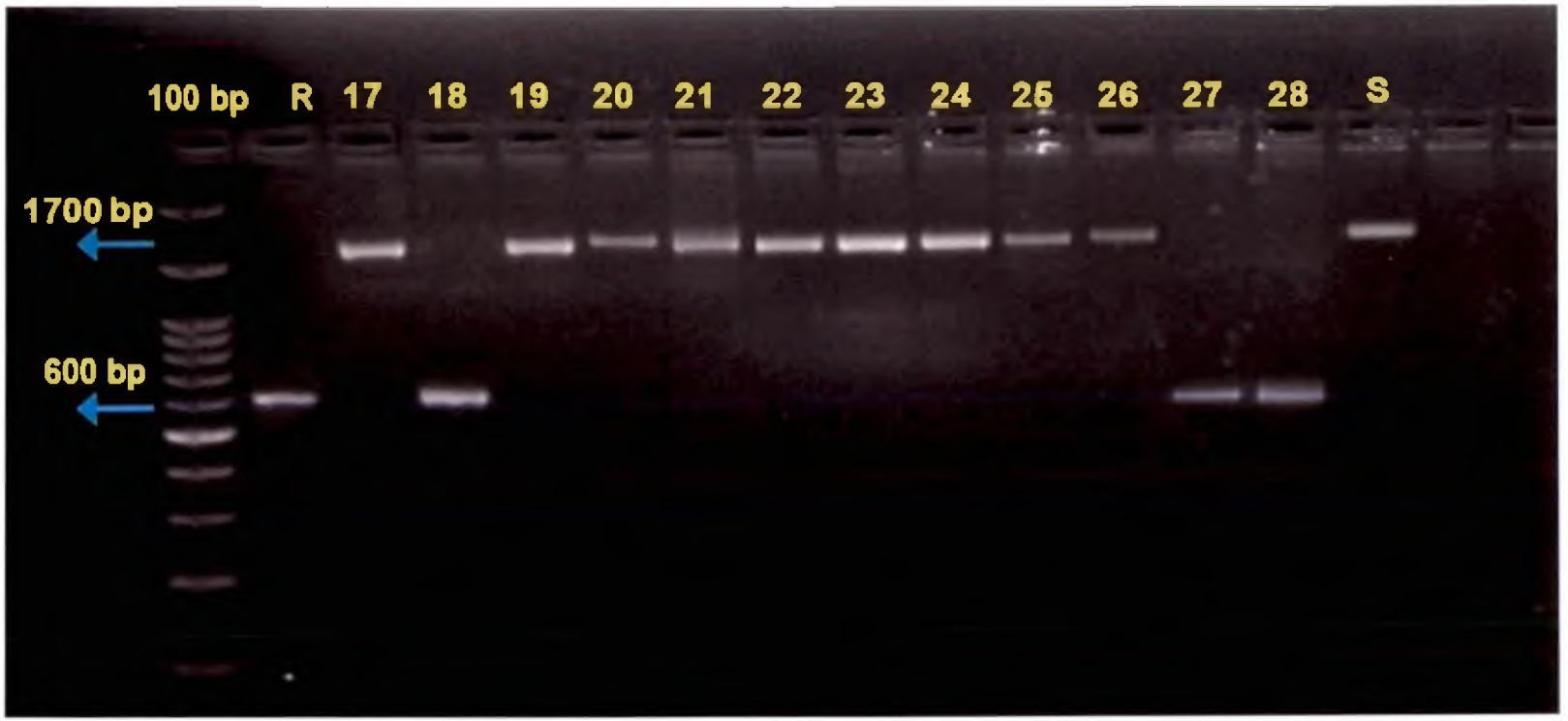
Sl. No.	Varieties	A 260 nm	A 280 nm	A260/A280	DNA Yield (ng/µl)
29	Urulan Kayama	0.023	0.012	1.92	690
30	Vellari	0.012	0.008	1.50	360
31	Veluthittaryan	0.017	0.009	1.89	510
32	Velutha Vattan	0.002	0.001	2.00	60
33	W 1263(Check *Gm1*)	0.016	0.009	1.78	480

Plate No.2a. Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PF10 (R-Phalguna, S-TN1).



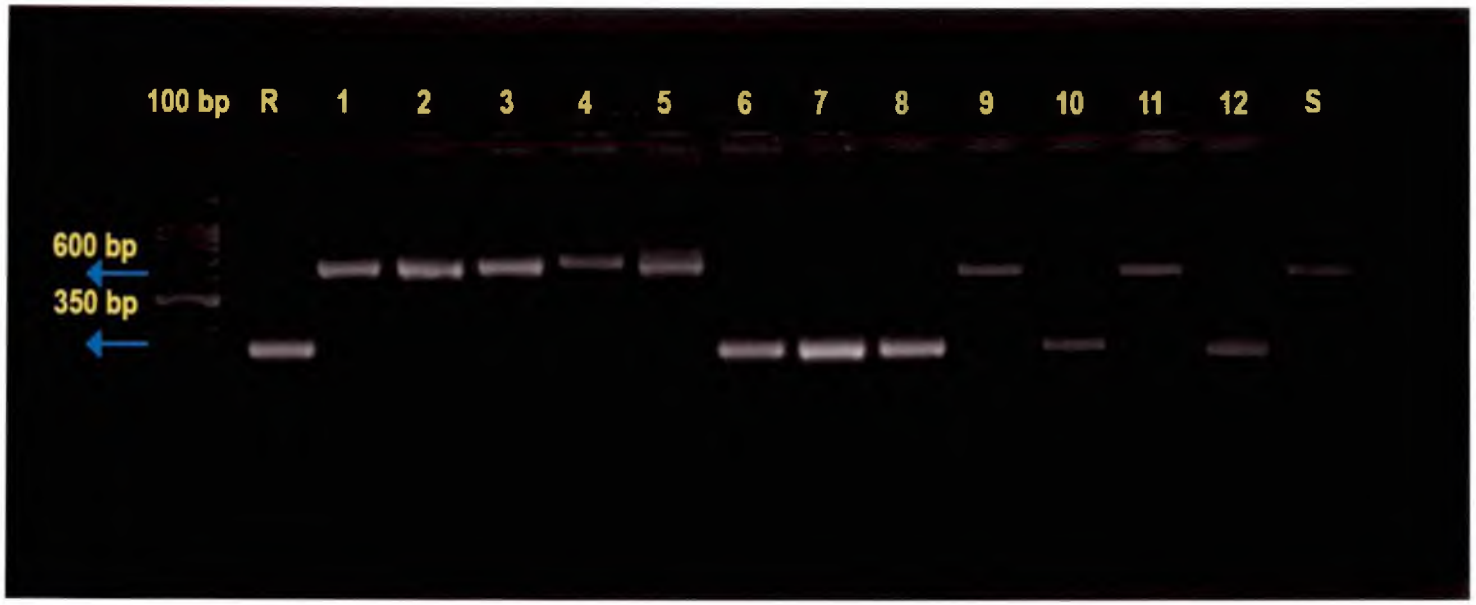
1. Arrikkirai **2. Aryan** 3. Athikkiraya **4. Bolgatti Kayama** **5. Chuvanna IR 8** 6. Vellari 7. Kalluruli 8. Velutha Vattan 9. Mattatriveni 10. Chettivirippu 11. Chitteni **12. Kaathikannan** 13. Palthondi 14. Paluveliyan 15. Urulan Kayama 16. Veluthittaryan.

Plate No.2b. Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PF10 (R-Phalguna, S-TN1)



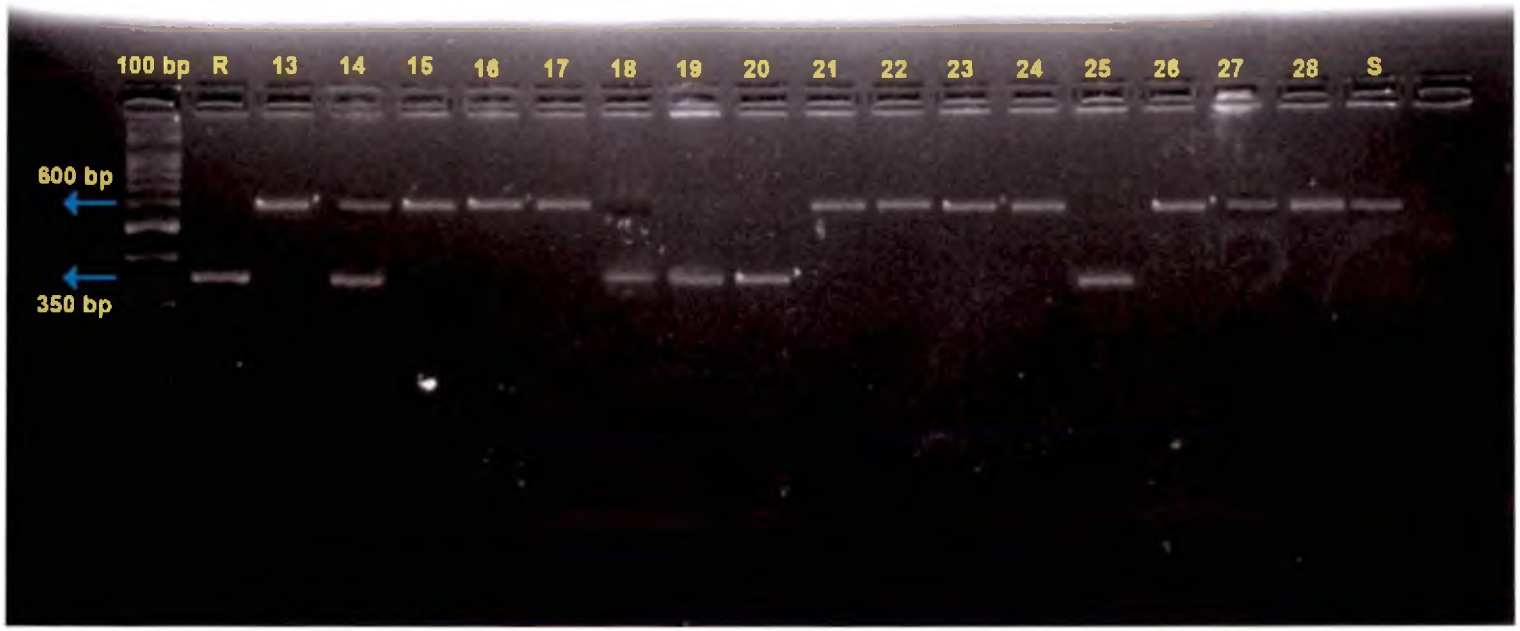
17.Chenkayama **18. Parambuvattan** 19. Thavalakkannan 20. Thekken Cheera 21. Aruvakkari 22.Chenellu 23. Chennelltondi 24. Ittikandappan 25. Njavara Kunnathoor 26. Karimundakan **27.Nooranvella 28.Kirwana**

Plate No.3a. Amplification pattern of genomic DNA of 12 varieties and two check varieties using the primer LRR del linked to *Gm4*.(R-Abhaya, S-TN1).



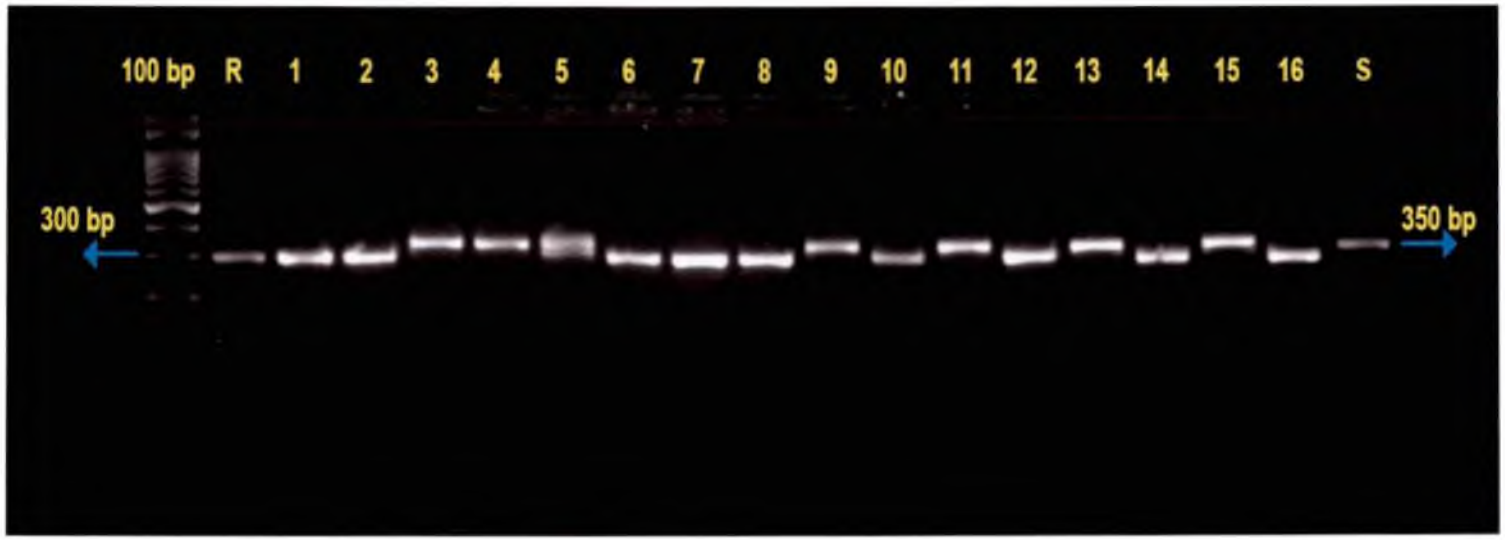
1. Arrikkirai 2. Aryan 3. Athikkiraya 4. Bolgatti Kayama 5. Chuvanna IR 8 **6. Vellari 7. Kalluruli 8. Velutha Vattan** 9. Mattatriveni **10. Chettivirippu** 11. Chitteni **12. Kaathikannan.**

Plate No.3b. Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer LRR del linked to *Gm4*.(R-Abhaya, S-TN1).



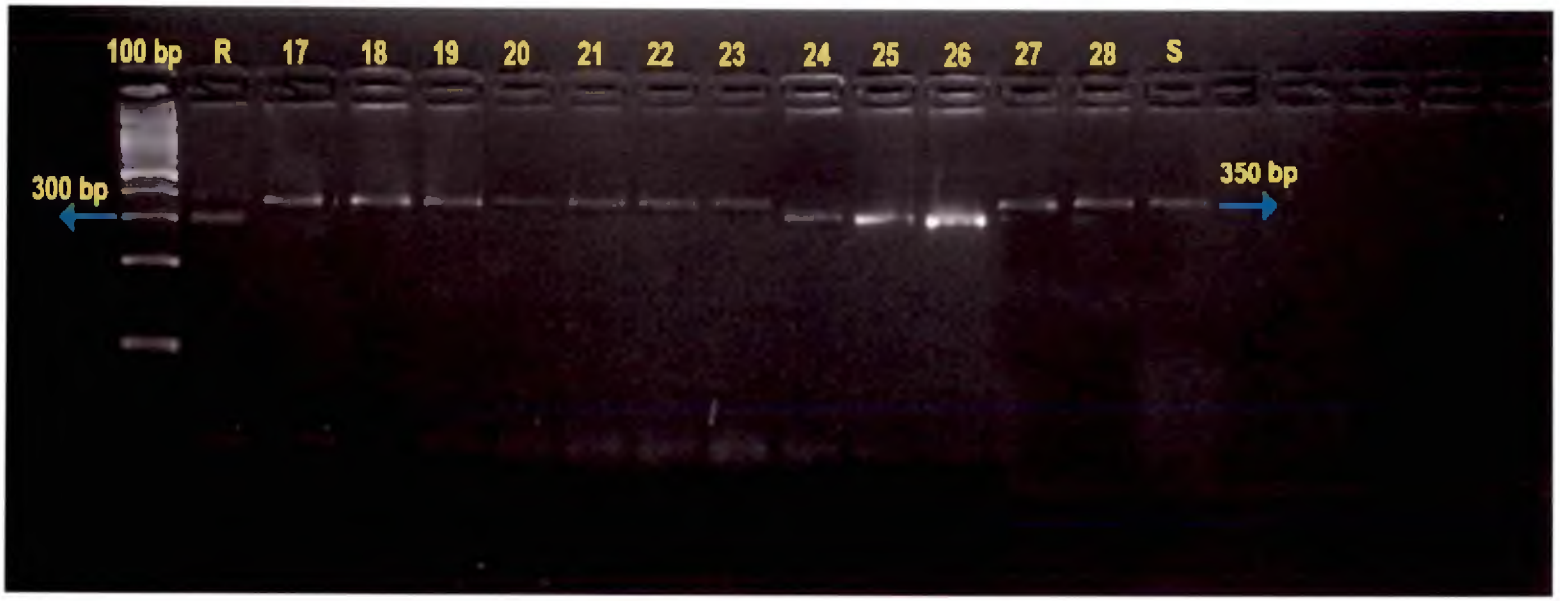
13.Palthondi **14. Paluveliyan** 15. Urulan kayama 16. Veluthittaryan 17. Chenkayama 18. **Parambuvattan** **19. Thavalkkannan** **20. Thekken Cheera** 21. Aruvakkari 22.Chenellu 23. Chennelltondi 24. Ittikandappan **25. Njavara kunnathoor** 26. Karimundakan 27.Nooranvella 28.Kirwana.

Plate No.4a Amplification pattern of genomic DNA of 16 varieties and two check varieties using the primer PRP for *Gm8* gene (R-Aganni, S-TN1).



1. Arrikkirai **2. Aryan** 3. Athikkiraya 4. Bolgatti Kayama 5. Chuvanna IR 8 **6. Vellari** **7. Kalluruli** **8. Velutha Vattan** 9. Mattatriveni **10. Chettivirippu** 11. Chitteni **12. Kaathikannan** 13. Palthondi **14. Paluveliyan** 15. Urulan Kayama **16. Veluthittaryan.**

Plate No.4b. Amplification pattern of genomic DNA of 12 varieties and two check varieties using the primer PRP for *Gm8* gene (R-Aganni, S-TN1).



17. Thavalakkannan 18. Thekken Cheera 19. Aruvakkari 20. Chennellu 21. Chennelltondi 22. Ittikandappan 23. Njavara Kunnathoor **24. Chenkayama 25. Parambuvattan 26. Karimundakan** 27. Nooranvella 28.Kirwana.

Table 7. Presence of various genes in selected varieties

Sl. No.	Varieties	Genes			
		1	*2*	*4*	*8*
1	Aruvakkari	▬			
2	Arrikkirai	▬	▬		▬
3	Aryan				▬
4	Athikiraya				
5	Bolgatti Kayama		▬		
6	Chenelthondi				
7	Chenkayma				▬
8	Chennellu				
9	Chettivirippu	▬		▬	▬
10	Chitteni				
11	Chuvanna IR8		▬		
12	Ittikandappan	▬			
13	Kaathikannan		▬	▬	▬
14	Kalluruli	▬		▬	▬
15	Mattatriveni				
16	Njavara Kunnathoor			▬	
17	Palthondi				
18	Paluveliyan			▬	▬
19	Karimundakan				▬
20	Parambuvattan	▬	▬	▬	▬
21	Thavalakkannan	▬		▬	
22	Veluthittaryan	▬			▬
23	Thekken Cheera	▬		▬	
24	Urulan Kayama	▬			
25	Vellari	▬		▬	▬
26	Velutha Vattan			▬	▬
27	Nooranvella		▬		
28	Kirwana		▬		
29	Abhaya (Check)			▬	
30	Aganni (Check)				▬
31	Phalguna (Check)		▬		
32	W 1263 (Check)	▬			
33	TN1 (Check)				

▬ Gene Present

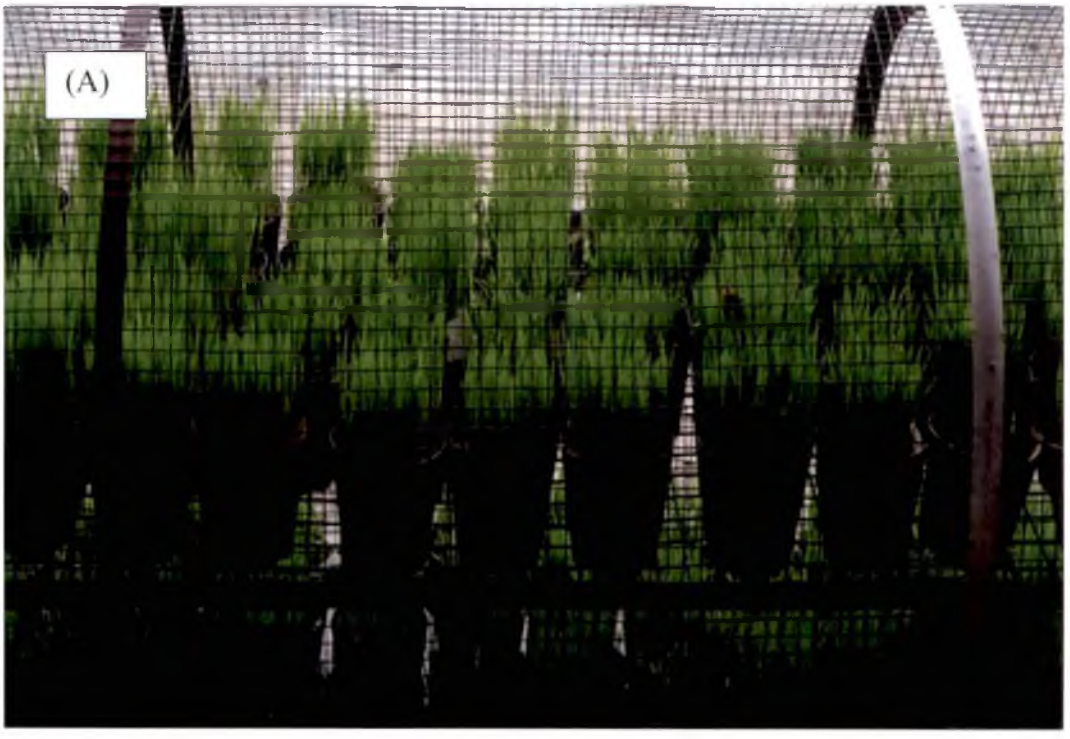
4.2 FIELD SCREENING OF GENOTYPES

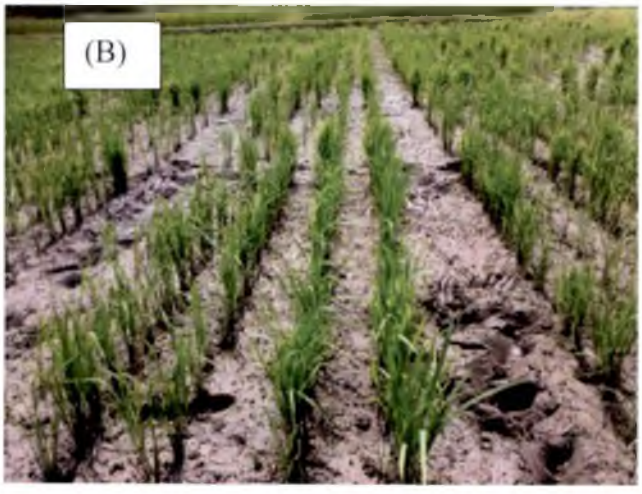
Field level screening of the varieties with genes for resistance, identified under the study by molecular markers (Table 7), was carried out at the Rice Research Station, Moncompu. Biotype 5 of gall midge is endemic to Moncompu region of Kerala state. Gall midge infestation is marked by onion shoot like appearance of tillers commonly called as 'Silver shoot' or Galls. Periodical observations were made for total number of tillers produced per plant and number of galls produced at 30 and 50 DAT (Table 8). Entries were scored in 0-9 SES scale (Table 9).

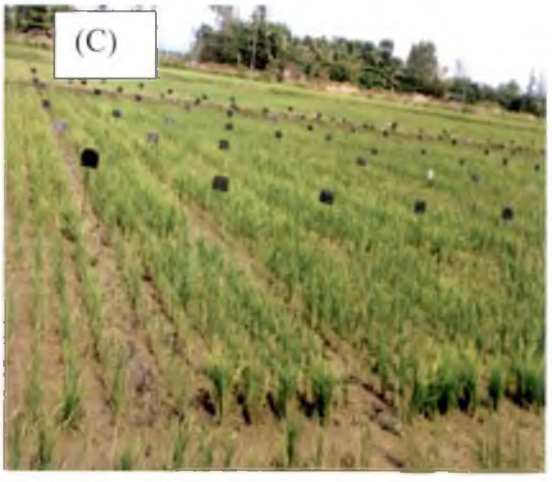
Four varieties namely, Aruvakkari, Urulan Kayama, Arrikkirai and Parambuvattan showed resistance response while, Bolgatti Kayama, Chettivirippu, Chuvana IR8, Ittiknandappan, Kaathikannan, Kalluruli, Kirwana, Nooranvella,Vellari, Veluthittaryan, Thavalakkannan and Thekken Cheera were moderately resistant in response. Veluta Vattan, Paluveliyan and Njavara Kunnathoor exhibited susceptibility reaction towards the gall midge biotype under study. None of the varieties were immune or highly susceptible.

Using the formula, percentage gall infestation was calculated and their variances were analyzed. Statistical analysis of percentage gall infestation, showed significant difference between the varieties with respect to reaction to gall midge infestation (Table 8). Variety W1263 showed least gall infestation of 0.345% followed by the varieties Arrikkirai (0.42), Urulan Kayama (0.51), Aruvakkari (0.67) and Parambuvattan (0.96). Variety TN1 was found to be with maximum gall infestation of 23.10 per cent and it was significantly different from all the remaining varieties. Paluveliyan showed maximum gall infestation after TN 1 with mean infestation of 15.17 per cent. Those varieties with *Gm4* and *Gm8* which had susceptible and moderately susceptible response, respectively, towards gall midge biotype 5 were found to be on par. While those varieties with *Gm1* and *Gm2* were also found to be on par.

Plate 5. (A) Rice nursery, (B) Field view just after transplanting, (C) Labelled field.







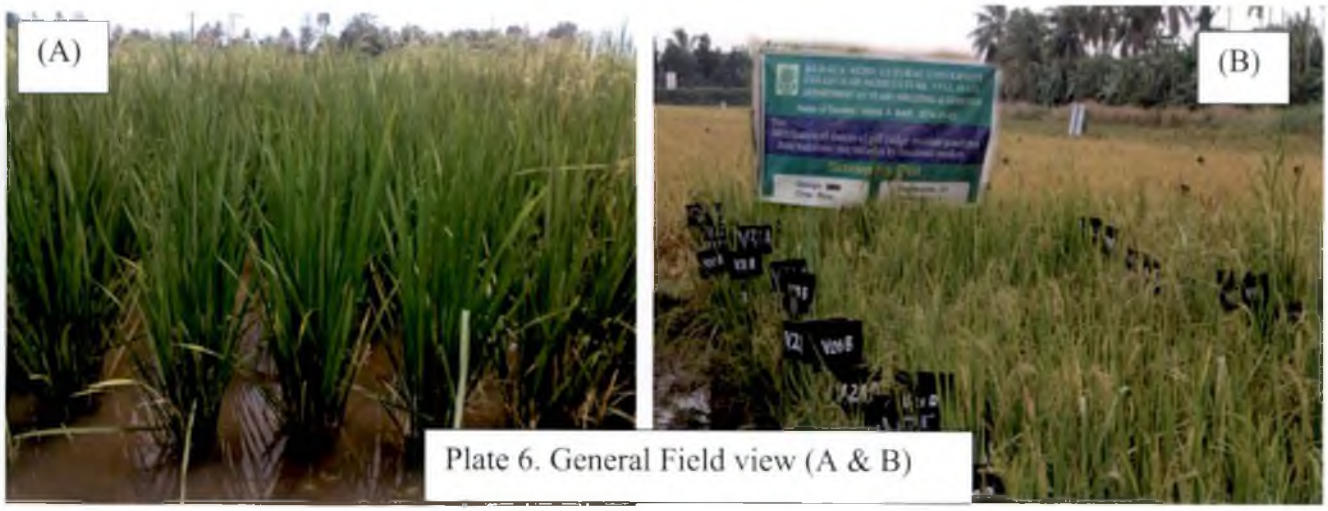


Plate 6. General Field view (A & B)

Plate 7. Silver shoot or Gall

Plate 8. Life cycle rice gall midge *Orseolia oryaze.*

(A) Eggs of gall midge
(B) Gall midge larvae
(C) Pupae
(D) Adult gall fly

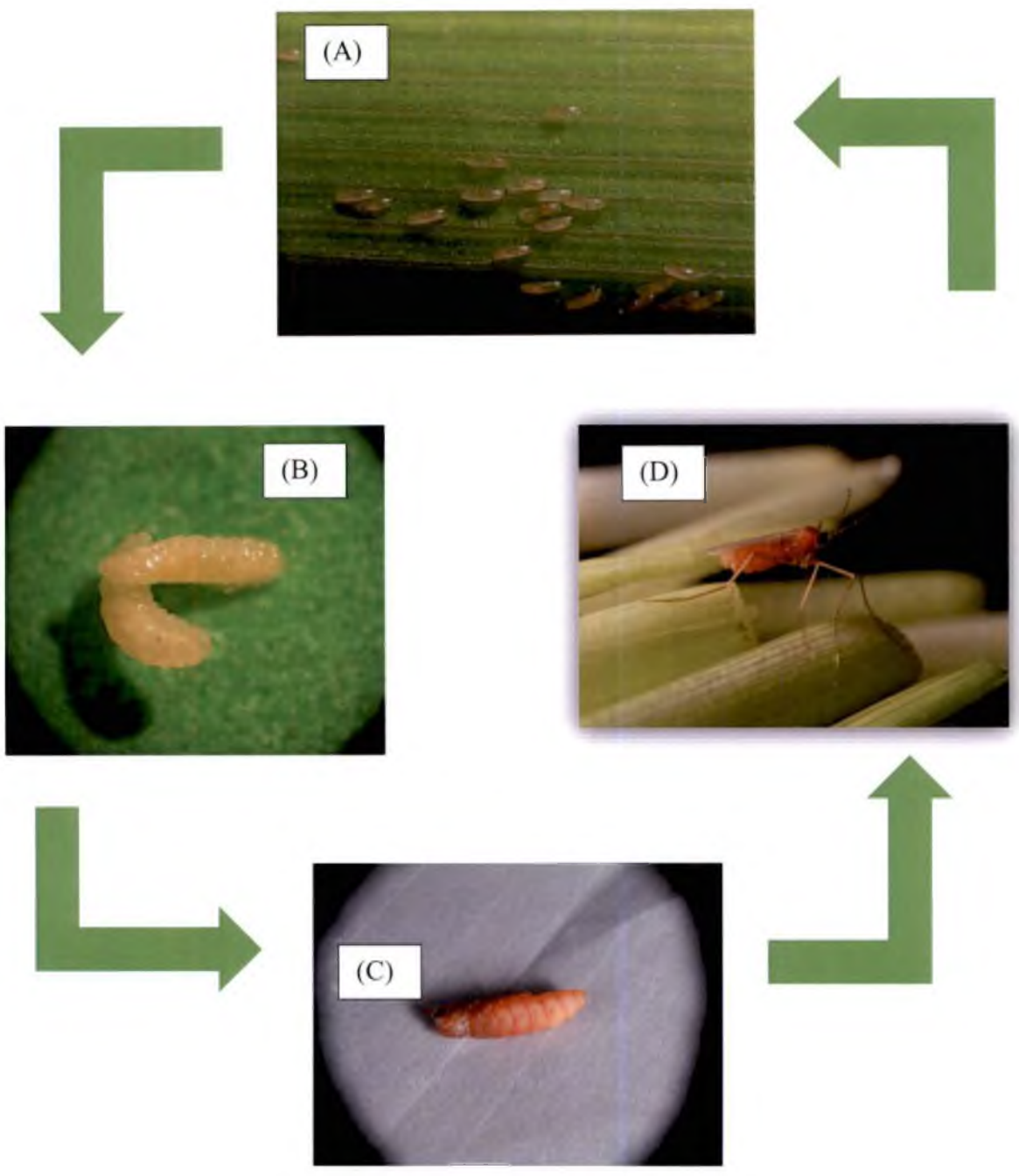


Table 8. Selected varieties and gall infestation percentage observed at 30 DAT & 50 DAT.

Varieties	30DAT	50 DAT	mean gall infestation at 30 DAT & 50 DAT
Abhaya (Check *Gm4*)	9.05	15.62	12.33
Aganni (Check *Gm8*)	9.47	8.2	8.83
Arrikirai	0.22	0.63	0.42
Bolgati Kayama	1.78	2.78	2.28
Chenkayama	6.74	8.36	7.55
Chettivirippu	1.29	2.73	2.01
Chuvana IR8	2.23	2.76	2.49
Ittikandappan	2.26	3.07	2.66
Kaathikannan	1.95	2.09	2.02
Kalluruli	1.97	2.12	2.04
Karimundakan	6.19	7.81	7
Kirwana	1.38	2.68	2.04
Njavara Kunnathoor	9.46	14.8	12.13
Nooranvella	1.09	3.38	2.23
Paluveliyan	14.81	15.53	15.17
Phalguna (Check *Gm2*)	3.95	6.05	4.89
Aruvakkari	0.6	0.74	0.67
Thavalakkannan	3.02	3.36	1.19
Parambuvattan	1.27	3.42	0.92
Thekken Cheera	1.53	3.2	1.06
Aryan	10	12.1	8.06
TN 1 (Susceptible check)	22.4	23.8	23.1
Urulan Kayama	0.26	0.77	0.51
Vellari	2.13	3.14	2.63

Table 8. Selected varieties and gall infestation percentage observed at 30 DAT & 50 DAT (Contd.).

Varieties	30DAT	50 DAT	mean gall infestation at 30 DAT & 50 DAT
Veluta Vattan	10.1	16.76	13.43
Veluthittaryan	1.5	3.6	1.55
W1263 (Check *Gm1*)	0	0.69	0.34
General mean	**4.49**	**6.09**	
S.E.M	**0.89**	**0.90**	
C.D (5%)	**2.553**	**2.561**	

Mean comparison of percentage gall infestation at 30 DAT, 50 DAT (Table 8) showed that, varieties with *Gm1* and *Gm2* were on par underlying their ability to combat the gall midge attack. While, varieties with *Gm4* and *Gm8* was significantly different from varieties with *Gm1* and *Gm2*, but were on par with each other. TN1, with none of genes resistant to gall midge, marked significantly different from rest of the varieties.

The traditional varieties under study was scored as per SES scale varying from 1-9. (Table 5). Varieties were classified into four different divisions based on their scale of reaction to gall midge biotype 5 under study. Four varieties, Arrikkirai, Urulan Kayama, Aruvakkari and Parambuvattan were resistant like the check variety W1263. Twelve varieties, Bolgatti Kayama, Chettivirippu, Chuvana IR8, Ittiknandappan, Kaathikannan, Kalluruli, Kirwana, Nooranvella, Vellari, Veluthittaryan, Thavalakkannan and Thekken Cheera were found moderately resistant, including the check variety Phalguna. Three varieties (Karimundakan, Chenkayama and Aryan) were moderately susceptible as in case with another check Aganni. Remaining four (Abhaya, Veluta Vattan, Paluveliyan, Njavara Kunnathoor) were categorized as

Table 9. Gall infestation scale scoring and reaction showed by the traditional varieties under study

Varieties	Gall infestation (%)	Scale	Reaction
Abhaya (Check *Gm4*)	12.33	7	S
Aganni (Check *Gm8*)	8.83	5	MS
Arrikkirai	0.42	1	R
Bolgati Kayama	2.28	3	MR
Chenkayama	7.55	5	MS
Chettivirippu	2.01	3	MR
Chuvana IR8	2.49	3	MR
Ittiknandappan	2.66	3	MR
Kaathikannan	2.02	3	MR
Kalluruli	2.04	3	MR
Karimundakan	7	5	MS
Kirwana	2.04	3	MR
Njavara Kunnathoor	12.13	7	S
Nooranvella	2.23	3	MR
Paluveliyan	15.17	7	S
Phalguna (Check *Gm2*)	4.89	3	MR
Arurvakkari	0.67	1	R
Thavalkkannan	1.19	3	MR
Parambuvattan	0.92	1	R
Thekken Cheera	1.06	3	MR
Aryan	8.06	5	MS
TN 1 (Susceptible check)	23.1	7	S
Urulan Kayama	0.51	1	R
Vellari	2.63	3	MR
Veluta Vattan	13.43	7	S
Veluthittaryan	1.55	3	MR
W1263 (Check *Gm1*)	0.34	1	R

R- Resistant; MR- Moderately Resistant; MS- Moderately Susceptible; S- Susceptible

susceptible along with check TN1. Overall categorization of varieties under study is represented in Table 10.

Table 10. Classification of the varieties based on the overall reaction to rice gall midge (Biotype 5) attack.

Class	Score	Varieties
Highly resistant	0	Nil
Resistant	<1%	W1263, Arrikkirai, Urulan Kayama, Aruvakkari, Parambuvattan
Moderately resistant	1-5 %	Bolgatti Kayama, Chettivirippu, Chuvana IR8, Ittiknandappan, Kaathikannan, Kalluruli, Kirwana, Nooranvella, Phalguna, Vellari, Veluthittaryan, Thavalakkannan and Thekken Cheera
Moderately Susceptible	6-10%	Karimundakan, Chenkayama, Aganni, Aryan
Susceptible	11-25%	TN1, Abhaya, Veluta Vattan, Paluveliyan, Njavara Kunnathoor,
Highly susceptible	>25 %	Nil

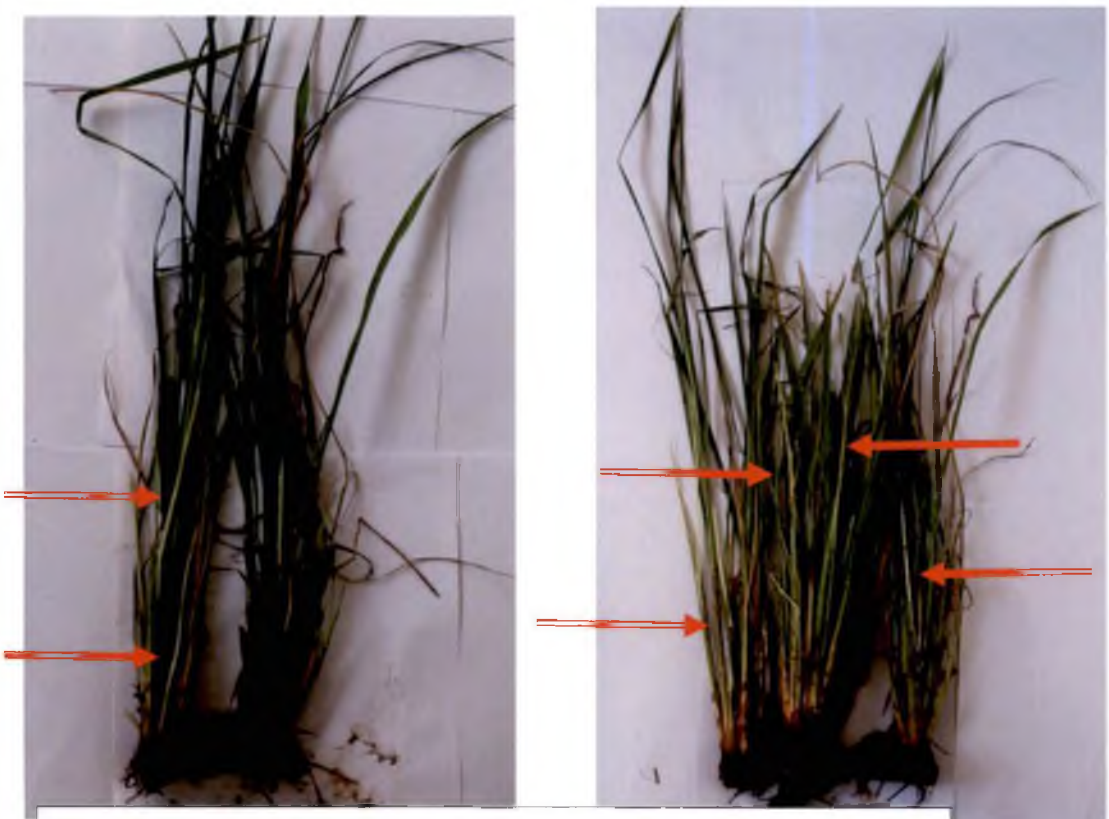
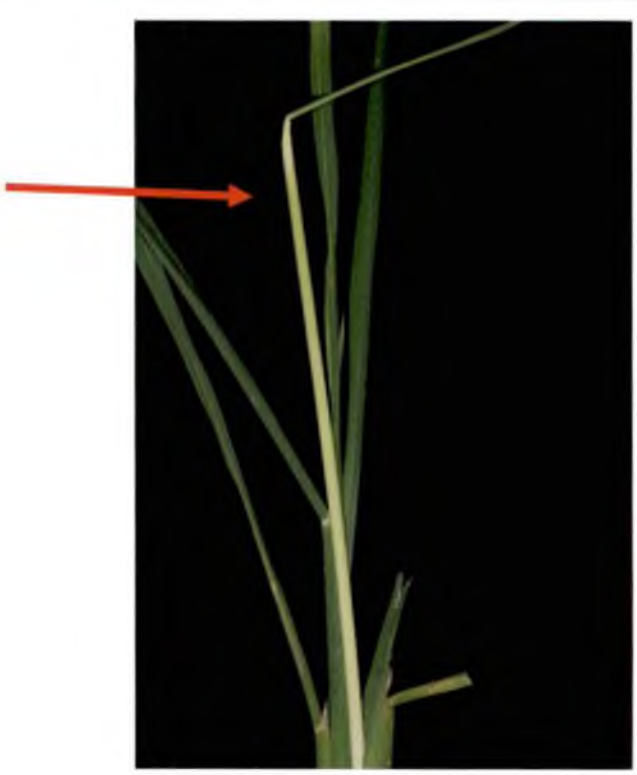


Plate 9. Intensity of gall midge infestation



Discussion

5. DISCUSSION

Day to day increasing world population is demanding an increased production of food grains. Zeigler and Savary (2009) reported that each year an additional 50 million rice consumers are added to the world population. In order to meet the growing demand of the ever increasing population, we need to produce one million tons more of rice every year. However rice production is affected by various biotic and abiotic stresses of which insect pest alone causes about 25 per cent loss accounting to Rs.240, 138 million (Dhaliwal *et al.*, 2007).

High humidity and temperature prevailing in Kerala state is conducive for increased incidence of pests and diseases. Among the various pest and disease complexes attacking rice, brown plant hopper, stem borer, gall midge, leaf roller and rice bug and minor pests like thrips, case worm, blue beetle, whorl maggot etc. are of major importance.

A severe outbreak of gall midge attack in Kuttanadu rice tract of Kerala state was observed during the years 1990 and 1996. This was followed by an economic loss of Rupees eight crores, damaging the crop in about 30,000 ha of land (Kumary, 2004). The strain of gall midge was identified as GM Biotype 5 (Nair and Devi, 1994). Research initiatives at Rice Research Station, Moncompu, could observe some of the varieties *viz.*, Uma, Pavithra and Panchami showing tolerance to gall midge attack (Devika *et al.*, 2004).

A rich diversity of traditional rice varieties which are the proven sources of pest and disease resistance is found in Kerala. The less exploited traditional germplasm could offer potent donors to combat the problem of pest and disease attack. The current study was designed to identify such potent sources of gall midge resistant genotypes from traditional rice varieties of Kerala by using functional markers. The results of this investigation are elaborated in this chapter.

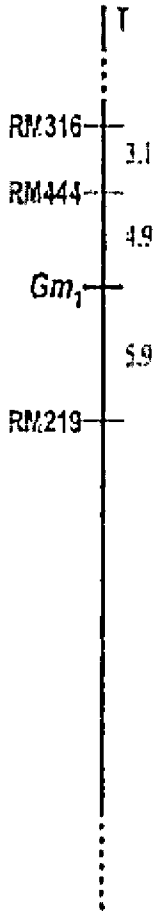


Figure 2. Map position of SSR marker RM444 on rice chromosome flanking gene Gm1 (Himabindu *et al.*, 2007)

5.1. LOCATING GENES FOR RESISTANCE

Twenty six traditional rice varieties were selected for assessing the presence or absence of gall midge resistance genes *Gm1, Gm2, Gm4* and *Gm8* using specific markers linked to them. These genes were reported to give durable resistance to different biotypes of gall midge (*Orseolia oryzae*).

5.1.1 Screening for *Gm1* Gene.

Among the various resistance sources used for gall midge resistance, *Gm1* gene is a dominant gene known to confer durable resistance. This gene was first reported from landraces of *Eswarakora* (Sastry *et al.*, 1975; Chaudhary *et al.*, 1985). Specificity of this gene towards biotypes 1, 3, 5 and 6 were reported by Bentur *et al.* (2003). It was Biradar *et al.* (2004) who mapped and validated the presence of *Gm1* gene in rice cultivar W1263 with 3 SSR markers RM444, RM316 and RM 219. He also reported that the gene is present in chromosome number 9 with these markers located at 4.9, 8, and 5.9 cM respectively from *Gm1*, thus making RM 444 a reliable marker (Fig. 2). The current study deployed RM 444 as the marker for identifying the genotypes with *Gm1*gene.

Among the 26 traditional rice varieties and two improved rice varieties screened, 11 varieties confirmed the presence of *Gm1* gene in their genome. Varieties like Arrikkirai, Vellari, Kalluruli, Chettivirippu, Urulan Kayama, Veluthittaryan and showed heterozygous loci pattern at 320bp while, Thavalakkannan, Thekken Cheera, Aruvakkari, Ittikandappan and Parambuvattan confirmed the presence of *Gm1* gene with homozygous loci for the resistant gene allele at 320bp with RM 444 primer. W1263, reported gene source of *Gm1* also have produced allele at 320bp on amplification with RM444 (Biradar *et al.*, 2004).

5.1.2 Screening for *Gm2* Gene

Mohan *et al.* (1994) reported *Gm2* gene conferring resistance against gall midge biotype 5 using RFLP markers. Nair *et al.* (1995) developed more reliable sequence characterized amplified regions (SCARs) marker, PF10, linked to *Gm2*. The reported variety *Phalguna* produced alleles at 600bp with PF10 marker. Lakshmi *et al.* (2006) compiled a report stating that variety *Phalguna* contains *Gm*2 gene capable of offering resistance against gall midge biotype GMB 1, 2 and 5.

The current PCR analysis with PF 10 primer revealed the presence of *Gm2* gene in seven samples under investigation. Varieties like, Aryan, Bolgatti Kayama, Chuvanna IR8, Kaathikannan, Parambuvattan, Nooranevella and Kirwana produced resistant allele at 600bp. Variety *Phalguna* with resistant gene *Gm2* also produced an allele at 600bp with PF 10 marker (Nair *et al.*,1995).

5.1.3 Screening for *Gm4* Gene

Abhaya genotype has been identified with the presence of *Gm4* gene and was mapped using RAPD (Nair *et al.*, 1996) and RFLP (Mohan *et al.*, 1997) markers on chromosome 8 within a region flanked by the SSR markers RM22551 and RM22562 (Himabindu, 2009). Later, Divya *et al.*(2015) revealed LRR-del as a candidate gene marker which can be used for detecting *Gm4* as this marker produces an allele at 620bp in TN1 (susceptible check) and 350bp in Abhaya (resistant check) (Fig. 3).

Varities Vellari, Kalluruli, Velutha Vattan, Chettivirippu, Kaathikannan, Paluveliyan, Njavara Kunnathoor, Thekken Cheera, Thavalakkannan, Parambuvattan were amplified at 350bp and confirming the presence of *Gm4* gene.

5.1.4 Screening for *Gm8* Gene

Tagging and mapping of *Gm 8* gene by making use of SCAR marker with variety Jhitpiti and TN 1 by Jain *et al.* (2004), located the gene on chromosome number 8. Also this gene has been tagged within 0.43-Mb region on chromosome 8 by using the

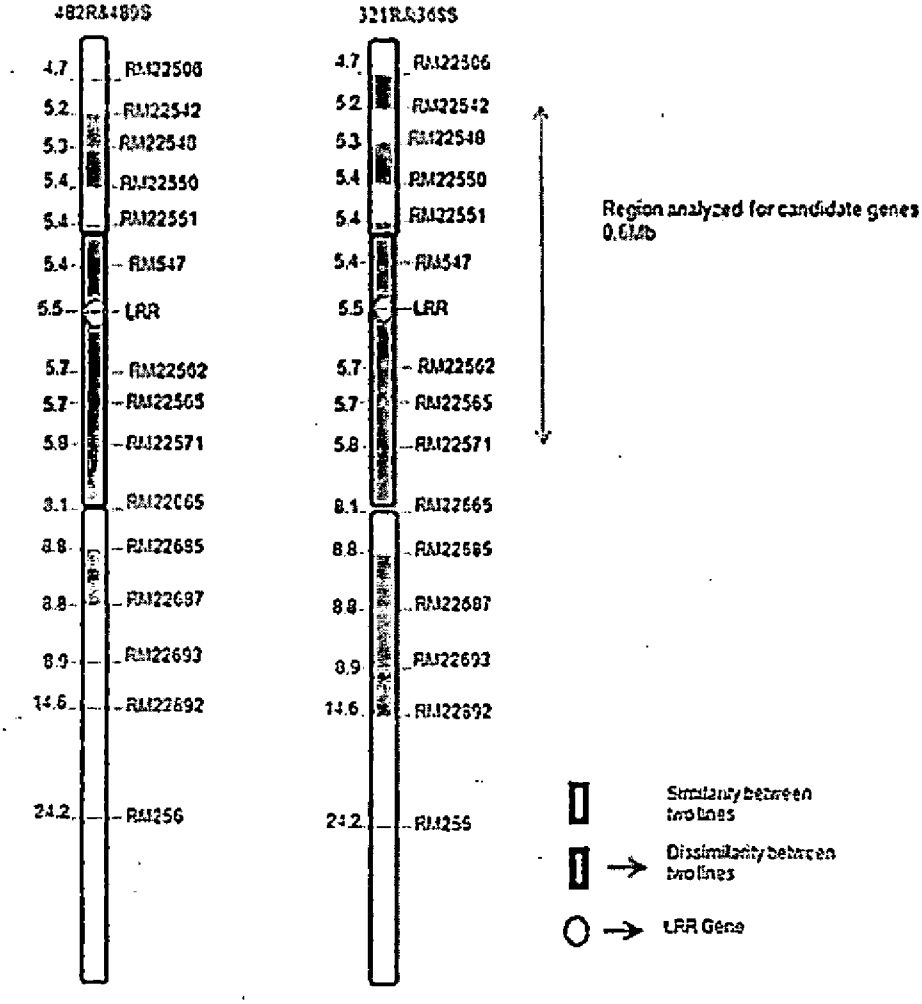


Figure 3. Map position showing the location of candidate gene LRR flanking the gall midge resistance gene Gm4 (Divya *et al.*, 2015)

bp-base pair, Chr.-chromosome, Trans.-transposon, retrotrans.-retrotransposon.

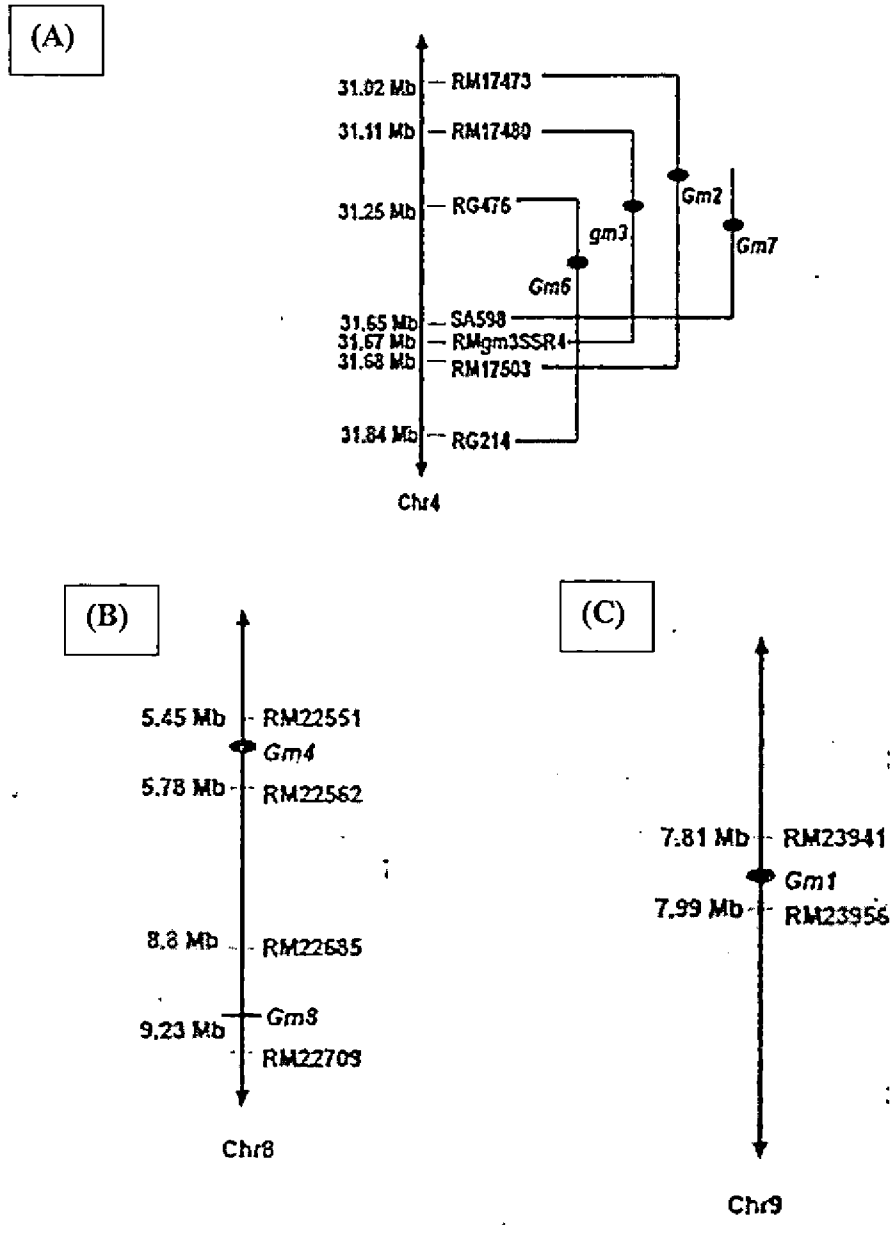


Fig. 4. Map positions of gall midge resistance genes *Gm1*, *Gm2*, *Gm4* and *Gm8* on respective chromosomes with reported markers on the right and their physical locations in Mb on the left. (Yasala *et al.*, 2012).

flanking SSR markers RM22685 and RM22709 by Sama *et al.* (2012). Further,*in-silico* analysis was made by Yasala *et al.* (2012) revealing several functional gene loci and later attempts narrowed down the search with a candidate gene coding for Proline Rich Protein as a marker for *Gm8,* which was validated by Divya *et al.* (2015). Using this marker-PRP, several genotypes were confirmed to have *Gm8* gene.

Divya *et al.,* (2016) reported that the indica rice cultivar Aganni, possess gall midge resistance gene *Gm8* and has wide range of resistance across gall midge biotypes 1, 2, 3 and 4 (Lakshmi *et al.,* 2006).

Arrikkirai, Aryan, Vellari, Kalluruli, Veluthavattan, Chettivirippu, Kaathikannan, Paluveliyan, Parambuvattan, Velutittaryan, Chenkayama and Karimundakan produced alleles similar to Aganni at 300bp confirming the presence of *Gm8* gene, while rest of the genotypes produced alleles at 350 bp in line with TN 1- the susceptible check.

These resistant lines can be used as resistant sources in pyramiding or breeding programmes as they confer resistance towards biotypes 1, 2, 3 and 4.

Certain varieties showed the presence of more than one gene of resistance under study. All the accessions with genes of resistance to gall midge biotype 5, prevailing in Moncompu were assessed further at field level to study the interactions with gall midge.

5.2. FIELD SCREENING

Field level screening for gall midge infestation was conducted at Moncompu, the gall midge endemic region in Alappuzha district, Kerala. The biotype of gall midge in Moncompu area was identified as GM Biotype 5 (Nair and Devi, 1994).

The varieties were classified into different groups as per the Standard Evaluation System (SES) of IRRI (IRRI, 2002). The data on field screening showed

that varieties, Arrikkirai, Urulan Kayama, Parambuvattan, Aruvakkari showed resistance reaction (infestation < 1%) comparable with the check W1263.

Bolgatti Kayama. Chuvanna IR 8, Vellari, Kalluruli, Velutittaryan, Chettivirippu, Kaathikannan, Thavalakkannan, Thekken Cheera, Ittikandappan, Nooranvella and Kirwana showed moderately resistant reaction with scale ranging from1-5 per cent.

Aryan, Chenkayama and Karimundakan were moderately susceptible while Velutha Vattan, Paluveliyan and Njavara Kunnathoor showed susceptible reaction. Among the checks, W1263 was resistant, Phalguna was moderately resistant, Aganni and Abhaya showed moderately susceptible and susceptible reaction respectively. TN1 showed a pronounced susceptibility (23.5%) to prevalent biotype of gall midge. TN1 is reported as susceptible check, as it is marked by presence of none of the genes for resistance (Bentur *et al.*, 2003).

Comparison of reaction to the pest and varieties with one gene, two genes, three genes and four genes for resistance are given in Tables 11, 12, 13 and 14 respectively.

Those varieties with single gene of resistance to gall midge are compared in Fig. 6.

Arrikkirai having *Gm1, Gm2* and *Gm8* showed resistance reaction (0.52%) to gall midge in field screening. Parambuvattan, which is having all the four genes (*Gm1, Gm2, Gm4* and *Gm8*) also showed resistance to gall midge at field level with a mean percentage gall infestation of 0.92%.

This study conclusively proves that *Gm1* is the gene conferring resistance to gall midge biotype 5 prevailing in Kuttandu rice tract of Kerala. Also the biotype of gall midge in Kuttanadu gives virulent reaction for the genes *Gm4* and *Gm8*. Different combinations of genes *Gm1* and *Gm2* can confer durable resistance.

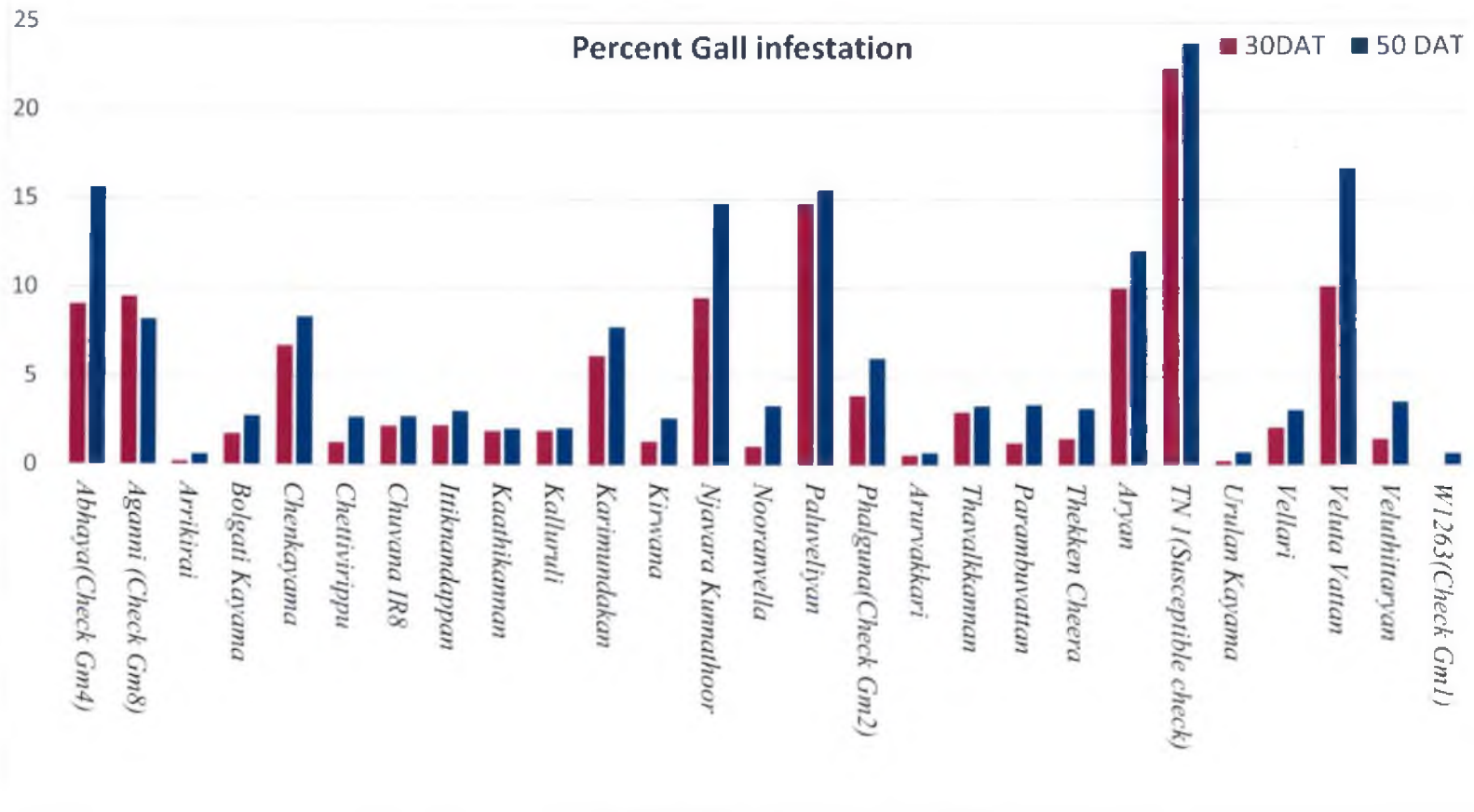


Figure 5. Per cent gall infestation at 30 DAT and 50 DAT.

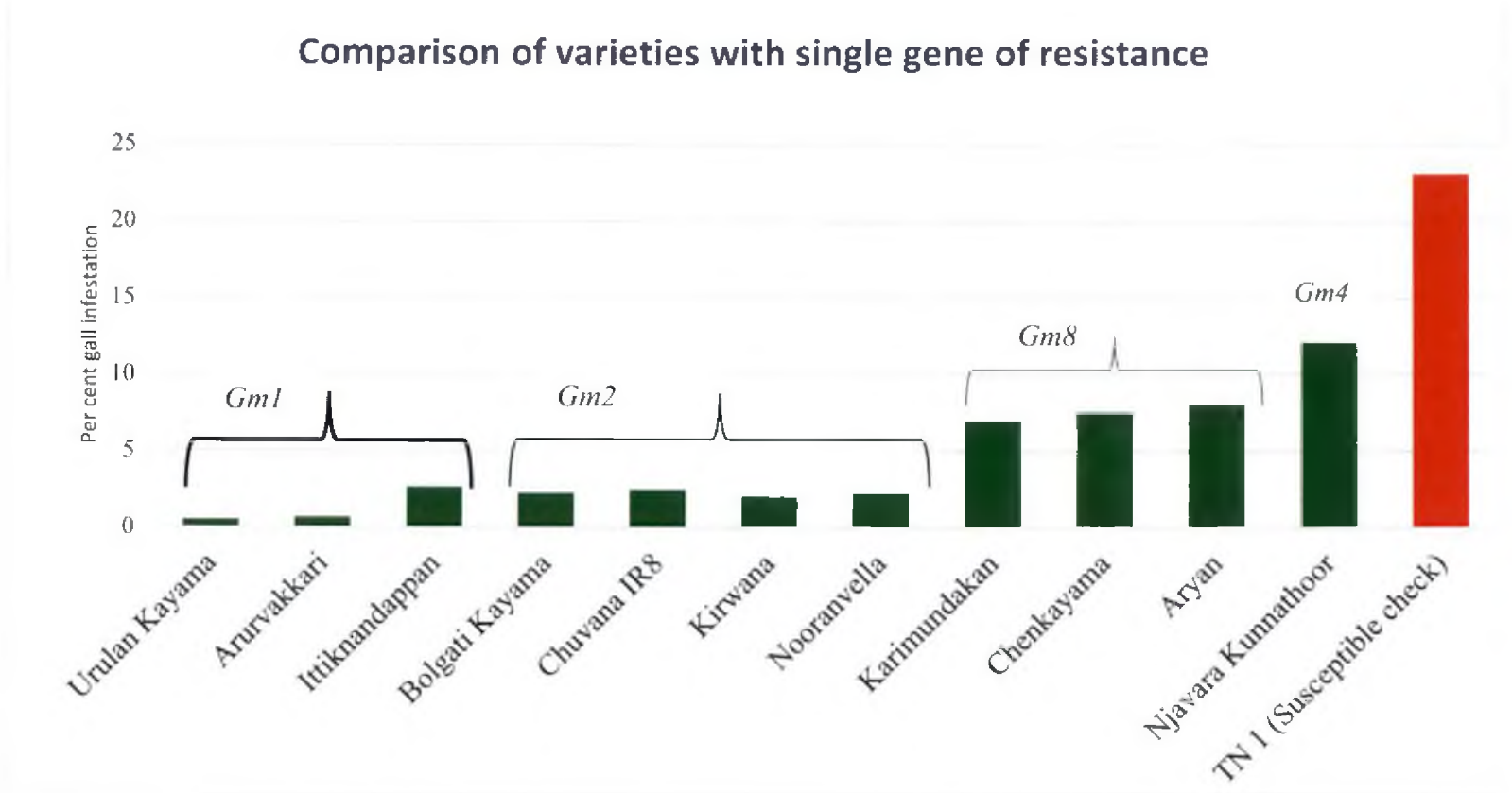


Figure 6. Comparison of gall infestation per cent in varieties with single gene *Gm1*, *Gm2*, *Gm4* and *Gm8*.

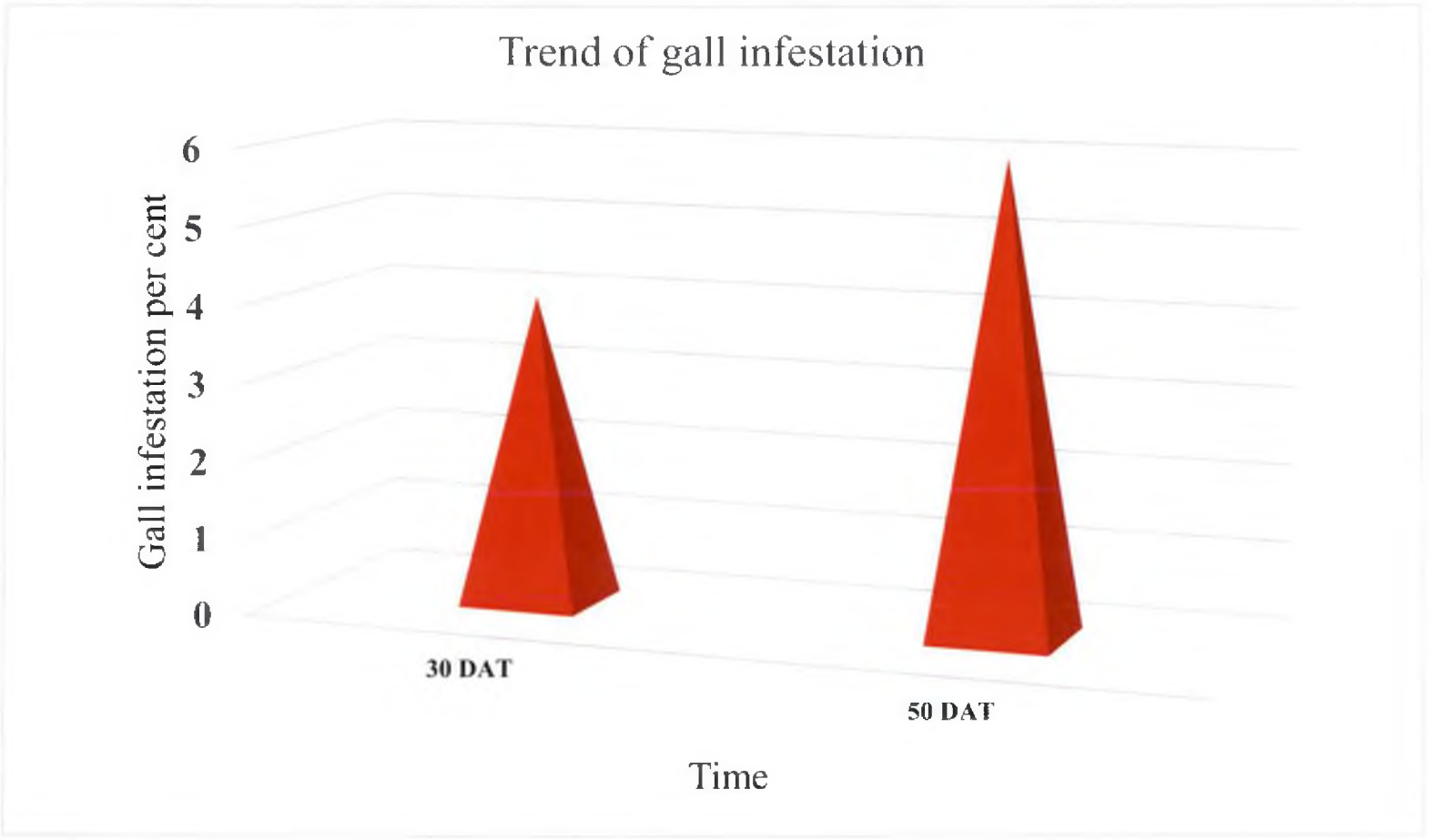


Figure 7. Trend of percentage gall infestation at 30 and 50 DAT.

TN 1 gave a mean gall infestation of 23.10 per cent, ranking it as susceptible, with more than 60 per cent of plants being infested.

Table 11. Varieties with one gene of resistance for gall midge.

Varieties	**Gene present**	**Reaction**
Aryan	*Gm8*	MS
Urulan Kayama	*Gm1*	R
Ittikanadappan	*Gm1*	MR
Aruvakkari	*Gm1*	R
Bolgatti Kayama	*Gm2*	MR
Chuvanna IR 8	*Gm2*	MR
Nooranvella	*Gm2*	MR
Kirwana	*Gm2*	MR
Karimundakan	*Gm2*	MR
Chenkayama	*Gm8*	MS
Njavara Kunnathoor	*Gm4*	S

Table 12. Varieties with two genes of resistance for gall midge

Varieties	Genes present	Reaction
Paluveliyan	*Gm4* and *Gm8*	S
Thavalakkannan	*Gm1* and *Gm4*	MR
Thekken Cheera	*Gm1* and *Gm4*	MR
Veluthittaryan	*Gm1* and *Gm8*	MR
Velutha Vattan	*Gm4* and *Gm8*	S

Table 13. Varieties with three genes of resistance for gall midge

Varieties	Genes present	Reaction
Arrikkirai	*Gm1*, *Gm2* and *Gm8*	R
Vellari	*Gm1*, *Gm4* and *Gm8*	MR
Kalluruli	*Gm1*, *Gm4* and *Gm8*	MR
Chettivirippu	*Gm1*, *Gm4* and *Gm8*	MR
Kaathikannan	*Gm2* , *Gm4* and *Gm8*	MR

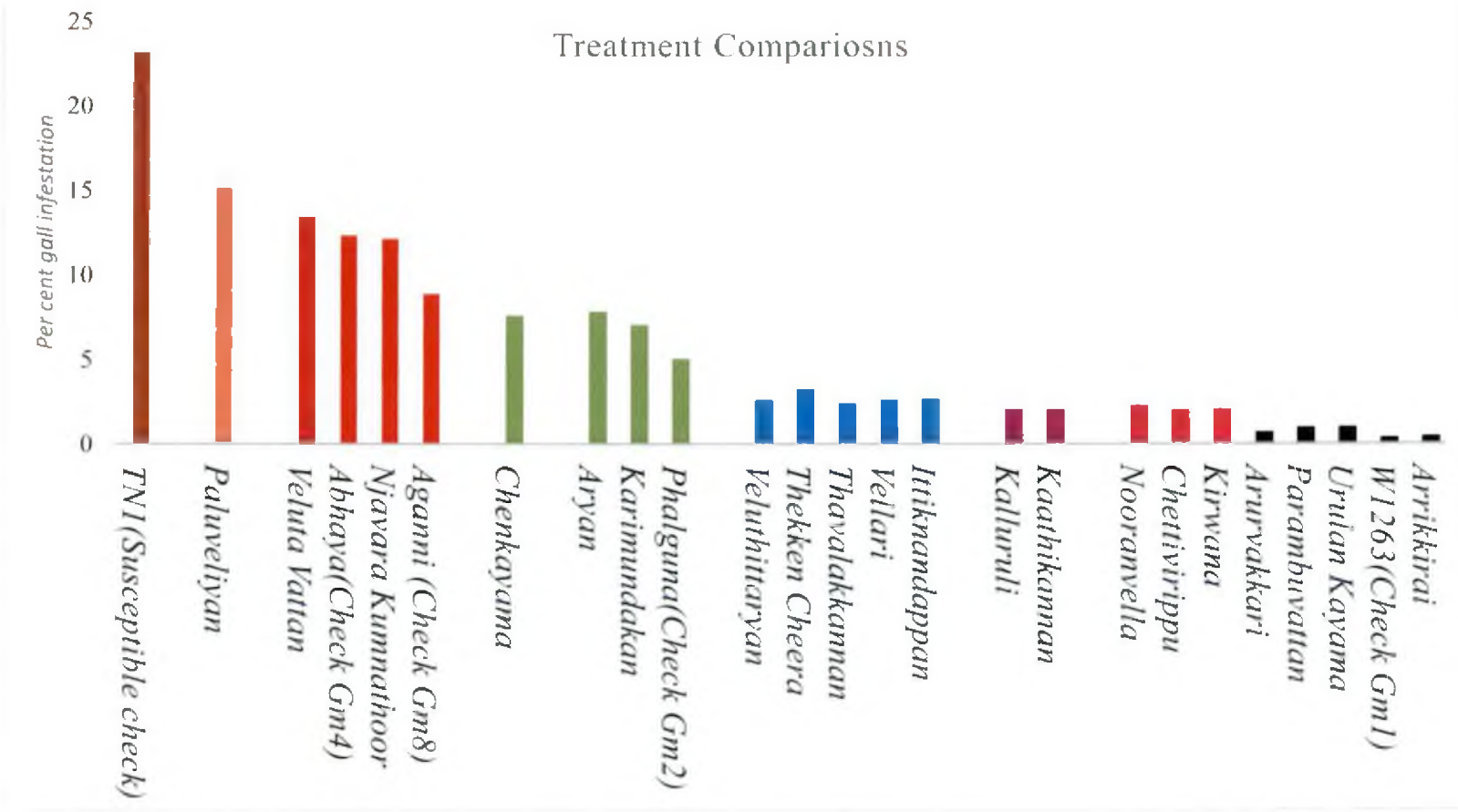


Figure 8. Treatment comparison of varieties under field screening.

(Similar coloured treatments are on par.)

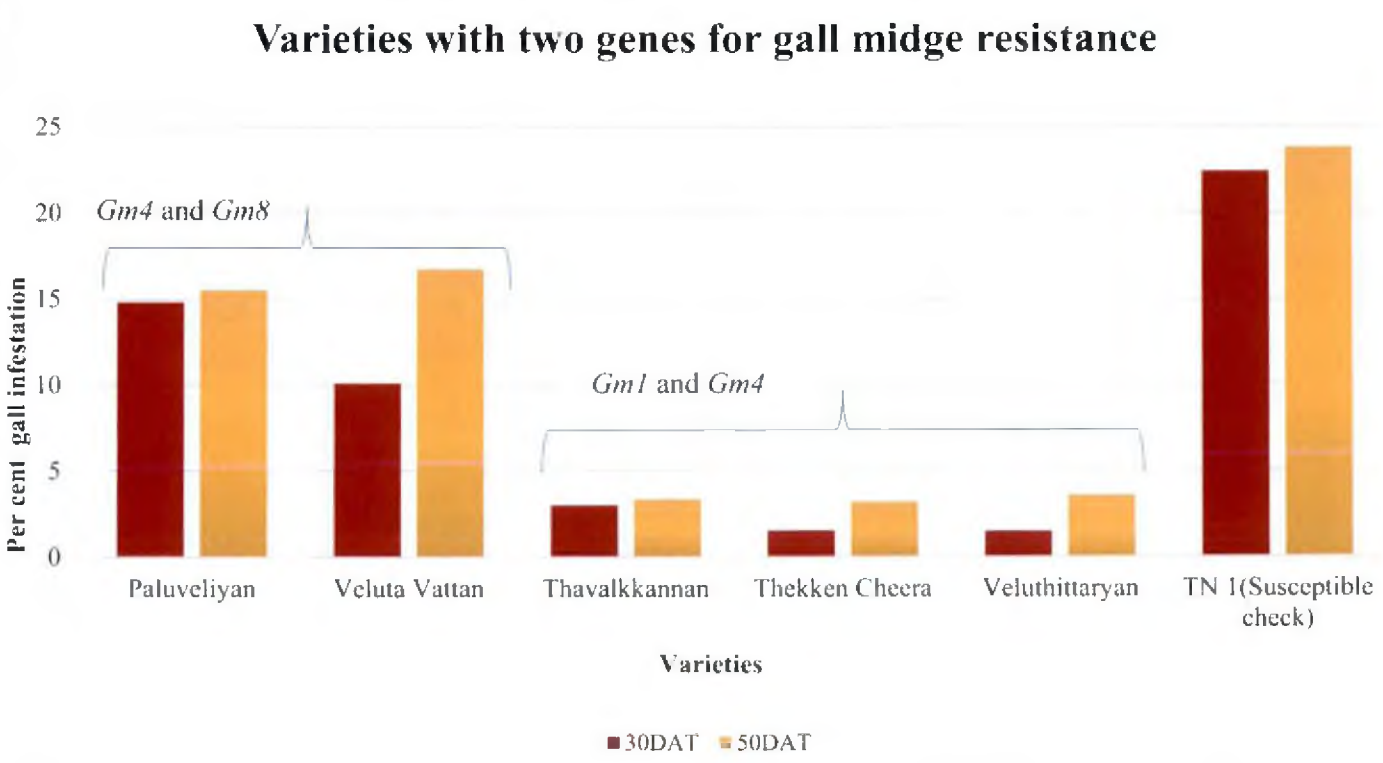


Figure 9. Comparison of gall infestation per cent in varieties with two genes for resistance.

5.2.1 Comparison of Infestation Score with Presence of Gene

The gall incidence score of the varieties with single gene *Gm1, Gm2, Gm4* and *Gm8* are compared in Fig.6. Results clearly showed that, the pest infestation in the varieties having the *Gm1* gene had less severity compared to the other genes. This was in agreement with earlier reports of Sastry *et al.* (1975), Chaudhary *et al.* (1985) and Reddy *et al.* (1997) stating that *Gm1* is a dominant gene present in the genome of W1263 and it offers durable resistance to gall midge biotypes. Later Bentur *et al.* (2003) demonstrated that *Gm1* confers multiple resistance not only against one biotype of the insect but against four Indian gall midge biotypes *viz.*, biotypes 1, 3, 5 and 6.

Varieties with *Gm2* scaled as moderately resistant with a range of 1-5 per cent gall infestation. *Gm4* and *Gm8* scored susceptible but in combination with *Gm1* or *Gm2* showed moderately resistant reaction. Underlying the reports of dominance of *Gm1* gene offering durable resistance against biotype 5.When these genes are taken in combination, the combination with the genes *Gm1* or *Gm2* showed least infestation score.

Gene pyramiding enables stacking of multiple genes in one variety leading to the simultaneous expression of more than one gene rendering durable resistance expression (Joshi and Nayak, 2010). Hence, the pyramiding of genes *Gm1* and *Gm2* can impart a better resistance to gall midge biotype 5 under Kerala condition.

Gall infestation per cent was observed at 30 DAT and 50 DAT showed a gradual increase at 50 DAT compared to the initial data (Fig. 7). The presence of *Gm1* or *Gm2* gene made the cultivars to remain in safer limits irrespective of other genetic counterparts. The varieties with *Gm1* and *Gm2* were on par in reaction, while those with *Gm4* and *Gm8* were similar in performance.

From this study on identifying donors of gall midge resistance from traditional rice varieties of Kerala, varieties with specific single gene alone for resistance as well as in combination were identified. These can be used for further crop improvement programmes. The varieties which have *Gm1* and *Gm2* genes can be used as donors for pyramiding these genes in the popular rice varieties by marker aided selection, to develop Essentially Derived Varieties (EDVs) to tackle the incidence of gall midge.

Summary

6. SUMMARY

The current study entitled "Identifying donors for gall midge resistance from traditional rice varieties by functional markers," was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Rice Research Station, Moncompu during the year 2015-16 with the objectives of identifying traditional rice varieties of Kerala with gall midge resistance genes *Gm1, Gm2, Gm4* and *Gm8* using associated functional markers and field level validation of the identified gene sources under pest stress condition.

This study made use of 26 traditional rice varieties of Kerala for identifying the resistance gene source at molecular and field levels. The varieties were screened for the presence of genes *Gm1, Gm2, Gm4* and *Gm8,* which are few major genes identified to confer resistance against different biotypes of rice gall midge. The Simple Sequence Repeats (SSR) markers RM444 linked to gene *Gm1*, a candidate gene marker LRR del linked to gene *Gm4*, PRP flanking *Gm8* and Sequence Characterized Amplified Region (SCAR) marker PF10 linked to gene *Gm2* were used to check the presence of these genes. Varieties like W1263, Phalguna, Abhaya and Aganni were used as resistant gene sources for these genes, *Gm1, Gm2, Gm4* and *Gm8* respectively.

Molecular marker analysis revealed the presence of *Gm1* gene in 11 varieties as they produced allele at 320bp as in the case with resistant gene source W1263. Seven varieties were confirmed with *Gm2* gene in their genome by producing an allele at 600bp, 10 varieties produced an allele at 350bp with LRR del primer confirming the presence of *Gm4* and 12 varieties confirmed the presence of *Gm8* gene with an allele at 300bp with PRP primer. Six varieties did not show the presence of any of these genes under study.

Field level screening of these identified varieties were done at Rice Research Station, Moncompu, which is an endemic region for gall midge biotype 5 of Kerala.

From the field screening results, four varieties, Aruvakkari, Urulan Kayama, Arrikkirai, Parambuvattan turned out to be resistant. Thirteen varieties viz, Bolgati Kayama, Chettivirippu, Ittikandappan, Kaathikannan, Chuvanna IR8 Kalluruli, Kirwana, Nooranvella, Phalguna, Vellari, Veluthittaryan, Thavalakkannan and Thekken Cheera were found to be moderately resistant. Three among them, Karimundakan, Chenkayama, and Aryan were moderately susceptible, while, Veluta Vattan, Paluveliyan, Njavara Kunnathoor seemed susceptible to prevalent biotype of Kerala. None of the varieties were highly resistant or highly susceptible.

Comparison of mean gall infestation data elucidated that the varieties with gene *Gm1* were showing resistance towards the gall midge biotype 5 of Kuttanadu region. Varieties with *Gm2*, showed moderate resistance while, varieties with *Gm8* and *Gm4* were moderately susceptible and susceptible respectively, thus proving that the biotype of gall midge in Kuttanadu has virulent action for the genes *Gm4* and *Gm8*.

Arrikkirai having *Gm1, Gm2* and *Gm8* showed resistance (0.52%) to gall midge in field screening. Parambuvattan, which is having all the four genes (*Gm1, Gm2, Gm4* and *Gm8*) also showed resistance to gall midge at field level with a mean percentage gall infestation of 0.92 per cent. Those varieties with *Gm1* gene were resistant and was on par in reaction with those with *Gm2* which offered moderate resistance towards the gall midge infestation.

When *Gm1* came along with *Gm4* and in combination with *Gm4* and *Gm8*, the varieties showed moderate resistance. When *Gm4* and *Gm8* genes were in combination, the varieties were susceptible. Also these varieties were on par in their mean gall infestation data.

This study conclusively proved that *Gm1* and *Gm2* genes can offer durable resistance to gall midge biotype 5 prevailing in Kuttanadu rice tract of Kerala. There are various traditional germplasm of rice which act as effective sources against various

insect biotypes all over the country. So by recognizing such resistant donors the existing cultivars can be improved by resorting to gene pyramiding programmes.

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**Originals not seen*

Identifying Donors for Gall midge Resistance from Traditional Rice Varieties by Functional Markers

by

ASHA A NAIR

(2014 – 11 - 122)

Abstract of the thesis
Submitted in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

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KERALA, INDIA

2016

ABSTRACT

Asian rice gall midge *Orseolia oryzae* Wood-Mason (Cecidomyiidae: Diptera) is regarded as a serious pest of rice with a number of biotypes. Genes *Gm1, Gm2, Gm4* and *Gm8* are few major genes identified to confer resistance against different biotypes of rice gall midge. The current study, entitled "Identifying donors for gall midge resistance from traditional rice varieties by functional markers," was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Rice Research Station, Moncompu during the year 2015-16 with the objectives of identifying traditional rice varieties of Kerala with gall midge resistance genes *Gm1, Gm2, Gm4* and *Gm8* using associated functional markers and field level validation of the identified gene sources under pest stress condition.

The Simple Sequence Repeats (SSR) markers RM444 linked to gene *Gm1*, LRR del linked to gene *Gm4*, PRP flanking *Gm8* and Sequence Characterised Amplified Region (SCAR) marker PF10 linked to gene *Gm2* were used to check the presence of these genes in the 26 traditional rice varieties under study. Varieties like W1263 for *Gm1* gene, Phalguna for *Gm2*, Abhaya for *Gm4* and Aganni for *Gm8* were used as checks for the presence of these genes and TN 1 was used as susceptible check for all the genes.

Among the 26 traditional rice varieties screened, 11 confirmed the presence of the gene *Gm1* by producing an allele at 320 bp with the primer RM444. Seven varieties confirmed *Gm2* gene in its genome by producing an allele at 600bp, 10 varieties produced an allele at 350bp with LRR del primer confirming the presence of *Gm4* and 12 varieties confirmed the presence of *Gm8* gene with an allele at 300bp with PRP primer.

Five varieties viz, Paluveliyan, Thavalakkanan, Thekken Cheera, Veluthittaryan and Velutha Vattan showed the presence of two genes in combination, another five varieties, Arrikkirai, Vellari, Kalluruli, Chettivirippu and Kaathikannan

confirmed the presence of three genes in combination and one variety, Parambuvattan showed the combination of all the four genes.

Screening of 22 rice varieties with resistance genes under pest stress condition at Rice Research Station, Moncompu, during kharif 2015 revealed that the varieties with gene *Gm1* showed resistance reaction towards the gall midge biotype 5 of Kuttanadu region. Varieties with *Gm2*, showed moderate resistance while, varieties with *Gm8* and *Gm4* showed moderately susceptible and susceptible reaction respectively. This shows that the biotype of gall midge in Kuttanadu has virulent action for the genes *Gm4* and *Gm8*.

Arrikkirai having *Gm1*, *Gm2* and *Gm8* showed resistance reaction (0.52%) to gall midge in field screening. Parambuvattan, which is having all the four genes (*Gm1*, *Gm2*, *Gm4* and *Gm8*) also showed resistance to gall midge at field level with a mean percentage gall infestation of 0.92%.

When *Gm1* came along with *Gm4* and in combination with *Gm4* and *Gm8*, the varieties showed moderate resistance whereas, *Gm4* and *Gm8* genes in combination produced susceptible reaction in the varieties.

This study conclusively proves that *Gm1* is the gene conferring resistance to gall midge biotype 5 prevailing in Kuttandu rice tract of Kerala. Different combinations of genes *Gm1* and *Gm2* can confer durable resistance. The varieties which have *Gm1* and *Gm2* genes can be used as donors for pyramiding these genes in the popular rice varieties by marker aided selection, to develop Essentially Derived Varieties (EDVs) to tackle the incidence of gall midge.