IDENTIFICATION OF BLAST TOLERANT RICE (*Oryza sativa* L.) GENOTYPES USING GENOME WIDE ASSOCIATION MAPPING

M. Sc. (Ag.) Thesis

by

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2018

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Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Anannya Susan

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science

in

Agriculture

(Genetics and Plant Breeding)

UE ID.20161725122 Student ID.120416006

JULY, 2018

CERTIFICATE-I

This is to certify that the thesis entitled "**Identification of Blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping**" submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), is a record of the bonafide research work carried out by **Anannya Susan** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or certificate course. All the assistance and help received during the course of the investigations have been duly acknowledged.

Co-Chairman
Date: 20.07.18

Chairman
Date: 20.07.18

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

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Member	(Dr. J. L. Salam)	
Member	(Dr. R. S. Netam)	
Member	(Dr. Ravi R. Saxena)	

CERTIFICATE-II

This is to certify that the thesis entitled "**Identification of Blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping**" submitted by **Anannya Susan** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the Department of **Genetics and Plant Breeding** has been approved by the external examiner and Student's Advisory Committee after an oral examination.

17/8/18

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Director of Instructions _____

ACKNOWLEDGEMENT

Towards the end of my endeavour, it's the right moment to express my profound etiquette to all those who have directly or indirectly helped me accomplish this job because research work and its documentation cannot be a single person's job, it needs assistance from all quarters of scientific community to keep oneself updated. I start with expressing my indebtedness to everyone who generously imparted their help without any faltering.

First and the foremost, praise and thanks to The Almighty, for bestowing me with health and courage throughout the course to complete this manuscript.

I feel the inadequacy of words to express my deep sense of gratitude and profound indebtedness to ***Dr. Sonali Kar****, Scientist, Section of Genetics and Plant Breeding and Chairperson of my Advisory Committee for her valuable guidance, constant supervision, constructive criticism, vivid encouragement and affectionate dealing throughout the period of investigation and during preparation of the thesis.*

I am also grateful to my co-chairperson, ***Dr. N. Umakanta****, Scientist, Department of Plant Biotechnology, National Rice Research Institute, Cuttack. His suggestions while planning and executing the experiments were helpful in bringing a sense of direction to the whole investigation. I am grateful to* ***Dr. A. K. Sarawgi,*** *Professor and Head, Department of Genetics and Plant breeding, College of Agriculture, IGKV for providing all sorts of departmental facilities and extending sincere help during the tenure of my research work. I am grateful to* ***Dr. S. C. Mukherjee****, Dean, S. G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur, for providing me the facilities for my thesis work and for all necessary help which led to the completion of my thesis. I would also like to thank my Advisory Committee members,* ***Dr. J. L. Salam****, Scientist & Head of Section, Section of Genetics and Plant Breeding,* ***Dr. R. S. Netam****, Senior Scientist, Plant Pathology, S. G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur, and* ***Dr. R. R. Saxena,*** *Professor, Department of Agricultural statistics and Social Science, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, for their timely*

suggestions, valuable counsel and keen interest from the beginning of this investigation which helped me shape this manuscript in its present form.

I express my heart-felt indebtedness to my co-supervisor ***Dr. M. K. Yadav****, Junior Scientist, National Rice Research Institute (NRRI), Cuttack, for his constant support and encouragement while carrying out the research work. His suggestions while planning and executing the experiments have been helpful in bringing a sense of direction to the whole investigation and I would also like to thank him profusely for his kind help and support while writing the thesis. I would like to record my special thanks to the Department of Plant Pathology, NRRI for providing all facilities during the course of my study.*

The support received from ***Mr. Kaanu*** *and* ***Mr. Pintu****, Technical assistants in carrying out the sporulation, lab and field work related to the research study were indeed significant and thankfully acknowledged. Thanks are also due to all other scientists, technical staff and administrative staff of NRRI for the help and support received.*

I express my warmest love and affection to my parents ***Mrs. Ligi Joy*** *and* ***Mr. Joy P. P.****, to my beloved sister* ***Angel Susan****, to my brother-in-law* ***Eldho Varghese*** *without whose inspiration, support and care I would have never achieved this level of education.*

I express my deep sense of honour and heartfelt regards to my friends, seniors and labmates (Fasna, Nisha mam, Sangeeta mam, Amrita, Kusumlata, Namrata, Madhu Di, Debarchana Di, Manjusha, Subhashree and Sashmita) for their support and help throughout my research work.

I apologize for the faux pass of the persons who have extended the help in a way or other and deserved such thanks. All are not mentioned, but none is forgotten.

Date

Anannya Susan

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LIST OF NOTATIONS/SYMBOLS

%	Percent
@	At the rate of
^{0}C	Degree centigrade
CG	Chhattisgarh
cm	Centimeter
et al.	And co-worker/ and others
G	Gram
i.e.	That is
Kg	Kilogram
L	Litre
cM	Centimorgan
mL	Millilitre
mg	Milligram
mm	Millimetre
ng	Nano gram
pg	Pico gram
µg	Microgram
No.	Number
Sr. No.	Serial number
µl	Micro litre
viz.,	Namely
bp	base pair
mM	Millimole
pM	Picomole
ha	hectare
psi	pounds per square inch
M	Molar
rpm	Revolutions per minute
λ	lambda
V	Volt
min	Minutes
sec	Seconds
∞	Infinity

LIST OF ABBREVIATIONS

SSR	Simple Sequence Repeats
SNP	Single Nucleotide Polymorphisms
GWAS	Genome Wide Association Studies
OMA	Oatmeal Agar
CTAB	Cetyl-Trimethyl Ammonium Bromide
DNA	De-oxy ribo Nucleic acid
NRRI	National Rice Research Institute
R	Resistant
QTL	Quantitative Trait Loci
bp	Base pair
AMOVA	Analysis of Molecular Variance
PcoA	Principal Cordinate Analysis
GLM	General Linear Mode
MLM	Mixed Linear Mode
NE	North-East
AD	Ad mixture
NRVs	NRRI Released Varieties
PIC	Polymorphic Information Content
PCR	Polymerase Chain Reaction
QQ	Quantile-Quantile
EDTA	Ethylene Diamine Tetra Acetate
TBE	Tris/Borate/EDTA
UBN	Uniform Blast Nursery
DAS	Days After Sowing
SES	Standard Evaluation System

शोध सार

शोध का शीर्षक	:	"जीनोम वाइड एसोसिएशन मैपिंग के द्वारा धान के झुलसा रोग प्रतिरोधक जीन प्रारूपों की पहचान"
छात्रा का पूरा नाम	:	अनन्या सूसन
प्रमुख विषय	:	अनुवांशिकी एवं पादप प्रजनन विभाग
प्रमुख सलाहकार का नाम एवं पता	:	डॉ० सोनाली कर, वैज्ञानिक, अनुवांशिकी एवं पादप प्रजनन प्रभाग, श० गु० कृषि महाविद्यालय एवं अनुसंधान केन्द्र, कुम्हरावंड, जगदलपुर, इ० गा० कृ० वि० वि० रायपुर (छ० ग०)
उपाधि से सम्मानित किया जाना है	:	एम ० एस सी० (कृषि)

प्रमुख सलाहकार के हस्ताक्षर

छात्रा के हस्ताक्षर

दिनांक 20.07.18

विभागाध्यक्ष के हस्ताक्षर

धान मे झुलसा रोग *मेगनापोरथे ओराइजे* नामक फफूंद द्वारा होता है जो धान के ऐसी हानिकारक रोगों मे प्रमुख है, जिससे पूरे विश्व मे हानि होती है। कम लागत व पर्यावरण मैत्रिक रोकथाम विधि से आर जीन का उपयोग कर, आर जीन के अनुवांशिकीय विविधता की झुलसा रोग में धान में समझ देशी किस्मों की विविधता को संजोने में महत्वपूर्ण होगी, जिनमें जैविक एवं अजैविक तनाव के प्रति गुण अंवेषण करना है।

highly resistant (123), moderately resistant (217) and susceptible (161). The landraces harbour a range of zero to nine blast resistance genes with the genetic frequencies of the eighteen major blast resistance genes varied from 6.25% to 27.43%. The cluster analysis grouped entire landraces into three major groups. Population structure along with other parameters was also analyzed to understand the evolution of blast resistance in rice. The population structure analysis and principal coordinate analysis classified the landraces into three sub–populations. Analysis of molecular variance showed maximum (97%) diversity within the population and least (3%) between populations. Among 19 blast resistance gene specific markers analyzed, six markers (Pi56, RM72, Tk59-2, pi21, RM1233 and RM6648) corresponding to six resistance genes (*Pi56, Pi33, Pit, pi21, Pi1* and *Pish*) were found to be significantly associated with the blast disease explaining a phenotypic variance of 1.1% to 6.4% which could be used in marker-assisted rice breeding programs for gene pyramiding against the blast disease.

The association of five SSR markers for leaf blast resistance was determined using genome-wide association analysis. The associated markers can be utilized for identification of minor QTLs for leaf blast resistance. The resistant landraces in the present study represents a valuable genetic resource for blast disease in rice to be utilized for future genomic studies, host–pathogen interaction, identification of novel *R* genes and rice improvement strategies.

THESIS ABSTRACT

Title of the Thesis	:	Identification of Blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping.
Full Name of the Student	:	Anannya Susan
Major Subject	:	Genetics and Plant Breeding
Name and Address of the Major Advisor	:	Dr. Sonali Kar Section of Genetics and Plant Breeding S.G. College of Agriculture and Research Station, Jagdalpur, IGKV, Raipur-492012
Degree to be Awarded	:	M.Sc. (Ag.)

Signature of Major Advisor

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Date: 20.07.18

16.07.2018
Signature of Head of the Department

ABSTRACT

Rice blast disease caused by the fungus *Magnaporthe oryzae* is one of the most devastating disease causing enormous losses worldwide. The utilization of *R* genes against the blast disease is the most widely, economically viable and environment friendly choice for the control of this disease. Therefore, understanding of genetic diversity of *R* genes is important to explore existing diversity preserved in the landraces which are well known reservoirs of important traits for biotic and abiotic stresses.

In the present study, **"Identification of Blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping"** the genetic diversity of eighteen major blast resistance gene loci and genome wide association study using SSR markers were investigated in landraces originated from Chhattisgarh and North Eastern India. Based on phenotypic evaluation, landraces were classified into three distinct groups:

वर्तमान में किए गए अध्ययन " जीनोम वाइड एसोसिएशन मैपिंग के द्वारा धान के झुलसा रोग प्रतिरोधक जीन प्रारूपी की पहचान" में झुलसा रोग के अठारह प्रमुख प्रतिरोधक जीन स्थान की अनुवांशिकीय विविधता तथा एस एस आर चिन्हक द्वारा जीनोम वाइड एसोसिएशन का अध्ययन छत्तीसगढ़ व उत्तर पूर्वी भारत के धान की देशी किस्मों में किया गया। इन देशी किस्मों का प्रारोपी विश्लेषण कर तीन भिन्न दलों मे वर्गीकृत किया : अति प्रतिरोधी (123), मध्यम प्रतिरोधी (217) व संवेदनशील (160)। देशी किस्मों में शून्य से नौ तक के झुलसा रोधक जीन पाए गएए जिनमें अठारह प्रमुख झुलसा रोग प्रतिरोधक जीन्स की आवृत्ति 6.25% से 27.43% के बीच थी। क्लस्टर विश्लेषण द्वारा समस्त किस्मों को तीन प्रमुख दलों में विभाजित किया। जनसंख्या संरचना विश्लेषण व प्रमुख तुल्य विश्लेषण द्वारा देसी किस्मों को तीन उप जनसंख्य दलों में विभाजित किया गया। आण्विक विभिन्नता के विश्लेषण से यह ज्ञात हुआ कि अधिकतम (97%) भिन्नता जनसंख्या के अन्तर्गत तथा निम्न (3%) जनसंख्या के मध्य थी। 20 झुलसा रोग प्रतिरोधक जीन विशिष्ट चिन्हको के विश्लेषण में छरू चिन्हक (Pi56, RM72, Tk59-2, pi21, RM1233 व RM6648) छ: अनुरूप प्रतिरोधक जीन्स (*pi56*, *pi33*, *pit*, *pi21*, *pit* व *pish*) से संबंधित पाए गएए जिससे यह पता चलता है कि प्रारूपिक विभिन्नता 1.1% से 6.4% तक हैए जिसे धान के प्रजनन कार्यों के चिन्हक सहाय कार्य के माध्यम से झुलसा रोग के लिए जीन पिरामिडिंग में प्रयोग किया जा सकता है।

पर्ण झुलसा रोग प्रतिरोधकता के प्रति पांच एस एस आर चिन्हको के साहचर्य को जीनोम वाइड एसोसिएशन विश्लेषण से ज्ञात किया गया। संबंधित चिन्हक को पर्ण झुलसा रोग प्रतिरोधकता के लिए लघु क्यू टी एल की पहचान के लिए किया जा सकता है। प्रस्तुत अध्ययन में प्रतिरोधक देशी किस्म के धानो का झुलसा रोग के लिए एक बहुमूल्य अनुवांशिकीय संसाधन हैं जिनका उपयोग भविष्य में अनुवांशिकीय अध्ययनों में पोषिता रोगजनक परस्पर क्रिया में नए आर जीन्स की पहचान में तथा धान सुधार कार्यों में किया जा सकता हैं।

CHAPTER – I
INTRODUCTION

The cultivated rice *(Oryza sativa* L.) belongs to the family *Poaceae* with diploid chromosome number, 2n=24. Cultivation of rice dates back to 3000 B.C. Rice is the most valuable and primary food crop for more than 50% of the world's population (Khush, 2005). More than 90% of the world's rice is grown and consumed in Asia where 60% of the Earth's people live. It accounts for 35–75% of the calories consumed by more than 3 billion Asians. It is planted on about 154 million hectares annually or on about 11% of the world's cultivated land (Khush and Jena, 2009). It contributes 23% of the calories consumed by the global human population and is the most important food product in Asia (Wilson and Talbot, 2009).

India is the second largest producer of rice and total production of rice in India in the year 2016-17 was estimated at 104.32 million tonnes which is lower by 1.17 million tonnes than the production of 105.48 million tonnes during the preceding year (Anonymous, 2017). It is grown in a wide range of climatic conditions and is probably the most diverse crop with the ability to be grown in a wide range of elevation of 300 m in Nepal to 3 m below mean sea level in Kerala, India. Even with its diverse nature the production and productivity of the crop is not enough for the ever growing population. The world will need about 25% more rice by the year 2030 to meet the estimated demand of an increasing global population (Wani and Sah, 2014). One way to meet this challenge is to grow rice on more area, which is difficult due to increasing urbanization and escalating population in underdeveloped countries. The other option is to improve varieties and increase per hectare yield by reducing loss in yield due to biotic and abiotic stresses. Rice productivity is adversely impacted by numerous biotic and abiotic stresses. Diseases and insect pests are the major biotic agents causing significant yield losses. An approximate 52% of the global production of rice is lost annually owing to the damage caused by biotic factors (Yarasi *et al.,* 2008).

Among the biotic factors disease is the most important factor which results in crop losses of $ 5 billion every year (Asghar *et al*., 2007). Of particular concern is rice blast caused by filamentous ascomycete *Pyricularia oryzae* Cavara (*Magnaporthe grisea* (Herbert) Barr) (Miah *et al.,* 2013). Rice blast disease can cause 10% to 30% yield loss depending on the susceptibility of the variety (Talbot, 2003) and up to 100% during an epidemic (Khush and Jena, 2009). This disease was recorded from 85 countries and it is estimated to cause 14-18% grain yield losses worldwide (Mew and Gonzales, 2002). The yield losses due to pests and diseases are estimated to be around 37% of which blast accounts to 14-18%.

Rice blast was first recorded in China (1637) later from Japan (1704). In India, the disease gained importance when a devastating epidemic occurred in Thanjavur (Tanjore) delta of Tamilnadu during 1919. It can infect rice plant right from seedlings to adult plant stages affecting leaves, nodes, collar, panicles and roots but causes the greatest losses when necks and panicles are infected, it has been leading to severe yield losses worldwide and threatening global food security (Liu *et al.*, 2014). This loss in rice yield should be minimized in order to help the marginal and poor farmers of developing countries (Latif *et al*., 2011). The disease can be managed by the use of fungicides, resistant cultivars, agronomic practices and biotechnological methods (Ribot *et al.*, 2008). The overuse of fungicides prompts the evolution of resistance in the disease, which in turn leads to disease resurgence. Therefore, the exploitation of host plant resistance has generally been considered as one of the most economical and environmentally friendly approaches to combat the disease (Khush and Jena, 2009).

Consequently, breeding blast resistant cultivar is an objective to stabilize the yield of rice. The first and foremost strategy in developing resistant varieties is identification of broad-spectrum durable resistance gene and incorporation of the gene/s in the background of the agronomically superior genotypes. Molecular breeding approaches facilitate the early and efficient selection for resistance genes. DNA marker-based technology is being increasingly used to overcome difficulties of classical plant breeding which mainly used phenotypic characters like disease resistance. It also paves the way for selecting the target gene based on DNA marker with a predictable rate of accuracy. Among all the DNA markers,

microsatellites or simple sequence repeats (SSRs) are efficient as they are co-dominant in nature; that shows high allelic diversity and are easily and economically assayed by PCR and can be automated. Many potential SSR markers have been identified in rice and over 25,000 have been developed as molecular markers (Temnykh *et al.*,2000; McCouch *et al.*,2002). These molecular markers have been effectively utilized for many purposes including genome mapping, assessment of the genetic diversity and relatedness among various cultivars and marker-aided selection breeding.

Regarding the genetic basis of the resistance to *M. oryzae*, more than 86 dominant R genes and approximately 350 QTLs for resistance to rice blast have been identified, and 23 of them have been molecularly characterized: i.e. *pb1, Pi-a, Pi-b, Pi-d2,Pi-d3, Pi-k, Pik-h/Pi-54, Pik-m, Pik-p, Pi-sh, Pi-t, Pi-ta, Piz-t, Pi-1, Pi-2/Piz-5, Pi5, Pi-9, pi-21, Pi-25, Pi-36, Pi-37, Pi-35* and *Pi-64* up to date (Ma *et al.*, 2015). However, as each of these R genes usually act only against a subset of existing pathogen races, the identification of new R genes/alleles is still essential to the breeding of endurably resistant varieties (Miah, 2013). The frequent change in pathogenicity or escape from host recognition requires the continuous identification of new sources of host disease resistance against continuously evolving and geographically diverse pathogen races.

Generally, R genes for *M. oryzae* are identified in landraces, cultivars or wild rice. The development of improved rice cultivars has led to the replacement of landraces and traditional varieties by modern cultivars, which has resulted in a decline in the diversity of agriculturally used rice (Yan *et al.*, 2017). However, the diversity lost in the elite materials is somewhat preserved in crop gene banks, wild rice collections and breeding resources, thus, these rice materials provide the basis for genetic improvement of crops for specific traits and represent rich sources of novel allelic variation. The landraces and wild relatives may not possess favorable agronomic traits (such as plant height or yield) suitable for modern breeding programs, but they are highly relevant sources of genes for specific traits, including resistance or tolerance to biotic and abiotic stresses (Vasudevan *et al.*, 2014).

Keeping these points in view, to identify candidate accessions that can be deployed as donors in rice breeding programs and as starting material for the isolation of novel rice blast resistance genes, various rice genotypes which include landraces and gene bank rice accessions have been taken to study the following objectives:

1. Screening of rice germplasm for resistance to blast in uniform blast nursery.
2. To map novel/known blast R genes/QTL in rice germplasm.
3. To characterize novel blast R genes/QTL in rice.

CHAPTER – II
REVIEW OF LITERATURE

The comprehensive survey of literatures relevant to experiments of this investigation is reviewed and presented under the following headings.

2.1 Rice and its Importance

2.2 Blast Disease

2.3 Molecular Markers for Blast resistance

2.4 Breeding for Resistance to Blast

2.5 Screening of rice germplasm for Blast resistance

2.6 Genome Wide Association Studies (GWAS) in rice

2.1 Rice and its Importance

Brar and Khush (2003) reported that rice belongs to the family poaceae (gramineae), tribe *Oryzeae.* This tribe has 11 genera (*Chikusiochloa, Hygroryza, Leersia, Luziola, Prosphytochloa, Rhynchorya, Zizania, Zizaniopsis, Porteresian, Potamophila* and *Oryzea*). The genus *Oryza* covers 25 recognized species, of which 23 are wild species and two, *O. sativa* and *O. glaberrima* are cultivated.

Linscombe (2006) reported that rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%.Rice grain contains on an average 7% protein, 62-65% starch, 0.7% fat & 1.3% fibre and rice is the main source of vitamin B1(thiamin), B2 (riboflavin), B3 (niacin) & B5 (panthothenic acid). The biological value of rice 63% whereas biological value of wheat and rice is 49% & 36%. The domesticated rice contains of two species of food crop in Poaceae ("true grass") family: *Oryza sativa* and *Oryza glabberima*.

Yarasi *et al.* (2008) reported that rice productivity is adversely impacted by numerous biotic and abiotic stresses. Diseases and insect pests are the major biotic agents causing significant yield losses. An approximate 52 per cent of the global production of rice is lost annually owing to the damage caused by biotic factors.

Khush (2013) reported that rice (*Oryza sativa* L.) is the staple food for more than half of the world's population.

Elert (2014) reported that rice provides more than 19% of the calories consumed by the world population. Global rice demand is estimated to rise from 6.76 × 108 t in 2010 to 8.52 × 108 t in 2035.

Anonymous (2017) stated that India is the second largest producer of rice and total production of rice in India in the year 2016-17 is estimated at 104.32 million tonnes which is lower by 1.17 million tonnes than the production of 105.48 million tonnes during the preceding year.

2.2 Blast Disease

Couch and Kohn (2002) studied Rice blast, caused by a fungus *Magnaporthe oryzae*, causes lesions to form on leaves, stems, peduncles, panicles, seeds and even roots. So great is the potential threat for crop failure from this disease, that it has been ranked among the most important crop diseases.

Oerke and Dehne (2004) reported that one of the main limitations in production of rice is rice blast disease caused by the fungus *Magnaporthe oryzae.* Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production.

Chandrasekhara *et al*. (2008) mentioned that rice blast caused by *P. oryzae* is one of the devastating disease of rice resulting in yield losses up to 65% in susceptible rice cultivars.

Nutsugah *et al.* (2008) reported that in West Africa, the largest area of African production, this pathogen is the main constraint to production with yield losses ranging from 3-77%. The fungus is able to infect plants at all stages of growth and development in both upland and lowland rice production systems. Lowland rice produced in temperate and subtropical climates of Asia are highly susceptible to the pathogen, while tropical upland areas are susceptible only under irrigation.

Mahesh *et al*. (2012) reported that under traditional system of rice cultivation and in System of Rice Intensification (SRI) methods, the damage of blast in terms of grain yield was recorded as 8.2 and 7.5% respectively. The yield losses due to

pests and diseases are estimated to be around 37% (IRRI, 2014) of which blast accounts to 14-18 per cent.

Hasan *et al.* (2015) stated that blast disease is distributed in about 85 countries in all continents in both lowland and upland conditions and it is considered the most destructive pathogen of rice worldwide and accounts for 50% of the yield losses.

2.2.1 Blast Pathogen

Kuyek (2000) reported that in its sexual state, the fungus *Magnaporthe oryzae* feeds on the rice plant, causing severe damage. It attacks different parts of the plant includes, the collar, which can ultimately kill the entire leaf blade; the stem, which turns blackish and breaks easily called node blast; the neck of the panicle, where the infected part is girdled by a grayish brown lesion, or in severe cases, causes the panicles to fall over; or on the branches of the panicles which exhibit brown lesions when infected.

Talbot *et al.* (2003) found that infection by the rice blast fungus starts when the three-celled conidium lands on a host leaf and anchors itself to the leaf cuticle with spore-tip mucilage. Germination proceeds with the extension of a germ tube, which undergoes hooking and swelling at its tip and then differentiates into an infection structure called the appressorium. During maturation, the appressorium becomes melanized, except for a well-defined pore between the appressorium and the rice leaf. The formation of this infection structure on the host surface marks the onset of the disease. A penetration peg is then driven through the host surface and the infection hypha invades and grows through the rice leaf. They also mentioned that plant diseases are often severe during periods of warm temperatures and high moisture. Cloudy overcast weather, dew drops encourage blast spread and the fungus sporulates profusely from disease lesions under conditions of high humidity

Dean *et al.* (2005) reported that the causal organism of blast, *P. oryzae* is a haploid filamentous *Ascomycete* with a relatively small genome of ~40 Mb divided into seven chromosomes.

Caracuel-Rios *et al*. (2007) stated that *P. oryzae* shares many characteristics associated with other important cereal pathogens, such as appressorium formation and intracellular tissue invasion.

Scheuermann *et al.* (2012) mentioned that the anamorph *P. oryzae* produces piriform shaped conidia with one to two transversal septa, slightly darked or hyaline, linked to conidiophore by its larger bottom. Conidiophores are septated, simple, rarely branched, showing sympodial growth and slightly browned. The teleomorph *P. oryzae* has not been found in nature, but it has been produced after crossing appropriate compatible isolates in laboratory. The teleomorph stage produces hyaline ascospores, typically fusiform shaped with three-septate and involved by a *Unitunicate asci.*

Ashkani *et al*. (2015) reported that rice blast disease, caused by *P.oryzae* is considered to be a major threats to rice production worldwide and it has recently emerged as a model organism for the investigation of plant diseases caused by fungi, largely because of its economic importance, but also owing to the experimental tractability of the fungus.

2.2.2 Blast symptoms

Seebold *et al.* (2004) reported that the fungus Pyricularia oryzae attacks at all stages of the crop and symptoms appear on leaves and nodes.

Sesma & Osbourn (2004) stated that blast is known to attack nearly all above ground parts as well as during all growth stages of plant. Recent reports have shown that the fungus has the capacity to infect plant roots also.

Ram *et al*. (2007) reported that leaf blast fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. When the last node is attacked, it causes partial to complete sterility.

Tebeest *et al.* (2007) found that the symptoms on leaves may vary according to the environmental conditions, age of the plant, and level of resistance of the host cultivars. On susceptible cultivars, lesions may initially appear gray-green and

water-soaked with a dark green border which expand rapidly to several centimeters in length often becoming light tan in color with necrotic borders. On resistant cultivars, lesions often remain small in size (1-2 mm) and brown to dark brown in color.

Nutsugah *et al*. (2008) stated that the fungus is able to infect plants at all stages of growth and development in both upland and lowland rice production systems. Lowland rice produced in temperate and subtropical climates of Asia are highly susceptible to the pathogen, while tropical upland areas are susceptible only under irrigation.

Castilla *et al.* (2009) reported that rice blast pathogen infects all the above ground parts of rice plants at different growth stages, *i.e.*, leaf, collar, nodes, internodes, base or neck and other parts like panicle and leaf sheath. They stated that a typical blast lesion on rice leaf is grey at the center with a dark border and is spindle shaped. The environment with frequent and prolonged dew periods and with cool temperature in day time is most favourable for the spread of the disease.

Srinivas prasad *et al*. (2011) reported that the neck blast infects the panicle causing failure of the seeds to fill or causing the entire panicle to fall over as it is rotted. Infection of the necks can be very destructive and directly reduces the economic value of the produce. The lesions are often greyish brown discoloration of the branches of the panicle and over time, the branches may break at the lesion. Out of three symptoms, neck blast is more destructive.

2.2.3 Isolation of Blast pathogen.

Silva *et al*. (2009) collected eight samples of rice leaves infected with blast from commercial fields of upland rice cultivars in the state of Goias, Brazil. Monoconidial isolates were obtained by directly transferring one conidium per lesion on 5% water agar from two to three lesions per leaf. The isolates from panicles in the majority of the cases were obtained from one conidium per panicle. The collected isolates were conserved on sterilized filter paper discs in a freezer at -20 ± 10C.

Motlagh and Javadzadeh, (2010) collected blast affected leaves of rice cultivars from rice fields in Guilan province of Iran. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed with sterile distilled water and placed on potato dextrose agar in Petri dishes at 25ºC for 2–3 days. Later, Petri dishes were incubated at 25ºC in the dark or artificial fluorescent light on a 12 h light/dark photoperiod for 15–25 days. Monoconidial isolates of the recovered fungi were maintained on half-strength potato dextrose agar slants in test tubes as stock cultures.

Priya Vanaraj *et al*. (2013) Blast lesions were surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28±2°C). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance. The causal organism was identified as *Pyricularia oryae* based on the spore morphology

2.2.4 Sporulation of Blast Pathogen

Meena (2005) came to the conclusion that colony colour of all the rice blast (*P. grisea*) isolates was usually buff with good growth on Oat meal agar, greyish black with medium growth on host seed extract + 2% sucrose agar, the raised mycelial growth with smooth colony margin on potato dextrose agar and raised mycelium with concentric ring pattern on Richard's agar medium. On host seed extract + 2% sucrose agar all the blast pathogenic isolates showed black to greyish black colour with smooth colony margin and good growth.

Ram *et al.* (2012) found isolates of the fungus from different hosts differed in their response in media for mycelial growth and sporulation. Radial mycelial growth and days of sporulation of *P. grisea* were studied by culturing three fungal isolates from rice, finger millet and *Panicum* sp. on six different media: prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA) and finger millet meal agar. The highest RMG was found in the isolates from finger millet and the lowest in the

isolates from rice. The shortest days of sporulation (1 week) was found in the isolate from rice and the longest (>2 weeks) in the isolate from finger millet. Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet.

Culturing of different isolates of *Pyricularia oryzae* was studied by Priya Vanaraj *et al.* (2013) and reported that colonies of *P. oryzae* appeared as white on oat meal, rice polish and malt extract agar, grey on potato dextrose agar and whitish grey on rice agar. Spore induction was hastened on maize stem pieces than on rice and *Panicum repens*. When spores of 11 isolates of *P. oryzae* were compared, conidia of the isolate from *Pennisetum purpureum* were significantly bigger than the other isolates. The spores of rice isolates from Erode and Gopichettipalayam were significantly smaller in length and width.

Srivastava *et al*. (2014) Blast fungal isolates produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff colour, greyish black to black colour.

Gashaw *et al.* (2014) reported that the colony diameters of different groups ranged from 67.40 to 82.50 mm and the conidial shape of the different groups was pyriform (pear-shaped) with rounded base and narrowed towards the tip which is pointed or blunt. On oat meal agar, colony colour of all the isolates was usually grey with good growth. All the isolates showed raised mycelial growth with smooth colony margin.

2.2.5 Pathological Variability

Chen *et al.* (2001) tested the pathogenicity reactions of 792 *M. grisea* isolates of rice using 13 host differentials consisting of six *indica* and seven *japonica* near-isogenic lines (NILs) and identified that 48 pathotypes with the indica NILs, 82 pathotypes with the *japonica* NILs, and a total of 344 pathotypes with both *indica* and *japonica* NILs. It is concluded that large differences in distribution of the pathotypes occur among the different rice growing areas of the world.

Sharma *et al.* (2002) grouped 119 isolates of *M. grisea* from north-western Himalayan region into 52 pathotypes on the basis of disease reaction on international differential rice lines and proved the set was inadequate to characterize the pathogen population.

Muralidharan *et al.* (2004) studied performance of BL 245 with two resistance genes (*Pi-2* and *Pi-4*) and C101LAC (*Pi-1*) was comparable to A57. The performance of these NILs was marginally superior to the resistant checks (Tadukan, Rasi, Tetep and IR 64) and the international blast differential Raminad Strain 3. Alleles for the genes identified as effective and durable in this study must be located in our cultivars besides discovering newer ones to effectively utilize resistance genes in future.

A study conducted by Rathour *et al.* (2004) stated the presence of high genotypic diversity and continuous DNA fingerprint variation in the *M. grisea* population in the northwestern Himalayan region and that no correlation was found between RAPD patterns and virulence characteristics of the pathogen.

2.2.6 Rice Blast Resistance

Eulgem (2005) and Benschop *et al*. (2007) reported that the genes involved in disease resistance are of two classes, the receptor genes, which include R genes and host pattern recognition receptor (HPRR) genes, and defense-responsive genes. The latter are characterized by responding to a pathogen attack via changing expression levels or post translational modification of the encoded proteins.

Robert *et al.* (2006) stated that, Pi-z is a disease resistance gene that has been effectively used to combat a broad-spectrum of races of the rice blast fungus *Magnaporthe grisea*. Although DNA markers have been reported for selection of the Pi2(t) and Pi-z resistance genes at the Pi-z locus, markers that are more tightly linked to the Pi-z locus would benefit rapid and effective cultivar development. Analysis of the publicly available genome sequence of Nipponbarenear the Pi-z locus revealed numerous SSRs that could be converted into markers. Three SSRs on rice PACAP005659 were found to be very tightly linked to the Pi-z locus, with one marker, AP5659-3, co-segregating with the Pi-z resistance reaction. Two SSR

marker haplotypes were unique for cultivars carrying the Pi-z gene, which indicates these markers are useful for selection of resistance genes at the Pi-z locus in rice germplasm.

Panstruga *et al*. (2009) reported that plant–pathogen recognition initiates the signal transduction pathways that interact with each other to form a complex network leading to defense responses by a classical gene-for-gene interaction, in which a pathogen strain expressing a single corresponding dominant avirulence (*AVR*) gene triggers the corresponding resistance gene mediated defense response.

Hayashi *et al.* (2010) mentioned that a functional marker for the blast resistance gene *Pit* has been developed and employed in the mining of this gene in diverse rice varieties or landraces. In the case of *Pik* gene locus in rice, markers, which reflect allele-specific blast resistance, were identified.

Wang *et al*. (2010) and Roy Chowdhury *et al*., (2012) found that single-locus resistance can prove short lived, often lasting for only two to four years. Therefore, the utilization of multiple R genes with overlapped resistance spectra is one of the most powerful strategies for managing blast disease.

Sharma *et al*. (2012) reported that so far, more than 100 blast resistance genes have been identified in both *indica* (51%), *japonica* (45%) rice cultivars and the remaining 4% are from wild species of rice. Out of these, 22 R-genes have been successfully cloned and characterized.

Ashkani *et al*. (2014) reported that most of the R-genes were detected on chromosomes 11, 12 and 6 (approximately 64% of the identified genes). Chromosome 11, with the maximum percentage of resistance genes (25%) and it has at least 27 genes and alleles, chromosome 12 (21%) has at least 22 resistance genes and alleles, and chromosome 6 (18%) has at least 19 genes and alleles. Out of these, several genes such as loci *Pi2/Piz*, *Piz-t*, and *Piz-5* on chromosome 6; *Pik*, *Pik-s*, *Pik-p*, *Pik-m*, *Pik-h*, *Pik-g* and *Pi1* on chromosome 11, with seven alleles; and *Pita* and *Pita-2* on chromosome 12, with two alleles are suggested to be allelic or tightly linked.

Li *et al.* (2014) reported that Some of the blast resistant varieties were short lived, while others were deployed over large geographical areas for several decades, including rice cultivar such as IR64 and katy and its derivatives.

Wang *et al.* (2014) reported that the use of tatep or taducan as the *Pi-ta* donar has led to the development of over a dozen *Pi-ta* carrying rice varieties worldwide.

Fukuoka *et al*. (2013) and Ma *et al*., (2015) studied that regarding the genetic basis of the resistance to *M. oryzae,* more than 86 dominant R genes and approximately 350 QTLs for resistance to rice blast have been identified, and 23 of them have been molecularly characterized: i.e. *pb1, Pi-a, Pi-b, Pi-d2, Pi-d3, Pi-k, Pik-h/Pi-54, Pik-m, Pik-p, Pi-sh, Pi-t, Pi-ta, Piz-t, Pi-1, Pi-2/Piz-5, Pi5, Pi-9, pi-21, Pi-25, Pi-36, Pi-37, Pi-35* and *Pi-64* upto date.

Khanna *et al.* (2015) reported that *Pi-ta* gene was identified and introduced from another landrace rice variety, Tatep, to breed blast resistance rice varieties through classical rice breeding.

Wenlong luo *et al*. (2016) have studied that, the two allelic R genes *Pi2* and *Pi9* confer very broad-spectrum resistance against blast isolates collected worldwide. However, the two genes have not yet been widely deployed in rice breeding programmes. The availability of specific markers for them would facilitate incorporating the two R genes into new rice lines through marker-assisted selection and they were able to transfer the *Pi2* into an elite restorer line through marker-assisted backcrossing, successfully obtained effective resistance to blast disease, by incorporate the *Pi2* and *Pi9* with two other R genes.

2.3 Molecular Markers

Gupta and Varshney, (2000) reported that RFLP, AFLP, RAPD, SSR, SNP, etc. among the most widely used markers in major cereals are called simple sequence repeats (SSRs).

McCouch *et al*. (2002) studied that the international Rice Microsatellite Initiative (IRMI) has developed a microsatellite map covering all 12 rice chromosomes, at least one microsatellite at the distance of 0.5 cM.

Fjellstrom *et al*. (2004b) reported that the application of DNA markers provides an effective and rapid tool to select for multiple blast resistance genes without needing to test the progeny or screen for inexact phenotypic disease. Currently, many useful DNA markers related to the major genes that confer race-specific resistance to blast have been identified

Collard *et al*. (2005) reported that DNA markers have developed into many systems based on different polymorphism detecting techniques like southern blotting, PCR, and DNA sequencing

Yang *et al.* (2008) found that MAS is used for screening of selected populations to track introgression of resistance genes *Pi-b, Pi-k, Pi-i, Pi-z,* and *Pi-ta*. Also, it is possible to pyramid *Pi-ta* with either of these major resistance genes to achieve broad spectrum resistance in the improved germplasm. In addition, the conserved DNA sequence variation has been used to develop *Pi-ta* and *Pi-b* dominant markers for MAS. Some of the genes *Pi-1(t), Pi2, Pi9, Pi20 (t), Pi27 (t), Pi39 (t), Pi40* (t) and *Pikh* are reported to have confers broadspectrum resistance (BSR) and some of them including *Pia, Pib, Pii, Pikm, Pi-t, Pi12 (t)* and *Pi19 (t)* confers race specific resistance (RSR). These information greatly advanced to understanding of molecular mechanisms that govern race specificity.

Song *et al*. (2010) reported that since the 1990s SSR markers have been extensively used in constructing genetic linkage maps, QTL mapping, marker-assisted selection and germplasm analysis in plants. In many species, plenty of breeder-friendly SSR markers have been developed and are available for breeders. For instance, there are over 35,000 SSR markers developed. Xu, (2010) reported that genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. The markers used in genetics and plant breeding can be classified into two categories, classical markers and DNA markers. Classical markers include morphological markers, cytological markers and biochemical markers.

Ashkani *et al.* (2011) studied that the multiline carrying more than one blast resistance gene can be developed with the help of SSRs markers, without screening against pathogens. Several studies found that six SSR markers, RM168, RM5961, RM413, RM1233, RM6836 and RM8225 directly associated with leaf blast resistance in a Malaysian rice variety. Further, the major blast resistance genes *Pi-b, Pi-kh* and *Pi-ta* linked SSR markers have been developed and successfully introgressed into different susceptible rice cultivars through MAS.

Selvaraj *et al.* (2011) reported that germplasm accessions from North East and Eastern India were validated for the presence of diverse blast resistant genes. Markers like SNPs, STS, Candidate gene markers were used (Imam *et al.*, 2014). Pita 3, YL155/187, YL183/YL 87, Pb 28, 195R-1 primers were linked to major genes of resistance. Molecular markers along with screening under artificial condition increase the precision of breeder in obtaining resistant genotypes. Polymorphic markers as RAPD, SSRs even help in characterizing the given land races and germplasm accessions for presence of diverse resistance genes. Three SSR markers RM5757, RM451 and RM492 on chromosome four and two are linked to leaf blast.

Sarah Brumlop *et al.* (2011) found that SSR primers are very useful for rapid and accurate detection of polymorphic loci and the information could be used for developing a physical map based on these sequence tags.

Xu *et al*. (2014) reported that the *Pi63*-mediated resistance was demonstrated to be dependent from its expression level, as assessed by comparison among resistant and susceptible lines and between different transgenic lines. In addition, the adult blast resistance conferred by *Pb1* was ascribed to its expression pattern that increases during plant development. During the early vegetative stages (two- and six-leaf stages) the level of *Pb1* transcripts is low and the plants are susceptible, while from the 10-leaf stages, *Pb1* expression is upregulated leading to blast resistance.

Yadav *et al.* (2017) used SNP markers linked to twelve major blast resistance (*R*) genes viz *Pib*, *Piz*, *Piz-t*, *Pik*, *Pik-p*, *Pikm Pik-h*, *Pita/Pita*-2, *Pi2*, *Pi9*, *Pi1* and *Pi5*. The study revealed that nineteen varieties (23.75%) showed resistance, twenty

one were moderately resistant (26.25%) while remaining forty varieties (50%) showed susceptible in uniform blast nursery.

2.4Breeding for resistance to Blast

Hittalmani *et al.* (2000) studied pyramided three major genes (Pi1, Piz-5 and Pita) using RFLP markers and combined genes originating from three parents for rice blast and stripe rust in barley, respectively. From the previous study, MAS application in rice shows that the target genes can be identified more efficiently in a segregating population at any plant growth stage with the use of tightly linked DNA markers.

Fjellstrom *et al.* (2004b) mapped the blast resistance genes *Pi-b*, *Pi-k*, and *Pi-ta2* on rice chromosomes 2, 11, and 12, respectively, using segregation information from hundreds of progeny in several crosses. Two microsatellite markers, RM208 and RM224, were found to cosegregate with the *Pi-b* and *Pi-k* genes, respectively, while additional microsatellites were found to closely flank these two genes and the *Pi-ta2* gene. The new markers were polymorphic in the narrow crosses characteristic of applied breeding programs and appear to be ideally suited for marker assisted selection for blast resistance in rice because of their tight linkage with resistance genes and ease of use through analysis of amplification products.

Jones *et al*. (2009) reported that most molecular marker technologies can be classified into hybridization-based or PCR-based systems. Restriction fragment length polymorphism (RFLP) is the first hybridization-based molecular marker system that was intensively used at the beginning of the molecular biology era in life science while hybridization-based marker methods such as microarrays and diversity array technology (DAT) are used currently to detect single nucleotide polymorphisms (SNP). In contrast, many PCR-based molecular marker detection methods have been developed. For example, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and sequence related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), sequence tagged site (STS), and sequence

characterized amplification region (SCAR), are commonly used in genomic analysis.

Jia *et al*. (2010) mentioned that blast resistance (*R*) genes *Pi-ta*, *Pi-ks*, *Pi-kh* have been effectively deployed in rice (*Oryza sativa* L.) in the southern USA for preventing disease caused by the predominant races of *Magnaporthe oryzae* Cav. [=*Magnaporthe grisea* (Herbert) Barr.]. In this study, the codominant single nucleotide length polymorphism DNA marker for the *Pi-ta* gene, and the simple sequence repeat markers, RM144 and RM224, that cosegregate with two alleles of the *Pi-k* gene, *Pi-k*$_s$ and *Pi-k*$_h$, were used for identification in a F_{10} recombinant inbred line population. One hundred eighty-two pure lines were identified from the population with the *Pi-ta*, *Pi-k*$_s$, and *Pik*$_h$ genes. A total of 56 had *Pi-ta* and *Pi-k*$_s$, 51 lines had *Pi-k*$_s$, 27 had *Pi-k*$_h$, and 48 lines had *Pi-k*$_h$ and *Pi-ta* These monogenic and digenic rice lines with the major blast *R* genes are expected to be useful for studying effects of each *R* gene singly and in combination for their epistatic interaction with yield and for introducing blast resistance with marker assisted selection. Ashkani *et al.,* (2011) studied the most virulent blast (*Magnaporthe oryzae*) pathotype, P7.2, which was used in screening of F2 population in order to understand the inheritance of blast resistance as well as linkage with SSR markers. The plants from F2 lines that showed resistance to blast pathotype P7.2 were linked to six alleles of SSR markers, RM 168 (116 bp), RM 8225 (221 bp), RM 1233 (175 bp), RM 6836 (240 bp), RM 5961 (129 bp), and RM 413 (79 bp). These diagnostic markers could be used in marker assisted selection programs to develop a durable blast resistant variety.

Sadegh *et al.* (2011) stated that, rice blast caused by the fungus M. grisea is one of the most devastating diseases of rice in nearly all rice growing areas of the world including Malaysia. To develop cultivars with resistance against different races of *M. oryzae*, availability of molecular markers along with marker-assisted selection strategies are essential. In this study, 11 polymorphic simple sequence repeat (SSR) markers with good fit of 1:2:1 ratio for single gene model in F2 population derived from the cross of Pongsuseribu 2 (Resistant) and Mahsuri (Susceptible) rice cultivars were analysed in 296 F3 families derived from

individual F2 plants to investigate association with Pi gene conferring resistance to *M. oryzae* pathotype. Association of SSR markers with phenotypic trait in F3 families was identified by statistical analysis. Four SSR markers (RM413, RM5961, RM1233 and RM8225) were significantly associated with blast resistance to pathotype 7.2 of *M. oryzae* in rice ($p \leq 0.01$).

Abedi *et al.* (2012) evaluated 58 rice genotypes for blast resistance phenotypic and molecular assessment. Phenotypic tests were conducted in a blast upland nursery and also in the greenhouse by using specific races of blast IA-82 and IA-90 in the greenhouse and local races for the nursery. The traits assessed consisted of infection type (IT), percent diseased leaf area (DLA) (in both nursery and green house), and lesion number (LN), lesion size (LS, mm2) only in greenhouse conditions. Molecular assessment was done by using three STS, JJ80, JJ81, and JJ113, and four microsatellite markers, RM224, RM277, RM463, and RM179 which were linked to resistance genes on rice chromosomes. Genotypes had different reactions against blast races in the phenotypic part of experiment. Consequently, all genotypes were divided into three groups with highly resistance and moderately resistance. Results indicated that partial resistant genotypes are preferable for achieving durable control. Eventually, the association test between molecular data and phenotypic results showed that there was a significant level for some of the SSR markers. This means there was at least one race-specific resistance gene in the genetic sources of these genotypes that bring about resistance functions to the blast races.

Ghaley *et al*. (2012) screened 352 landraces against 32 R gene lines. They reported 19 lines as completely resistant with zero disease score and 203 and 163 lines showed partial resistance to early leaf and panicle blast respectively. All microsatellite markers were polymorphic with 2 - 21 different alleles per marker with high polymorphic information content value of 0.6. The identified blast resistance races were genetically diverse origin from different rice cultivation zones. Molecular markers that are tightly linked to resistant genes could be used for the efficient selection of parents with resistance to leaf blast disease and their utilization in disease resistance breeding program.

Roychowdhury *et al.* (2012) studied the blast resistance gene Pib in 164 rice germplasm accessions from a core subset of the National Small Grains Collection utilizing DNA markers and pathogenicity assays. The presence of Pib was evaluated with two simple sequence repeat (SSR) markers and a dominant marker (Pib-dom) derived from the Pib gene sequence. Pathogenicity assays using two avirulent races (IE1k and IB1) and a virulent race (IB54) were performed to verify the resistance responses of accessions. Of the 164 accessions evaluated, 109 contained the Pib gene as determined using both SSR markers and pathogenicity assays, albeit different haplotypes were detected. The remaining 52 germplasm accessions were different in their responses to the blast races IB54, IE1k, and IB1, thus indicating the presence of R gene(s) other than Pib. The accessions characterized in this study could be used for marker-assisted breeding to improve blast resistance in indica and japonica cultivars worldwide.

Jayawardana *et al.* (2014) studied detection of blast resistance genes in phenotypically evaluated, selected rice cultivars in Sri Lanka based on simple sequence repeats (SSR) DNA markers. In the present study blast pathogen was identified by morphological characters, molecular techniques which confirmed the identity as M. grisea. Hence, resulted polymorphism findings can be used in future studies to differentiate the resistant and susceptible varieties.

Ramadevi *et al*. (2015) characterized that introgression lines for leaf and neck blast resistance, 326 introgression lines were developed using various accessions of six different AA genome wild species in the genetic background of elite Indian varieties like PR114 and Pusa 44 and were screened for blast resistance. Molecular characterization of these introgression lines using genome-wide simple sequence repeat (SSR) markers revealed the presence of small percentage of wild *Oryza* genome introgrssion. So these lines can be used for mapping and identification of novel leaf and neck blast resistance genes. Thus, these four introgression lines can be considered as new genetic resources for blast resistance.

Singh *et al.* (2015a) proved that using resistant rice varieties would be the most effective way to control rice blast disease. Molecular screening and genetic diversities of major rice blast resistance genes were determined in 192 rice

germplasm accessions using simple sequence repeat (SSR) markers. The genetic frequencies of the 10 major rice blast resistance genes varied from 19.79% to 54.69%. Seven accessions IC337593, IC346002, IC346004, IC346813, IC356117, IC356422 and IC383441 had maximum eight blast resistance gene, while FR13B, Hourakani, Kala Rata 1-24, Lemont, Brown Gora, IR87756-20-2-2-3, IC282418, IC356419, PKSLGR-1 and PKSLGR-39 had seven blast resistance genes. Twenty accessions possessed six genes, 36 accessions had five genes, 41 accessions had four genes, 38 accessions had three genes, 26 accessions had two genes, 13 accessions had single R gene and only one accession IC438644 does not possess any one blast resistant gene. Out of 192 accessions only 17 accessions harboured 7 to 8 blast resistance genes.

Thippeswamy *et al.* (2015) investigated that, 312 indigenous and 65 exotic germplasm lines were evaluated against blast resistance at RARS, Jagtial. More percentage (83%) of exotic germplasm showed resistance to rice blast disease compared to indigenous germplasm (46%). Three genotypes (JGL23710, JGL23713 and JGL23714) in indigenous germplasm and two genotypes (IR09N500 and IR12M101) in exotic germplasm were immune to rice blast disease. "These can be used as donor genetic stock for development of highly resistant rice cultivars with high yields". Among five linked markers studied for Pi-1 gene, one marker RM6094 was able to identify resistant genotypes at allelic level and for Pi-2 gene; RM527 was validated in four genotypes out of six genotypes used. This information will help rice breeders to improve the resistance to rice blast by marker assisted selection.

Wenlonglu *et al*. (2016) have studied that, the two allelic R genes *Pi2* and *Pi9* confer very broad-spectrum resistance against blast isolates collected worldwide. However, the two genes have not yet been widely deployed in rice breeding programmes. The availability of specific markers for them would facilitate incorporating the two R genes into new rice lines through marker-assisted selection and they were able to transfer the *Pi2* into an elite restorer line through marker-assisted backcrossing, successfully obtained effective resistance to blast disease, by incorporate the *Pi2* and *Pi9* with two other R genes.

Anupam *et al*. (2017) genotyped 74 rice germplasms using molecular markers for genetic diversity, drought QTLs, and blast resistance genes. The number of alleles per locus ranged from 2 to 5 with an average of 2.9. The polymorphic information content value per locus ranged from 0.059 (RM537) to 0.755 (RM252) with an average of 0.475. Cluster analysis based on 30 simple sequence repeat markers revealed 5 clusters and also indicated the presence of variability within the rice accessions. The drought QTL *qDTY2.1* was found in 56.0% of germplasms and *qDTY1.1* was detected in only 6.8% of the germplasms. Among 74 rice germplasms, only three accessions, Releng, RCPL1-82 and Buh Vubuk (Lubuk), possessed both drought-related QTLs and blast resistance genes whereas two rice varieties, RCPL-1-82.

2.5 Screening of germplasm for Blast resistance

Vossen *et al*. (2003) and Foster *et al*. (2009) found that the landraces and wild relatives may not possess favorable agronomic traits such as plant higher yield suitable for modern breeding programs, but they are highly relevant sources of genes for specific traits, including resistance or tolerance to biotic and abiotic stresses.

Variar *et al*. (2009) studied that Eastern India is considered to be a rich pocket of rice genetic resources in the world owing to the extremely diverse rice growing conditions as compared to other parts of the country. Genetic frequency of the nine major rice blast resistance genes, *Piz, Piz-t, Pik, Pik-p, Pik-h, Pita/Pita-2, pita, Pi9* and *Pib*, ranged from 6 to 97 % in the select set of germplasm that exhibited different level of resistance in the uniform blast nursery. Further, multi-location evaluation of a set of isogenic lines carrying 24 major blast resistance genes had earlier indicated that Pi9, Piz-t and Pita-2 were resistant at most locations, indicating their broader spectrum of resistance to pathotypes prevalent in Eastern India.

Liu *et al.* (2010) reported that Philippines, India, Vietnam and Thailand contributed more broad-spectrum blast resistant genotypes (29.4, 22.8, 13.1, and 11.8%, respectively) among the 289 genotypes identified as broad-spectrum resistant of which 39.4% are landraces and these genotypes were found resistant to

blast, both in the uniform blast nursery and when inoculated with the five individual rice blast isolates. To support progress, Three genes (*Pi2*, *Pi9*, and *Piz-t*) have been cloned and another four genes (*Pi40 (t)*, *Pigm*, *Pi26,* and *Piz*) have been mapped to confer broad spectrum resistance against rice blast, but at the same time these genes known to be vary in the irresistance pattern against different *M. oryzae* isolates.

Vasudevan *et al.* (2014) reported that resistance breeding requires continuous efforts of enriching the reservoir of resistance genes to effectively tackle the disease. Seed banks represent a rich stock of genetic diversity, however, there still under explored for identifying novel genes and their functional alleles.

Imam *et al.* (2014) reported that the genetic frequency of *Pi-z, Piz-t*, *Pi-k*, *Pik-p*, *Pik-h*, *Pi-ta*/*Pi-ta2*, *Pi-ta*, *Pi-9* and *Pi-b* ranged from 6% to 97% in the select set of rice germplasms. The less frequently detected R genes in germplasm accessions always be effective in pathogenicity assays.

Singh *et al.* (2015b) reported that the genotyping of rice blast resistance genes suggests that the available DNA markers are linked to the major blast resistance gene is a valuable tool in confirming, identifying and screening these specific genes among the rice germplasm. They also reported that the genetic frequencies of *Piz-5*, *Pi-9*, *Pi-tp*(t), *Pi-1*, *Pi5*(t), *Pi-33*, *Pi-b*, *Pi-27*(t), *Pik-h* and *Pi-ta* in 192 rice accessions ranged from 19.79% to 54.69%, and only 17 accessions harbored 7–8 blast resistance genes.

Scheuermann and Jia (2016) reorted the development of a dominant marker to identify *Pi9*- containing rice germplasm. It is shown that the marker which simultaneously distinguishes *Pi2* and *Pi9* from other alleles might be promising in breeding new rice varieties with broad-spectrum resistance to blast disease.

Tian *et al*. (2016) reported the development of an InDel marker and a CAPS marker for genotyping of *Pi2* and *Pi9* that derived the polymorphisms within the *Pi2/Pi9* locus.

Yan *et al.* (2017) reported that the development of improved rice cultivars has led to the replacement of landraces and traditional varieties by modern cultivars, which has resulted in a decline in the diversity of agriculturally used rice and the diversity lost in the elite materials is somewhat preserved in crop gene banks, wild

rice collections and breeding resources. The field evaluations and genotyping of the germplasm with allelic related markers were conducted to help identifying 11 major blast resistant genes *Pi-d2*, *Pi-z*, *Piz-t*, *Pi-9*, *Pi-36*, *Pi-37*, *Pi5*, *Pi-b*, *Pik-p*, *Pik-h* and *Pi-ta*2 with the genetic frequencies ranging from 9.4% to 100.0% in 32 rice germplasms resistant to *M. oryzae*.

2.6 Genome-Wide Association Studies (GWAS) in Rice

Huang *et al.* (2010) sequenced 517 rice landraces using next generation sequencing approach and identified large number of single nucleotide polymorphism (SNPs). A total of 3,625,200 non-redundant SNPs were identified, which spans in an average of 9.32 SNPs per Kb and constructed a high-density haplotype map. They performed GWAS for 14 agronomic traits in the population of *Oryza sativa indica* subspecies by employing both simple model and the compressed mixed linear model (MLM). Peak SNPs at the identified loci explained ~36% of the phenotypic variance on an average. The peak signals at six loci were tied closely to previously identified genes and have demonstrated GWAS can be used as powerful complementary strategy for classical biparental mapping for dissecting complex agronomically important traits in rice.

Famoso *et al*. (2011) screened 383 diverse rice accessions for aluminium (Al) tolerance and conducted GWAS. Forty-eight regions were found to be associated with Al tolerance and four of these regions were co-localized with *a priori* candidate genes, and two highly significant regions were co-localized with previously identified QTLs. Finally, they opined that GWAS discovered more phenotype-genotype associations and provided higher resolution.

A set of 241 recombinant inbred lines (RILs) derived from cross between two elite *indica* subspecies of rice, Zhenshan 97 and Minghui 63 were sequenced and SNPs were identified by Yu *et al.* (2011). The SNP map was used to perform QTL analysis for yield and three yield component traits. Sequencing approach helped them to identify more number of QTLs especially for grain weight, with precise map locations and construction of ultra-high density map.

Zhao *et al.* (2011) showed the utility of GWAS based on genotyping using Affymetrix SNP array containing 44,100 SNP variants across 413 diverse

accessions of rice collected from 82 countries and phenotyping them for 34 traits. They identified several common variants influencing numerous complex traits.

The origin and domestication process of rice a controversial debate till date. So Huang *et al*. (2012) sequenced 446 geographical diverse accessions of *Oryza rufipogon* and 1083 accessions of cultivated *indica* and *japonica* varieties. They constructed comprehensive map of rice genome variation and deciphered the origin of rice.

Huang *et al.* (2012) generated sequence data for 950 rice varieties and conducted GWAS. They identified 32 new loci associated with flowering time and 10 grain related traits. Additionally, they identified candidate genes for 18 associated loci through detailed annotation. Their study showed that the integrated approach of sequence based GWAS and functional genome annotation has a potential role to match complex traits to their causal polymorphism in rice.

Xu *et al.* (2012) resequenced 40 cultivated rice accessions and 10 accessions of their wild progenitors (*Oryza rufipogon* and *Oryza nivara*) of >15x sequence depth. The study revealed genome-wide variations and obtained 6.5 million high quality SNPs. From this SNP data, they identified thousands of genes with significantly lower diversity in cultivated but not in wild rice, which represent candidate genomic regions selected during domestication process.

Vilhjalmsson and Nordborg (2013) argued that population structure *per se* is not a problem in GWAS. Instead, environment and the genetic background are the major sources of variation. Finally, they concluded that mixed model of GWAS would address these issues.

Wang *et al*. (2014) extensively conducted GWAS for 16 rice blast strains by sequencing 366 diverse *indica* accessions and identified thirty associated rice blast resistance loci.

Begum *et al*. (2015) performed GWAS for 19 agronomic traits in breeding population of elite irrigated tropical rice breeding lines. They identified 52 QTLs for 11 agronomic traits and proposed the utility of these resources for genomic-assisted selection models.

Salinity tolerance in rice is a complex trait and was dissected effectively by deploying GWAS in 220 rice accessions (Kumar *et al*., 2015). A custom-based

array comprising of 6K SNPs distributed across stress-responsive genes was used to genotype 220 rice accessions using Infinium high-throughput platform.

Ueda *et al*. (2015) attempted to dissect genetics of ozone tolerance in rice through GWAS. Genotyping and association based on 30K SNPs revealed 195 candidate genes associated with various biomass related traits.

A set of 270 rice accessions from China was screened for mesocotyl lengths of seedlings grown in water in darkness and sand culture (Wu *et al.*, 2015). Re sequencing these accessions identified around one million SNPs. They mapped several candidate loci on rice chromosomes 1, 3, 4, 5, 6 and 9 by GWAS approach.

Yang and co-workers (2015) demonstrated utility of GWAS to dissect genetic bases of leaf traits in 533 rice accessions. They identified nine associated loci contained known leaf-related genes in addition other loci. In addition, they claim that effective high throughput phenotyping of leaf traits and GWAS will help to identify genetic loci controlling rice leaf traits.

Patishtan *et al.* (2017) used a diversity panel of 306 rice accessions and collected phenotypic data after short (6 h), medium (7 d) and long (30 d) salinity treatment (50 mm NaCl). A genome-wide association study (GWAS) performed, identified around 1200 candidate genes from many functional categories. Further analysis showed the presence of cation transporters and transcription factors with a known role in salinity tolerance and those that hitherto were not known to be involved in salt stress.

Zheng *et al.* (2017) used genomic and germplasm resources available for rice (*Oryza sativa*) to perform a large-scale genome-wide association study (GWAS) of grain width. Using a filtered dataset of >1.9 million genome-wide SNPs in a sample of 570 cultivated and wild rice accessions, they performed GWAS with two complementary models, GLM and MLM. The models yielded 10 and 33 significant associations, respectively, and jointly yielded seven candidate locus regions, two of which have been previously identified. The results provide a 50% increase in the total number of rice grain width loci mapped to date and support a polygenic model whereby grain width is shaped by gene-by-environment interactions.

CHAPTER - III
MATERIALS AND METHODS

The present investigation "**Identification of Blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping**" was carried out during *Kharif* 2017. The details of the planting material used, the methods followed, the protocols and statistical tools employed for analyses in this experiment are presented in this chapter as follows:

3.1 Experimental Site

The experiment was carried out at ICAR-National Rice Research Institute (NRRI). The Institute is located at Cuttack in the state of Odisha between 85º55′48″ E to 85º56′48″ E longitudes and 20º26′35″ N to 20º27′35″ N latitudes and has a general elevation of 23.5 m above mean sea level (MSL). The annual rainfall at Cuttack is about 1500 mm and is received mostly during June to October (*Kharif* season). Minimal rainfall is received between November to May (*Rabi* season) from south west monsoon.

Molecular studies were performed at Biotechnology and Tissue Culture laboratory of Crop Improvement Division at ICAR-NRRI, Bidyadharpur, Cuttack, Odisha.

3.2 Experimental Season and Weather

The experiment was conducted during *Kharif* 2017. During, *Kharif* 2017 the temperature ranges between 12.1ºC to 35.8ºC throughout the crop growth season. The total rainfall received during crop growing season was 1039 mm. The maximum rainfall (189 mm) was received between 16 July and 22 July, 2017. During *Kharif* 2017, the maximum temperature 33.8°C was recorded between 9 October and 16 October, 2017. The data pertaining to weekly rainfall, minimum and maximum temperatures, relative humidity, evaporation, wind velocity and bright sunshine hours of entire crop growing period have been presented in Appendix A and Fig.3.1.

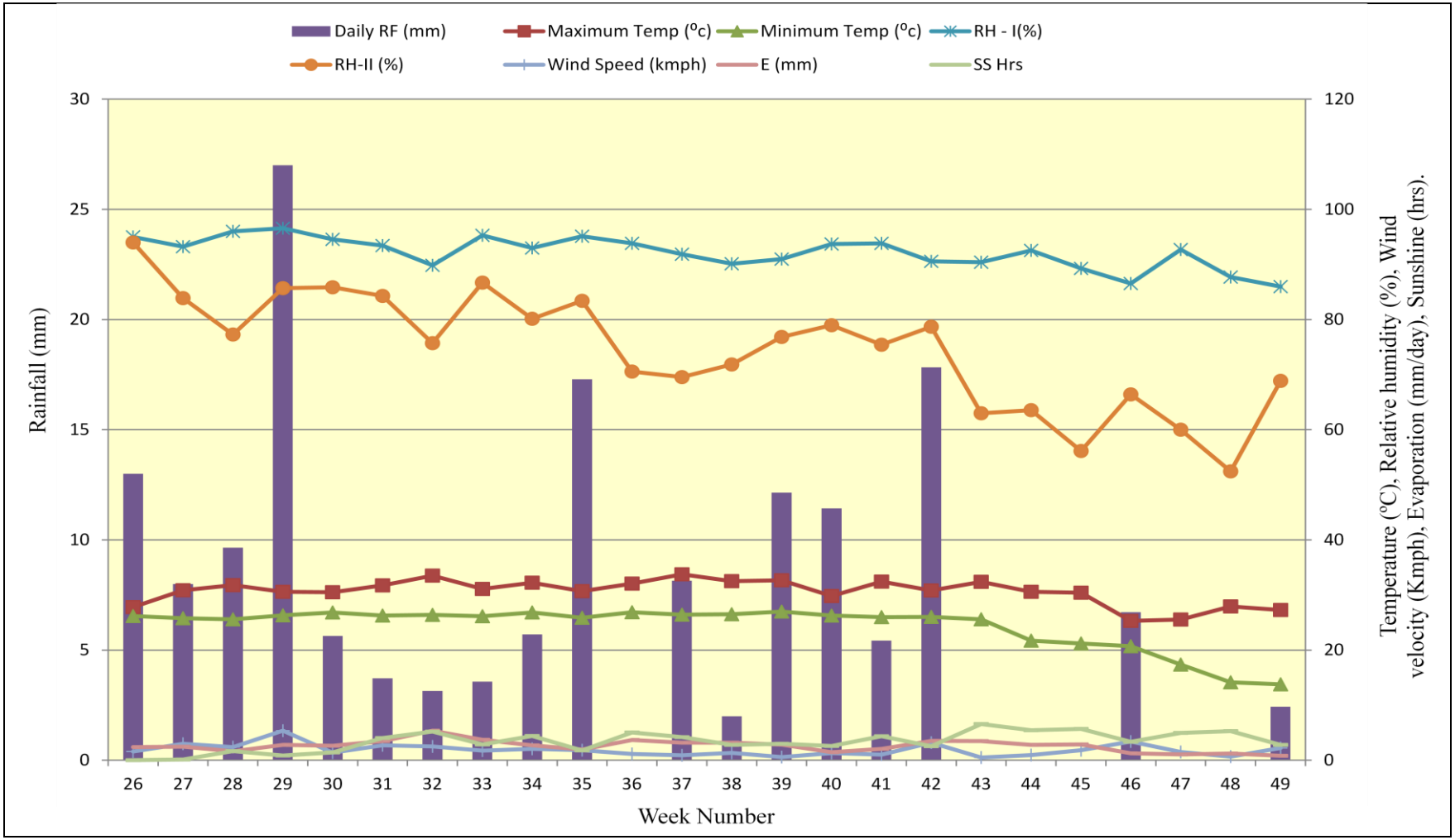

Figure 3.1: Weekly meteorological data during crop growth period (2017-18)

3.3Experimental Materials

The experimental materials comprised of 260 landraces from Bastar, Chhattisgarh (Table 3.1), 240 germplasm collections from gene bank of ICAR-NRRI (Table3.2) and HR-12, Co-39 were used as standard susceptible checks. The experimental materials were received from rice breeding section of S.G. College of Agriculture and Research Station, Jagdalpur, Bastar, Chhattisgarh and the gene bank of ICAR-NRRI, Cuttack, Odisha.

According to the phenotypic observations, 240 landraces of Chhattisgarh and 48 germplasm collections of ICAR-NRRI were selected for molecular studies (Table3.1 and Tables 3.2).

Table 3.1: List of 260 Bastar landraces of rice used in the present study.

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
1	CG-1*	1001	SGCARS1	17	CG-17*	1020	SGCARS5
2	CG-2*	1002	SGCARS2	18	CG-18*	1022	Badsa Bhog
3	CG-3*	1003	Pakhiya Dhaan	19	CG-19*	1023	Chpti Khuji
4	CG-4*	1004	Gurmutiya	20	CG-20*	1024	Aajan Dhaan
5	CG-5*	1006	Kurdaful	21	CG-21*	1025	Kurlu Dhaan
6	CG-6*	1007	SGCARS3	22	CG-22*	1026	Kabro Dhaan
7	CG-7*	1008	Pandri Lochai	23	CG-23*	1027	Kharla Muha
8	CG-8*	1010	Kurlu Dhaan	24	CG-24*	1028	Turej Gada Khuta
9	CG-9*	1012	Kakad kado	25	CG-25*	1029	Mutiya Dhhan
10	CG-10*	1013	Kata mehar	26	CG-26*	1030	Gadur Sela
11	CG-11*	1014	Hathi Panjaro	27	CG-27*	1031	SGCARS6
12	CG-12*	1015	Bariya Dhaan	28	CG-28*	1032	Gada Khuta
13	CG-13*	1016	Godavari	29	CG-29*	1033	Bhata Dubraaj
14	CG-14*	1017	SGCARS4	30	CG-30*	1034	Lakhechi
15	CG-15*	1018	Jira Dhaan	31	CG-31*	1035	Gurmutiya
16	CG-16*	1019	Aalag Dhaan	32	CG-32*	1037	Bandkari

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
33	CG-33*	1038	Pat Dhaan	58	CG-58*	1070	Yami Gali
34	CG-34*	1039	Goydi	59	CG-59*	1072	Temru Mudi
35	CG-35*	1040	Titir Pakhi	60	CG-60*	1073	Lal Baso
36	CG-36*	1041	Karmuri Bhog	61	CG-61*	1074	Badi Chudi
37	CG-37*	1043	Kale Tude Masino	62	CG-62*	1075	SGCARS10
38	CG-38*	1045	Dumar Ful	63	CG-63*	1076	Manki Dhaan
39	CG-39*	1046	Hansa Dubraaj	64	CG-64*	1077	Dhgda Dhaan
40	CG-40*	1047	BhaluDubraaj	65	CG-65*	1078	Banspati
41	CG-41*	1048	Aajam Lali	66	CG-66*	1079	SGCARS11
42	CG-42*	1049	Dongar Kabri	67	CG-67*	1080	Bhvar Gedi
43	CG-43*	1050	Huldi Chudi	68	CG-68*	1081	Chind Jhopa
44	CG-44*	1051	Temru Mudi	69	CG-69*	1082	Badsa Bhog
45	CG-45*	1052	SGCARS7	70	CG-70*	1084	Godandi Dhaan
46	CG-46*	1053	Kata mehar	71	CG-71*	1086	Pandko Guda
47	CG-47*	1054	SGCARS8	72	CG-72*	1087	SGCARS12
48	CG-48*	1057	SGCARS9	73	CG-73*	1088	Nani chudi
49	CG-49*	1058	Pote Khuji	74	CG-74*	1089	Shivnath
50	CG-50*	1060	Bhata Kandai	75	CG-75*	1090	Pote Khuji
51	CG-51*	1061	Sagi Pareta	76	CG-76*	1091	Bash Mukhi
52	CG-52*	1062	Gadur Sela	77	CG-77*	1092	SGCARS13
53	CG-53*	1063	Hardiful	78	CG-78*	1093	Kadam Ful
54	CG-54*	1065	Bako Dhaan	79	CG-79*	1095	Rang Gada Khuta
55	CG-55*	1066	Bakti Chudi	80	CG-80*	1097	Hare Krishna
56	CG-56*	1067	Bhata Mokdo	81	CG-81*	1098	Shivnath
57	CG-57*	1068	Sona Sari	82	CG-82*	1099	Tiki Chudi

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
83	CG-83*	1000	Rani Kanjar	111	CG-111*	2019	SGCARS21
84	CG-84*	1101	DokaraMecha	112	CG-112*	2021	Pandri Satka
85	CG-85*	1102	Rakhi Dhaan	113	CG-113*	2024	Jira Dhaan
86	CG-86*	1103	Surmatiya	114	CG-114*	2026	Bhayar Dhaan
87	CG-87*	1104	UmariChudi	115	CG-115*	2027	SGCARS22
88	CG-88*	1109	Ratan Chudi	116	CG-116*	2028	SGCARS23
89	CG-89*	1110	Adga Dhaan	117	CG-117*	2029	Jeera dhaan
90	CG-90*	1111	Surmatiya	118	CG-118*	2030	Masuri Desi
91	CG-91*	1112	Hiruya Dhaan	119	CG-119*	2031	SGCARS24
92	CG-92*	1113	Goydi	120	CG-120*	2032	SGCARS25
93	CG-93*	1114	SGCARS14	121	CG-121*	2033	SGCARS26
94	CG-94*	1115	Lal Baso	122	CG-122*	2035	SGCARS27
95	CG-95*	1116	Mayur Fada	123	CG-123*	2036	SGCARS28
96	CG-96*	1117	Kari Graas	124	CG-124*	2037	Masur Dhaan
97	CG-97*	1118	SGCARS15	125	CG-125*	2038	SGCARS29
98	CG-98*	1119	Kava Paadi	126	CG-126*	2039	SGCARS30
99	CG-99*	1120	Bariya Dhaan	127	CG-127*	2040	SGCARS31
100	CG-100*	1122	Sonpuri	128	CG-128*	2043	SGCARS32
101	CG-101*	1124	Sorchu Badi	129	CG-129*	2044	Farsa Ful
102	CG-102*	1125	Sendur senga	130	CG-130*	2045	Ram Laxman
103	CG-103*	2001	SGCARS16	131	CG-131*	2046	Alti Mijo
104	CG-104*	2003	Dhadhar Dhaan	132	CG-132*	2047	Laycha
105	CG-105*	2004	SGCARS17	133	CG-133*	2048	Sela Dhaan
106	CG-106*	2011	SGCARS18	134	CG-134*	2049	Masur Dhaan
107	CG-107*	2015	SGCARS19	135	CG-135*	2050	Chatiya Dhaan
108	CG-108*	2016	Kari Graas	136	CG-136*	2051	Kandai
109	CG-109*	2017	Jhumra	137	CG-137*	2052	Khutbadi
110	CG-110*	2018	SGCARS20	138	CG-138*	2053	Vishnu bhog

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
139	CG-139*	2054	SGCARS33	168	CG-168*	2086	SGCARS43
140	CG-140*	2055	Meso Dhaan	169	CG-169*	2087	Pundri Satka
141	CG-141*	2056	Lodhyari	170	CG-170*	2088	SGCARS44
142	CG-142*	2057	SGCARS34	171	CG-171*	2089	Kukadi Mudi
143	CG-143*	2059	SGCARS35	172	CG-172*	2090	SGCARS45
144	CG-144*	2060	Jodra Nakti	173	CG-173*	2091	Kala Mali
145	CG-145*	2061	SGCARS36	174	CG-174*	2093	Motilur
146	CG-146*	2062	Sirodi Bako	175	CG-175*	2094	Moha Dhaan
147	CG-147*	2063	SGCARS37	176	CG-176*	2096	Mukukuda
148	CG-148*	2064	Kata Barangi	177	CG-177*	2097	Noni Dhaan
149	CG-149*	2065	SGCARS38	178	CG-178*	2100	Kala Umari
150	CG-150*	2066	Luchai Dhaan	179	CG-179*	3001	SGCARS46
151	CG-151*	2067	Umari Dhaan	180	CG-180*	3002	SGCARS47
152	CG-152*	2068	Gaada Khuta	181	CG-181*	3003	SGCARS48
153	CG-153*	2070	Khuti Dhaan	182	CG-182*	3004	SGCARS49
154	CG-154*	2071	Kari Gudi	183	CG-183*	3005	Aasan Chudi
155	CG-155*	2072	Tama Koni	184	CG-184*	3007	SGCARS50
156	CG-156*	2073	Kata Nakti	185	CG-185*	3008	SGCARS51
157	CG-157*	2074	SGCARS39	186	CG-186*	3009	SGCARS52
158	CG-158*	2075	Tiki Chudi	187	CG-187*	3010	SGCARS53
159	CG-159*	2076	SGCARS40	188	CG-188*	3011	SGCARS54
160	CG-160*	2078	Neem Chudi	189	CG-189*	3012	SGCARS55
161	CG-161*	2079	Nani Chudi	190	CG-190*	3013	SGCARS56
162	CG-162*	2080	KusumJhopa	191	CG-191*	3014	SGCARS57
163	CG-163*	2081	SGCARS41	192	CG-192*	3015	SGCARS58
164	CG-164*	2082	Kukda Bhour	193	CG-193	3016	Umari chudi
165	CG-165*	2083	SGCARS42	194	CG-194	3017	SGCARS59
166	CG-166*	2084	Kapoor Saay	195	CG-195	3018	SGCARS60
167	CG-167*	2085	Bhata Mokdo	196	CG-196*	3020	SGCARS61

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
197	CG-197*	3021	SGCARS62	226	CG-226*	3056	Dhotiya Dhaan
198	CG-198*	3022	Mudariya	227	CG-227*	3058	Sonasari
199	CG-199*	3023	LokatiMachi	228	CG-228*	3059	Bahiya Khuta
200	CG-200*	3024	Ajuniya	229	CG-229*	3060	Ganga Baru
201	CG-201	3025	UmariChudi	230	CG-230*	3062	Bghal Bijo
202	CG-202*	3027	UmariChudi	231	CG-231*	3063	Kurlu Kabri
203	CG-203*	3028	SGCARS63	232	CG-232*	3064	SGCARS72
204	CG-204	3029	Bode Bargi	233	CG-233	3065	Huldi Gadi
205	CG-205	3031	NaniChudi	234	CG-234*	3066	SGCARS73
206	CG-206*	3032	Bhaispat	235	CG-235*	3067	SGCARS74
207	CG-207*	3033	Badsabhog	236	CG-236*	3068	SGCARS75
208	CG-208	3035	SGCARS64	237	CG-237*	3071	UmariChudi
209	CG-209*	3036	SGCARS65	238	CG-238*	3072	Bhanvargedi
210	CG-210	3038	Kera Ful	239	CG-239*	3073	SGCARS76
211	CG-211*	3039	SGCARS66	240	CG-240	3075	Gaadha khuta
212	CG-212*	3041	Degichudi	241	CG-241*	3076	Sargiful
213	CG-213*	3042	Huldi Godi	242	CG-242*	3077	Kursobhog
214	CG-214*	3043	SGCARS67	243	CG-243	3079	Kush Dhaan
215	CG-215*	3044	Masuri Desi	244	CG-244	3080	Rangovati
216	CG-216*	3045	Kera Ful	245	CG-245	3081	Kaani Chudi
217	CG-217*	3046	Baadi Lochai	246	CG-246	3082	Dubraaj
218	CG-218*	3047	Karmuri Bhog	247	CG-247*	3083	Huldi Gathi
219	CG-219*	3048	BadiLochai	248	CG-248*	3084	Muthiya
220	CG-220*	3049	SGCARS68	249	CG-249*	3085	Huldi Gathi
221	CG-221*	3050	Mokdodhaan	250	CG-250	3086	Bagdi Chudi
222	CG-222*	3051	SGCARS69	251	CG-251	3087	Olesar
223	CG-223*	3052	SGCARS70	252	CG-252*	3088	Milkor Mel
224	CG-224*	3053	Dhgdikaaj	253	CG-253	3090	Kumhadaful
225	CG-225*	3055	SGCARS71	254	CG-254	3092	SGCARS77

Sr. No.	Code No.1	Code No.2	Vernacular Name	Sr. No.	Code No.1	Code No.2	Vernacular Name
255	CG-255*	3095	Gogal	258	CG-258*	3106	UmariChudi
256	CG-256	3098	SGCARS78	259	CG-259*	3107	Bhaiya Khuta
257	CG-257*	3105	Assam Chudi	260	CG-260	3108	Gada Khuta

Asterisks * indicate the genotypes used for molecular studies.

Table 3.2: List of 120 ICAR-NRRI germplasm collections of rice.

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
1	RSG-1*	41586	18	RSG-18*	41718
2	RSG-2*	41588	19	RSG-19*	41723
3	RSG-3*	41589	20	RSG-20*	41728
4	RSG-4*	41592	21	RSG-21*	41734
5	RSG-5*	41593	22	RSG-22*	41743
6	RSG-6*	41596	23	RSG-23*	41744
7	RSG-7*	41601	24	RSG-24*	41745
8	RSG-8*	41661	25	RSG-25*	41747
9	RSG-9*	41666	26	RSG-26*	41752
10	RSG-10*	41668	27	RSG-27*	41754
11	RSG-11*	40670	28	RSG-28*	41756
12	RSG-12*	41671	29	RSG-29*	41763
13	RSG-13*	41675	30	RSG-30*	41766
14	RSG-14*	41676	31	RSG-31*	41784
15	RSG-15*	41683	32	RSG-32*	41787
16	RSG-16*	41695	33	RSG-33*	41792
17	RSG-17*	41712	34	RSG-34*	41796

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
35	RSG-35*	41797	58	RSG-58	41602
36	RSG-36*	41798	59	RSG-59	41603
37	RSG-37*	41799	60	RSG-60	41604
38	RSG-38*	41800	61	RSG-61	41605
39	RSG-39*	41808	62	RSG-62	41606
40	RSG-40*	41826	63	RSG-63	41607
41	RSG-41*	41827	64	RSG-64	41608
42	RSG-42*	41830	65	RSG-65	41609
43	RSG-43*	41832	66	RSG-66	41610
44	RSG-44*	41846	67	RSG-67	41611
45	RSG-45*	41854	68	RSG-68	41612
46	RSG-46*	41855	69	RSG-69	41613
47	RSG-47*	41863	70	RSG-70	41614
48	RSG-48*	41868	71	RSG-71	41615
49	RSG-49	41587	72	RSG-72	41616
50	RSG-50	41590	73	RSG-73	41617
51	RSG-51	41591	74	RSG-74	41618
52	RSG-52	41594	75	RSG-75	41619
53	RSG-53	41595	76	RSG-76	41620
54	RSG-54	41597	77	RSG-77	41656
55	RSG-55	41598	78	RSG-78	41658
56	RSG-56	41599	79	RSG-79	41659
57	RSG-57	41600	80	RSG-80	41660

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
81	RSG-81	41662	103	RSG-103	41691
82	RSG-82	41663	104	RSG-104	41692
83	RSG-83	41664	105	RSG-105	41693
84	RSG-84	41665	106	RSG-106	41694
85	RSG-85	41667	107	RSG-107	41696
86	RSG-86	41669	108	RSG-108	41697
87	RSG-87	41672	109	RSG-109	41698
88	RSG-88	41673	110	RSG-110	41699
89	RSG-89	41674	111	RSG-111	41700
90	RSG-90	41677	112	RSG-112	41701
91	RSG-91	41678	113	RSG-113	41702
92	RSG-92	41679	114	RSG-114	41703
93	RSG-93	41680	115	RSG-115	41704
94	RSG-94	41681	116	RSG-116	41705
95	RSG-95	41682	117	RSG-117	41706
96	RSG-96	41684	118	RSG-118	41707
97	RSG-97	41685	119	RSG-119	41708
98	RSG-98	41686	120	RSG-120	41709
99	RSG-99	41687	121	RSG-121	41710
100	RSG-100	41688	122	RSG-122	41711
101	RSG-101	41689	123	RSG-123	41713
102	RSG-102	41690	124	RSG124	41714

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
125	RSG 125	41715	148	RSG 148	41749
126	RSG 126	41716	149	RSG 149	41750
127	RSG 127	41717	150	RSG 150	41751
128	RSG 128	41719	151	RSG 151	41753
129	RSG 129	41720	152	RSG 152	41757
130	RSG 130	41722	153	RSG 153	41758
131	RSG 131	41724	154	RSG 154	41761
132	RSG 132	41725	155	RSG 155	41762
133	RSG 133	41726	156	RSG 156	41764
134	RSG 134	41727	157	RSG 157	41765
135	RSG 135	41729	158	RSG 158	41768
136	RSG 136	41730	159	RSG 159	41769
137	RSG 137	41731	160	RSG 160	41770
138	RSG 138	41732	161	RSG 161	41771
139	RSG 139	41733	162	RSG 162	41772
140	RSG 140	41735	163	RSG 163	41773
141	RSG 141	41736	164	RSG 164	41774
142	RSG 142	41737	165	RSG 165	41775
143	RSG 143	41740	166	RSG 166	41776
144	RSG 144	41741	167	RSG 167	41777
145	RSG 145	41742	168	RSG 168	41778
146	RSG 146	41746	169	RSG 169	41779
147	RSG 147	41748	170	RSG 170	41780

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
171	RSG 171	41781	194	RSG 194	41814
172	RSG 172	41782	195	RSG 195	41815
173	RSG 173	41785	196	RSG 196	41816
174	RSG 174	41786	197	RSG 197	41817
175	RSG 175	41788	198	RSG 198	41818
176	RSG 176	41789	199	RSG 199	41819
177	RSG 177	41790	200	RSG 200	41820
178	RSG 178	41791	201	RSG 201	41821
179	RSG 179	41793	202	RSG 202	41822
180	RSG 180	41794	203	RSG 203	41823
181	RSG 181	41795	204	RSG 204	41824
182	RSG 182	41801	205	RSG 205	41825
183	RSG 183	41802	206	RSG 206	41828
184	RSG 184	41803	207	RSG 207	41829
185	RSG 185	41804	208	RSG 208	41831
186	RSG 186	41805	209	RSG 209	41834
187	RSG 187	41806	210	RSG 210	41835
188	RSG 188	41807	211	RSG 211	41836
189	RSG 189	41809	212	RSG 212	41837
190	RSG 190	41810	213	RSG 213	41869
191	RSG 191	41811	214	RSG 214	41870
192	RSG 192	41812	215	RSG 215	41871
193	RSG 193	41813	216	RSG 216	41872

Sr. No.	Code No.1	Code No.2	Sr. No.	Code No.1	Code No.2
217	RSG 217	41874	229	RSG-229	41867
218	RSG 218	41875	230	RSG-230	41888
219	RSG 219	41876	231	RSG-231	41889
220	RSG 220	41878	232	RSG-232	41890
221	RSG 221	41879	233	RSG-233	41891
222	RSG 222	41880	234	RSG-234	41893
223	RSG 223	41881	235	RSG-235	41894
224	RSG 224	41883	236	RSG-236	41895
225	RSG 225	41884	237	RSG-237	41896
226	RSG 226	41885	238	RSG-238	41897
227	RSG 227	41886	239	RSG-239	41898
228	RSG 228	41887	240	RSG-240	41899

Asterisks* indicate the genotypes used for molecular studies.

3.4 Experimental Methods

3.4.1 Nursery Preparation

Rice germplasm were evaluated for their resistance spectrum against the leaf blast at the Uniform Blast Nursery (UBN) at the research farm of NRRI, Cuttack, Division of Plant Pathology, Cuttack.

3.4.1.1 Layout

Uniform Blast Nursery (UBN) pattern (Fig.3.2) was followed. Each Germplasm were sown in a single row of 50 cm with row to row spacing of 10 cm. After every 5 and 10 germplasm, local susceptible check (HR 12 and CO-39) was planted. The entire nursery was surrounded on all sides by two rows of susceptible check varieties.

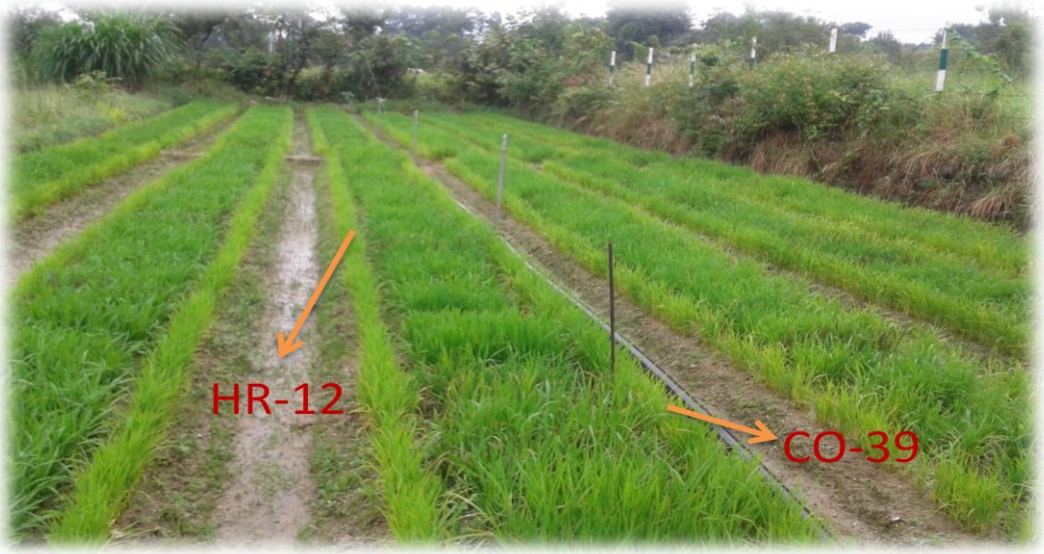

Figure 3.2: Blast nursery at ICAR-NRRI, Cuttack with HR-12 and CO-39 as susceptible checks.

Figure 3.3: Steps followed in preparation and spraying of Inoculum. 1.Blast culture prepared in OMA medium. 2.Addition of Tween solution to the culture plate. 3.Filtering the spores using miracloth. 4.Spraying of the inoculum by glass atomizer.

3.4.1.2 Fertilizers

A high level of farmyard manure was incorporated into the UBN nursery before sowing. Nitrogen fertilizer was applied at rate of 120 kg/ha**.** While half of the nitrogen was given as basal dose at the time of sowing, remaining half was applied 15 days after sowing (DAS). Other fertilizers were applied as per recommended package of practice *i.e.* 50 kg/ha of both Phosphorous and Potassium.

3.4.1.3 Inoculum

It is necessary to sow the nursery during blast favorable weather conditions. To create severe blast incidence additional inoculum was provided. This operation was carried out during prolonged wet weather by continuous supply of water through sprinklers to facilitate infection and polycyclic development of disease.

3.4.2 Culture Preparation and Inoculation

Virulent isolates were collected from rice field and they were used to evaluate the set of germplasm for identification of blast resistance reaction. Preparations of *M. oryzae* spore suspension were done in following steps:

1. The 3% (3 grams of powdered unprocessed oats and 1.5 grams of Agar) Oatmeal Agar (OMA) medium was prepared.
2. Then, OMA medium was cooled to below room temperature and added 1 mL of Kanamycin (100 mg/mL) to avoid bacterial contamination. Medium was mixed thoroughly and poured aseptically in a laminar hood.
3. *M. oryzae* culture (single spore culture) was inoculated on OMA and incubated under 4 days of dark and 4 days of blue light to induce sporulation at 26-28 C.

   Note: Parafilm should be removed from petridish before transferring to light chamber.
4. *M. oryzae* cultures were removed to aseptic laminar hood from incubator.
5. Prepare Tween solution by adding 20 µL of Tween 20 to 100 mL of sterilized distilled water.
6. The above solution was added to *M. oryzae* culture plate and scrubbed the culture using hand or 1.5mL sterilized eppendorf micro-centrifuge tube to release the *M. oryzae* spores from conidiophores (Figure 3.3).

7. Spores were filtered using sterilized miracloth and spores suspension was transferred to sterilized 50 mL Falcon tube.
8. The spore concentration was checked using Haemocytometer and spore concentration was adjusted to $1x10^5$ spores/mL using Tween-20 added distilled water (Step 5).
9. Now, spore suspension is ready for spraying.

The prepared spore suspension was sprayed onto 21 days old seedlings using a glass atomizer (Figure 3.3). The seedlings were grown in nursery. Several measures were taken to create and maintain an environment that is conducive for the blast pathogenesis that includes maintaining of proper humidity by creating mist. The mist was created at least 3-4 times a day using over-head sprinklers. The inoculated seedlings were observed for blast lesions at regular intervals after the inoculation.

3.4.3 Field Screening and Disease Scoring

Observations were recorded, 25 days after sowing and/or after 7^{th} day post inoculation and plants were scored based on leaf blast severity by following Standard Evaluation System (SES) scale, International Rice Research Institute (IRRI) as given in Table 3.3.

Table 3.3: Description of SES Scale (IRRI 2002) for blast disease scoring

0-9	Scale	Disease severity
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size (smaller than 0.5 mm in diameter)	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Moderately Resistant
3	Lesion type same as in 2 with 1-3 mm in diameter, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical spindle shaped susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately Susceptible

5	Typical susceptible blast lesions of 3 mm or longer infecting 4- 10% of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

3.4.4 Genotypic Characterization of Germplasm for Blast Resistance

3.4.4.1 Genotyping and Molecular Marker Analysis

Marker validations of different germplasms were carried out using gene specific SSR markers for blast and Genome Wide Association Studies (GWAS). About 20 blast resistance gene markers and 96 SSR markers for GWAS were used which are listed in Table 3.4 and Table 3.5, respectively.

3.4.4.2 Isolation of Genomic DNA

Genomic DNA was isolated from young leaf tissue following the Cetyl Trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) with minor modifications.

1. Extraction Buffer

Cetyl-Trimethyl Ammonium Bromide (CTAB) extraction buffer (100 mL) was used. Preparation of stock solutions for CTAB buffer:

A. 1M Tris HCl (pH 8.0):

Tris base of 12.114 g was dissolved in 40 mL of distilled water. The pH was adjusted to 8.0 with concentrated HCl and the solution was cooled to room temperature. After cooling the final volume was made to 100 mL with distilled water and the solution was autoclaved at 121˚C temperature and 15 psi pressure of about for 20 minutes and stored in a transparent reagent bottle.

Table 3.4: List of 20 Gene Specific Markers used in the study.

Sr. No	Gene	Marker used	Forward (5`-3`)	Reverse (5`-3`)	Type of marker	Reference
1	**Pib**	**Pb28**	GACTCGGTCGACCAATTCGCC	ATCAGGCCAGGCCAGATTTG	SNP	Hayashi et al., 2006
2	**Piz**	**Z56592**	GGACCCGCGTTTTCCACGTGTAA	AGGAATCTATTGCTAAGCATGAC	SNP	Hayashi et al., 2006
3	**Piz-t**	**Zt56591**	TTGCTGAGCCATTGTTAAACA	ATCTCTTCATATATATGAAGGCCAC	SNP	Hayashi et al., 2006
4	**Pik**	**K39512**	GCCACATCAATGGCTACAACGTT	CCAGAATTTACAGGCTCTGG	SNP	Hayashi et al., 2006
5	**Pik-p**	**K3957**	ATAGTTGAATGTATGGAATGGAAT	CTGCGCCAAGCAATAAAGTC	SNP	Hayashi et al., 2006
6	**Pikm**	**K6441**	CGTGCTGTCGCCTGAATCTG	CACGAACAAGAGTGTGTCGG	InDel	Ashikawa et al 2008
7	**Pi54**	**Pikh**	CAATCTCCAAAGTTTTCAGG	GCTTCAATCACTGCTAGACC	FM	Ramkumar et al., 2011
8	**Pi9**	**Pi9-i**	GCTGTGCTCCAAATGAGGAT	GCGATCTCACATCCTTTGCT	FNP	Yang et al., 2017
9	**Pi2**	**Pi2-i**	CAGCGATGGTATGAGCACAA	CGTTCCTATACTGCCACATCG	FNP	Yang et al., 2017
10	**Pita/Pita2**	Pita3	AGTCGTGCGATGCGAGGACAGAAAC	GCATTCTCCAACCCTTTTGCATGCAT	FM	Hayashi et al. (2006)
11	**Pi5**	**40N23R**	TGTGAGGCAACAATGCCTATTGCG	CTATGAGTTCACTATGTGGAGGCT	InDel	Jeon et al., 2003
12	**Pit**	**tk59-1**	ATGATAACCTCATCCTCAATAAGT	GTTGGAGCTACGGTTGTTCAG	FM	Hayashi et al., 2010
13	**Pit**	**tk59-2**	ATGATAACCTCATCCTCAATAAGT	CCAAGGGATTAGGTCCTAGTG	FM	Hayashi et al., 2010
14	**Pish**	**RM6648**	GATCGATCATGGCCAGAGAG	ACAGCAGGTTGATGAGGACC	LM	Koide et al. 2010

Sr. No	Gene	Marker used	Forward (5`-3`)	Reverse (5`-3`)	Type of marker	Reference
15	**Pi33**	**Rm72**	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	LM	Berruyer et al. 2003
16	**Pia**	**Pia-STS**	CTTTTGAGCTTGATTGGTCTGC	CTATTGCACCAGAGGGACCAG	FM	Okuyama et al 2011
17	**Pi1**	**RM1233**	GTGTAAATCATGGGCACGTG	AGATTGGCTCCTGAAGAAGG	SSR	Fuentes et al. 2008
	Pi1	**RM224**	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	SSR	Jiang et al. 2012
18	**pi21**	**pi21_79-3**	GATCCTCATCGTCGACGTCTGGC	AGGGTACGGCACCAGCTTG	Indel	Fukuoka et al. 2009
19	**Pi56**	**CRG4-2**	CCTGTCAGTCTTTCCGAGAG	GAATCCGGTAGCTCAAGGTG	Gene specific	Liu et al. 2013
20	**Pi65**	**SNP_3**	TGCCACCAGCCATCTTCAACAT	ACCACATCACTCATCGCCATCC	Indel	Zheng et al. 2016

Table 3.5: List of 96 SSR markers used for Genome-Wide Association Studies.

Sr. No.	Chr. no.	Marker used	Primer sequence		Physical location (bp)
			Forward (5`-3`)	Reverse (5`-3`)	
1.	**1**	RM495	ATGATGATGGACGACGACAACG	TGAATCCAAGGTGCAGAGATGG	215,956
2.	**1**	RM10123	ACACATCCCTCATCATCATCTCC	GAATCCGATCTTGTTCTGATACCC	2,282,947
3.	**1**	RM5443	TACGGCTTACCCATAGCAGC	AAACGGAGGGAGTATTTCCC	6,224,260
4.	**1**	RM10820	ACCCTAGCTAGCCACCATGAACC	GAAAGCCCTTCTTTCTCCACTCTACC	13,261,114
5.	**1**	RM11184	GCGGAGAAGTAGGAGTCCAAGG	GTCAACCTCCGCTTCCATCG	21,542,145
6.	**1**	RM11486	AGCAGACCAATGAACCATGAACC	GGTTTGGCTAAACTCATGAGAGAGG	27,544,962

Sr. No.	Chr. no.	Marker used	Primer sequence		Physical location (bp)
			Forward (5`-3`)	Reverse (5`-3`)	
7.	**1**	RM11943	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	37,851,779
8.	**1**	RM12233	CTTGAGTTCGAAGCGAGAAGACG	CACTTGAGCTCGAGACGTAGCC	42,415,653
9.	**2**	RM12349	CCGATTAGCGATTGATATGGAGTAGG	AGTGCACAGCCATGGAATTATGC	977,704
10.	**2**	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	2,882,052
11.	**2**	RM12634	GGGTTTATAACCGGGAGTAAAGG	GGCTTATCAGTCATACAACAGACG	5,083,063
12.	**2**	RM12806	CACTGCTTTGCTCTCCAGTCTCC	CAACATCTCCGTCAGAATCAAGC	7,706,704
13.	**2**	RM550	CTGAGCTCTGGTCCGAAGTC	GGTGGTGGAAGAACAGGAAG	12,464,295
14.	**2**	RM13199	AATCCCTCTCCCTGGCGAAGTCC	CGTCCTCACCACCAATCCCTTCC	16,462,458
15.	**2**	RM13477	ACGGAGAAGGCGAGGAGGATGG	ATGGCGCCTGCTACGACTGTCC	21,922,781
16.	**2**	RM14188	GTGTGCTCCTTGGGAAGAAAGG	GGGAGAAAGGTGGACATGTAAGC	35,241,945
17.	**3**	RM14320	CACCTGTAAATTAGGACACTGG	CAGTGTACTTTGAACTGCCTAGC	1,341,631
18.	**3**	RM14602	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	6,167,252
19.	**3**	RM14860	GGAAGGTGATTTCATCCGGTAGC	TGGCATGTTTAATGCTGGTTCG	11,653,160
20.	**3**	RM15203	ATGGTGAGACGACAGAACAGATGC	GACGCGCTTTGTGTTTCTTGG	16,919,911
21.	**3**	RM15490	GTGGGAGAGGCGAGGGTTTAAGG	CAGGGCCCATATGTGAGTTGAGG	23,822,358
22.	**3**	RM15761	TCCACGTGTTATCCTCTCTTTGC	CCAGATTCCTGCGTTGTACAGG	28,322,531
23.	**3**	RM15952	TGCTCCTGTTGTCATTCTTTGG	ATAAGATCGACTTTGCCGACAGC	31,399,285
24.	**3**	RM16238	GCAGCGCATCATTGTATTAAGG	GGACACTAAATCAGAAACCCATGC	36,268,979
25.	**4**	RM16284	ACACTGCTCGGGAGTTACTGACC	CAATAAGCAGGGCTACAACATGC	523,445
26.	**4**	RM16459	TCCAGGAGTTTGCCTTGTAGTGC	TAGCGAAGTCAGGATGGCATAGG	5,209,425
27.	**4**	RM16575	CACCAACTACACTCCTACACTCC	CTAGATCATAGGCGGTCACG	10,021,327

Sr. No.	Chr. no.	Marker used	Primer sequence		Physical location(bp)
			Forward (5`-3`)	Reverse (5`-3`)	
28.	**4**	RM16706	AGCAGGCTCACAGCAGCACAGC	CTGTCGTCGAAGTCAATTCCGAATCC	15,211,518
29.	**4**	RM16926	CGACTTGCCAAAGGTCAACG	GATTCTACGGGCCACAAGTCC	20,032,520
30.	**4**	RM17201	GATCGTTGCTGCTTTCAATGAGG	AGTGTTTCACCTTGGACCCATGC	25,220,310
31.	**4**	RM17438	CTTCCGAGTGCCAAATAAACTGC	TCTAGACGCGTATGGTGATTTCG	30,022,550
32.	**4**	RM17682	GCCAGCAGCAAACTCGTTGTTCC	TGCAGTACCACGTGGAGAACTGAGG	34,930,479
33.	**5**	RM17753	CGCTACGGAAGGGTAATAATGC	AGCGTGGGAAGAAGGATACACC	471,657
34.	**5**	RM17903	CCTCCTCAGGGCCAAACATTGC	TTGAGTGGCTCTGGACCTTCTTCAGC	2,915,930
35.	**5**	RM18004	CTCGAAGCTATTAGCCGGGATCG	ATCTTCTTCCTCGCCGTCTTCC	5,030,702
36.	**5**	RM18222	TGATTCCTCTATATGCAGCCTTGG	TATCGTGGTTTCATCGTGTGTGC	10,123,637
37.	**5**	RM18334	TCCAACAAAGCAAGAGCAAGAGG	CTGGCCTTCCAGAAATATCAAGG	13,474,576
38.	**5**	RM18655	GGTAGGCACCAAAGAGTTTGACG	GGCATCACCTTATCCAATCACC	19,975,045
39.	**5**	RM18948	GTGATGGTTGCTTTCTTTCTCTCG	TCACGTTGCTCAGCTCACTATCC	24,989,636
40.	**5**	RM19160	AGTAGTGCAGACGCGAGGAACC	GAACAGGAGAGAGGGAGAGAAACG	28,858,690
41.	**6**	RM19255	TTAAGCTAGGGAATCAGCGGTTAGC	GGAGTTGCAGTGTGGTGTGTGG	534,683
42.	**6**	RM19364	TTCCCATCTGCACTACCATAATCC	GAGCAGAGATGTGCTTTGCTACC	2,292,801
43.	**6**	RM19552	TGCTGCCACATGTTTGTCATGG	AAGAAAGGGATAGTTGCGGAGTGG	5,212,353
44.	**6**	RM19850	GATGTTCTCTCGGTTTGGACTTCG	CGACGAACAACAACTCAACTTTCACC	11,247,523
45.	**6**	RM20046	AACGAGCGGCTGAGATTTATGG	ATGTACCAGCCCTTTGAGAGTGG	16,092,252
46.	**6**	RM20228	TTTCAGCATGACGCAGTTGTCC	GATCCCATTGAGTATTGTACCTCACG	21,033,706
47.	**6**	RM20462	CTAGTACGATTGCTGTGGCTGTCG	GGGAAGGGAGCCATGAAATAGG	25,888,951
48.	**6**	RM20724	GGCATATATGTGTACAAGGGATGG	CCAAATCCAAGATTCCAAGTCC	30,702,259
49.	**7**	RM20810	TGGCTCACCGAGTTCCCTTTGC	CGCCATTGTTCAGCTCCTCTGC	306,306
50.	**7**	RM20948	GCAAGCTGGAAGAACATCGTACC	TGCTTATGGTTCTGGTCACTTCG	2,493,623

Sr. No.	Chr. no.	Marker used	Primer sequence		Physical location (bp)
			Forward (5`-3`)	Reverse (5`-3`)	
51.	**7**	RM21258	TATCATTCCGGTCCAAAGTGTCG	TCCGGTCCAAAGTCTCATTTGC	7,179,993
52.	**7**	RM21421	CGCTCCTTTCTAGCTCCATCTCC	AACTGCAACGAGTAAGGCAGAGG	12,713,008
53.	**7**	RM21640	CACCTGCAGGACTGGATTTGG	CGTGGGAAAGTATGAGCATTCAGC	18,730,408
54.	**7**	RM21842	GAACGGGAGGAGGAGTTGTAGG	GACTTCATTTCAACTCGACGATGG	22,776,600
55.	**7**	RM22085	CCGCCTAGAAACACTGAACTATTGG	GACTACCGGAGGGCCATCTACG	27,786,294
56.	**7**	RM22181	ATTCTGGGACTGGAGGCTCTTGAGG	TCGCCTCCATCCATGTGATTCC	29,592,242
57.	**8**	RM22212	TCCTCTGAATCTTCACAGTTGG	GAAGAAGAACTCGAAGCATGG	189,294
58.	**8**	RM22383	ACTTATCAGCCGCCTCCTCTCG	ATGAGCTCGTCCTCCTGGATGC	2,923,219
59.	**8**	RM22554	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC	5,592,398
60.	**8**	RM22659	GTCGTCGGAGACCACGATAGTCC	CGGCGCGCGACTACTATTACG	7,880,637
61.	**8**	RM22870	CCCGGTAGTAGTGGGTTATGTCC	CTAGTCGCCCTGAGAAGAAGACC	13,916,781
62.	**8**	RM23026	CTCTGACCTTGAGAAAGGAAATCG	GCATGATCTAATACCGGTGATGC	18,472,776
63.	**8**	RM23292	GGAGGAGAGCCAAGCGATGG	ACCGTCTTGACGCTGAGAGTGC	23,231,815
64.	**8**	RM23578	AGCGATTCAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC	27,473,038
65.	**9**	RM23662	GAGAGGACGATGGCACTATTGG	CGAGGAACTTGATTCGCATGG	430,978
66.	**9**	RM23744	CTTAATACTCCGACGTAACAGTGG	CCTGACTAAATGGAGCTTCTTCC	2,913,810
67.	**9**	RM23835	TTCCGCTGTTTCTCTTCTTGTGC	CTGGTTCTGCTGGTTCTGTAGTTGG	5,527,971
68.	**9**	RM23937	CACATTGAAACCATCTGGGCTTGG	GAATGGACGGCTTCTCTGTGTTGC	7,771,951
69.	**9**	RM24199	CTCGTAAGCCTAGGCCATCAAGC	AAACTTGAGCTTGGCTCGTTTCC	12,811,434
70.	**9**	RM24542	ATCCACAAGAGCACCGATGAGG	TGACCTGGTAGTGGTGAGTGTGC	18,173,373
71.	**9**	RM24683	CAGTGGCGTGGAGAGAAATTTGG	CTCACCTGCGACAGCAAGATCG	20,547,878
72.	**9**	RM24814	CGAACGAACTTGAACGAGCTAGG	GTCACTTGCGTGCTCCAATTCC	22,475,800
73.	**10**	RM24878	TCAGAGTGCATCTCGCTCACTACC	CGCATGCATATGTTGTCGATGG	314,293
74.	**10**	RM24999	AGATGAGGAGATGAGGAGCAGGAAC	CCGGGCACCATGCTATCTAATGC	3,007,026

Sr. No.	Chr. no.	Marker used	Primer sequence		Physical location (bp)
			Forward (5`-3`)	Reverse (5`-3`)	
75.	**10**	RM25092	CTATCTCCCTTGATGCGTACATGC	AAATCAGCGCGTGACAATTCG	5,348,752
76.	**10**	RM25262	CAATGCAAAGTCTTGTACGG	GCTACATTGCATAGATCACTCG	10,899,469
77.	**10**	RM25516	CCCATACACGTGGTTGCTCACC	CGACGAAGAAGCCAGCATTCG	16,805,238
78.	**10**	RM25679	GAAGCCTCCTTGATGTTGACTGG	CAACGATTATGCGTCCTTAGATGC	19,415,921
79.	**10**	RM25817	GCCTCGAATCAACCAAATAGTGG	GGGCTGAGACCTTGTGGTATGG	21,644,427
80.	**10**	RM25940	CGGTGCCTTCACCACACATCG	GAAAGCAAAGGGAGTGAGGGAGATGG	23,115,932
81.	**11**	RM26002	CGATTGATCCCGTGCAAGTAGG	CATGCTAGTGCATTCTGCGTAGG	904,685
82.	**11**	RM26118	CACCCTACATAGGTGTTCACATCG	CTCCCTAGGAAGCTGAGAAGAGG	3,025,904
83.	**11**	RM26241	TTGATCACCTACACGTACACATGC	AAGGTCCTTTGAGCATTCAGTCG	5,481,301
84.	**11**	RM26362	ATCCGTCCTAGAATACTTGTCG	CATCCGTCCTAAATTAGTAGCC	8,023,301
85.	**11**	RM26603	GATTCCGATAGAACGGAAGAGAGC	GAAGACCTCCTCACCAGTGAACC	13,857,650
86.	**11**	RM26823	CGACACAGTTACAAAGCCACAGC	TGGGAGGACGTTGATATGTCTCG	19,082,766
87.	**11**	RM27096	AGTTAGGATCGCTTCCAGGTTCC	TCCAACTGGAATATCGTCTTGTAGGC	23,866,321
88.	**11**	RM27387	TAGGTCCATCCAAATCTCGATCC	TGGCAGAGGAGATTAGAGTAATACGG	28,957,562
89.	**12**	RM27400	CTCGTTCCTCCTTCCTCATCACC	GGTGAAGTACTCACATTCCGATGG	104,894
90.	**12**	RM27534	GAACGTGAAAGAGAAGCTCATGG	TCTCCTTCTCTCCCAACATCTCG	2,611,621
91.	**12**	RM27819	CCACATATCAATGGCCTATCTTCC	AGCAGCAACTTCCTCATGTTGG	7,579,529
92.	**12**	RM27926	GCCATCACTGTACCTTGTTCTTGG	CAATGCCGACGAGTTCTTCTCC	10,254,738
93.	**12**	RM27982	CTGGTGAAAGCTTGAGGATCTCG	ATCAGTTCGTACAGGCCCAACC	12,628,094
94.	**12**	RM28207	TTTGCTGATCTAGTACGTGTGTGG	GTAGTGCTTCTTCCCGTTGATCC	18,306,763
95.	**12**	RM28367	CGTATCTCCACCTCCCGAGAAGC	GCCAAATCTCACGGATCGAAGC	21,173,248
96.	**12**	RM28722	TCGCCTTCTCTGCTCGCTACTGC	TTGCATTTGGGTCAGCAACTCTGC	26,119,421

B. 0.5M EDTA (pH 8.0):

Disodium Ethylene Diamine Tetra Acetate (EDTA) of 18.612 g was added to 40mL of distilled water and the pH adjusted to 8.0 by adding NaOH pellets and stirred vigorously on a magnetic stirrer. The total volume was adjusted to 100 mL with distilled water, sterilized by autoclaving and stored in transparent reagent bottle.

C. **4M NaCl**:

23.37 g of NaCl was dissolved in 40 mL of distilled water and the final volume was made upto 100 mL with distilled water. It was sterilized in autoclave and stored in room temperature in transparent reagent bottle.

CTAB 2% (w/v)

Tris HCl (pH 8.0) 1M

Sodium chloride 4M

EDTA 0.5M

(Tris, sodium chloride and EDTA were autoclaved and 2% CTAB was added after autoclaving and preheated before using the buffer)**.**

3. **Phenol : Chloroform : Isoamyl alcohol 25:24:1 (v/v)**
4. **Chloroform : Isoamylalcohol 24:1 (v/v)**
5. **3 M Sodium acetate**

40.82 g of Sodium acetate was added to 40 mL distilled water and thoroughly mixed using magnetic stirrer. The pH was measured and adjusted to 5.2 using glacial acetic acid. Finally, the volume was made upto 100 mL.

6. **Ethanol (70% and 100%)**
7. ***RNase* A - 10 mg/mL**

DNA Extraction Protocol

The leaf sample was prepared for DNA isolation by surface sterilization with 70% ethanol. DNA was extracted from the frozen leaf samples (–80°C) using CTAB protocol of Murray and Thompson (1980) with extraction buffer (2% CTAB, 1M Tris, 0.5M EDTA, 4M NaCl and 0.02% β-mercaptoethanol)and it was carried out as follows:

1. About two grams of healthy leaf samples were collected, cut into small bits with the help of sterile scissor and transferred to pre chilled mortar.

2. The leaf tissues were frozen using liquid nitrogen and ground to fine powder using a pestle.
3. The fine powder was transferred to 2 mL polypropylene centrifuge tubes into which 800 µL of pre-warmed CTAB (add β-Mercaptoethanol prior to heating the buffer) buffer was added. The tubes were then incubated for 60 minutes at 65°C in water bath with intermittent shaking at 15 minutes interval.
4. The tubes were mounted on stands and left to cool down at room temperature, after which almost equal volume of Phenol: Chloroform: Isoamyl Alcohol (PCI) (25:24:1) was added using a micro-pipette. Solution was mixed by gentle inversion of tubes for 5 minutes.
5. The content was then centrifuged at 12000 rpm for 10 minutes at 4ºC. The supernatant was carefully transferred to a fresh tube with wide bore tip to avoid DNA shearing.
6. 3µl of RNase-A solution was added into the aqueous supernatant to remove the RNA. It was then mixed properly and incubated in water bath at 37°C for 1 hour.
7. After incubation, the tubes were mounted on stands and left to cool down at room temperature, after which almost equal volume of Chloroform: Isoamyl alcohol (24:1) was added using a micro-pipette. Solution was mixed by inversion of tubes for 5 minutes.
8. The content was then centrifuged at 10000 rpm for 10 minutes at 4ºC. The supernatant was carefully transferred to a fresh tube into which double the volume of chilled absolute alcohol (about 800 µl) was added followed by gentle inversion until fibrous mass was visible. Tubes were incubated at -20ºC overnight.
9. After storing it overnight, about 100 µl sodium acetate ($1/10^{th}$ volume) was added followed by gentle inversion for proper mixing and then the solution was stored at -20ºC for 2 hours.
10. After 2 hours, the tubes were then put to centrifugation at 10000 rpm for 10 minutes at 4ºC to obtain a precipitate. The supernatant was drained by gently inverting the tubes. The tubes were left inverted with lids open on blotting paper to drain the residual absolute alcohol.

11. After a while, the DNA pellet was washed twice with 70% ethanol by centrifuging @7000 rpm at 4ºC for 7 min.
12. The supernatant was drained out and the pellet was air dried and later dissolved using 100µL 1xTE (Tris-EDTA) buffer and was kept overnight at room temperature.
13. The dissolved DNA was stored at 4ºC for immediate uses or -20ºC for future uses.
14. The extracted DNA was subjected for checking quality and quantification using 0.8 per cent agarose gel electrophoresis.

3.4.4.3 Quantification of Genomic DNA

The quality and quantity of DNA were analyzed by running the genomic DNA samples on agarose gels (Figure3.2). This step would give us an idea on the extent of DNA shearing.

Materials

Loading dye

Glycerol 50% (V/V)

Bromophenol blue 0.5% (W/V)

Xylene cyanol Solution

10X TBE (Tris Boric EDTA buffer)

Tris base – 121 gm

Boric acid – 51.30 gm

EDTA – 3.7 gm

(Volume made up to 1000 mL with 8.0 pH)

Protocol

1. The gel casting plates were placed and care was taken to ensure that there was no leakage and the combs were placed properly in casting plate kept on a perfectly horizontal platform.
2. Agarose 0.8 % (0.8 g/100 mL) was added to 1x TBE buffer, boiled until the agarose dissolved completely and then allowed to cool. Ethidium bromide was added thereafter at 5 µL/100 mL to give fluorescence property to the DNA under UV light.
3. The dissolved gel was poured into the gel mould and allowed to solidify.

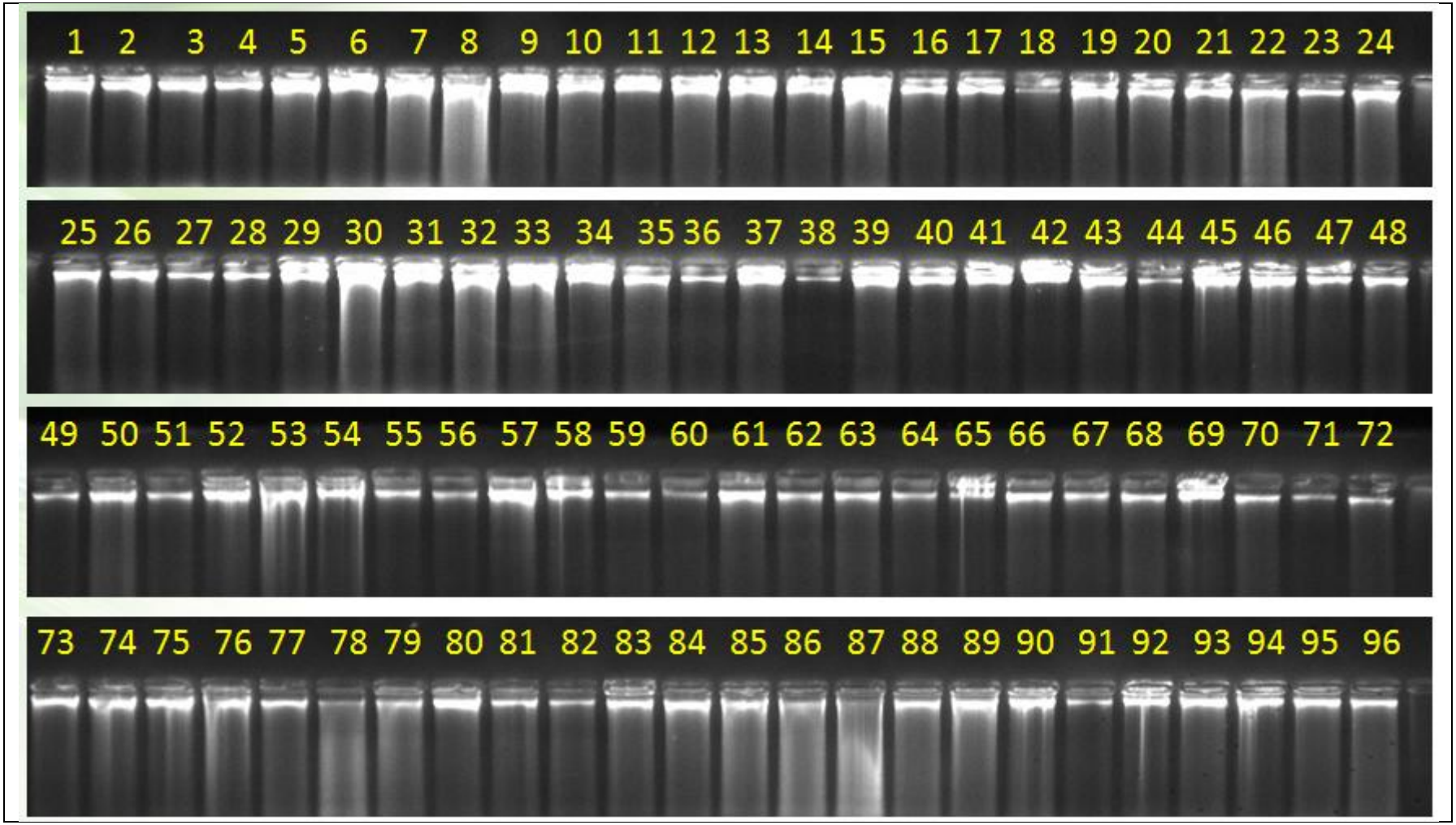

Plate 3.1 DNA quantification of 96 genotypes using 0.8% agarose gel. (1-96 represents genotypes CG1-CG96)

4. After solidification of the agarose, the gel is transferred to electrophoresis unit with wells towards the cathode and submerged with 1X TBE to a depth of about 1cm.
5. The combs are then removed carefully.

Loading the DNA samples

1. To quantify the DNA, a standard cut lambda (λ) DNA was loaded in gel electrophoresis along with about 2 µL of DNA sample mixed with 2 µL of loading dye.
2. The mixture was loaded on to 0.8% agarose gel.
3. The electrophoresis was carried out at 120V (Larger tank) for 30-45 minutes to run the samples.
4. After 45 minutes, bands were visualized and documented using a gel documentation system.
5. DNA samples were diluted to a final concentration of 1 ng/µL and used for amplification reaction. The diluted DNA was subsequently used for PCR amplification.

The amount of DNA in a sample was quantified by using the formula;

Genomic DNA concentration in µg/µL= OD at 260nm x 50µg/µL x dilution factor.

(Sambrook and Russel, 2001).

3.4.4.4 PCR Analysis

Polymerase chain reaction (PCR) is very simple method for *in vitro* amplification of specific nucleic acid using Taq DNA polymerase and short stretch of oligonucleotides (primers), which are specific to the DNA to be amplified and the DNA amplification involves repeated rounds of DNA synthesis.

Twenty blast resistance genes specific markers (Table 3.4) and ninety six SSR markers for GWAS (Table 3.5) of rice were amplified by PCR (Polymerase Chain Reaction) using unique flanking sequences as forward and reverse primers with the reaction volume of 10µL. The primer sequences were obtained from www.gramene.org and/or other previously published research work on blast resistance genes associated markers. The DNA amplification was performed with Eppendorf thermo cycler by adding different components as mentioned in Table 3.6.

Table 3.6: List of PCR components

PCR components	Concentration	Quantity
Template DNA	40 ng/µL	1.0 µL
PCR buffer	10x	1.0 µL
dNTP mix	2mM	0.5 µL
Forward primer	5pM	0.25µL
Reverse primer	5pM	0.25µL
Taq. DNA polymerase	3U/µL	0.1 µL
Water	-	6.9 µL
Total		**10µL**

Procedure

1. 1µL of the genomic DNA of each genotype was dispensed into the wells of PCR plates. The master mixture was prepared in a 1.5mL Eppendorf tube by adding 6.9µL of sterile distilled water, 1µL of buffer,0.5µL dNTP mix, 0.1µL Taq polymerase enzyme and 0.25µL each of forward and reverse primers.
2. 9µL of master mix was dispensed into each well of the PCR plate to create a reaction volume of 10µL.
3. The PCR plate was set up in programmable thermal cycler for DNA amplification. Once the reaction is setup, the components were mixed well and the mixture was given a short spin. The PCR reaction was started by placing the PCR plate in a thermal cycler. The PCR reaction in a thermal cycler was programmed under the following conditions (Table 3.7).

Table3.7: Temperature profile used for PCR amplification using SSR primers

Profile	Activity	Temperature	Duration	Cycle
1	Initial Denaturation	95ºC	5 min	1
	Denaturation	94ºC	30 sec	
2	Annealing	55ºC	45sec	35
	Extension	72ºC	45 sec	
3	Final Extension	72ºC	10 min	1
4	Storage	4ºC	∞	-

3.4.4.5 Resolution of Amplified products on Agarose gels by Electrophoresis

The PCR products were analyzed by electrophoresis using 2.5% agarose gel for gene specific markers (blast resistance) and 4% agarose gel for SSR markers (GWAS) in a horizontal gel electrophoresis unit.

3.4.4.5.1 Agarose gel preparation

Procedure

1. Agarose gel was prepared by weighing about 10 g of agarose for 2.5% and 16 g of agarose for 4%. These were transferred to reagent bottles containing 400mL of 0.5X TBE buffer and mixed well.
2. The contents were then boiled gently in a microwave oven with intermittent mixing until complete melting of agarose was achieved and the solution became crystal clear.
3. The gel-casting tray was washed with water and wiped with ethanol. When the boiled agarose solution had cooled down substantially, 10µL of Ethidium bromide (EtBr, 10 mg/mL) was added, mixed thoroughly and poured into the gel-casting tray.
4. The combs were immediately arranged in the slots on the gel-casting tray and the solution was allowed to solidify at room temperature for 20-30 minutes. Care was taken so that no air bubble was present.
5. Once the gel gets solidified, it was transferred into the electrophoresis unit containing sufficient buffer and the combs were gently removed.

3.4.4.5.2 Loading of PCR products and gel documentation

1. In 10 µL of PCR amplified product 4µL of loading dye was added.
2. 10 µL of the amplified PCR products were loaded into the wells by using micropipette.
3. To know and compare the expected size of the DNA, a standard low range DNA ruler of 100 bp was loaded along with the amplified PCR products.
4. The gel was run at 120 volts for 2.5-3.0 hours for 2.5% gels and 4-4.5 hours for 4% gels. The bands were visualized and documented in gel documentation system (Alpha Manager)
5. The viewed picture was photographed and saved for further scrutiny.

3.5 Statistical Analysis

3.5.1 Allele scoring and diversity analysis

The binary matrix representing different alleles of the ninety six SSR markers were scored as binary data; the presence (1) or absence (0) was used for estimation of genetic distance and similarity coefficients. An unweighted neighbor joining un-rooted tree was constructed in the DARwin5 program (Perrier and Jacquemoud-Collet, 2006) with bootstrap value of 1000 and dissimilarity index was calculated by using NEI coefficient (Nei, 1973). Polymorphism information content (PIC) value of the marker was estimated using the program POWERMARKER Ver3.25.

3.5.2 Data analysis

The data set of 96 SSRs markers were scored for the presence or absence of the alleles for each accession. The binary data was used to assess the genetic similarities using Jaccard's coefficient. The allele number, allele frequency and polymorphism information content (PIC) for all the markers was estimated using the program POWERMARKER Ver3.25 (Liu and Muse, 2005). The Principal Coordinate analysis of the genotypes was computed using the binary data matrix of 135 SSR markers through GenAlEx 6.5. The molecular variance within and between the population was assessed using analysis of molecular variance (AMOVA) using GenAlEx (Peakall and Smouse, 2012).

3.5.3 Statistical analysis for blast resistance genes

Analysis of the population structure was computed using a model based approach in the software program STRUCTURE version 2.3.4 (Pritchard *et al.*, 2012). The program was run using the admixture model and correlated allele frequencies at different K values (from K = 1 to K = 10), with 5 independent iterations per K value. The program was run with 200,000 burn-in period and 200,000 Markov Chain Monte Carlo (MCMC). The ΔK value was used to estimate the optimal K value using STRUCTURE HARVESTER programme (Earl, 2012) according to the method described by Evanno *et al.* (2005). A total of 96 linked markers distributed on 12 chromosomes were used to dissect the selected accessions into different groups with the threshold value of 0.55. Those accessions with <0.55 membership probabilities were retained in the admixed group. The

population structure (Q matrix) estimated from STRUCTURE was used as covariate for the GLM and MLM approach. The genetic association markers with the blast resistance was estimated using a general linear model (GLM) and mixed linear model (MLM) in the program TASSEL 5 (Bradbury et al., 2007). The kinship matrix (K) obtained using Tassel was used in the MLM approach. The *p*-value distributions (observed *vs.* cumulative *p*-values on a $-\log_{10}$ scale) were displayed on a quantile-quantile plot drawn Tassel.

CHAPTER - IV
RESULTS AND DISCUSSION

Rice is the main staple crop in India with the large number of varieties released every year. The effect of major blast resistance genes is often influenced by environmental factors and stages of plant. DNA markers tightly linked to resistant genes offer an efficient and quick way to choose for many types of blast resistance genes without performing phenotype-based screening. In the present study, 500 landraces originated from Chhattisgarh (260) and North Eastern states (240) were phenotyped and genotyped (selected 288 landraces) for nineteen major blast resistant genes which could provide valuable information for its further use in gene deployment and gene pyramiding in different rice growing regions of India and specifically in Chhattisgarh for blast disease control and can be directly used as a variety or donor for marker assisted selection (MAS). The extensive use of high–yielding varieties has narrowed down the genetic base of plant breeding material of food crops, which limit their future improvements (Tanksley and McCouch, 1997; Warschefsky *et al.*, 2014). Cultivation of genetically uniform varieties over large scale imposed high selection pressure on the pathogen populations which makes them vulnerable to biotic and abiotic stresses. Due to climate change and emergence of new virulent races global food security is at high risk. This threat can be evaded through the identification of new resistance genes and alleles from landraces and wild relatives. In the present study, the genetic diversity of nineteen major blast resistance gene and genome wide association study to identify major blast resistance *R* gene(s) was carried out in Chhattisgarh and North-East collections.

4.1 Evaluation of the Landraces against leaf blast resistance

In order to breed new varieties or pyramiding of existing varieties with genes for addressing the blast disease problem, the gene pool of cultivars must be broadened by introducing wild species, landraces and exotic germplasm into breeding programs. Traditional rice varieties whose seeds are maintained by farmers or scientists contain useful resistant genes. These genes are preserved in landraces from long time and they are of great interest in breeding programs to develop disease resistant varieties.

A total of 500 landraces (including 260 landraces from Chhattisgarh and 240 landraces from North-East) were selected for phenotypic evaluation for their reaction to blast pathogen. The phenotypic screening for blast resistance was conducted at National Rice Research Institute, Cuttack, Odisha during *kharif* 2017. The results of this evaluation are presented below.

The test genotypes were sown in a uniform blast nursery (UBN). Reaction of a set of 500 landraces along with suitable checks such as HR 12 and CO-39 (Susceptible checks) were evaluated under controlled conditions with artificial inoculation of *Magnaporthe oryzae* isolate (which was previously collected from rice field, NRRI, Cuttack, Odisha) by spraying the inoculums on 21 days old seedlings and the disease was allowed to develop. All the measures were taken to ensure the occurrence of maximum disease pressure that includes maintaining optimum humidity with the use of over head sprinklers and mist creators, covering the nursery with polythene covers during night and planting of susceptible check (HR 12 and CO-39) all along the borders and after every five test genotypes. The disease reactions were scored at 40 days after sowing, with a scale ranging from 0 to 9, when the susceptible spreader HR 12 and CO-39 were completely killed. The results of leaf blast disease evaluation are presented in Table 4.1.

Among the 500 landraces, 123 (24.69%) were highly resistant (with a score of 0 to 3), 217 (43.42%) were found to be moderately resistant (score of 4-5), while 160 (32.29%) were highly susceptible (score of 6-9)(Figure 4.1). The ratio of resistant landraces to the total landraces was observed to be 123/500 (24.60). The screening score varied from score of 0 (SGCARS 74, Bhaiya Khuta, and 41586) to score of 9 (Khutbadi, SGCARS36, etc.) as given in Table 4.1.

The observations also note that the range of disease severity varied from lesions infecting 0% (resistant) to more than 75% (highly susceptible) of leaf area affected (Figure 4.2). This result indicates that, experimental setup was robust and performed as expected and the location chosen was suitable for evaluation of Landraces for blast disease incidence. The main reason for moderate susceptibility of the large numbers of Landraces could be due to existence of

Table 4.1: Phenotyping of the landraces for blast resistance in Uniform Blast Nursery using 0-9 disease scale (SES, IRRI).

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
CG-1	SGCARS1	4	CG-25	Mutiya Dhhan	6	CG-49	Pote Khuji	5
CG-2	SGCARS2	5	CG-26	Gadur Sela	8	CG-50	Bhata Kandai	7
CG-3	Pakhiya Dhaan	6	CG-27	SGCARS6	5	CG-51	Sagi Pareta	5
CG-4	Gurmutiya	5	CG-28	Gada Khuta	6	CG-52	Gadur Sela	5
CG-5	Kurdaful	6	CG-29	Bhata Dubraaj	5	CG-53	Hardiful	4
CG-6	SGCARS3	6	CG-30	Lakhechi	4	CG-54	Bako Dhaan	3
CG-7	Pandri Lochai	4	CG-31	Gurmutiya	8	CG-55	Bakti Chudi	3
CG-8	Kurlu Dhaan	4	CG-32	Bandkari	3	CG-56	Bhata Mokdo	5
CG-9	Kakad kado	5	CG-33	Pat Dhaan	6	CG-57	Sona Sari	5
CG-10	Kata mehar	5	CG-34	Goydi	8	CG-58	Yami Gali	6
CG-11	Hathi Panjaro	5	CG-35	Titir Pakhi	3	CG-59	Temru Mudi	5
CG-12	Bariya Dhaan	6	CG-36	Karmuri Bhog	4	CG-60	Lal Baso	5
CG-13	Godavari	6	CG-37	Kale Tude Masino	6	CG-61	Badi Chudi	5
CG-14	SGCARS4	6	CG-38	Dumar Ful	6	CG-62	SGCARS10	4
CG-15	Jira Dhaan	4	CG-39	Hansa Dubraaj	5	CG-63	Manki Dhaan	4
CG-16	Aalag Dhaan	4	CG-40	BhaluDubraaj	5	CG-64	Dhgda Dhaan	4
CG-17	SGCARS5	5	CG-41	Aajam Lali	4	CG-65	Banspati	5
CG-18	Badsa Bhog	6	CG-42	Dongar Kabri	6	CG-66	SGCARS11	3
CG-19	Chpti Khuji	6	CG-43	Huldi Chudi	7	CG-67	Bhvar Gedi	5
CG-20	Aajan Dhaan	5	CG-44	Temru Mudi	6	CG-68	Chind Jhopa	6
CG-21	Kurlu Dhaan	7	CG-45	SGCARS7	4	CG-69	Badsa Bhog	7
CG-22	Kabro Dhaan	7	CG-46	Kata mehar	7	CG-70	Godandi Dhaan	6
CG-23	Kharla Muha	6	CG-47	SGCARS8	4	CG-71	Pandko Guda	4
CG-24	Turej Gada Khuta	5	CG-48	SGCARS9	5	CG-72	SGCARS12	3

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
CG-73	Nani chudi	5	CG-99	Bariya Dhaan	5	CG-125	SGCARS29	4
CG-74	Shivnath	2	CG-100	Sonpuri	3	CG-126	SGCARS30	5
CG-75	Pote Khuji	6	CG-101	Sorchu Badi	5	CG-127	SGCARS31	6
CG-76	Bash Mukhi	6	CG-102	Sendur senga	5	CG-128	SGCARS32	7
CG-77	SGCARS13	4	CG-103	SGCARS16	4	CG-129	Farsa Ful	6
CG-78	Kadam Ful	3	CG-104	Dhadhar Dhaan	3	CG-130	Ram Laxman	7
CG-79	Rang Gada Khuta	6	CG-105	SGCARS17	6	CG-131	Alti Mijo	6
CG-80	Hare Krishna	5	CG-106	SGCARS18	5	CG-132	Laycha	5
CG-81	Shivnath	4	CG-107	SGCARS19	5	CG-133	Sela Dhaan	7
CG-82	Tiki Chudi	4	CG-108	Kari Graas	4	CG-134	Masur Dhaan	7
CG-83	Rani Kanjar	5	CG-109	Jhumra	4	CG-135	Chatiya Dhaan	6
CG-84	DokaraMecha	3	CG-110	SGCARS20	5	CG-136	Kandai	6
CG-85	Rakhi Dhaan	6	CG-111	SGCARS21	6	CG-137	Khutbadi	9
CG-86	Surmatiya	6	CG-112	Pandri Satka	5	CG-138	Vishnu bhog	2
CG-87	UmariChudi	5	CG-113	Jira Dhaan	6	CG-139	SGCARS33	6
CG-88	Ratan Chudi	7	CG-114	Bhayar Dhaan	5	CG-140	Meso Dhaan	6
CG-89	Adga Dhaan	5	CG-115	SGCARS22	4	CG-141	Lodhyari	4
CG-90	Surmatiya	7	CG-116	SGCARS23	3	CG-142	SGCARS34	6
CG-91	Hiruya Dhaan	5	CG-117	Jeera dhaan	5	CG-143	SGCARS35	4
CG-92	Goydi	5	CG-118	Masuri Desi	5	CG-144	Jodra Nakti	6
CG-93	SGCARS14	4	CG-119	SGCARS24	4	CG-145	SGCARS36	9
CG-94	Lal Baso	4	CG-120	SGCARS25	4	CG-146	Sirodi Bako	7
CG-95	Mayur Fada	5	CG-121	SGCARS26	3	CG-147	SGCARS37	7
CG-96	Kari Graas	4	CG-122	SGCARS27	4	CG-148	Kata Barangi	6
CG-97	SGCARS15	4	CG-123	SGCARS28	5	CG-149	SGCARS38	6
CG-98	Kava Paadi	5	CG-124	Masur Dhaan	4	CG-150	Luchai Dhaan	5

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
CG-151	Umari Dhaan	3	CG-177	Noni Dhaan	5	CG-203	SGCARS63	5
CG-152	Gaada Khuta	2	CG-178	Kala Umari	6	CG-204	Bode Bargi	7
CG-153	Khuti Dhaan	5	CG-179	SGCARS46	3	CG-205	NaniChudi	6
CG-154	Kari Gudi	5	CG-180	SGCARS47	2	CG-206	Bhaispat	5
CG-155	Tama Koni	4	CG-181	SGCARS48	3	CG-207	Badsabhog	6
CG-156	Kata Nakti	4	CG-182	SGCARS49	4	CG-208	SGCARS64	3
CG-157	SGCARS39	6	CG-183	Aasan Chudi	4	CG-209	SGCARS65	3
CG-158	Tiki Chudi	6	CG-184	SGCARS50	4	CG-210	Kera Ful	5
CG-159	SGCARS40	6	CG-185	SGCARS51	4	CG-211	SGCARS66	5
CG-160	Neem Chudi	6	CG-186	SGCARS52	3	CG-212	Degichudi	6
CG-161	Nani Chudi	9	CG-187	SGCARS53	3	CG-213	Huldi Godi	6
CG-162	KusumJhopa	9	CG-188	SGCARS54	4	CG-214	SGCARS67	6
CG-163	SGCARS41	5	CG-189	SGCARS55	4	CG-215	Masuri Desi	6
CG-164	Kukda Bhour	4	CG-190	SGCARS56	4	CG-216	Kera Ful	5
CG-165	SGCARS42	5	CG-191	SGCARS57	6	CG-217	Baadi Lochai	5
CG-166	Kapoor Saay	5	CG-192	SGCARS58	2	CG-218	Karmuri Bhog	6
CG-167	Bhata Mokdo	4	CG-193	Umari chudi	-	CG-219	BadiLochai	6
CG-168	SGCARS43	4	CG-194	SGCARS59	5	CG-220	SGCARS68	6
CG-169	Pundri Satka	5	CG-195	SGCARS60	8	CG-221	Mokdodhaan	4
CG-170	SGCARS44	5	CG-196	SGCARS61	6	CG-222	SGCARS69	4
CG-171	Kukadi Mudi	5	CG-197	SGCARS62	4	CG-223	SGCARS70	6
CG-172	SGCARS45	4	CG-198	Mudariya	5	CG-224	Dhgdikaaj	5
CG-173	Kala Mali	5	CG-199	LokatiMachi	5	CG-225	SGCARS71	4
CG-174	Motilur	2	CG-200	Ajuniya	5	CG-226	Dhotiya Dhaan	6
CG-175	Moha Dhaan	4	CG-201	UmariChudi	7	CG-227	Sonasari	4
CG-176	Mukukuda	6	CG-202	UmariChudi	5	CG-228	Bahiya Khuta	4

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
CG-229	Ganga Baru	6	CG-255	Gogal	4	RSG 21	41734	1
CG-230	Bghal Bijo	7	CG-256	SGCARS78	6	RSG 22	41743	1
CG-231	Kurlu Kabri	7	CG-257	Assam Chudi	7	RSG 23	41744	1
CG-232	SGCARS72	6	CG-258	UmariChudi	2	RSG 24	41745	1
CG-233	Huldi Gadi	5	CG-259	Bhaiya Khuta	2	RSG 25	41747	1
CG-234	SGCARS73	5	CG-260	Gada Khuta	8	RSG 26	41752	1
CG-235	SGCARS74	7	RSG 1	41586	0	RSG 27	41754	1
CG-236	SGCARS75	5	RSG 2	41588	1	RSG 28	41756	1
CG-237	UmariChudi	5	RSG 3	41589	0	RSG 29	41763	0
CG-238	Bhanvargedi	4	RSG 4	41592	0	RSG 30	41766	0
CG-239	SGCARS76	7	RSG 5	41593	1	RSG 31	41784	1
CG-240	Gaadha khuta	8	RSG 6	41596	0	RSG 32	41787	0
CG-241	Sargiful	6	RSG 7	41601	0	RSG 33	41792	0
CG-242	Kursobhog	6	RSG 8	41661	1	RSG 34	41796	1
CG-243	Kush Dhaan	7	RSG 9	41666	0	RSG 35	41797	2
CG-244	Rangovati	6	RSG 10	41668	0	RSG 36	41798	1
CG-245	Kaani Chudi	7	RSG 11	40670	0	RSG 37	41799	1
CG-246	Dubraaj	5	RSG 12	41671	0	RSG 38	41800	1
CG-247	Huldi Gathi	5	RSG 13	41675	1	RSG 39	41808	1
CG-248	Muthiya	6	RSG 14	41676	1	RSG 40	41826	1
CG-249	Huldi Gathi	6	RSG 15	41683	1	RSG 41	41827	1
CG-250	Bagdi Chudi	7	RSG 16	41695	0	RSG 42	41830	1
CG-251	Olesar	8	RSG 17	41712	0	RSG 43	41832	1
CG-252	Milkor Mel	9	RSG 18	41718	1	RSG 44	41846	2
CG-253	Kumhadaful	7	RSG 19	41723	0	RSG 45	41854	1
CG-254	SGCARS77	5	RSG 20	41728	0	RSG 46	41855	1

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
RSG 47	41863	1	RSG 73	41617	7	RSG 99	41687	5
RSG 48	41868	1	RSG 74	41618	6	RSG 100	41688	6
RSG 49	41587	2	RSG 75	41619	8	RSG 101	41689	5
RSG 50	41590	4	RSG 76	41620	7	RSG 102	41690	4
RSG 51	41591	5	RSG 77	41656	8	RSG 103	41691	8
RSG 52	41594	5	RSG 78	41658	7	RSG 104	41692	6
RSG 53	41595	5	RSG 79	41659	5	RSG 105	41693	7
RSG 54	41597	5	RSG 80	41660	4	RSG 106	41694	6
RSG 55	41598	5	RSG 81	41662	4	RSG 107	41696	6
RSG 56	41599	5	RSG 82	41663	4	RSG 108	41697	5
RSG 57	41600	6	RSG 83	41664	6	RSG 109	41698	7
RSG 58	41602	6	RSG 84	41665	4	RSG 110	41699	6
RSG 59	41603	6	RSG 85	41667	6	RSG 111	41700	9
RSG 60	41604	6	RSG 86	41669	5	RSG 112	41701	7
RSG 61	41605	8	RSG 87	41672	7	RSG 113	41702	8
RSG 62	41606	5	RSG 88	41673	6	RSG 114	41703	4
RSG 63	41607	6	RSG 89	41674	6	RSG 115	41704	5
RSG 64	41608	7	RSG 90	41677	5	RSG 116	41705	7
RSG 65	41609	6	RSG 91	41678	5	RSG 117	41706	5
RSG 66	41610	8	RSG 92	41679	6	RSG 118	41707	5
RSG 67	41611	4	RSG 93	41680	7	RSG 119	41708	5
RSG 68	41612	4	RSG 94	41681	7	RSG 120	41709	6
RSG 69	41613	7	RSG 95	41682	7	RSG 121	41710	6
RSG 70	41614	6	RSG 96	41684	4	RSG 122	41711	6
RSG 71	41615	7	RSG 97	41685	4	RSG 123	41713	6
RSG 72	41616	5	RSG 98	41686	6	RSG 124	41714	4

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
RSG 125	41715	5	RSG 151	41753	6	RSG 177	41790	4
RSG 126	41716	5	RSG 152	41757	5	RSG 178	41791	4
RSG 127	41717	4	RSG 153	41758	5	RSG 179	41793	5
RSG 128	41719	6	RSG 154	41761	4	RSG 180	41794	5
RSG 129	41720	6	RSG 155	41762	6	RSG 181	41795	6
RSG 130	41722	4	RSG 156	41764	7	RSG 182	41801	4
RSG 131	41724	5	RSG 157	41765	4	RSG 183	41802	4
RSG 132	41725	7	RSG 158	41768	4	RSG 184	41803	7
RSG 133	41726	5	RSG 159	41769	4	RSG 185	41804	5
RSG 134	41727	6	RSG 160	41770	4	RSG 186	41805	4
RSG 135	41729	6	RSG 161	41771	4	RSG 187	41806	5
RSG 136	41730	7	RSG 162	41772	5	RSG 188	41807	5
RSG 137	41731	4	RSG 163	41773	5	RSG 189	41809	4
RSG 138	41732	4	RSG 164	41774	5	RSG 190	41810	4
RSG 139	41733	4	RSG 165	41775	5	RSG 191	41811	5
RSG 140	41735	5	RSG 166	41776	4	RSG 192	41812	5
RSG 141	41736	5	RSG 167	41777	5	RSG 193	41813	6
RSG 142	41737	4	RSG 168	41778	5	RSG 194	41814	6
RSG 143	41740	5	RSG 169	41779	4	RSG 195	41815	6
RSG 144	41741	4	RSG 170	41780	4	RSG 196	41816	4
RSG 145	41742	7	RSG 171	41781	5	RSG 197	41817	4
RSG 146	41746	6	RSG 172	41782	6	RSG 198	41818	5
RSG 147	41748	4	RSG 173	41785	7	RSG 199	41819	6
RSG 148	41749	4	RSG 174	41786	6	RSG 200	41820	5
RSG 149	41750	5	RSG 175	41788	5	RSG 201	41821	6
RSG 150	41751	5	RSG 176	41789	6	RSG 202	41822	6

Code No	Name	Disease Score	Code No	Name	Disease Score	Code No.	Name	Disease Score
RSG 203	41823	4	RSG 216	41872	9	RSG 229	41867	9
RSG 204	41824	6	RSG 217	41874	8	RSG 230	41888	9
RSG 205	41825	6	RSG 218	41875	8	RSG 231	41889	9
RSG 206	41828	5	RSG 219	41876	9	RSG 232	41890	7
RSG 207	41829	5	RSG 220	41878	7	RSG 233	41891	7
RSG 208	41831	6	RSG 221	41879	8	RSG 234	41893	8
RSG 209	41834	6	RSG 222	41880	7	RSG 235	41894	7
RSG 210	41835	8	RSG 223	41881	9	RSG 236	41895	9
RSG 211	41836	4	RSG 224	41883	7	RSG 237	41896	8
RSG 212	41837	7	RSG 225	41884	7	RSG 238	41897	7
RSG 213	41869	**7**	RSG 226	41885	7	RSG 239	41898	7
RSG 214	41870	**7**	RSG 227	41886	8	RSG 240	41899	9
RSG 215	41871	8	RSG 228	41887	9			

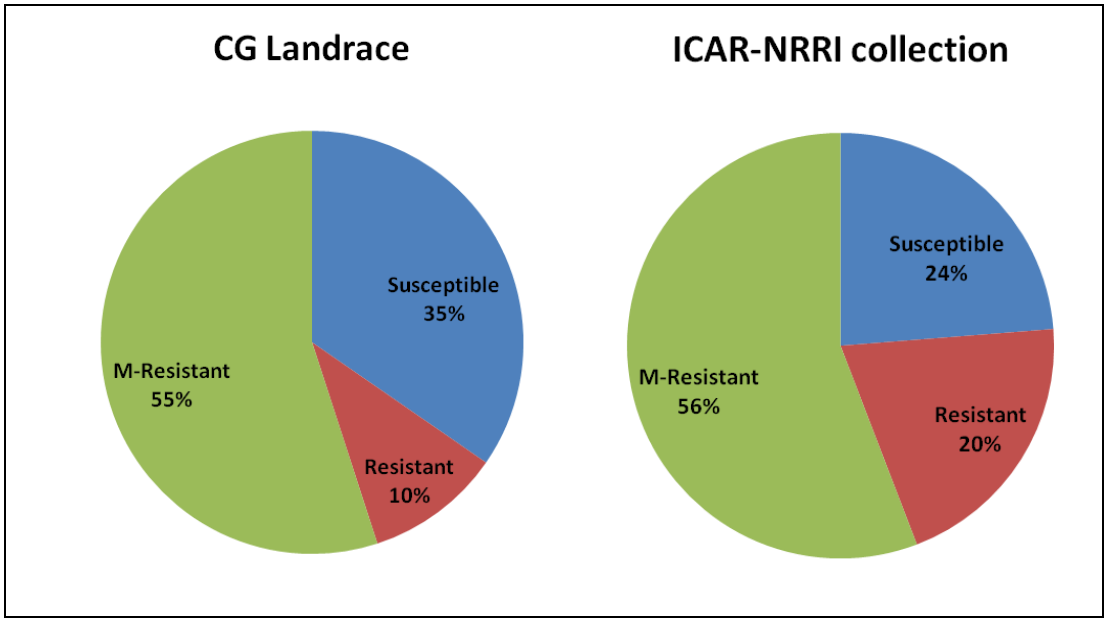

Figure 4.1: Graph depicting Blast disease reaction of the 500 rice accessions used in the present study.

Figure 4.2: Disease severity at 0% (left) and at >75% lesion (right) observed at the Uniform blast Nursery in NRRI, Cuttack.

highest disease pressure with artificial inoculation and also absence of major broad spectrum resistance genes in those landraces.

Multi-location blast screening increases the reliability of phenotyping as different virulent blast isolates have diverse pathogenic behavior (Madan *et al.*, 2012)., Previously, same approach was used for identification of best donors for blast resistance (Devi *et al.*, 2015; Aglawe *et al.*, 2017). Furthermore, the Chhattisgarh and North Eastern regions are endemic for rice blast.

4.2 Genetic diversity of blast resistant R genes

In order to effectively address the problems of Blast disease, there is need to understand the molecular mechanism involved in the gene-for-gene type of pathogen resistance. R-gene mediated resistance offers an environmentally sustainable solution for management of this important disease of rice. Therefore, mining of the resistant alleles might be important fundamental work in the breeding program, by studying different markers linked to resistant genes.

Molecular markers increase the precision of identification and incorporation of resistant genes in breeding programme. Many PCR-based markers have been developed to screen and identify different resistant genes.

In the present investigation, validation of blast resistant genes was carried out by using reported molecular markers which were linked to these resistant genes and even utilized by different workers with different set of genotypes at different locations. SSR marker that were either gene based or gene linked to blast resistant genes were employed in the genotypic evaluation of the selected 288 landraces. These landraces were screened with 20 known gene linked markers for blast resistant genes and the list of markers, associated genes, used for this study are given in Table 3.4 and its position is given in Figure 4.3. The genotypes which harbored different numbers and types of resistant genes are listed in Table 4.2 and Table 4.3 respectively.The present study aims to study the genetic diversity of major blast resistant genes in unexplored germplasm for identifying the best donors and markers eighteen major blast resistance genes varied from 6.25% to 27.43%. The scoring data indicated that frequency of the positive allele of *R* gene ranges

from zero genes (23) to nine genes (2) in the 288 landraces (Figure 4.4 and 4.5). Only two rice accessions (41676 and 41745) possess maximum number of the positive allele of nine resistant genes. Fifty seven landraces (19.79%) showed positive bands for one *R* genes, sixty seven (23.26%) were positive for two *R* genes, Sixty (20.83%) for three *R* genes, forty four (22.50%) for four *R* genes, twenty two (7.6%) for five *R* genes, six (2.08%) for six *R* genes, three (1.04%) for eight genes, and two (0.69%) for nine *R* genes.

Anupam *et al.* (2017) identified the genetic frequency of seven blast resistant genes ranging from 0 to 80% in 74 local landraces collected from Tripura. Similarly, the gene frequency of the twelve major blast resistant gene in NRRI released varieties varied from 0 to 100%, and the genetic frequency of nine and ten major rice blast resistant genes varied from 6 to 97% in the in North East and 19.79 to 54.69% in Eastern germplasm (Yadav *et al.* 2017; Imam *et al.* 2014; Singh *et al.* 2015a).

Interestingly, twenty six resistant landraces did not show the presence of any of the tested genes that may harbour the novel. Identification of new donor for durable resistance and transferring resistant genes into popular varieties could lead to increase in resistance for blast disease (Das *et al.*, 2012).

The presence of *Pib* gene was determined by visualization of amplicons of 388 bp fragments using SNP marker Pb28 along with the positive control IRBLB-b. This gene was found to be present in 23 genotypes, showed gene frequency of 7.98%. The *Pib* gene was also detected in NRRI released varieties, North East and Eastern germplasm and Manipur rice accessions (Yadav *et al.*, 2017; Imam *et al*., 2014 and Mahender *et al*., 2012). The presence of *Pish* gene was estimated by visualization of PCR product with the linked markers, RM6648. The average gene frequency was found to be 15.62%. The estimation of Pit *R* gene localized on chromosome 1 was determined through visualization of 733 bp and 530 bp amplicons corresponding to the tk59-1 and tk59-2 markers, respectively. Twenty eight landraces found to be positive for *Pit* gene with 9.72% gene frequency.

Table 4.2 List of Genotypes harbouring different number of *R* genes.

Code No.	Name	No. of gene	Code No.	Name	No. of gene
CG-1	SGCARS1	2	CG-38	Dumar Ful	3
CG-2	SGCARS2	2	CG-39	Hansa Dubraaj	2
CG-3	Pakhiya Dhaan	4	CG-40	BhaluDubraaj	5
CG-4	Gurmutiya	3	CG-41	Aajam Lali	0
CG-5	Kurdaful	3	CG-42	Dongar Kabri	0
CG-6	SGCARS3	4	CG-43	Huldi Chudi	1
CG-7	Pandri Lochai	3	CG-44	Temru Mudi	3
CG-8	Kurlu Dhaan	4	CG-45	SGCARS7	2
CG-9	Kakad kado	4	CG-46	Kata mehar	2
CG-10	Kata mehar	5	CG-47	SGCARS8	4
CG-11	Hathi Panjaro	2	CG-48	SGCARS9	4
CG-12	Bariya Dhaan	5	CG-49	Pote Khuji	4
CG-13	Godavari	1	CG-50	Bhata Kandai	2
CG-14	SGCARS4	2	CG-51	Sagi Pareta	1
CG-15	Jira Dhaan	2	CG-52	Gadur Sela	0
CG-16	Aalag Dhaan	4	CG-53	Hardiful	2
CG-17	SGCARS5	5	CG-54	Bako Dhaan	2
CG-18	Badsa Bhog	3	CG-55	Bakti Chudi	2
CG-19	Chpti Khuji	5	CG-56	Bhata Mokdo	4
CG-20	Aajan Dhaan	3	CG-57	Sona Sari	3
CG-21	Kurlu Dhaan	3	CG-58	Yami Gali	1
CG-22	Kabro Dhaan	2	CG-59	Temru Mudi	0
CG-23	Kharla Muha	4	CG-60	Lal Baso	2
CG-24	Turej Gada Khuta	4	CG-61	Badi Chudi	2
CG-25	Mutiya Dhhan	4	CG-62	SGCARS10	1
CG-26	Gadur Sela	4	CG-63	Manki Dhaan	2
CG-27	SGCARS6	7	CG-64	Dhgda Dhaan	3
CG-28	Gada Khuta	2	CG-65	Banspati	2
CG-29	Bhata Dubraaj	3	CG-66	SGCARS11	2
CG-30	Lakhechi	5	CG-67	Bhvar Gedi	2
CG-31	Gurmutiya	1	CG-68	Chind Jhopa	1
CG-32	Bandkari	6	CG-69	Badsa Bhog	2
CG-33	Pat Dhaan	3	CG-70	Godandi Dhaan	1
CG-34	Goydi	3	CG-71	Pandko Guda	1
CG-35	Titir Pakhi	3	CG-72	SGCARS12	2
CG-36	Karmuri Bhog	5	CG-73	Nani chudi	2
CG-37	Kale Tude Masino	4	CG-74	Shivnath	2

Code No.	Name	No. of gene	Code No.	Name	No. of gene
CG-75	Pote Khuji	4	CG-119	SGCARS24	0
CG-76	Bash Mukhi	3	CG-120	SGCARS25	1
CG-77	SGCARS13	3	CG-121	SGCARS26	0
CG-78	Kadam Ful	5	CG-122	SGCARS27	1
CG-79	Rang Gada Khuta	3	CG-123	SGCARS28	3
CG-80	Hare Krishna	3	CG-124	Masur Dhaan	3
CG-81	Shivnath	3	CG-125	SGCARS29	3
CG-82	Tiki Chudi	1	CG-126	SGCARS30	0
CG-83	Rani Kanjar	2	CG-127	SGCARS31	1
CG-84	DokaraMecha	3	CG-128	SGCARS32	5
CG-85	Rakhi Dhaan	3	CG-129	Farsa Ful	1
CG-86	Surmatiya	2	CG-130	Ram Laxman	0
CG-87	UmariChudi	5	CG-131	Alti Mijo	1
CG-88	Ratan Chudi	3	CG-132	Laycha	3
CG-89	Adga Dhaan	2	CG-133	Sela Dhaan	0
CG-90	Surmatiya	3	CG-134	Masur Dhaan	0
CG-91	Hiruya Dhaan	5	CG-135	Chatiya Dhaan	2
CG-92	Goydi	3	CG-136	Kandai	0
CG-93	SGCARS14	5	CG-137	Khutbadi	2
CG-94	Lal Baso	3	CG-138	Vishnu bhog	0
CG-95	Mayur Fada	1	CG-139	SGCARS33	5
CG-96	Kari Graas	0	CG-140	Meso Dhaan	3
CG-97	SGCARS15	0	CG-141	Lodhyari	0
CG-98	Kava Paadi	2	CG-142	SGCARS34	3
CG-99	Bariya Dhaan	2	CG-143	SGCARS35	1
CG-100	Sonpuri	1	CG-144	Jodra Nakti	2
CG-101	Sorchu Badi	2	CG-145	SGCARS36	5
CG-102	Sendur senga	6	CG-146	Sirodi Bako	1
CG-103	SGCARS16	1	CG-147	SGCARS37	1
CG-104	Dhadhar Dhaan	3	CG-148	Kata Barangi	1
CG-105	SGCARS17	2	CG-149	SGCARS38	1
CG-106	SGCARS18	2	CG-150	Luchai Dhaan	4
CG-107	SGCARS19	3	CG-151	Umari Dhaan	4
CG-108	Kari Graas	3	CG-152	Gaada Khuta	3
CG-109	Jhumra	1	CG-153	Khuti Dhaan	2
CG-110	SGCARS20	2	CG-154	Kari Gudi	1
CG-111	SGCARS21	2	CG-155	Tama Koni	1
CG-112	Pandri Satka	3	CG-156	Kata Nakti	2
CG-113	Jira Dhaan	3	CG-157	SGCARS39	2
CG-114	Bhayar Dhaan	2	CG-158	Tiki Chudi	1
CG-115	SGCARS22	1	CG-159	SGCARS40	1
CG-116	SGCARS23	1	CG-160	Neem Chudi	1
CG-117	Jeera dhaan	0	CG-161	Nani Chudi	3
CG-118	Masuri Desi	4	CG-162	KusumJhopa	3

Code No.	Name	No. of gene	Code No.	Name	No. of gene
CG-163	SGCARS41	3	CG-215	Masuri Desi	0
CG-164	Kukda Bhour	1	CG-216	Kera Ful	3
CG-165	GCARS42	0	CG-217	Baadi Lochai	4
CG-166	Kapoor Saay	0	CG-218	Karmuri Bhog	1
CG-167	Bhata Mokdo	1	CG-219	BadiLochai	0
CG-168	SGCARS43	3	CG-220	SGCARS68	3
CG-169	Pundri Satka	1	CG-221	Mokdodhaan	1
CG-170	SGCARS44	2	CG-222	SGCARS69	1
CG-171	Kukadi Mudi	0	CG-223	SGCARS70	2
CG-172	SGCARS45	1	CG-224	Dhgdikaaj	2
CG-173	Kala Mali	3	CG-225	SGCARS71	2
CG-174	Motilur	2	CG-226	Dhotiya Dhaan	0
CG-175	Moha Dhaan	2	CG-227	Sonasari	1
CG-176	Mukukuda	1	CG-228	Bahiya Khuta	2
CG-177	Noni Dhaan	1	CG-229	Ganga Baru	4
CG-178	Kala Umari	5	CG-230	Bghal Bijo	2
CG-179	SGCARS46	2	CG-231	Kurlu Kabri	1
CG-180	SGCARS47	1	CG-232	SGCARS72	0
CG-181	SGCARS48	3	CG-234	SGCARS73	1
CG-182	SGCARS49	1	CG-235	SGCARS74	4
CG-183	Aasan Chudi	1	CG-236	SGCARS75	2
CG-184	SGCARS50	4	CG-237	UmariChudi	2
CG-185	SGCARS51	2	CG-238	Bhanvargedi	4
CG-186	SGCARS52	2	CG-239	SGCARS76	4
CG-187	SGCARS53	2	CG-241	Sargiful	1
CG-188	SGCARS54	1	CG-242	Kursobhog	1
CG-189	SGCARS55	1	CG-247	Huldi Gathi	1
CG-190	SGCARS56	1	CG-248	Muthiya	3
CG-191	SGCARS57	1	CG-249	Huldi Gathi	2
CG-192	SGCARS58	2	CG-252	Milkor Mel	1
CG-196	SGCARS61	3	CG-255	Gogal	4
CG-197	SGCARS62	2	CG-257	Assam Chudi	3
CG-198	Mudariya	3	CG-258	UmariChudi	0
CG-199	LokatiMachi	1	CG-259	Bhaiya Khuta	4
CG-200	Ajuniya	0	RSG-1	41586	2
CG-202	UmariChudi	4	RSG-2	41588	5
CG-203	SGCARS63	4	RSG-3	41589	1
CG-206	Bhaispat	3	RSG-4	41592	2
CG-207	Badsabhog	4	RSG-5	41593	3
CG-209	SGCARS65	2	RSG-6	41596	4
CG-211	SGCARS66	2	RSG-7	41601	4
CG-212	Degichudi	4	RSG-8	41661	4
CG-213	Huldi Godi	2	RSG-9	41666	2
CG-214	SGCARS67	2	RSG-10	41668	8

Code No.	Name	No. of gene	Code No.	Name	No. of gene
RSG-11	40670	3			
RSG-12	41671	5			
RSG-13	41675	4			
RSG-14	41676	9			
RSG-15	41683	4			
RSG-16	41695	5			
RSG-17	41712	4			
RSG-18	41718	1			
RSG-19	41723	4			
RSG-20	41728	4			
RSG-21	41734	6			
RSG-22	41743	5			
RSG-23	41744	3			
RSG-24	41745	9			
RSG-25	41747	2			
RSG-26	41752	4			
RSG-27	41754	4			
RSG-28	41756	5			
RSG-29	41763	5			
RSG-30	41766	4			
RSG-31	41784	7			
RSG-32	41787	2			
RSG-33	41792	6			
RSG-34	41796	3			
RSG-35	41797	3			
RSG-36	41798	3			
RSG-37	41799	3			
RSG-38	41800	3			
RSG-39	41808	7			
RSG-40	41826	2			
RSG-41	41827	3			
RSG-42	41830	4			
RSG-43	41832	4			
RSG-44	41846	4			
RSG-45	41854	6			
RSG-46	41855	3			
RSG-47	41863	6			
RSG-48	41868	5			

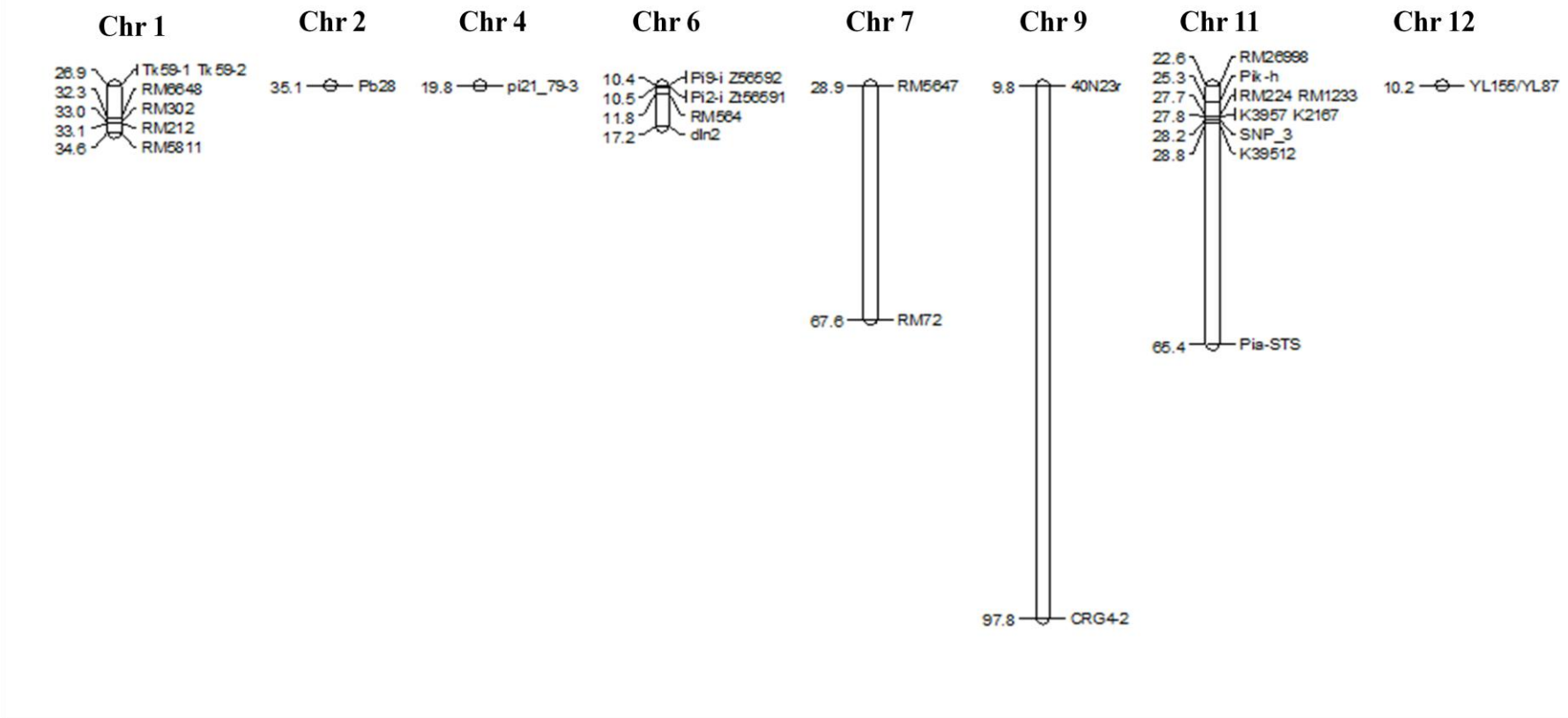

Figure 4.3: Graphical presentation of 20 markers used for the study of blast resistant genes.

Table 4.3. List of landraces that harboured blast resistant genes.

Markers	Genotypes
Pi56	Pakhiya dhan, SGCARS3, Kata meher, Kurlu Dhaan, Kharla mul, kabro dhan, Turej Gada Khuta, Muthiya Dhan, Kale Tude Masino, Dumar Phul, SGCARS9, Pote Khuji, Nani Chudi, Kadam phool, Adga dhan, Goydi, Surmatiya, SGCARS14, SCARS18, SGCARS19, Kari Grass,Jhumra, SGCARS28, Masoor dhan, Pharsa Phul, SGCARS33, Umari Dhan, Khuti Dhan, Motilur, SGCARS55, SGCARS63, Bhaispat, Badshah bhog, SGCARS65, SGCARS66, Degichudi, SGCARS76, Muthiya, 41586,41592, 41661, 40670, 41676, 41718, 41734, 41745, 41752, 41754, 41766, 41792, 41798, 41808, 41830, 41854, 41868.
Pi2450	Kurlu Dhan, SGCARS5, Karmuri Bhog, SGCARS9, Yamigali, SGCARS13, Kadamphul, Umari Chudi, Ratan Chudi, Sorchu badi, SGCARS23, Altimijo, Laicha, SGCARS34, SGCARS36, KAta Barangi, SGCARS38, Tama Koni, Kaata nakti, SGCARS39, SGCARS44, Kaala Mali, Kaala umari, Mudaria, Degi Chudi, Kera phul, SGCARS68, SGCARS70, Bahiy khuta, SGCARS74, Kumari Chudi, Bhaavar gedi, SGCARS76, Gogal, 41588, 41596, 40670, 41676, 41728, 41745, 41756, 41784, 41796, 41808, 41830, 41932,41846, 41854, 41855, 41863, 41868.
PiaSTS	Kurda phul, SGCARS4, Kurlu dhan, SGCARS6, Lakheji, Pat dhan, Kurmuri Bhog, Tiki Chudi, Mayur Phada, Kavapadi, Bariya dhan, Sonpuri, Masur Dhan, SGCARS29, SGCARS31, SGCARS32, Meso Dhan, SGCARS34, SGCARS35, SGCARS36, Gada Khuta, Bhata mukdo, SGCARS43, Kala Umari, SGCARS50, SGCARS58, Degi Chudi, Haldi kodi, SGCARS75, 41676, 41683, 41695, 41743,41744, 41745, 41747, 41756, 41766, 41792, 41799, 41827, 41846, 41868.

Markers	Genotypes
RM72	Kurda dhan, Haathi Panjaro, Bariya dhan, Alag Dhan, Chepti Khuji, Ajan Dhan, Turej Gada khuta, gadur sela,Lakheji, Karmuri Bhog, Kale Tude Masino, Dumar phul, SGCARS7, Bhata kandi, Hardi phul, Bhata Mokdo, Lal Baso, Manti Dhan,SGCARS11, Badshah bhog, SGCARS12, Pote Khuji, Kadam Phul, Shivnath, Dokra mecha, Umari Chudi, Sumatiya, SGCARS14, Lalbaso, Sochubadi, Sendur Senga, SGCARS20, Bhayar Dhan, SGCARS22,Khuti Dhan, Kari Chudi, Kusum jhopa, SGCARS41, Kaal mali, Motilur, SGCARS51, SGCARS52, Bhaispat, Badshah bhog, Badi lochai, Baghal bijo, SGCARS23, SGCARS76, Kurso bhog, Bilkormel, Bhaiya kuta, 41588, 41592, 41593, 41596, 41661, 41666, 41668, 40670, 41671, 41676,41695, 41712, 41723,41728, 41734, 41743, 41745, 41754, 41766, 41787, 41797, 41800, 41808, 41830, 41832, 41854, 41855, 41863.
Tk591	Kata meher, Bariya Dhan, Chepti Khuji, Ajan Dhan, SGCARS6, Kadam Phul, Hare Krishna, Kumari chudi, Sendur senga, Pandri sakta, Chatiya Dhan, Lochai Dhan, Gada khuta, Pundri Satka, Kaala Umari, Mundariya, 41588, 41601, 41683, 41752, 41763, 41784, 41832, 41863.
Tk592	Bariya Dhan, SGCARS5, Chepti Khuji, SGCARS6, Dumar Phul, SGCARS13, HareKrishna, Umari Chudi, KavaPadi, SGCARS16, SGCARS21, Gada Khuta, SGCARS50, SGCARS61, Degi chudi, Kurlu Kabri, 41593, 41661, 41668, 41676, 41712, 41743, 41752, 41792, 41796, 41830, 41863, 41868.

Markers	Genotypes
K6441	Kakad kad, Badshah Bhpg, Turej gada Khuta, Gada Khuta, Bhaata Dubraj, Gurmutiya, Bandkari, Titir Paakhi, Kale Ture Masino, Bhalu dubraj, Temru Mudi, Chind Jhopa, Badshah Bhog, pote khuji, Rakhi Dhan, hiruviya Dhan, sendur senga dhadar dhan, kari Grass, jeera dhan, Masuri desi, Masur Dhan, SGCARS32, Khutbadi, SGCARS33, SGCARS34, jodra nakti, Sirodi Bako, Kukda Bhor, SGCARS43, SGCARS45, SGCARS53, SGCARS62, Mudariya, Umari Chudi, SGCARS63, SGCARS66, Haldi godi, Kera phul, Badi Lochai, SGCARS68, Mokdo Dhan, SGCARS71, Sonasari, Baghalbijo, SGCARS74, Sargi Phul, Gogal, Asaam Chudi, 41588, 41601, 41668, 41675, 41695, 41723, 41734, 41745, 41747, 41752, 41756, 41763, 41784, 41792, 41826, 41846, 41855.
pi21	SGCARS1, Kata Meher, Jeera Dhan, Chepti Khuji, Muthiya dhan, Bandkari,Temrumudi, Pote Khuji, Bakti Churi, Lal Baso, Shivnath, Hiruya Dhan, SGCARS14, Sendhur Senga, Dhadhar Dhan, Pandri satka,SGCARS28, laicha, Meso Dhan, Umari dhan, Kusum Jhopa, Noni Dhan, SGCARS56, SGCARS63, SGCARS68, Muthiya, Bhaiya khuta, 41601, 41675, 41676, 41683, 41723, 41734, 41744, 41745, 41754, 41763, 41784, 41787, 41792, 41797, 41798, 41799, 41826, 41827.
Pita3	Pandri lochai, karla Muha, Gadusela, Lakhechi, bandkari, Bhalu dubraj, SGCARS7, SGCARS8, Sagi paretha, Bhata mokdo, Badi Chudi, Dagda Dhan, Baasmukhi, Lalbaso, SGCARS18, Jeera Dhan, SGCARS25, SGCARS32, SGCARS36, Lochai Dhan, SGCARS43, SGCARS49, SGCARS53, Lokti Machi, umari Chudi, SGCARS67, Badi Lochai, Dagdikaj, SGCARS74, SGCARS75, Bhaiyakhuta, 41586, 41668, 41671, 41675, 41712, 41728, 41754, 41796, 41808, 41854.

Markers	Genotypes
RM1233	Gurmutiya, SGCARS3, Pandri Lochai, kurlu Dhan, Kakad kado, SGCARS5, Badshah Bhog, Chepti khuji, Turej gada Khuta, SGCARS6, Bandkari, Goydi, Karmuri Bhog, SGCARS8, SGCARS9, Pote Khuji, Bakti Chudi, Bhata Mokdo, Adga Dhan, Hiruya Dhan, SGCARs17, Karigrass, Masuri Desi, SGCARS29, Chatiya Dhan, SGCARS33, Jodra Nakti, SGCARS36, Nani Chudi, Moha dhan, Kaala umari, Asaam Chudi, SGCARS52, SGCARS57, Badshah Bhog, kera phul, SGCARS69, Ganga Baru, SGCARS74, Bhanwargedi, muthiya, Haldi Ghaati, Bhaiya Khuta, 41589, 41668, 41671, 41695, 41723, 41743, 41763, 41766, 41784, 41799, 41800, 41808, 41863.
RM6648	SGCARS1, SCARS2, Pakhiya dhan, Gurmutiya, kurda phul, SGCARS5, Badshah Bhog, Gada Khuta, Lakhechi, Karmuri Bhog, Bhaalu Dubraj, Haldi chudi, Dhagda Dhan, Banspati, Rani Knjar, Dokra mecha, Surmatiya, SGCARS14, Sendur senga, Pandri Satka, Jeera Dhan, Masuri desi, SGCARS29, SGCARS33, SGCARS36, Umari dhan, SGCARS40, SGCARS41, SGCARS48, SGCARS50, SGCARS61, Umari Chudi, Badi Lochai, Ganga Baru, Bhanwargedi, Haldi Gathi, 41601, 41668, 41675, 41683, 41728, 41745, 41763, 41792, 41846.
Pi65	Kurlu dhan, Alag dhan, Goydi, Kata meher, SGCARS11, Rang Gada Khuta, Dokra Mecha, Surmatiya, Dhadar dhan, SGCARS20, SGCARS28, SGCARS33, SGCARS39, Kaala umari, SGCARS51, Kurmuri Bhog, 41596, 41668, 41676, 41745, 41784, 41832
40N23R	Gadur sela, Lakhechi, Goydi, Hansa Dubraj, Bako dhan, SGCARS10, SGCARS12, Ratan Chudi, Bariya dhan, Masuri Desi, Laicha, meso Dhan, SGCARS37, Tiki chudi, Neem Chudi, SGCARS44, Mukukuda, SGCARS50, SGCARS63, SGCARS67, SGCARS70, Haldi Gathi, 41671, 41676, 41744, 41808, 41863.

Markers	Genotypes
K3957	Pakhiya Dhan, Bariya Dhan, Bandkari, Bako dhan, Banspati, Rang Gada Kuta, Goydi, Sendur Senga, SGCARS19, Bhayar dhan, SGCARS27, SGCARS32, Umari dhan, Lochai Dhan, Nani Chudi, Kusum jhopa, SGCARS41, SGCARS46, SGCARS47, SGCARS48, Bhaispat, Badshah Bhog, SGCARS65, Bahiya Khuta Ganga Baru, Bhanwargedi, SGCARS76, Gogal, Asam Chudi, 41593, 41596, 41666, 41668, 41676, 41695, 41734, 41745, 41756, 41784, 41798, 41808, 41854.
K39512	Kakad Kado, Bariya Dhan, SGCARS6, Paat Dhan, Titir Pakhi, Bhalu Dubraj, SGCARS8, Sonasari, Bhanwargedi, Pote Khuji, Bashmukhi, Rani kanjer, Hiruya dhan.
Z56591	SGCARS3, Kurlu Dhan, Kale Tude Masino, Bhata Mokdo, Shivnath, SGCARS17, SGCARS19, Khutbadi, Kata Nakti, Moha Dhan, SGCARS48, Dagdikaj, Asam Chudi, 41671, 41734, 41756, 41827
Pb28	SGCARS2, Kata Meher, Kharla Muha, bhalu Dubraj, Sonasari, SGCARS13, SGCARS21, SGCARS32, Lochai dhan, Kala mali, SGCARS46, SGCARS58, SGCARS62, Umari Chudi, SGCARS71, Ganga Baru, 41588, 41661, 41712, 41743, 41797, 41854, 41868.
Pi9	Pakhiya dhan, gurmutiya, Kata Meher, Haathi Panjaro, Godavari, SGCARS4, Jeera Dhan, Alag Dhan, SGCARS5, Ajan Dhan, Kabro Dhan, Kharla Muha, Mutiya Dhan, Gadur sela, SGCARS6, Bhata Dubraj. Bandkari, Hansa Dubraj, temru mudi, kata Meher, SGCARS9, POte Khuji, Bhata Kandayi, Hardiphul, Sonasari, Dagda Dhan, Manki Dhan, Godandi Dhan, Padko Guda, Nani Chudi, Pote Khuji, Kadam Phul, Rang Gada Khuta, Rakhi Dhan, surmatiya, Umari Chudi, Ratan chudi, hiruya Dhan, goydi, SGCARS14
Pikh	SGCARS3, PAndri Lochai, Kurlu Dhan, kakad Kado, Alag dhan, Mutiya Dhan, SGCARS6, Bhata Dubraj, Pat Dhan, Titir pakhi, SGCARS8, Badi Chudi, Bhanwargedi, Shivnath, Bashmukhi, HareKrishna, Shivnath, Rakhi Dhan

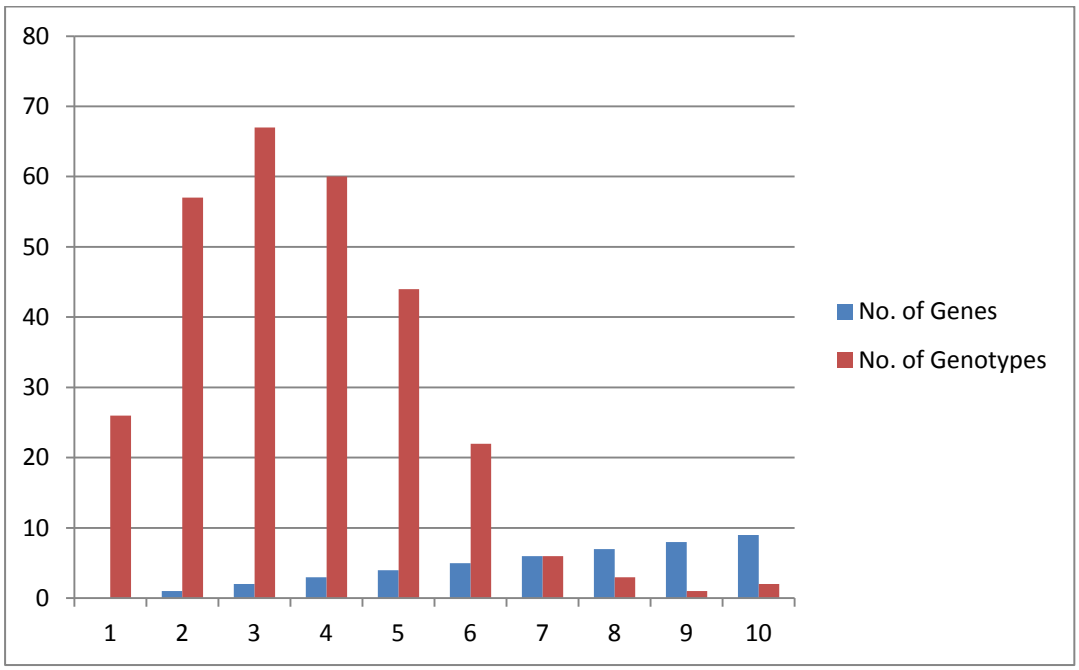

Figure 4.4: Graph depicting number of blast resistance genes with respect to genotypes.

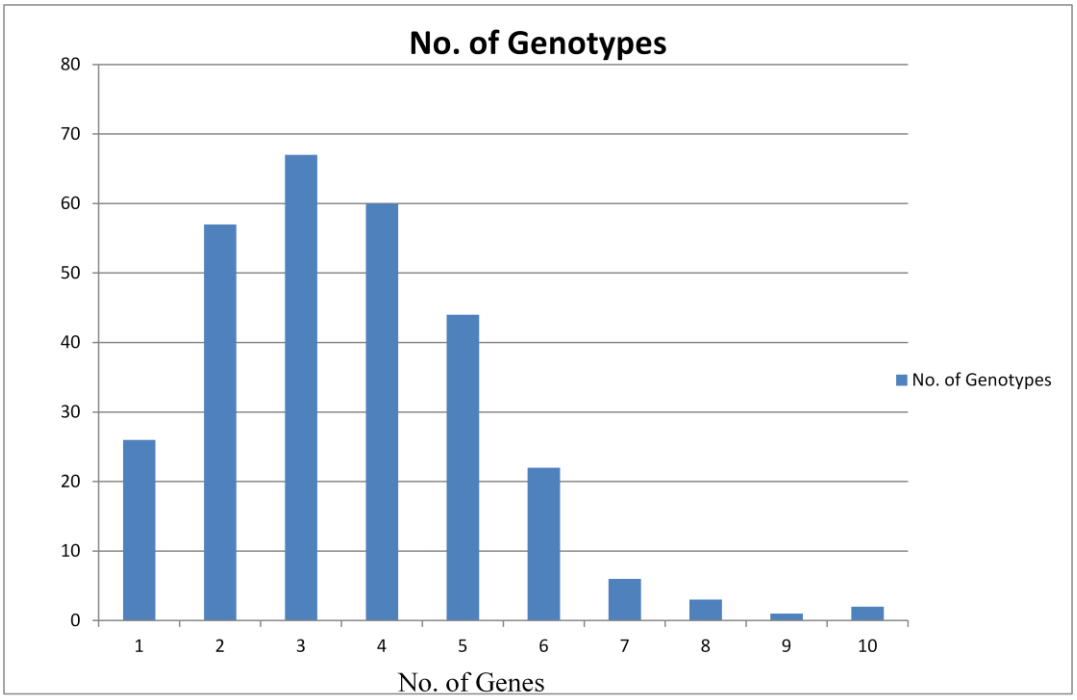

Figure 4.5: Graph depicting Genotypes with their corresponding number of *R* genes.

Similar study was carried out by Yang *et al.*, (2017) which showed the presence of *Pit* gene in 14 (3.9%) among 358 rice accessions. The recessive blast resistant gene *pi21* which is located on chromosome 4 was detected by using the InDel marker, pi21_79-3. It was observed that forty seven landraces were found as positive for the *pi21* gene with a gene frequency of 16.31%. The functional marker Pi9-i marker was able to detect the presence of *Pi9* gene (Plate 4.1). The *Pi9* is constant in landraces from a long time as it was originally isolated from the wild species (*Oryza minuta*) of rice.

Forty landraces were found positive with a gene frequency of 13.88%. Similarly, it was detected in 15 NRVs, 2 North East and East Indian rice (Yadav *et al.*, 2017; Imam *et al.*, 2014). Likewise, the presence of *Pi2* gene was noticed detected using the functional marker Pi2-i. Interestingly, fifty one landraces were found positive for the *Pi2* gene with a gene frequency of 17.70%. Similarly, the *Pi2* gene was detected in twenty (12.42%) landraces whereas, it was observed in 14 accessions of the tested 358 accessions (Yang *et al.*, 2017). The SNP primers zt56591 was used to ascertain the presence of *Piz-t* gene was visualized which demonstrated the presence of *Piz-t* gene in eighteen genotypes with a gene frequency of 6.25%. Previous reports showed that these two genes conferred partial resistance to the tested entries (Yadav *et al.*, 2017; Imam *et al.*, 2014).

The rice blast *R* gene *Pi33* on chromosome 8 was amplified using the linked marker RM72 that showed its presence in 79 landraces with gene frequency of 27.43%. Similarly, *Pi33* gene was observed in 77 (40.10%) of tested accessions (Singh *et al.*, 2015a). The *Pi5* gene located on chromosome 9 was determined with the marker 40N23r. Twenty seven landraces were found to harbour the blast resistant gene *Pi5* whereas previous study showed its presence in 60 landraces from Karnataka, 4 from Manipur and 26 NRVs (Ingole *et al.*, 2014; Mahender *et al.*, 2012; Yadav *et al.*, 2017). Likewise, the presence of *Pi56(t)* gene was detected in fifty six landraces determined by using the InDel marker CRG4_2.

The multiple gene complex loci was provided by the *Pik* locus on chromosome 11, where at least five genes, *Pik*, *Pikm*, *Pikh*, *Pikp*, and *Piks*, have

been identified (Hayashi *et al.*, 2006). Employment of the SNP primer, K39512 (Plate 4.2) and K3957 could be helpful for determining the presence of *Pik* and *Pik-p* genes by visualizing a product with the fragment size of 112 bp and 148 bp respectively. Based on these markers, distribution of *Pik* and *Pik-p* were found in 13 and 42 landraces, with a genetic frequency of 4.58 and 14.58%, respectively. Likewise, these genes were found to be present in maximum frequency in the NRVs (Yadav *et al.*, 2017). Interestingly, the *Pik* gene was detected in majority of the accessions tested (Imam *et al.* 2014; Yadav *et al.* 2017). The InDel marker, *Pikm*, showed its presence with a fragment size of 619 bp in 67 landraces.

The broad spectrum resistance gene *Pikh* isolated from Tetep variety was scored in eighteen (6.25%) landraces (Plate 4.3). Similarly, this gene was detected in fifty–six (70%) NRVs and in another study it was observed in 18 and 52 accessions (Yadav *et al.*, 2017; Imam *et al.*, 2014; Singh *et al.*, 2015a). The broad spectrum blast resistant gene *Pi1* was detected by using linked markers, RM1233 in 57 landraces. In another study, it was observed in 39 landraces and 20 NRVs (Ingole *et al.*, 2014; Yadav *et al.*, 2017). The InDel and STS primer, SNP_3, and Pia-STS were used for the detection of *Pi65(t*) and *Pia* genes, respectively. The present study showed the presence of *Pi65(t)* gene in 22 landraces with a gene frequency of 7.63%, whereas, *Pia* was present in 44 landraces with a gene frequency of 15.27%. The presence of broad spectrum *Pita/Pita-2* on chromosome 12 was detected with Pita3 primer pairs. The *Pita/Pita-2* gene was present in forty one landraces and showed gene frequency of 14.23%. Similarly, other workers reported its presence in 32.50%, 19.29%, 6.25% and 27% accessions (Yadav *et al.*, 2017; Singh *et al.*, 2015; Imam *et al.*, 2014; Shikari *et al.*, 2014). In all the PCR amplification, HR12 was used as a negative control.

4.3 Genetic Diversity

Genetic diversity study was carried out to study the diverse nature of the landraces and to differentiate closely related individuals. A total of 288 landraces consisted of resistant and susceptible genotypes were genotyped using 75 polymorphic SSR markers that cover the whole genome to assess the genetic diversity for blast resistance (Plate 4.4, Plate 4.5, Plate 4.6). Alleles generated with

Plate 4.1: Blast resistant gene profiling of landraces using gene specific Pi9 marker with L as 50 bp gene ladder.

The numbers 1- 96 signifies landraces CG-1 to CG-96. + sign signifies the genotypes carrying blast resistant genes.

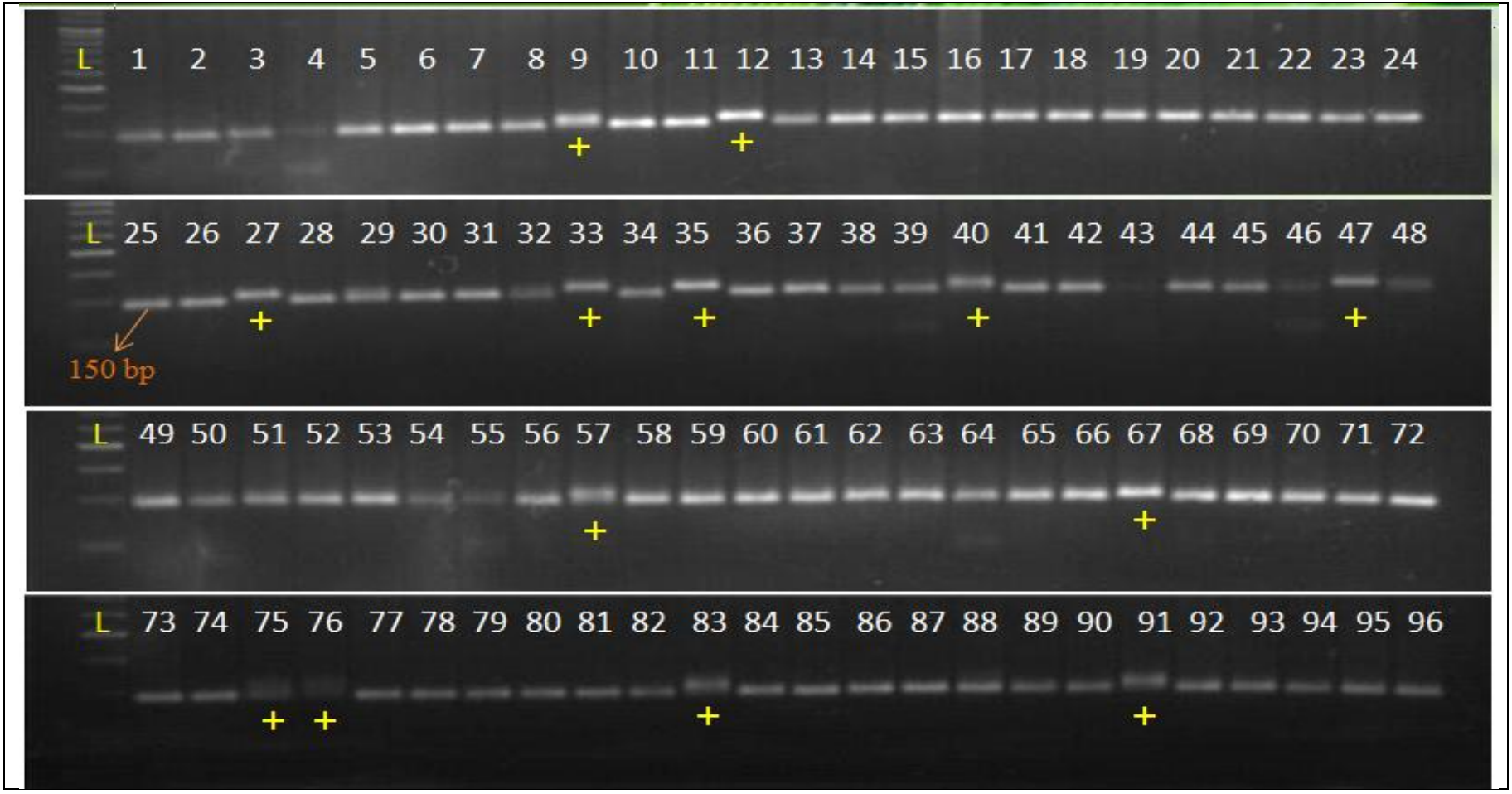

Plate 4.2: Blast resistant gene profiling of landraces using gene specific K39512 marker with L as 100 bp gene ladder.

The numbers 1- 96 signifies landraces CG-1 to CG-96. + sign signifies the genotypes carrying blast resistant genes.

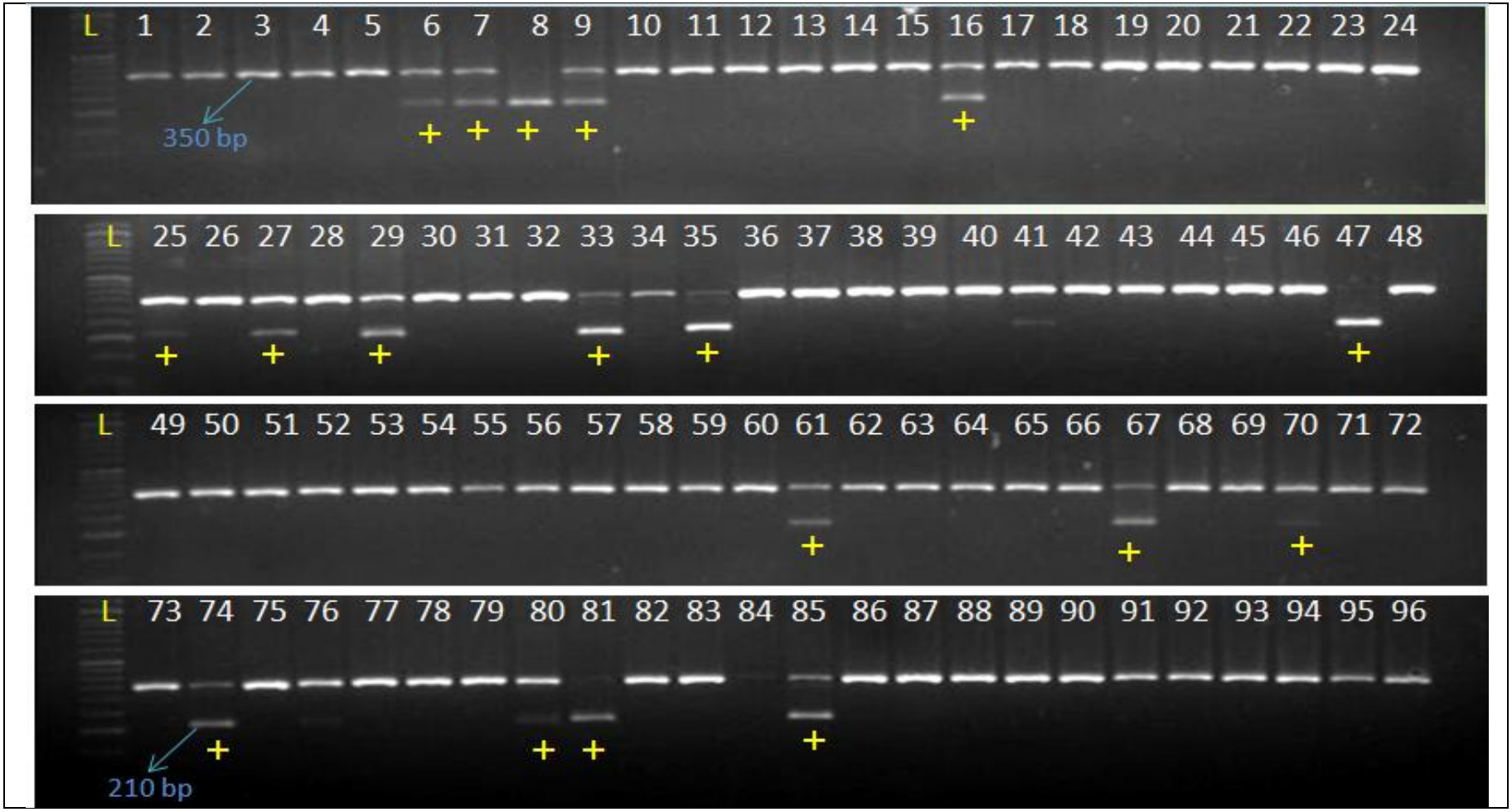

Plate 4.3: Blast resistant gene profiling of landraces using gene specific Pikh marker with L as 50 bp gene ladder.

The numbers 1- 96 signifies landraces CG-1 to CG-96. + sign signifies the genotypes carrying blast resistant genes.

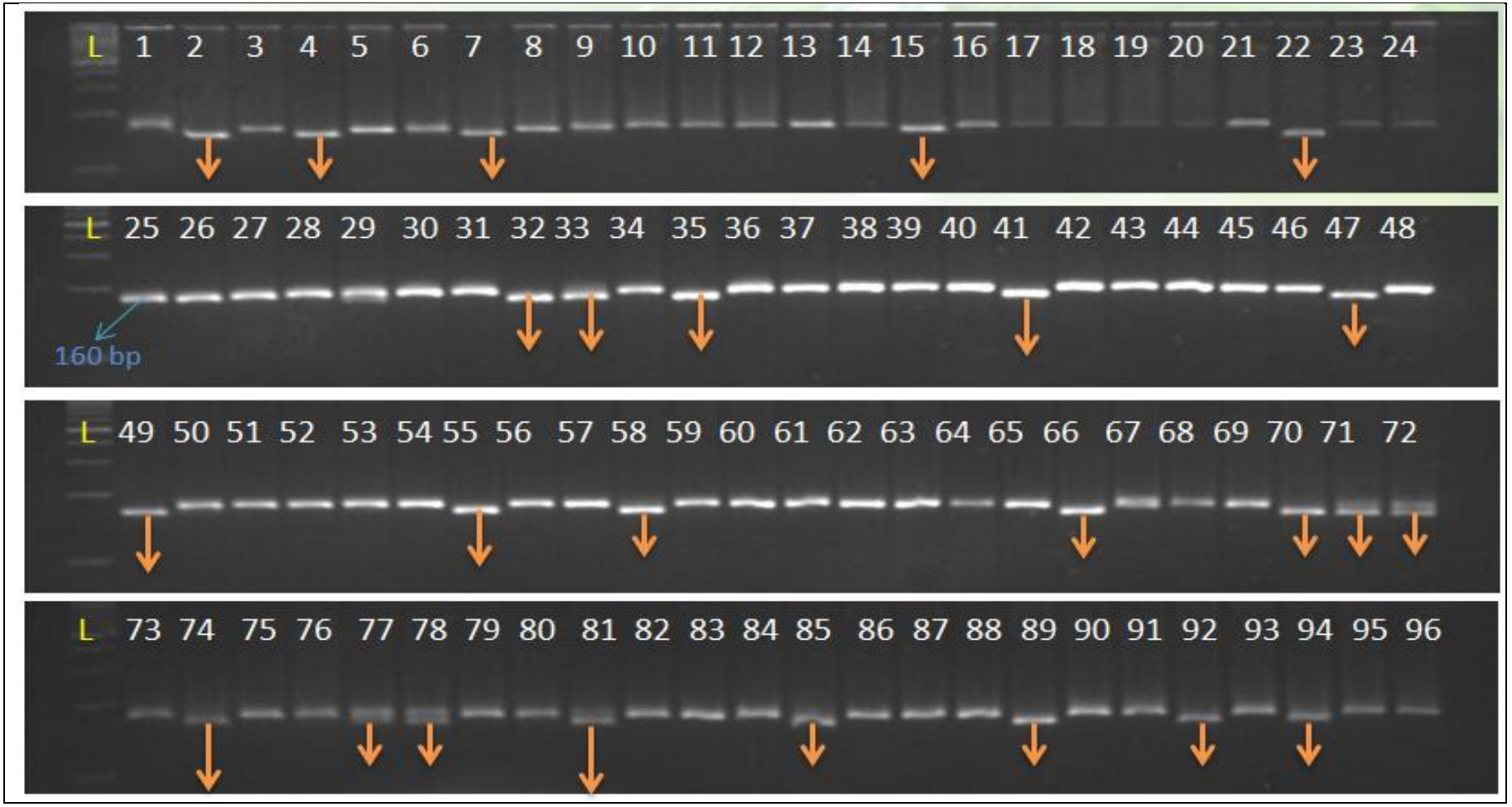

Plate 4.4: Agarose gel photograph of 96 accessions of the selected 288 genotypes, at the locus RM495. L: 100 bp gene ladder.

The numbers 1- 96 signifies landraces CG-1 to CG-96.

Plate 4.5: Agarose gel photograph of 96 accessions of the selected 288 genotypes, at the locus RM17753. L: 100 bp gene ladder.
The numbers 1- 96 signifies landraces CG-1 to CG-96.

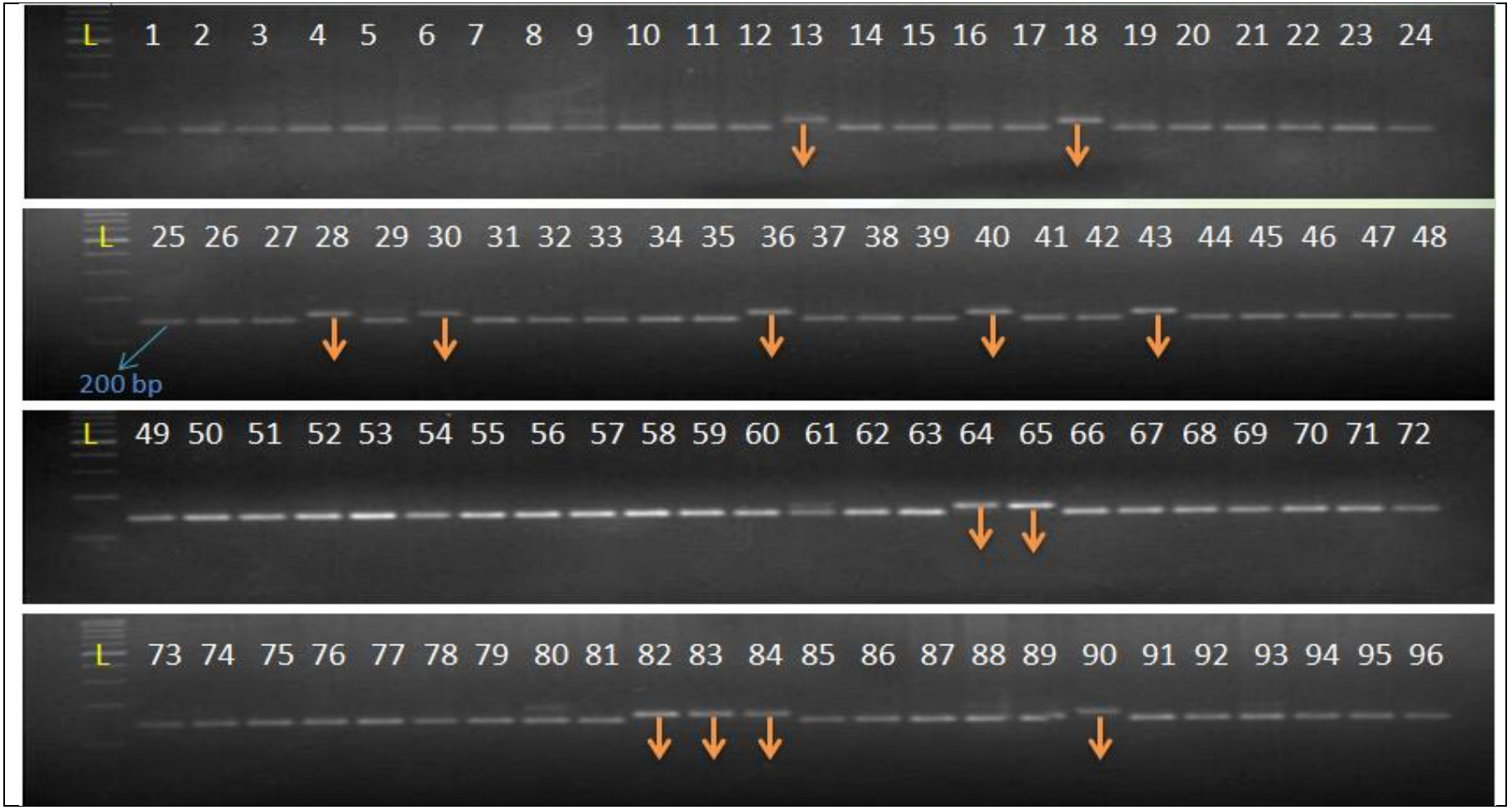

Plate 4.6: Agarose gel photograph of 96 accessions of the selected 288 genotypes, at the locus RM22659. L: 100 bp gene ladder.
The numbers 1- 96 signifies landraces CG-1 to CG-96.

all polymorphic SSR markers were scored to study the genetic diversity. The details of all the markers used including their genetic diversity parameters are given in Table3.4 and the position is given in Figure 4.6 (a) and (b). The degree of polymorphism was detected by calculating PIC values, allelic number and genetic diversity was calculated. The mean value of major allele frequency was found to be 0.72 and varied from 0.50 (RM16284) to 0.94 (RM27926). The polymorphism information content of 96 markers varied from 0.10 (RM27926) to 0.37 (RM14320, RM15203 and RM16284) with an average of 0.30. The mean PIC value of 0.47 was recorded in 74 Tripura rice landraces by using 30 SSR markers (Anupam *et al.*, 2017). Similarly, Roy *et al.* (2016) observed a PIC value of 0.62 with the NE Himalayan landraces. Giarrocco *et al.* (2007) also reported an average PIC value of 0.69 in 68 accessions from Argentina. Yang et al. (1994) observed mean PIC value of 0.58 in 238 landraces collected from India, IRRI China, and Japan. The gene diversity varied from 0.11 (RM27926) to 0.49 (RM14320, RM15203 and RM16284) (Table4.4). The average gene diversity was observed to be 0.38 which is lower than to most of the gene diversity panel (0.5–0.7) consisting of global accessions (Ni *et al.*, 2002; Garris *et al.*, 2005; Ali *et al.*, 2011; Roy *et al.*, 2015). The present study showed the SSR markers were informative and can be used to assess the genetic diversity of diverse germplasm.

4.4 Estimation of population genetics through AMOVA analysis

AMOVA denotes Analysis of Molecular VAriance and it allows genetic variation (measured as the genetic distance among alleles or haplotypes) to be partitioned into various hierarchical levels utilizing molecular markers. The genetic relationships among the three populations of 288 landraces based on disease reaction were determined through AMOVA analysis. The results of the AMOVA confirmed the presence of phylogeographic structure. The results of this analysis considering all populations as one demonstrated that most of the molecular variation was distributed within populations (97%) while minimum variance existed among populations (3%) (Figure 4.7, Table 4.5). The F_{IS} and F_{IT} value for

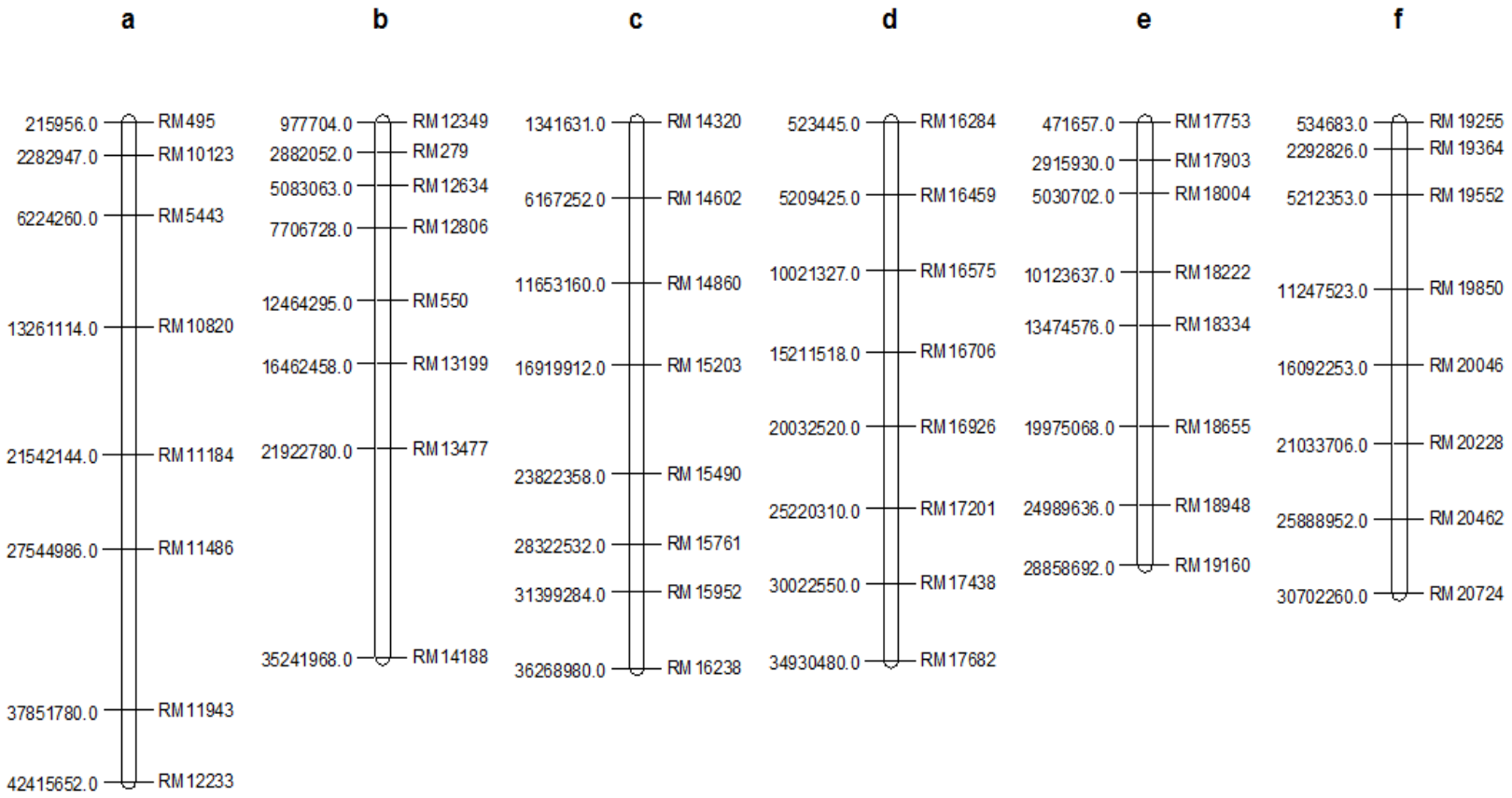

Figure 4.6(a): Graphical presentation of 96 SSR markers used for GWAS for blast resistance genes.(a, b, c, d, e, f, represents Chromosomes 1, 2, 3, 4, 5 and 6 respectively.

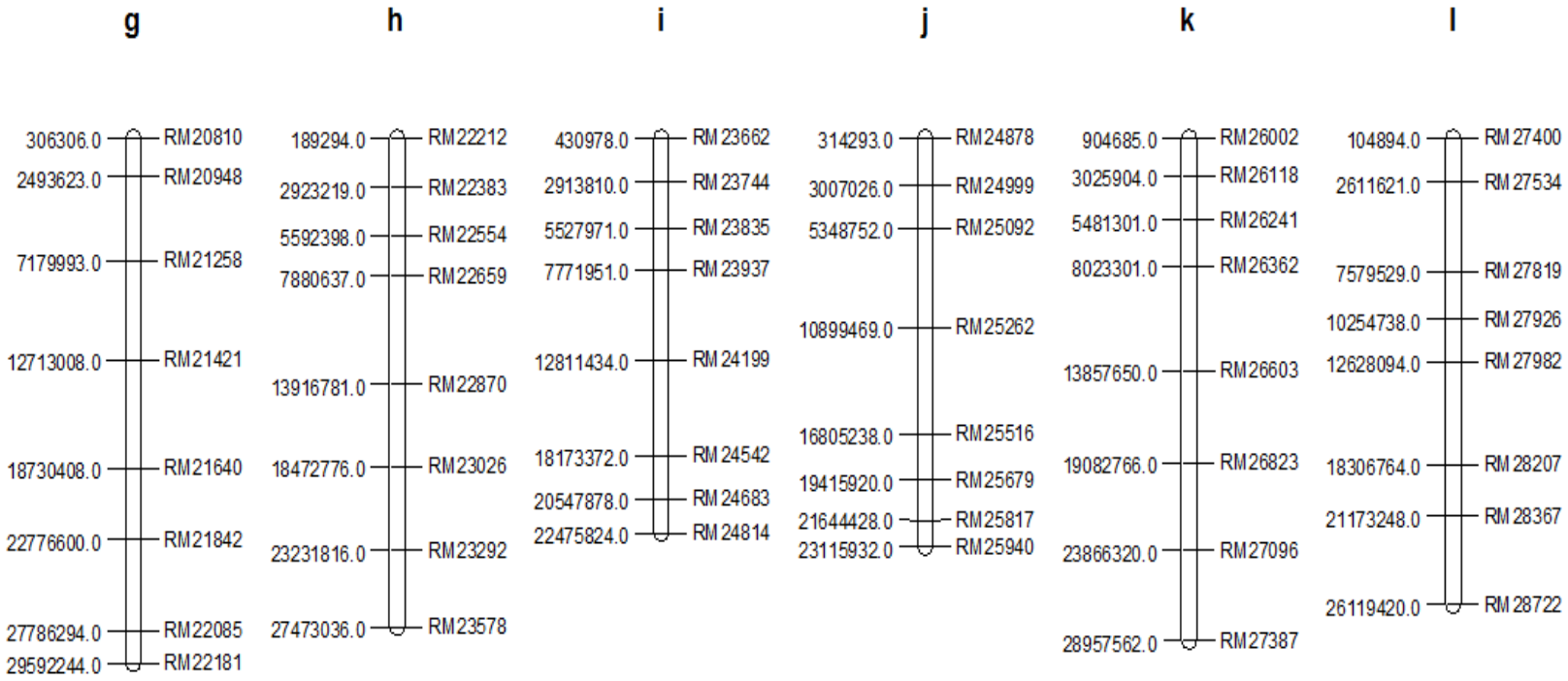

Figure 4.6(b): Graphical presentation of 96 SSR markers used for GWAS for blast resistance genes.(g, h, I, j, k, l represents Chromosomes 7, 8, 9, 10, 11 and 12 respectively.

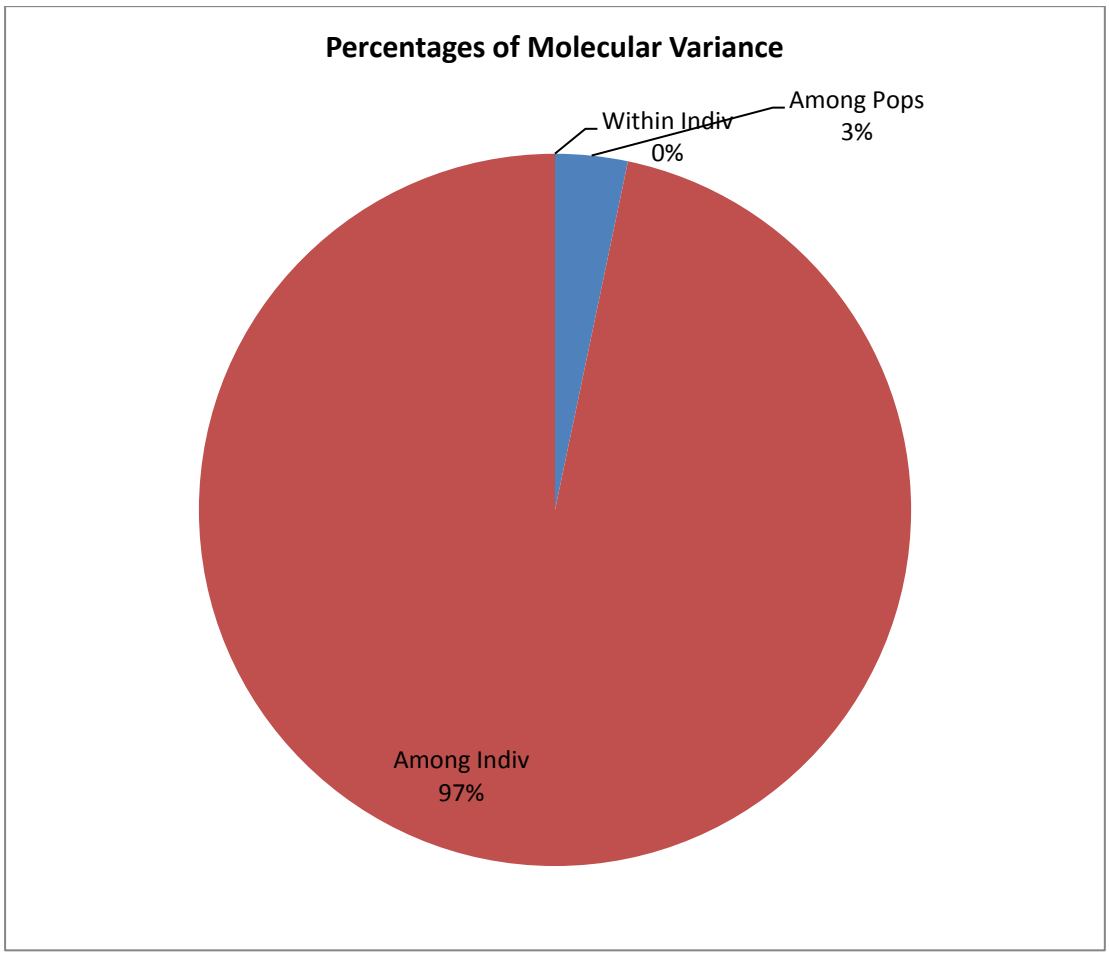

Figure 4.7: Analysis of molecular variance in rice genotypes using SSR molecular markers.

Table 4.4: Details of SSR loci used for genotyping a set of 288 rice landraces and their genetic diversity parameters.

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
RM11184	0.5903	2.0000	0.4837	0.3667
RM495	0.7188	2.0000	0.4043	0.3226
RM5443	0.6979	2.0000	0.4217	0.3328
RM10123	0.7188	2.0000	0.4043	0.3226
RM10820	0.7813	2.0000	0.3418	0.2834
RM11486	**0.8889**	2.0000	0.1975	0.1780
RM11943	0.5903	2.0000	0.4837	0.3667
RM12233	0.6042	2.0000	0.4783	0.3639
RM550	0.5938	2.0000	0.4824	0.3661
RM12806	0.7361	2.0000	0.3885	0.3130
RM13199	0.6493	2.0000	0.4554	0.3517
RM13477	0.6632	2.0000	0.4467	0.3469
RM14320	0.5174	2.0000	0.4994	0.3747
RM14860	0.7813	2.0000	0.3418	0.2834
RM15203	**0.5069**	2.0000	0.4999	0.3750
RM15490	0.6736	2.0000	0.4397	0.3430
RM15761	0.7188	2.0000	0.4043	0.3226
RM15952	0.6042	2.0000	0.4783	0.3639
RM16238	0.6667	2.0000	0.4444	0.3457
RM14602	0.6875	2.0000	0.4297	0.3374
RM16284	**0.5069**	2.0000	0.4999	0.3750
RM16459	0.7118	2.0000	0.4103	0.3261
RM16575	0.6806	2.0000	0.4348	0.3403
RM16926	0.6215	2.0000	0.4705	0.3598
RM17201	0.6250	2.0000	0.4688	0.3589
RM17438	0.6910	2.0000	0.4271	0.3359
RM17682	0.6597	2.0000	0.4490	0.3482
RM17753	0.7188	2.0000	0.4043	0.3226
RM17903	0.8542	2.0000	0.2491	0.2181

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
RM18004	0.7535	2.0000	0.3715	0.3025
RM18334	0.8750	2.0000	0.2188	0.1948
RM18655	**0.8819**	2.0000	0.2082	0.1866
RM18948	0.8299	2.0000	0.2824	0.2425
RM19255	0**.8889**	2.0000	0.1975	0.1780
RM19552	0.7222	2.0000	0.4012	0.3207
RM19850	0.6250	2.0000	0.4688	0.3589
RM20462	0.8264	2.0000	0.2869	0.2458
RM20046	0.8611	2.0000	0.2392	0.2106
RM21421	0.8611	2.0000	0.2392	0.2106
RM21640	0.8056	2.0000	0.3133	0.2642
RM20810	0.7743	2.0000	0.3495	0.2884
RM22085	0.6701	2.0000	0.4421	0.3444
RM22181	0.7396	2.0000	0.3852	0.3110
RM22212	0.7049	2.0000	0.4161	0.3295
RM22554	0.6979	2.0000	0.4217	0.3328
RM23578	0.6910	2.0000	0.4271	0.3359
RM23026	**0.8854**	2.0000	0.2029	0.1823
RM23292	0.6701	2.0000	0.4421	0.3444
RM22383	0.7049	2.0000	0.4161	0.3295
RM22659	0.8056	2.0000	0.3133	0.2642
RM23937	0.7326	2.0000	0.3918	0.3150
RM23744	0.6597	2.0000	0.4490	0.3482
RM23662	0.7326	2.0000	0.3918	0.3150
RM24199	0.6806	2.0000	0.4348	0.3403
RM24542	0.7292	2.0000	0.3950	0.3170
RM24683	0.6042	2.0000	0.4783	0.3639
RM24878	0.6875	2.0000	0.4297	0.3374
RM24999	0.6806	2.0000	0.4348	0.3403
RM25516	0.7465	2.0000	0.3784	0.3068

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
RM25679	0.7014	2.0000	0.4189	0.3312
RM25940	0.8056	2.0000	0.3133	0.2642
RM25817	0.7569	2.0000	0.3680	0.3003
RM26823	0.8090	2.0000	0.3090	0.2613
RM20796	0.7257	2.0000	0.3981	0.3189
RM26002	0.7882	2.0000	0.3339	0.2781
RM26241	0.8056	2.0000	0.3133	0.2642
RM26362	0.6944	2.0000	0.4244	0.3343
RM26603	0.6736	2.0000	0.4397	0.3430
RM27387	0.7292	2.0000	0.3950	0.3170
RM27400	0.8542	2.0000	0.2491	0.2181
RM27534	0.8611	2.0000	0.2392	0.2106
RM27819	0.8889	2.0000	0.1975	0.1780
RM27926	**0.9410**	2.0000	0.1111	0.1049
RM27982	0.6840	2.0000	0.4323	0.3388
RM28367	0.6528	2.0000	0.4533	0.3506
Mean	0.7248	2.0000	0.3808	0.3043

Table 4.5: Summary Table of Analysis of Molecular Variance (AMOVA) for SSR markers

Source	Degree of freedom	Sum of square	Mean sum of squares	Estimated Variance	Percentage variation
Among Populations	2	233.95	116.980	0.47	3%
Among Individual	285	7991.89	28.042	14.02	97%
Within Individual	288	0.00	0.000	0.00	0%
Total	575	8225.85		14.49	100%

all the 135 markers loci were observed to be 1.0 whereas F_{ST} was found to be 0.033. The pair-wise fixation indices (F_{ST}) among the four populations were presented in the Table 4.6. The highest pair wise F_{ST} was observed between

susceptible and resistant while, the least was observed between resistant and moderately resistant populations. The F_{ST} value of fixation indices showed that there is a weak population structures with no clear cut differentiation of sub populations. These results suggested that populations were still structured within at least one group.

Table 4.6: Pair-wise population F_{ST} estimates among the three populations of Rice genotypes.

Populations	Highly Resistant	M-Resistant	Susceptible
Highly Resistant	0.000	0.001	0.001
M-Resistant	0.022	0.000	0.001
Susceptible	0.076	0.016	0.000

F_{ST} values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.

F-Statistics	Value	P- value
$\mathbf{F_{ST}}$	0.033	0.001
$\mathbf{F_{IS}}$	1.000	0.001
$\mathbf{F_{IT}}$	1.000	0.001

Yadav *et al.* (2017) reported 4% and 96% variation among and within population in a set of 80 NRVs. Nachimuthu *et al.* (2015) analyzed 192 rice accessions and grouped them in two subgroups and reported 14% variations among the groups and 86% within the group. Similarly, Zhang *et al.* (2009) also grouped the 3024 rice accessions, into two subgroups and the variation among the groups which was little higher (36.65%) and 40.80% among individuals within populations.

4.5 Genetic relatedness through cluster analysis

Genetic distance refers to the genetic divergence among populations, which can be measured by a variety of parameters in relation to the frequency of a particular trait. The dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar types clustered together. The un-weighted neighbor joining

tree was constructed using DARwin software to evaluate the genetic diversity among the landraces originated from Chhattisgarh and North East states (Figure 4.8).

The genetic distance was assessed to construct a dendrogram by using the 75 polymorphic SSR markers data led to the clustering of 288 landraces into three major clusters, I, II and III (Figure 4.9). Major cluster I consisted of 75 landraces, was divided into two sub-clusters IA and IB. The sub-cluster IA included 65 landraces, of which, 16 (24.61%) are highly resistant. Sub-cluster IB consists of only ten landraces with only one resistant genotype. The average disease score of major cluster was observed to be 4.34. Major Cluster II observed to be the largest cluster (166 landraces) which is further divided into two sub-clusters. Sub-cluster IIA consisted of 127 landraces, of which 22 genotypes (17.33%) were resistant. Similarly, Sub-cluster IIB contained 39 genotypes, in which 14 (35.89%) are highly resistant. The mean disease scores of - IIA and IIB were observed to be 4.8 and 3.7. Major sub-cluster III possessed of 45 landraces with a disease score of 2.97. It contained 22 resistant landraces (approx 50%). Interestingly, majority of resistant genotypes were observed Major cluster III and Sub-cluster IIB whereas, susceptible genotypes were clustered in Major cluster 1 and Sub-cluster IIB. Interestingly, the cluster analysis could discriminate all the resistant and susceptible landraces tested which is similar to the study of Yadav *et al.*, (2017).

Similarly, (Vanniarajan *et al.*, 2012) classified the rice genotypes from Tamil Nadu, into *indica* and *japonica* types based on the distance based clustering. The resistant landraces from different locations belonged to all the three clusters. On the contrary, each cluster consisted of genetically similar genotypes but landraces of diverse ecologies.

4.6 Genetic relatedness by Principal coordinate and population structure

The SSR markers data was used to determine the Principal coordinate analysis (PCoA) to estimate the genetic relatedness among the rice landraces (Figure 4.10). In PCoA, the resistant genotypes are depicted in the blue dots and mostly observed in 1st quadrant, moderately resistant genotypes were mostly grouped in first and fourth quadrant while susceptible genotypes are clustered in

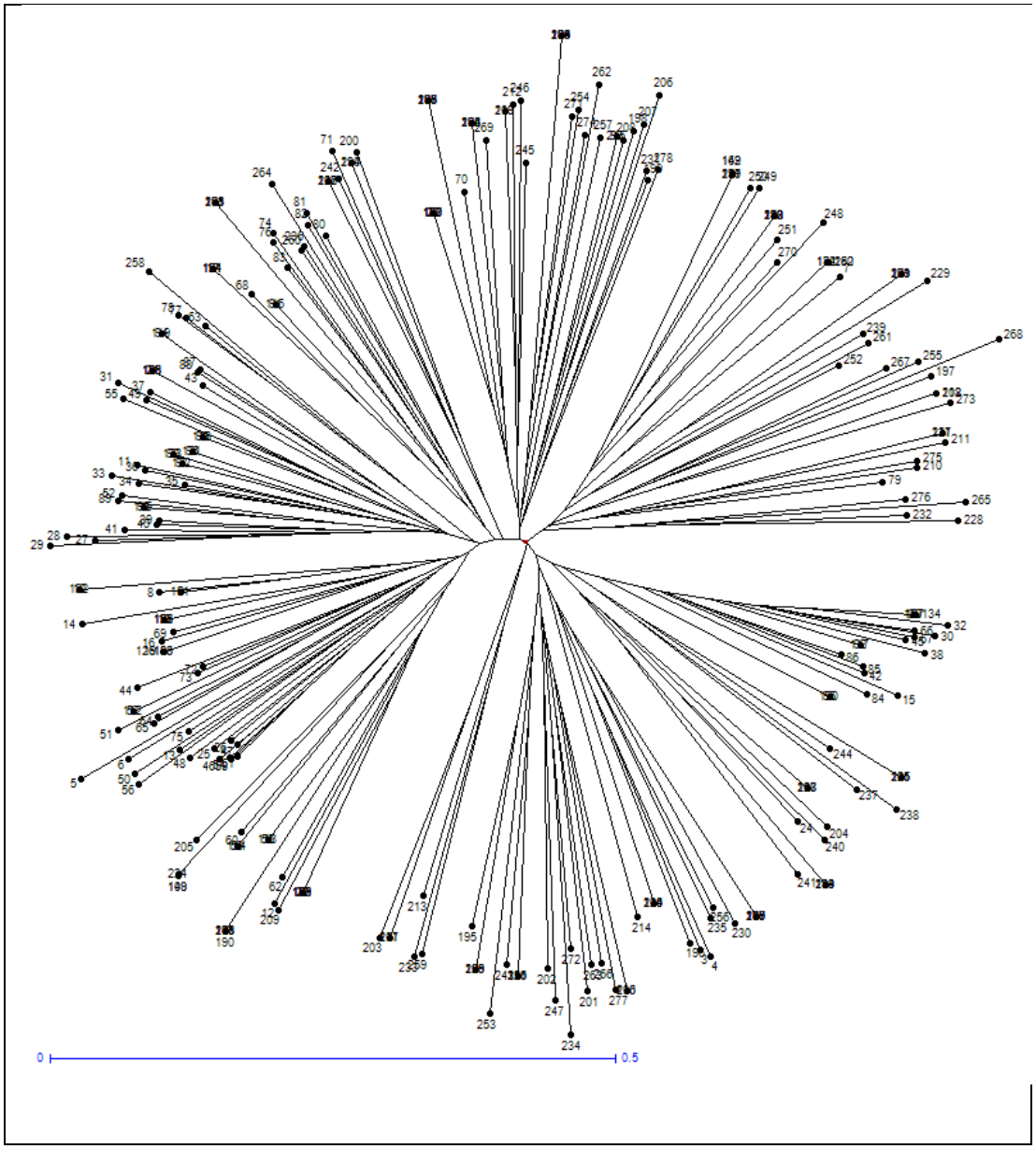

Figure 4.8: Unrooted tree using UPGMA method showing the genetic relationship among 288 landraces based on 75 SSR markers

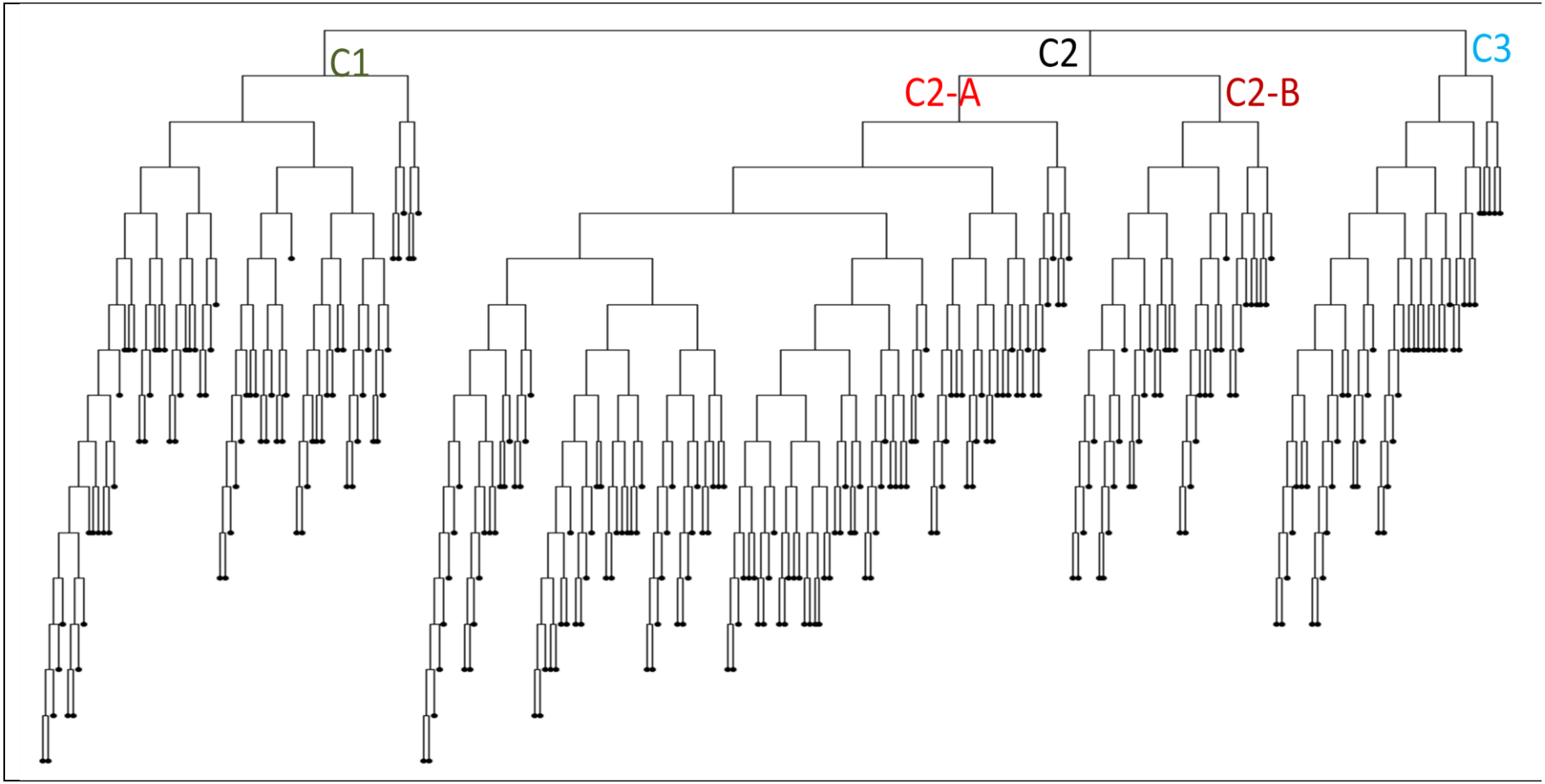

Figure 4.9: Dendrogram Depicting 3 cluster formation in the group of 288 genotypes.

3rd and 4th quadrant. The distribution pattern of rice landraces represented a clear grouping based on resistance to rice blast. Interestingly, most of the highly resistant landraces were clustered together in 1st quadrant (Figure 4.10). The first two axes in the scatter plot generated from the PCoA analysis explained 7.31% and 5.65% of the total genetic variation, respectively contributing a total of 12.96% of genetic variation.

The genetic structure of 288 NRVs for blast resistance using 75 SSR markers was determined on the basis of analysis by STRUCTURE software. The Bayesian clustering approach was used to analyze genetic structure and selecting highest plateau of adhoc measure ΔK (Evanno *et al.*, 2005). The membership of each landrace was run from K=1 to K=10 for all the accession to estimate the number of populations. Structure Harvester was used to establish final number of populations. A high ΔK peak value was observed to be K=3 as per the Evano table output (Figure 4.11) which differentiated the rice genotypes into three subpopulations (SP1 SP2 and SP3). Based on minimum probability of 0.55, all the rice genotypes were divided into three subpopulations with four admixtures (Table 4.7). The subpopulation 1 (SP1) consisted of 159 landraces representing mostly resistant population (60 highly resistant landraces). A total of 107 rice genotypes are present in subpopulation 2 (SP2) with only 13 resistant genotypes. The subpopulation 3 (SP3) consisted of only 18 landraces with only one resistant genotypes. The SP1 population consisted of more numbers of resistant genotypes as compared to SP2 and SP3 subpopulations.

Therefore SP1 can be considered as dominated by resistant genotypes; SP2 is represented by susceptible genotypes while SP3 is represented by moderately resistant genotypes. Hence overall, the study represented that the structure analysis was able to distinguish resistant, M-resistant and susceptible genotypes into three different subpopulations, SP1, SP3 and SP2. Maximum allele frequency divergence among populations was observed in SP1 and SP3 (0.042). Interestingly, all the resistant North east collection was grouped in SP1 whereas Chhattisgarh landraces were fairly distributed in all the three subpopulations. The minimum and maximum average distance between individuals in same cluster was observed to be

0.25 and 0.36. Similarly, fixation index values (F_{ST}) of the sub-populations were found to be 0.052, 0.52, and 0.21 for SP1, SP2, and SP3, respectively.

Similar results were obtained while grouping 3024 Chinese rice landraces and 64 landraces of NE Himalayan region of India, and 192 rice accessions of varied origin (Zhang *et al.,* 2009; Roy *et al.,* 2016; Nachimuthu *et al.,* 2015). However other studies grouped the rice accessions into two to eight sub-groups (Garris *et al*., 2005; Agrama *et al.*, 2007; Zhang *et al*., 2007, 2009; Ali *et al*., 2011; Chakhonkaen *et al.,* 2012). Similarly, 107 landraces from NE region were divided into three subgroups using 40 SSRs and 91 rice lines were grouped into three sub-populations (Roy *et al.,* 2015: Das *et al.,* 2013).

Similarly, the degree of admixture (alpha) was calculated from the data. When alpha approaches zero, it signified that most individuals are essentially from one population or another, while alpha > 1 denotes that most individuals are admixed. The mean value of alpha was observed to be 0.043 that tells that population if divided into three groups with only four admixtures.

4.7 Association of gene specific marker alleles with blast resistance

Association analysis of blast resistance gene specific marker was conducted using the generalized linear model (GLM) to find out any significance association of the blast disease. Among 19 markers analyzed, six markers (Pi56, RM72, Tk59-2, pi21, RM1233 and RM6648) corresponded to six resistance genes (*Pi56, Pi33, Pit, pi21, Pi1* and *Pish*) and found significantly associated with the blast disease (Table 4.8). The phenotypic variance of these six markers varied from 1.1% to 6.4%. Out of these six markers, RM72 of *Pi33* gene showed the largest phenotypic variance (6.4%) followed by RM6648 of *Pish* gene with phenotypic variance of 1.86%.

Another marker of the *Pi56* gene also showed significant association with the phenotypic variance of 1.83%. The markers associated with the genes *pi21, Pi1* and *Pit* showed a phenotypic variance of 1.5%, 1.3% and 1.1%, respectively, while the rest of the markers for thirteen resistance genes did not show significant association at P value < 0.01.

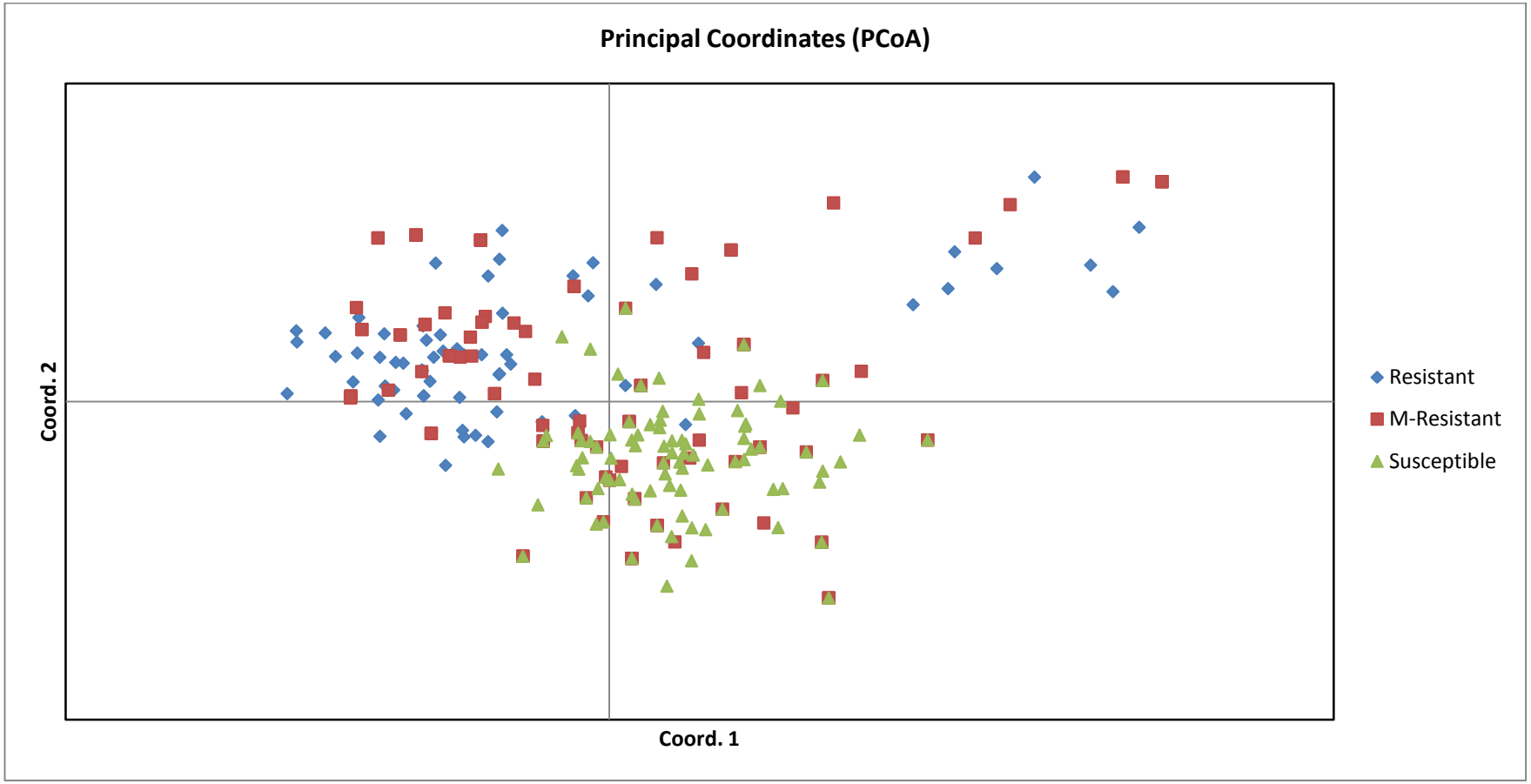

Figure 4.10: Two dimensional Principal coordinate analysis display of 288 rice landraces based on 75 SSR markers.

Coord 1 and Coord 2 represent first and second coordinates, respectively.

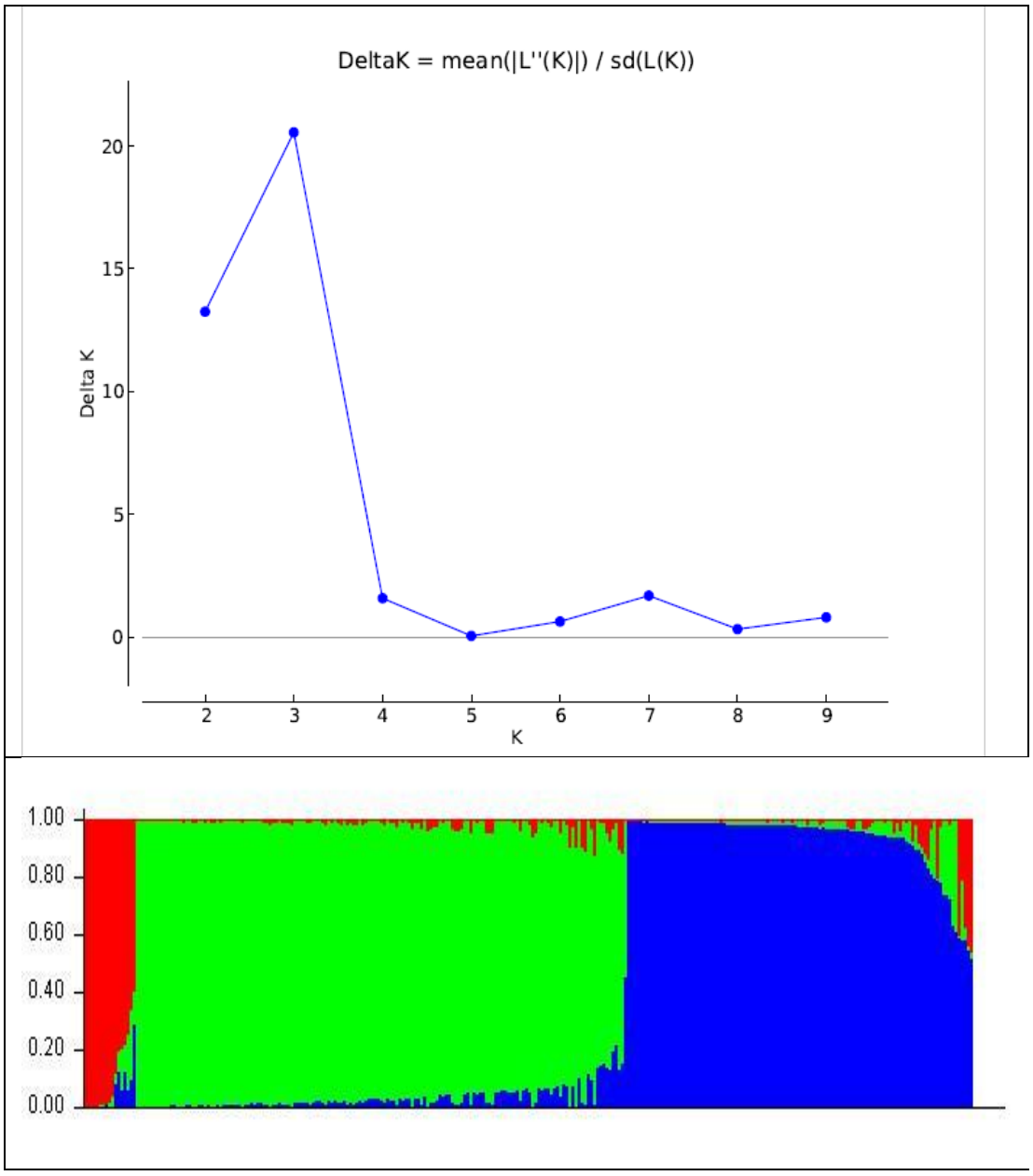

Figure 4.11: Population structure of 288 landraces based on 96 SSR markers (K = 3) and graph of estimated membership fraction for K = 3.

Table 4.7: Population structure group of landraces based on inferred ancestry values based on SSR markers.

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-1	SGCARS1	0.185	0.806	0.009	M-Resistant	S2
CG-2	SGCARS2	0.979	0.015	0.007	M-Resistant	S1
CG-3	Pakhiya Dhaan	0.058	0.935	0.008	Susceptible	S2
CG-4	Gurmutiya	0.205	0.792	0.003	M-Resistant	S2
CG-5	Kurdaful	0.854	0.086	0.06	Susceptible	S1
CG-6	SGCARS3	0.015	0.967	0.017	Susceptible	S2
CG-7	Pandri Lochai	0.007	0.99	0.003	M-Resistant	S2
CG-8	Kurlu Dhaan	0.028	0.592	0.38	M-Resistant	S2
CG-9	Kakad kado	0.037	0.877	0.086	M-Resistant	S2
CG-10	Kata mehar	0.044	0.95	0.006	M-Resistant	S2
CG-11	Hathi Panjaro	0.04	0.954	0.006	M-Resistant	S2
CG-12	Bariya Dhaan	0.124	0.864	0.011	Susceptible	S2
CG-13	Godavari	0.14	0.183	0.677	Susceptible	S3
CG-14	SGCARS4	0.027	0.927	0.046	Susceptible	S2
CG-15	Jira Dhaan	0.048	0.951	0.002	M-Resistant	S2
CG-16	Aalag Dhaan	0.001	0.995	0.003	M-Resistant	S2
CG-17	SGCARS5	0.015	0.974	0.011	M-Resistant	S2
CG-18	Badsa Bhog	0.002	0.001	0.997	Susceptible	S3
CG-19	Chpti Khuji	0.007	0.984	0.009	Susceptible	S2
CG-20	Aajan Dhaan	0.004	0.994	0.002	M-Resistant	S3
CG-21	Kurlu Dhaan	0.009	0.986	0.004	Susceptible	S2
CG-22	Kabro Dhaan	0.069	0.923	0.008	Susceptible	S2
CG-23	Kharla Muha	0.003	0.992	0.005	Susceptible	S2
CG-24	Turej Gada Khuta	0.005	0.993	0.002	M-Resistant	S2
CG-25	Mutiya Dhan	0.006	0.988	0.006	Susceptible	S2

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-26	Gadur Sela	0.008	0.99	0.002	Susceptible	S2
CG-27	SGCARS6	0.017	0.982	0.001	M-Resistant	S2
CG-28	Gada Khuta	0.01	0.009	0.981	Susceptible	S3
CG-29	Bhata Dubraaj	0.009	0.88	0.11	M-Resistant	S2
CG-30	Lakhechi	0.016	0.01	0.975	M-Resistant	S3
CG-31	Gurmutiya	0.02	0.977	0.004	Susceptible	S2
CG-32	Bandkari	0.005	0.993	0.002	Resistant	S2
CG-33	Pat Dhaan	0.015	0.89	0.095	Susceptible	S2
CG-34	Goydi	0.014	0.984	0.002	Susceptible	S2
CG-35	Titir Pakhi	0.019	0.945	0.036	Resistant	S2
CG-36	Karmuri Bhog	0.068	0.117	0.815	M-Resistant	S3
CG-37	Kale Tude Masino	0.007	0.991	0.002	Susceptible	S2
CG-38	Dumar Ful	0.004	0.989	0.006	Susceptible	S2
CG-39	Hansa Dubraaj	0.014	0.984	0.002	M-Resistant	S2
CG-40	BhaluDubraaj	0.276	0.031	0.692	M-Resistant	S3
CG-41	Aajam Lali	0.042	0.934	0.024	M-Resistant	S2
CG-42	Dongar Kabri	0.013	0.983	0.003	Susceptible	S2
CG-43	Huldi Chudi	0.049	0.072	0.879	Susceptible	S3
CG-44	Temru Mudi	0.004	0.993	0.002	Susceptible	S2
CG-45	SGCARS7	0.013	0.985	0.002	M-Resistant	S2
CG-46	Kata mehar	0.015	0.98	0.006	Susceptible	S2
CG-47	SGCARS8	0.038	0.869	0.093	M-Resistant	S2
CG-48	SGCARS9	0.009	0.985	0.007	M-Resistant	S2
CG-49	Pote Khuji	0.072	0.925	0.003	M-Resistant	S2
CG-50	Bhata Kandai	0.014	0.981	0.004	Susceptible	S2
CG-51	Sagi Pareta	0.01	0.988	0.003	M-Resistant	S2

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-52	Gadur Sela	0.002	0.993	0.005	M-Resistant	S2
CG-53	Hardiful	0.043	0.955	0.002	M-Resistant	S2
CG-54	Bako Dhaan	0.006	0.99	0.004	Resistant	S2
CG-55	Bakti Chudi	0.02	0.975	0.005	Resistant	S2
CG-56	Bhata Mokdo	0.011	0.985	0.004	M-Resistant	S2
CG-57	Sona Sari	0.016	0.981	0.003	M-Resistant	S2
CG-58	Yami Gali	0.197	0.799	0.003	Susceptible	S2
CG-59	Temru Mudi	0.005	0.993	0.002	M-Resistant	S2
CG-60	Lal Baso	0.013	0.98	0.007	M-Resistant	S2
CG-61	Badi Chudi	0.719	0.25	0.03	M-Resistant	S1
CG-62	SGCARS10	0.012	0.985	0.003	M-Resistant	S2
CG-63	Manki Dhaan	0.026	0.97	0.004	M-Resistant	S2
CG-64	Dhgda Dhaan	0.004	0.002	0.994	M-Resistant	S3
CG-65	Banspati	0.003	0.007	0.99	M-Resistant	S3
CG-66	SGCARS11	0.013	0.984	0.003	Resistant	S2
CG-67	Bhvar Gedi	0.296	0.532	0.171	M-Resistant	AD
CG-68	Chind Jhopa	0.306	0.685	0.01	Susceptible	S2
CG-69	Badsa Bhog	0.357	0.64	0.004	Susceptible	S2
CG-70	Godandi Dhaan	0.005	0.991	0.004	Susceptible	S2
CG-71	Pandko Guda	0.016	0.982	0.002	M-Resistant	S2
CG-72	SGCARS12	0.048	0.948	0.004	Resistant	S2
CG-73	Nani chudi	0.014	0.983	0.003	M-Resistant	S2
CG-74	Shivnath	0.019	0.972	0.009	Resistant	S2
CG-75	Pote Khuji	0.016	0.979	0.004	Susceptible	S2
CG-76	Bash Mukhi	0.007	0.989	0.004	Susceptible	S2
CG-77	SGCARS13	0.891	0.078	0.031	M-Resistant	S1
CG-78	Kadam Ful	0.23	0.733	0.038	Resistant	S2
CG-79	Rang Gada Khuta	0.052	0.936	0.012	Susceptible	S2

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-80	Hare Krishna	0.019	0.975	0.006	M-Resistant	S2
CG-81	Shivnath	0.032	0.96	0.008	M-Resistant	S2
CG-82	Tiki Chudi	0.084	0.339	0.577	M-Resistant	S3
CG-83	Rani Kanjar	0.17	0.042	0.787	M-Resistant	S3
CG-84	DokaraMecha	0.122	0.108	0.77	Resistant	S3
CG-85	Rakhi Dhaan	0.056	0.78	0.165	Susceptible	S2
CG-86	Surmatiya	0.033	0.96	0.006	Susceptible	S2
CG-87	UmariChudi	0.01	0.988	0.002	M-Resistant	S2
CG-88	Ratan Chudi	0.012	0.962	0.026	Susceptible	S2
CG-89	Adga Dhaan	0.312	0.677	0.011	M-Resistant	S2
CG-90	Surmatiya	0.004	0.003	0.993	Susceptible	S3
CG-91	Hiruya Dhaan	0.009	0.987	0.003	M-Resistant	S2
CG-92	Goydi	0.006	0.985	0.009	M-Resistant	S2
CG-93	SGCARS14	0.013	0.539	0.448	M-Resistant	AD
CG-94	Lal Baso	0.005	0.993	0.002	M-Resistant	S2
CG-95	Mayur Fada	0.012	0.985	0.003	M-Resistant	S2
CG-96	Kari Graas	0.004	0.991	0.005	M-Resistant	S2
CG-97	SGCARS15	0.951	0.046	0.003	M-Resistant	S1
CG-98	Kava Paadi	0.965	0.006	0.029	M-Resistant	S1
CG-99	Bariya Dhaan	0.934	0.063	0.003	M-Resistant	S1
CG-100	Sonpuri	0.985	0.011	0.004	Resistant	S1
CG-101	Sorchu Badi	0.968	0.023	0.009	M-Resistant	S1
CG-102	Sendur senga	0.879	0.118	0.003	M-Resistant	S1
CG-103	SGCARS16	0.927	0.006	0.067	M-Resistant	S1
CG-104	Dhadhar Dhaan	0.965	0.028	0.007	Resistant	S1
CG-105	SGCARS17	0.956	0.005	0.038	Susceptible	S1
CG-106	SGCARS18	0.981	0.014	0.005	M-Resistant	S1
CG-107	SGCARS19	0.981	0.015	0.004	M-Resistant	S1
CG-108	Kari Graas	0.986	0.004	0.011	M-Resistant	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-109	Jhumra	0.965	0.021	0.013	M-Resistant	S1
CG-110	SGCARS20	0.006	0.987	0.007	M-Resistant	S2
CG-111	SGCARS21	0.007	0.992	0.002	Susceptible	S2
CG-112	Pandri Satka	0.008	0.989	0.003	M-Resistant	S2
CG-113	Jira Dhaan	0.007	0.959	0.034	Susceptible	S2
CG-114	Bhayar Dhaan	0.273	0.703	0.024	M-Resistant	S2
CG-115	SGCARS22	0.003	0.003	0.994	M-Resistant	S3
CG-116	SGCARS23	0.005	0.992	0.004	Resistant	S2
CG-117	Jeera dhaan	0.01	0.982	0.008	M-Resistant	S2
CG-118	Masuri Desi	0.006	0.541	0.453	M-Resistant	AD
CG-119	SGCARS24	0.003	0.993	0.003	M-Resistant	S2
CG-120	SGCARS25	0.024	0.969	0.007	M-Resistant	S2
CG-121	SGCARS26	0.003	0.994	0.003	Resistant	S2
CG-122	SGCARS27	0.014	0.985	0.001	M-Resistant	S2
CG-123	SGCARS28	0.006	0.988	0.006	M-Resistant	S2
CG-124	Masur Dhaan	0.014	0.974	0.012	M-Resistant	S2
CG-125	SGCARS29	0.002	0.001	0.997	M-Resistant	S3
CG-126	SGCARS30	0.013	0.98	0.007	M-Resistant	S2
CG-127	SGCARS31	0.013	0.984	0.002	Susceptible	S2
CG-128	SGCARS32	0.007	0.991	0.002	Susceptible	S2
CG-129	Farsa Ful	0.028	0.97	0.002	Susceptible	S2
CG-130	Ram Laxman	0.003	0.994	0.003	Susceptible	S2
CG-131	Alti Mijo	0.014	0.97	0.016	Susceptible	S2
CG-132	Laycha	0.002	0.002	0.997	M-Resistant	S3
CG-133	Sela Dhaan	0.007	0.991	0.003	Susceptible	S2
CG-134	Masur Dhaan	0.013	0.985	0.002	Susceptible	S2
CG-135	Chatiya Dhaan	0.005	0.992	0.003	Susceptible	S2
CG-136	Kandai	0.949	0.049	0.002	Susceptible	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-137	Khutbadi	0.989	0.009	0.002	Susceptible	S1
CG-138	Vishnu bhog	0.922	0.057	0.022	Resistant	S1
CG-139	SGCARS33	0.902	0.091	0.007	Susceptible	S1
CG-140	Meso Dhaan	0.963	0.006	0.031	Susceptible	S1
CG-141	Lodhyari	0.981	0.017	0.003	M-Resistant	S1
CG-142	SGCARS34	0.986	0.007	0.007	Susceptible	S1
CG-143	SGCARS35	0.877	0.118	0.005	M-Resistant	S1
CG-144	Jodra Nakti	0.903	0.014	0.083	Susceptible	S1
CG-145	SGCARS36	0.966	0.029	0.005	Susceptible	S1
CG-146	Sirodi Bako	0.95	0.01	0.04	Susceptible	S1
CG-147	SGCARS37	0.978	0.02	0.002	Susceptible	S1
CG-148	Kata Barangi	0.984	0.01	0.006	Susceptible	S1
CG-149	SGCARS38	0.981	0.007	0.012	Susceptible	S1
CG-150	Luchai Dhaan	0.009	0.99	0.001	M-Resistant	S2
CG-151	Umari Dhaan	0.007	0.99	0.003	Resistant	S2
CG-152	Gaada Khuta	0.057	0.942	0.002	Resistant	S2
CG-153	Khuti Dhaan	0.003	0.993	0.003	M-Resistant	S2
CG-154	Kari Gudi	0.007	0.975	0.019	M-Resistant	S2
CG-155	Tama Koni	0.002	0.001	0.997	M-Resistant	S3
CG-156	Kata Nakti	0.007	0.991	0.002	M-Resistant	S2
CG-157	SGCARS39	0.003	0.994	0.003	Susceptible	S2
CG-158	Tiki Chudi	0.006	0.993	0.001	Susceptible	S2
CG-159	SGCARS40	0.992	0.005	0.003	Susceptible	S1
CG-160	Neem Chudi	0.971	0.026	0.003	Susceptible	S1
CG-161	Nani Chudi	0.93	0.06	0.01	Susceptible	S1
CG-162	KusumJhopa	0.986	0.008	0.006	Susceptible	S1
CG-163	SGCARS41	0.957	0.04	0.003	M-Resistant	S1
CG-164	Kukda Bhour	0.993	0.005	0.002	M-Resistant	S1
CG-165	SGCARS42	0.934	0.041	0.025	M-Resistant	S1
CG-166	Kapoor Saay	0.947	0.047	0.007	M-Resistant	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-167	Bhata Mokdo	0.976	0.01	0.014	M-Resistant	S1
CG-168	SGCARS43	0.987	0.011	0.003	M-Resistant	S1
CG-169	Pundri Satka	0.99	0.005	0.005	M-Resistant	S1
CG-170	SGCARS44	0.901	0.086	0.013	M-Resistant	S1
CG-171	Kukadi Mudi	0.916	0.066	0.017	M-Resistant	S1
CG-172	SGCARS45	0.989	0.009	0.001	M-Resistant	S1
CG-173	Kala Mali	0.932	0.067	0.001	M-Resistant	S1
CG-174	Motilur	0.991	0.005	0.004	Resistant	S1
CG-175	Moha Dhaan	0.94	0.038	0.021	M-Resistant	S1
CG-176	Mukukuda	0.97	0.024	0.006	Susceptible	S1
CG-177	Noni Dhaan	0.977	0.014	0.009	M-Resistant	S1
CG-178	Kala Umari	0.979	0.013	0.007	Susceptible	S1
CG-179	SGCARS46	0.019	0.979	0.003	Resistant	S2
CG-180	SGCARS47	0.012	0.59	0.398	Resistant	AD
CG-181	SGCARS48	0.99	0.005	0.005	Resistant	S1
CG-182	SGCARS49	0.963	0.03	0.007	M-Resistant	S1
CG-183	Aasan Chudi	0.911	0.074	0.014	M-Resistant	S1
CG-184	SGCARS50	0.993	0.005	0.003	M-Resistant	S1
CG-185	SGCARS51	0.972	0.025	0.003	M-Resistant	S1
CG-186	SGCARS52	0.978	0.019	0.002	Resistant	S1
CG-187	SGCARS53	0.901	0.078	0.021	Resistant	S1
CG-188	SGCARS54	0.897	0.099	0.004	M-Resistant	S1
CG-189	SGCARS55	0.976	0.014	0.011	M-Resistant	S1
CG-190	SGCARS56	0.987	0.008	0.005	M-Resistant	S1
CG-191	SGCARS57	0.99	0.004	0.006	Susceptible	S1
CG-192	SGCARS58	0.833	0.088	0.079	Resistant	S1
CG-196	SGCARS61	0.896	0.012	0.092	Susceptible	S1
CG-197	SGCARS62	0.975	0.022	0.003	M-Resistant	S1
CG-198	Mudariya	0.986	0.009	0.005	M-Resistant	S1
CG-199	LokatiMachi	0.857	0.139	0.005	M-Resistant	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-200	Ajuniya	0.952	0.044	0.004	M-Resistant	S1
CG-202	UmariChudi	0.982	0.014	0.004	M-Resistant	S1
CG-203	SGCARS63	0.992	0.004	0.004	M-Resistant	S1
CG-206	Bhaispat	0.912	0.036	0.053	M-Resistant	S1
CG-207	Badsabhog	0.964	0.019	0.016	Susceptible	S1
CG-209	SGCARS65	0.904	0.005	0.091	Resistant	S1
CG-211	SGCARS66	0.976	0.014	0.01	M-Resistant	S1
CG-212	Degichudi	0.968	0.005	0.027	Susceptible	S1
CG-213	Huldi Godi	0.974	0.013	0.013	Susceptible	S1
CG-214	SGCARS67	0.937	0.059	0.004	Susceptible	S1
CG-215	Masuri Desi	0.845	0.146	0.009	Susceptible	S1
CG-216	Kera Ful	0.95	0.003	0.047	M-Resistant	S1
CG-217	Baadi Lochai	0.918	0.071	0.011	M-Resistant	S1
CG-218	Karmuri Bhog	0.981	0.014	0.005	Susceptible	S1
CG-219	BadiLochai	0.964	0.029	0.007	Susceptible	S1
CG-220	SGCARS68	0.956	0.036	0.008	Susceptible	S1
CG-221	Mokdodhaan	0.959	0.039	0.002	M-Resistant	S1
CG-222	SGCARS69	0.968	0.007	0.026	M-Resistant	S1
CG-223	SGCARS70	0.972	0.025	0.003	Susceptible	S1
CG-224	Dhgdikaaj	0.986	0.009	0.005	M-Resistant	S1
CG-225	SGCARS71	0.968	0.018	0.014	M-Resistant	S1
CG-226	Dhotiya Dhaan	0.939	0.058	0.003	Susceptible	S1
CG-227	Sonasari	0.865	0.003	0.132	M-Resistant	S1
CG-228	Bahiya Khuta	0.961	0.036	0.003	M-Resistant	S1
CG-229	Ganga Baru	0.938	0.01	0.051	Susceptible	S1
CG-230	Bghal Bijo	0.978	0.015	0.007	Susceptible	S1
CG-231	Kurlu Kabri	0.991	0.007	0.001	Susceptible	S1
CG-232	SGCARS72	0.983	0.008	0.009	Susceptible	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
CG-234	SGCARS73	0.991	0.005	0.004	M-Resistant	S1
CG-235	SGCARS74	0.959	0.005	0.037	Resistant	S1
CG-236	SGCARS75	0.968	0.008	0.024	M-Resistant	S1
CG-237	UmariChudi	0.942	0.046	0.013	M-Resistant	S1
CG-238	Bhanvargedi	0.841	0.127	0.032	M-Resistant	S1
CG-239	SGCARS76	0.679	0.319	0.003	Susceptible	S1
CG-241	Sargiful	0.973	0.024	0.003	Susceptible	S1
CG-242	Kursobhog	0.993	0.005	0.002	Susceptible	S1
CG-247	Huldi Gathi	0.951	0.043	0.006	M-Resistant	S1
CG-248	Muthiya	0.647	0.333	0.02	Susceptible	S1
CG-249	Huldi Gathi	0.964	0.026	0.009	Susceptible	S1
CG-252	Milkor Mel	0.974	0.019	0.007	M-Resistant	S1
CG-255	Gogal	0.992	0.004	0.004	M-Resistant	S1
CG-257	Assam Chudi	0.967	0.027	0.006	M-Resistant	S1
CG-258	UmariChudi	0.908	0.03	0.062	Resistant	S1
CG-259	Bhaiya Khuta	0.961	0.029	0.01	Resistant	S1
RSG-1	41586	0.953	0.02	0.026	Resistant	S1
RSG-2	41588	0.982	0.013	0.005	Resistant	S1
RSG-3	41589	0.984	0.009	0.007	Resistant	S1
RSG-4	41592	0.989	0.006	0.005	Resistant	S1
RSG-5	41593	0.965	0.032	0.003	Resistant	S1
RSG-6	41596	0.986	0.01	0.003	Resistant	S1
RSG-7	41601	0.993	0.005	0.003	Resistant	S1
RSG-8	41661	0.988	0.005	0.008	Resistant	S1
RSG-9	41666	0.976	0.01	0.014	Resistant	S1
RSG-10	41668	0.97	0.006	0.024	Resistant	S1
RSG-11	40670	0.983	0.011	0.006	Resistant	S1
RSG-12	41671	0.98	0.018	0.002	Resistant	S1
RSG-13	41675	0.984	0.013	0.004	Resistant	S1
RSG-14	41676	0.971	0.013	0.016	Resistant	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
RSG-15	41683	0.968	0.027	0.004	Resistant	S1
RSG-16	41695	0.951	0.044	0.004	Resistant	S1
RSG-17	41712	0.978	0.018	0.005	Resistant	S1
RSG-18	41718	0.881	0.112	0.007	Resistant	S1
RSG-19	41723	0.98	0.017	0.003	Resistant	S1
RSG-20	41728	0.978	0.021	0.002	Resistant	S1
RSG-21	41734	0.989	0.008	0.003	Resistant	S1
RSG-22	41743	0.935	0.011	0.055	Resistant	S1
RSG-23	41744	0.985	0.005	0.01	Resistant	S1
RSG-24	41745	0.99	0.007	0.003	Resistant	S1
RSG-25	41747	0.989	0.008	0.003	Resistant	S1
RSG-26	41752	0.983	0.015	0.002	Resistant	S1
RSG-27	41754	0.985	0.007	0.008	Resistant	S1
RSG-28	41756	0.973	0.022	0.005	Resistant	S1
RSG-29	41763	0.991	0.006	0.003	Resistant	S1
RSG-30	41766	0.976	0.016	0.008	Resistant	S1
RSG-31	41784	0.937	0.06	0.003	Resistant	S1
RSG-32	41787	0.96	0.029	0.01	Resistant	S1
RSG-33	41792	0.895	0.075	0.03	Resistant	S1
RSG-34	41796	0.971	0.024	0.006	Resistant	S1
RSG-35	41797	0.989	0.005	0.007	Resistant	S1
RSG-36	41798	0.939	0.038	0.023	Resistant	S1
RSG-37	41799	0.992	0.003	0.005	Resistant	S1
RSG-38	41800	0.97	0.024	0.006	Resistant	S1
RSG-39	41808	0.939	0.052	0.009	Resistant	S1
RSG-40	41826	0.99	0.005	0.005	Resistant	S1
RSG-41	41827	0.954	0.045	0.001	Resistant	S1
RSG-42	41830	0.987	0.009	0.004	Resistant	S1
RSG-43	41832	0.949	0.026	0.025	Resistant	S1
RSG-44	41846	0.933	0.057	0.01	Resistant	S1

Code no.	Name	Q1	Q2	Q3	Disease reaction	Cluster
RSG-45	41854	0.963	0.018	0.018	Resistant	S1
RSG-46	41855	0.979	0.016	0.005	Resistant	S1
RSG-47	41863	0.987	0.007	0.006	Resistant	S1
RSG-48	41868	0.769	0.145	0.086	Resistant	S1

In this study, only six markers were established to be considerably associated with the blast disease. The present study showed that the selection of individual markers for selected blast resistance genes makes them a suitable marker for genotyping of rice blast resistant genes in the rice germplasm. Association mapping is an important approach used for identifying genes controlling important traits which has been successfully used in the identification of new traits. Association study of blast resistance in *indica* rice and finger millet blast resistant genes showed its importance in identification of markers linked to the loci or QTls conferring blast resistance (Babu *et al.*, 2014; Wang *et al.* 2014). Moreover, the association between the number of resistance gene(s) and the disease reaction were not completely understood in our study which could be explained by addition of more number of markers or these landraces needs to be tested for new resistance genes/alleles or QTLs.

The genome-wide association approach (GWAS) overcomes several limitations of traditional gene mapping viz; providing higher resolution, using samples from previously well-studied populations in which commonly occurring genetic variations can be associated with phenotypic variation. The GWAS has been successful plant studies in identifying loci that explain large portions of phenotypic variation. The genome wide association study (GWAS) for marker-trait association was performed using GLM and MLM (Q+K) model in TASSLE5 software. The association was filtered with $p < 0.01$. Overall, 75 comparisons with GLM and MLM were tested for association. Among 75 comparisons only five markers were found to be significantly associated for blast disease at $p < 0.05$ and $r2 > 0.01$. The r^2-values (phenotypic variance) for five markers varied from 0.0061 to 0.03298 with an average of .015847 through GLM, while in MLM, it varied from 0.01103 to 0.01759 with a mean value of 0.01458 (Table 4.9).

Table 4.8: Association of blast specific resistant genes with blast disease in 288 landraces.

Marker	Marker_F	Marker_p	Marker_R^2
Pi56	5.33875	0.02157	0.01832
Pi2450	2.38316	0.12376	0.00826
PiaSTS	0.723	0.39587	0.00252
RM72	19.78153	1.24E-05	0.06469
Tk591	1.83822	0.17623	0.00639
Tk592	3.35155	0.06818	0.01158
K6441	0.07006	0.79144	2.45E-04
Pi21	4.36182	0.03764	0.01502
Pita3	0.88277	0.34824	0.00308
RM1233	3.92194	0.04862	0.01353
RM6648	5.43146	0.02047	0.01864
Pi65	1.35022	0.24621	0.0047
40N23R	0.20353	0.65223	7.11E-04
K3957	1.61161	0.2053	0.0056
K39512	0.09628	0.75656	3.37E-04
Z56591	0.02568	0.87279	8.98E-05
Pb28	0.18516	0.6673	6.47E-04
Pi9	1.452	0.2292	0.00505
Pikh	0.53391	0.46557	0.00186

Table 4.9: Association of marker through GLM and MLM analysis

Marker	Marker_ F	Marker_ p	Marker_R^2	Marker_ F	Marker_ p	Marker_R^2
RM10123	3.13893	0.07751	0.01089	2.6273	0.10617	0.01103
RM17753	9.72082	0.00201	0.03298	3.28652	0.07093	0.01379
RM23026	3.00616	0.08403	0.01044	4.19098	0.04158	0.01759
RM20796	1.74929	0.18703	0.0061	4.08876	0.04413	0.01716
RM26241	5.4563	0.02019	0.01879	3.17529	0.07585	0.01333
			0.01584			0.01458

Five markers namely, RM10123, RM17753, RM23026, RM20796, and RM26241, were associated with blast disease $R^2 > 0.005$ with GLM and MLM analysis (Table 4.9). Lower p-value, higher F-value and high R^2 indicated the significant association with the blast disease. Additionally, MLM analysis was performed to attain more accurate association, considering the kinship value. The marker RM17753 showed highest phenotypic variance of 3.29% through GLM while the marker RM23026 showed highest phenotypic variance of 1.75% through MLM analysis. Manhattan plot was constructed using Tassel5 software for association of 75 SSR markers with average disease score on 288 landraces (Figure 4.12). The QQ plot also confirmed significant association of markers for the blast resistance (Figure 4.13). These newly identified SSR markers associated with blast disease can be used for the discovery of novel minor genes/QTLs. Associations detected through GLM were also detected in MLM analysis where kinship analysis plays a major role.

Umakanth *et al.* (2017) reported the association of eight SSR markers for important agronomic traits which includes leaf and neck blast resistance using genome-wide association analysis. Anandan *et al.* (2016) reported the association of 16 markers in 629 rice genotypes using 39 SSR markers for early seedling vigor using GLM. Borba *et al.*, (2010) found the association of eight markers among 86 SSR markers for yield and grain quality traits in Embrapa Rice Core Collection (ERiCC) from Brazil. Lou *et al.*, (2011) studied the association grain metabolites rom the Chinese core collection and found the association of 29 markers among 218 SSR markers. Wu *et al.*, (2016) reported twenty-six SSR markers associated with blast resistance in a set of 276 indica landraces. These associated markers may be helpful for mapping minor genes for leaf blast with durable resistance in the landraces.

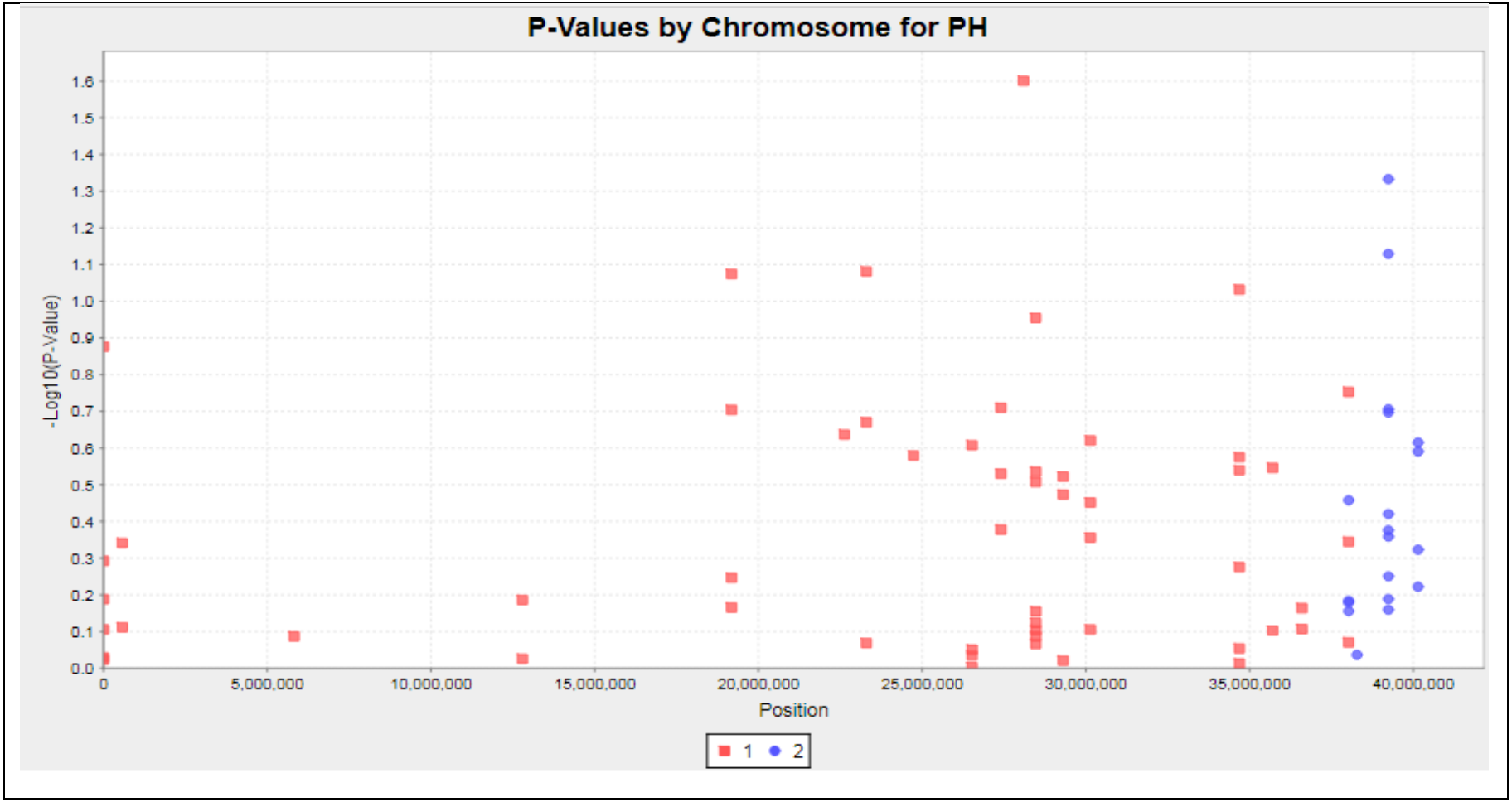

Figure 4.12: Manhattan plots of 76 SSR markers.

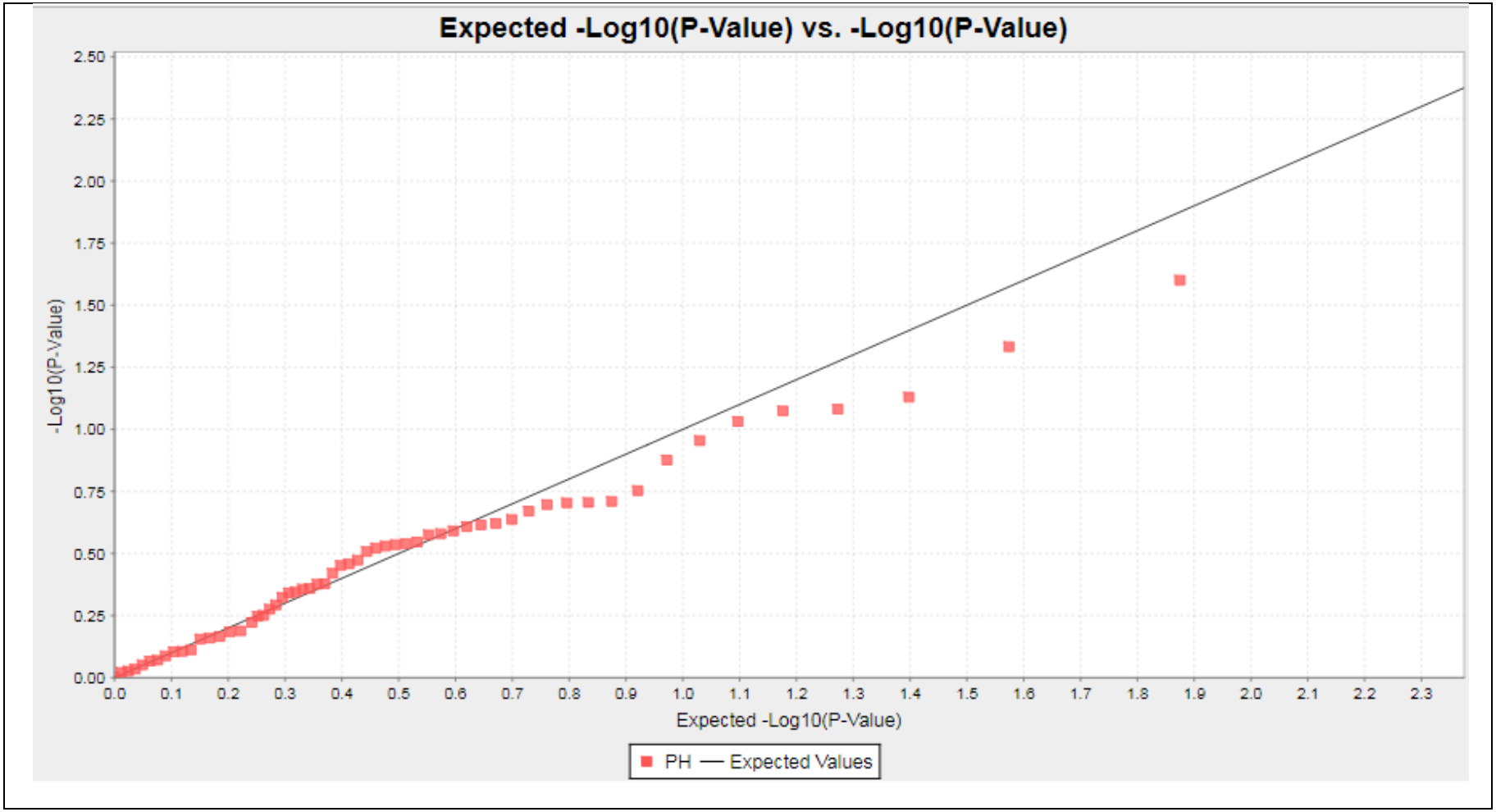

Figure 4.13: QQ plot/ Regional association (LD) plot for 76 SSR markers

CHAPTER - V
SUMMARY AND CONCLUSION

The present study entitled **"Identification of blast tolerant rice (*Oryza sativa* L.) genotypes using Genome Wide Association mapping"** was carried out at ICAR-National Rice Research Institute (NRRI), Cuttack and Research Farm, S.G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur, Bastar, Chhattisgarh, India. The phenotyping for blast disease was performed during *Kharif* 2017 at the Uniform blast nursery (UBN) of Division of Plant Pathology, NRRI, Cuttack and S.G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur. The experimental materials comprised of 500 landraces from Chhattisgarh and North eastern states.

1. Among 500 landraces (including 288 landraces from Chhattisgarh and 212 landraces from North-east) screened for leaf blast resistance; 123 (24.69%) were highly resistant, 217 (43.42%) found to be moderately resistant and 161 (32.29%) were highly susceptible. The ratio of resistant landraces to the total landraces was observed to be 123/500 (24.60). The disease score varied from score of 0 to 9.
2. Selected 288 landraces representing Chhattisgarh and North Eastern region were used in association mapping for the blast disease.
3. The genetic frequencies of the eighteen major blast resistant genes varied from 6.25% to 27.43%.
4. The frequency of the *R* gene varied from zero genes (23) to nine genes (1) in the 288 landraces. Only two rice landraces (41676 and 41745) possess maximum number of the positive allele of nine resistance genes, one landrace(41668) has eight resistant genes, three landraces (SGCARS6, 41808, 41784) have seven resistant genes and five landraces (Bandkari, Sedur Senga, 41863, 41854, 41734) have six resistant genes. Fifty seven landraces (19.79%) showed positive bands for one R genes, sixty seven (23.26%) were positive for two *R* genes, sixty (20.83%) for three *R* genes, forty four (22.50%) for four *R* genes, twenty two (7.6%) for five *R* genes, six (2.08%) for six *R* genes, three (1.04%) for eight genes.

5. The mean value of major allele frequency was observed to be 0.72 and varied from 0.50 (RM16284) to 0.94 (RM27926). The polymorphism information content of 96 markers varied from .10 (RM27926) to 0.37 (RM14320, RM15203 and RM16284) with an average of 0.30. The average gene diversity was observed to be 0.38 and varied from 0.11 (RM27926) to 0.49 (RM14320, RM15203 and RM16284).
6. The results of the AMOVA analysis demonstrated that most of the molecular variation was distributed within populations (97%) while minimum variance existed among populations (3%). The F_{IS} and F_{IT} value for all the 135 markers loci were observed to be 1.0 whereas F_{ST} was found to be 0.033.
7. The highest pair wise F_{ST} was observed between susceptible and resistant while, the least was observed between resistant and M-resistant populations.
8. The cluster analysis grouped the 288 landraces into three major clusters, I, II and III. Major cluster I consisted of 75 landraces, was divided into two sub-clusters IA and IB. The sub-cluster IA included 65 landraces, of which, 16 (24.61%) are highly resistant. Sub-cluster IB consists of only ten landraces with only one resistant genotype. Major Cluster II observed to be the largest cluster (166 landraces) which is further divided into two sub-clusters. Sub-cluster IIA consisted of 127 landraces, of which 22 genotypes (17.33%) were resistant. Similarly, Sub-cluster IIB contained 39 genotypes, in which 14 (35.89%) are highly resistant. It contained 22 resistant landraces (approx 50%).
9. The SSR markers data was used to determine the Principal Coordinate Analysis (PCoA) to estimate the genetic relatedness among the rice landraces. In PCoA, the resistant genotypes are mostly observed in 1^{st} quadrant, moderately resistant genotypes were mostly grouped in first and fourth quadrant while susceptible genotypes are clustered in 3^{rd} and 4^{th} quadrant. The first two axes in the scatter plot generated from the PCoA analysis explained 7.31% and 5.65% of the total genetic variation, respectively contributing a total of 12.96% of genetic variation.
10. A high ΔK peak value was observed to be K=3 and minimum probability of 0.55, differentiated the rice landraces into three subpopulations (SP1 SP2 and SP3) with four admixtures. The subpopulation 1 (SP1) consisted of 159

landraces representing mostly resistant population (60 highly resistant landraces). A total of 107 rice genotypes are present in subpopulation 2 (SP2) with only 13 resistant genotypes. The subpopulation 3 (SP3) consisted of only 18 landraces with only one resistant genotypes. The fixation index values (F_{ST}) of the sub-populations were found to be 0.052, 0.52, and 0.21 for SP1, SP2, and SP3, respectively. Similarly, the degree of admixture (alpha) was calculated from the data. The mean value of alpha was observed to be 0.043 that divided the population into three groups with only four admixtures.

11. Among gene specific markers (19) analyzed, six markers (Pi56, RM72, Tk59-2, pi21, RM1233 and RM6648) corresponded to six resistance genes (*Pi56, Pi33, Pit, pi21, Pi1* and *Pish*) were found to be significantly associated with the blast disease. They explained a phenotypic variance of 1.1% to 6.4%.
12. Among 75 SSR markers, only five markers (RM10123, RM17753, RM23026, RM20796, and RM26241) were found to be significantly associated for blast disease at $p < 0.05$ and $r2 > 0.01$. The r^2-values for five markers varied from 0.0061 to 0.03298 with an average of 0.015847 through GLM, while in MLM, it varied from 0.01103 to 0.01759 with a mean value of 0.01458

CONCLUSION

The present study showed the SSR markers were informative and can be used to assess the genetic diversity of diverse germplasm.

The results of the AMOVA (Analysis of Molecular Variance) confirmed the presence of phylogeographic structure. The results of this analysis considering all populations as one demonstrated that most of the molecular variation was distributed within populations (97%) while minimum variance existed among populations (3%).

The F_{ST} value of fixation indices showed that there is a weak population structures with no clear cut differentiation of sub populations. These results suggested that populations were still structured within at least one group.

Majority of resistant genotypes were observed Major cluster III and Sub-cluster IIB whereas, susceptible genotypes were clustered in Major cluster 1 and Sub-cluster IIB. The resistant landraces of different locations belonged to all the three

clusters. On the contrary, each cluster consisted of genetically similar genotypes but landraces of diverse ecologies.

The distribution pattern of rice landraces through PCoA, represented a clear grouping based on resistance to rice blast. Interestingly, most of the highly resistant landraces were clustered together in 1st quadrant.

The SP1 population consisted of more numbers of resistant genotypes as compared to SP2 and SP3 subpopulations. Therefore SP1 can be considered as dominated by resistant genotypes; SP2 is represented by susceptible genotypes while SP3 is represented by moderately resistant genotypes. Maximum allele frequency divergence among populations was observed in SP1 and SP3. The resistant North East landraces was grouped in SP1 whereas Chhattisgarh landraces were fairly distributed in all the three subpopulations.

The mean value of alpha was observed to be 0.043 that depicts that population consisted of very few admixtures. The markers associated with the blast disease may be helpful for mapping minor genes for leaf blast in the landraces.

FUTURE PROSPECTS

- Based on the results, the resistant genotypes can be used for fine mapping to find novel genes responsible for Blast Resistance.
- High density mapping through SNP can reveal the identification of novel resistance loci.
- These resistant landraces can be screened for other biotic and abiotic stresses for multiple resistance
- These landraces can be used to study host-pathogen interaction

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Appendix A.

Weekly weather data of NRRI, Cuttack during *kharif* 2017.

Week	Maximum Temp (^{0}c)	Minimum Temp (^{0}c)	Daily RF (mm)	RH - I(%)	RH-II (%)	Wind Speed (kmph)	E (mm)	SS Hrs
26	27.8	26.2	13	95	94	1.6	2.4	0
27	30.85	25.8	8	93.25	83.8	2.97	2.4	0.13
28	31.78	25.57	9.6	96	77.2	2.4	1.6	1.65
29	30.58	26.3	27	96.57	85.7	5.38	2.7	0.87
30	30.51	26.81	5.64	94.57	85.8	1.4	2.6	1.42
31	31.72	26.25	3.71	93.42	84.2	2.7	3.4	4.02
32	33.51	26.4	3.14	89.85	75.7	2.5	5.3	5.2
33	31.08	26.12	3.57	95.28	86.7	1.72	3.7	2.8
34	32.21	26.82	5.71	93	80.1	2.07	2.7	4.4
35	30.68	25.88	17.28	95.14	83.4	1.82	1.9	1.8
36	32.08	26.85	0	93.85	70.5	1.14	3.6	5.03
37	33.77	26.44	8.14	91.85	69.5	0.91	3.2	4.1
38	32.51	26.53	2	90.14	71.8	1.32	3.2	2.7
39	32.65	26.98	12.14	91	76.8	0.55	2.8	3
40	29.8	26.25	11.42	93.71	79	1.31	1.4	2.57

Week	Maximum Temp (^{0}c)	Minimum Temp (^{0}c)	Daily RF (mm)	RH - I(%)	RH-II (%)	Wind Speed (kmph)	E (mm)	SS Hrs
41	32.44	25.98	5.42	93.85	75.42	1.01	2.07	4.3
42	30.81	26.04	17.8	90.57	78.71	3.2	3.5	2.5
43	32.4	25.58	0	90.42	63	0.48	3.47	6.5
44	30.58	21.72	0	92.57	63.57	0.94	2.8	5.4
45	30.4	21.21	0	89.28	56.14	1.82	2.87	5.6
46	25.3	20.71	6.71	86.57	66.42	3.41	1.24	3.3
47	25.54	17.38	0	92.71	60	1.48	1.07	4.9
48	27.91	14.12	0	87.71	52.42	0.67	1.21	5.3
49	27.27	13.78	2.42	86	68.85	2.24	0.8	2.8

Appendix B

Scoring of 288 genotypes for 19 Gene Specific markers

	CG-1	CG-2	CG-3	CG-4	CG-5	CG-6	CG-7	CG-8	CG-9	CG-10	CG-11	CG-12	CG-13	CG-14	CG-15	CG-16	CG-17	CG-18	CG-19	CG-20	CG-21
Pi56	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Pi2450	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
PiaSTS	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
RM72	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0
Tk591	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0
Tk592	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0
K6441	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
pi21	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0
Pita3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM1233	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0
RM6648	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Pi65	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K3957	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
K39512	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pi9	0	0	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	1	0
Pikh	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0

	CG-22	CG-23	CG-24	CG-25	CG-26	CG-27	CG-28	CG-29	CG-30	CG-31	CG-32	CG-33	CG-34	CG-35	CG-36	CG-37	CG-38	CG-39	CG-40	CG-41
Pi56	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Pi2450	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
PiaSTS	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0
RM72	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0
Tk591	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
K6441	0	0	1	0	0	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0
pi21	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pita3	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0
RM1233	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0
RM6648	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0
Pi65	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
40N23R	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
K3957	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
K39512	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	1	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pi9	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0
Pikh	0	0	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0

	CG-42	CG-43	CG-44	CG-45	CG-46	CG-47	CG-48	CG-49	CG-50	CG-51	CG-52	CG-53	CG-54	CG-55	CG-56	CG-57	CG-58	CG-59	CG-60	CG-61
Pi56	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Pi2450	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM72	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K6441	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pi21	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0
Pita3	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1
RM1233	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0
RM6648	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi65	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
K3957	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
K39512	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Pi9	0	0	1	0	1	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0
Pikh	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1

	CG-62	CG-63	CG-64	CG-65	CG-66	CG-67	CG-68	CG-69	CG-70	CG-71	CG-72	CG-73	CG-74	CG-75	CG-76	CG-77	CG-78	CG-79	CG-80	CG-81
Pi56	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Pi2450	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM72	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
K6441	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
pi21	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pita3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
RM1233	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM6648	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi65	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
40N23R	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
K3957	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
K39512	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Pi9	0	1	1	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	0	0
Pikh	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1

	CG-82	CG-83	CG-84	CG-85	CG-86	CG-87	CG-88	CG-89	CG-90	CG-91	CG-92	CG-93	CG-94	CG-95	CG-96	CG-97	CG-98	CG-99	CG-100	CG-101
Pi56	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
Pi2450	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
PiaSTS	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0
RM72	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1
Tk591	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
K6441	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
pi21	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
Pita3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
RM1233	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
RM6648	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Pi65	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
K3957	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
K39512	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi9	0	0	0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0
Pikh	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-102	CG-103	CG-104	CG-105	CG-106	CG-107	CG-108	CG-109	CG-110	CG-111	CG-112	CG-113	CG-114	CG-115	CG-116	CG-117	CG-118
Pi56	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
Pi2450	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM72	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0
Tk591	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Tk592	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
K6441	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1
pi21	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pita3	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
RM1233	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1
RM6648	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1
Pi65	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
K3957	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-119	CG-120	CG-121	CG-122	CG-123	CG-124	CG-125	CG-126	CG-127	CG-128	CG-129	CG-130	CG-131	CG-132	CG-133	CG-134	CG-135
Pi56	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0
Pi2450	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
PiaSTS	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0
RM72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K6441	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
pi21	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
Pita3	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
RM1233	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
RM6648	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Pi65	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
K3957	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-136	CG-137	CG-138	CG-139	CG-140	CG-141	CG-142	CG-143	CG-144	CG-145	CG-146	CG-147	CG-148	CG-149	CG-150	CG-151	CG-152
Pi56	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Pi2450	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0
PiaSTS	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	1
RM72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
K6441	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0
pi21	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
Pita3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
RM1233	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0
RM6648	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
Pi65	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
K3957	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-153	CG-154	CG-155	CG-156	CG-157	CG-158	CG-159	CG-160	CG-161	CG-162	CG-163	CG-164	CG-165	CG-166	CG-167	CG-168	CG-169
Pi56	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pi2450	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
RM72	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K6441	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
pi21	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pita3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
RM1233	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
RM6648	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Pi65	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
K3957	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-171	CG-172	CG-173	CG-174	CG-175	CG-176	CG-177	CG-178	CG-179	CG-180	CG-181	CG-182	CG-183	CG-184	CG-185	CG-186	CG-187
Pi56	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi2450	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
PiaSTS	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
RM72	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Tk591	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
K6441	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
pi21	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Pita3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
RM1233	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0
RM6648	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Pi65	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
40N23R	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
K3957	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-189	CG-190	CG-191	CG-192	CG-196	CG-197	CG-198	CG-199	CG-200	CG-202	CG-203	CG-206	CG-207	CG-209	CG-211	CG-212	CG-213
Pi56	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
Pi2450	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
PiaSTS	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1
RM72	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Tk591	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
K6441	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	1
pi21	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pita3	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
RM1233	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
RM6648	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Pi65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
K3957	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-214	CG-215	CG-216	CG-217	CG-218	CG-219	CG-220	CG-221	CG-222	CG-223	CG-224	CG-225	CG-226	CG-227	CG-228	CG-229	CG-230
Pi56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi2450	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM72	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K6441	0	0	1	1	0	0	1	1	0	0	0	1	0	1	0	0	1
pi21	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Pita3	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
RM1233	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0
RM6648	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Pi65	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
K3957	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-231	CG-232	CG-234	CG-235	CG-236	CG-237	CG-238	CG-239	CG-241	CG-242	CG-247	CG-248	CG-249	CG-252	CG-255	CG-257	CG-258
Pi56	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Pi2450	0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0
PiaSTS	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RM72	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Tk591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tk592	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K6441	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0
pi21	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pita3	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
RM1233	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0	0
RM6648	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Pi65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
K3957	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	CG-259	RSG-1	RSG-2	RSG-3	RSG-4	RSG-5	RSG-6	RSG-7	RSG-8	RSG-9	RSG-10	RSG-11	RSG-12	RSG-13	RSG-14	RSG-15	RSG-16	RSG-17	RSG-18
Pi56	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1
Pi2450	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
PiaSTS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
RM72	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
Tk591	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Tk592	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0
K6441	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0
pi21	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0
Pita3	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0
RM1233	1	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
RM6648	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0
Pi65	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0
40N23R	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
K3957	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	1	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	RSG-19	RSG-20	RSG-21	RSG-22	RSG-23	RSG-24	RSG-25	RSG-26	RSG-27	RSG-28	RSG-29	RSG-30	RSG-31	RSG-32	RSG-33	RSG-34	RSG-35	RSG-36
Pi56	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1
Pi2450	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0
PiaSTS	0	0	0	1	1	1	1	0	0	1	0	1	0	0	1	0	0	0
RM72	1	1	1	1	0	1	0	0	1	0	0	1	0	1	0	0	1	0
Tk591	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0
Tk592	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0
K6441	1	0	1	0	0	1	1	1	0	1	1	0	1	0	1	0	0	0
pi21	1	0	1	0	1	1	0	0	1	0	1	0	1	1	1	0	1	1
Pita3	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
RM1233	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0
RM6648	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0
Pi65	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
40N23R	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
K3957	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1
K39512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pi9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	RSG-37	RSG-38	RSG-39	RSG-40	RSG-41	RSG-42	RSG-43	RSG-44	RSG-45	RSG-46	RSG-47	RSG-48
Pi56	0	0	1	0	0	1	0	0	1	0	0	1
Pi2450	0	0	1	0	0	1	1	1	1	1	1	1
PiaSTS	1	0	0	0	1	0	0	1	0	0	0	1
RM72	0	1	1	0	0	1	1	0	1	1	1	0
Tk591	0	0	0	0	0	0	1	0	0	0	1	0
Tk592	0	0	0	0	0	1	0	0	0	0	1	1
K6441	0	0	0	1	0	0	0	1	0	1	0	0
pi21	1	1	0	1	1	0	0	0	0	0	0	0
Pita3	0	0	1	0	0	0	0	0	1	0	0	0
RM1233	1	1	1	0	0	0	0	0	0	0	1	0
RM6648	0	0	0	0	0	0	0	1	0	0	0	0
Pi65	0	0	0	0	0	0	1	0	0	0	0	0
40N23R	0	0	1	0	0	0	0	0	0	0	1	0
K3957	0	0	1	0	0	0	0	0	1	0	0	0
K39512	0	0	0	0	0	0	0	0	0	0	0	0
Z56591	0	0	0	0	1	0	0	0	0	0	0	0
Z56592	0	0	0	0	0	0	0	0	0	0	0	0
Pb28	0	0	0	0	0	0	0	0	1	0	0	1
Pi9	0	0	0	0	0	0	0	0	0	0	0	0
Pikh	0	0	0	0	0	0	0	0	0	0	0	0

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