MOLECULAR CHARACTERIZATION OF RICE GENOTYPES FOR BROWN PLANT HOPPER AND BACTERIAL BLIGHT RESISTANCE GENES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
PLANT BREEDING AND GENETICS
(Minor Subject: Biotechnology)

By

Yin Yin Aye
(L-2019-A-377-M)

Department of Plant Breeding and Genetics
College of Agriculture
©PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA- 141004

2022

CERTIFICATE - I

This is to certify that the thesis entitled, **“Molecular Characterization of Rice Genotypes for Brown Plant Hopper and Bacterial Blight Resistance Genes’’** submitted for the degree of **Master of Science,** in the subject of **Plant Breeding & Genetics (**Minor subject: **Biotechnology)** of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Yin Yin Aye (L-2019-A-377-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. G.S. Mangat)
Major Advisor
Additional Director of Research
(Crop Improvement)
Punjab Agricultural University
Ludhiana - 141004

CERTIFICATE - II

This is to certify that the thesis entitled, **“Molecular Characterization of Rice Genotypes for Brown Plant Hopper and Bacterial Blight Resistance Genes”** submitted by **Ms. Yin Yin Aye (Admission No. L-2019-A-377-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirement for the degree of **Master of Science,** in the subject of **Plant Breeding & Genetics (**Minor subject: **Biotechnology)** has been approved by the Student’s Advisory Committee along with External Examiner after an oral examination on the same.

(Dr. G.S. Mangat)
Major Advisor

(Dr. M.S. Gill)
External Examiner
Professor (Retd.)
Department of Plant Breeding and Genetics
Punjab Agricultural University
Ludhiana

(Dr. R.S. Sohu)
Head of the Department

(Dr. Sandeep Bains)
Dean Postgraduate Studies

ACKNOWLEDGEMENTS

I bow my head with utmost reverence before the Almighty whose eternal blessing has enabled me to accomplish this noble effort.

I feel proud being a student of **Punjab Agricultural University** *and at the same time I acknowledge the help provided by the* **Department of Plant Breeding & Genetics** *and the university. The financial assistance provided by* **ICCR** *in the form of Junior Research Fellowship during the tenure is gratefully acknowledged. I also would like to express my sincere gratitude to* **Dr. Naing Kyi Win, Director General, Department of Agricultural Research (DAR)** *for kind permission and encouragement to study this programme.*

I feel it my profound privilege to record my deep sense of gratitude and sincere thanks from the core of my heart to my esteemed and worthy advisor **Dr. G.S. Mangat***, Additional Director of Research, Department of Plant Breeding & Genetics, for his invaluable guidance, constant encouragement, friendly attitude, immense patience, useful discussion and peerless criticisms during the course of investigation which helped me to learn something from him at each and every moment of my degree. He has always been a fountain of inspiration to me. Today, at the moment of successful completion of my degree, I am feeling lack of words to convey my heartfelt reverence for him. I am also grateful to* **Dr. R.S. Sohu** *(Head, Department of Plant Breeding & Genetics) for providing necessary facilities and moral support for successful completion of this work.*

It is a great privilege for me to express my esteem and profound sense of gratitude to **Dr. Rupinder Kaur***, Principal Rice Breeder, Department of Plant Breeding & Genetics and member of my Advisory Committee, for her valuable suggestion. She was always there in all my needs and helped his best whenever I sought for it. I am equally indebted to other members of my advisory committee* **Dr. Preetinder S. Sarao** *Principal Entomologist, Department of Plant Breeding and Genetics and* **Dr. Johar Singh Saini***, Principal Wheat Breeder (Plant Breeding), Dean PGS Nominee for their valuable suggestions, considerate behavior, guidance and critical appraisal of the manuscript.*

I accolade my high respect and profound gratitude to **Dr. Renu Khanna***, Plant Breeder, Department of Plant Breeding & Genetics, PAU, for her excellent guidance and providing me this golden opportunity to work at Molecular Biology Laboratory, Department of Plant Breeding & Genetics.*

I wish to express sincere thanks and heartfelt gratitude to **Dr. Satwinder Kaur Dhillion, Dr. Pritpal Singh***, Statistician and* **Dr. Dharminder Bhatia,** *Quantitative Geneticist, Department of Plant Breeding & Genetics for their unfailing encouragement and valuable suggestions at every phase of the study.*

I owe my parents, **Mr. U Pho Kha and Ms. Sein Yin***, who have raised me to be the*

person I am today. The words at my command are not adequate in form and spirit to convey the depth of my gratefulness to my loving parents for their ever encouraging moral support, constant concern and selfless sacrifices for my future. My vocabulary utterly fails in expressing my dear brother, ***Myo Hlaing****, sisters,* ***Khin Mar Aye****,* ***Thida Aung, Uncle, Aunt*** *for their support and affection.*

I feel no words in expressing my profound gratitude to ***Ms. Padma Angmo, Dechan Angmo, Kunzang Lamo*** *for their constant encouragement, pain taking help in completion of this investigation.*

I express my deep sense of affection to my dear friends ***Aung Lwin Oo, Than Naing****, who have been a constant source of inspiration to me. I owe my gratitude to my seniors,* ***Mr. Harpreet Singh, Mr. Gurjeet, Ms. Harsimran, Ravneet, Kiran, Navkiran*** *and* ***Naveet, Rajwant, Pavneet*** *for their extensive support in my research Lab work. It gives me immense pleasure to mention the name of my batch mates: my heartfelt feeling coupled with sincere thanks goes to all my friends, seniors and juniors whose constant support, help and collective efforts have been reflected in the completion of this venture. I extend my heartfelt thanks to the supervisors and workers in the field, Teaching and not teaching Staff, Department of Plant Breeding & Genetics for their help.*

Last but not the least, I duly acknowledge my sincere thanks to all who love and care for me.

Date: **(Yin Yin Aye)**

Title of Thesis : Molecular characterization of rice genotypes for brown plant hopper and bacterial blight resistance genes

Name of the Student and Admission No. : Yin Yin Aye
(L-2019-A-377-M)

Major Subject : Plant Breeding and Genetics

Minor Subject : Agricultural Biotechnology

Name and Designation of Major Advisor : Dr. G.S. Mangat
Additional Director of Research (Crop Improvement)

Degree to be Awarded : M.Sc.

Year of award of Degree : 2022

Total Pages in Thesis : 69 + Appendices (v) + VITA

Name of University : Punjab Agricultural University, Ludhiana – 141 004,
Punjab, India

ABSTRACT

Rice, an important staple of Asian diet, is devastated by biotic stresses caused by Brown Plant Hopper and *Xanthomonas oryzae*. Thus, an important mandate of rice breeding programs is to find host plant resistance rather than utilization of pesticides as the former approach is environment friendly and economical. The present study aimed at molecular characterization of advanced rice breeding lines for BPH and BB in order to identify lines which could serve as potential germplasm resource for breeding programs. The complete set of ABLs were sown for two years 2019-20 and 2020-21 at two locations, Ludhiana and Rauni, in order to cater for environmental and location effects. Phenotypic data analysis indicated variation between two locations to be statistically significant for EBT, TGW, DTF, yield and Fertility%. Analysis of variance for various morpho-physiological traits and milling quality traits revealed significant variations among different genotypes, environments and the interaction between genotypes and environments at both the locations. Similarly, pooled ANOVA over both locations also determined the effect of genotypes and locations to be significant for all the traits. The 64 genotypes under study were also showing resistance to BPH and BB pathogens when screened phenotypically and by using molecular markers. Five best performing lines along with BPH and BB resistance were selected from the study which can be taken further in future rice breeding programmes.

Keywords: Brown planthopper, Bacterial blight, Ear bearing tillers, Days to flowering, 1000-grain weight

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਦਾ ਸਿਰਲੇਖ	:	ਭੂਰੇ ਪੌਦੇ ਦੇ ਹੌਪਰ ਅਤੇ ਬੈਕਟੀਰੀਆ ਦੇ ਝੁਲਸ ਪ੍ਰਤੀਰੋਧ ਵਾਲੇ ਜੀਨਾਂ ਲਈ ਚੌਲਾਂ ਦੇ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਅਣੂ ਵਿਸ਼ੇਸ਼ਤਾ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਜਿਨ ਜਿਨ ਅਏ (ਐਲ-2019-ਏ-337-ਡੀ)
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਪਲਾਂਟ ਬ੍ਰੀਡਿੰਗ ਅਤੇ ਜੈਨੇਟਿਕਸ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਐਗਰੀਕਲਚਰਲ ਬਾਇਓਟੈਕਨਾਲੋਜੀ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁੱਦਾ	:	ਡਾ. ਜੀ.ਐੱਸ. ਮਾਂਗਟ ਅਡੀਸ਼ਨਲ ਡਾਇਰੈਕਟਰ ਆਫ ਰਿਸਰਚ (ਫ਼ਸਲ ਸੁਧਾਰ)
ਡਿਗਰੀ	:	ਐੱਮ.ਐੱਸਸੀ
ਡਿਗਰੀ ਨਾਲ ਸਨਮਾਨਿਤ ਕਰਨ ਦਾ ਸਾਲ	:	2022
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	69 + ਅੰਤਿਕਾ (v) + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ-141 004 ਪੰਜਾਬ, ਭਾਰਤ

ਸਾਰ ਅੰਸ਼

ਚਾਵਲ, ਏਸ਼ੀਅਨ ਖੁਰਾਕ ਦਾ ਇੱਕ ਮਹੱਤਵਪੂਰਨ ਮੁੱਖ ਹਿੱਸਾ, ਭੂਰੇ ਪੌਦੇ ਹੌਪਰ ਅਤੇ ਜ਼ੈਂਥੋਮੋਨਸ ਓਰੀਜ਼ਾ ਦੇ ਕਾਰਨ ਬਾਇਓਟਿਕ ਤਣਾਅ ਦੁਆਰਾ ਤਬਾਹ ਹੋ ਜਾਂਦਾ ਹੈ। ਇਸ ਤਰ੍ਹਾਂ ਚਾਵਲ ਦੇ ਪ੍ਰਜਨਨ ਪ੍ਰੋਗਰਾਮਾਂ ਦਾ ਇੱਕ ਮਹੱਤਵਪੂਰਨ ਆਦੇਸ਼ ਕੀਟਨਾਸ਼ਕਾਂ ਦੀ ਵਰਤੋਂ ਦੀ ਬਜਾਏ ਮੇਜ਼ਬਾਨ ਪੌਦਿਆਂ ਦੇ ਪ੍ਰਤੀਰੋਧ ਨੂੰ ਲੱਭਣਾ ਹੈ ਕਿਉਂਕਿ ਪਹਿਲਾਂ ਦੀ ਪਹੁੰਚ ਵਾਤਾਵਰਨ ਅਨੁਕੂਲ ਅਤੇ ਆਰਥਿਕ ਹੈ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਦਾ ਉਦੇਸ਼ ਬੀ ਪੀ ਐੱਚ ਅਤੇ ਬੀ ਬੀ ਲਈ ਅਡਵਾਂਸਡ ਰਾਈਸ ਬਰੀਡਿੰਗ ਲਾਈਨਾਂ ਦੀ ਅਣੂ ਦੀ ਵਿਸ਼ੇਸ਼ਤਾ ਹੈ ਤਾਂ ਜੋ ਉਹਨਾਂ ਲਾਈਨਾਂ ਦੀ ਪਛਾਣ ਕੀਤੀ ਜਾ ਸਕੇ ਜੋ ਪ੍ਰਜਨਨ ਪ੍ਰੋਗਰਾਮਾਂ ਲਈ ਸੰਭਾਵੀ ਜਰਮਪਲਾਜ਼ਮ ਸਰੋਤ ਵਜੋਂ ਕੰਮ ਕਰ ਸਕਦੀਆਂ ਹਨ। ਏ ਬੀ ਐੱਲ ਦਾ ਪੂਰਾ ਸੈੱਟ ਦੋ ਸਾਲਾਂ 2019-20 ਅਤੇ 2020-21 ਲਈ ਦੋ ਸਥਾਨਾਂ, ਲੁਧਿਆਣਾ ਅਤੇ ਰੌਣੀ ਵਿਖੇ ਬੀਜਿਆ ਗਿਆ ਸੀ, ਤਾਂ ਜੋ ਵਾਤਾਵਰਨ ਅਤੇ ਸਥਾਨ ਦੇ ਪ੍ਰਭਾਵਾਂ ਨੂੰ ਪੂਰਾ ਕੀਤਾ ਜਾ ਸਕੇ। ਫੀਨੋਟਾਈਪਿਕ ਡੇਟਾ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਸੰਕੇਤ ਦਿੱਤਾ ਹੈ ਕਿ ਦੋ ਸਥਾਨਾਂ ਦੇ ਵਿਚਕਾਰ ਭਿੰਨਤਾ ਈ ਬੀ ਟੀ, ਟੀ ਜੀ ਡਬਲਯੂ, ਡੀ ਟੀ ਐੱਫ, ਉਪਜ ਅਤੇ ਉਪਜਾਊਤਾ ਦੀ ਪ੍ਰਤੀਸ਼ਤ ਲਈ ਅੰਕੜਾਤਮਕ ਤੌਰ ‘ਤੇ ਮਹੱਤਵਪੂਰਨ ਹੈ। ਵੱਖ-ਵੱਖ ਮੋਰਫੋ-ਸਰੀਰਕ ਗੁਣਾਂ ਅਤੇ ਮਿਲਿੰਗ ਗੁਣਾਂ ਲਈ ਵਿਭਿੰਨਤਾ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਵੱਖ-ਵੱਖ ਜੀਨੋਟਾਈਪਾਂ, ਵਤਾਵਰਣਾਂ ਅਤੇ ਦੋਨਾਂ ਸਥਾਨਾਂ ’ਤੇ ਜੀਨੋਟਾਈਪਾਂ ਅਤੇ ਵਾਤਾਵਰਣਾਂ ਵਿਚਕਾਰ ਆਪਸੀ ਤਾਲਮੇਲ ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਭਿੰਤਾਵਾਂ ਦਾ ਖੁਲਾਸਾ ਕੀਤਾ। ਇਸੇ ਤਰ੍ਹਾਂ, ਦੋਵਾਂ ਸਥਾਨਾਂ ’ਤੇ ਪੂਲ ਕੀਤੇ ਅਨੋਵਾ ਨੇ ਜੀਨੋਟਾਈਪਾਂ ਅਤੇ ਸਥਾਨਾਂ ਦੇ ਪ੍ਰਭਾਵ ਨੂੰ ਸਾਰੇ ਗੁਣਾਂ ਲਈ ਮਹੱਤਵਪੂਰਨ ਹੋਣ ਲਈ ਵੀ ਨਿਰਧਾਰਤ ਕੀਤਾ। ਅਧਿਐਨ ਅਧੀਨ 64 ਜੀਨੋਟਾਈਪ ਵੀ ਬੀ ਪੀ ਐੱਚ ਅਤੇ ਬੀ ਬੀ ਜਰਾਸੀਮ ਦੇ ਪ੍ਰਤੀ ਵਿਰੋਧ ਦਿਖਾ ਰਹੇ ਸਨ ਜਦੋਂ ਫੀਨੋਟਾਈਪ ਅਤੇ ਅਣੂ ਮਾਰਕਰਾਂ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਸਕ੍ਰੀਨ ਕੀਤੀ ਗਈ ਸੀ। ਅਧਿਐਨ ਤੋਂ ਬੀ ਪੀ ਐੱਚ ਅਤੇ ਬੀ ਬੀ ਪ੍ਰਤੀਰੋਧ ਦੇ ਨਾਲ ਪੰਜ ਸਭ ਤੋਂ ਵਧੀਆ ਪ੍ਰਦਰਸ਼ਨ ਕਰਨ ਵਾਲੀਆਂ ਲਾਈਨਾਂ ਦੀ ਚੋਣ ਕੀਤੀ ਗਈ ਸੀ ਜਿਨ੍ਹਾਂ ਨੂੰ ਭਵਿੱਖ ਦੇ ਚਾਵਲ ਪ੍ਰਜਨਨ ਪ੍ਰੋਗਰਾਮਾਂ ਵਿੱਚ ਅੱਗੇ ਲਿਆ ਜਾ ਸਕਦਾ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਭੂਰਾ ਹੌਪਰ ਪੌਦਾ, ਬੈਕਟੀਰੀਆ ਦਾ ਝੁਲਸ, ਕੰਨਾਂ ਨੂੰ ਪੈਦਾ ਕਰਨ ਵਾਲੇ ਟਿਲਰ, ਫੁੱਲ ਆਉਣ ਦੇ ਦਿਨ, 1000 ਦਾਣੇ ਵਜ਼ਨ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER-I

INTRODUCTION

Rice (*Oryza sativa* L.) being the staple diet of an enormous populace caters to day-by-day dietary needs of close to half of the total populace. The world rice production need to be increased markedly over the next 30 years to keep up with population growth. It is cultivated in diverse climatic condition. Rice is an important cereal crop of India which is grown on an area of 43.79 million ha with a production of 168.50 million ton (http://www.fao.org) during 2018. Rice production worldwide including India is threatened by a number of biotic and abiotic stresses (Richa *et al* 2016). These stresses are reported to have a significant negative impact on rice growth, development and yield. Among the biotic stresses, Brown plant hopper (BPH) and Bacterial blight (BB) are the most devastating diseases which cause severe yield losses. Ect rice productivity. However Due to the sustained efforts by reading approaches significant progress has been made over the year in the development of suitable cultivate effectively combating different types of constraints that affect rice productivity. However, the occurrence of new stress demands the sticking of several genes in HY background enabling the crop to sustain attacks from several pathogens at a time.

BPH is considered as one of the most important monophagous insect pest which is highly destructive and causes huge yield losses annually throughout the tropical, subtropical and temperate areas in Asia (Park *et al* 2008). It damages the plants directly by removal of plant sap but it also indirectly transmits numerous rice viruses viz. ragged stunt virus (Rivera *et al* 1996) and grassy stunt virus (Ling *et al* 1978). It can consume more than 28% of the total dry matter of rice plants at reproductive stage (Sogawa *et al* 1994). Under severe infestations, plant hoppers often result in hopper burn that results in discoloration as well as dehydration of the rice plant (Backus *et al* 2005).Intensive chemical control of BPH can result in serious issues such as toxicity to the natural enemies of the BPH like *Anagrus nilaparvatae* (Wang *et al* 2008), increased cost of production and long run agro-ecological damage. Moreover, it imparts many adverse effects on human health. Breeding programmes focusing on the development of new rice genotypes possessing genetic resistance to insect pests should therefore complement or replace the conventional control approaches.

BB is another major disease of concern in India. The severity of the disease can be estimated from the fact that it has been reported to cause upto 50% of production losses (Kulkarni and Jahagirdar 2011). Host plant resistance is one of the most effective, economical as well as environmentally friendly option for management of BB pathogen (Khush *et al* 1989). Last two decades have seen an upsurge in understanding the genetics of BB resistance. World over, thirty-eight BB resistance genes have been identified from various sources

(Bhasin *et al* 2012) and eleven genes have been cloned.

Identification of novel sources for disease resistance is a primary requisite followed by its introgression into elite cultivars. Nowadays, marker-assisted selection (MAS) is being routinely used for the identification as well as introgression of BPH resistance genes into susceptible rice cultivars (Li *et al* 2019, Renganayaki *et al* 2002, Jairin *et al* 2010). Several molecular markers like amplified fragment length polymorphisms (AFLPs), rapid amplified polymorphisms DNAs (RAPDs), rapid fragment length polymorphisms (RFLPs), sequence-tagged sites (STSs), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and insertion deletions (InDels) (Sani Haliru *et al* 2020) have been successfully used for detection and further introgression of BPH resistance genes rice. In order to create BPH resistant rice cultivars, several investigations aimed at discovering novel resistance genes have been carried out. BPH resistance comes from a variety of sources, and genetic study has shown 19 dominant and 7 recessive genes to influence BPH resistance (Ishii *et al* 1994). More than 37 genes controlling BPH resistance have been reported till date (Kumar *et al* 2018). Majority of the identified genes have been successfully designated to different locations on chromosomes 1, 2,3, 4, 6, 7, 8, 9, 10, 11 and 12 (Jean and Kim 2010, Chen *et al* 2013, Shabanimofrad *et al* 2017).

The present research work was, therefore, planned with an aim to evaluate various yield attributes, quality traits, BPH damage as well as BB reaction of a set of advance genotypes of rice in the Punjab state. The study has been useful in identifying new high yielding, BPH and BB resistant cultivars of varying duration with suitable grain quality. The study was conducted under the following objectives

1. Evaluation of a set of advance breeding lines of rice carrying BPH resistance genes for various yield attributing traits.
2. Assessment of physicochemical and milling quality traits of advance breeding lines of rice.
3. Molecular characterization of advance breeding lines for known BPH and BB resistance genes.

CHAPTER-II

REVIEW OF LITERATURE

The pertinent of literature related to the objectives of the study has been reviewed under the following heads

Identification of new donors and resistance genes

Pathak *et al* 1969 reported that germplasm assessment for BPH obstruction is fundamental for understanding the hereditary qualities of BPH opposition as well with respect to the ID of new benefactors and obstruction qualities. Different screening techniques, for example, normal separating field populaces, seed box strategy including enormous scope screening of germplasm or rearing lines by overrunning seedlings at a few leaves stage with BPH fairies (second instars) under nursery conditions, altered seedbox test (Panda and Heinrichs 1983) have been utilized by a few specialists. The primary BPH-safe quality source was laid out in 1967.

Subsequently, Athwal *et al (*1971) also reported the BPH1 and BPH2 resistance loci in Mudgo and ASD7, respectively. Moreover, the four resistant loci viz: BPH4, BPH2, BPH3 and BPH1 have been broadly used in majority of the breeding programmes (Khush and Virk 2005). Twenty-eight (28) rice genotypes, phenotypically as well as genotypically have been determined using SSR markers (Harini *et al* 2013). The genetic variability was studied using closely linked simple sequence repeat (SSR) marker of known BPH resistant loci present all over the genome (chromosomes 2, 3, 4, 5, 6, 8, 10, 11 and 12), A set of 155 alleles were found with the use of 30 polymorphic markers having a mean of 4.6 per locus. The genetic diversity, polymorphic information content (PIC) ranging 0.15 to 0.89 and 0.13 to 0.88, respectively whereas the allele frequency varies from 0.21 to 0.89. The rice genotypes were grouped into three clusters further having sub groups and sub sub groups.

Four biotypes of BPH have been reported so far out of which biotypes 1, 2, and 3 are broadly distributed in southeastern and eastern Asia, whereas in the Indian subcontinent, biotype 4 exists (Khush and Brar 1991). It is observed that biotype 4 can survive on plants having *Bph1* or *Bph2* in South India, but shows a distinct reaction pattern on BPH resistant cultivars that are maintained at IRRI (Velusamy *et al* 1984).

In 1967, the discovery of a BPH resistant gene source was first initiated (Pathak *et al* 1969). BPH1 and BPH2 resistance loci were discovered in Mudgo and ASD7 respectively (Athwal *et al* 1971). The genetic studies were carried out for 20 BPH resistant varieties of rice, it is observed that in three varieties, two genes controlled the resistance, seven varieties possessed BPH3 and 10 varieties carried the locus controlling BPH4 resistant (Sidhu and Khush 1978). The BPH3 resistant locus obtained from Rathu Heenati segregates independently of BPH1. According to Jairin *et al* (2007) the broad-spectrum resistance gene

Bph3 is one of the major BPH resistance genes determined until now in cultivated rice. It also has been broadly utilized in rice improvement programs. Likely, BPH4 segregates independently from BPH2, the former is the gene locus for resistance in the variety Babawee. It was discovered in a trisomic analysis that the genetic loci of BPH3 and BPH4 genes were found on chromosome 10 (Ikeda and Kaneda 1981). Khush *et al* 1985 found that bph5 locus was detected from cultivar ARC10550. Ali and Chowdhary (2014) reported that resistance genes *Bph5, Bph6, Bph7* are only resistant to biotype 4 whereas *Bph8, Bph9, Bph10 (t)* genes shows resistance towards biotypes 1, 2 and 3.

Molecular markers are an extremely important tool facilitating the incorporation of desired genes in different genetic backgrounds (Tanweer *et al* 2015). For the detection and introgression of genes, various molecular markers have been used that impart BPH resistance in rice. These include sequence tagged sites (STSs) (Qiu *et al* 2010), simple sequence repeats (SSRs) (Yang *et al* 2019), amplified fragment length polymorphisms (AFLPs) (Murata *et al* 1998), single nucleotide polymorphisms (SNPs) (Balachiranjeevi *et al* 2019), rapid amplified polymorphisms DNAs (RAPDs) (Renganayaki *et al* 2002), rapid fragment length polymorphisms (RFLPs) (Murata *et al* 1998) and insertion deletions (InDels) (Hu *et al* 2018).

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most serious production constraints of rice worldwide. Deployment of resistant cultivars is the most economical and effective method to control the disease due to the non-availability of effective bactericidal agents. Ten pathotypes have been identified in Punjab based on near isogenic lines, standard international differentials and local rice cultivars (Singh *et al* 2012). Numerous resistance genes have been identified and used for disease management so far (Dinh *et al* 2010). The molecular markers usage in plant breeding has earned much attention in the recent past years. The microsatellite or simple sequence repeat (SSR) marker loci are widely distributed in the rice genome and can be easily analyzed using the polymerase chain reaction (McCouch *et al* 2002).

SSR markers are useful for wide application range in plants due to their reproducibility, multiallelic nature, co-dominance nature of inheritance and wide genomic coverage (Powell *et al* 1996). Keeping these observations in mind, the present research was carried out to characterize the advance breeding lines for BB resistance and to look for presence of any known BB resistance gene(s).

The *Bph33* resistance gene was identified as 60-kb region ranging 0.91-0.97 Mb which was carried on the short arm of chromosome 4 (4S) by Hu *et al* 2018. From seedling stage to tillering stage, the gene showed continuous and stable resistance exhibiting both antixenosis as well as antibiosis effects on BPH. Kumar *et al* 2018 identified a new 91 Kb BPH-resistant locus named as Bph34 in *Oryza nivara* acc. IRGC104646 and mapped it on the long arm of chromosome 4 with the use of high-resolution mapping using 50 K SNP chip.

Two SSR markers, RM16994 and RM17007, also co-segregated with the Bph34 which can be used effectively for markers assisted transfer into elite rice cultivars.

In the recent years, the use of marker assisted gene pyramiding approach for improving the resistance of elite rice cultivars to BPH is increasing (Fujita *et al* 2006) as combining large number of genes in one genetic background hampers insect pest adaptability thereby improving the durability of resistance (Joshi and Nayak 2010). The success of any approach related to gene pyramiding depends upon many considerations like the nature of genetic materials, the number of genotypes selected in various generations, the linkage between the flanking markers and target gene and the number of genes to be stacked (Wang *et al* 2014). MAS have been widely used for the identification and introgression of BPH resistance genes into susceptible rice cultivars (Li *et al* 2019).

Source of genes for BPH resistance

The gene designated, *Bph1, bph2, Bph3, bph4, bph5, Bph6,* and *bph7* have been identified from different sources through genetic segregation analysis (Lakshminarayana and Khush 1977) and *bph8* and *Bph9* through allelism tests (Nemoto *et al* 1989). Since then, many donors for resistance to *N. lugens* have been identified and used in breeding *N. lugens*-resistant varieties and exploring more resistance source of rice continues.

Breeding a resistant cultivar with major resistance genes was highly successful. However, *N. lugens* itself also successfully adapts to feed on the resistant cultivars by changing their biotypes. Resistance breakdown due to the occurrence of new virulent biotypes has been a serious problem in breeding programs for *N. lugens* resistance. *N. lugens* biotypes differ in their ability to infest and feed on rice cultivars with specific major *N. lugens* resistance genes (Panda and Khush 1995). Four *N. lugens* biotypes have been reported so far. Biotypes 1 and 2 widely distributed in Southeast Asia; Biotype 3 laboratory biotype produced in Philippines; Biotype 4 occurs in the Indian subcontinent but we apprehended that another new biotype may be developed in Bangladesh because previously reported major resistant rice varieties now showed susceptible (Ali *et al* 2012a). 37 BPH resistance genes have been reported from different resistance sources (Li and Yang *et al* 2019).

Notably, the majority of the BPH resistance genes are mapped on six of the 12 chromosomes (2, 3, 4, 6, 11, and 12) and, reportedly, four clusters of BPH resistance loci are located on three chromosomes. Cluster A is located on the long arm of chromosome 12 and contains eight loci. Clusters B and D are located on the short and long arm of chromosome 4 containing 13 and four QTL/gene loci, respectively, including the recently identified BPH34, BPH30, and BPH36 resistance genes located on chromosome 4 (Li *et al* 2019). Cluster C is located on the short arm of chromosome 6, which includes six gene loci (Fujita *et al* 2013). Out of 34 BPH resistance genes, 20 genes were fine mapped and only eight were cloned and characterized (BPH14, BPH17, BPH18, BPH26, BPH29, BPH9, BPH32, and BPH6) (Guo *et* *al* 2018). Wang *et al* (2018) mapped BPH30 by using F2:3 populations AC1613/ 9311 and fine mapped by using backcrossed population on the short arm of the chromosome 4.

Later, population derived from Kang wenging zhan/RBPH16 and HHZ/RBPH17 (lines RBHH16 and RBPH17 derived from *O. rufipogon* accession GX2183) were used to map BPH36 and BPH27 on chromosome 4 (BPH27 already reported by Huang *et al* (2013) on Chromosome 4) and BPH36 were fine mapped by using backcross population (Li *et al* 2019). Recently, Yang *et al* (2019) mapped a novel QTL BPH37 by using F2:3 population derived from KWQZ/IR64 on chromosome 1, between RM 302 and YM35 markers.

Evaluation of BPH resistance genes needed

Panda and Heinrichs (1983) were reported that appropriate evaluation of germplasm for BPH resistance is the key to studying the genetics of BPH resistance and to identifying resistance genes correctly. There are several types of screening methods such as natural field populations mass screening of germplasm/breeding materials in seed box by infesting plants at the seedling stage (two to three-leaf stage) with BPH nymphs (second instars) in a greenhouse, modified seed box test. However, the major limitations of evaluating germplasm in the field are seasonality, unpredictability, and uneven distribution of BPH, rendering field screening unreliable.

The mass screening using seed box test in the greenhouse uses BPH nymphs with free-choice of plant materials at seedling stage and sometimes the test is extended to different stages of plant growth. Panda and Khush (1995) was studied that the modified seed box test is sometimes used to identify germplasm tolerance to BPH. In contrast to conventional seedbox test, the modified seed box test assesses seedling damage caused by the progeny of the initially infested nymphs.

However, due to economy of time and space, the seedbox test is most commonly used with free-choice method to determine genotypes for resistance and susceptibility in the greenhouse (Heinrichs *et al* 1985). Regardless of screening methods, maximum caution should be taken for the purity of BPH population used in the evaluation. Additionally, the standard seedbox test can also be modified with no-choice of the insect to understand the mechanism of resistance caused by antibiosis.

It is important to evaluate BPH resistance by seedbox test in the greenhouse using free-choice method (antixenosis) as well as by testing in BPH hotspot areas to reconfirm the phenotypes for resistance and susceptibility.

Biotypes of BPH are defined as a population or an individual that differs from other populations or people based on non-morphological characteristics such as adaptability and resistance to infection. Adaptation to a certain host, host preference for feeding, oviposition, or both is possible. The biotypes of BPH are as follows: On rice cultivars, there are clear variances in virulence patterns. Rice has four different BPH biotypes.

In Southeast and East Asia, 1 and 2 are abundantly dispersed. Biotype 3 was created in a laboratory setting by raising insects on the resistant ASD7 strain, which has the resistance gene bph2 (Panda and Heinrich 1983).

Biotype 4, also known as South Asian biotype, is the most destructive biotype. It is found in the Indian subcontinent. Bph1 gene-carrying cultivars are resistant to Biotypes 1 and 3 are resistant to biotype 2, although they are susceptible to biotype 2. Resistance to biotypes 1 and 2 is conferred by this gene, but not resistance to biotype 3. Biotype 3, as well as the Bph3 and Bph4genes, as well as the Bph8, and Bph9 genes. All four biotypes will be resistant. Genes like bph5 only biotype 4 resistance is conferred by Bph6, and Bph7 (Khush and Brar, 1991). Significantly, a new locus on chromosome 1 delimited by markers RM488 and RM11522 has emerged as the site of a new gene that we designate as Bph33(t). None of the reported BPH genes has been mapped in this region. This gene accounted for 18-22% of phenotypic variation in terms of damage score and HDA. Besides another locus on chromosome 4 encompassing markers RM16569, RM7279 and RM16757 and covering 4 Mb region could be carrying gene(s)/QTLs influencing these traits. It is tempting to suggest that these genes may have pleiotropic effects on the traits. However, biologically more relevant view is that seedling damage in SSST is the manifestation of BPH response to feeding and preference. As nymphs initiate feeding randomly on seedlings, gustatory response to feed quality results in continued feeding or cease feeding and move from the plant. First response leads to increased feeding and honey dew excretion and seedling mortality. However, such response is not seen under no-choice setup like NS test. Forced feeding may not lead to knockdown mortality suggesting presence of acute toxic antibiotic factors, but retards growth and development and delayed mortality. These studies indicate resistance to be associated with phloem sap composition and chemistry, plant surface chemistry, volatiles and inducible responses to planthopper attack. The resistance mechanism could be due to either diet-related primary metabolites or defense related secondary metabolites of volatile or plant-surface characteristics. Ghaffar *et al* (2011) reported that through their studies with electroneurography, that insect feeding behavior can be an indicator of plant resistance. Du *et al* (2009) report action of Bph14 through induction of salicylic acid signaling pathway, deposition of callus in phloem cells and production of trypsin inhibitors. Bph29 is also reported to in a recent global gene expression study through microarray analysis also revealed induction of salicylic acid pathway genes in rice variety Rathu Heenati in comparison to TN1 rice under BPH infestation (Li *et al* 2017). Thus, it is likely that several components, both constitutive and induced, are involved in rice resistance to BPH which in-turn may be controlled by different sets of genes. Kuang *et al* (2017) found a class II small heat shock protein gene, OsHsp18.0 to be involved in resistance against bacterial blight in rice. HSP class of genes is part of salicylic acid mediated resistance pathway and is associated with

NBS-LRR genes (Wang *et al* 2015).

Xoo survives primarily in rice stubble and on weed hosts, notably *Leersia oryzoides*, *Zizania latifolia Leptocholoa chinensis*, *L. panicea* and *Cyperus rotundis*. In Australia, the bacterium is known to survive in wild *Oryza* species (*O. rufipogon* and *O. australiensis*). Xoo can also survive for short periods on infected seed and in soil, but these have not been demonstrated to be important sources of inoculum. In tropical areas, the bacterium may also survive in irrigation water (Mew *et al* 1992).

Successful infection involves the movement of the bacterium towards the host, contact between the two, penetration of the host and proliferation of the bacterium inside the host immediately following entry. The BB pathogen mainly enters through hydathodes as suggested by electron microscopic studies (Horino 1984 and Mew *et al* 1984). Wounds on rice leaves are also favourable avenues for entry of the pathogen. The infection seems more successful in the case of entry of the pathogen through wound sites than natural openings. However, new wounds are more conducive to infection than old wounds.

Kiryu *et al* (1954) demonstrated that abundant inoculum results in higher percentage of infection. Xoo enters the rice leaf typically through the hydathodes at the leaf tip and leaf margin. Bacterial cells on the leaf surface may become suspended in guttation fluid as it exudes at night and then enter the plant by swimming, or passively as the fluid is withdrawn into the leaf in the morning. Bacteria multiply in the intercellular spaces of the underlying epithelial cells. It then enters and spreads into the plant through the xylem.

Xoo may likewise get to the xylem through injuries or openings brought about by arising roots at the foundation of the leaf sheath. Inside the xylem, Xoo apparently associates with the xylem parenchyma cells. The microorganism moves upward through the leaf by means of the essential veins. It might advance horizontally through commissural veins. Inside a couple of days, bacterial cells and EPS (extracellular polysaccharide) fill the xylem vessels and ooze out from hydathodes, forming beads or strands of exudate on the leaf surface, a characteristic sign of the disease and a source of secondary inoculum (Nino-Liu *et al* 2006). Webster & Gunnell (1992) reported that bacterial blight, the more common syndrome, generally occurs from tillering stage to onward, but in our research seedling stage was very susceptible to BB. As compared to maximum tillering stage and leaf flag stage.

On the other hand, Mew *et al* 1993 reported that infection at the tillering stage can lead to total crop losses, more commonly however, plants are affected at the maximum tillering stage. When the plants were inoculated at the maximum tillering and leaf flag stage, it was found that the young plants were more susceptible than the older ones. The disease incidence of all the three-basmati varieties was less at maximum tillering stage than the seedling stage and most of varieties, which were susceptible at seedling stage gained resistance from tip to leaf flag stage against different bacterial strains.

Cho (1975) also reported that the disease incidence was higher in younger plants than older ones. There was a direct relationship between the resistance and the age of host plant. Plant age greatly influenced the varieties that were susceptible at seedling stage and these varieties showed more pronounced resistant response in the later stages. Super basmati had the highest disease incidence against PXO 340 with 89.5% that reduced to 56.21 at leaf flag stage. On the other hand, response of varieties which were resistant to different bacterial strains at seedling stage was not greatly influenced by the host age. Zhang *et al* (1984) and Mazzola *et al* (1993) reported similar results that plants gained resistance with the age against BB disease. Mariappan *et al* (1980) reported that a cultivar ASD 5, was resistant until the tillering stage, but became infected. Tu *et al* (1998) reported that the transformation of *Elite indica* rice variety IR72 with *Xa21* gene confers resistance to the BB pathogen and this resistance was shown to be stably inherited in subsequent generations.

BB is a vascular disease resulting in a systemic infection (Mew *et al* 1987) that produces tannish-grey to white lesions along the veins. Symptoms are observed at the tillering stage, disease incidence increases with plant growth, peaking at the flowering stage (Mew *et al* 1992). Kresek is the more destructive manifestation of the disease, where in the leaves of the entire plant turn pale yellow and wilt during the seedling to the early tillering stage, resulting in a partial or total crop failure. Plants less than 21 days old are the most susceptible and temperatures between 28 and 34°C favour kresek development (Mew *et al* 1979 and Mizukami and Wakimoto 1969).

BB is characteristic of yellow lesions with wavy margins on leaf blades that may extend to the sheath. The occurrence of bacterial ooze from infected leaves has been observed in warm and humid climates, which contributes to the spread of this disease. While damage is extensive when kresek precedes BB, post flowering infections have very little effect on grain yield. BB was favoured by warm temperatures (25 to 30°C), high humidity, rain, and deep water. The disease is more prevalent in wetland areas where these conditions often occur. Winds severe enough to cause wounds and excess nitrogen also favour the disease. The severity of the disease is in part dependent on the virulence of the bacterial isolates present. Bacterial blight is severe in susceptible rice varieties under high nitrogen fertilization (Annonymous *et al* 2010). The bacterium can be disseminated by irrigation water, by splashing or windblown rain, by plant-to-plant contact, by trimming tools used in transplanting, and by handling plants during transplanting (Mew *et al* 1992).

Fine mapping of the BB resistance genes

The significant resistance gene, xa13, was found in rice variety BJ1 and localised to the long arm of rice chromosome 8 (Ogawa *et al* 1986). Fine-mapping of the xa13 gene has been completed (Sanchez *et al* 1999). In the putative gene's promoter region, a series of insertions and deletions have been done to the cloned DNA (InDels). The bacteria Os8N3 has been found to be the source of the gene's activity (Chu *et al* 2006a). Compatible strains of Xoo expressing the transcription activator-like (TAL) effector pthXo1, which bind to the promoter of Os8N3 to activate its expression, generally cause the expression of the dominant allele of the gene, Xa13, which encodes a sugar transporter termed Os8N3 (Chu *et al* 2006b).

In rice genotypes bearing the gene's recessive allele, Due to the InDels, pthXo1, i.e. xa13, is unable to bind to the promoter of Os8N3 and hence cannot activate the rice sugar transporter to establish infection (Antony *et al* 2010). We created four sets of primer pairs that target the In Dels in the promoter of Os8N3 as functional markers for xa13 in this investigation because xa13 has been cloned and a functional nucleotide polymorphism specific for the gene has been clearly found. The gene, which was discovered in DZ192, confers strong resistance to a wide spectrum of Xoo races (Suh *et al* 2013). On chromosome 5, xa5 has been fine-mapped (Blair *et al* 2003). The resistant allele (xa5) has a 2-bp alteration in the second exon, resulting in a single amino acid change (valine to glutamiate) at position 39, interrupting the transcription factor's function and resulting in Xoo resistance in rice types with xa5 homozygous. Even though the CAPS marker RG556, which is very close to the gene (Huang *et al* 1997), has been widely used for marker-assisted transfer of xa5 (Rajpurohit *et al* 2011), it is cumbersome to use and of limited utility for routine marker-assisted breeding programmes.

CHAPTER-III

MATERIALS AND METHODS

The present study was conducted to evaluate a set of advanced breeding lines of rice carrying BPH resistance genes for various yield contributing traits, assessment of their physicochemical, milling quality traits and molecularly characterizes for BPH and BB resistance genes.

To accomplish the objectives of the study, two experiments were conducted. The Experiment I was designed for Morpho- Physiological, milling quality evaluation of advance breeding lines for yield as well as reaction to BPH at two different locations that is Punjab Agricultural University, Ludhiana and Regional Research Station, Rauni, Patiala (Plate 1). The experiment II was aimed at screening and molecular characterization of a set of rice genotypes for known BPH and BB resistance genes by using molecular markers (gene specific as well as SSRs).

Experiment I

3.1.1 Morpho- Physiological evaluation of advance breeding lines for yield and reaction to BPH.

The experiment was conducted at two different locations that are the Rice Experimental Area, B Block, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana and Regional Research Station, Rauni, Patiala during *kharif* season 2021. The tested genotypes comprised of 55 backcross progenies in the background of PR 121 and PR 122 including donors and checks varieties. The details of the genotypes along parentage donors and checks varieties are given in the Table 3.1.

During *kharif* 2021, complete set of 64 genotypes was planted in Randomized complete block design (RBD) with three replications at both locations to evaluate these genotypes for yield attribute and reaction to BPH. The nursery was sown at PAU, Ludhiana a plot size was 4.62 m^2 and 2.5 m^2 are the plot size are one month old nursery, we transplanted at Ludhiana as well Rauni with a row to row spacing of 20 cm and plant to plant spacing of 15 cm at Ludhiana and Rauni replication. The standard package of practices was followed to raise a healthy crop.

Observations recorded

The following observations were recorded for the following characters:

a) Days to 50% flowering

The number of days was recorded on the whole plot basis from the date of sowing in the nursery to the date when 50% of tillers for each entry flowered on plot basis.

b) Plant height (cm)

Five plants were randomly chosen from the center of plots. Plant height was measured from the ground level to the tip of the primary panicle at the time of maturity and expressed in cm.

c) Ear bearing tillers (EBT/m^2)

The number of productive tillers was counted from five randomly selected plants at the time of maturity. The number of ears bearing tillers/m^2 was computed.

d) Spikelet sterility (%)

At physiological maturity, five plants were selected randomly avoiding the border plants. Primary panicle was sampled from each tagged plant and used for spikelet fertility count. The total numbers of filled and sterile spikelet's were added which gives the total number of spikelet's per panicle. Spikelet fertility percentage was calculated using the formula given below and expressed in percentage. Average of spikelet fertility of five panicles was considered.

$$\text{Spikelet sterility (SS)\%} = \frac{\text{Total number of unfilled spikelets per panicle}}{\text{Total number of spikelets per panicle}} \text{ x } 100$$

e) Thousand grain weight per panicle

Random grain sample was taken from each entry and 100 grains were counted. They were weighed using weighing balance and thousand grain weight in grams was calculated

f) Grain yield (kg/ plot)

The dried and cleaned grains from each plot was weighed and recorded, the data were expressed in kg and converted to kg/ha.

g) Total Rice Recovery (TRR)

The weighed samples (125 g) of paddy having moisture content not more than 13-14% were taken and then dehusked by using Satake Rubber Roll Laboratory Sheller (Satake Engineering Co., Japan). The brown rice was obtained after shelling and brown rice samples were milled in McGill Miller No. 2, USA (Plate 2). The time of the milling was selected to obtain a 6% degree of polish in brown rice samples. The remaining rice sample after milling was total rice including broken rice grains and recovery percentage was calculated by using formula.

Total Rice Recovery (TRR) % = (Weight of milled rice/ Weight of paddy) ×100%

h) Head Rice Recovery (HRR)

The head rice is the milled rice which includes broken kernels that have 75% or more of the whole kernel. The total rice obtained after polishing was graded for 2 minutes by using test rice grader machine to separate the head rice from broken. It was calculated by using formula.

Head Rice Recovery % = (Weight of head rice/ Weight of paddy) ×100%

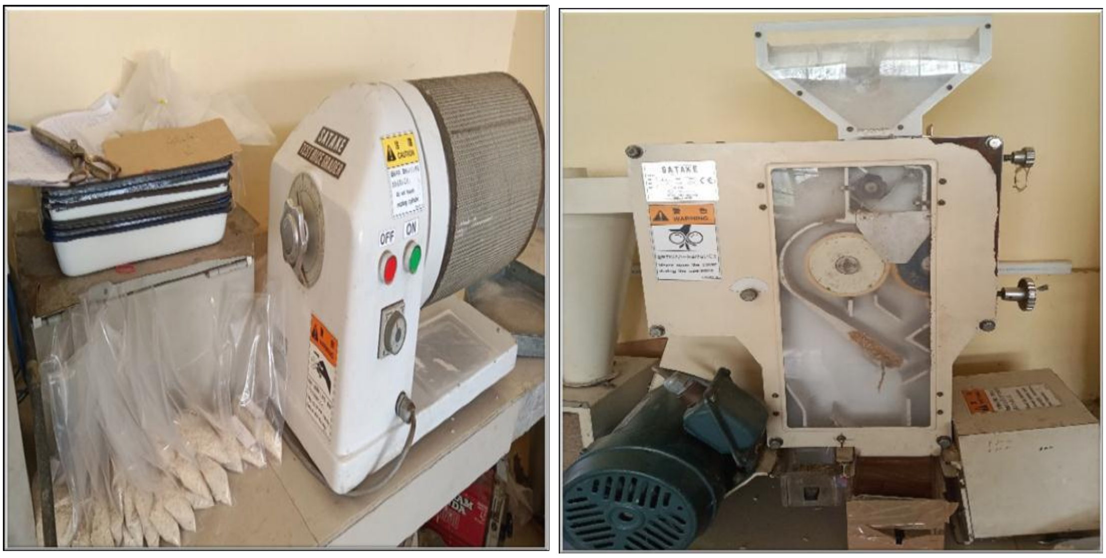
i) BB reaction (Bacterial Blight Reaction)

The disease reaction was scored 14 days post inoculation as per 0-9 Standard

Ludhiana Trial *Kharif* Season

Rauni Trial *Kharif* Season

Plate 1: General Overview of Experiment at different two locations



Dehusker machine Polisher machine (30 seconds)

Grading rice by using Test rice grader Length and Breadth machine

Plate 2: Grain quality analysis of advances breeding lines

Evaluation Scale (SES 2013, IRRI).

j) BPH damage score

The BPH damage was recorded under artificial conditions and was scored as per SES 0-9 scale.

k) Length breadth/ ratio

Ten whole kernels were selected randomly. Their length and breadth were recorded and the ratio of length to breadth of the brown rice grains was determined.

Table3.1 List of genotypes included in the study *kharif* 2020

Sr. No.	Designation	Cross
1	PAU9711-1	PR121*^{2}//PR121/*O.nivara* CR100204]
2	PAU 9711-2	PR121*^{2}//PR121/*O.nivara* CR100204]
3	PAU 9711-3	PR121*^{2}//PR121/*O.nivara* CR100204]
4	PAU 9711-4	PR121*^{2}//PR121/*O.nivara* CR100204]
5	PAU 9711-5	PR121*^{2}//PR121/*O.nivara* CR100204]
6	PAU 9712-1	PR121*^{2}//PR121/*O.nivara* CR100204]
7	PAU 9712-2	PR121*^{2}//PR121/*O.nivara* CR100204]
8	PAU 9712-3	PR121*^{2}//PR121/*O.nivara* CR100204]
9	PAU 9712-4	PR121*^{2}//PR121/*O.nivara* CR100204]
10	PAU 9712-5	PR121*^{2}//PR121/*O.nivara* CR100204]
11	PAU 97221- 1	PR121*^{2}//PR121/*O.nivara* CR100204]
12	PAU 97221-2	PR121*^{2}//PR121/*O.nivara* CR100204]
13	PAU 97221-3	PR121*^{2}//PR121/*O.nivara* CR100204]
14	PAU 97221-4	PR121*^{2}//PR121/*O.nivara* CR100204]
15	PAU 9723-1	PR121*^{3}//PR121/RP2068]
16	PAU 9723-2	PR121*^{3}//PR121/RP2068]
17	PAU9723-3	PR121*^{3}//PR121/RP2068]
18	PAU 9723-4	PR121*^{3}//PR121/RP2068]
19	PAU 9723-5	PR121*^{3}//PR121/RP2068]
20	PAU 9724-1	PR121*^{3}//PR121/RP2068]
21	PAU 9724-2	PR121*^{3}//PR121/RP2068]
22	PAU 9724-3	PR121*^{3}//PR121/RP2068]
23	PAU 9724-4	PR121*^{3}//PR121/RP2068]
24	PAU 9724-5	PR121*^{3}//PR121/RP2068]
25	PAU 9725-1	PR121*^{3}//PR121/RP2068]
26	PAU 9725-2	PR121*^{3}//PR121/RP2068]

Sr. No.	**Designation**	**Cross**
27	PAU 9725-3	PR121*^{3}//PR121/RP2068]
28	PAU 9725-4	PR121*^{3}//PR121/RP2068]
29	PAU 9725-5	PR121*^{3}//PR121/RP2068]
30	PAU 9726-1	PR121*^{3}//PR121/RP2068]
31	PAU 9726-2	PR121*^{3}//PR121/RP2068]
32	PAU 9726-3	PR121*^{3}//PR121/RP2068]
33	PAU 9726-4	[PR121*^{3}//PR121/RP2068]
34	PAU 9726-5	[PR121*^{3}//PR121/RP2068]
35	PAU 9729-1	[PR121*^{3}//PR121/RP2068]
36	PAU 9729-2	[PR121*^{3}//PR121/RP2068]
37	PAU 9729-3	[PR121*^{3}//PR121/RP2068]
38	PAU 9729-4	[PR121*^{3}//PR121/RP2068]
39	PAU 9729-5	[PR121*^{3}//PR121/RP2068]
40	PAU 9730-1	[PR121*^{3}//PR121/RP2068]
41	PAU 9730-2	[PR121*^{3}//PR121/RP2068]
42	PAU 9730-3	[PR121*^{3}//PR121/RP2068]
43	PAU 9731-1	[PR121*^{3}//PR121/RP2068]
44	PAU 9731-2	[PR121*^{3}//PR121/RP2068]
45	PAU 9731-3	[PR121*^{3}//PR121/RP2068]
46	PAU 9731-4	[PR121*^{3}//PR121/RP2068]
47	PAU 9731-5	[PR121*^{3}//PR121/RP2068]
48	PAU 9737-1	[PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
49	PAU 9737-2	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
50	PAU 9737-3	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
51	PAU 9737-4	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
52	PAU 9737-5	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
53	PAU 9738-1	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
54	PAU 9738-2	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
55	PAU 9738-3	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
56	PR114	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
57	PR121	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
58	PR122	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
59	PR126	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]

Sr. No.	Designation	Cross
60	IR 71033	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
61	IR54842	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
62	Kaum182-1	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
63	RP2068-295	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]
64	RP2068-303	PR122*^{2}//PR122/*O.rufiopogon* (CR100441)]

3.1.2 Screening for bacterial blight resistance by leaf clipping method

The set of 64 advance breeding lines along with check varieties were screened for bacterial blight resistance under artificial inoculation conditions at Rice Experimental Area, B Block, Department of Plant Breeding and Genetics, PAU, Ludhiana. Inoculation with bacterial culture was done at maximum tillering stages using clip method. A sterilized scissor dipped in bacterial suspension was used for inoculation. Leaves of all the tillers in a single plant were taken and the top 1-2 inches of 3-5 leaves were clipped off simultaneously and the same procedure was repeated for all the plots in a replication (Plate 3). The inoculum was utilized within two hours of its preparation as the pathogen losses its viability very quickly. The disease severity was then recorded 14 days after inoculation according to SES scale (0-9) of IRRI (Anonymous 1996) as given in Table 3.2.

Table 3.2: Scale for reaction to bacterial Blight (Anonymous 1996)

Infection%	Score	Disease Reaction
0%	0	HR(Highly Resistant)
>1-10%	1	R(Resistant)
>10-30%	3	MR(Moderately Resistant)
>30-50%	5	MS(Moderately susceptible)
>50-75%	7	S (susceptible)
>75-100%	9	HS(Highly Susceptible)

3.1.3 Screening for Brown Plant hopper resistance (BPH resistance)

The plant material was tested along checks was screened using single seed box. Second and third instar nymphs of BPH were released on twelve day old rice seedlings. Scoring was done according to Standard Evaluation System (SES), IRRI (2014) when 90% to 100% plants of susceptible check TN1 were dead each. The scale used for scoring is given in Table 3.3 (Plate 4).

Table 3.3: Scale for reaction to brown plant hopper (IRRI 1998)

Score	Reaction
0	No damage
1	Very slight damage
3	The first and second leaves of most plants turn partially yellow
5	Pronounced yellowing and stunting or about half of the plants wilting or dead
7	More than half of the plants show wilting or are dead
9	All plant are dead

3.1.4 Statistical Analysis

The study was conducted in a Randomized Block Design with three replications each at both the locations. The pearson corelation co-efficient was calculated between locations for all the traits and significance of corelation was determined at p-value < 0.05. Similarly, the value of corelation coefficient was determined between the traits within a location. Correlation co-efficients and plots were constructed using corrplot package (Wei T *et al* 2021) and psych package (Revelle W (2021) in R. Graphical representation of data for comparing the effect of replications within and across locations was done using boxplot function in R. Similarly, significance of difference in mean values of trait at two locations was computed using paired t-test in R.

On the basis of mean phenotypic performance, a set of ten lines was selected, referred to as “A” set and the remaining lines were allocated to “B” subset. Kruskal-Wallis rank sum test was done to compare two groups in order to determine the level of significance using kruskal.test function in R.

The linear model for analyzing combined data over two locations for the year 2021 was done in R using the lmer function of lme4 package. The linear model assumed genotypes and replication within locations to be random effects. The linear model assumed trait value to be determined by genotypes, locations, replication within location and genotype x location, where all the traits were random effects. The variance obtained after fitting linear mixed model was used to calculate broad-sense heritability for all the traits using the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 \ + \ (\sigma_{gl}^2/nl) + (\sigma_{rl}^2/nr) + (\sigma_r^2/nl * nr)}$$

Data recorded for all characters was subjected to analysis of variance (ANOVA). ANOVA was calculated location wise as well as pooled analysis was done. Following equation was taken into account for carrying out the statistical analysis

Bacterial Colonies on nutrient agar slant **Artificial Inoculation**

Scoring for BB disease **Field view of Trial at PAU, Ludhiana**

Plate 3: Screening of advance breeding lines against ten pathotypes (PbXo-1 to PbXo-7) of BB under artificial conditions

Seed box technique method **Seed box nursery method**

Seedling plants **Sowing of seeds**

Plate 4: Screening of advance breeding lines BPH under artificial condition

$Y_{ij} = m + g_i + b_j + e_{ij}$

Where,

Y_{ij}= phenotypic value of the i^{th} genotype of a generation grown in the j^{th} replication

m = general population mean

g_i = effect of the i^{th} genotype, where i=1 g

b_j= effect of the j^{th} replication, where j=1r

e_{ij} = environmental effect

Analysis of variance based on the above model led to the following components of varianc**e** as stated in Table 3.4

Table 3.4 Analysis of variance

Source of variation	**DF**	**SS**	**MSS**		**F value**
			Observed	**Expected**	
Replication	r - 1	$Sr=\Sigma r^2/g-(\Sigma x)^2/N$	$M_r= S_r/r-1$	$\sigma_e+ g\sigma_r$	M_r/ M_e
Genotypes	g - 1	$Sg=\Sigma g^2/r- (\Sigma x)^2/N$	$M_g= S_g/g-1$	$\sigma_e+ r\sigma_g$	M_g/M_e
Error	**(**r - 1) (g - 1)	$S_e= S_t-S_r-S_g$	$M_e=S_e/(r-1) (g-1)$	σ_e	
Total	gr - 1	S_t			

Where,

r = the number of replications

g = number of tested genotypes

N = total number of observations taken

S_r= replication sum of squares

S_g= genotype sum of squares

S_e= error sum of squares

S_t= total sum of squares

σ_r= replication variance

σ_g= genotypic variance

σ_e= error variance

The genotypic variance was tested against error variance by F test for (g-1) and (r-1)(g-1) degrees of freedom. Similarly, the replication variance was tested against error variance for (r-1) and (r-1)(g-1) degrees of freedom.

The standard error of difference between the genotypic means was estimated as follows:

$$SD (d) = \pm \sqrt{\frac{2Me}{r}}$$

Critical Difference (CD) = SE (d) × t $_{(r-1)\,(g-1)}$ at 5% level of significance.

Experiment II: Screening and molecular characterization of a set of rice genotypes for known BPH and BB resistance genes

The present investigations were carried out at Molecular Biology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The genotypes were subjected to molecular analysis to identify the know BPH and BB resistance genes.

3.1.5 DNA Extraction

The total genomic DNA of the advance breeding lines was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method by Murray and Thompson (1980). About 5-7 g of leaves from each young plant was harvested and placed in white butter paper bag marked giving appropriate number. The samples were collected in ice box from the field and were later transferred to the deep freezer at -80℃. The DNA extraction was done by crushing the leaf tissue into fine powder with liquid nitrogen in a mortar and pestle. The fine leaf powder was then transferred to the 2 ml Eppendorf tubes and was suspended in 800 μl pre-warmed CTAB buffer by inverting the tubes gently. The composition of CTAB buffer is given in Table 3.5.

Table 3.5: Composition of CTAB extraction buffer

Components	**Final concentration**
CTAB	2%
Tris base(pH)	100 mM
NaCl	1.4 M
EDTA (pH 8.0)	10mM
β Mercaptoethanol	0.2%

Thereafter, the homogenate was incubated at 65℃ for one hour in a water bath and the contents were mixed by inverting the tubes at regular intervals. After removing the tubes from water bath, 800 μl of chloroform: isoamyl alcohol (24: 1) solution was added to the homogenate and the tubes were placed on a shaker for 20 to 40 min for the uniform and constant mixing of the contents, till it made a dark green emulsion of the organic phase. The tubes were then centrifuged at 10,000 rpm for 10 min (Eppendorf Centrifuge 5415D). After centrifugation, the supernatant (aqueous phase) was transfer into another 1.5ml Eppendorf tubes using micropipette, care was taken to avoid debris inclusion. To this, heated RNase A (Genetrix) was added to a final concentration of 10ul/ml. These tubes were incubated at 37 ℃ in water bath for one hour. Then 600 μl chilled isopropanol was added to the supernatant placed in 1.5ml fresh tubes and tubes were gently inverted several times. To allow proper precipitation of DNA tubes were placed at 4℃ for one to two hours. The DNA formed a white cotton thread -like precipitate and good quality DNA floated at the top. Tubes were then centrifuged at 10,000 rpm for 8 min. After discarding the supernatant washings with 70 percent ethanol were given and DNA was air dried by simply inverting the tubes. The purified DNA pallet was finally dissolved into 200ul of 1X TE buffer (Tris base-50mM,1 mM EDTA, pH 8.0)

3.1.5 DNA quantification

The agarose gel electrophoresis method was used on DNA quantification. A 0.8% agarose dissolved in 100 ml of 1X TBE electrophoresis buffer (45mM Tris base, 45 mM Boric acid and 1mM EDTA, pH 8.0) was used for gel electrophoresis. The mixture was then subjected to heatting until the agarose dissolved completely i.e when the solution became transparent. Then the solution was allowed to cool to about 60℃ and up to 5 μl ethidium bromide (10mg/ml) was added to final concentration of 0.5 ug/ μl buffer. The agarose solution was then poured into gel moulds with combs and was then left for 30-40 minutes for solidification. DNA sample were prepared by adding loading dye (6X loading dye consist of 0.25%w/v bromophenol blue, 0.4% w/v sucrose or 30% glycerol in sterile water) to the DNA so that the final concentration of loading dye was 1X. After solidification, combs were removed gently, the gel was placed in the gel tank for electrophoresis. DNA sample were loaded into the wells. After loading, the gel was subjected to electrophoresis at constant voltage of 5V/cm for about 1 hour. After this, the gel was visualized under UV trans-illuminator and recorded with gel documentation system (Alpha Imager HP, USA). The observations of DNA samples revealed the quality of the DNA. If DNA forms a smear, it indicates that DNA is degraded.

3.1.6 Selection of SSR markers

A total of 8 SSRs markers either gene-specific or closely linked to the BPH and BB resistance genes (one each for Xa4, xa13 and Xa21 and two for xa 5) and (BPH 33, BPH 34) were used for identification of resistance genes present in each line. However, xa 5 had primer for both the resistant and susceptible alleles. The details of the primers used for molecular analysis are given in (Table 3.6).

3.1.7 PCR amplification and gel electrophoresis

In-vitro amplification using Polymerase Chain Reaction (PCR) was performed using 96 well microtitre plate in Eppendorf (Applied Biosystem, GeneAmpPCR system 9700) Master cycler for confirmation of Xa4, xa5, xa13 and Xa21. The reaction mixture contained 0.5 mM of each primer (forward and reverse), 0.25mM of each dNTP mix, 1.5mM $MgCl_{2,}$ *and* 0.2 μl of Taq polymerase, 1.0X PCR buffer and about 50ng/ μl of template DNA as given in Table 3.7. PCR analysis was carried out using Xa4, xa5, xa13 and Xa21 gene linked specific primers and the PCR profile applied is mentioned in Table 3.8.

3.1.8 Visualization of amplified PCR products

The amplified product was visualized by electrophoresis on 2.5% agarose gel except for *Xa*4 primer which was visualized on 4% agarose gel to separate the DNA bands. For the preparation of 2.5% or 4% aagarose gel,12.5 or 20 gm of agarose powder (Electrophoresis matrix, G Biosciences, USA) respectively was dissolved in 500ml of 0.5X TBE buffer. The mixture was then heated for some time to dissolve the contents completely to obtain a transparent solution. The solution was then allowed to cool to about 60℃; visualization or fluorescence dye was then added to the solution with constant stirring. The solution was then poured into gel mound onto which the combs were adjusted and the gel was then left for solidification. After PCR was finished, 4 μl of 6X loading dye was added to each of the amplified products and thoroughly mixed. The PCR products (10/ μl well) were then loaded into the wells after removing the comb. A standard DNA ladder 100bp was loaded along with sample. The PCR products were resolved by running gel at 5V/cm for 3-5 hours and then the bands were visualized under UV light and photographed using gel documentation system (Alpha Imager HP, USA) with Alpha view software programme.

Table 3.6: Chromosome location and primer sequence of gene specific markers for Xa4, xa5, xa13 and Xa21 genes

BB resistance	Chromosome location	Marker	Primer Pair	Resistant and size (base pairs)	Susceptible band size (base pairs)	Reference
Xa4	11	Mp4	F:5′-ATCGATCGATCTTCACGAGG-3′ R:5′	150	50	Wang *et al* (2001)
			dTGCTATAAAAGGCATTCGGG-3′			
xa13	8	xa13 promoter	F:5′-GGCCATGGCTCAGTGTTTAT-3′	500	250	Sundarm *et al* (2008)
			R:5′ CAGCTCCAGCTCTCCAAATG-3′			
Xa21	11	pTA248	F:5′ AGACGCGGAAGGGTGGTTCCCGGA3′	1000	650	Huang *et al* (1997)
			R:5′ AGACGCGGTAATCGAAGATGAAA-3′			
xa5	5	xa5FM-SF	GTCTGTAATTTGCTCGCGTTCG	Null allele	190	Hajira *et al* (2016)
		xa5 FM-SR	TGGTAAAGTACATACCTTATCAAACTGGA			
		xa5 FM-RF	AGCTCGCCATTCAAGTTCTTGAG	190	Null allele	
		xa5FM-RR	TGACTTGGTTCTCCAAGGCTT			

Table 3.7: Concentration of different PCR components in 20μl reaction

Components	**Stock Concentration**	**Volume (μl)**	**Working concentration**
Sterile water	-	11.2	-
$MgCl_2$	10mM	0.4	1.5 Mm
PCR buffer	5X	4	1.0X
dNTP mix	25 mM	0.2	0.25mM
Primer Forward	10 mM	1	0.5Mm
Primer Reverse	10 mM	1	0.5Mm
Taq Polymerase	3 units/ μl	0.2	1.0 unit
DNA Template	25ng/ μl)	2	50 ng/ μl
Total	-	20μl	

Table: 3.8: PCR profile for gene specific primers of BPH & BB resistance genes BPH33, BPH34, Xa4, xa5, xa13, Xa21

Sr. No	**Steps**	**Temp.**	**Time**				
			MP4(Xa4) and xa5(xa5Randxa5S)	**xa13 (xa13p)**	**Xa21 (pTA248)**	**BPH 33**	**BPH34**
1	Initial denaturation	94℃	4 min	4min	4min	4min	4min
2	Denaturation	94℃	1 min	1min	30sec	30sec	30sec
3	Annealing	55℃	1 min	1min	30sec	30sec	30sec
4	Extension	72℃	2 min	1min	1min	1min	1min
5	Go to step II		30 cycles	35cycles	30cycles	35cycles	35cycles
6	Final extension	72℃	7 min	7 min	7min	7min	7min
7	Hold	4℃					

CHAPTER-IV

RESULTS AND DISCUSSION

The present study was conducted to evaluate a set of advanced breeding lines of rice carrying BPH resistance genes for various yield attributing traits and milling quality traits further these lines were arterially exceed for BPH resistance and characterized and BB resistance genes by using molecular markers. Experiment I included morpho-physiological evaluation of advance breeding lines for yield and reaction to BPH at two different locations while Experiment II aimed at screening and molecular characterization of a set of rice genotypes for known BPH and BB resistance genes using molecular markers. The results of the study are presented below:

Experiment I: Morpho-Physiological evaluation of advanced breeding lines for yield and reaction to BPH

In order to determine the strength and nature of linear relationship between variables at different locations and within a location, Pearson correlation co-efficient was calculated and its significance was determined. A descriptive correlogram presenting frequency distribution curves along with correlation values has been presented via Figure 4.1. High degree of correlation was observed for days to 50% flowering between two locations. Similarly, the value of Pearson correlation co-efficient between locations determined yield and plant height to be moderately correlated between two locations. On the contrary, EBT and fertility showed very little correlation between two locations. Correlograms depicting correlation between traits at each location have been presented via heat maps (Figure 4.2). Within Ludhiana, the co-efficient values determined all the traits to have very little correlation except PH and EBT where moderate values of significant correlation were obtained. Correlation analysis between traits at Rauni determined moderate values of correlation co-efficient for fertility with yield and PH but correlation was insignificant. Negative correlation was obtained between PH and DF, EBT and PH but only the later was significant. In general, higher co- efficient of correlation between traits were obtained at Rauni relative to Ludhiana.

Graphical representation for determining difference in mean values of traits between locations is given via box-plots presented in Figure 4.3. The significance of difference between trait values was determined using t-test. t-test results for various traits are presented in Table 4.1. The variation in yield between two locations was found to be statistically significant for all the traits as determined using paired t test. Box-plots were plotted in order to visualize the effect of replications at both locations (Figure 4.4).

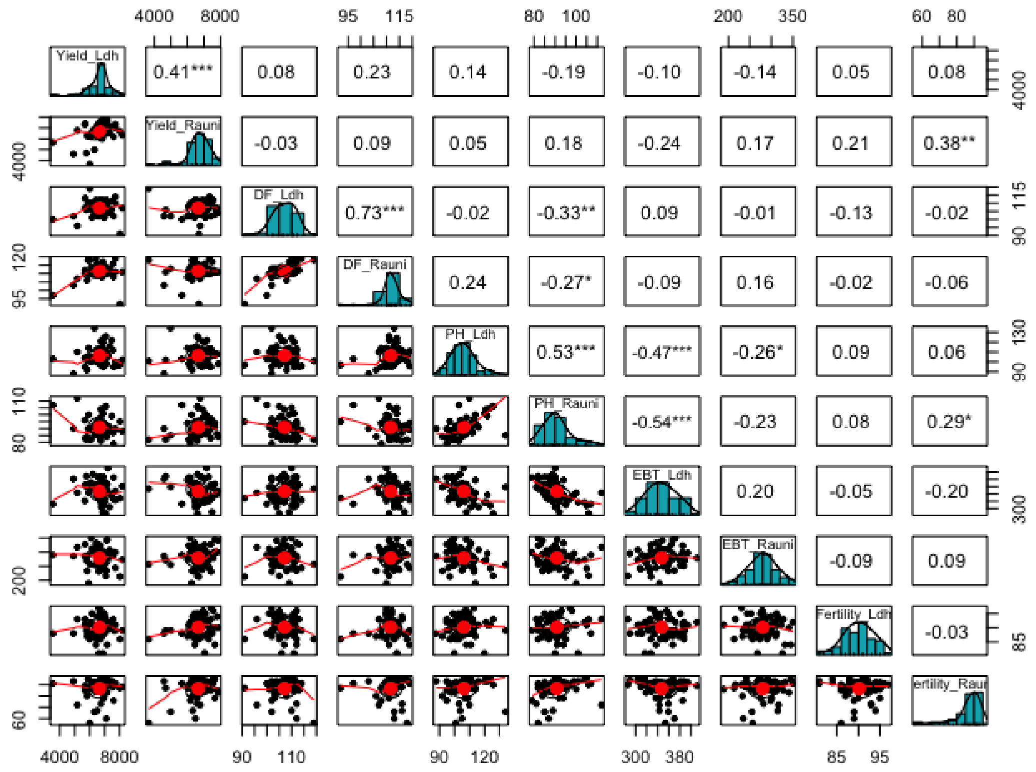


Figure 4.1: Correlogram showing the distribution of phenotypic data for various traits and pairwise correlations between various traits.

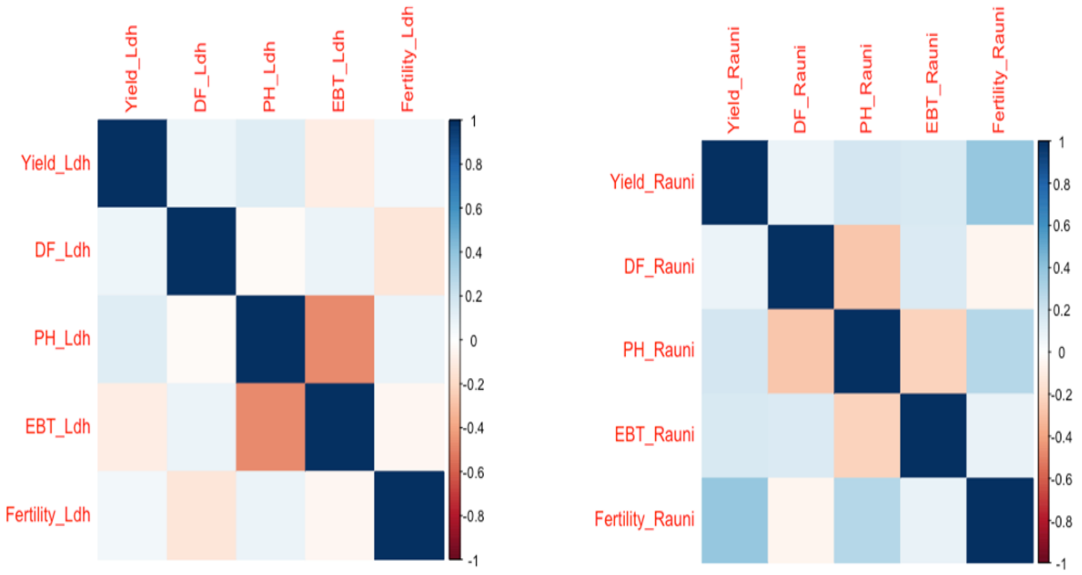


Figure 4.2: Heat map depicting correlation between traits at Ludhiana and Rauni respectively

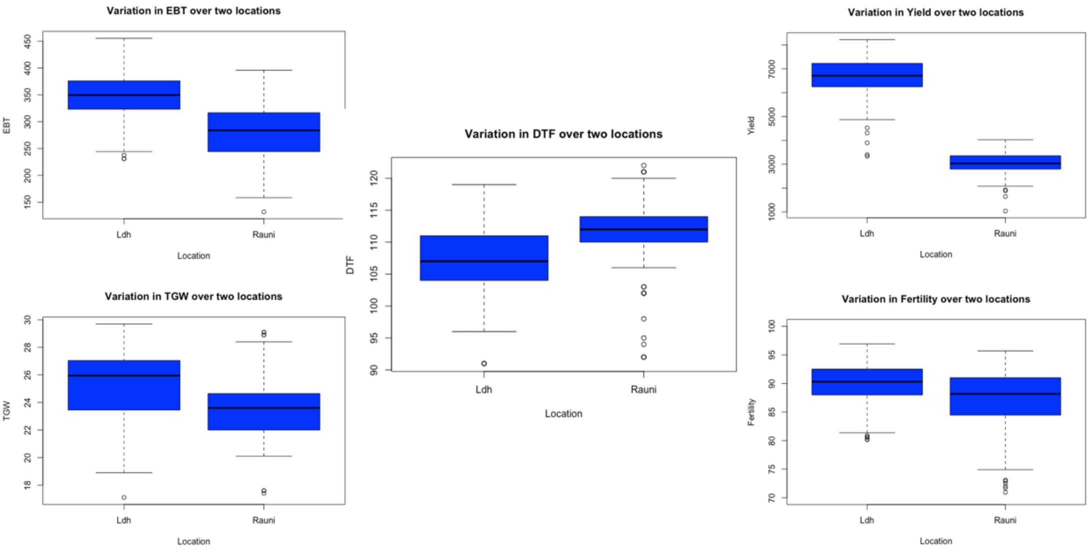


Figure 4.3 :Box-plots comparing trait values over two locations

Table 4.1: Paired t-test determined p-values for various traits at two locations

	Mean_Ldh	**Mean_Rauni**	**p-value**
DTF	107.0781	109.3177	4.314e-07
PH	106.30729	98.60156	2.766e-13
EBT	346.7062	313.1906	7.95e-13
Yield	6650.095	4840.875	< 2.2e-16
Fertility%	90.14071	88.11121	8.851e-05
TGW	25.33542	24.41328	2.644e-05

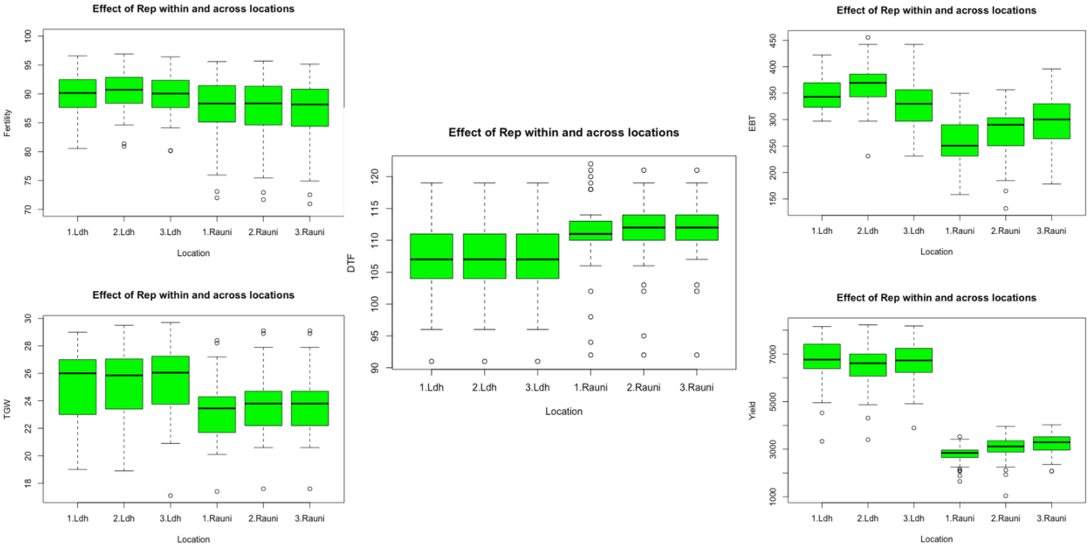


Figure 4.4: Box-plots presenting effect of replications on trait values over two locations where the numbers on y-axis refer to replications.

A set of 10 lines were selected based on their phenotypic performance and were determined to be best performing lines. The details of the lines along with phenotypic data has been given in Table 4.2. Pictorial representation depicting difference between two sets, i.e., Best performing lines and remaining lines, designated as A and B, for various traits have been presented via box plots (Figure 4.5). Chi-Square and p-values obtained from Kruskal-wallis rank sum test have been given in Table 4.3. However, KW test determined the difference between two groups to be statistically significant for yield only, suggesting effective selection to have taken place. Descriptive statistics for the two groups have been given in Table 4.4.

Table 4.2: Agronomic performance of best lines based on average phenotypic data

Genotype	Yield (kg/ha)	DTF	PH (cm)	EBT/m^2	Sterile Fertility (%)	BB	BPH	HRR (%)
9712-3	7325	109	111	316	92	5	3	65.0
9724-1	7328	109	97	319	90	3	1	51
9724-2	7416	109	100	356	90	3	3	6.56
9724-3	7500	108	91	344	87	3	3	58.4
9724-4	7286	107	101	319	84	5	1	63.7
9724-5	7523	117	93	284	90	5	1	58.0
9726-1	7184	113	100	293	91	3	3	62.7
9726-5	7324	114	99	306	91	3	3	66.0
9737-2	7183	110	98	345	90	3	9	62.0
9737-3	7356	110	95	323	91	3	7	57.7
PR 121	6672	110	92	319	86	3	9	63.5
PR 126	7364	92	96	271	87	5	9	62.0

Table 4.3: Kruskal-Wallis rank sum test determined chi-square and p-value for different traits

Trait	Kruskal-Wallis chi-square	p-value
Yield	22.688	1.906e-06*
Days to 50% flowering	0.14043	0.7079
Plant height	0.14013	0.7082
EBT/m^2	0.25742	0.6119
Fertility percentage	0.03992	0.8416

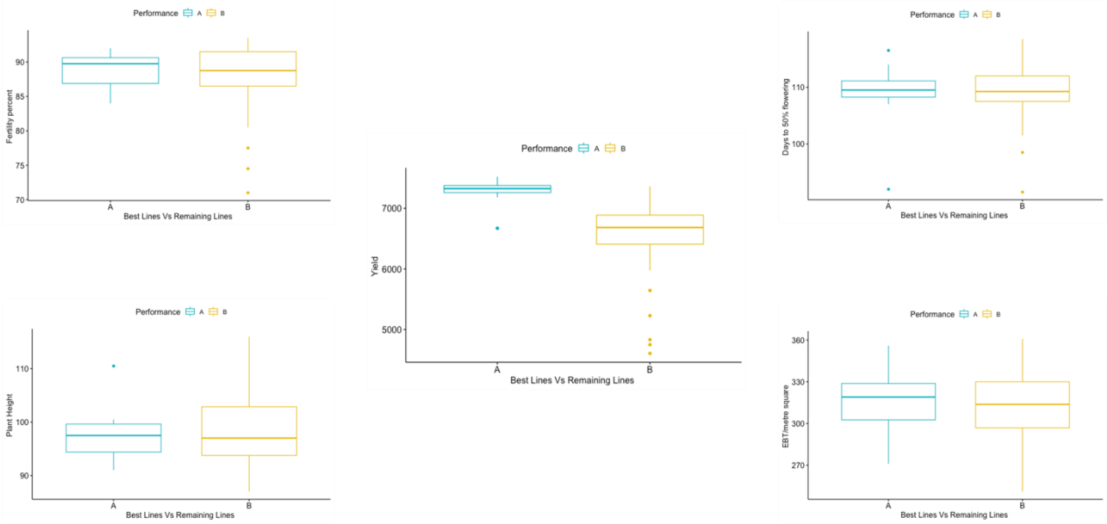


Figure 4.5: Box-plots depicting phenotypic difference between best lines and the remaining set. Here A represents set of 10 lines with better phenotypic performance and B represents remaining lines under study.

Table 4.4: Basic statistic measures determining difference between best performing lines (A) and remaining lines under study (B)

Trait	**Performance**	**Count**	**Mean**	**sd**	**median**	**IQR**
Yield	A	12	7288	220	7326	116
	B	54	6533	586	480	480
DTF	A	12	109	6.01	110	2.88
	B	54	109	4.3	108	4.5
PH	A	12	97.6	5.21	97.5	5.25
	B	54	98.6	6.87	97	9.12
EBT	A	12	316	5.4	319	26.1
	B	54	312	23.2	314	33.1
Fertility%	A	12	89	2.47	89.8	3.75
	B	54	88.1	4.66	88.8	5

The analysis of variance for Randomized block design revealed significant variations for various morpho-physiological traits and milling quality traits among different genotypes, environments and the interaction between genotypes and environments at both the locations (Table 4.5 and 4.6). Similarly, most of the variables showed non-significant replication and block effects except few parameters viz. plant height (cm), ear bearing tillers (EBT) /m^2, grain yield per plot, thousand grain weights (g). The variance in the two environments i.e., Ludhiana and Rauni were tested for homogeneity by F test and as it was homogenous the pooled analysis was done for both the locations. Pooled analysis of variance (Table 4.7) for both locations for the year 2021 determined effect of genotypes and locations to be significant for all the traits. For EBT, yield and TBW, the effect of replications was also found to be significant as evident from p-values < 0.01.

Days to 50% flowering

The days to 50% flowering the number of days to reach maturity play a significant role in the cropping system. Variation in days to flowering in rice shows its adaptation to different agro-climatological zones. The varieties of different maturity duration are cultivated in different areas depending on crop rotation, irrigation facilities and land scenario. The optimum growth duration involves a balance of growth processes throughout the life of the plant. This balance should result in the production of the maximum number of matured panicles, filled spikelets, and mean grain weight.

Significant genotypic variation was observed in the breeding lines for days to 50% flowering at both locations (Tables 4.5, 4.6, 4.7). Weiya *et al* (2008) also observed variation in heading days among several genotypes and identified a regulatory gene responsible for this variation. The mean days to 50% flowering were 91 days at Ludhiana location ranging from 91 to 119 days while at Rauni, the mean days to 50% flowering was 92 days varying from 92 to 119 days. Most of genotypes (43) were having maturity and flowered between 101 to 110 days at Ludhiana and flowered between 111 to 120 days after seeding. The falling in long duration allegory PR-126 (92 days), IR54842 (97 days), RP 2068-295 (102 days) and 9723-5 (102 days) lines flowered early and 9729-1 (111 days) 9737-3 (110days), 9725-2 (112 days) flowered late. In Punjab, medium to short duration varieties are mostly preferred over the long duration varieties as it helps in saving irrigation water and other resources. Moreover, medium and early maturing varieties vacate the field timely for the sowing of wheat crop.

Plant height (cm)

Plant height is the main factor that determines the plant architecture and has a direct effect on yield and lodging. The tall genotypes are not preferred as they are respond to lodging which reduces grain yield (Shahidullah *et al* 2009). In the current breeding programmes, the dwarf and semi-dwarf plant types are the most preferred. Bhadru *et al* (2011) reported that plant height is highly correlated with the level of lodging and ease of harvest. Therefore, it is one of the most important characters in influencing the acceptability of the variety by the farmer.

Table 4.5: ANOVA for different morpho-physiological traits at Ludhiana

TRAITS												
Source	**DF**	**DTF**	**PTH (cm)**	**EBT/m^2**	**Spikelet (%)**	**Yield kg/ha**	**1000gwt**	**Sterility (%)**	**Br(%)**	**Mill(%)**	**Hr(%)**	**LB**
Mean sum of squares												
Genotype	**63**	**68.86****	**215.80****	**2191.93****	**36.02****	**0.33****	**17.89****	**34.18****	**136.46****	**91.88****	**123.70****	**0.25****
Replication	**2**	**5.77**	**7.91**	**26283.97**	**9.01**	**0.131**	**3.89**	**7.58**	**0.02**	**1.91**	**1.03**	**0.01**

** = significant different at 5%, *** = significant different at 1%, ns= non-significant, DF= degree of freedom, DF= Days to fifty percent flowering, PH = Plant height, EBT/ m^2 = Ear bearing tiller/ m^2, GY= Grain yield per plot and TGW= Thousand grain weight BRR = Brown rice Recovery, MRR= Milled rice Recovery, HRR= Head rice recovery

Table 4.6: ANOVA for yield contributing traits at Rauni

TRAITS								
Source	**DF**	**DF days**	**PTH (cm)**	**EBT/m^2**	**Spf (%)**	**Ste (%)**	**1000gwt**	**Yield kg/ha**
	Mean sum of squares							
Genotype	**63**	**54.900050****	**132.411376****	**3227.8190****	**209.67590****	**9.0582011****	**13.2710979****	**0.11320711****
Replication	**2**	**9.411458**	**135.630208**	**30918.7519**	**6.27949**	**13.0781250**	**1.8984896**	**0.00928086**

** = significant different at 5%, *** = significant different at 1%, ns= non-significant, DF= degree of freedom, DF= Days to fifty percent flowering, PH = Plant height, EBT/ m^2 = Ear bearing tiller/ m^2, GY= Grain yield per plot and TGW= Thousand grain weight BRR = Brown rice Recovery, MRR= Milled rice Recovery, HRR= Head rice recovery

Table 4.7: Combined Anova for various morpho- physiological yields attribution trait over two locations

PH		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	16689	265	5.355	<2e-16***
	Rep	2	157	78	1.583	0.207
	Location	1	22801	22801	460.884	<2e-16***
	Residuals	317	15683	49		
DTF		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	6504	103.2	20.82	<2e-16***
	Rep	2	9	4.7	0.949	0.388
	Location	1	1926	1926	388.423	<2e-16***
	Residuals	317	1572	5		
EBT		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	204666	3249	1.863	0.000285***
	Rep	2	21351	10675	6.122	0.002463**
	Location	1	431346	431346	247.36	<2e-16***
	Residuals	317	552784	1744		
Yield		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	7.89E+07	1.25E+06	4.223	<2e-16***
	Rep	2	2.62E+06	1.31E+06	4.412	0.0129*
	Location	1	1.26E+09	1.26E+09	4240.696	<2e-16***
	Residuals	317	9.40E+07	2.96E+05		
TGW		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	1537.4	24.4	16.867	<2e-16***
	Rep	2	10.1	5	3.478	0.0321*
	Location	1	326.5	326.5	225.692	<2e-16***
	Residuals	317	458.6	1.4		
Fertility %		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	7617	120.9	4.846	<2e-16***
	Rep	2	14	7	0.279	0.756
	Location	1	1582	1581.6	63.392	3.05e-14***
	Residuals	317	7909	25		

In the present study, plant height showed significant variation among different genotypes at both the locations i.e., Ludhiana and Rauni, Patiala (Table 4.5, 4.6 and 4.7). According to IRRI (2002), plant height is divided into three categories, namely short (<110 cm), medium (110-130 cm), and tall (>130 cm). Most of the genotypes in this study were present in medium category. The genotypes had a mean plant height of 102 cm which ranged from 88 cm to 102 cm along with coefficient of variation of 7.94%.RP 2068 (88 cm) and 9738-1 (79 cm) had the minimum plant height at both Ludhiana and Rauni respectively. However, PR114 was the tallest genotype with the maximum plant height of 102 cm at Ludhiana and IR71033 (112 cm) at Rauni. Similarly, RP2068 (88cm) and PR 114(102 cm) were the shortest and tallest check varieties, respectively. Most of the genotypes were semi dwarf and did not lodge. According to Khush (1984), the dwarf and semi-dwarf cultivars normally have high yield, are resistant to lodging and show a positive response to fertilizer application along with good water use efficiency, contrary to very short plants that have poor yield due to low biomass production.

Ear bearing tillers (EBT)/m^2

In rice, the number and size of panicles is closely related to number of tillers. Tillering ability is one of the most important traits of rice, since it can have a significant influence on the future production of panicles (Miller *et al* 1991), which in turn is highly correlated with grain yield. Moderate tillering greatly contributes to rice yield but excessive tillering leads to poor grain setting and smaller panicle size. Most of the genotypes showed moderate tillering ability (6 to 8tillers/plant). The number of tillers also has been reported to have a positive association with plant biomass and economic yield in rice (Deng *et al* 2015). The genotypes had mean ear bearing tillers of 411 EBT/ m^2 that is about 10-11 panicles per plant. However, the genotype PAU minimum and maximum range of ear bearing tiller were 286/m^2 and 411/m^2 respectively. 9712-1 had minimum tillering with 286 EBT /m^2 which is equal to 7 panicles per plant at Ludhiana. Similarly, Kaum-182-1 had the lowest tillering (189 EBT /m^2) at Rauni. The miximum tillering was recorded for PAU 9737- 2 (411 EBT /m^2) at Ludhiana location and 9730-3 (349EBT /m^2) in Rauni. The optimum tillering is very important to develop primary and secondary tillers having healthy panicles (Pawar *et al* 2016) as it plays a major role in determining grain yield. According to new plant type concept 6-10 tillers per plant give higher yield than the profusely tillering varieties with 20-25 tillers per plant.

In the present study, the genotypes showed significant differences for ear bearing tillers at both locations (Table 4.5, 4.6 and 4.7). Peng *et al* (2008) also reported that the number of panicles per plant, number of spikelets per panicle, weight of spikelet and spikelet fertility and/or sterility are strongly linked to the yield performance of rice and hence determine the potential grain yield.

Grain yield (kg/ha)

Grain yield (kg/ha) is the major criterion which determines the future of varieties and is a primary concern for farmers. In the present study, the grain yield showed a strong and significant variation between the genotypes as evident from their mean performance given in Table 4.5. Results from single location anova and multi-location anova also reversed the variation among genotypes to be highly significant (Tables 4.5, 4.6 and 4.7).

The average grain yield of the test genotypes ranged from 3543 to 8175 kg/ha. Genotypes 9731-5 (8175 kg/ha) had the highest yield at Ludhiana and 9724-3 (7857 kg/ha) was the highest yielding genotype at Rauni with yields better than or comparable to check varieties PR 121, PR 122. However, IR 54842 and 9726-4 yielded low i.e. 3543 kg/ha and 3667 kg/ha in Ludhiana and Rauni respectively. Genotypes 9712-3, 9724-2, 9724-4, 9724-5, 9726-1, 9726-5, 9724-1 and 9724-3 at PAU performed well at both the locations. The variation in grain yield across the locations might be due to environmental effects or due to variation in different yield contributing traits like number of spikelets per panicle, grain weight, spikelet fertility etc. Zhao *et al* 2020 reported that the differences in grain yield across environments were mostly due to the combination effect of various agronomic traits that were greatly affected by the different climate conditions. The grain yield was significantly and positively correlated with plant height, tiller number , filled grain number per panicle, filled grain percentage as well as grain weight.

Spikelet Fertility (%)

The economic grain yield of rice is determined by several factors, including the number of productive tillers, number of potential grains per panicle and spikelet fertility (Sheehy *et al* 2001). The harvested spikelets can either be fertilized or sterile (empty). Similarly fertilized spikelets may either be adequately or inadequately filled. Adequately filled grains in fertilized spikelets are the main contributor to higher yield in rice cultivars (Mohapatra *et al* 2011).

In the present study, pooled anova as well as single location anova for each location indicated significant genotypic variation for spikelet fertility at Ludhiana (Table 4.5). Anis *et al* (2016) reported significant differences in 100 rice genotypes for number of spikelets per panicle and spikelet fertility. The mean spikelet fertility was 86.6-90% at both locations. It ranged from 81% to 97% along with 0.26 coefficient of variation at Ludhiana and 56% to 96 % along with 0.47 coefficient of variation at Rauni. Genotypes 9726-2 (81%) and 9726-4 (56%) had low spikelet fertility % and 9722-4 (97%) and 9712-4 (90%) had highest spikelet fertility at both locations. The genotype 9726-4 (56 fertile seeds per panicle) had least number of fertility percent of while 9722-4 (97% fertile seeds per panicle) had the maximum number of spikelets per panicles. Amegan *et al* (2020) also reported high phenotypic variation in a set of 130 rice accessions from South Korea and Africa for spikelet number and fertility. The genotypes having more than 85% spikelet fertility are desirable.

Thousand grain weight (g)

A higher thousand grain weight and high spikelet fertility not only improves the grain yield but also the head rice ratio (Xu *et al* 2015). In recent decade, the improvement of rice productivity by selection for superior yield component and ideal plant architecture have become an important focus of rice breeding research (Peng *et al* 2008). Hongthong *et al* (2012) compared grain yield between two super hybrid cultivars differing in grain weight and suggested that developing rice cultivars with high grain weight is a possible approach to achieve high grain yield. Grain weight is the product of the duration and rate of grain filling. Jones *et al* (1979) determined the correlation between grain weight with the duration and rate of grain filling in fifteen rice cultivars and reported that grain weight was positively and significantly related to grain-filling rate.

In this study, significant genotypic variation was present for thousand grain weight both at Ludhiana, Rauni as well as in combined analysis. The mean thousand grain weight of the genotypes, PR-126 (20.12 g) and 9726- 4 (26.40), was found to be lowest at Ludhiana and Rauni, respectively. However, 9731-2 (29.50 g) and 9712-5 (28.40 g) recorded the highest thousand grain weight both at Ludhiana and Rauni respectively. The average TGW of check varieties was 23.90 g. Conley and John (2013) reported test weight as an important factor when selecting a variety as both environment and pests may greatly affect test weigh. Thus, selecting a variety that has a high-test weight potential in any area is critical to maximizing economic gain.

Head Rice Recovery (%)

The mean HRR ranged from 36.52% to 67.54% . The genotypes with minimum HHR were IR 54842 (36.53 %) at Ludhiana. However, 9714-4 (67.54%) had maximum HRR at Ludhiana and was comparable to best check, PR122. Parallel to enhancing paddy yield, improving the milling quality of rice is essential in ensuring food security by mitigating the impact of significant losses during the postharvest processing of rice grains. From an industrial standpoint, maximizing the milling recovery of whole grain polished rice is crucial in fetching higher revenues to rice farmers. L/B ratio is an important determinant of the quality of rice grains. Based on the L/B ratio, grains were classified into long slender (LS), short slender (SS) and medium selender (MS) (Ramaiah, 1985). In this study, significant genotypic variation was present for L/B ratio at Ludhiana. The mean L/B of the genotypes ranged from Kaun 182-1 (2.82) and PR 114 (4.41) at Ludhiana.

Experiment II: Screening and molecular characterization of a set of rice genotypes for known BPH and BB resistance genes

Screening of advanced breeding lines for BB resistance.

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious production constraints of rice worldwide. Deployment of resistant cultivars is the most economical and effective method to control the disease due to the non-availability of effective bactericidal agents. Ten pathotypes have been identified in Punjab on the basis of near isogenic lines, standard international differentials and local rice cultivars (Singh *et al* 2012). Numerous resistance genes have been identified and used for disease management so far (Dinh *et al* 2010). The molecular markers usage in plant breeding has earned much attention in the recent past years. The microsatellite or simple sequence repeat (SSR) marker loci are widely distributed in the rice genome and can be easily analyzed using the polymerase chain reaction (McCouch *et al* 2002). SSR markers are useful for wide application range in plants due to their reproducibility, multiallelic nature, co-dominance nature of inheritance and wide genomic coverage (Powell *et al* 1996). Keeping these observations in mind, the present research was carried out to characterize the advance breeding lines for BB resistance and to look for presence of any known BB resistance gene(s).

Considering BB disease as a threat to rice production, sixty-four advance breeding lines along with checks were screened during Kharif 2021 against all the seven prevalent pathotypes of BB disease present in the Punjab state using clip inoculation method. The results indicated that most of the genotypes (32) showed resistance to the BB pathotype (Table 4.8). The resistance in these lines was either due to known genes or combination of known genes or as yet undescribed gene(s). Twenty-six lines showed moderate resistance reaction against BB pathogen. The genotypes IR 71033, IR 54842 and RP 2068-295 showed susceptible reaction against BB resistant. The genotypes PR 114, Kaun 182-1 and RP 2068-303 were highly susceptible. Table 4.8 shows the genotypes and their disease reaction towards BB pathogen seven along with the checks.

The advanced breeding lines were further subjected to molecular analysis to identify the presence or absence of resistance genes using gene-linked or gene-specific markers for BB resistance genes viz. *Xa4*, *xa13* and *Xa21*. PR 121 was used as positive control during PCR analysis as it has been reported to carry *Xa4*, *xa5* and *xa13* (Bharaj *et al* 2014). The resistance gene *Xa4* was first characterized on long arm of rice chromosome 11 in the rice variety TKM 6 by Wang *et al* (2001). Amplification of sequence tagged site (STS) marker MP4 which is linked to this BB resistance gene revealed the presence of a 150bp fragment specific for *Xa4* mediated BB resistance in the positive control PR 121 and 120bp fragment corresponding to absence of this gene. This can be easily resolved on 4% agarose gel using 50bp DNA ladder. Based on the banding pattern of advanced breeding lines, BB resistance gene *Xa4*, was found to be present in ten lines (Table 4.8). Khush *et al* (1989) reported that the exploitation of gene *Xa4* resulted in development of many BB resistant rice cultivars that played significant role in protecting rice from Xoo. It has been reported by many workers that the pyramided lines with *Xa4* and other bacterial blight resistance genes showed a wider spectrum and a higher level of resistance than the lines with single resistance gene (Huang *et al* 1997; Arif *et al* 2008).

xa13 gene was first discovered in the rice variety BJ1 (Sanchez *et al* 1999) and was fine mapped to a region less than 4 cM on the long arm of chromosome 8 between two RFLP markers, RG136 and R2027. Similarly, the amplified product with primer *xa13* promoter from resistant line PR121 was of 500bp, while that from susceptible lines was about 250bp which could be easily resolved on 2.5% agarose gel by using standard 100bp DNA ladder to confirm proper size of amplified product. Based on the banding pattern of germplasm lines, it was observed that in case of *xa13* BB resistance gene, 48 lines showed presence of this gene. Plate 5 shows the agarose gel picture of *xa13* marker on 64 genotypes of rice.

The gene *Xa21* was introgressed from *Oryza longistaminata*, a wild relative of the cultivated species *Oryza sativa* (Khush and Angeles 1999). Genetic and physical analysis of *Xa21* localized it in an 8.3 cM interval on chromosome 11 (Ronald *et al* 1992). Screening of dominant *Xa21* resistance gene by the amplification of pTA248 marker revealed 1000bp band as presence of *Xa21* allele and 750bp band as absence of the allele. Based on the banding pattern of germplasm lines, it was observed, 20 lines showed presence of this gene. Ramalingam *et al* (2001) performed similar type of molecular survey for the presence of bacterial blight resistance genes *xa13* and *Xa21* in Chinese rice germplasm. It was also observed that out of the 64 advance lines tested, some of the lines showed presence of two gene combination, respectively. Thirty lines showed presence of single gene while one line showed none of the known gene tested. Among the three gene combination, three lines showed presence of *Xa4+xa13+Xa21*. None of the lines showed all the four gene tested in combination. On the other hand, in case of two gene combination, 17 lines showed presence of *xa13+Xa21* also included lines showed presence of *Xa4+xa13.* Similar work has been reported by Mangat *et al* (2012) and Khanna *et al* (2014) in which they identified *Xa4*, *xa13* and *Xa21* genes through molecular analysis in elite non-basmati rice breeding lines and showed effectiveness of multiple BB resistance genes in rice varieties. Goel *et al* (1998) reported that none of the known genes were effective fully against all the pathotypes of BB pathogen from northern India. So, the resistance in these advance breeding lines may be attributed to presence of additional known or novel genes and/or modifiers. The narrow genetic base of cultivated rice will cause vulnerability to BB because of an increased frequency of newly evolved pathotypes of greater virulence. As a result, increasing attention has focused on the accumulation of major disease resistance genes in crop plants. Pyramided lines carrying more resistance genes showed broad spectrum and higher resistance than the lines with a single resistance gene (Suh *et al* 2009; 2013).

Several BB resistance genes have been identified and characterized in non- aromatic rice and incorporated and pyramided through MAS to develop resistant cultivars

(Perumalsamy *et al* 2009; Rajpurohit *et al* 2011). Shehzad *et al* (2012) screened different commercial rice varieties in order to exploit their resistant potential against bacterial leaf blight and identified resistance sources for breeding programs. Singh *et al* (2014) evaluated a set of uncharacterized aromatic germplasm lines along with four cultivated varieties for resistance against two pathotypes of BB pathogen. Raj Purohit *et al* (2011) reported that pyramiding of more than one major resistance gene has proven to deliver durable resistance against BB disease. In the present study all 64 genotypes showed resistance to more than half of the pathotypes present in Punjab. Therefore, gene pyramiding is the corner stone for controlling all the pathotypes of BB disease present in Punjab. The pyramided lines showed a wider spectrum and higher level of resistance than lines with only a gene because of the pathogens ability to rapidly overcome major genes (Mew 1987). One of the most challenging and important areas of research will be to develop a sound strategy for deployment of resistance gene carrying varieties in order to maximize the durability of resistance in a particular location. The information gained in this study has a significant importance in identifying potential donors for BB resistance to be used in rice hybridization programme.

Table 4.8: List of 64 genotypes showing the presence and absence of *Xa4, xa13* and *Xa21* genes individually or in combination.

Sr no	**Genotype**	***Xa*4**	***xa*13**	***Xa*21**	**Remarks**
1	9711-1		+	-	*xa*13
2	9711-2		+	+	*xa*13+*Xa*21
3	9711-3		+	+	*xa*13+*Xa*21
4	9711-4		+	+	*xa*13+*Xa*21
5	9711-5		+	-	*xa*13
6	9712-1		+	+	*xa*13(H)+*Xa*21
7	9712-2		+	NA	*xa*13
8	9712-3		+	+	*xa*13+*Xa*21
9	9712-4		+	-	*xa*13
10	9712-5		+	-	*xa*13
11	9722-1		+	-	*xa*13
12	9722-2		+	-	*xa*13
13	9722- 3		+	-	*xa*13
14	9722-4		+	-	*xa*13
15	9723-1		+	-	*xa*13
16	9723-2		+	-	*xa*13
17	9723-3		NA	-	-

18	9723-4		+	-	*xa*13
19	9723-5		+	-	*xa*13
20	9724-1		+	-	*xa*13
21	9724-2		+	-	*xa*13
22	9724-3		+	-	*xa*13
23	9724-4		+	-	*xa*13
24	9724-5		+	-	*xa*13
25	9725-1		NA	-	-
26	9725-2		+	-	*xa*13
27	9725-3		+	-	*xa*13
28	9725-4		+	-	*xa*13
29	9725-5		+	+	*xa*13+*Xa*21
30	9726-1		+	-	*xa*13
31	9726-2		+	-	*xa*13
32	9726-3		+	-	*xa*13
33	9726-4		+	+	*xa*13+*Xa*21
34	9726-5		+	+	*xa*13+*Xa*21
35	9729-1		+	-	*xa*13
36	9729-2		+	-	*xa*13
37	9729-3		+	-	*xa*13
38	9729-4		+	-	*xa*13
39	9729-5		+	-	*xa*13
40	9730-1		+	-	*xa*13
41	9730-2		NA	+	*Xa*21
42	9730-3		NA	+	*Xa*21
43	9731-1		+	-	*xa*13
44	9731-2		+	+	*xa*13+*Xa*21
45	9731-3		NA	+	*Xa*21
46	9731-4		+	+	*xa*13+*Xa*21
47	9731-5		+	+	*xa*13+*Xa*21
48	9737-1		NA	+	*Xa*21
49	9737-2		NA	+	*Xa*21
50	9737-3		NA	+	*Xa*21
51	9737-4	-	+	+	*xa*13+*Xa*21

52	9737-5	+	+	-	*Xa*4+*xa*13
53	9738-1	+	+	+	*Xa*4+*xa*13+*Xa*21
54	9738-2	NA	+	+	*xa*13+*Xa*21
55	9738-3	+	+	-	*Xa*4+*xa*13
56	PR 114	NA	-	-	-
57	PR 121	+	+	+	*Xa*4+*xa*13+*Xa*21
58	PR 122	+	+	+	*Xa*4+*xa*13+*Xa*21
59	PR 126	+	-	-	*Xa*4
60	IR 71033	+	-	-	*Xa*4
61	IR 54842	+	-	+	*Xa*4+*Xa*21
62	Kaum 182-1	+	-	-	*Xa*4
63	RP 2068-295	NA	NA	-	-
64	RP 2068-303	+	-	-	*Xa*4

Screening advance breeding lines for BPH resistance

Screening of 64 genotypes that is the advanced breeding lines for the Brown plant hopper (BPH) using the molecular marker RM279 (SSR marker, Hu *et al* 2018) revealed the presence of *Bph33* gene in 24 lines along with the check RP 2068-295 which is the donor of *Bph33*. *Bph33* was mapped on chromosome 4 using the two resistant Sri Lankan rice cultivars (KOLAYAL and POLIYAL) using F2:3 populations and near-isogenic lines (NILs). *Bph33* was fine mapped to a 60-kb region ranging 0.91-0.97 Mb on the short arm of chromosome 4. The gene exhibited continuous and stable resistance from seedling stage to tillering stage, showing both antixenosis and antibiosis effects on BPH (Naik *et al* 2018). Plate 6 shows the agarose gel picture illustrating the presence and absence of *Bph33* gene in some of the rice genotypes.

In addition to this, the material was also screened for the presence of *Bph34* using SSR markers RM16994 and RM17104 and none of the lines showed the presence of the resistance gene *Bph34.* BPH resistant locus Bph34 had been identified from the wild source of rice *O.nivara* on chromosome 4 of rice using 50K SNP chip (Kumar *et al* 2019). In addition to SNP markers two SSR markers RM16994 and RM17007 also co-segregated with the BPH resistance which are being implemented in rice breeding programmes using marker assisted selection. Plate 7 shows the agarose gel picture of few rice genotypes screened for the presence of Bph*34* allele. The lines were showing presence of band corresponding to the PR122 recipient parent and not the band of *Oryza nivara* acc. IRGC104646, the donor of *Bph34*. It was quite interesting to observe that some of the rice genotypes did not contain either *Bph33* and *Bph34* but showing resistance against BPH when screened phenotypically.

These would mean that these lines may carry novel uncharacterized genes or carry other *Bph* resistance genes which can be explored further and hence can be utilised in rice breeding programmes. Table 4.12 lists all the 64 genotypes of rice showing the presence or absence of *Bph34* and *Bph33* along with phenotypic score of disease reaction against BPH.

Conclusion

From the present study, it is concluded that there are some lines that perform best agronomically at both the locations and also carried genes for bacterial blight and brown plant hopper. Some of these lines carried single gene for BB/BPH while some other carried multiple genes for BB. These advanced breeding lines or genotypes characterized for BB and BPH would serve as great source for germplasm enhancement. The best performing lines with single genes for BB and BPH resistance can be crossed among each other to produce lines with pyramided genes that will provide durable resistance against these devastating pathogens. These lines would serve as an important germplasm resource as potential lines identified can be employed in varietal improvement programmes.

Table 4.9- Disease reaction recorded on 64 genotypes against Bacterial Blight

Disease score (Reaction	**Number of advanced lines**
1.0(HR)	**0**
1.1 -3(R)	**32**
3.1-5(MR)	**26**
5.1-7(S)	**3**
7.1-9(HS)	**3**
Total	64

Table 4.10 - Disease reaction recorded on 64 genotypes against Brown Plant hopper

Disease score (Reaction)	**Number of advance lines**
1.0(HR)	**0**
1.1 -3(R)	27
3.1-5(MR)	**26**
5.1-7(S)	**3**
7.1-9(HS)	8
Total	64

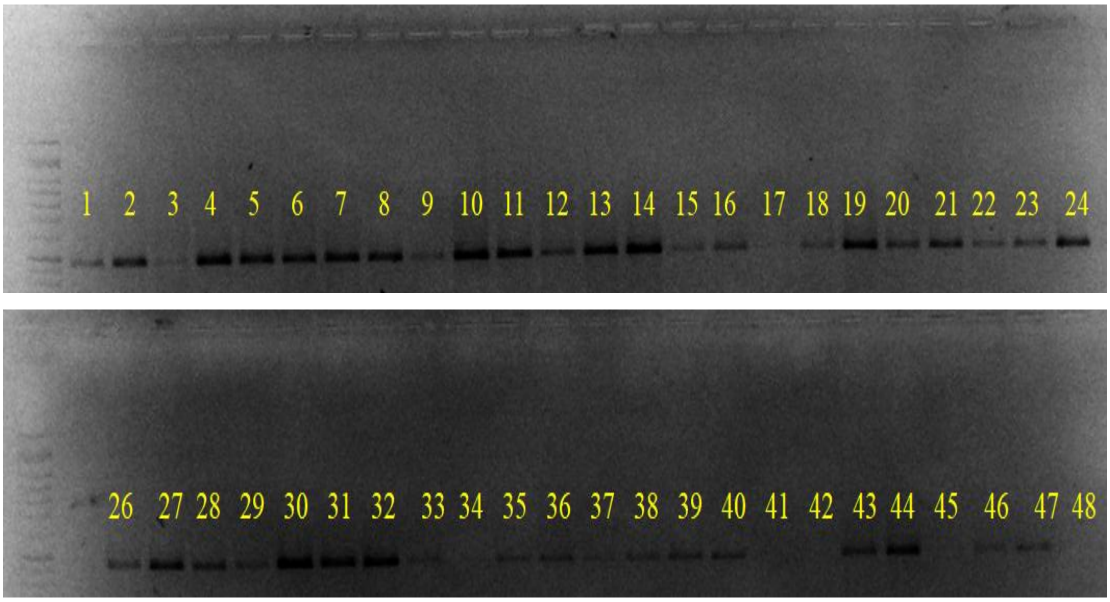


Plate 5: **Agarose Gel picture showing the presence of *Xa13* gene in some of the advanced breeding lines of rice.**

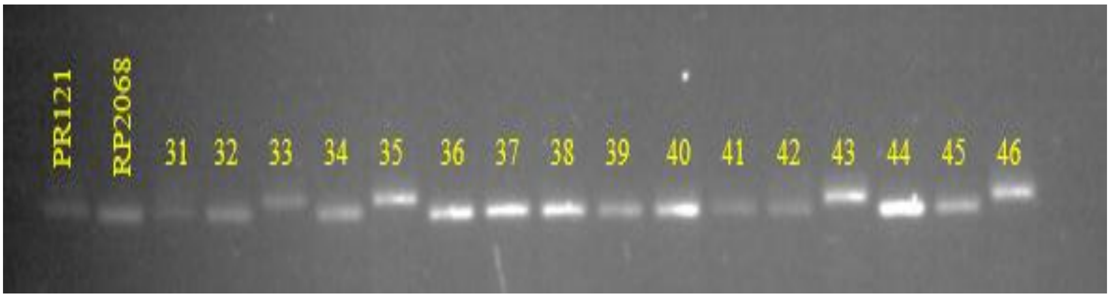


Plate 6: **Agarose Gel picture showing the presence of *Bph33* gene in some of the advanced breeding lines of rice.**

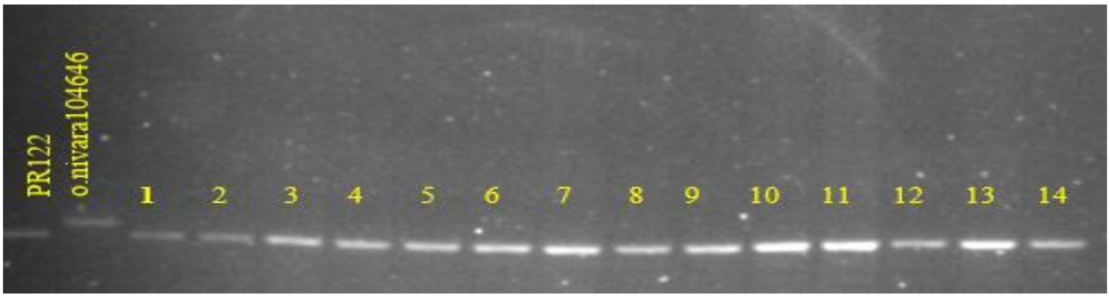


Plate 7: **Agarose Gel (2.5%) picture showing the absence of *Bph34* gene using SSR marker RM16994 in some of the genotypes. The genotypes (1-14) carried the band corresponding to PR122 (one of the recipient parents).**

Table 4.11- Summary statistics of Molecular characterization of BB resistant genes on 64 genotypes

Gene	No. of genotypes
*Xa*4	4
*xa*13	31
*Xa*21	6
*Xa*4+*xa*13	2
*Xa*4+*Xa*21	1
*xa*13+*Xa*21	14
*Xa*4+*xa*13+*Xa*21	3
Unknown genes or putative new genes	3

Table 4.12- List of Genotypes showing presence and absence of *Bph33* and *Bph34* disease resistance genes along with the phenotypic score of disease reaction observed against BPH

No.	Genotype	BPH Disease reaction	BPH 33	BPH 34
1	9711-1	1		-
2	9711-2	1		-
3	9711-3	3		-
4	9711-4	1		-
5	9712-1	3		-
6	9712-2	1		-
7	9712-3	3		-
8	9712-4	3		-
9	9712-5	7		-
10	9722-1	1		-
11	9722-2	3		-
12	9722-3	1		-
13	9722-4	1		-

14	9722-1	1		-
15	9723-1	3	+	-
16	9723-2	3	+	-
17	9723-3	3	-	-
18	9723-4	3	+	-
19	9723-5	1	-	-
20	9724-1	1	+	-
21	9724-2	3	+	-
22	9724-3	3	+	-
23	9724-4	1	+	-
24	9724-5	1	+	-
25	9725-1	3	+	-
26	9725-2	3	+	-
27	9725-3	3	-	-
28	9725-4	3	+	-
29	9725-5	3	+	-
30	9726-1	3	-	-
31	9726-2	5	-	-
32	9726-3	7		-
33	9726-4	5	+	-
34	9726-5	3	+	-
35	9729-1	1	+	-
36	9729-2	5	-	-
37	9729-3	3	-	-
38	9729-4	3	+	-
39	9729-5	3	+	-
40	9730-1	3	+	-
41	9730-2	1	+	-
42	9730-3	3	+	-
43	9731-1	3	-	-

44	**9731-2**	**5**	+	**-**
45	**9731-3**	**3**	+	**-**
46	**9731-4**	**5**	+	**-**
47	**9731-5**	**3**	+	**-**
48	**9737-1**	**5**	**-**	**-**
49	**9737-2**	**9**	**-**	**-**
50	**9737-3**	**7**	**-**	**-**
51	**9737-4**	**5**	**-**	**-**
52	**9737-5**	**9**	**-**	**-**
53	**9738-1**	**5**	**-**	**-**
54	**9738-2**	**5**	**-**	**-**
55	**9738-3**	**3**	**-**	**-**
56	**PR114**	**9**	**-**	**-**
57	**PR121**	**9**	**-**	**-**
58	**PR122**	**9**	**-**	**-**
59	**PR126**	**9**		**-**
60	**IR 71033**	**7**		**-**
61	**IR 54842**	**7**		**-**
62	**Kaum 182-1**	**5**		**-**
63	**RP 2068-295**	**5**	+	**-**
64	**RP 2068-303**	**5**		**-**

Table 4.13: Combined Anova over two locations for the year 2021 has been given in Table 4.7

PH		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	16689	265	5.355	<2e-16***
	Rep	2	157	78	1.583	0.207
	Location	1	22801	22801	460.884	<2e-16***
	Residuals	317	15683	49		
DTF		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	6504	103.2	20.82	<2e-16***
	Rep	2	9	4.7	0.949	0.388
	Location	1	1926	1926	388.423	<2e-16***
	Residuals	317	1572	5		
EBT		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	204666	3249	1.863	0.000285***
	Rep	2	21351	10675	6.122	0.002463**
	Location	1	431346	431346	247.36	<2e-16***
	Residuals	317	552784	1744		
Yield		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	7.89E+07	1.25E+06	4.223	<2e-16***
	Rep	2	2.62E+06	1.31E+06	4.412	0.0129*
	Location	1	1.26E+09	1.26E+09	4240.696	<2e-16***
	Residuals	317	9.40E+07	2.96E+05		
TGW		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	1537.4	24.4	16.867	<2e-16***
	Rep	2	10.1	5	3.478	0.0321*
	Location	1	326.5	326.5	225.692	<2e-16***
	Residuals	317	458.6	1.4		
Fertility %		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
	Genotype	63	7617	120.9	4.846	<2e-16***
	Rep	2	14	7	0.279	0.756
	Location	1	1582	1581.6	63.392	3.05e-14***
	Residuals	317	7909	25		

Table 4.14: Best Performing lines at Rauni

No.	Genotype	DTF	PH (+)	EBT	NSP	Sterile(%)	1000w.t
22	9724-3	111 (-)	84 (+)	341	83	17	21.80
20	9724-1	111 (-)	86	299	85	15	22.30
21	9724-2	111 (-)	91	345	95	5	23.20
23	9724-4	111 (-)	94	301	78	22	21.60
17	9723-3	112 (-)	93	279	91	9	22.60
30	9726-1	117 (-)	92	295	94	7	24.50
14	9722-1-PR 121-4	110	97	231	90	10	26.50
26	9725-2	112	83	328	85	15	20.50
24	9724-5	119	89	244	91	10	22.60
27	9725-3	110	96	286	89	11	23.90

Table 4.15: Best Performing lines at Ludhiana

No.	Genotype	Yield (kg/ha)	DTF	EBT/m^2	NSP (%)	StE (%)	1000.wt	BB	BPH	HRR (%)	LB ratio
47	9731-5	8175	107	361	89	11	27.00	5	3	54.15	3.83
59	PR 126	8030	91	328	86	14	18.90	5	9	62.04	4
24	9724-5	7713	114	323	89	11	25.10	5	1	58.00	4.04
50	9737-3	7713	110	348	91	9	20.50	3	7	57.69	3.32
53	9738-1	7597	102	385	89	11	24.00	3	5	63.59	3.96
34	9726-5	7569	112	326	93	7	26.30	3	3	66.50	3.85
8	9712-3	7475	106	337	91	9	28.10	5	3	65.10	3.63
12	9722-1-PR 121-2	7316	104	304	91	9	26.40	5	1	63.56	3.69
49	9737-2	7208	110	411	92	8	21.60	3	9	56.24	3.49
21	9724-2	7165	107	367	84	16	25.30	3	3	65.62	3.83

Table 4.16: Mean Performance of 64 Genotypes

Sr No.	Genotype	Yield (kg/ha)		Days to 50% flowering		Plant height (cm)		EBT/m^2		Fertility (%)	
		Ldh	Rauni	Ldh	Rauni	Ldh	Rauni	Ldh	Rauni	Ldh	Rauni
1	9711-1	6890	7095	105	113	107	92	319	264	91	89
2	9711-2	6999	6444	103	113	107	89	319	264	92	83
3	9711-3	6746	6937	105	110	108	98	334	279	96	90
4	9711-4	6955	7095	101	110	110	92	337	282	95	91
5	9711-5	6797	6508	112	114	113	95	310	240	90	88
6	9712-1	7078	6603	105	111	126	106	286	216	93	90
7	9712-2	6732	6905	107	113	121	104	317	273	92	95
8	9712-3	7475	7175	106	111	122	99	337	295	91	93
9	9712-4	6941	6635	110	114	124	105	308	242	91	96
10	9712-5	6876	7063	111	114	120	101	317	268	81	93
11	9722-1	6926	6587	103	111	109	92	345	275	95	78
12	9722-2	7316	6540	104	113	116	91	304	308	91	93
13	9722-3	6147	6444	101	110	103	91	359	312	95	86
14	9722-4	6602	7429	102	110	113	97	326	231	97	90
15	9723-1	6811	7063	103	111	111	91	350	249	91	87
16	9723-2	6760	6476	111	115	111	90	304	273	88	95
17	9723-3	6667	7556	112	112	107	93	356	279	96	91
18	9723-4	6869	6889	112	115	113	92	326	238	90	91

Sr No.	Genotype	Yield (kg/ha)		Days to 50% flowering		Plant height (cm)		EBT/m^2		Fertility (%)	
		Ldh	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**
19	9723-5	6833	6000	102	108	114	94	378	275	87	87
20	9724-1	6861	7794	107	111	108	86	339	299	94	85
21	9724-2	7165	7667	107	111	108	91	367	345	84	95
22	9724-3	7143	7857	104	111	98	84	348	341	90	83
23	9724-4	6905	7667	103	111	107	94	337	301	90	78
24	9724-5	7713	7333	114	119	96	89	323	244	89	91
25	9725-1	6948	6603	111	112	106	85	352	262	88	84
26	9725-2	5685	7413	113	112	95	83	365	328	93	85
27	9725-3	5671	7302	107	110	104	96	321	286	91	89
28	9725-4	6746	6587	112	116	103	81	359	282	93	73
29	9725-5	6703	7286	112	113	98	87	387	273	86	90
30	9726-1	6876	7492	109	117	108	92	290	295	88	94
31	9726-2	6609	7032	106	114	100	90	328	326	81	91
32	9726-3	5895	6286	110	118	106	90	367	290	88	95
33	9726-4	5996	3667	119	118	101	82	354	257	86	56
34	9726-5	7569	7079	112	116	112	87	326	286	93	88
35	9729-1	6760	6635	111	112	106	85	337	244	95	92
36	9729-2	6753	6571	111	114	105	84	367	293	87	87
37	9729-3	6508	7238	108	112	113	94	334	268	88	87

Sr No.	Genotype	Yield (kg/ha)		Days to 50% flowering		Plant height (cm)		EBT/m^2		Fertility (%)	
		Ldh	Rauni	Ldh	Rauni	Ldh	Rauni	Ldh	Rauni	Ldh	Rauni
38	9729-4	6501	6317	110	116	104	89	343	315	94	67
39	9729-5	6486	6270	110	113	107	89	365	279	89	88
40	9730-1	6760	7095	105	113	97	86	328	306	92	84
41	9730-2	6703	6079	108	112	98	82	385	323	91	90
42	9730-3	6089	6190	110	114	100	82	372	350	92	91
43	9731-1	6465	6476	105	110	103	91	345	290	86	85
44	9731-2	6219	6794	110	112	105	89	359	312	88	92
45	9731-3	7107	6492	112	113	105	87	370	235	89	60
46	9731-4	6212	6603	107	111	104	89	394	297	91	92
47	9731-5	8175	6349	107	111	102	88	361	273	89	88
48	9737-1	6724	7048	105	110	99	88	381	253	95	87
49	9737-2	7208	7159	110	110	106	90	411	279	92	89
50	9737-3	7713	7000	110	111	100	90	348	299	91	91
51	9737-4	6905	6873	111	110	95	90	387	273	92	93
52	9737-5	7006	6841	103	110	101	92	337	284	94	92
53	9738-1	7597	6190	102	108	95	79	385	299	89	76
54	9738-2	6364	6937	105	110	101	88	350	310	87	84
55	9738-3	5772	4683	106	111	102	82	389	271	89	87
56	PR 114	6306	4984	102	113	102	85	359	255	89	66

Sr No.	Genotype	Yield (kg/ha)		Days to 50% flowering		Plant height (cm)		EBT/m^2		Fertility (%)	
		Ldh	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**	**Ldh**	**Rauni**
57	PR 121	6551	6794	106	113	99	85	387	251	87	86
58	PR 122	6414	6460	112	119	111	94	337	332	84	92
59	PR 126	8030	6698	91	92	97	95	328	213	86	87
60	IR 71033	5173	6778	96	107	110	112	343	317	93	72
61	IR 54842	3543	5667	100	97	103	107	312	290	88	91
62	Kaum 182-1	5924	6254	109	110	114	100	343	189	91	86
63	RP 2068-295	4913	4587	102	103	88	90	381	286	88	93
64	RP 2068-303	5823	6286	108	106	92	84	359	233	87	80
Mean		6650	6670	107	112	106	91	347	280	90	86
Se		0.06	0.06	1.33	4.17	8.44	10.4	32.79	14.86	0.05	0.16
Range		3543-8175	3667-7857	91-119	92-119	88-134	79-112	286-411	189-350	81-97	56-96

Table 4.17: Performance of 64 Genotypes

No.	Genotype	BB	BPH	TGW (g)	LB Ratio	HRR (%)
		Ldh	Ldh	Ldh	Ldh	Ldh
1	9711-1	3	1	26.00	3.38	57.77
2	9711-2	3	1	22.10	3.35	65.22
3	9711-3	5	3	22.40	3.09	65.96
4	9711-4	5	1	26.60	3.51	57.28
5	9711-5	5	3	23.20	3.64	66.18
6	9712-1	3	1	27.00	3.07	60.50
7	9712-2	3	3	25.00	3.39	65.52
8	9712-3	5	3	28.10	3.63	65.10
9	9712-4	3	7	25.90	3.21	67.54
10	9712-5	5	1	26.30	3.55	65.46
11	9722-1	5	3	23.90	3.83	62.14
12	9722-2	5	1	26.40	3.69	63.56
13	9722-3	3	1	25.30	3.58	52.10
14	9722-4	5	1	29.40	3.85	59.73
15	9723-1	5	3	28.20	3.66	59.55
16	9723-2	5	3	27.30	3.55	65.78
17	9723-3	5	3	23.60	3.85	58.73
18	9723-4	5	3	26.80	3.63	44.74

No.	**Genotype**	**BB**	**BPH**	**TGW (g)**	**LB Ratio**	**HRR (%)**
		Ldh	**Ldh**	**Ldh**	**Ldh**	**Ldh**
19	9723-5	5	1	25.40	3.78	60.78
20	9724-1	3	1	25.80	3.77	51.02
21	9724-2	3	3	25.30	3.83	65.62
22	9724-3	3	3	26.50	3.56	58.42
23	9724-4	5	1	28.00	3.92	50.23
24	9724-5	5	1	25.10	4.04	58.00
25	9725-1	5	3	26.50	3.94	59.20
26	9725-2	3	3	23.10	3.94	65.32
27	9725-3	5	3	25.20	3.79	56.58
28	9725-4	3	3	24.00	3.71	66.25
29	9725-5	3	3	27.10	3.72	63.50
30	9726-1	3	3	28.10	3.71	62.74
31	9726-2	3	5	26.90	3.63	61.82
32	9726-3	3	7	27.10	3.65	51.70
33	9726-4	5	5	26.40	3.69	63.76
34	9726-5	3	3	26.30	3.85	66.50
35	9729-1	3	1	29.40	3.66	62.88
36	9729-2	3	5	25.50	3.94	55.92
37	9729-3	3	3	28.00	3.82	53.98

No.	**Genotype**	**BB**	**BPH**	**TGW (g)**	**LB Ratio**	**HRR (%)**
		Ldh	**Ldh**	**Ldh**	**Ldh**	**Ldh**
38	9729-4	3	3	26.90	4.10	59.74
39	9729-5	3	3	28.40	3.31	59.91
40	9730-1	3	3	25.00	3.83	55.20
41	9730-2	3	1	23.10	3.56	59.30
42	9730-3	3	3	21.60	3.64	66.04
43	9731-1	5	3	28.10	3.64	47.44
44	9731-2	5	5	29.50	3.99	55.37
45	9731-3	3	3	28.50	3.86	57.13
46	9731-4	5	5	27.10	3.71	56.44
47	9731-5	5	3	27.00	3.83	54.15
48	9737-1	3	5	24.00	3.69	60.41
49	9737-2	3	9	21.60	3.49	56.24
50	9737-3	3	7	20.50	3.32	57.69
51	9737-4	5	5	23.10	3.68	60.98
52	9737-5	5	9	22.50	3.61	50.15
53	9738-1	3	5	24.00	3.96	63.59
54	9738-2	3	5	22.50	4.04	53.50
55	9738-3	5	3	25.90	3.92	54.51
56	PR 114	9	9	21.40	4.41	58.98

No.	Genotype	BB	BPH	TGW (g)	LB Ratio	HRR (%)
		Ldh	Ldh	Ldh	Ldh	Ldh
57	PR 121	3	9	23.90	3.94	63.53
58	PR 122	3	9	26.30	3.89	65.50
59	PR 126	5	9	20.12	4.00	62.04
60	IR 71033	7	7	22.70	3.92	56.08
61	IR 54842	7	7	23.50	3.84	36.52
62	Kaum 182-1	9	5	21.20	2.82	53.41
63	RP 2068-295	7	5	23.30	2.90	41.33
64	RP 2068-303	9	5	28.10	3.06	67.87
Mean			25	3.68	59	
SE			0.02	0.07	0.98	
Range			18.9 - 29.5	2.82-4.41	36.52-67.54	

Table 4.18: Best performing lines

Sr. No.	Genotype	Yield (kg/ha)	DTF	PH (cm)	EBT/m^2	Fertility (%)	BB	BPH	HRR (%)
8	9712-3	7325	109	111	316	92	5	3	65.0
20	9724-1	7328	109	97	319	90	3	1	51.0
21	9724-2	7416	109	100	356	90	3	3	65.6
22	9724-3	7500	108	91	344	87	3	3	58.4
23	9724-4	7286	107	101	319	84	5	1	63.7
24	9724-5	7523	117	93	284	90	5	1	58.0
30	9726-1	7184	113	100	293	91	3	3	62.7
34	9726-5	7324	114	99	306	91	3	3	66.0
49	9737-2	7183	110	98	345	90	3	9	56.2
50	9737-3	7356	110	95	323	91	3	7	57.7
57	PR 121	6672	110	92	319	86	3	9	63.5
59	PR 126	7364	92	96	271	87	5	9	62.0

Table 4.19: Scoring list of Xa-4, xa-13 and XA21 for 64 genotypes

Sr. No.	Genotype	Xa4	Xa 13	Xa21	Remarks
1	9711-1		+	-	*Xa*13
2	9711-2		+	+	*xa*13+*Xa*21
3	9711-3		+	+	*xa*13+*Xa*21
4	9711-4		+	+	*xa*13+*Xa*21
5	9711-5		+	-	*xa*13
6	9712-1		±	+	*xa*13(H)+*Xa*21
7	9712-2		+	NA	*xa*13
8	9712-3		+	+	*xa*13+*Xa*21
9	9712-4		+	-	*xa*13
10	9712-5		+	-	*xa*13
11	9722-1		+	-	*xa*13
12	9722-2		+	-	*xa*13
13	9722- 3		+	-	*xa*13
14	9722-4		+	-	*xa*13
15	9723-1		+	-	*xa*13
16	9723-2		+	-	*xa*13
17	9723-3		NA	-	-
18	9723-4		+	-	*xa*13
19	9723-5		+	-	*xa*13
20	9724-1		+	-	*xa*13
21	9724-2		+	-	*xa*13
22	9724-3		+	-	*xa*13
23	9724-4		+	-	*xa*13
24	9724-5		+	-	*xa*13
25	9725-1		NA	-	-
26	9725-2		+	-	*xa*13
27	9725-3		+	-	*xa*13
28	9725-4		+	-	*xa*13
29	9725-5		+	+	*xa*13+*Xa*21
30	9726-1		+	-	*xa*13
31	9726-2		+	-	*xa*13
32	9726-3		+	-	*xa*13

33	9726-4		+	+	*xa*13+*Xa*21
34	9726-5		+	+	*xa*13+*Xa*21
35	9729-1		+	-	*xa*13
36	9729-2		+	-	*xa*13
37	9729-3		+	-	*xa*13
38	9729-4		+	-	*xa*13
39	9729-5		+	-	*xa*13
40	9730-1		+	-	*xa*13
41	9730-2		NA	+	*Xa*21
42	9730-3		NA	+	*Xa*21
43	9731-1		+	-	*xa*13
44	9731-2		+	+	*xa*13+*Xa*21
45	9731-3		NA	+	*Xa*21
46	9731-4		+	+	*xa*13+*Xa*21
47	9731-5		+	+	*xa*13+*Xa*21
48	9737-1		NA	+	*Xa*21
49	9737-2		NA	+	*Xa*21
50	9737-3		NA	+	*Xa*21
51	9737-4	-	+	+	*xa*13+*Xa*21
52	9737-5	+	+	-	*Xa*4+*xa*13
53	9738-1	+	+	+	*Xa*4+*xa*13+*Xa*21
54	9738-2	NA	+	+	*xa*13+*Xa*21
55	9738-3	+	+	-	*Xa*4+*xa*13
56	PR 114	NA	-	-	-
57	PR 121	+	+	+	*Xa*4+*xa*13+*Xa*21
58	PR 122	+	+	+	*Xa*4+*xa*13+*Xa*21
59	PR 126	+	-	-	*Xa*4
60	IR 71033	+	-	-	*Xa*4
61	IR 54842	+	-	+	*Xa*4+*Xa*21
62	Kaum 182-1	+	-	-	*Xa*4
63	RP 2068-295	NA	NA	-	-
64	RP 2068-303	+	-	-	*Xa*4

Table 4.20: Molecular analysis of Bph resistance genes-*Bph*33 &*Bph*34

Sr. No.	Genotype	BPH	BPH 33	BPH 34
1	9711-1	1		-
2	9711-2	1		-
3	9711-3	3		-
4	9711-4	1		-
5	9711-5	3		-
6	9712-1	1		-
7	9712-2	3		-
8	9712-3	3		-
9	9712-4	7		-
10	9712-5	1		-
11	9722-1	3		-
12	9722-2	1		-
13	9722- 3	1		-
14	9722-4	1		-
15	9723-1	3	+	-
16	9723-2	3	+	-
17	9723-3	3	-	-
18	9723-4	3	+	-
19	9723-5	1	-	-
20	9724-1	1	+	-
21	9724-2	3	+	-
22	9724-3	3	+	-
23	9724-4	1	+	-
24	9724-5	1	+	-
25	9725-1	3	+	-
26	9725-2	3	+	-
27	9725-3	3	-	-
28	9725-4	3	+	-
29	9725-5	3	+	-
30	9726-1	3	-	-
31	9726-2	5	-	-
32	9726-3	7		-

33	9726-4	5	+	-
34	9726-5	3	+	-
35	9729-1	1	+	-
36	9729-2	5	-	-
37	9729-3	3	-	-
38	9729-4	3	+	-
39	9729-5	3	+	-
40	9730-1	3	+	-
41	9730-2	1	+	-
42	9730-3	3	+	-
43	9731-1	3	-	-
44	9731-2	5	+	-
45	9731-3	3	+	-
46	9731-4	5	+	-
47	9731-5	3	+	-
48	9737-1	5	-	-
49	9737-2	9	-	-
50	9737-3	7	-	-
51	9737-4	5	-	-
52	9737-5	9	-	-
53	9738-1	5	-	-
54	9738-2	5	-	-
55	9738-3	3	-	-
56	PR 114	9	-	-
57	PR 121	9	-	-
58	PR 122	9	-	-
59	PR 126	9		-
60	IR 71033	7		-
61	IR 54842	7		-
62	Kaum 182-1	5		-
63	RP 2068-295	5	+	-
64	RP 2068-303	5		-

CHAPTER V

SUMMARY

Rice is one of the major cereal food crops of the world that forms the staple diet of about half of the total world population. However, recently, the dramatic increase in the potential grain yield of rice has slowed down and has apparently reached a plateau. Since the economic grain yield of the rice plants is largely connected with various physiological traits, a better understanding of these traits will be useful in planning future strategies for breeding and crop management.

Punjab is a major rice growing state of India. Although it occupies only 1.56% of the total geographical area of the country, but it is the main contributor of rice to the central pool of food grains in the country. Since the inception of rice research at Punjab Agricultural University (PAU), Ludhiana in the year 1962; tremendous achievements have been made in the varietal improvement and standardization of production and protection technologies. Previous popular rice varieties had longer duration of 150-160 days with an average yield of 7.5 to 8.0 tonnes per ha; producing 45-50 kg per day and consumed a lot of water. In the present scenario of lowering water table, facilitation of proper straw management and timely sowing of wheat, the breeding strategies need to be reoriented towards development of short duration varieties with higher per day productivity, resistance to numerous abiotic and biotic factors and acceptable grain quality. The new cultivars need to have better resource use efficiency. Thus, there is a need to understand the physiological and yield attributes of rice for further improvement. Along with yield and quality, BB resistance is one of the most important traits in the varietal breeding programme of PAU.

The study found genotypes 9712-3, 9724-2, 9724-4 to be promising as these lines had high yield, medium maturity, BPH & BB resistance as well as good HRR. Genotypes 9724-5, 9726-1 and 9726-5 although high yielding, having BPH &BB resistance but had longer duration. Genotypes like 9724-1 and 9724-3 had high yield coupled with BPH and BB resistance. However, their HRR was low. Genotypes 9737-2 and 9737 -3 had low HRR and were susceptible to BPH. None of the lines harbored *Bph34*. However, 24 lines showed presence of *Bph33* gene. In addition, check line RP 2068-295 also showed presence of Bph 33 which is donor of this gene. But a few lines showed Bph resistance despite absence of *Bph 33* and *Bph 34,* indicative of presence of some other known Bph resistance genes or as yet uncharacterized genes. The entries 9724-2 and carry 9724-4 *Bph*33 gene. Their BB resistance is attributable to BB resistance gene *xa13*. Genotype 9712-3 does not carry *Bph33* and *Bph34* genes. The resistance may be due to a novel gene in the donor *O. nivara acc* CR 100204. It also possesses two BB resistance genes *xa 13* and *Xa 21*. These entries can be further evaluated in multi-location trials. The observed wide agro-morphological variations among the rice genotypes and high genetics polymorphism suggest ample opportunity for improvement of the crops through the next generation.

REFERENCES

Ali M and Chowdhary T (2014) Tagging and mapping of genes and QTLs of *Nilaparvata lugens* resistance in rice. *Euphytica* **195:**1 -30.

Ali M P, Alghamdi S S, Begum M A, Uddin A B M A, Alam M Z and Huang D (2012a) Screening of rice genotypes for resistance to the brown planthopper, *Nilaparvata lugens Stal. Cereal Resear Commun* **40:**502-08

Amegan E, Efisue A, Akoroda M, Shittu A and Tonegnikes F (2020) Genetic Diversity of Korean Rice (*Oryza sativa* L.) Germplasm for Yield and Yield Related Traits for Adoption in Rice Farming System in Nigeria. *J Int Genet Genom* **8(1)**:19-28.

Anis G B, Namaky R A, Ashkar I M, Barutçular C and Sabagh A (2016) Yield potential and correlation analysis of some rice hybrids for yield and its component traits. *J Animal Plant Sci* **30(2**): 4748-57.

Annonymous (2010) International Rice Research Institute, Rice Fact Sheet, Bacterial Blight

Anonymous (1996) Standard evaluation system for rice. International Rice Research Institute Manila, Philippines.

Antony G, Zhou J H, Huang S, Li T, Liu B, White F and Yang B (2010) Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. *Plant Cell* **22**: 3864-76

Arif M, Jaffar M, Babar M and Sheikh A M (2008) Identification of bacterial blight resistance gene *Xa4* in Pakistani rice germplasm using PCR. *Afr J Biotechnol* **7:** 541-45.

Athwal D S, Pathak M D, Bacalangco E H and Pura C D (1971) Genetics of resistance to brown plant hopper and green leafhoppers in *Oryza sativa* L. *Crop Sci* **11:**747-50.

Backus E A, Serrano M S and Ranger C M (2005) Mechanisms of hopper burn: an overview of insect taxonomy, behavior and physiology. *Annu Rev Entomol* **50**:125-51.

Balachiranjeevi C H, Prahalada G D, Mahender A, Jamaloddin M, Sevilla M A L, Marfori-Nazarea C M, Vinarao R, Sushanto U, Baehaki S E and Li Z K (2019) Identification of a novel locus, BPH38(t), conferring resistance to brown plant hopper (*Nilaparvata lugens Stal*.) using early backcross population in rice (*Oryza sativa* L.). *Euphytica* **215:**1-12.

Bhadru D, Reddy D L and Ramesha M S (2011) Correlation and path coefficient analysis of yield and yield contributing traits in rice hybrids and their parental lines. *Elect J Plant Breed* **2(1):**112-16.

Bharaj T S, Mangat G S, Rupinder K, Singh K and Naveen S (2014) PR 121: A new semi-dwarf high yielding variety of rice (*Oryza sativa* L.). *J Res Punjab Agric Univ* **51(2):** 202-03.

Bhasin H, Bhatia D, Raghuvanshi S, Lore S J, Gurpreet K, Sahi K G, Kaur B, Vikal Y and Singh K (2012) New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice. *Mol Breed* **30**:607-11

Blair M W, Garris A J, Iyer A S, Chapman B, Kresovich S and McCouch S R (2003) High resolution genetic mapping and candidate gene identification at the *xa5* locus for bacterial blight resistance in rice (*Oryza sativa* L.). *Theor Appl Genet* **107**: 62-73

Chen X, Zhu L and He G (2013) Towards understanding of molecular interaction between rice and the brown plant hopper. *Mol Plant* **6:**621-34.

Cho Y S (1975) Studies on the relationship between Inoculum concentration and disease development in bacterial leaf blight of rice. *Kor J Plant Prot* **14**: 1-5.

Chu Z H, Fu B Y, Yang H, Xu C G, Li Z K, Sanchez A, Park Y J, Bennetzen J L, Zhang Q F and Wang S P (2006a) Targeting *xa13*, a recessive gene for bacterial blight resistance in rice. *Theor Appl Genet* **112**: 455-61.

Chu Z H, Yuan M, Yao J L, Ge X J, Yuan B, Xu C G, Li X H, Fu B Y, Li Z K, Bennetzen J L, Zhang Q F and Wang S P (2006b) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev* **20**: 1250-55.

Conley S and John G (2013) Top 7 Recommendations for Winter Wheat Establishment, University of Wisconsin agronomy, Soybean Research, University of Wisconsin-Extension

Deng F, Wang L, Ren W J, Mei X F and Li S X (2015) Optimized nitrogen managements and polyaspartic acid urea improved dry matter production and yield of indica hybrid rice. *Soil Till Res* **145:**1-9.

Dinh H D, Du P V and Loan L C (2010) Study on the use of combination resistance genes in rice lines against *Xanthomonas oryzae* pv *oryzae* in cuulong river delta. *Omonrice* **17:** 147-51.

Du B, Zhang W and Liu B (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown plant hopper in rice. *Proc Natl Acad Sci USA* **106:**163-68.

Fujita D, Doi K, Yoshimura A and Yasui H (2006) Molecular mapping of a novel gene, Grh5,conferring resistance to green rice leafhopper (Nephotettixcincticeps Ulher) in rice. *Oryza sativa* L. *Theor Appl Genet* **113:**567-73.

Fujita D, Kohli A and Horgan F G (2013) Rice resistance to planthoppers and leafhoppers. *Crit Rev Plant Sci* **32**:162-91.

Ghaffar MBA, Pritchard J and Ford-Lloyd B (2011) Brown planthopper *(N. lugens Stal)* feeding behaviour on rice germplasm as an indicator of resistance. *Plos One* **6:**37

Goel R K, Kaur L and Saini R G (1998) Effectiveness of different *Xa* genes against *Xanthomonas oryzae* pv. *oryzae* population causing bacterial blight of rice in Punjab (India). *Rice Gen News* **15**: 131-33.

Guo J P, Xu C X and Wu D (2018) Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. *Res Crops* **7:**55-67.

Harini A S, Kumar S S, Balaravi P, Sharma R, Dass M A and Shinoy V (2013) Evaluation of rice genotypes for brown plant hopper (BPH) resistance using molecular markers and phenotypic methods. *Afr J Biotechnol* **12**: 2515-25.

Heinrichs E A, Medrano F G and Rapusas H R (1985) Genetic evaluation for insect resistance in rice. Int. *Rice Res. Inst., Los* tructure of water pores in *Leersia japonica* Mikino and *Oryza sativa* L. its correlation wi*Banos,* pp 1-56

Hongthong P, Huang M, Xia B, Cao F, Jiang P and Zou Y (2012) Yield formation strategies of a loose-panicle super hybrid rice. *Res Crops* **13(3):**781-89.

Horino O. (1984) Ultra sth the resistance to hydrathodal invasion of *Xanthomonas campestris* pv. *oryzae*. *Ann Phytopath Soc Japan* **50**: 72-76.

Hu J, Chang X, Zou L, Tang W and Wu W (2018) Identification and fine mapping of Bph33, new brown plant hopper resistance gene in rice (*Oryza sativa* L.). *Rice* **11:** 1-12.

Huang D, Qiu Y and Zhang Y (2013) Fine mapping and characterization of BPH27, a brown planthopper resistance gene from wild rice (*Oryza rufipogon Griff.*). *Theor Appl Genet* **126**: 219-29.

Huang N, Angeles E R, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J and Khush G S (1997) Pyramiding of bacterial blight resistance genes in rice: Marker aided selection using RFLP and PCR. *Theor Appl Genet* **95**: 313-20.

Ikeda R and Kaneda C (1981) Genetic analysis of resistance to BPH *Nilaparvata lugens Stal* in rice. *Jpn J Breed* **31:** 279-85.

IRRI (1998) Standard Evaluation System for Rice (SES). Los Baños,Philippines: International Rice Research Institute.

Ishii T, Brar D S, Multani D S and Khush G S (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from Oryza australiensis into cultivated rice, *O. sativa*. *Genome* **37**: 217-21

Jairin J, Sansen K, Wongboon W and Kothcharerk J (2010) Detection of a brown planthopper resistance gene bph4 at the same chromosomal position of Bph3 using two different genetic backgrounds of rice. *Breed Sci* **60**:71-75.

Jairin J, Teangdeerith S, Leelagud S, Phengrat P, Apichart K, Vanavichit A and Toojinda T (2007) Physical mapping of Bph3, a brown plant hopper resistance locus in rice. *Maejo Int J Sci Tech* **1:** 166-77.

Jena K K and Kim S K (2010) Current status of brown planthopper (BPH) resistance and genetics. *Rice* **3**:161-71

Jones D B, Peterson M L and Geng S (1979) Association between grain filling rate and duration and yield components in rice. *Crop Sci* **19:** 641-44.

Joshi R K and Nayak S (2010) Gene pyramiding- A broad spectrum technique for developing durable resistance in crops. *Biotech Mol Biol Rev* **5:** 51-60.

Khanna R, Kaur R, Sidhu N, Gill R S and Mangat G S (2014) Characterization of elite rice breeding lines for bacterial blight resistance genes using molecular markers. In: Tomar S M S, Talukdar Akshay and Tyagi R K (eds.), *Proceedings of National Symposium on Crop Improvement for Inclusive Sustainable Development.* Pp 727-29. Nov. 7-9, 2014, Ludhiana,

Khush G S (1984) IRRI breeding program and its worldwide impact on increasing rice production. In: Gustofron J P (ed) *Gene Manipulation in Plant Improvement*. Pp 16.

Stadler Genetics Symposium, Columbia, Missouri.

Khush G S and Angeles E R (1999) A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. *Rice Genet Newslett* **16:** 92-93.

Khush G S and Brar D S (1991) Genetics of resistance to insects in crop plants. *Adv Agron* **45:** 223-74.

Khush G S and Virk P S (2005) *IRRI Varieties and Their Impact*. Pp. 163. International Rice Research Institute: Los Banos, CA, USA; Manila, Philippines.

Khush G S Mackill D J and Sidhu G S (1989) Breeding rice for resistance to bacterial blight. In: IRRI (ed) *Bacterial blight of rice*. Pp 207-17. IRRI, Manila, Philippines

Khush G S, Karim A R and Angeles E R (1985) Genetics of resistance of rice cultivar ARC 10550 to Bangladesh brown plant hopper biotype. *J Genet* **64:** 121-25.

Kiryu, T, Nishizawa T and Kuhara S (1954) On the relation of time passing after injuring of rice plant leaves to the infection of bacterium *Xanthomonas oryzae*. *Bull Kyushu Agric Expt Stn* **2**: 125-29.

Kuang J, Liu J, Mei J, Wang C, Hu H, Zhang Y, Sun M, Ning X, Xiao L and Ling Yang L (2017) A Class II small heat shock protein OsHsp18.0 plays positive roles in both biotic and abiotic defense responses in rice. *Sci Rep* **7:**33.

Kulkarni S and Jahagirdar S (2011) Evaluation of new molecules in the management of bacterial leaf blight of paddy in India. *Int J Plant Protec* **4(2):** 289-91.

Kumar K, Sarao P S, Bhatia D, Neelam K, Kaur A, Mangat G S, Brar D S and Singh K (2018) High-resolution genetic mapping of a novel brown plant hopper resistance locus, Bph 34 in *Oryzasativa L*.X *Oryza nivara* (Sharma & Shastry) derived interspecific F2 population. *Theor Appl Genet* **131:** 1163-71.

Lakashminarayana A and Khush G S (1977) New genes for resistance to the brown planthopper in rice. *Crop Sci* **17**: 96-100.

Li C, Luo C, Zhou Z, Wang R, Ling F, Xiao L, Lin Y and Chen H (2017) Gene expression and plant hormone levels in two contrasting rice genotypes responding to brown planthopper infestation. *BMC Plant Biol* **17**:57

Li Z, Xue Y, Zhou H, Li Y, Usman B, Jiao X, Wang X, Liu F, Qin B and Li R (2019) High-resolution mapping and breeding application of a novel brown plant hopper resistance gene derived from wild rice *(Oryza rufipogon* Griff). *Rice* **12:** 1-13.

Ling K C, Tiongco E R and Aguiero V M (1978) Rice ragged stunt, a new virus disease. *Plant Dis Rep* **62:** 701-05.

Mangat G S, Rupinder K, Lore J S, Jasdeep K, Sidhu N and Khanna R (2012) Molecular characterization of elite rice breeding lines for bacterial blight resistance genes. In: Sandhu S.K., Navjot Sidhu and Allah Rang (eds). International Conference on Sustainable Agriculture for Food and Livelihood Security, November 27-29, *Crop Int Soci India* pp **125**-26

Mariappan V, Paridasan V and Durairaj P (1980) Resistance to bacterial blight. *Int Rice Res Newslett* **5**: 6.

Mazzola M, Leach J E, Mew T W and White F F (1993) Effect of plant age on IR-BB21 resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). *Int Rice Res Notes* **18**: 21-22.

McCouch S R, Teytelman L, Xu Y, Lobos K B, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D and Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa L.*). *DNA Res* **9**:199-07

Mew T W (1984) Current status and future prospects of research on bacterial blight of rice. *Annu Rev Phytopathol* **25**: 359-82.

Mew T W (1987) Current status and future prospects of research on bacterial blight of rice. *Annu J Res Phytopathol* **25**: 359-82.

Mew T W (1992) Bacterial blight. In: *Compendium of rice diseases*. Webster R K and Pamela S G eds. St. Paul. *Amer Phytopathol Soc* pp. 10-11.

Mew T W, Alvarez A M, Leach J E and Swings J (1993) Focus on Bacterial blight of Rice. *Plant Dis Int J Appl Plant Pathol* **77**: 5.

Mew T W, Vera Cruz C M, Reyes R C and Zaragoza B A (1979) Study on kresek (wilt) of the rice bacterial blight syndrome. *IRRI Res Pap Ser* **39**: 1-8.

Miller B C, Hill J E and Roberts S R (1991) Plant population effects on growth and yield in water -seeded rice. *J Agron* **83**: 291-97.

Mizukami T and Wakimoto S (1969) Epidemiology and control of bacterial leaf blight of rice. *Ann Rev Phytopathol* **7**: 51-72.

Mohapatra P K, Panigrahi R and Turner N C (2011) Physiology of spikelet development on the rice panicle: Is manipulation of apical dominance crucial for grain yield improvement? *Adv Agron* **110**: 333-59.

Murata K, Fujiwara M, Kaneda C, Takumi S, Mori N and Nakamura C (1998) RFLP mapping of a brown plant hopper (*Nilaparvata lugens* Stal) resistance gene bph2 of indica rice introgressed into a japonica breeding line Norin-PL40 . *Genes Genet Syst* **73:** 359-64.

Murray M G and Thompson W F (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* **8:** 4321-25.

Naik S B , Dhanasekar D, Nihar S, Raman M S, Preetinder S S, Kuldeep S, Jhansi L V and Jagadish S B (2018) A new gene Bph33 conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. *Euphytica* **214:** 53.

Nemoto H, Ikeda R and Kenada C (1989) New genes for resistance to brown planthopper *Nilaparvata lugens* Stål in rice. *Jpn J Breed* **39**: 23-28.

Niño Liu D O, Darnielle L and Bogdanove A J (2006) *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Mol Plant Pathol* **7**: 303-24.

Ogawa T and Yamamoto T (1986) Inheritance of resistance to bacterial blight in rice. *In*: *Rice Genetics.* Manila, the Philippines: *International Rice Research Institute* **471**: 80.

Panda N and Heinrichs E A (1983) Levels of tolerance and antibiosis in rice varieties having moderate resistance to the brown plant hopper, *Nilaparvata lugens* (Stal) (Hemiptera:

Delphacidae). *Environ Entomol* **12:**1204-14.

Panda N and Khush G S (1995) *Host plant resistance to insects*. Pp. 431. Wallingford (UK): CAB International.

Park D S, Song M Y, Park S K, Lee S K and Lee J H (2008) Molecular tagging of the Bph locus for resistance to brown plant hopper (*Nilaparvata lugens Stal*) through representational divergence analysis. *Mol Genet Genomics* **280**: 16-72.

Pathak M D, Cheng C H and Furtuno M E (1969) Resistance to Nephotettixcincticeps and *Nilaparvata lugens* in varieties of rice. *Nat* **223:** 502-04.

Pawar S Y, Radhakrishnan V V and Mohanan K V (2016) The importance of optimum tillering in rice-an overview. *South Indian J Biolog Sci* **2(1):**125-27.

Peng S, Khush G, Virk P, Tang Q and Zou Y (2008) Progress in ideotype breeding to increase rice yield potential. *Field Crops Res* **108**: 32-38.

Perumalsamy S, Bharani M, Sudha M, Nagarajan P, Loganathan A, Saraswathi R, Ponnuswami B and Ramalingam (2009) Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.*). Plant Breed* **129(4):**400 -06

Petpisit V, Khush G S and Kauffman H E (1977) Inheritance of resistance to bacterial blight in rice. *Crop Sci* **17:**551-54.

Powell W, Machray G C and Provan J (1996) Polymorphism revealed by simple sequence repeats. *Trends Plant Sci* **1**:215-22.

Qiu Y, Guo J, Jing S, Zhu L and He G (2010) High resolution mapping of the brown plant hopper resistance gene Bph6 in rice and characterizing its resistance in 9311 and *Nipponbare* near isogenic backgrounds. *Theor Appl Genet* **121:** 1601-11.

Rajpurohit D, Kumar R, Kumar M, Paul P, Awasthi A, Basha P O, Puri A, Jhang T, Singh K and Dhaliwal H S (2011) Pyramiding of two bacterial blight resistance and a semidwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* **178**: 111-26.

Ramaiah K (1985) Grain Classification Rice Research in India, ICAR Publication -629

Ramalingm J, Vera C C M, Kukereja K, Chittoor J M, Wu J L, Lee S W, Baroidan M, George M L, Cohen M, Hulbert S H, Leach J E and Leung H (2001) Candiate resistance genes from rice, barley and maize and their quantitative resistance in rice. *Mol Plant Micro Interact* **16**:14-24.

Renganayaki K, Fritz A K, Sadasivam S, Pammi S, Harrington S E, McCouch S R, Kumar S M and Reddy A S (2002) Mapping and progress toward map-based cloning of brown plant hopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice *O. sativa. Crop Sci* **42:** 2112-17.

Revelle W, Elizabeth M, Dworak D M and Condon (2021) Exploring the persome: The power of the item in understanding personality structure. *Personality and Individual Differences* pp. 169

Richa K, Tiwari I M, Kumari M, Devanna B N, Sonah H and Kumari A (2016) Functional characterization of novel chitinase gene present in the sheath blight resistance QTL: qSBR11-1 in rice line tetep. *Front Plant Sci* **7**:244.

Rivera C T, Ou S H and Lida T T (1966) Grassy stunt disease of rice and its transmission by *Nilaparvata lugens* (Stal). *Plant Dis Rep* **50:** 453-56.

Ronald P C, Albano B, Tabien R, Abenes L, Wu K S, McCouch S and Tanksley S D (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Mol General Genet* **236**:113-20.

Sai Harini A, Santhana Lakshmi S and Sai Kumar S (2010) Validation and fine-mapping of genetic locus associated with resistance to brown planthopper *Nilaparvata lugens* (Stal.) in rice (*Oryza sativa* L.). *Asian J Bio Sci* **5**:32-37.

Sanchez A C, Ilag L L, Yang D, Brar D S, Ausubel F, Khush G S, Yano M, Sasaki T, Li Z and Huang N (1999) Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. *Theor Appl Genet* **98**: 1022-28.

Sani Haliru B, Rafii M Y, Mazlan N, Ramlee S I, Muhammad I, Silas Akos I, Halidu J, Swaray S and Rini Bashir Y (2020) Recent Strategies for Detection and Improvement of Brown Plant hopper Resistance Genes in Rice: A Review. *Plant* **9:** 1202.

Shabanimofrad M, Rafii M Y, Ashkani S, Hanafi M M, Adam N A, Harun A R, Latif M A, Miah G, Sahebi M and Azizi P (2017) Mapping of QTLs conferring resistance in rice to brown plant hopper, *Nilaparvata lugens*. *Entomol Exp Appl* **162:** 60-68.

Shahidullah S M, Hanafi M M, Ashrafuzzaman M, Uddin M K and Meon S (2009) Analysis of lodging parameters in aromatic rice. *Ach Agron Soil Sci* **55**: 525-33

Sheehy J E, Dionora M J A and Mitchell P L (2001) Spikelet numbers, sink size and potential yield in rice. *Field Crops Res* **71**:77-85.

Shehzad F I D, Farhatullah, N I, Shah M A and Ahmad M (2012) Screening of local rice germplasm against bacterial leaf blight caused by *Xanthomonas oryzae pv. oryzae*. *Sarhad J Agric* **28:** 565-69.

Sidhu G S and Khush G S (1978) Genetic analysis of brown plant hopper resistance in twenty varieties of rice, *Oryza sativa*. *Theor Appl Genet* **53:** 199-203.

Singh N, Khanna R, Kaur R, Lore J S, Neelam K, Rani N S, Mangat G S and Singh K (2014) Evaluation of aromatic rice germplasm against emerging pathotypes of *Xanthomonas oryza* pv. *oryzae* causing bacterial blight in Punjab. *In: Proceedings of National Symposium on Crop Improvement for Inclusive Sustainable Development*, pp 314-15.

Singh S P, Goel R K, Hunjan M S, Vikal Y and Lore J S (2012) Screening of land races of rice (*Oryza sativa*) for bacterial blight resistance and marker assisted surveying of known *Xa/xa* gene. *Plant Dis Res* **27(2)**:209-15.

Sogawa K, Gallagher K D and Kenmore P E (1994) Pest management in tropical paddy ecosystems. *Jpn J Trop Agric* **38**: 361 -68.

Suh J P, Jeung J U, Noh T H, Cho Y C, Park S H, Park H S, Shin M S, Kim C K and Jena K K (2013) Development of breeding lines with three pyramided resistance genes that confer broadspectrum bacterial blight resistance and their molecular analysis in rice. *Rice* **6**: 5.

Tanweer F A, Rafii M Y, Sijam K, Rahim H A, Ahmed F and Latif M A (2015) Current advance methods for identification of blast resistance genes in rice. *Comptes Rendus Biol* **338**: 321-34.

Tu J, Ona I, Zhang Q, Mew T W, Khush G S and Datta S K (1998) Transgenic rice variety *IR72* with *Xa-21* is resistant to bacterial blight. *Theor Appl Genet* **97***:* 31-36.

Velusamy R, Chelliah S, Heinrichs E A and Medrano F (1984) Brown plant hopper biotypes in India. *Int Rice Res Notes* **9**: 19.

Wang B, Zha Z, Li J and Yin D (2014) Development of elite rice restorer lines in the genetics background of R022 possessing tolerance to brown plant hopper, stem borer, leaf folder and herbicide through marker-assisted breeding. *Euphytica* **195**:129-42.

Wang H Y, Yang Y, Su J Y, Shen J L and Gao C F (2008) Assessment of the impact of insecticides on Anagrusnilaparvatae (Pang et Wang) (Hymenoptera: Mymanidae), an egg parasitoid of the rice plant hopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). *Crop Prot* **27**: 514-22.

Wang H, Shi S and Guo Q (2018) High-resolution mapping of a gene conferring strong antibiosis to brown planthopper and developing resistant near-isogenic lines in 9311 background. *Mol Breed* **38:**07.

Wang W, Zhai W, Luo M, Jiang G, Chen X, Li X, Wing R A and Zhu L (2001) Chromosome landing at the bacterial blight resistance gene *Xa4* locus using a deep coverage rice BAC library. *Mol Genet Genom* **265:**118-25.

Wang Y, Cao L and Zhang Y (2015) Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. *J Exp Bot* **66**:035-45.

Webster and Gunnell (1992) Compendium of rice Diseases University of California, Davis APS Press America P: 10.

Wei T and Simko V (2021) R package 'corrplot': Visualization of a Correlation Matrix (Version 0.90)

Weiya X, Yongzhong X, Xiaoyu W, Yu Z, Weijiang T, Lei W and Hongju Z (2008) Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nature Genet* **40**: 761-67.

Xu Q, Wenfu Chen W and Xu Z (2015) Relationship between grain yield and quality in rice germplasms grown across different growing areas. *Breed Sci* **65**: 226-32

Yang M, Cheng L, Yan L, Shu W, Wang X and Qiu Y(2019) Mapping and characterization of a quantitative trait locus resistance to the brown plant hopper in the rice variety IR64. *Hereditas* **156**: 1-9.

Zhang D P , Xie Y F and Mew T W (1984) Studies on adult plant resistance to bacterial blight in rice. *Sci Agric Sin* **1**: 40-50.

Zhao H, Mo Z, Lin Q, Pan S, Duan M, Tian H, Wang S and Tang X (2020) Relationships between grain yield and agronomic traits of rice in southern China. *Chil J Agric Res* **80**:1.

APPENDIX-I

Mean and Standard deviation, range and coefficient of variation

Variables	**Mean ± SD**	**Range**		**CV (%)**
		Minimum	**Maximum**	
DF (days)		91	119	1.24
PTH (cm)		88	134	7.94
EBT/ m2		286	411	9.45
GY(kg/ha)		3543	8175	8.20
SPF (%)		81	97	0.26
STE(%)		3	19	6.66
TGW (g)		18.9	29.5	0.66
HRR (%)		36.52	47.87	1.66
L/B(%)		2.8	4.4	2.41

CV= coefficient of variation, SD = standard deviation, DF= Days to 50**%** flowering, PTH= Plant height, EBT/ m2 = Ear bearing tiller/ m2, GY= Grain yield per plot, SF (%) =Spikelet fertility, STE(%)=sterility,TGW= Thousand grain weight, HRR= Head rice recovery and LB=Length/Breadth ratio

APPENDIX-II

The mean agronomic performance of advance breeding lines for the yield and quality analysis

No.	Genotype	DTF(days)		PH(cm)		EBT/m2		Fertility(%)		Sterile(%)		1000wt(g)		BB	BPH	LB Ratio	HRR(%)	Yield kg/ha	
		LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	LUD	LUD	LUD	LUD	Rauni
1	9711-1	105	113	107	92	319	264	91	89	9	11	26.00	25.70	3	1	3.38	57.77	6890	7095
2	9711-2	103	113	107	89	319	264	92	83	8	17	22.10	25.60	3	1	3.35	65.22	6999	6444
3	9711-3	105	110	108	98	334	279	96	90	4	10	22.40	21.50	5	3	3.09	65.96	6746	6937
4	9711-4	101	110	110	92	337	282	95	91	5	9	26.60	23.30	5	1	3.51	57.28	6955	7095
5	9711-5	112	114	113	95	310	240	90	88	10	12	23.20	21.50	5	3	3.64	66.18	6797	6508
6	9712-1	105	111	126	106	286	216	93	90	7	10	27.00	25.70	3	1	3.07	60.50	7078	6603
7	9712-2	107	113	121	104	317	273	92	95	8	5	25.00	24.80	3	3	3.39	65.52	6732	6905
8	9712-3	106	111	122	99	337	295	91	93	9	7	28.10	25.50	5	3	3.63	65.10	7475	7175
9	9712-4	110	114	124	105	308	242	91	96	9	5	25.90	25.00	3	7	3.21	67.54	6941	6635
10	9712-5	111	114	120	101	317	268	81	93	19	8	26.30	28.40	5	1	3.55	65.46	6876	7063
11	9722-1-PR 121-1	103	111	109	92	345	275	95	78	5	22	23.90	23.60	5	3	3.83	62.14	6926	6587
12	9722-1-PR 121-2	104	113	116	91	304	308	91	93	9	7	26.40	25.90	5	1	3.69	63.56	7316	6540
13	9722-1-PR 121-3	101	110	103	91	359	312	95	86	5	14	25.30	23.90	3	1	3.58	52.10	6147	6444
14	9722-1-PR 121-4	102	110	113	97	326	231	97	90	3	10	29.40	26.50	5	1	3.85	59.73	6602	7429
15	9723-1	103	111	111	91	350	249	91	87	9	14	28.20	27.20	5	3	3.66	59.55	6811	7063
16	9723-2	111	115	111	90	304	273	88	95	12	6	27.30	24.30	5	3	3.55	65.78	6760	6476
17	9723-3	112	112	107	93	356	279	96	91	4	9	23.60	22.60	5	3	3.85	58.73	6667	7556

No.	**Genotype**	**DTF(days)**		**PH(cm)**		**EBT/m2**		**Fertility(%)**		**Sterile(%)**		**1000wt(g)**		**BB**	**BPH**	**LB Ratio**	**HRR(%)**	**Yield kg/ha**	
		LUD	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**LUD**	**LUD**	**LUD**	**LUD**	**Rauni**
18	9723-4	112	115	113	92	326	238	90	91	10	9	26.80	24.20	5	3	3.63	44.74	6869	6889
19	9723-5	102	108	114	94	378	275	87	87	13	14	25.40	22.20	5	1	3.78	60.78	6833	6000
20	9724-1	107	111	108	86	339	299	94	85	6	15	25.80	22.30	3	1	3.77	51.02	6861	7794
21	9724-2	107	111	108	91	367	345	84	95	16	5	25.30	23.20	3	**3**	3.83	65.62	7165	7667
22	9724-3	104	111	98	84	348	341	90	83	10	17	26.50	21.80	3	3	3.56	58.42	7143	7857
23	9724-4	103	111	107	94	337	301	90	78	10	22	28.00	21.60	5	1	3.92	50.23	6905	7667
24	9724-5	114	119	96	89	323	244	89	91	11	10	25.10	22.60	5	1	4.04	58.00	7713	7333
25	9725-1	111	112	106	85	352	262	88	84	12	16	26.50	23.60	5	3	3.94	59.20	6948	6603
26	9725-2	113	112	95	83	365	328	93	85	7	15	23.10	20.50	3	3	3.94	65.32	5685	7413
27	9725-3	107	110	104	96	321	286	91	89	9	11	25.20	23.90	5	3	3.79	56.58	5671	7302
28	9725-4	112	116	103	81	359	282	93	73	7	27	24.00	20.60	3	3	3.71	66.25	6746	6587
29	9725-5	112	113	98	87	387	273	86	90	14	10	27.10	23.70	3	3	3.72	63.50	6703	7286
30	9726-1	109	117	108	92	290	295	88	94	12	7	28.10	24.50	3	3	3.71	62.74	6876	7492
31	9726-2	106	114	100	90	328	326	81	91	19	10	26.90	23.50	3	5	3.63	61.82	6609	7032
32	9726-3	110	118	106	90	367	290	88	95	12	5	27.10	23.60	3	7	3.65	51.70	5895	6286
33	9726-4	119	118	101	82	354	257	86	56	14	46	26.40	17.40	5	5	3.69	63.76	5996	3667
34	9726-5	112	116	112	87	326	286	93	88	7	13	26.30	24.10	3	3	3.85	66.50	7569	7079
35	9729-1	111	112	106	85	337	244	95	92	5	8	29.40	25.60	3	1	3.66	62.88	6760	6635
36	9729-2	111	114	105	84	367	293	87	87	13	14	25.50	23.40	3	5	3.94	55.92	6753	6571

No.	Genotype	DTF(days)		PH(cm)		EBT/m2		Fertility(%)		Sterile(%)		1000wt(g)		BB	BPH	LB Ratio	HRR(%)	Yield kg/ha	
		LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	Rauni	LUD	LUD	LUD	LUD	LUD	Rauni
37	9729-3	108	112	113	94	334	268	88	87	12	14	28.00	24.30	3	3	3.82	53.98	6508	7238
38	9729-4	110	116	104	89	343	315	94	67	6	33	26.90	23.10	3	3	4.10	59.74	6501	6317
39	9729-5	110	113	107	89	365	279	89	88	11	12	28.40	22.60	3	3	3.31	59.91	6486	6270
40	9730-1	105	113	97	86	328	306	92	84	8	17	25.00	22.50	3	3	3.83	55.20	6760	7095
41	9730-2	108	112	98	82	385	323	91	90	9	11	23.10	20.30	3	1	3.56	59.30	6703	6079
42	9730-3	110	114	100	82	372	350	92	91	8	10	21.60	21.10	3	3	3.64	66.04	6089	6190
43	9731-1	105	110	103	91	345	290	86	85	14	15	28.10	23.20	5	3	3.64	47.44	6465	6476
44	9731-2	110	112	105	89	359	312	88	92	12	8	29.50	24.10	5	5	3.99	55.37	6219	6794
45	9731-3	112	113	105	87	370	235	89	60	11	42	28.50	23.60	3	3	3.86	57.13	7107	6492
46	9731-4	107	111	104	89	394	297	91	92	8	8	27.10	23.80	5	5	3.71	56.44	6212	6603
47	9731-5	107	111	102	88	361	273	89	88	11	12	27.00	23.50	5	3	3.83	54.15	8175	6349
48	9737-1	105	110	99	88	381	253	95	87	5	14	24.00	22.70	3	5	3.69	60.41	6724	7048
48	9737-1	105	110	99	88	381	253	95	87	5	14	24.00	22.70	3	5	3.69	60.41	6724	7048
49	9737-2	110	110	106	90	411	279	92	89	8	12	21.60	20.40	3	9	3.49	56.24	7208	7159
50	9737-3	110	111	100	90	348	299	91	91	9	10	20.50	20.20	3	7	3.32	57.69	7713	7000
51	9737-4	111	110	95	90	387	273	92	93	8	7	23.10	21.10	5	5	3.68	60.98	6905	6873
52	9737-5	103	110	101	92	337	284	94	92	6	8	22.50	20.30	5	9	3.61	50.15	7006	6841
53	9738-1	102	108	95	79	385	299	89	76	11	25	24.00	25.60	3	5	3.96	63.59	7597	6190
54	9738-2	105	110	101	88	350	310	87	84	13	17	22.50	22.40	3	5	4.04	53.50	6364	6937

No.	Genotype	DTF(days)		PH(cm)		EBT/m2		Fertility(%)		Sterile(%)		1000wt(g)		BB	BPH	LB Ratio	HRR(%)	Yield kg/ha	
		LUD	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**Rauni**	**LUD**	**LUD**	**LUD**	**LUD**	**LUD**	**Rauni**
55	9738-3	106	111	102	82	389	271	89	87	11	14	25.90	27.10	5	3	3.92	54.51	5772	4683
56	PR 114	102	113	134	85	359	255	89	66	11	35	21.40	20.10	9	9	4.41	58.98	6306	4984
57	PR 121	106	113	99	85	387	251	87	86	13	14	23.90	24.10	3	9	3.94	63.53	6551	6794
58	PR 122	112	119	111	94	337	332	84	92	16	8	26.30	22.80	3	9	3.89	65.50	6414	6460
59	PR 126	91	92	97	95	328	213	86	87	14	13	18.90	21.30	5	9	4.00	62.04	8030	6698
60	IR 71033	96	107	110	112	343	317	93	72	7	28	22.70	20.60	7	7	3.92	56.08	5173	6778
61	IR 54842	100	97	103	107	312	290	88	91	12	10	23.50	23.10	7	7	3.84	36.52	3543	5667
62	Kaum 182-1	109	110	114	100	343	189	91	86	9	15	21.20	20.70	9	5	2.82	53.41	5924	6254
63	RP 2068-295	102	103	88	90	381	286	88	93	12	7	23.30	23.60	7	5	2.90	41.33	4913	4587
64	RP 2068-303	108	106	92	84	359	233	87	80	13	20	28.10	28.20	9	5	3.06	67.87	5823	6286

DF= Days to fifty percent flowering, PTH= Plant height, EBT/ m2 = Ear bearing tiller/ m2, GY= Grain yield per plot, SF (%) =Spikelet fertility, STE(%)=Sterility, TGW= Thousand grain weight, HRR= Head rice recovery ,LB= length and Breadth ratio

VITA

Name of the student : Yin Yin Aye

Father’s name : U Pho Kha

Mother name : Daw Sein Yin

Nationality : Myanmar

Date of birth : 26th September, 1980

Permanent home Address : Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw, Myanmar

Email Address : yinyinayedar@gmail.com

EDUCATIONAL QUALIFICATION

University : Yezin Agricultural University (YAU), Yezin, Nay Pyi Taw, Myanmar

Year of award : 2009

OCPA : 7.3/10.00

Master’s of degree : M.Sc. (Plant Breeding and Genetics)

University : Punjab Agricultural University, Ludhiana, Punjab -141004

Year of award : 2022

OCPA : 6.90/10.00

Title of Master’s Thesis : Molecular characterization of rice genotypes for brown plant hopper and bacterial blight resistance genes

Awards/ Distinction : The Indian Council for Cultural Relations (ICCR) during M.Sc.