IDENTIFICATION OF DONORS/QTLs FOR FOOT ROT RESISTANCE IN RICE AND STUDIES ON ASSOCIATION OF GIBBERELLIC ACID REGULATED/SYNTHESIS GENES WITH THE DISEASE

Thesis

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

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

By

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2020

CERTIFICATE – I

This is to certify that the thesis entitled, "**Identification of Donors/QTLs for foot rot resistance in rice and studies on association of gibberellic acid regulated/synthesis genes with the disease**" submitted for the degree of **M. Sc.**, in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Baljeet Kaur (L-2017-A-109-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.

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CERTIFICATE II

This is to certify that the thesis entitled, **"Identification of Donors/QTLs for foot rot resistance in rice and studies on association of gibberellic acid regulated/synthesis genes with the disease"** submitted by **Baljeet Kaur (L-2017-A-109-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.,** in the subject of **Plant Breeding and Genetics** (Minor Subject: **Biotechnology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ACKNOWLEDGEMENT

Words definitely stand nowhere to acknowledge the ***"Almighty God"*** for his blessings at every step of my life. My immense gratitude to the merciful ***"God"*** by whose kindness I am able to complete this part of my academic career, successfully.

The sense of veneration and gratitude through words would hardly suffice my feelings of respect for my esteemed major advisor and mentor, ***Dr. R. S. Gill***, Principal Rice Breeder, Department of Plant Breeding and Genetics, PAU, Ludhiana, for his sagacious guidance, ceaseless encouragement and suggestions and helped me to make this manuscript worthy of presentation. It has been my great privileged to work under his guidance.

I also wish to express my sincere thanks to ***Dr. G. S. Mangat***, Head, Department of Plant Breeding and Genetics PAU, Ludhiana for providing necessary facilities during my research work.

I am highly indebted to my co-advisor ***Dr. Dharminder Bhatia***, Quantitative Geneticist, Department of Plant Breeding and Genetics for his constant encouragement, valuable suggestions, constructive comments, endless efforts and careful supervision throughout the research to bring this work in the present form and he, who would be my source of inspiration throughout my life.

I wish to extend my due regards and thankfulness to ***Dr. (Mrs) Jyoti Jain***, Plant Pathologist, Department of Plant Breeding and Genetics, for her continuous support, immaculate guidance and help while carrying out the field related work of the research.

I feel great elation in expressing my thanks to ***Dr. Amandeep Mittal***, Fruit Molecular Geneticist for his ever-willing help, cooperation and suggestions while carrying out the molecular related work of the research.

I also acknowledge the help rendered by ***Dr. (Mrs) Yogesh Vikal***, Senior Geneticist, School of Agricultural biotechnology, ***Dr. (Mrs) S. K. Dhillon***, Professor (Dean, PGS' Nominee), Department of Plant Breeding and Genetics for the continuous support, immaculate guidance and help throughout the course of my study and research.

I express my feelings of deep appreciation to my friends, ***Ramandeep Kaur, Savneet Kaur, Saundrya Kumari, Sukhvir Kaur, Pawandeep Kaur, Namita, Archana Mukta*** *and* ***Mr. Maneesh Jindal*** for their support, help and cooperation. I am immensely grateful to my friend ***Harmandeep Kaur Gill*** for her constant encouragement, help, cooperation and suggestions throughout my research. No words can suffice my feelings of gratitude to my father ***Gurjant Singh***, my mother ***Balveer Kaur***, my sisters ***Daljeet Kaur*** and my brother ***Jagjeet Singh*** whose unconditional love, constant moral support and encouragement are always a source of motivation and inspiration in every walk of life.

I am thankful to ***Alps Computers*** for helping me in preparing and bringing this dissertation in the present form. All may not be mentioned but none is forgotten at my heart, words may be due but thoughts remain with me.

Place: Ludhiana

Date: (Baljeet Kaur)

Title of the Thesis	**:**	Identification of donors/QTLs for foot rot resistance in rice and studies on association of gibberellic acid regulated/synthesis genes with the disease
Name of the Student and Admission No.	**:**	Baljit Kaur L-2017-A-109-M
Major Subject	**:**	Plant Breeding and Genetics
Minor Subject	**:**	Biotechnology
Name and Designation of Major Advisor	**:**	Dr. R.S. Gill Principal Rice Breeder
Degree to be Awarded	**:**	M.Sc.
Year of award of degree	**:**	2020
Total pages in thesis	**:**	65 + Appendices (xxiii) + VITA
Name of the University	**:**	Punjab Agricultural University, Ludhiana – 141 004, Punjab, India

ABSTRACT

To identify resistant sources for foot rot disease of rice, a total of 275 diverse germplasm lines consisting of 159 lines from rice 2K diversity panel, elite germplasm developed at Punjab Agricultural University, Ludhiana (RYT lines) and ANP lines (diverse collection of aromatic germplasm), accessions from *O. glaberrima* and cultivated basmati and non-basmati varieties were screened through artificial inoculations technique for two crop seasons. Out of 275 lines, eighteen lines of 2K diversity panel, five lines of *O. glabberrima*, two ANP lines and ten released varieties (one basmati &nine non- basmati varieties) were identified as completely resistant to foot rot disease of rice. Among the susceptible varieties the foot rot symptoms varied with dry and humid weather conditions during evaluation causing stunting and elongation respectively. Genome wide association studies (GWAS) using 159 lines, a subset from rice 2k diversity panel identified significant QTLs associated with foot rot resistance based on disease severity index. On the basis of *Kharif* 2019 crop season evaluation during April when significant rate of stunting was observed, significant associations were observed on 4, 6 and 8 chromosomes. On the basis of *Kharif* 2019 season evaluation done in July when significant elongation was observed the QTLs on chromosomes 1, 7 and 8 were found to be highly significant. On the basis of combined data analysis of both of the evaluations, significant QTLs were identified on chromosome 6 and 9.The expression analysis of some GA regulated/synthesis genes which included *sd1, OsXTH8, OsGAE1, EUI, G alpha (D1)* and *GID1* using qRT-PCR in two susceptible (Pusa Basmati 1121 and Pusa Basmati 1509) and one resistant variety (PR127) was studied at four different stages 10DAS, 17DAS, 24DAS and 31DAS. Though the expression pattern of these genes varied at different stages in the selected set but no association of these genes with foot rot resistance/susceptibility could be observed. In general all the genes showed significant change in expression either in susceptible or resistant variety at 24DAS and it seems to be the most important stage for further such studies. The identified novel sources for resistance to foot rot can be used in breeding foot rot resistant varieties. Further studies on the identification of major QTLs associated with foot rot disease and research unravelling the mechanism of foot rot resistance/susceptibility may be helpful for the integrated use of modern molecular biology techniques for the speedy and targeted trait transfer into desirable backgrounds.

Keywords: Foot rot, *Fusarium moniliforme*, 2k diversity panel, GWAS, QTL, Gibberellic, Fusaric, RNA, Expression.

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

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ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਪਲਾਂਟ ਬ੍ਰੀਡਿੰਗ ਅਤੇ ਜੈਨੇਟਿਕਸ
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ਡਿਗਰੀ	:	ਐਮ.ਐੱਸ ਸੀ.
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2020
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	65 + ਅੰਤਿਕਾਵਾਂ (xxiii) + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਐਗਰੀਕਲਚਰਲ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ - 141 004, ਪੰਜਾਬ, ਭਾਰਤ

ਸਾਰ-ਅੰਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ ਦੋ ਫ਼ਸਲੀ ਮੌਸਮਾਂ ਵਿੱਚ ਬਨਾਵਟੀ ਇਨੋਕੁਲੇਸ਼ਨ ਵਿਧੀ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਝੋਨੇ ਵਿੱਚ ਝੰਡਾ ਰੋਗ ਦੇ ਪ੍ਰਤੀਰੋਧਕ ਸਰੋਤਾਂ ਦੀ ਪਹਿਚਾਣ ਕਰਨ ਲਈ, ਕੁੱਲ 275 ਵਿਭਿੰਨ ਜਰਮਪਲਾਜ਼ਮ ਲਾਈਨਾਂ (ਜਿਹਨਾਂ ਵਿੱਚ 159 ਲਾਈਨਾਂ ਝੋਨਾ 2ਕੇ ਵਿਭਿੰਨਤਾ ਪੈਨਲ ਨਾਲ ਸਬੰਧਤ ਸਨ), ਪੰਜਾਬ ਐਗਰੀਕਲਚਰਲ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਵਿੱਚ ਵਿਕਸਤ ਕੀਤੇ ਗਏ ਵਿਲੱਖਣ ਜਰਮਪਲਾਜ਼ਮ (RYT ਲਾਈਨਾਂ) ਅਤੇ ANP ਲਾਈਨਾਂ (ਐਰੋਮੈਟਿਕ ਜਰਮਪਲਾਜ਼ਮ ਦੇ ਵਿਭਿੰਨ ਸੰਗ੍ਰਹਿ), *O. glaberrima* ਦੇ ਐਕਸੇਸ਼ਨ ਅਤੇ ਕਾਸ਼ਤ ਕੀਤੀਆਂ ਜਾ ਰਹੀਆਂ ਬਾਸਮਤੀ ਅਤੇ ਨਾਨ-ਬਾਸਮਤੀ ਕਿਸਮਾਂ ਦੀ ਜਾਂਚ ਕੀਤੀ ਗਈ। 275 ਲਾਈਨਾਂ ਵਿੱਚੋਂ, 2ਕੇ ਵਿਭਿੰਨਤਾ ਪੈਨਲ ਨਾਲ ਸਬੰਧਤ 18 ਲਾਈਨਾਂ, *O. glabberrima* ਦੀਆਂ ਪੰਜ ਲਾਈਨਾਂ, ਦੋ ANP ਲਾਈਨਾਂ ਅਤੇ ਦਸ ਕਿਸਮਾਂ (ਇੱਕ ਬਾਸਮਤੀ ਅਤੇ ਨੌਂ ਨਾਨ-ਬਾਸਮਤੀ) ਦੀ ਪਹਿਚਾਣ ਝੋਨੇ ਵਿੱਚ ਝੰਡਾ ਰੋਗ ਦਾ ਟਾਕਰਾ ਕਰਨ ਵਾਲੀਆਂ ਕਿਸਮਾਂ ਵਜੋਂ ਹੋਈ। ਸੰਵੇਦਨਸ਼ੀਲ ਕਿਸਮਾਂ ਵਿੱਚ ਰੋਗ ਦੇ ਲੱਛਣ ਸੁੱਕੇ ਅਤੇ ਨਮੀ ਵਾਲੇ ਮੌਸਮੀ ਹਲਾਤਾਂ ਅਧੀਨ ਬਦਲਦੇ ਰਹੇ। 2K ਵਿਭਿੰਨਤਾ ਪੈਨਲ ਨਾਲ ਸਬੰਧਿਤ 159 ਲਾਈਨਾਂ ਦੀ ਵਰਤੋਂ ਕਰਕੇ GWAS ਅਧਿਐਨ ਕੀਤਾ ਗਿਆ ਅਤੇ ਰੋਗ ਤੀਬਰਤਾ ਸੂਚਕਾਂਕ ਦੇ ਅਧਾਰ ਤੇ ਝੰਡਾ ਰੋਗ ਨਾਲ ਸਬੰਧਿਤ QTLs ਦੀ ਪਹਿਚਾਣ ਕੀਤੀ ਗਈ। ਸੰਨ 2019 ਦੀ ਸਾਉਣੀ ਦੀ ਰੁੱਤ ਦੌਰਾਨ ਅਪ੍ਰੈਲ ਮਹੀਨੇ ਪਰਖ ਸਮੇਂ ਸੰਵੇਨਸ਼ੀਲ ਲਾਈਨਾਂ ਵਿੱਚ ਵਿਕਾਸ ਦਰ ਵਿੱਚ ਕਮੀ ਦਰਜ ਕੀਤੀ ਗਈ ਅਤੇ ਉਸ ਸਮੇਂ 4, 6 ਅਤੇ 8 ਗੁਣਸੂਤਰਾਂ ਉਪਰ ਰੋਗ ਨਾਲ ਸਬੰਧਿਤ QTLs ਦਾ ਪਤਾ ਚੱਲਿਆ। ਸੰਨ 2019 ਦੀ ਸਾਉਣੀ ਰੁੱਤ ਦੌਰਾਨ ਜੁਲਾਈ ਮਹੀਨੇ ਪਰਖ ਸਮੇਂ ਸੰਵੇਨਸ਼ੀਲ ਲਾਈਨਾਂ ਦੀ ਵਿਕਾਸ ਦਰ ਵਿੱਚ ਤੇਜ਼ੀ (ਜ਼ਿਆਦਾ ਲੰਬਾਈ) ਦਰਜ ਕੀਤੀ ਗਈ ਅਤੇ ਉਸ ਸਮੇਂ 1, 7 ਅਤੇ 8 ਗੁਣਸੂਤਰਾਂ ਉਪਰ QTLs ਦਾ ਪਤਾ ਚੱਲਿਆ। ਸਾਂਝੇ ਆਂਕੜਿਆਂ ਦੇ ਅਧਾਰ ਤੇ ਦੋਨਾਂ ਮੁਲਾਂਕਣਾਂ ਦੇ ਕੀਤੇ ਗਏ ਅਧਿਐਨ ਤੋਂ ਗੁਣਸੂਤਰ 6 ਅਤੇ 9 ਉਪਰ ਅਰਥਪੂਰਨ QTLs ਦੀ ਪਹਿਚਾਣ ਹੋਈ। qRT-PCR ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਝੰਡਾ ਰੋਗ ਨਾਲ ਸਬੰਧਤ ਦੋ ਸੰਵੇਦਨਸ਼ੀਲ ਕਿਸਮਾਂ (ਪੂਸਾ ਬਾਸਮਤੀ 1121 ਅਤੇ ਪੂਸਾ ਬਾਸਮਤੀ 1509) ਅਤੇ ਇੱਕ ਸਹਿਣਸ਼ੀਲ ਕਿਸਮ (ਪੀ ਆਰ 127) ਵਿੱਚ ਬੀਜਾਈ ਤੋਂ 10, 17, 24 ਅਤੇ 31 ਦਿਨਾਂ ਮਗਰੋਂ ਕੁੱਝ GA ਰੇਗੂਲੇਟਿਡ/ਸਿੰਥੇਸਿਸ ਜੀਨਾਂ (*sd1, OsXTH8, OsGAE1, EUI, G alpha (D1)* ਅਤੇ *GID1*) ਦੇ ਵਿਹਾਰ (ਐਕਸਪ੍ਰੇਸ਼ਨ) ਦਾ ਅਧਿਐਨ ਕੀਤਾ ਗਿਆ। ਹਾਲਾਂਕਿ ਚੋਣਵੇਂ ਸੈੱਟ ਵਿੱਚ ਵੱਖੋ-ਵੱਖਰੇ ਪੜਾਵਾਂ ਉਪਰ ਇਹਨਾਂ ਜੀਨਾਂ ਦੇ ਵਿਹਾਰ (ਐਕਸਪ੍ਰੇਸ਼ਨ) ਵਿੱਚ ਵਿਭਿੰਨਤਾ ਸੀ ਪਰ ਝੋਨੇ ਵਿੱਚ ਝੰਡਾ ਰੋਗ ਦੀ ਪ੍ਰਤੀਰੋਧਕਤਾ/ਸਹਿਣਸ਼ੀਲਤਾ ਨਾਲ ਇਹਨਾਂ ਜੀਨਾਂ ਦਾ ਕੋਈ ਸਬੰਧ ਵੇਖਣ ਨੂੰ ਨਹੀਂ ਮਿਲਿਆ। ਬੀਜਾਈ ਦੇ 24 ਦਿਨਾਂ ਮਗਰੋਂ ਸੰਵੇਦਨਸ਼ੀਲ ਅਤੇ ਸਹਿਣਸ਼ੀਲ ਕਿਸਮਾਂ ਵਿੱਚ ਇਹਨਾਂ ਸਾਰੇ ਜੀਨਾਂ ਨੇ ਵਿਹਾਰ (ਐਕਸਪ੍ਰੇਸ਼ਨ) ਵਿੱਚ ਅਰਥਪੂਰਨ ਤਬਦੀਲੀਆਂ ਦਰਸਾਈਆਂ ਅਤੇ ਇਹ ਪੜਾਅ ਅਗਲੇਰੀਆਂ ਖੋਜਾਂ ਲਈ ਅਹਿਮ ਹੈ। ਝੰਡਾ ਰੋਗ ਦਾ ਟਾਕਰਾ ਕਰਨ ਵਾਲੀਆਂ ਕਿਸਮਾਂ ਵਿਕਸਤ ਕਰਨ ਵਿੱਚ ਇਸ ਅਧਿਐਨ ਦੇ ਨਤੀਜਿਆਂ ਦੀ ਵਰਤੋਂ ਕੀਤੀ ਜਾ ਸਕਦੀ ਹੈ। ਝੰਡਾ ਰੋਗ ਨਾਲ ਸਬੰਧਤ ਅਗਲੇਰੀ ਖੋਜ ਵਿੱਚ ਇਸਨੂੰ ਰੋਕਣ ਵਾਲੇ ਪ੍ਰਮੁੱਖ QTLs ਅਤੇ ਪ੍ਰਤੀਰੋਧਕਤਾ/ਸਹਿਣਸ਼ੀਲਤਾ ਦੀ ਕਿਰਿਆ ਅਤੇ ਹੋਰ ਅਧਿਐਨ ਦੀ ਸਿਫਾਰਿਸ਼ ਕੀਤੀ ਜਾਂਦੀ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਝੰਡਾ ਰੋਗ, *ਫੁਸੇਜ਼ੇਰੀਅਮ ਮੋਨੀਲੀਫੋਰਮੇ*, 2ਕੇ ਵਿਭਿੰਨਤਾ ਪੈਨਲ, GWAS, QTL, ਜ਼ਿਬਰੈਲਿਕ, ਫੁਜ਼ੇਰਿਕ, RNA, ਐਕਸਪ੍ਰੇਸ਼ਨ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is a main food for about 3.5 billion people who take more than 20 percent of their daily calories from it. During 2017, it was cultivated on 167.24 million hectares area with production of 769.65 million tonnes in the world and on 43.799 million hectares area with a total production of 117.45 million tonnes in India (FAO 2019). In the Punjab, the crop occupied an area 30.65 lakh hectares with a total production of 199.72 lakh tonnes (Anonymous 2019). The demand of rice is expected to rise from 723 million tons in 2015 to 852 million tons by 2035 (Brar and Khush 2017). Depending upon taste preference, the demand for high quality rice has been increased substantially.

Among the wide variety of rices grown around the world the 'Basmati rice' is a nature's gift to Indian sub-continent, where it has been grown in the foothills of Himalayas from ancient times. The rice grown in this area has unique quality features and pleasant aroma which endowed by climate of this region. India is the leading exporter of basmati rice in the world. Basmati rice is grown on about 1.8 million hectares area with production of 8.2 million tonnes in India. An export of 4.4 million tons of basmati rice amounting to 3280430.16 crores has been made from India (Agricultural and Processed Food Products Export Development Authority, APEDA Report Dec, 2018-19).

Basmati rice is also attacked by various types of abiotic and biotic stresses. Among these, foot rot of rice has emerged as an alarming disease (Bashyal *et al* 2016). The intensive cultivation of susceptible varieties, poor management strategies, changes occurring in climate and cropping pattern have contributed towards the spread of disease and depending upon severity, it is reported to cause 3-95.4 per cent loss in the various parts of the world. This disease has been occurred in various regions of the world like Korea, China, Japan, Pakistan, India, Philippines, Iran and Thailand etc. This disease is known by different names like "elongation disease", "white stalk", "bakanae" disease in the different regions of the world. It was reported first time in Japan since 1828 and derives its name from a Japanese word meaning "foolish seedling. It is known by "foot rot" or bakanae disease in India (Fiyaz *et al* 2014). It was caused by many species which belong to Liseola section of fungus (Desjardins *et al* 2000, Wulff *et al* 2010) and from this three species complex of *Fusarium* viz.; *F. fujikuroi* (FFSC), *F. proliferatum* (FPSC) and *F. verticilloides* (FVSC) is involved in the development of typical symptoms of the disease. Among them *F. fujikuroi* has been reported to be more virulent and its strain *F. moniliforme* being the major causal organism (Gupta *et al* 2015). Many isolates of *F. moniliforme* have been identified from the different parts of the world on the basis of morphology, pathogenecity and molecular characterization etc.

The various kind of symptoms are observed from pre-emergence seedling death to grain infection at maturity which include the high growth of the seedling, rotting of the base of seedling, fainted grain and sterility. Fungus produces two different chemicals fusaric and gibberellic acid. Fusaric acid is responsible for stunting while gibberellins increase the growth of the seedling. Other important features of the disease are whitish/pinkish mycelial growth, small roots like growth on basal nodes and more leaf angles. The fungus is responsible for causing necrotic lesions on various parts of plant including leaves, stem and kernel, rotting of inflorescence in which light brownish spots are formed on glumes. Dead heart is resulted due to sneering of shoots.

The disease is seed as well as soil-borne, infection becomes easily taken place when infected seeds are sown in non-infested fields or healthy seeds in fungus infested fields and infected seeds in infested fields. The high recovery of pathogen was reported in hulled grains as compared to caryopsis, highest being in lemma followed by palea, the basal glumes, embryo and endosperm.

There is no chemical control available to the control of disease; seed treatment is the only recommended option. But, the use of chemicals also raises concerns of pesticide residues and meeting MRL (minimum residue limits) specified by different importing countries is nowadays a chief concern for trouble free basmati export. Development of new foot rot resistant basmati cultivars is the highly effective, efficient, eco-friendly and also a significant way of reducing losses in the crop yield and for maintaining the sanctity of basmati rice international trade. For initiating a sound breeding program, there is need of the availability of such type of donors which gave complete resistance to bakanae disease. This is a major limitation at present and the present studies was focused on identification of foot rot resistant/tolerant lines from a selected set of basmati germplasm and to study the role of gibberellic acid synthesis pathway genes in the development of bakanae disease.

The identification of donors for bakanae disease resistance warrants the attention of rice researchers. Efforts on identification of germplasm with foot rot resistance/tolerance have already been initiated (Khokhar and Jafferey, 2002, Hossain *et al* 2013, Iqbal *et al* 2011, Pannu *et al* 2013, Kim *et al* 2014, Fiyaz *et al* 2016, Puyam *et al* 2017). The identification of genes/QTLs governing resistance/tolerance against the disease can be a major break through which can aid the precise transfer and quick development of foot rot resistance/tolerance genes. Some QTLs for foot rot resistance are already identified in various studies like *qBK1* (Hur *et al* 2015), *qBK1.1, qBK1.2* and *qBK1.3* (Fiyaz *et al* 2016), *qBK1_628091* and *qBK4_31750955* (Volante *et al* 2017) and *qBK1WD* (Lee *et al* 2018). In the present study, the germplasm set used against screening of foot rot disease is a subset of 2K panel, the sequence

data for which is publically available. The modern approaches like GWAS may help to associate the genes/QTLs associated with the trait.

The interaction between rice and fungus is very complex and involves various biological and physiological processes. To understand the molecular mechanism of disease, comparative transcriptomic profiling of both healthy and fungus infected plant tissues of susceptible and resistant genotypes is probably an effective method. Expression studies also can help in the identification of differentially expressed genes between the fungal infected tissue of both susceptible and resistant varieties. It is reported that the genes of defence mechanism are generally expressed in both varieties. But the genes of gibberellic acid pathway, chitinase and salicylic acid pathways are upregulated while the genes of jasmonic acid pathway, PR1, germin like proteins, *cytochrome P450 monooxygenases* and MAP (Mitogen activated proteins) kinases are downregulated in susceptible varieties as compared to resistant varieties (Matic *et al* 2016). The most important phenomenon associated with this disease is that increased the level of gibberellic acid which externally provided by the fungus to plant, responsible for abnormal elongation and death of the infected plants. The *sd1* gene is semi- dwarfing gene which present in most of the present day cultivated semi-dwarf rice germplasm. It has been reported that *sd1* allele responsible for loss of function of this gene which encodes *GA 20 oxidase* enzyme, an important gibberellic acid biosynthetic pathway enzyme and may have some association with the disease occurrence. The study of GA regulated/synthesis pathway genes may provide deeper insights into the defence mechanism & role of *sd-1* gene in context to severity of foot rot disease. With this vision we laid out the objectives as below:

- To screen diverse set of basmati and non- basmati rice germplasm for foot rot resistance.
- To study genome wide association to identify QTLs for foot rot resistance.
- To find association of GA regulated/synthesis genes with foot rot disease by expression analysis.

CHAPTER II

REVIEW OF LITERATURE

Rice ranks second in terms of area under cultivation and more than half of the world's population depends upon rice for food. It possesses enormous genetic diversity in which basmati rice is the aromatic rice which is a nature's gift to India and is cultivated on the foot hills of the Himalayas where the whole ambience of environment bestows is responsible for its unique properties to this specialty rice. The sustainable cultivation of basmati rice is threatened by many biotic and abiotic stresses among which foot rot of rice caused by *Fusarium moniliforme* has emerged as a serious problem nowadays. The present study was focused on identification of foot rot resistance/tolerance basmati germplasm and transcriptome profiling of GA (Gibberellic acid) synthesis/regulated genes. The GA is the major secondary metabolite associated with the foot rot disease. The relevant review of literature discussed under following sub-heads:

2.1 Foot rot of rice: Historical aspect

2.2 Distribution and Economic losses

2.3 Symptoms

2.4 Predisposition factors

2.5 Disease Cycle

2.6 Control measures

2.7 Artificial screening techniques

2.8 Identification of resistant sources/QTLs

2.9 Transcriptomic analysis

2.10 Gibberellic acid pathway and foot rot disease

2.1 Foot rot of rice: Historical aspect

Foot rot/Bakanae of rice was first recorded in Japan in 1828. The culture, of fungi collected from 126 infected plants was prepared separately and its identification was done on the basis of colour of conidia. It was first recognized that foolish seedling like symptoms were induced by *F. heterosporum* Nees (Hori 1898). It was suggested by Sawada (1917) that some stimulus provided by hyphae of *Fusarium* fungus may be responsible for the elongation of infected plants. He also identified the ascigerous stage (perfect stage) of fungus and gave the name *Lisea fujikuroi* Sawada.

Kurosawa (1926) reported that culture filtrate which was obtained from dried infected plants also caused the elongation of rice and some sub-tropical grasses. He concluded that the

chemical secreted by the fungus was responsible for culms elongation, restricted growth of root and chlorotic appearance and wilting like symptoms of diseased plants. Similar results were also observed by Hemmi and Seto (1928) and they gave 'Bakanae Byo' name to this disease. There has been conflict among plant Pathologists for the nomenclature of bakanae fungus. Later on, the name of the organism was amended to *Gibberella fujikuroi* and it was put under the genus *Gibberella* by Ito and Kimura (1931). They also identified the asexusal stage of fungus, *F. moniliforme.*

During 1935, Wollenweber and Reinking published their work on *Fusarium* taxonomy. They organized 1000 named spp. of *Fusarium* into 16 sections and also described the *F. moniliforme* as imperfect stage and *G. fujikuroi* (Sawada) Wollenweber as perfect stage of the fungus. The terms '*Fujikuroi'* and 'Saw' in *G. fujikuroi* (Sawada) Wollenweber were derived from the name of two famous plant pathologists from Japan, Yosaburo Fujikuro and Kenkichi Sawada (Watanabe and Umehara 1997). *G. fujikuroi* was a species complex in which *F. fujikuroi, F. proliferatum* and *F. verticilloides* was found to be associated with disease (Leslie and Summerell 2006). *F. proliferatum* var. *proliferatum* in Iran and *F. moniliforme* var. *zhejiangensis* in China was found to be responsible for the bakanae disease (Singh and Sunder 1997). It was reported that *F. proliferatum* and *F. verticilloides* grow only as saprophytes on the infected plants but *F. fujikuroi* is hemibiotrophic fungi, which behaves as biotrophic at initial infection and as necrotrophic at the time of further development of disease by destruction of host cells (Ma *et al* 2013). In vitro conditions, the most of strains of *F. proliferatum* and *F. verticilloides* produced only fumonisins but not gibberellins, while huge amount of gibberellins was produced only by *F. fujikuroi.* It was reported that *F. fujikuroi* was mainly associated with the diseased plants and infected seeds in Asia (Wulff 2010).

Ou (1985) reported that *Fusarium* produced hyaline, septate and highly branched hyphae of 1-8um in thickness. Basically it was found to produce macro conidia and micro conidia, the macro conidia (8.4-66 x 2.4-3.5um) are delicate, sickle shaped with narrow ends or somewhat straight along with 3-5 septa and the micro conidia being short (5-12 x 3-3.5um), pyriform shaped having 0-1 septa and formed in chain of 15-32 spores on host. Leslie and Summerell (2006) reported perithecia are generally oval shaped with size of 200-400 x 150-400 µm and wall thickness 20-54um. It was bluish black in color and consisted of several layers of cells, asci and paraphyses which are emerging at its bottom. Asci are long and cylindrical having 8-ascospores arranged in single row but sometimes these are arranged in two rows. Ascospores are elliptical in shape with size of 15-18 x 6.7 µm and one or two (rarely) septate, but slightly constricted at it ends. Paraphyses (120-135 x 15µm) obclavate

shaped with 3-5 septate and constricted at septa and the chlamydospores are usually absent but rarely pseudochlamydospores may form in some species.

It was observed that occasionally, spherical to globose shaped, dark blue coloured sclerotia and yellowish, brownish or violet colored stroma are also developed. So, the different species of *Fusarium* genus can be identified on the basis of shapes, sizes and formation of macroconidia and micro-conidia, formation of conidiophores and presence or absence of chlamydospores and pigmentation presence in culture or also on molecular, biochemical and physiological characters (Hsuan *et al* 2010).

The disease also has been recognised by many other names like Root rot and White head disease, *Fusarium* blight, Elongation disease, Fusariosis, White stalk in China, Palaylalake (man rice) in British Guiana, Foot rot in the Philippines, Otokenae in Japan, bakanae in USA, Africa and Australia, Foot rot & Bakanae in French Equitorial, Africa and Ceylon and Foolish plant or Foot rot in India (Dodan *et al* 1997).

2.2 Distribution and Economic losses

The biotic stresses have always remained a challenge for researchers threatening the sustained cultivation of agriculturally important crops. In rice, more than 10 per cent of rice yield has been reported to be lost because of diseases (Gupta *et al* 2015). With time, the minor diseases like elongation disease, false smut, and sheath rot has emerged as alarming diseases and create danger to rice cultivation. In basmati rice, foot rot caused by *Fusarium moniliforme* (teleomorph: *Gibberella fujikuroi*, Sawada, Wollenweber) has emerged as a potential threat and caused more than 20% yield losses in rice growing areas of Asia including Philippines, Thailand, Japan, China, Bangladesh, India, Pakistan and Nepal. It is also gaining importance in newer regions of the world like Africa, Australia, British Guiana, Cameroon, Italy, Ivory Coast, Japan, Kenya, Nigeria, Surinam, Tanzania, Trinidad, Uganda, USA, Venezuela, Vietnam, Brazil, Ceylon, Indonesia, Mexico, Malaysia, Taiwan, Iran, Spain (Singh and Sunder, 2012). In India, Gupta *et al* (2014) observed highest incidence of disease in Punjab (10.5-40%) followed by Rajasthan (2.4-13.6%), Uttar Pardesh (1.2-11.7%), Bihar (1.8-8.7%), and Haryana (1.2-2.8%). Bashyal *et al* (2016) reported 3-20 per cent incidence of disease in Uttar Pradesh, 1-20 per cent in Haryana and 1-10 per cent in Punjab. Within the Punjab state, the varying levels of incidence has been observed in different districts by Sandhu and Dhaliwal (2016) viz.; Fatehgarh Sahib (1%), Amritsar (2-3%), Ferozpur (3-5%), Patiala (5%), Sangrur (2-10%), Tarantaran (3-10%), Kapurthala (10%), Sri Muktsar Sahib Districts (10%) and lowest in Jalandhar (1-3 %). More than 10 per cent incidence of this disease was found in south-western districts of Punjab.

The disease has been reported to cause extensive losses in grain yield of crop. Sunder *et al* (1997**)** reported that 4.17 per cent (minimum) and 96.25 per cent (maximum) disease

incidence caused 3.4 per cent and 95.45 per cent grain yield loss in basmati variety Taraori. He also observed the linear regression between disease and yield losses. Suparyono *et al* (2009) reported that highest yield loss occurred in Japan (20-50%) followed by Thailand (15%) and India (3.7%). Yield reduction was reported upto 20-50 per cent in Japan by Ito and Kimura (1931), 40 per cent in Nepal by Desjardins *et al* (2000), 10-50% in Pakistan by Khokhar and Jaffrey (2002), 75 per cent in Iran by Saremei *et al* (2008), 25 per cent in Bangladesh by Hossain *et al* (2011) and 15-25 per cent in India by Gupta *et al* (2015). It has also been reported to deteriorate the quality of seed along with grain yield losses. The export of basmati rice which has been the extensive source of earning the foreign exchange is reduced due to deterioration of seed quality by this disease (Singh and Sunder 2012). The disease has been reported a potential threat to successful cultivation of basmati rice in Punjab (Pannu *et al* 2013b).

2.3 Symptoms

Normally, this disease become visible at seedling stage just 20-25 days after infection but it is also observed in the field conditions after transplanting. The infection occurs through crown or roots of the plant either in nursery or after transplanting. Then, fungus grows systemically through the plant and finally infects the panicle (Bashyal *et al* 2012).

F. moniliforme causes different types of symptoms starting from pre-emergence seedling death to grain infection at maturity which includes elongation than normal growth, stunted growth and no growth (Ou 1985). Elongation of rice plant depends on the ratio of GA_3 and fusaric acid (Thakur 1974). The diseased plants show less tillering, drying of leaves, wilting and die in small time. If the plant survives upto maturity, it has empty chaffy panicles. The vascular bundles mainly xylem and large pitted vessels are extensively filled with mycelium and conidia. The fungus produces abundant spores at the base of the infected plant near the water level which becomes visible as white or pink mycelial growth on drainage of water. Sometimes, appearance of pink colored sporodochia has been observed at the junction of lemma and palea of infected grain (Gupta *et al* 2015). Under favorable conditions perithecia are formed at the base of the diseased plant and it turns black in color. Other important features of the disease are, like more leaf angle near about 90^0 (Ilija *et al* 2009), rotting of plant starts at crown level and formation of adventitious roots from the lower internodes showed small root like structures (Singh and Sunder 2012).

2.4 Predisposition factors

Development of the disease depends on the various factors like climatic conditions mainly temperature and relative humidity, planting time, stage of crop, soil moisture, types of seeds or variety and inoculums potential. High rate of infection (7.2%) was reported in early

planting (June 19) while it remained low (2.5%) in late planting (July 31). This is due to the presence of high temperature at early planting of crop. The maximum development of disease occurs at tillering (20 days after transplanting) and boot stage (Bagga *et al* 2007). It was observed by Ilija *et al* (2009) that bakanae like symptoms are produced from infected seeds which do not show any surface discoloration and the suppressive growth (stunting) like symptoms were produced from discolored seeds. It was reported further, that chlorotic and stunted seedling symptoms appeared due to high inoculum level and elongated seedling developed under low inoculums level and the abnormal growth of seedlings occurs under wet soil conditions and stunting in dry soils (Singh and Sunder 2012).

Pannu *et al* (2013a) reported that incidence was mainly found in basmati cultivars as compared to non basmati cultivars. Fiyaz *et al* (2014) reported that 27-30^0 C temperature is required for infection and 35^0 C for the development of disease along with 60-80% relative humidity.

2.5 Disease Cycle

Foot rot of rice is monocyclic disease. Both infected seed and soil are the main source of primary infection but seed borne infection is more important. It was reported that hulls are the main site of infection in seed. The maximum infection of pathogen was observed from lemma followed by palea, endosperm and embryo (Kumar *et al* 2015).

The pathogen was found to enter into the plant through basal/crown region or roots, and the critical time for development of disease is 72 hours after germination of seed when it exudates large amount of sugars and amino acids (Matic *et al* 2017). High humidity, 27^0 to 30^0 C temperature and heavy amount of nitrogen in soil were found favorable for proper development of disease (Gupta *et al* 2015).

Then, the fungus was found to grow systemically in plant, vascular bundles showing the superficial growth of fungus, in which xylem gaps and large vessels having high infection rate as compared to phloem and parenchyma. The mycelium starts concentrating in leaf blade, sheath and in adventitious roots (Sun 1975). Secondary infection occurred through wind and water borne conidia/ascospores. Dead or infected plants were found to produce a large amount of conidia at basal region, from flowering to late maturation phase of crop. It was reported that after germination, the hyphae grow intercellularly in stigma and anthers and finally reached to the embryo within 48 hours under favorable conditions (Yu and Sun, 1976). The infected seed shows slight reddish color but highly infected seed give discolored appearance. So, wind and rain was found to play an important role in dispersion of conidia and ascospores. Fungus was reported to overwinter either in various parts of infected seed or in form of conidia, ascospores, sclerotia and mycelium in crop residue in soil (Matic *et al* 2017).

Manadhar (1999) reported that *F. moniliforme* survives in the various parts of the seed. He took three varieties IR50 (resistant), IR43 (moderately resistant), IR841 (susceptible) and planted them in normal field, spray of conidial suspension was done up to thrice during anthesis to increase the rate of infection. Detection of infected seeds was done by using specialized medium (FMSM). The number of infected seeds was very less in uninoculated plots as compared to inoculated plots. The infected seed percentage was highest in IR841 then by IR43 and IR50. Maximum infection was found in hull followed by lemma, palea, basal glumes, embryo and endosperm. Embryo infection was highest in IR43 (41%) followed by IR841 (16%) and IR50 (2%). Under storage conditions of 40% relative humidity and 10^0 C temperature, pathogen survived for 22 to 24 months in seeds. Number of infected seeds was high in seed lots when harvesting done during wet season as compared to dry season.

Kumar *et al* (2015) indicated that foot rot is an internally and externally seed borne disease. They took two varieties Pusa basmati 1121 and Basmati CSR 30 and planted in bakanae infected field, the spray of conidial suspension ($2*10^6$/ml) was done to increase the infection rate and then the collected grains were stored at 5.3°C – 41.2°C temperature under shade conditions. After one month sample of grains were tested, in which Basmati CSR 30 and Pusa Basmati 1121 showed 96.25% and 90% infections respectively by *F. moniliforme*. In Basmati CSR 30, lemma (91.25%), palea (78.75%), endosperm (58.75%) and embryo (35%) infection of *Fusarium* fungus was observed. This study thus reported that *Fusarium* fungus survives in all the parts of the grain.

Pannu *et al* (2012) has reported that after thirteen months of storage of bits of infected plants and infected seeds in refrigerator and at room temperature, the pathogen showed 5.5% and 16.6% survival in stored grains and 33.3% and 22.5% in infected straw bits under room temperature and refrigerator condition respectively. But under natural field conditions of Punjab, the pathogen survived for eight months and its amount continuously declined and was not able to cause infection during next year.

2.6 Control measures

Lee (1990) reported that the use of few *Pseudomonas* and *Bacillus* bacterial strains is valuable to control the infection due to this fungus. However, the technique has been reported to show effectiveness only when other management practices are used along with it.

Rosales and Mew (1997) also reported that some strains of *Pseudomonas* bacteria were capable of controlling the bakanae disease by inhibiting the mycelia growth or germination of conidia through unknown mechanism. The 250gm seeds of IR-58 variety were soaked in bacterial suspension (10^9 CFU per ml) for 24 hours and then sown in infested seed beds with *Fusarium* inoculums. The range of disease control was from 71.7- 96.4 per cent as compared to control.

Manadhar and Yami (2008) used various types of composts prepared from tea like aerated vermicompost tea (ACTV), non aerated vermicompost tea (NCTV), aerated compost tea (ACTC) and non aerated compost tea (NCTV) etc. to test their effect on bakanae disease and compared there results with carbendazium (2.5gm per kg of seeds). Among them ACTV was found to give maximum control with 25.6 per cent healthy seedlings and minimum control was found with NCTC having 13.6 per cent healthy seedling.

Pannu *et al* (2013a) tested nine different fungicides to check their efficacy on the growth of fungus under in vitro conditions from these, Bavistin 50 WP (94.4%), Nativo 80 FS (89.99%), Raxil 50FS (80.16%) were found to be the best while Vitavax (59.52%) showed poor performance. On the basis of this it was suggested that both seed treatment and seedling root dip (Bavistin @ 0.2%) used together is a good measure to reduce the incidence of disease. On the basis of these results Punjab Agricultural University, Ludhiana gave recommendation of soaking the seeds in carbendazim 50 WP @ 0.2 per cent + Streptocycline 0.01 per cent for 12 hrs followed by the seedlings treatment with carbendazim 50 WP (0.2%) for 6 hours. However, no chemical is recommended to check the secondary infection caused by the fungus and also the use of chemicals sometimes raises safety concerns especially with basmati being it an export commodity and keeping in view the stringent restrictions imposed by the importers.

As an alternate to bavistin, use of talc formulation of *Trichoderma harzianum* @ 15 g/kg before sowing and seedling root dip for 6 hrs with *T. harzianum* @ 15 g/litre of water before transplanting has also been recommended by Punjab Agricultural University, Ludhiana (Anonymus 2019).

For the efficient control of this disease in an eco-friendly manner, the development of foot rot resistant varieties is the most viable and practical solution. For this purpose the availability of effective donors providing stable resistance against the disease is a basic pre-requisite for any breeding program aiming at the development of foot rot resistant varieties.

2.7 Artificial screening techniques

Toledo *et al* (1975) reported that the fungus can be easily cultured on various media and show maximum growth on PDA (potato dextrose agar) and sporulation on oat meal agar. These studies helped in developing artificial screening techniques for the identification of germplasm possessing resistance against foot rot disease.

Ahmed *et al* (1986) standardized the test tube inoculation method which involved soaking of grains for 48 h in water and incubating 10 sprouted grains with 5 drops of spore suspension (125×10^3 spores ml) in test tubes at room temperature for 7-10 days.

Khokhar and Jaffery (2002) in an attempt identify resistant sources against foot rot disease, used seed inoculation method for artificial screening process. The seeds of each test entry were treated with inoculum of *F. moniliforme* spore suspension containing 2000 spores/ml for two hours followed by planting of infected seeds in trays at 35^0C in green house.

Several varietal screening methods against foot rot disease were used which included the inoculation of seeds with spore suspension of the fungus and artificial spraying of gibberellic acid (GA_3). On application of different concentrations of GA_3 (Gibberellic acid) and spore suspensions of fungus to the different stages of variety BR-26, it was concluded that effect of GA_3 and spore suspension on growth of the seedlings was almost similar.It was suggested that the GA_3 treatment is best method to test the susceptibility of the large number of germplasm lines as it does not involves maintaining of the inoculum of fungus (Hossain *et al* 2013).

Kim *et al* (2014) reported a fast and reproducible inoculation method, using a tissue imbedding cassette and seedling tray for large scale evaluation of rice accessions for bakanae resistance. It was recommended that 80 seeds of each accession present in seedling tray should be surface sterilized in hot water bath at 57^0 C for 13 minutes and then 40 seeds should be soaked in spore suspension and 40 seeds in GA_3 (50ppm) for three days at 26^0C. Then the plants should be grown in a greenhouse (23°C-28°C, 12 h light). Evaluation was suggested after one month on the basis of disease severity index (percentage of healthy plants in each plot).

Further, Fiyaz *et al* (2014) reported a standard protocol that can be used to test the susceptibility of varieties. Two varieties Pusa Basmati 1121 (highly prone to disease) and Pusa 1342 (highly resistant) were infected with fungus by seed inoculation method and seedling dip method on the basis of results, it was recommended that the seed treatment method in which seeds were dipped in spore suspension ($1*10^6$ spores/ml) for one day at room temperature was more effective technique for artificial screening of germplasm against foot rot disease.

Chung *et al* (2016) suggested use of morphological traits like seedling length or width, leaf angle and color, second-internode length, second and third leaf length, aspect ratio (seedling height divided by the seedling width) etc for differentiation of healthy and infected seedling. The Images were taken by flatbed scanners and results were obtained from support vector machine (SVM). SVM1 classifiers were able to distinguish between inoculated or controlled seedling and SVM2 classifiers were to check the level of infection. The diseased and healthy seedlings of two varieties Tainan 11 and Toyonishiki screened after three week of incubation. This method was reported to have 87.9% accuracy.

2.8 Identification of resistant sources/QTLs

Using the seed inoculation method, Khokhar and Jaffery (2002) screened thirteen (10 coarse and 3 fine) rice varieties for their response towards foot rot disease. From these nine coarse grain cultivars showed 1% infected tillers while the fine cultivars Basmati 370 (20%), Super Basmati (65%), Basmati 385 (70%) infected tillers were found to have higher infection rates. It was concluded that fine cultivars were more susceptible than coarse cultivars.

Iqbal *et al* (2011) screened three coarse (IR-6, KS-133 and KS-282) and six fine cultivars (Bas-198, Bas-2000, Bas-Kernel, Bas-Super, Bas-370 and Bas-385) to test them against foot rot disease of rice. Seeds were sown in infested soil with inoculums of *F. moniliforme*. Among them, Bas-385 and Bas-Super were susceptible, IR-6 and KSK-133 were resistant, Bas-kernel, Bas-198 and Bas-370 were found to be moderately resistant.

Seven basmati varieties and eight non- basmati varieties against the foot rot disease by using seed inoculation method by Pannu *et al* (2013b). The basmati varieties were found susceptible, showing varying rates of incidence of the disease like 48 per cent on Pusa basmati 1121, 39 per cent on Punjab basmati 2, 24 per cent on Basmati 370, 22 per cent on Basmati 386, 2.5 per cent on Pusa basmati 1 while Punjab Mehak did not show any incidence. All non-basmati rice varieties except PR120 showed high resistance response.

Hur *et al* (2015) had reported the identification of a QTL associated with foot rot resistance on the basis of screening of 168 near isogenic lines developed form Shingwang (highly resistant) and Ilpum (highly susceptible) parents belonging to *indica* and *japonica* rice respectively. The QTL (*qBK1*) was present between the markers RM8144 and RM11295 on chromosome 1 & it explained 65% phenotypic variation in these lines.

Jain *et al* (2016) also reported the screening of 291 germplasm lines of aromatic germplasm using the technique developed by Fiyaz *et* al (2014). Only one entry, BPT 5204 showed resistant reaction, whereas, twenty two entries were observed to be moderately resistant. Seven genotypes showed moderately susceptible reaction while eight genotypes were found susceptible to the disease. Three genotypes namely Basmati 385, Type 3 and Tilak Chandan were found highly susceptible.

Similarly, Fiyaz *et al* (2016) used 168 F_{14} recombinant inbred lines (RILs) derived from Pusa 1342 (resistance) and Pusa Basmati 1121(Susceptible) for QTL mapping. Three QTLs, *qBK1.1*, *qBK1.2* and *qBK1.3* which explained 4.76, 24.74 and 6.49 percent of phenotypic variation were used for composite interval mapping. The 0.26 Mb regions between RM5336 and RM10153 markers showed the presence of *qBK1.2*. It was reported that these QTLs may be useful for the production of resistance varieties to foolish seedling disease.

Puyam *et al* (2017) also identified resistant sources against foot rot disease from the screening of one hundred and thirty three germplasm lines of basmati. The results revealed that, 21 lines were found moderately susceptible, 6 as susceptible, 6 as highly susceptible, 24 as moderately resistant, 7 as resistant, and 5 lines were identified as highly resistant.

Ji *et al* (2018) performed the QTL analysis based on the genetic map and mortality rate data. It revealed a major QTL, *qFfR1* which is located at 89.8 cM on chromosome1. QTL mapping was carried out using F2 and F3 plants derived from a cross between resistant variety, Nampyeong and a susceptible Korean japonica line, DongjinAD. There were three markers at this point: JNS01033, JNS01037, and JNS01041. A total of 15 genes were identified with annotations related to defence against plant diseases among the 179 genes in the *qFfR1* interval at 95% probability.

Kang *et al* (2019) used F2 and F3 population derived from crossing a BD-resistant and a BD-susceptible Korean japonica rice variety, 'Samgwang' and 'Junam', for QTL mapping. The degree of BD susceptibility of each F2 plant was evaluated on the basis of the mortality rate measured with corresponding F3 progeny seedlings by in vitro screening. A major QTL, *qFfR9* was discovered at 30.1 (cM) on chromosome 9 with 95% probability, it lies within a 7.24–7.56 Mbp interval. It was found that eight genes exhibited non-synonymous single nucleotide polymorphisms (SNPs) by comparing the 'Junam' and 'Samgwang' genome sequence data.

Lee *et al* (2019) identified the locus *qBK1* (35-kb region) which confers the resistance to bakanae disease by using recombinant inbred lines of two rice varieties, Shingwang (resistant) and Ilpum (susceptible). Sequence analysis of this region revealed four candidate genes, *LOC_Os01g41770, LOC_Os01g41780, LOC_Os01g41790, and LOC_Os01g41800*. There were many non-synonymous SNPs in *LOC_Os01g41770* and the transcript of *LOC_Os01g41790* was early terminated in Shingwang, whereas there were no differences in both *LOC_ Os01g41780* and *LOC_Os01g41800* sequences between Ilpum and Shingwang. Expression profiling of the four candidate genes showed the up-regulation of *LOC_Os01g41770, LOC_Os01g41780, and LOC_Os01g41790* in Ilpum and of *LOC_Os01g41800* in Shingwang after inoculation of *G. fujikuroi*.

There is another powerful tool, GWAS (genome wide association studies) which is useful to understand the genetics of complex traits. It has high resolution as compared to QTL mapping because it exploits thousands of recombination events happened in natural population over its evolutionary history. But, the success of GWAS depends upon genetic makeup and synthesis of association mapping population or panel, population structure, LD (Linkage disequilibrium) and LD decay within population.

The GWAS technique has been used in many major crops, such as maize, soybean, barley, wheat, tomato, sorghum and potato. Wang *et al* (2014) had reported the screening of 366 different accessions of *indica* rice against blast disease with 16 different strains by using GWAS technique and 30 QTLs resistant to blast disease were identified. Among these, one showed the strongest signal within blast resistant gene *Os11g0225100* on chromosome 11.

Similarly, the collection of *japonica* rice was screened against foot rot disease of rice which gave high to moderate resistance results (Volante *et al*, 2017). Using GWAS approach, some QTLs which showed resistance to foot rot disease in *japonica* rice were identified. Two regions, one on 1S (named as *qBK1_628091*) and second on 4L (named as *qBK4_31750955*) which were responsible for high phenotypic variation for foot rot.

2.9 Transcriptomic analysis

To understand the molecular mechanism of fungal diseases, transcriptomic analysis of both fungus-infected and healthy plant tissues is a powerful and effective approach as compared to traditional microarray analysis. It gives accurate quantification of gene expression with low background, high sensitivity, the capability to detect differential expression over a large dynamic range and high reproducibility for both technical and biological replicates (Nagalakshmi *et al* 2008).

Matic *et al* (2016) screened twelve genotypes of rice against foot rot disease and selected the two genotypes, Dorella and Selenio as susceptible and moderately resistance for comparative transcriptome profiling to identify the differentially activated genes. RNA was isolated from inoculated and uninoculated seedlings at one and three weeks after germination. Significant results were obtained from three week old seedlings. It was observed that 3119 transcripts were differentially expressed between the control and the infected seedling in Selenio and 5095 transcripts in Dorella. On sequencing, it was observed that basic defence mechanism genes like peroxidases, PR (pathogenesis related), glucanases are generally expressed in both genotypes. Upon infection germin-like proteins, glycoside hydrolases, cytochrome P450 monooxygenases, jasmonic acid biosynthetic pathway, MAP (Mitogen-activated protein) kinases, and WRKY genes showed high expression in Selenio genotype. The genes related to chitinase, gibberellins metabolic pathway and salicylic acid signaling pathway was upregulated in susceptible genotype.

Ji *et al* (2016) conducted transcriptomic analysis on 93-11 (resistant) and Nipponbare (susceptible) genotypes for bakanae disease of rice. Inoculum treatment was given to three days old seedling for twenty four hours and RNA was isolated seven days after treatment. Upon transcriptome analysis, it was found that 1152 and 1052 differentially expressed genes were present between the untreated and the treated seedlings of 93-11 and Nipponbare

genotypes. Further it was observed that WRKYs, WAK (wall associated kinases) and MAP3Ks genes in resistant genotype and five genes of POEI (Pollen_Ole_e_I) family were upregulated upon infection of *F. moniliforme.*

Ji *et al* (2019) used tandem mass tag (TMT) approach for relative quantitative proteomic comparison of infected and uninfected rice seedlings 7 days post-inoculation with two genotypes: the resistant genotype 93–11 and the susceptible genotype Nipponbare. In which total, 123 (77.2% up-regulated, 22.8% down regulated) and 91 (94.5% up-regulated, 5.5% down-regulated) differentially expressed proteins (DEPs) accumulated in 93–11 and Nipponbare, respectively. Only 11 DEPs were both shared by the two genotypes. Further analysis showed that a noticeable aquaporin, PIP2–2, was sharply upregulated in 93–11, which might be related to pathogen defense and the execution of bakanae disease resistance. Certain antifungal proteins were regulated in both 93–11 and Nipponbare with moderate FCs. Correlation analysis between the transcriptome and proteome revealed a significant positive correlation only in the resistant genotype, while no correlation was found in the susceptible genotype.

2.10 Gibberellic acid pathway and foot rot disease

The enhanced level of gibberellin production causing abnormal elongation of the seedlings leading to ultimate death is one of the characteristic features of this disease. Majority of the semi-dwarf rice germplasm harbours *sd1* gene as a dwarfing source. Ashikari *et al* (2002) reported the physiological, molecular genetics and biochemical characterisation of this gene. The *sd1* gene was reported to encode gibberellin biosynthetic pathway enzyme *GA20 oxidase* while the mutants of *sd1* gene showed the loss or substitutions of some nucleotide, leading to internal stop codons or substitutions of some amino acids which makes the GAs pathway defunct.

So the *sd1* gene may have some role in imparting resistance to foot rot. Ma *et al* (2008) tested the reaction of various rice genotypes to this fungus and GA_3 which have different dwarfing genes. They observed that genotypes having *sd1*gene were sensitive to GA_3 as well as susceptible to foot rot, while genotypes carrying *d1* dwarfing gene was insensitive to GA_3 but sensitive to foot rot. Genotypes carrying dwarfing genes like *d29, sd6, sdq(t)*were found to show resistance to foot rot.

he function of some GA regulated/synthesis genes have been characterised. Change in expression of these genes in foot rot resistant & susceptible varieties may provide some useful information, like GA regulated gene *OsXTH8* (Jan 2004) is responsible for elongation and cell wall organisation. In response to gibberellic acid *OsXTH8* gene helps in formation of primary cell wall using Xyloglucan by catalysing the xyloglucan endotransglucosylases

enzyme and more expression in root nodes and leaf sheath. Another GA regulated gene *OsGAE1* was identified by Jan (2006) whose expression was found to increase by rise in GA_3 concentration from 1uM to 50uM, starting from 30 min to 24 h after GA_3 treatment. From in-situ hybridization, they concluded that it showed high expression in leaf sheath, callus and meristematic region of shoot apex. *OsGAE1* gene produce N- terminal signal peptide (Tusnady and Simmon 2001) which causes the breakdown of cell membrane and release of apoplast.

OsWOX3A gene is a GA responsive gene which acts as negative regulator in gibberellic acid biosynthetic pathway. It directly interacts with promoter of *KAO* gene which encodes a GA biosynthetic enzyme, ent-kaurenoic acid oxidase and reduces its expression. Its main function is to maintain the level of GA pathway intermediates like GA_{20} and bioactive GA_1 throughout the development. But its expression is down regulated by exogenous application of paclobutrazol and upregulated by GA_3 (Cho *et al* 2015).

The other gene, *EUI* (Elongated uppermost internode) is a stage-specific gene which shows its expression in uppermost internode at heading stage and its product is responsible for the deactivation of biological active GAs. *Eui* gene encodes a previously uncharacterized cytochrome *P450 monooxygenase* which catalyses 16a,17-epoxidation of non-13-hydroxylated GAs and 16a,17-epoxidation reduces the biological activity of GA_4 in rice (Zhu *et al* 2006).

Galpha is alpha subunit of heteromeric G protein which plays an important role in GA signaling pathway. It is responsible for the induction of alpha amylase activity in aleurone cells and internode elongation. But *d1* mutant produced defective alpha subunit of heteromeric G protein which reduces the activity of alpha amylase activity in aleurone cells and results in dwarf rice mutant reported by Tanaka *et al* (2000). He also proposed another GA signaling pathway which is independent of *Galpha* protein but it shows less response to GA signaling as compared to *Galpha* protein dependent pathway.

GID1 (Gibberellin insensitive dwarf1) gene acts as functional receptor of GAs and plays an important role in its signaling pathway. It binds 16,17- dihydro-GA_4 in a saturable manner and shows ten times more affinity for biologically active GAs as compared to inactive GAs (Hartweck and Olszewski, 2016).

Sang *et al* (2015) further study the expression of *EUI, GID1, GID2, Galpha and SLR1* genes in two japonica varieties, Nampyeong and Hopum which is resistant and susceptible to foot rot disease of rice. Out of which, *EUI* and *GID1* genes showed low expression in susceptible variety as compared to resistant variety in the inoculum treated

plants but the expression of *Ga, SLR1* and *GID2* genes were low in resistant variety than susceptible one in infected plants.

Functional characterization of such genes in foot rot resistant and susceptible genotypes could reveal vital information on association of Gibberellic acid pathway with foot rot disease.

CHAPTER III

MATERIAL AND METHODS

The research was carried out to identify resistant donors /QTLs for foot rot disease and association of gibberellic acid regulated/synthesis genes with the disease. The screening for foot rot was carried out under artificial inoculation conditions in Rice Experimental Area, Department of Plant breeding and Genetics, PAU, Ludhiana for two consecutive years *kharif* 2018 and *kharif* 2019. In *kharif* 2019 the experiment was repeated two times by doing first sowing in April and second in July. A detailed description of the materials and the methodologies followed to conduct the experiments is given below:

3.1 Plant material

A diverse set of rice germplasm comprising of 278 lines (159 lines from rice 2k diversity panel, 10 accessions of *Oryza glaberrima*, 24 PAU released varieties, 67 aromatic lines, 17 elite breeding lines with RYT numbers along with one resistant and two susceptible checks) was evaluated under field condition at PAU, Ludhiana, Punjab. Pusa Basmati 1121 variety being highly susceptible to foot rot disease was used as susceptible check and CSR30, a moderately resistant variety was used as resistant check. The details of the material are given in Appendix I.

Table 3.1: Lines of 2k panel belonging to different subpopulations

Subpopulation*	**Number of genotypes**
Admixed	13
Admixed japonica	27
Aromatic	2
Aus	7
Indica	22
Temperate japonica	12
Tropical japonica	76
Grand Total	**159**

*Subpopulation classification is as per McCouch *et al* (2016)

3.2 Screening for foot rot resistance at Rice experimental area, PAU, Ludhiana

3.2.1 Experimental set up: The screening for foot rot resistance was done according to a high throughput screening protocol developed by Fiyaz *et al* (2014). For the purpose of artificial inoculations, culture of virulent isolate of *Fusarium moniliforme* was procured from Rice Pathology Lab, Dept. of Plant Breeding and Genetics, PAU, Ludhiana. Spore suspension

was prepared and 50 seeds of each test entry along with highly susceptible and resistant checks were soaked in 30 ml of inoculums suspension for 24 hours at room temperature. Untreated checks were soaked in sterile water. The inoculated seeds were then sown in rows on required beds in the trays containing sterilized soil. After 25 days seedlings were uprooted and dipped in inoculum for 24 hours and transplanted in field.

3.2.2. Data recording for disease traits: All the lines were evaluated for foot rot resistance using following characters:

1. Germination data was recorded one week after sowing.
2. Height of all inoculated and un-inoculated plants and the number of discolored, elongated, stunted and dead seedlings was recorded daily starting from 7^{th} day to 25^{th} day after inoculation.

The percent infected seedlings (including elongated and dead seedlings) were calculated by following formula:

$$\text{Disease incidence} = \frac{\text{Number of infected seedling}}{\text{Total number of plants}} \times 100$$

The data on percent infected seedlings was used to assess the level of resistance or susceptibility of each test genotype by using following disease rating scale:

Table 3.2: Screening based on percentage infected seedlings

Disease incidence(% infected seedlings)	Disease reaction
0-10	Highly Resistant
11-20	Resistant
21-40	Moderately Resistant
41-60	Moderately Susceptible
61-80	Susceptible
>80	Highly Susceptible

Disease severity index was calculated on the basis of 0-4 scale (Table 3.3) with following formula:

$$\text{DSI} = \frac{\Sigma \text{ numbers of plants in the specific scale} \times \text{disease scale}}{\text{Total number of plants observed}}$$

$$\text{DSI} = \frac{\Sigma\ (n\times0) + (n\times1) + (n\times2) + (n\times3) + (n\times4)}{\text{Total number of plants observed}}$$

Table 3.3: Disease scale and disease symptoms for seedling scoring

Disease Scale	Disease Symptoms
0	healthy and uninfected plants (no external symptoms)
1	normal growth but leaves beginning to show yellowish-green content
2	abnormal growth (shorter or taller than normal), thin and yellowish green leaves
3	abnormal growth (shorter or taller than normal), chlorotic, thin and brownish leaves
4	seedlings with fungal mass on the surface of infected plants or died

On the basis of disease severity index, the germplasm under evaluation was then categorised as given as below (Table 3.4)

Table 3.4: Screening based on Disease severity index (Halim *et al* 2015)

Disease severity index	Disease reaction
0-0.29	Resistant
0.30-1.99	Slightly Susceptible
2-2.99	Moderately Susceptible
3-4	Susceptible

3.3 Genome-wide association studies to identify QTL's for foot rot resistance

The high throughput SNP data of 159 germplasm lines used in evaluation is publically available at rice diversity project website (www.ricediversity.org). The "Rice diversity" is a NSF (National Science Fund), USA funded project which is a collaborative effort to understand the genetics of variation in rice. The project website hosts the SNP genotyping data of 1568 germplasm accessions of rice 2K panel. The genotypic data of the selected 159 accessions was extracted from the collective SNP genotyping data of 1568 rice accessions. The phenotypic and genotypic data of 159 accessions was used for genome wide association studies (GWAS) to identify significant QTLs (Quantitative Trait Loci) for resistance to foot rot disease. The disease severity index scale which was designed on the basis of phenotypic data used for GWAS is given below in Table 3.5.

Table 3.5: Disease severity index scale used for GWAS

DSI	Score	Reaction
0-0.29	1	HR
0.3-0.99	2	R
1-1.99	3	SS
2-2.99	4	MS
3-4	5	HS

The SNP data filtered with ≤ 20 per cent missing data and 0.05 minor allele frequency (MAF). GWAS was done in R (R Core Team 2018) package, GAPIT (Genome association and prediction integrated tool) version 3.0 (Lipka *et al* 2012) using FarmCPU (Fixed and random model circulating unification) model to calculate estimated *p* values of each SNP. FarmCPU is a multi-locus model that addresses the confounding problem of mixed linear models (MLM) by using population structure and multiple associated markers in fixed effect model (Liu *et al* 2016). The multiple associated markers estimated by incorporating kinship values in the random effect model (Liu *et al* 2016). Population structure estimated with principle component analysis (PCA). The associated *p* values of each SNP distributed on each chromosome were plotted as manhattan plots by using –log(p) values. To identify significant SNP-trait associations, significant threshold estimated by finding number of uncorrelated independent tests using linkage disequilibrium (LD) estimate (Zhang *et al* 2019).

3.4 RNA extraction, quantification and cDNA synthesis

The Pusa Basmati 1121, Pusa Basmati 1509 as susceptible varieties and PR127 as resistant variety were selected to study GA synthesis/regulated genes expression. RNA extraction was done through Trizol method (Chomczynski and Sacchi, 1987). It was extracted from seedlings of selected genotypes collected in liquid nitrogen at four different stages (10, 17, 24, 31 DAS) and was stored in -80^{0}C until processed. For RNA extraction, tissue samples were crushed in liquid nitrogen to fine powder. Trizol reagent (900µl) was added to 100mg of fine powder followed by inverting/tapping it for one or two times and then incubation for 5 minutes on ice. The mixture was centrifuged at 12000rpm for 10min at 0^{0}C. The supernatant was transferred to fresh tube. Then 200µl chloroform in each tube was added followed by vigorous shaking for 30secconds and incubation for 3-5 minutes on ice. Then, the mixture was centrifuged at 12000rpm for 15 minutes at 0^{0}C. The aqueous layer was transferred into fresh tube and equal amount of isopropanol was added .The solution was left at -20^{0}C for at least one hour. The solution was centrifuged at 12000rpm for 10 minutes at 0^{0}C and the isopropanol was removed. The pellets were washed with 1ml ethanol (75%) followed by centrifugation at 7500rpm at 0^{0}C for 5 minutes. This step was repeated twice. Pellets formed

were dried for three minutes on tissue paper. Finally, pellets were suspended in 50ul DEPC water and final purified RNA was stored at -20^{0}C.

3.4.1 RNA Quantification

RNA quantification was done on 1.2% agarose formaldehyde denaturing MOPS gel.

3.4.1.1 10X MOPS buffer preparation

For the preparation of 1000 ml of 10X RNA gel buffer, 41.84g of MOPS and 10.88 g of Sodium acetate was added in beaker containing 500 ml of DEPC water. During constant stirring, 20 ml of 0.5 M EDTA was added to the solution and its pH was adjusted to 7 using NaOH pellets. Final volume was made to 1000 ml with DEPC treated water (Table 3.6).

Table 3.6: 10X MOPS buffer preparation

Reagent	Final Concentration	Amount
MOPS	0.2M	41.84g
Sodium Acetate	80mM	10.88g
EDTA	10mM	20ml of 0.5M
Adjusted pH to 7 with NaOH Pellets and DEPC treated water was added to make the final volume upto 1000 ml.		

3.4.1.2 1X RNA gel Buffer

500 ml of 1X RNA gel buffer was prepared by adding 50ml of 10X RNA gel buffer in 450 ml of DEPC water.

3.4.1.3 1.2% denaturing agarose gel

For the preparation of 200 ml gel, 2.4g of agarose was added to 105ml of DEPC water and then the solution was boiled for 40 seconds intervals until agarose was completely dissolved. Solution was allowed to cool down to 65^{0}C under fume hood and 12 ml of 10X RNA gel buffer was added with 3ml of formaldehyde into the gel solution (Table 3.7). Gel was casted and allowed to solidify for 45 minutes. 1X RNA gel buffer was poured into gel apparatus so as to cover the gel and comb was removed. The gel was pre-run for 10 minutes at 70-100 V for equilibration with buffer.

Table 3.7: 1.2% denaturing agarose gel

Components	Final concentration	Amount
Agarose	1.2%	1.2g
*MOPS Buffer	1 X	10ml of 10X
*Formaldehyde	1%	3ml of 37%(w/v)
DEPC Water	Added to 100ml	

*Formaldehyde and 10 X MOPS buffer was added after boiling the agarose gel.

3.4.1.4 RNA Sample Preparation

The mixture was prepared by adding 1.25 µl 5X loading buffer to 2.5 µl of RNA sample and mixed properly. Then it was incubated at 65°C for 5-10 min for breaking the secondary structure of RNA. After that it was incubated on ice for 5 minutes, spinned briefly & then loaded onto equilibrated gel which was run for 1hr 30 min at 70-80 V (5 V/cm).

3.4.2 cDNA synthesis:

cDNA was synthesized using Takara first strand cDNA synthesis kit. The kit uses Prime Script Reverse Transcriptase (RT), an advanced enzyme derived by *in vitro* evolution of M-MLV (Moloney Murine) RT. The enzyme is capable of synthesizing cDNA upto12kb in length efficiently at 42^0C, even from the RNA templates that contain GC rich regions or complex secondary structures. Table 3.8 shows the different components of Template RNA Primer mixture.

Table 3.8: Template RNA Primer mixture

Reagent	**Volume (10 µl)**
Oligo dT primer or Random 6 mers	1µl
dNTP mixture	1µl
Template RNA	1-2µg
Nuclease free water	*µl
Total	10 µl

All the components were mixed gently and incubated for 5 minutes at 65 °C for denaturation of secondary structure. Then it was cooled immediately on ice. The cDNA synthesis reaction was set up which contained different components (Table 3.9).

Table 3.9: cDNA synthesis reaction set up

Reagent	**Volume (10 µl)**
Template RNA Primer mixture	10 µl
5X PrimeScript buffer	4µl
RNase Inhibitor	0.5µl
PrimeScript RTase	1µl
Nuclease free water to 20 µl reaction volume	

The RNase Inhibitor effectively protects RNA template from degradation by RNases A, B and C at temperatures up to 50°C. Nuclease-free water provided with the kit was used to make-up the reaction volume.

All the components were added to sterile RNase free tube, mixed gently and centrifuged. Then the reaction mixture was put into PCR tubes and incubated in a thermocycler at 42 ºC for 60 minutes and finally 5 minutes at 95 ºC to deactivate the enzyme. The synthesized cDNA was diluted to 50 µl and was stored at -20 ºC until qPCR analysis.

3.5 Quantitative PCR analysis

Real time quantitative PCR (RT-qPCR) is a very powerful approach for relative and absolute quantification of gene expression. It combines the two approaches, polymerase chain reaction and fluorescence signal detection. The output is received in the form of critical threshold (CT) or quantitation cycle (CQ). This CT/CQ is the cycle of amplification during PCR, when a minimum threshold fluorescence signal has been detected. The range of CT value lies between the numbers of amplification cycles specified during the PCR run. Generally lower CT value means higher the gene expression or vice-versa. This technique is basically used to estimate the copy number and fold change in the expression of a gene among treatments. For relative quantification, it requires a reference gene (a housekeeping gene) with equal expression among all developmental stages and tissues for normalization. In the relative quantification experiment four reactions were required to be set viz: control sample with housekeeping gene (HC), control sample with target gene (TC), experimental sample with housekeeping gene (HE) and experimental sample with target gene (TE). Reactions were performed thrice/triplicates. After taking the averages, 4 CT values came which were used for following calculations,

$$\Delta\mathrm{CT}\ (\mathrm{control}) = \mathrm{TC} - \mathrm{HC}$$

$$\Delta\mathrm{CT}\ (\mathrm{experimental}) = \mathrm{TE} - \mathrm{HE}$$

$$\Delta\mathrm{CT}\ (\mathrm{experimental}) = \mathrm{TE} - \mathrm{HE}$$

$$\Delta\Delta\mathrm{CT} = \Delta\mathrm{CT}\ (\mathrm{experimental}) - \Delta\mathrm{CT}(\mathrm{control})$$

$$\text{Fold change} = 2^{-\Delta\Delta CT}$$

The ΔCT is normalized value and ΔΔCT is actual difference of gene expression between the two samples at control and experimental conditions. A negative value indicates higher gene expression while a positive value indicates lower gene expression in experimental conditions as compared to control conditions. It is very powerful measure as we can observe the difference as lower as 0.5-fold (Livak *et al* 2001).

In present experiments, Ubiquitine 1 was used as an internal control for normalization. The treatment and control samples cDNA was synthesised from samples collected at 10 DAS, 17 DAS, 24DAS and 31 DAS. The reaction was set up using the reaction mixture as given in Table 3.10

Table 3.10: qRT-PCR reaction set up

Component	Volume (15 µl/well)
Template	1 µl (out of 50 µl)
Takara Sybr® green	5.5µl
Primer F/R (5 µM)	1.25 µl
Nuclease free water	7.25 µl
Total	15 µl

qRT-PCR was preincubated at 50°C for 2 minutes, followed by denaturation at 95°C for 3 minutes, then run for 40 cycles with denaturation at 95°C for 15 seconds followed by primer annealing and extension at 60°C for 1 minute. Primer specificity was tested using melt curve analysis by melting the final PCR product at 95°C for 15 seconds and then cooling down slowly to 60°C for reannealing of products. Multiple peaks in this analysis indicate multiple PCR products and non-specificity of the primer. Contrarily, a single peak indicates that the product accumulated is specific. The reaction was carried out in Roche LightCycler® 96 instrument and data was analyzed using LightCycler® 96 1.1 software and Micro Soft Excel.

CHAPTER IV

RESULTS AND DISCUSSION

The current research was undertaken to identify the novel sources for foot rot resistance in a set of 275 rice germplasm lines which consisted of 159 lines of 2K diversity panel, 10 *O. glaberrima*, 22 released varieties, 67 ANP lines and 17 RYT lines (Appendix I). Another aspect of the research was aimed at finding out QTLs associated with foot rot resistance through Genome Wide Association Studies (GWAS). A subset of 159 lines of rice belonging to 2k diversity panel collected from IRRI, Philippines was used for this purpose. The third aspect of the research was focused on studying association of gibberellic acid regulated/synthesis genes with foot rot disease by expression analysis from two extreme susceptible and resistant germplasm lines. Results obtained from the current research programme are summarized below under the following sub-headings:

4.1 Evaluation of rice germplasm for foot rot resistance.

4.2 Genome Wide Association Studies to map the QTLs for disease variables.

4.3 Association of GA regulated/synthesis genes with the foot rot disease.

4.1 Evaluation of rice germplasm for foot rot resistance:

The evaluation of rice germplasm for foot rot disease was carried out at Punjab Agricultural University, Ludhiana for two crop seasons viz. *kharif* 2018 & *kharif* 2019. The methodology described by Fiyaz *et al* (2014) was used for artificial inoculations and evaluation of germplasm.

In the month of July 2018, inoculated seeds of all lines were sown in portrays with 15 plants for each entry. Germination data was recorded 7 days after sowing of seeds. The germination percent of 159 lines varied from 40 to 100 percent, *O. glaberrima* lines 73.33 to 100 percent and of 22 released varieties from 80 to 100 percent. Out of 67 aromatic lines, 11 lines showed no germination and germination range of other lines varied from 13.33 to 100 percent. Its range varied from 13.33 to 100 percent in RYT lines. Data of different parameters like seedling height, elongated, stunted, pale and dead seedlings was recorded at four days interval from 8 to 24 days after sowing and used to check occurrence of foot rot disease. On the basis of disease severity index, 113 lines were found to be resistant, 43 lines were slightly susceptible, four lines were moderately susceptible out of 159 germplasm lines. All *O. glaberrima* lines showed resistant reaction. Out of 22 released varieties, 16 were found to be resistant and 6 were slightly susceptible. Out of 67 ANP lines, 11 lines did not germinate and from the rest, 37 lines were resistant, 34 lines were slightly susceptible and 2 lines were susceptible. Among RYT lines 6 were found to be resistant and 11 were slightly susceptible

(Table 4.1). However, despite of proper care, the material suffered from nutrient deficiencies which also caused drying and mortality. To authenticate the observations, the material was again evaluated in the year 2019 *kharif* season.

Table 4.1: Disease reaction of germplasm based on disease severity index evaluated during *kharif* 2018 crop season

Germplasm Group	Disease Reaction	Disease Severity Index	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	0.000-0.285	113
	Slightly Susceptible	0.307- 1.538	42
	Moderately Susceptible	2.000-2.600	4
O. glaberrima	Resistant	0.000-0.266	10
Released varieties	Resistant	0.000-0.285	16
	Slightly Susceptible	0.533-0.923	6
ANP lines	Resistant	0.000-0.285	37
	Slightly Susceptible	0.307-1.733	34
	Susceptible	4.000	2
RYT lines	Resistant	0.00-0.285	6
	Slightly Susceptible	0.307-1.636	11

Two sowings were done during this crop season, one in the month of April and other in July. During April 2019, inoculated seeds of all lines were sown in two replications along with control and mean value were taken for different parameters. In the subset of 159 lines of 2k panel, two lines did not germinate while the germination percentage among other 157 lines varied from 10 to 100. The germination percentage of checks plants with this subset varied from 0 to 100. In the subset of 22 released varieties, two failed to germinate and the germination percentage in the remaining set varied from 10 to 100 in both inoculated and checks. In subset of 10 O. *glaberrima* lines, three lines did not germinate while the germination percentage of rest of the lines varied from 20 to 85 and in its checks. It varied from 10 to 90. Out of 67 ANP lines, five lines failed to germinate and in rest of the lines it ranged from 15 to 95 percent in inoculated plants and 10 to 100 percent in control plants. One RYT line did not germinate and in other lines germination percentage ranged from 15 to 90 percent.

Disease reaction was calculated on the basis of disease severity index. Out of 159 lines, 19 lines were found to be resistant, 102 were slightly susceptible, 24 were moderately susceptible and 10 were susceptible to the disease (Table 4.2).

Table 4.2: Disease reaction of germplasms based on disease severity index evaluated during April 2019 crop season

Germplasm Group	Disease Reaction	Disease Severity Index	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	0.000-0.285	19
	Slightly Susceptible	0.292- 1.982	104
	Moderately Susceptible	2.000-2.857	24
	Susceptible	3.000-4.000	10
O. glaberrima	Resistant	0.000-0.166	5
	Slightly Susceptible	0.485-0.971	2
Released varieties	Resistant	0.000	8
	Slightly Susceptible	0.633-1.833	10
	Moderately Susceptible	2.000-2.333	2
ANP lines	Resistant	0.000-0.225	3
	Slightly Susceptible	0.667-1.889	28
	Moderately Susceptible	2.000-2.900	16
	Susceptible	3.000-4.000	15
RYT lines	Resistant	0.222	1
	Slightly Susceptible	0.525-1.694	12
	Moderately Susceptible	2.000-2.133	2
	Susceptible	3.222	1

In the *O. glaberrmia* five lines were found to be resistant and two were slightly susceptible. Among the released varieties eight varieties were found to be resistant, 10 were slightly susceptible whereas two were moderately susceptible. In the ANP germplasm subset, three lines gave resistant reaction, 28 were found to be slightly susceptible, 16 were moderately susceptible and 15 showed susceptible reaction to the disease. Among RYT lines, one lines gave resistant reaction, 12 were found to be slightly susceptible, 2 were moderately susceptible and one showed susceptible reaction to the disease (Table 4.2**).** The frequency distribution of 2K rice diversity penal germplasm for disease severity index revealed normal distribution which varied from 0 to 4 (Fig. 4.1).

Plate 1: *Fusarium moniliforme* grown on gram culture to harvest microconidia for inoculum

Plate 2: Soaking of seeds in microconidial suspension

Plate 3: Stunted plants of Basmati 386 at 17DAS

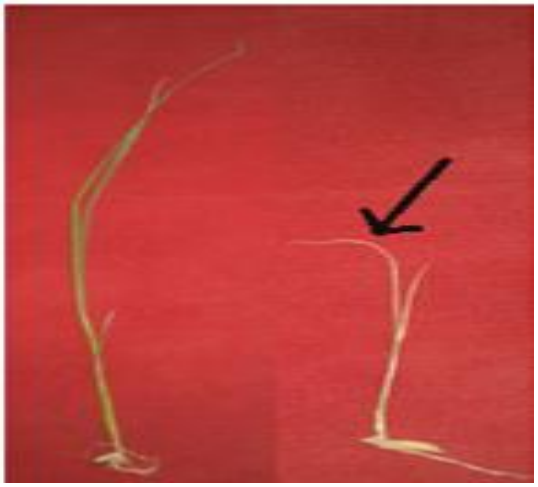

Plate 4: Healthy and stunted plants of PB 1121 at 17DAS

Plate 5: Fungal growth on inoculated plants as compared to uninoculated plants

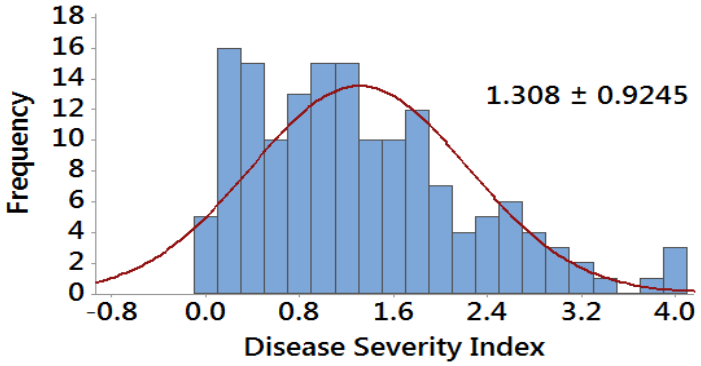

Fig. 4.1: Frequency distribution of disease severity index

Data of seedling length was recorded 24 days after sowing. Stunting was observed in most of the lines. It may be due to dry weather conditions and less relative humidity in open environment during April month. It was reported in literature that elongated seedlings developed only under damp soil or high humidity (60 to 80 %) conditions while arrested growth and stunting occurs in dry soil or less humid conditions (Seto 1933, Singh and Sunder 2012). Disease reaction based on the mean elongation/stunting was divided into two categories: Resistant and Susceptible. Those lines which showed significant elongation/ stunting over control plants were regarded as susceptible and others as resistant. Range of seedling height varied from 4.78 to 32.45 cm (Fig. 4.2).

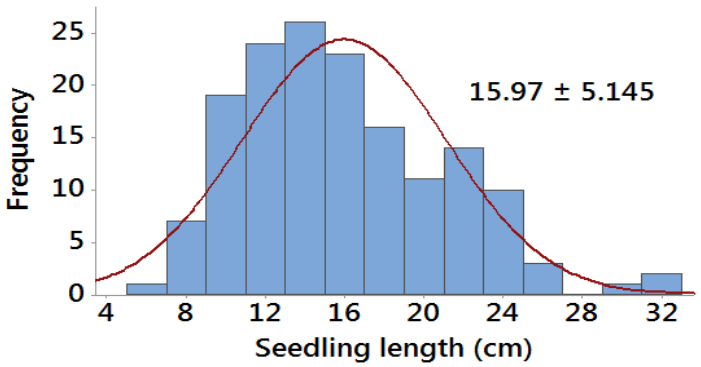

Fig. 4.2: Frequency distribution of mean seedling length (cm)

On the basis of these observations 79 lines of 2K panel, 7 released varieties and 42 ANP lines showed significant stunting. Only six lines showed significant elongation in which four were ANP lines and two lines belonged to 2K panel germplasm. In total, 78 lines from 2K diversity panel, 13 released varieties, 7 lines of *O. glaberrima*, 22 ANP lines and 10 RYT lines showed resistant reaction (Table 4.3). Some uninoculated plants were also dead. For its

confirmation, dead plant samples were taken from both inoculated and uninoculated plants. After surface sterilization with 0.1 percent $Hgcl_2$, put it on PDA media separately. After seven days it was observed that mycelial growth occurs only on dead samples which were taken from inoculated plants. And samples from uninoculated plants were free from mycelia growth (Plate 5). It means Plant died among the uninoculted plants may be due to nutrient deficiency or any other reason.

Table 4.3: Disease reaction on the basis of mean stunting evaluated during April 2019 crop season:

Germplasm Group	Disease reaction	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	78
	Susceptible	79
O. glaberrima	Resistant	7
	Susceptible	0
Released varieties	Resistant	13
	Susceptible	7
ANP lines	Resistant	22
	Susceptible	36
RYT lines	Resistant	10
	Susceptible	6

The material under evaluation was also characterized for another parameter of foot rot evaluation. On the basis of disease incidence, disease reaction was divided into six categories as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (Fiyaz *et al* 2014). From the material under evaluation, 49 lines were found to be highly resistant, 42 were resistant, 82 were moderately resistant, 55 were moderately susceptible, 26 were susceptible and 9 lines were highly susceptible. Most of the resistant germplasm lines belonged to 2K rice germplasm. It exhibited a complete range of diversity for foot rot disease as the incidence varied from 0 to 100 percent (Fig. 4.3). This in turn reflects the importance of genetic diversity in crop improvement programs. Among the *O. glaberrima* lines, the disease reaction varied from 0 to 36.42 percent, in released varieties it was varying from 0 to 61.905 percent whereas the range of disease incidence was 0 to 100 percent in ANP lines. Among the RYT lines it varied from 5.556 to 62.22 percent. The disease incidence was 71.429 percent in Pusa Basmati 1121 and 64.286 percent in Pusa Basmati 1509, which were the susceptible checks. Resistant check CSR30 was free from disease.

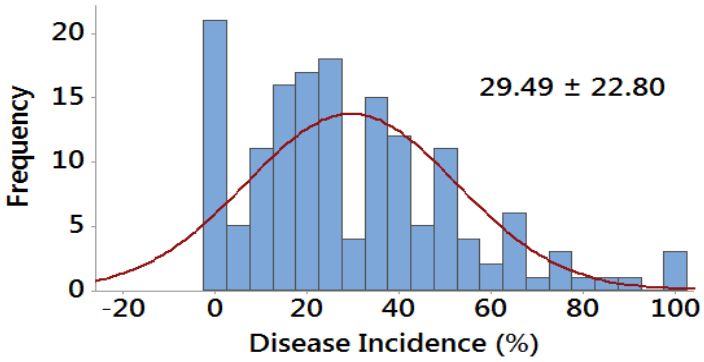

Fig. 4.3: Frequency distribution of percent disease incidence

Seedling mortality is another parameter which has also been used to check the susceptibility of lines to foot rot disease. Fiyaz *et al* (2016) recorded seedling mortality in 168 F_{14} RILs and reported that it ranged from 0-100% in which frequency of class 31-40% was highest. The seedling mortality was recorded 24 days after sowing and was found to be range from 0 to 100 percent among 159 lines of 2K rice diversity penal (Fig. 4.4). Among *O. glaberrima* lines, it varied from 0 to 12.143 percent. Among released varieties seedling mortality varied from 0 to 58.333 percent and among ANP lines, the range was varied from 0 to 91.667 percent. In RYT lines it varied from 5.556 to 62.22 percent. Among the susceptible checks, 57.143 and 42.857 percent seedling mortality was observed in Pusa Basmati 1121&Pusa Basmati 1509 respectively. No mortality was observed in resistant check CSR30.

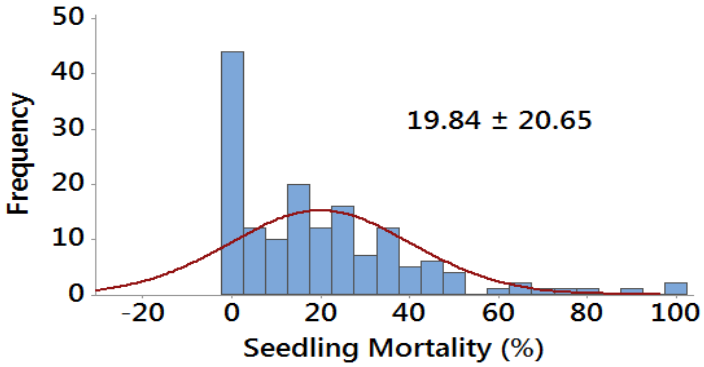

Fig. 4.4: Frequency distribution of percent seedling mortality

During further confirmation the material was sown in the month of July 2019. Forty eight ANP and 15 RYT lines which gave susceptible response during two consecutive testing's skipped from the testing. List of these lines was sown in Appendix II. But, some susceptible and resistant were included to take observations on disease appearance as during

the April sowing, climatic conditions were dry while July sowing coincided with onset of monsoons. A total of 159 lines were selected for evaluation during this trial. Also, contrary to use of portrays was carried out during the two previous screenings, third screening was done in the nursery beds under field conditions. During this final stage of testing, the same set of uninoculated lines was also planted in parallel beds for actual comparison in terms of elongation/stunting. The germination percentage of 159 lines varied from 10 to 100 percent in inoculated and 30 to 100 percent in control plants. Among *O. glaberrima* lines it varied from 10 to 93.33 percent (inoculated) and 73.33 to 100 percent (control plants). In released varieties, it varied from 11.66 to 100 percent (inoculated) and 86.66 to 100 percent (control plants). It was observed that in general the range of germination in inoculated plants was less as compared to control plants in all the germplasm groups. In ANP lines germination ranged from 8.33 to 88.33 percent (inoculated) and 13.33 to 100 percent (control plants). In RYT lines, germination was observed 10 percent in inoculated and from 30 to 33.33 percent in control plants.

Range of disease severity index varied from 0 to 1.84 percent in 157 lines of 2K diversity panel (Fig. 4.5). On its basis, out of total 159 lines, 109 lines were found to be resistant, 48 were slightly susceptible whereas 2 were susceptible to foot rot disease (Table 4.4).

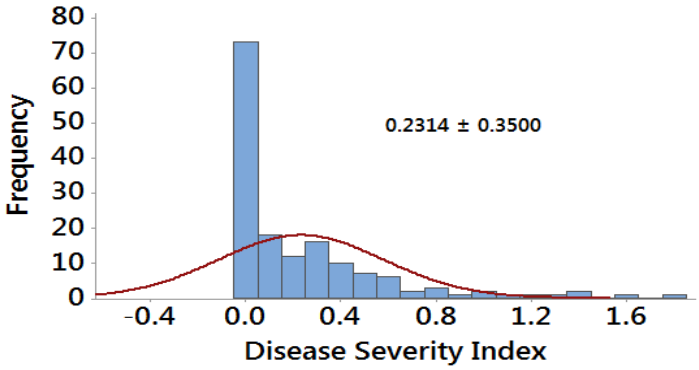

Fig. 4.5: Frequency distribution of disease severity index

Seven lines of *O. glaberrima* showed the resistant reaction and three were slightly susceptible to the disease. Eleven released varieties showed resistant, 8 slightly susceptible and one moderately susceptible reaction. Among the ANP lines included in the set, 9 were found to be resistant, 9 were slightly susceptible whereas one was susceptible. Both RYT lines included in the set showed slightly susceptible response (Table 4.4).

Table 4.4: Disease reaction of germplasms based on disease severity index evaluated during July 2019 crop season:

Germplasm Group	Disease Reaction	Disease Severity Index	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	0.000-0.285	109
	Slightly Susceptible	0.293 - 1.625	48
	Susceptible	4.000	2
O. glaberrima	Resistant	0.000-0.190	7
	Slightly Susceptible	0.500-0.678	3
Released varieties	Resistant	0.000	11
	Slightly Susceptible	0.333-1.828	8
	Moderately Susceptible	2.181	1
ANP lines	Resistant	0.000-0.250	9
	Slightly Susceptible	0.333-1.652	9
	Susceptible	3.100	1
RYT lines	Slightly Susceptible	0.500-0.666	2

Further classification of 2K diversity panel indicated that resistance was mostly observed in tropical japonica, admixed and indica germplasm lines. Seven temperate japonica lines and 14 admixed japonica lines were also found to be resistant to foot rot disease (Table 4.5).

Table 4.5: Disease reaction of different subpopulations belonging to 2k panel germplasm

Sub-populations*	No. of genotypes	Resistant	Susceptible
Admixed	13	11	2
Admixed japonica	27	14	13
Aromatic	2	0	2
Aus	7	3	4
Indica	22	18	4
Temperate japonica	12	7	5
Tropical japonica	76	71	5

*Subpopulation classification is as per McCouch *et al* (2016)

Height of 157 lines of 2K diversity panel varied from 26.02 to 49.1 cm (Fig. 4.6). The data of mean elongation revealed that 48 lines of 2K diversity penal, 3 lines of *O. glaberrima*, 8 ANP lines and 10 released varieties were susceptible to the disease (Table 4.6).

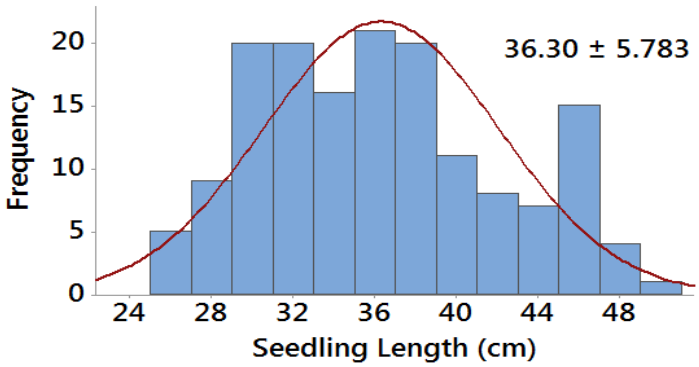

Fig. 4.6: Frequency distribution of seedling length (cm)

Table 4.6: Disease reaction on the basis of mean seedling length (cm) evaluated during July 2019 crop season:

Germplasm Group	Disease reaction	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	109
	Susceptible	48
O. glaberrima	Resistant	7
	Susceptible	3
Released varieties	Resistant	10
	Susceptible	10
ANP lines	Resistant	11
	Susceptible	8
RYT lines	Resistant	2

Observations on disease incidence indicated that 142 lines were highly resistant, 37 lines were resistant, 16 lines were moderately resistant, 7 lines were moderately susceptible, 6 lines were susceptible and 2 lines were highly susceptible. Among 159 lines of 2K diversity panel range of the disease incidence varied from 0 to 67.13 percent (Fig. 4.7). Among *O. glaberrima* lines, it ranged from 0 to 30.09 percent, in released varieties from 0 to 78.11 percent. In set of ANP lines, the range of disease incidence was varied from 0 to 63.33 percent. In RYT lines it varied from 12.6 to 16.66 percent. Among the susceptible checks, 71.875 percent disease incidence in Pusa Basmati 1121 and 78.636 percent in Pusa Basmati 1509 was observed. No incidence was observed in resistant check CSR30.

Plate 6: Elongation symptoms at 24 DAS

Plate 7: Significant difference between heights of inoculated and uninoculated plants of PB1121 at 24DAS

Plate 8: No significant difference between heights of uninoculated and inoculated plants of PR127 at 24DAS

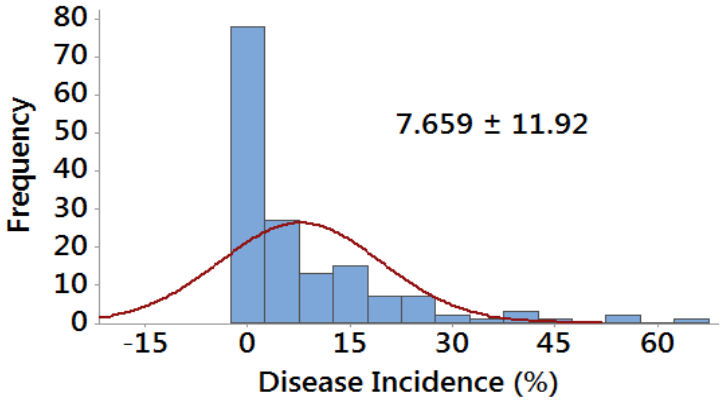

Fig. 4.7: Frequency distribution of percent disease incidence

Seedling mortality was observed to vary from 0 to 33.333 percent in 159 lines of 2K diversity panel (Fig. 4.8). In *O. glaberrima* lines, it varied from 0 to14.285 percent, in released varieties mortality ranged from 0 to 29.946 percent and in ANP lines from 0 to 45 percent. In RYT lines it varied from 12.6 to 16.66 percent. The mortality rate was observed to be 28.125 percent in Pusa Basmati 1121 and 21.09 percent in Pusa Basmati 1509 which were the susceptible checks. No mortality was observed in resistant check CSR30. Ji *et* al (2018) observed 0-10 percent mortality rate in resistant variety Nampyeong and 90-100 percent in susceptible variety DongjinAD. Wide variation of mortality rate in the F3 families from the cross between DongjinAD and Nampyeong varieties.Kang *et al* (2019) conducted also in vitro screening of F2 population derived from Junam (susceptible) and Samgwang (resistant) cultivars along with parents. It was observed that mortality rate ranged from 0% to 100% (32 days after inoculation), in which frequency of 50–60% class was the highest.

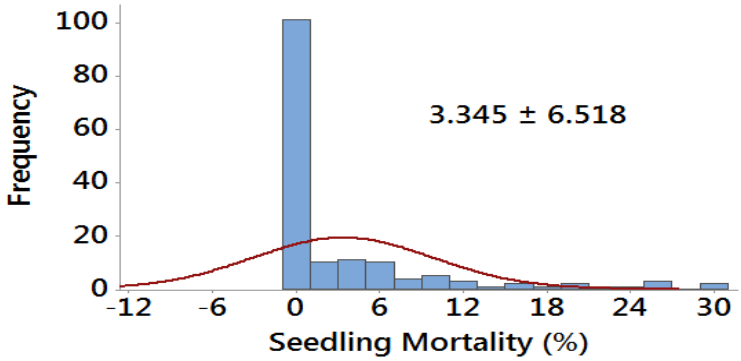

Fig. 4.8: Frequency distribution of percent seedling mortality

After taking observations in nursery, the surviving material was transplanted to field for further observations after 25 days. Two lines of 2k penal germplasm died in nursery and rest 157 lines were transplanted in field. Range of disease severity index varied from 0 to 4 (Fig. 4.9). Out of which 43 lines were found to be resistant, 107 were slightly susceptible, 5 were moderately susceptible whereas 2 lines were susceptible to the disease (Table 4.7).

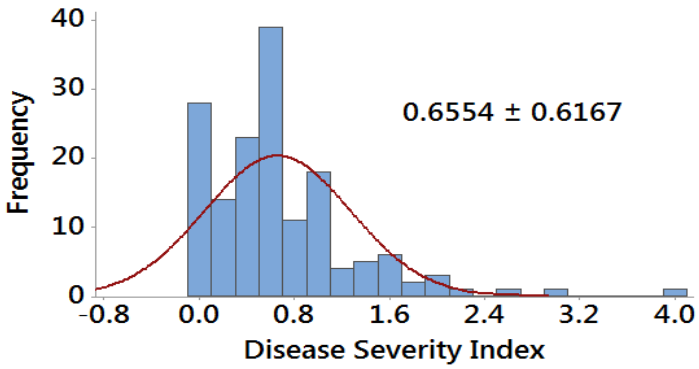

Fig. 4.9: Frequency distribution of disease severity index

In *O. glaberrima* set, 6 lines were found to be resistant and 4 were slightly susceptible. Among the released varieties, 12 varieties were resistant, 6 were slightly susceptible, one was moderately susceptible to the disease. One RYT line gave slightly susceptible response. Out of ANP lines, 5 lines were found to be resistant, 14 slightly susceptible to foot rot disease based on disease severity index (Table 4.7).

Table 4.7: Disease reaction of germplasm based on disease severity index evaluated during July 2019 (transplanted) crop season:

Germplasm Group	Disease Reaction	Disease Severity Index	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	0.000-0.285	43
	Slightly Susceptible	0.333-1.777	107
	Moderately Susceptible	2.000-2.500	5
	Susceptible	3.000-4.000	2
O. glaberrima	Resistant	0.000	6
	Slightly Susceptible	0.333-1.200	4
Released varieties	Resistant	0.000-0.167	13
	Slightly Susceptible	0.333-1.167	5
	Moderately Susceptible	2.000	1
ANP lines	Resistant	0.000-0.167	5
	Slightly Susceptible	0.333-1.500	14
RYT line	Slightly Susceptible	0.333	1

Among 43 lines of 2K diversity panel, showing resistant reaction under field conditions, most of the lines belong to tropical japonica and indica groups. Four lines of each admixed, admixed japonica and temperate were found to resistant (Table 4.8).

Table 4.8: Disease reaction of sub-population of 159 lines belonging to 2K penal germplasm

Sub-population*	No. of genotypes	Resistant	Susceptible
Admixed	13	4	9
Admixed japonica	27	4	23
Aromatic	2	0	2
Aus	7	2	5
Indica	22	14	8
Temperate japonica	12	4	8
Tropical japonica	76	15	61

*Subpopulation classification is as per McCouch *et al* (2016)

The observations on plant height were taken 45 days after transplanting. It varied from 51.5 to 137.5 cm in 157 lines of 2K diversity panel (Fig. 4.10). Fifty eight lines of 2K penal showed elongation but one line (YANCAOUSSA::IRGC16071-C1) which belonged to tropical japonica group showed significant stunting. One line of *O. glaberrima*, 5 released varieties, 11 ANP lines and one RYT line showed significant elongation (Table 4.19) as compared to control. The height of plants showing resistant reaction was comparable both in inoculated & uninoculated sets.

Table 4.9: Disease reaction on the basis of mean plant height (cm) evaluated during July 2019 (transplanted) crop season:

Germplasm Group	Disease reaction	No. of lines
159 lines derived from Rice 2k diversity panel	Resistant	97
	Susceptible	59
O. glaberrima	Resistant	9
	Susceptible	1
Released varieties	Resistant	14
	Susceptible	5
ANP lines	Resistant	8
	Susceptible	11
RYT line	Susceptible	1

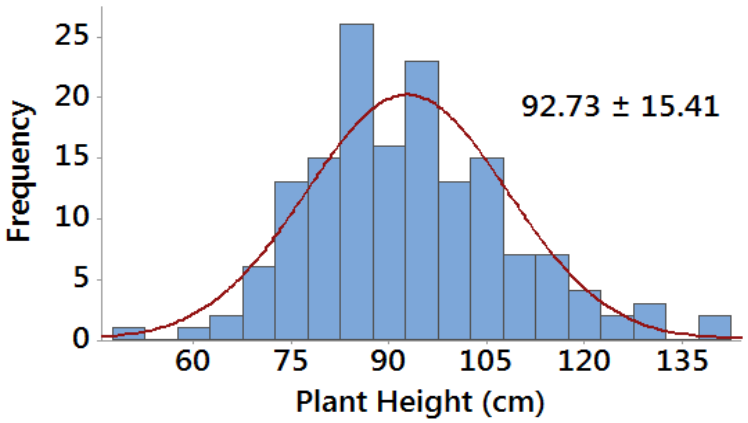

Fig. 4.10: Frequency distribution of plant height (cm)

On the basis of disease incidence, 89 lines were found to be highly resistant, 50 lines were resistant, 53 lines were moderately resistant, 12 lines were moderately susceptible, 2 lines were susceptible and one line was highly susceptible. Most of the resistant lines were belongs to 2K panel in which its range varied from 0 to 100 percent (Fig. 4.11) Among *O. glaberrima* lines, the range varied from 0 to 30 percent, in released varieties from 0 to 50 percent, in ANP lines from 0 to 50 percent and 16.67 percent in RYT lines. The disease incidence was 75 percent & 50 percent in Pusa Basmati 1121 and Pusa Basmati 1509 which were the susceptible checks. No incidence was observed in resistant check CSR30.

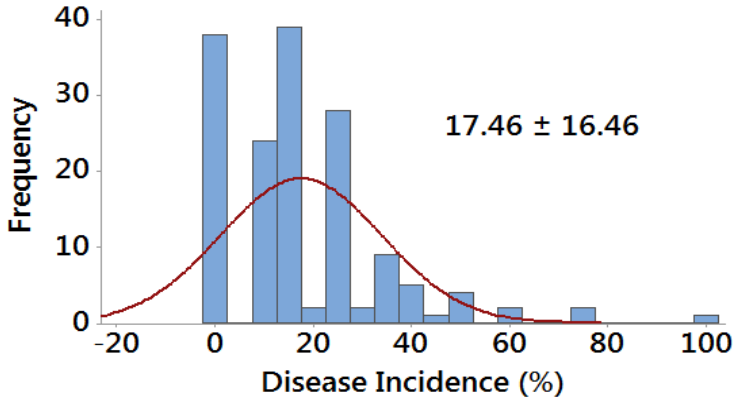

Fig. 4.11: Frequency distribution of percent disease incidence

Further observations seedling mortality indicated variation from 0 to 100 percent among 159 lines of 2K diversity penal (Fig. 4.12). In *O. glaberrima* lines, it varied from 0 to 30 percent, in released varieties from 0 to 50 percent, in ANP lines from 0 to 37.5 percent and 0 percent in RYT line. Seedling mortality was observed 50 percent and 41.96 percent in Pusa Basmati 1121 & Pusa Basmati 1509 which were the susceptible checks. No mortality was observed in resistant check CSR30.

Plate 9: Significant elongation after transplanting

Plate 10: Significant stunting after transplanting

Plate 11: Formation of adventitious roots

Plate 12: White mycelial growth on base of plant

Plate 13: Partially drying of foot rot infected plant

Plate 14: Partial filled sterile/empty or normal panicle

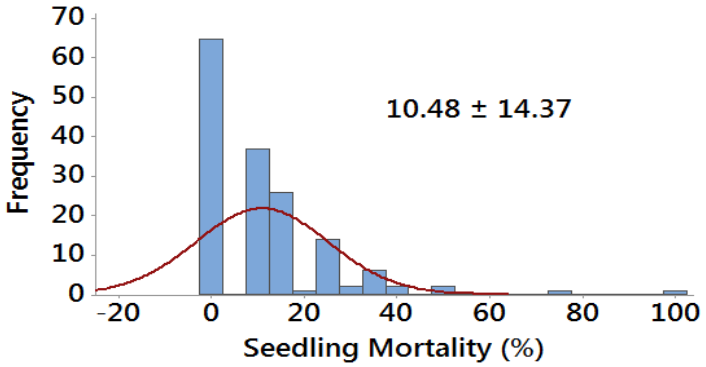

Fig. 4.12: Frequency distribution of percent seedling mortality

Overall on the basis of nursery and transplanted data evaluation, it was concluded that 25 lines showed completely resistant reaction to the disease, out of which 18 lines belongs to 2K diversity penal, 5 lines to *O. glaberrima*, 9 released varieties and 3 ANP lines (Appendix III). Among the 18 lines of 2K diversity penal showing resistant reaction, most of the lines belong to indica and tropical japonica groups (Table 4.10).

Table 4.10: Disease reaction of sub-population of 159 lines belonging to 2K penal germplasm

Sub-population*	No. of genotypes	Resistant	Susceptible
Admixed	13	2	11
Admixed japonica	27	1	26
Aromatic	2	0	2
Aus	7	0	7
Indica	22	7	15
Temperate japonica	12	0	12
Tropical japonica	76	8	68

*Subpopulation classification is as per McCouch *et al* (2016)

On the basis of overall evaluation for two seasons and three sowings, it found that 18 lines of 2K diversity panel, 5 lines of *O. glabberrima*, 2 ANP lines and 10 released varieties (Table 4.11) were identified as completely resistant to foot rot disease of rice which can serve as a novel sources for developing foot rot resistant germplasm coupled with agronomic desirability & acceptable quality.

Different cultivars of basmati rice viz., Basmati 386, Basmati 370, Punjab Basmati 2, Punjab Basmati 3 Punjab Basmati 4, Punjab Basmati 5, Pusa 1637, Pusa 1718, BPT 5204 gave susceptible except Pusa Basmati 1 which gave resistant response. All non- basmati

varieties gave resistant reaction except PR 120 to the disease. The results are in concurrence with the report of Pannu *et al* (2013) who also observed similar reaction in Basmati 386, Basmati 370, Pusa Basmati 1, Punjab Basmati 2, PR 113, PR 114, PR 115, and PR 120 rice varieties.

Table 4.11: Resistant lines belonging to whole germplasm

Germplasm group	Resistant lines
2K rice diversity penal	CHIGYUNGDO::IRGC55466-1, ZHENSHAN 97B, CO 39, GOGO LEMPAK::IRGC43392-C1, WAB 368-B-1-H1-HB::IRGC117359-1, NSICRC 106::IRGC117370-1, GUAYQUIRARO P A::IRGC116987-1, NS 1288::IRGC68930-1, CANA ROXA, HAWM OM, IR64-21, 63-104::IRGC15100-C1, PERLA::IRGC117021-1, GANIGI::IRGC48698-C1, OS 4::IRGC11335-C1, ICTA POLOCHIC::IRGC116997-1, CT 9993-5-10-1-M::IRGC116974-1, KAKANI 2::IRGC13373-C1
O. glaberrima	W 14 *(IR102512)*, W 16 *(IR102526)*, W 26 *(IR102615)*, W 30 *(IR103292)*, W 35 *(IR103545)*
ANP Lines	Sukala Phool, NDR8497-2
Released Varieties	Pusa Basmati1, PR113, PR114, PR115, PR121, PR122, PR123, PR124, PR126, PR127

Different parameters of disease evaluation have been used by various researchers to evaluate germplasm for foot rot resistance like disease severity (Halim *et al* 2015), disease incidence (Khokhar and Jaffery 2002, Fiyaz *et al* 2014, Puyam *et al* 2017) and seedling mortality rate (Fiyaz *et al* 2016, Ji *et al* 2018, Kang *et al* 2019). Significant stunting/elongation were considered as the most important factor to check the disease reaction of any line against foot rot disease of rice. Few cultivars were tested in natural infected fields of Zanjan province to identify resistant sources by Saremi *et al* (2008). On the basis of pathogenicity test, infected fields were found to have high density of pathogen (1575 colony forming propagule unit in one gram of soil) as compared to other fields (145cfu/g) where healthy crop was grown. It was observed that mostly diseased plants were found to be stunted. Then, it reported that stunting may be due high density of pathogen in that naturally infected field. Significant elongation was observed by Fiyaz *et al* (2016) in 168 F_{14} RILs (1.7 to 68.5%) under controlled conditions and by Kang *et al* (2019) in the in-vitro screening of F2 population derived from Junam (susceptible) and Samgwang (resistant) cultivars as compared to control.

Generally, foot rot disease is characterized by two major symptoms: elongation or

stunting of infected plants. Infact, rate of elongation or stunting depends on several factors like climatic conditions mainly temperature, relative humidity and rainfall, planting time, soil moisture, types of seeds or variety, kind of pathotypes and inoculum potential (Nyvall 1999, Sharma and Bagga 2007, Amtaulli *et al* 2010, Kaur *et al* 2014). Gibberellins hormone makes rice seedlings and plants to grow tall, thin, yellow and dead. However, high level of fusaric acid causes stunted and chlorotic seedlings followed by root and crown rots, which eventually die (Amoah *et al* 1995, Karov *et al* 2009, Fiyaz *et al* 2016, Pyuam *et al* 2017). Basically stunting was found to occur under dry and elongation under high soil moisture or humid conditions (Singh and Sunder 2012). It was observed that rate of disease incidence and the elongation and stunting type of symptoms also depends upon different isolates of *Fusarium moniliforme* (Pyuam *et al* 2017). So in the present studies also, mainly the stunting types of symptoms were observed during April 2019 screening as the dry weather considerations prevailed at that time. During July 2019 crop season, the elongation types of symptoms were mainly observed as the period coincided with monsoon leading to increased humidity. Overall the experiment revealed interesting results giving a range of variation for foot rot disease in tested germplasm and varieties under different climatic conditions. The studies also led to the identification of resistant sources for foot rot disease for further use in breeding programmes.

4.2 Genome Wide Association Studies (GWAS) to map QTLs for disease resistance

Genome wide association studies (GWAS) has emerged as a powerful approach to identify genes/QTLs underlying complex traits. The success of GWAS largely depends upon combining geographical diversity existing within a species to generate a diverse association mapping population/panel. Once these panels are thoroughly genotyped, these can be used for studying the variation for traits of interest. In rice, two such panels: rice 2K panel (McCouch *et al* 2016) and rice 3K panel (Li *et al* 2014) representing most of the geographical diversity exiting in *O. sativa* have been generated. Both of the panels have been thoroughly genotyped and genotypic data has been made publically available (www.ricediversity.org; http:/snp-seek.irri.org).

In the present investigation, GWAS was conducted to identify QTLs for resistance to foot rot disease in 159 diverse germplasm lines belonging to rice 2K panel. The SNP genotypic data of 159 lines was taken from www.ricediversity.org. The SNP data was filtered for ≤ 20 per cent missing data and 0.05 minor allele frequencies (MAF). A total of 208486 filtered SNPs distributed on 12 chromosomes of rice were used for further analysis. Highest number of SNPs were obtained on chromosome 1 and lowest on chromosome 9 (Fig. 4.13).

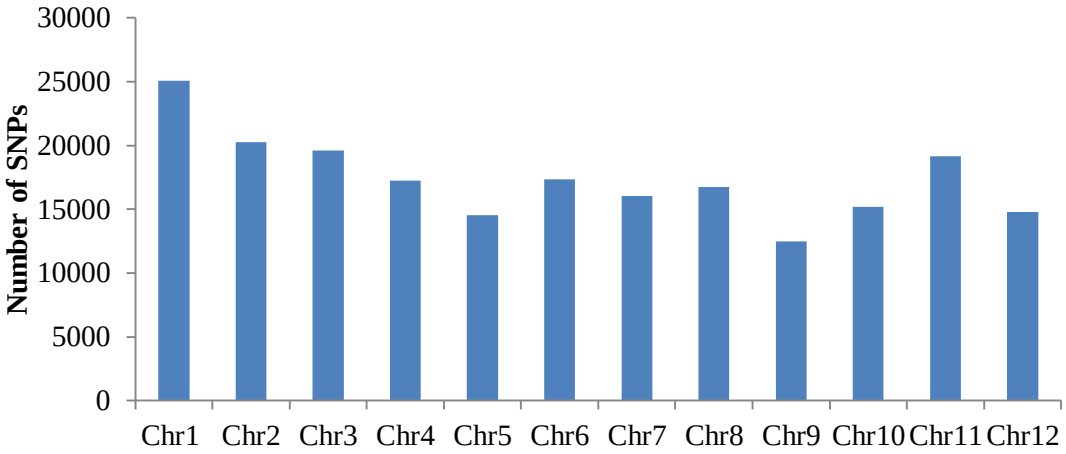

Fig. 4.13: Number of SNPs on each chromosome

The results of the GWAS are discussed under subheads of "population structure" and 'GWAS for resistance to foot rot" as follows:

4.2.1 Population structure:

Mapping QTLs without knowing the population structure may cause spurious associations between phenotype and genotype. These spurious associations might be due to association of subpopulation specific alleles with the phenotype, resulting in the false positives (Thornsberry *et al* 2001). In order to avoid the false positives, 208,486 SNPs were used to analyse population structure using principle component analysis (PCA). The first three principle components (PC) were then plotted on PCA plot. The first three PC could unfold the variation in population to form different sub populations (Fig. 4.14). Therefore, three PCs sufficiently can act as covariate in the GWAS.

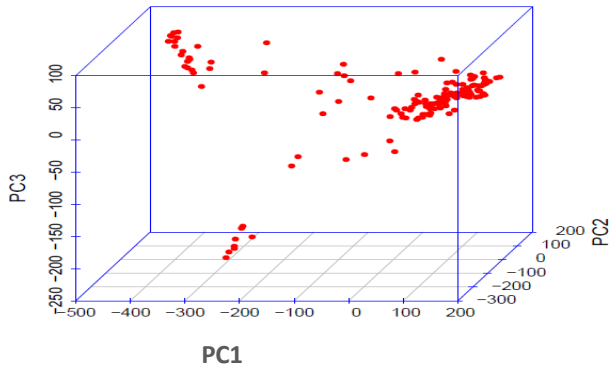

Fig 4.14: Principle component analysis

4.2.2 Estimation of linkage disequilibrium (LD) and significant threshold:

GWAS exploits LD to find association of phenotype with genotype. The extent of LD or LD decay in the population determines the resolution of mapping QTL in the population and number of markers required to find the associations (Flint-Garcia *et al* 2003). Genome

wide LD was estimated at 250 kb (Purohit, 2019) and $p < 3.35 \times 10^{-5}$ was calculated as GWAS threshold, which was used to identify significant associations. A lower threshold of p <0.002 equivalent to LOD = 3.0 (as suggested by Zhang *et al* 2019) was also kept to identify any significant associations due to relatively smaller size of association panel.

4.2.3 GWAS for foot rot resistance:

Disease severity score was used for GWAS analysis and identification of QTLs for resistance to foot rot. On the basis of *Kharif* 2019 April data when significant rate of stunting was observed, significant associations could be observed on 4, 6 and 8 chromosomes (Table 4.12, Fig 4.15), based on lower threshold limit. However no significant association was observed with upper threshold limit.

Table 4.12: SNPs associated with foot rot resistance based on disease severity index score for April 2019 scoring

SNP	Chromosome	Position	*p value*	MAF
SNP-6.3040024.	6	3041024	3.43E-05	0.270701
SNP-4.31484104.	4	31669212	4.98E-05	0.461783
SNP-8.17612511.	8	17615225	5.33E-05	0.171975
SNP-8.17608714.	8	17611428	6.11E-05	0.165605

On the basis of *Kharif* 2019 July data when elongation was observed as a typical symptom of foot rot, significant associations were observed on chromosomes 1, 3, 4, 5, 7, 8, 9 and 11. However, QTL on chromosomes 1, 7 and 8 were found to be highly significant (Fig. 4.14).

Table 4.13: SNPs associated with foot rot based on disease severity index score for July 2019 scoring

SNP	Chromosome	Position	*p value*	MAF
SNP-1.26170918.	1	26171963	2.31E-08	0.169811
SNP-10.2916212.	10	2917236	7.50E-07	0.179245
SNP-3.12543086.	3	12544369	5.96E-06	0.154088
SNP-9.6334692.	9	6335693	8.10E-06	0.103774
SNP-7.9674613.	7	9675608	1.09E-05	0.122642
SNP-6.4546073.	6	4547073	1.19E-05	0.150943
SNP-4.31078880.	4	31263991	2.46E-05	0.141509
SNP-11.19242622.	11	19708935	2.91E-05	0.157233
SNP-5.27348976.	5	27411621	3.12E-05	0.154088

A large number of SNPs were associated based on lower threshold limit (Table 4.13, Fig 4.15).On the basis of combined data of disease severity index (April & July), significant QTLs were identified on chromosome 6 and 9 (Table 4.14, Fig 4.15).

Table 4.14: SNPs associated with foot rot resistance based on combined analysis for April and July 2019 scorings

SNP	Chromosome	Position	*p value*	MAF
SNP-6.304	6	3041024	8.90E-07	0.267296
SNP-6.309	6	3095131	1.84E-05	0.336478
SNP-6.306	6	3067266	2.30E-05	0.235849
SNP-9.779	9	780864	3.31E-05	0.147799

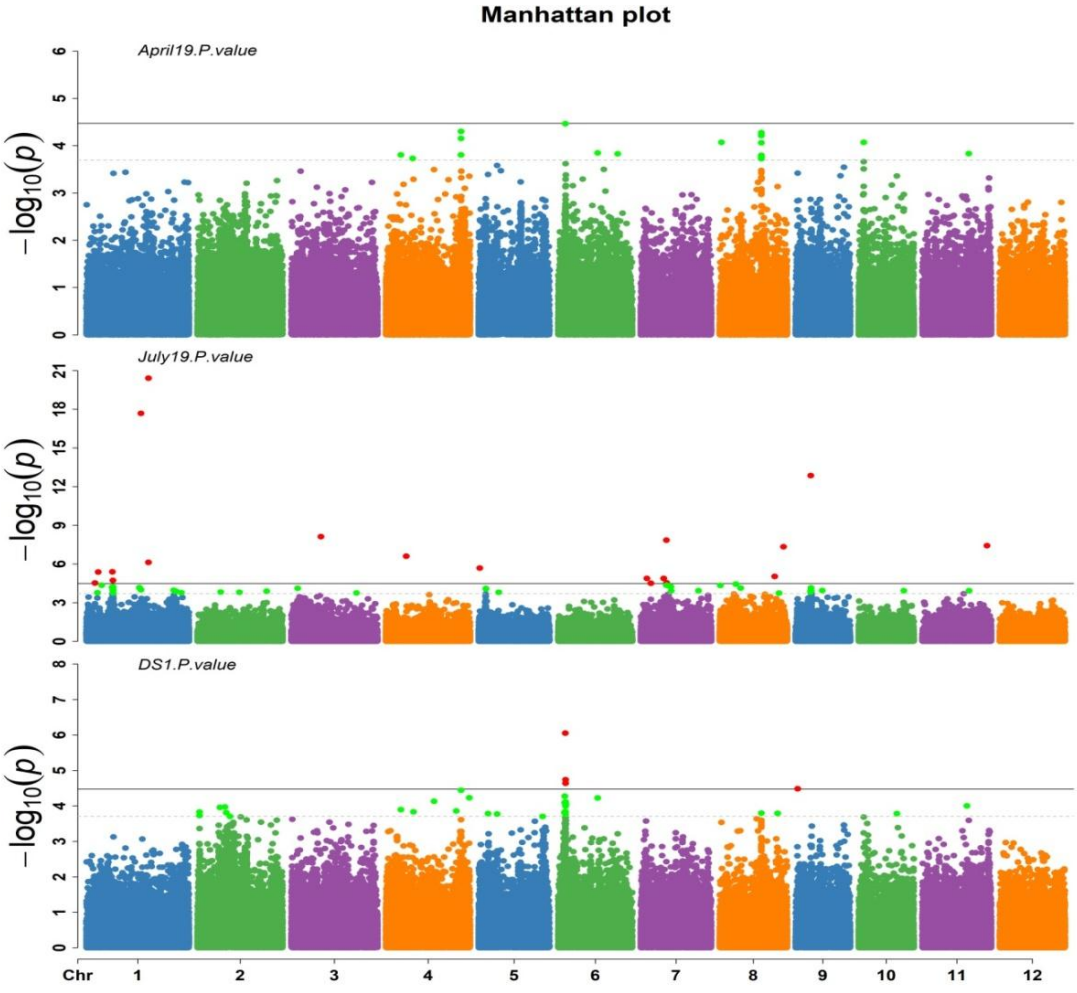

Fig 4.15: Manhattan Plot Genome wise– disease severity index score (GAPIT)

Few reports on the mapping of QTL's for resistance to foot rot are available in literature. Hur *et al* (2015) identified QTL, *qBK1* which was mapped in 520 kb region (19.30 Mb to 23.72 Mb) between RM8144 and RM 1129 and explained 65% phenotypic variation on

chromosome 1.Fiyaz *et al* (2016)identified the significant QTLs on chromosome 1, *qBK1.1, qBK1.2* and *qBK1.3* (which explained 4.76, 24.74 and 6.49 percent of phenotypic variation) using composite interval mapping. The major QTL, *qBK1.2* was mapped on 0.26 Mb from position 3,176,544bp to 3,355,066bp.Volante *et al* (2017) identified two new QTLs,*qBK1_628091*which were mapped on 0.41 Mb regions (628,091bp to1,040,823bp) on short arm of chromosome 1 and *qBK4_31750955*mapped on 0.59 Mb region (from position 31,162,467bp to 31,757,436bp) on long arm of chromosome 4. Another QTL, *qFfR1* which is located at 89.8 cM (from 22,879,904bp to 23,981,901bp) was identified by Ji *et al* (2018) and *qBK1* which was mapped in 35-kb region (from position 13,542,347bp to 15,132,528bp) explained 20.0% phenotypic variation has been reported by Lee *et al* (2019). A significant QTL, *qFfR9* was discovered at 30.1 (cM) on chromosome 9 (7.24–7.56 Mbp) interval by Kang *et al* (2019).In the present study, QTLs were mapped on chromosome 4, positioned 31,669,212 (April) and 31,263,991 (July). It indicates that these detected positions relate to the locus *qBK4_3175095 5*(31,162,467bp to 31,757,436bp) identified by Volante *et al* (2017). Moreover, previous studies (Ma *et al*2008) indicated that rice varieties with the *sd1* gene, a semi-dwarf gene resulting in defective 20-oxidase GA biosynthetic enzyme and localized from 38,382,382 to38,385,504, are susceptible to bakanae disease. So, QTL identified on chromosome 1 (position 26,171,963bp) in present study does not relates to locus of *sd1* allele. The QTLs on chromosomes 1, 6, 7, 8 and 9 may be the new identified regions associated with the disease.

4.3 Association of GA regulated/synthesis genes with the disease:

The genomic and tanscriptomic sequences of all genes were taken from rice genome innotation project. Seven genes which are GA regulated/synthesised were used for designing the qPCR primers with Primer 3+ software. The details are given in table 4.15.

Table 4.15: Forward and reverse primers for the transcripts/ genes with product size

Name of gene	Forward primer	Reverse primer	Amplicon length
Sd1	AGCTGGACATGCCCGTGGTC	TTGAGCTGCTGTCCGCGAAG	414
OsXTH8	CCAAGGACACCTACCTGTT	CAGAGGTAGAACTGGTGCTC	222
OsGAE1	CTTCCACGCCTTCTGGCGGCGGCT	CGTTGCGTACGTCGTCGTCGTCGT	468
EUI	GGCTTGCTTTGGGAGTGATTAC	GCGAAGGGATGCTGAAGATG	106
GID1	GCTGCTTGGGATCGCAAT	CAACCGAGTCACCATTTGATTAGA	98
Gα	GGTTCTTACTGTGGGCTGTTCAG	TCAAGGAAAATTTGCACAATGG	75
OsWOX3A	GCAACTGCTGCTGACATGGC	AAGAGGAGGACTTGGAGCTGC	126

4.3.1 RNA extraction and Normalization

Three varieties taken were selected based its response to disease under screening in previous experiment. These three varieties consisted of Pusa Basmati 1121, Pusa Basmati 1509 as susceptible varieties and PR127 as resistant variety. RNA was extracted from seedlings of three cultivars at 10, 17, 24, 31 DAS and quantified on 1.2% agarose gel. After quantification RNA was normalized to 600ng (Fig. 4.16).

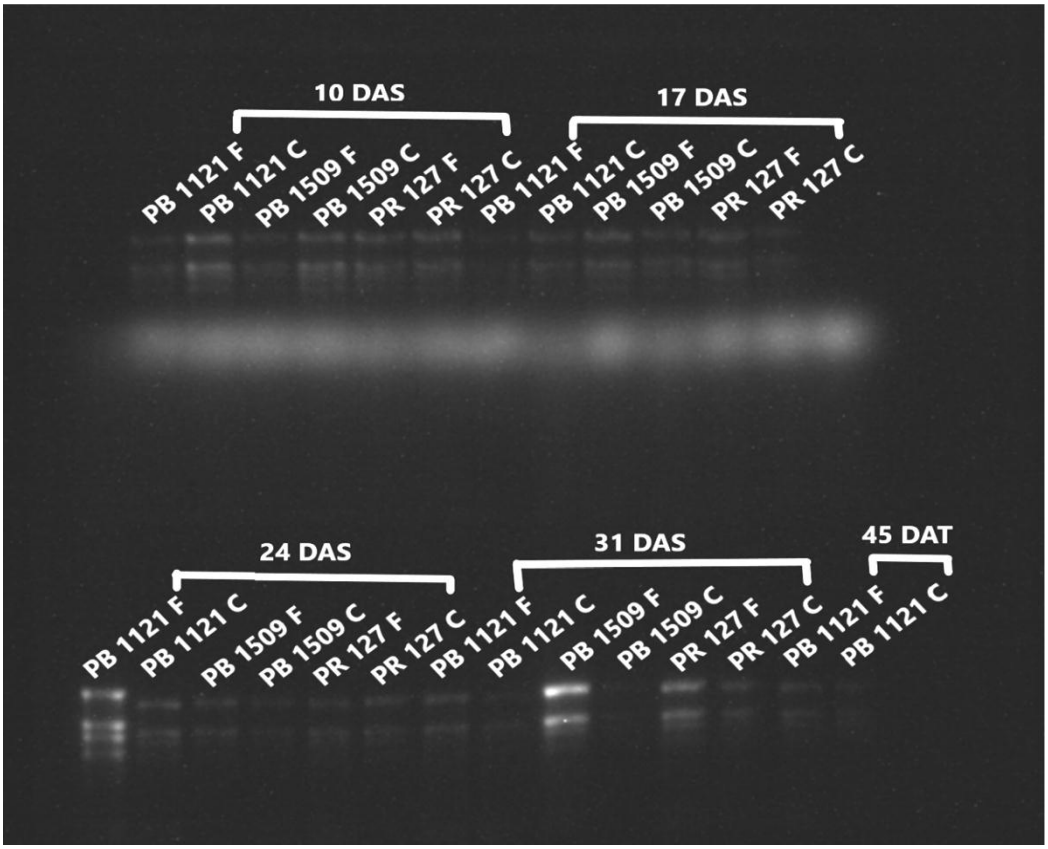

Fig. 4.16: RNA Normalisation (600ng)

qRT-PCR analysis of all the seven genes was carried out on the selected three varieties of rice at four different stages in nursury and one sample of Pusa Basmati 1121 at 45 DAT from natural infection. First, all the primers were validated on pooled cDNA of PB1121 and PB1509. Among the tested primer of only *OsWOX3A* gene showed no amplification, so rest of all the primers were used for further analysis. Rest of the genes did have specific PCR product as indicated by their melting curve analysis (Fig.4.17). From the qPCR results we obtained the threshold/quantitation cycle (CT/CQ) value. This CQ value was utilized for calculating the fold change in expression among different varieties. For the analysis Ubiquitine was used as the housekeeping control to normalize gene expression. To analyse the general trend of gene expression among the different cultivars during seedling stage fold change was calculated using respective control of all the genotypes.

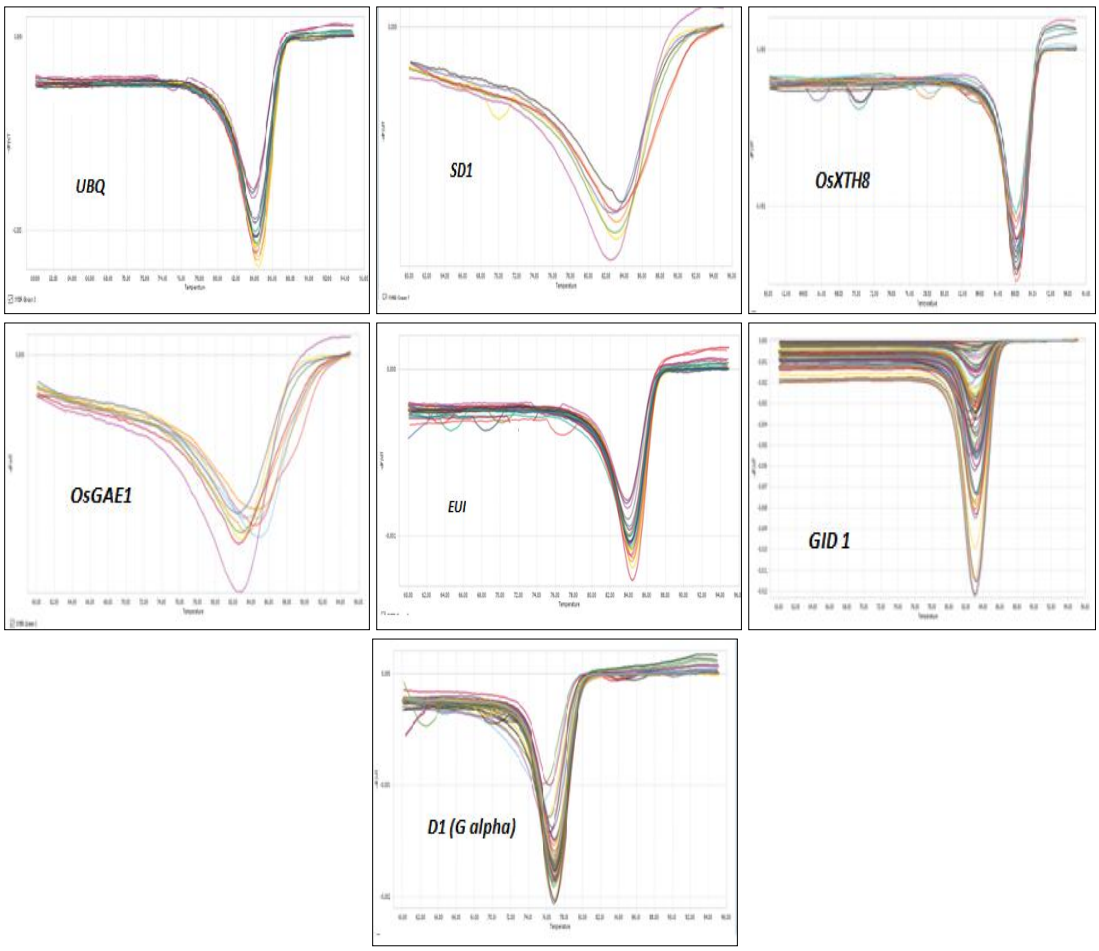

Fig. 4.17: Melting curves of different genes

4.3.2 Expression analysis of various genes

4.3.2.1 *SD1* (Semi dwarf1) gene

It is a GA synthesized pathway gene. It encodes gibberellin biosynthetic pathway enzyme GA_{20} oxidase 2 which is responsible for the conversion of GA_{12} to GA_9 and GA_{53} to GA_{20}. But its mutant allele (*sd1*) which GA insensitive show the loss or substitutions of some nucleotide, leading to internal stop codons or substitutions of some amino acids which makes the GAs pathway defunct. Earlier, we expected that its expression should be high in susceptible varieties and expected no change in resistant variety. Interestingly, its expression was very low in Pusa Basmati 1121, due to this reason it was excluded from further analysis. Its expression was reduced in PB 1509 at all stages except at 24DAS, where it is slightly increased. Similarly, highly reduced expression was observed in natural infection of PB1121 at 45 DAT (Table 4.16, Fig. 4.18). But its expression was high in PR127 at all stages except at 10DAS. It was concluded that *sd1* give response to the GA which is externally provided by fungus. Similar results were observed by Ma *et al* (2008). They tested response of different cultivars carried different dwarfing genes *sd1,d1, d29, sd6, sdq(t)* to GA treatment and

bakanae infection. Rice materials carrying dwarf gene such as *sd1* were not only sensitivity to GA3 but also susceptive to rice bakanae disease. Materials carrying dwarf gene *d1* were insensitive to GA3 but susceptive to bakanae. On the other hand, all materials carrying *d29*, *sd6* or *sdq*(t) genes showed resistance to bakanae.

Table 4.16: Critical threshold, fold change and standard error data (Infected and Control) of *SD1* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1509	7.22	5.55	0.314253	0.244207	0.526809
	PR 127		5.263333			
17 DAS	PB 1509	3.468333	2.383333	0.471392	0.036743	0.68663
	PR 127	2.428333	4.143333	3.282966	0.713179	0.008819
24 DAS	PB 1509	3.443333	3.6	1.114709	0.165769	0.948383
	PR 127	2.136667	4.263333	4.367073	0.221014	0.379543
31 DAS	PB 1509	4.016667	-0.58333	0.041235	0.105886	0.192388
	PR 127	1.33	2.286667	1.94082	0.261542	0.173882
45DAT	PB 1121	3.726667	1.563333	0.22324	0.384993	0.361166

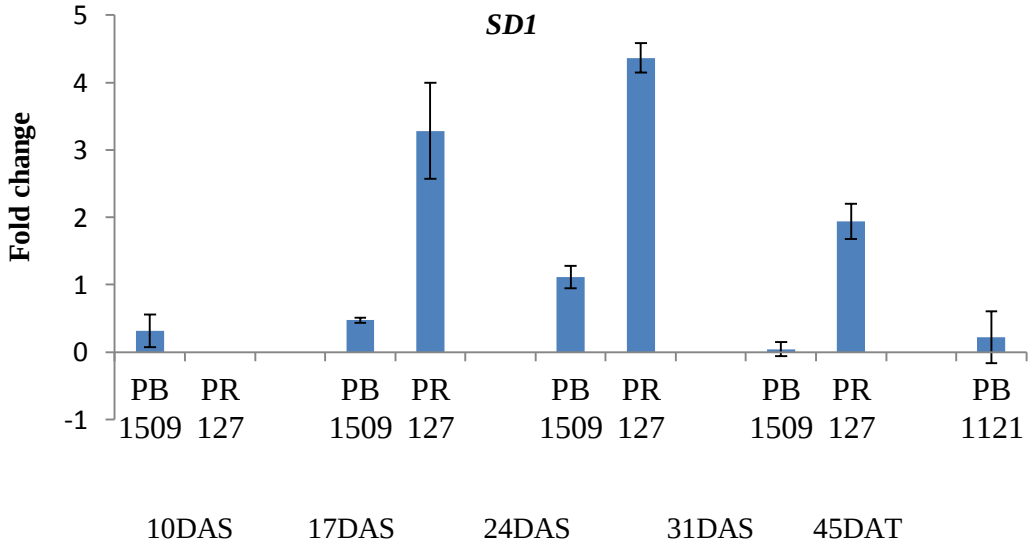

Fig. 4.18: Fold change diagram of *SD1* gene at different stages

4.3.2.2 *OsXTH8* gene

It is GA regulated gene *OsXTH8* (Jan 2004) which is responsible for elongation and cell wall organisation. In response to gibberellic acid *OsXTH8* gene helps in formation of primary cell wall using Xyloglucan by catalysing the xyloglucan endotransglucosylases/ hydolysaes enzyme and more expression in root nodes and leaf sheath. Earlier, we expected that its expression should be high in susceptible varieties and no change showed be there in

resistant variety. But its expression was comparatively same in resistant as well as susceptible varieties except that it was high at 24DAS in PR127 and in PB 1121 in natural infected sample at 45 DAT (Table 4.17, Fig. 4.19). So, it may be concluded that this gene might not be involved in bakanae disease.

Table 4.17: Critical threshold, fold change and standard error data (Infected and Control) of *OsXTH8* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1121	5.026667	5.275	0.841868	0.113189	0.554403
	PB 1509	5.656667	4.896667	1.693491	0.121155	0.086861
	PR 127	5.476667	4.883333	1.508729	0.525193	0.136914
17 DAS	PB 1121	6.143333	6.1125	1.021602	0.162725	0.639454
	PB 1509	6.483333	5.416667	2.094588	0.228504	0.066585
	PR 127	4.94	6.076667	0.454809	0.076886	0.402104
24 DAS	PB 1121	6.876667	5.956667	1.892115	0.135035	0.125481
	PB 1509	5.06	5.593333	0.690956	0.153011	0.118466
	PR 127	6.773333	4.533333	4.723971	0.10398	0.262453
31 DAS	PB 1509	7.19	7.143333	1.032876	0.043718	0.10398
	PR 127	6.226667	6.496667	0.82932	0.154313	0.207317
45 DAT	PB 1121	6.143333	2.483333	12.64066	0.145186	0.333893

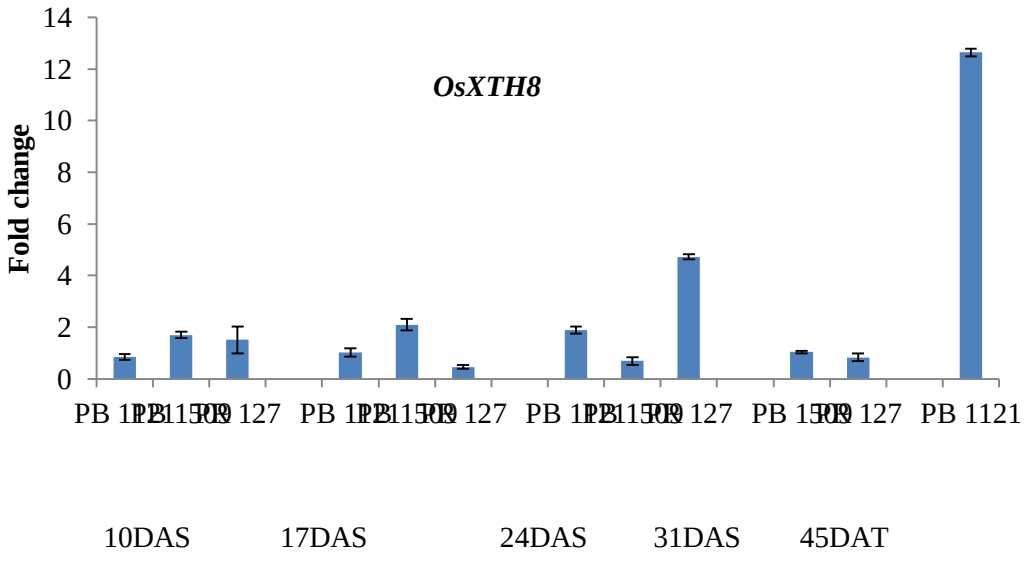

Fig. 4.19: Fold change diagram of *OsXTH8* gene at different stages

4.3.2.3 *OsGAE1*(Gibberellic acid enhanced) *gene*

It is GA regulated gene. Earlier, we expected that its expression should be high in susceptible varieties and might not effect in resistant variety. But its expression was reduced in both the susceptible varieties at all the stages and it showed high expression in PR127 at 17 DAS and 24 DAS (Table 4.18, Fig. 4.20). It concluded that this gene might not be associated with the disease.

Table 4.18: Critical threshold, fold change and standard error data (Infected and Control) of *OsGAE1* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1121	-2.58667	-2.37333	1.159364	0.148478	0.531188
	PB 1509	-2.37333	-1.93	1.359742	0.21988	0.242517
	PR 127	-1.94333	-1.69333	1.189207	0.291041	0.16344
17 DAS	PB 1121	-2.13333	-2.94	0.571701	0.115522	0.31534
	PB 1509	-1.69	-1.95667	0.831238	0.29169	0.360196
	PR 127	-2.52	-1.09667	2.682045	0.260285	0.14769
24 DAS	PB 1121	-2.02333	-1.78667	1.178267	0.182245	0.07219
	PB 1509	-1.28	-2.25667	0.508152	0.127719	0.209158
	PR 127	-3.69	-1.01333	6.393769	0.250074	0.196418
31 DAS	PB 1509	-2.28333	-3.27333	0.503478	0.260861	0.174584
	PR 127	-1.95667	-2.30333	0.786399	0.281097	0.149336
45 DAT	PB 1121	-1.20333	-2.16	0.515246	0.347205	0.335486

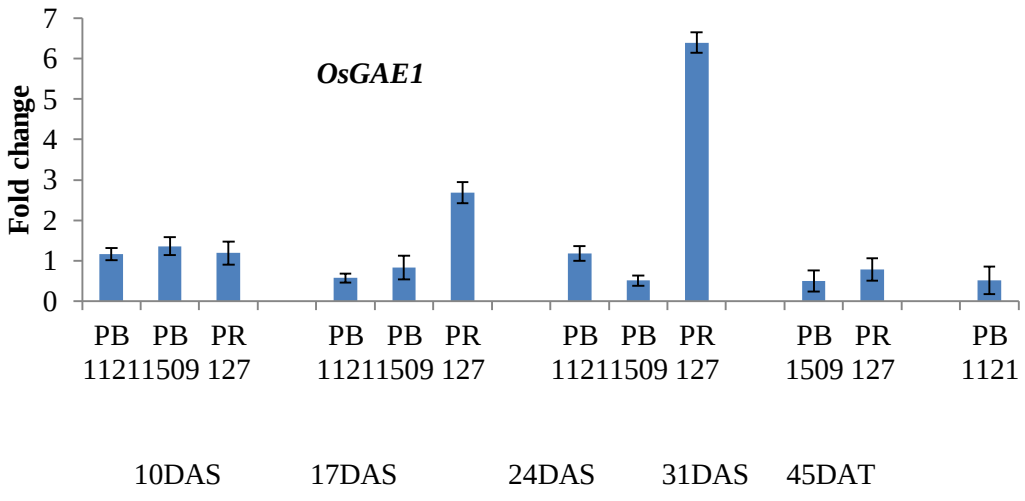

Fig. 4.20: Fold change diagram of *OsGAE1* gene at different stages

The expression of this gene was found to be increased by rise in GA_3 concentration from 1uM to 50uM, starting from 30 min to 24 h after GA_3 treatment Jan (2006). From in-situ hybridization, they concluded that it showed high expression in leaf sheath, callus and meristematic region of shoot apex. *OsGAE1* gene produce N- terminal signal peptide (Tusnady and Simmon 2001) which causes the breakdown of cell membrane and release of apoplast.

4.3.2.4 *EUI* (Elongated uppermost internode) gene

It is a stage-specific gene which shows its expression in uppermost internode at heading stage and its product is responsible for the deactivation of biological active GAs. *Eui* gene encodes a previously uncharacterized cytochrome P450 monooxygenase which catalyses 16a,17-epoxidation of non-13-hydroxylated GAs and 16a,17-epoxidation reduces the biological activity of GA_4 in rice (Zhu *et al* 2006).

It was expected that its expression should be reduced in susceptible varieties and no change/might be increased in resistant variety. It was observed that its expression was increased in PB1121 and PR127 at 17DAS and 24DAS (Table 4.19, Fig 4.21). But, its expression was reduced in PR127 at 31DAS and highly increased in PB1509. Thus no conclusive results in connection with foot rot disease could be obtained from the expression analysis of this gene.

Table 4.19: Critical threshold, fold change and standard error data (Infected and Control) of *EUI* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1121	4.006667	2.226667	0.291183	0.273829	0.108988
	PB 1509	3.556667	3.15	0.754364	0.19152	0.159831
	PR 127	3.013333	3.55	1.450617	0.178549	0.176168
17 DAS	PB 1121	2.276667	3.416667	2.20381	0.288818	0.217134
	PB 1509	3.11	3.246667	1.099362	0.431148	0.361965
	PR 127	2.193333	3.07	1.836128	0.222717	0.252396
24 DAS	PB 1121	1.333333	2.65	2.490899	0.247865	0.060829
	PB 1509	3.393333	2.456667	0.522439	0.124413	0.251072
	PR 127	2.343333	3.576667	2.351096	0.277877	0.317569
31 DAS	PB 1509	1.756667	3.64	3.689265	0.256219	0.241829
	PR 127	2.416667	1.736667	0.624165	0.334591	0.134292
45 DAT	PB 1121	2.69	2.51	0.882703	0.244343	0.194027

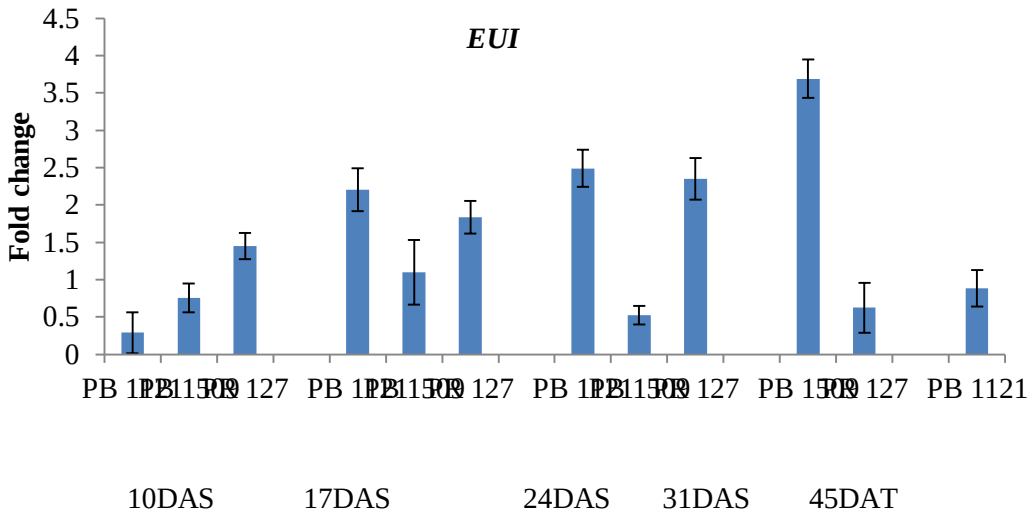

Fig. 4.21: Fold change diagram of *EUI* gene at different stages

4.3.2.5 *GID1*(Gibberellin insensitive dwarf1) gene

It acts as functional receptor of GAs and plays an important role in its signaling pathway. It binds 16,17- dihydro-GA_4 in a saturable manner and shows ten times more affinity for biologically active GAs as compared to inactive GAs (Hartweck and Olszewski, 2016). It was expected that its expression should be high in susceptible varieties and there should not be any change in resistant variety. But its expression was almost same in all the varieties at different intervals. The high expression was observed in PB1509 at 17DAS and in PB1121, PR127 at 24 DAS, thus revealing no conclusive pattern (Table 4.20, Fig. 4.22). It concluded that it might not be associated with disease.

Table 4.20: Critical threshold, fold change and standard error data (Infected and Control) of *GID1* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1121	-3.59	-4.76667	0.442372	0.060829	0.181878
	PB 1509	-4.03667	-3.92667	1.079228	0.074239	0.039301
	PR 127	-3.75	-3.46667	1.217004	0.26972	0.0809
17 DAS	PB 1121	-3.64333	-4.89667	0.419478	0.092078	0.088383
	PB 1509	-3.52667	-2.69667	1.777685	0.23545	0.040001
	PR 127	-3.38333	-3.62333	0.846745	0.064379	0.068882
24 DAS	PB 1121	-4.01667	-3.39667	1.536875	0.04933	0.146216
	PB 1509	-2.56333	-3.89333	0.397768	0.075721	0.078105
	PR 127	-4.31333	-3.1	2.318728	0.25784	0.045827
31 DAS	PB 1509	-4.16	-5.49	0.397768	0.060094	0.063598
	PR 127	-3.51667	-3.75	0.850667	0.031799	0.415918
45 DAT	PB 1121	-2.96667	-3.42333	0.728668	0.082935	0.133212

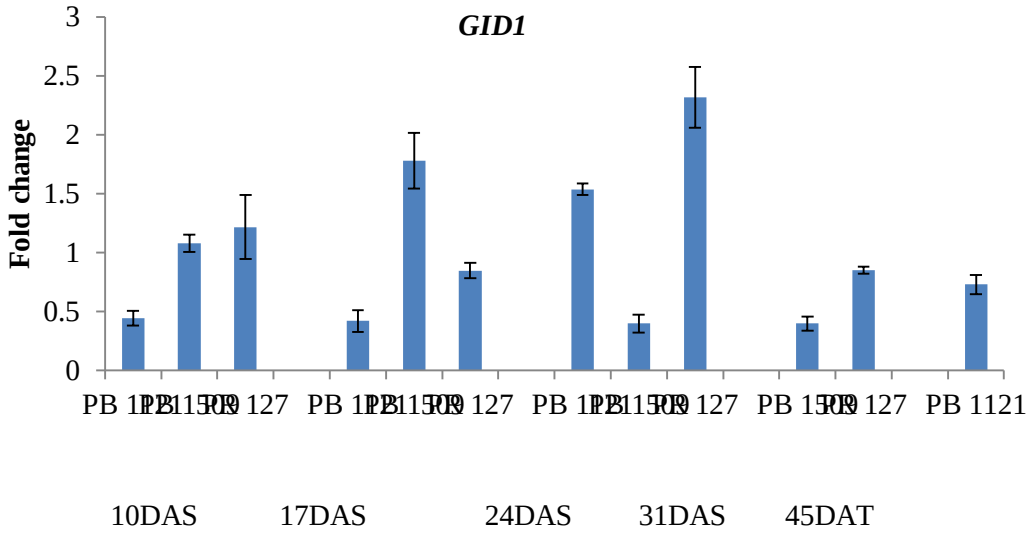

Fig. 4.22: Fold change diagram of *GID1* gene at different stages

4.3.2.6 *DI (G alpha)*

Galpha is alpha subunit of heteromeric G protein which plays an important role in GA signaling pathway. It is responsible for the induction of alpha amylase activity in aleurone cells and internode elongation. But *d1* mutant produced defective alpha subunit of heteromeric G protein which reduces the activity of alpha amylase activity in aleurone cells and results in dwarf rice mutant reported by Tanaka *et al* (2000). He also proposed another GA signaling pathway which is independent of *Galpha* protein but it shows less response to GA signaling as compared to *Galpha* protein dependent pathway.

Table 4.21: Critical threshold, fold change and standard error data (Infected and Control) of *D1 (G alpha)* gene

Stage	Varieties	Δ CT	Δ CT	FC	SE (In)	SE (C)
10 DAS	PB 1121	-2.18333	-2.49667	0.80478	0.298077	0.431843
	PB 1509	-2.22	-2.29667	0.948246	0.360566	0.013334
	PR 127	-1.47	-1.57	0.933033	0.467679	0.073713
17 DAS	PB 1121	-2.63667	-3.4	0.589134	0.092918	0.254238
	PB 1509	-2.36667	-2.13333	1.175548	0.026458	0.163848
	PR 127	-2.52	-2.15667	1.286395	0.381689	0.328254
24 DAS	PB 1121	-2.97667	-1.75333	2.334856	0.070002	0.086669
	PB 1509	-1.54667	-2.25667	0.61132	0.04631	0.048421
	PR 127	-2.95	-1.19333	3.379165	0.557182	0.292374
31 DAS	PB 1509	-2.75667	-2.79	0.97716	0.287932	0.453236
	PR 127	-2.40667	-3.00333	0.66128	0.068882	0.281492
45 DAT	PB 1121	-1.63333	-1.64	0.99539	0.077962	0.29169

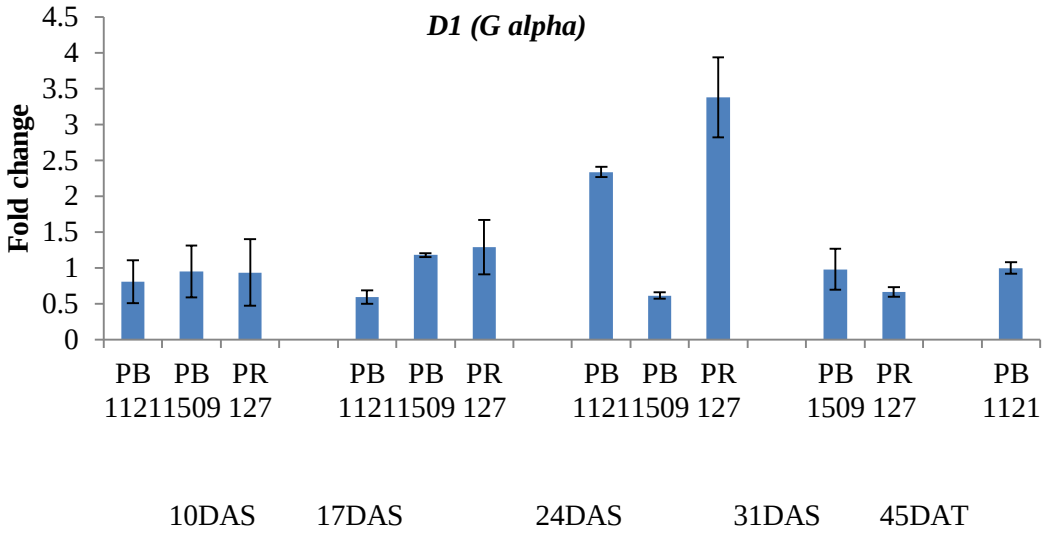

Fig. 4.23: Fold change diagram of *D1(G alpha)* gene at different stages

It was expected that its expression should be high in susceptible varieties and no change in resistant variety. But the low activity of this gene was observed at different stages of analysis in all the varieties under study. The only exception was found at 24 DAS where it was increased in PB1121 and also in PR127 (Table 4.21, Fig 4.23). However no conclusive pattern of variations in association with foot rot disease could be observed.

In this experiment the GA synthesis/regulated genes were chosen for studies with a view that *sd1* gene controls GA synthesis and germplasm carrying *sd1* allele is insensitive to gibberellic acid. Earlier reports (Pannu *et al* 2013) from PAU had found that non-basmati varieties released by PAU are tolerant to foot rot & being semi dwarf they are expected to carry *sd1* allele. So association of GA synthesis/regulated pathway genes were studied. But the present results could not reveal any significant association of these genes with the foot rot disease**.** In general the genes showed significant changes in expression either in susceptible or resistant variety at 24DAS. Therefore, one important conclusion could be drawn from this experiment is that 24DAS is the most important stage to study bakanae disease.

4.4 Further suggestions:

- As foot rot is a serious problem particularly in basmati rice, identification of novel sources with foot rot resistance is of prime importance for developing foot rot resistance/tolerance basmati cultivars.
- Efforts should be made to develop a high-through and efficient protocol of screening of foot rot resistance in rice.
- Identification of major genes/QTLs associated with foot rot resistance may help breeding the varieties with foot rot resistance.

- The prebreeding efforts aimed at transferring foot rot resistance/tolerance from non-basmati to basmati backgrounds by employing MAS accelerated breeding approaches is desired.
- Emphasis should be given to understand the mechanism of foot rot disease, disease establishment and host-pathogen interaction.

CHAPTER V

SUMMARY

In the current research project was aimed at identifying novel germplasm sources of foot rot resistance in rice, which could act as a donors for the developing foot rot resistant/tolerant varieties. In fact, foot rot disease, caused by *Fusarium moniliforme* has emerged as a disease of major concern particularity in basmati rice which holds a prestigious position among different types of rice and is a major export item of our country. The other objective of the study was to identify QTLs for foot rot resistance through Genome Wide Association Studies (GWAS). The third aspect of the studies was focused on studying the association of gibberellic acid regulated/synthesis genes with the foot rot disease by expression analysis from two extreme susceptible and resistant germplasm lines.

For the identification of resistant germplasm sources, a set of 275 rice germplasm lines were selected which consisted of 159 lines of 2K diversity panel, 10 accessions of *O. glaberrima*, 24 released varieties, 67ANP lines **(**collection of diverse aromatic rice germplasm) and 17 elite lines with RYT number developed at Punjab Agricultural University, Ludhiana during the ongoing rice breeding program and designated as RYT lines. The evaluation of rice germplasm for foot rot disease was carried out at Punjab Agricultural University, Ludhiana for two crop seasons viz. *kharif* 2018 &*kharif* 2019(two evaluations in April and July). Five parameters viz: seedling height, number of elongated, stunted, pale and dead plants was recorded to access the resistance reaction in germplasm lines. Different parameters of disease scoring which included the disease severity index, disease incidence, seedling mortality rate, seedling length were studied for the evaluation of germplasm. The first two screenings were conducted in portrays while the final screening was done by sowing the inoculated material on beds followed by transplanting of the surviving material to field after 31 days after sowing. During this season, the uninnoculated set of the germplasm was also sown in parallel to the inoculated set for actual comparison of height/stunting.

The symptoms of foot rot disease were found to vary depending upon the environmental conditions. During the *Kharif* 2019 crop season, in the evaluation conducted during the April month the susceptible lines showed significant stunting while during the evaluation in July, almost same lines showed elongation symptoms which may be attributed to the prevailing dry and humid weather conditions, leading to the production of fusaric and gibberellic acid during April and July months respectively.

On the basis of overall, evaluation, a total of 35 foot resistant lines were identified. Among 2K rice diversity panel, 18 lines namely CHIGYUNGDO::IRGC55466-1,

ZHENSHAN 97B, CO 39, GOGO LEMPAK::IRGC43392-C1, WAB 368-B-1-H1-HB::IRGC117359-1, NSICRC 106::IRGC117370-1, GUAYQUIRARO P A::IRGC116987-1, NS 1288::IRGC68930-1, CANA ROXA, HAWM OM, IR64-21, 63-104::IRGC15100-C1, PERLA::IRGC117021-1, GANIGI::IRGC48698-C1, OS 4::IRGC11335-C1, ICTA POLOCHIC::IRGC116997-1, CT 9993-5-10-1-M::IRGC116974-1, KAKANI 2::IRGC13373-C1 were found to be resistant on the basis of disease severity index. Five *O. glaberrima* lines (*IR102512, IR102526, IR102615, IR103292, IR103545*), two ANP lines (Sukala Phool, NDR8497-2) and ten released varieties (Pusa Basmati1, PR113, PR114, PR115, PR121, PR122, PR123, PR124, PR126, PR127) were found to be resistant to the disease. But all RYT lines showed susceptible response to the foot rot disease and this material belonged to basmati germplasm. The identified resistant lines can be used in breeding programme for transferring resistance to foot rot disease. The availability of recent biotechnological techniques has made the task easier. Marker assisted backcrossing can used to ensure the retention of unique quality traits of basmati and ensuring the background recovery.

Genome Wide Association Studies (GWAS) is a powerful technique to identify genes/QTLs under lying complex traits. The availability of high through-put SNP genotypic data of germplasm lines from rice 2K panel provided an opportunity to conduct GWAS to identify QTLs associated with foot rot resistance. A subset of 159 lines of rice belonging to 2k diversity panel collected from IRRI, Philippines was used for this purpose.

The SNP data was filtered for $\leq$ 20 per cent missing data and 0.05 minor allele frequencies (MAF). A total of 208486 filtered SNPs distributed on 12 chromosomes of rice were used for further analysis. GWAS was done in R (R Core Team 2018) package, GAPIT (Genome association and prediction integrated tool) version 3.0 using FarmCPU (Fixed and random model circulating unification) model to calculate estimated *p* values of each SNP. FarmCPU is a multi-locus model that addresses the confounding problem of mixed linear models (MLM) by using population structure and multiple associated markers in fixed effect model. The multiple associated markers were estimated by incorporating kinship values in the random effect model. Population structure was estimated with principle component analysis (PCA). The associated *p* values of each SNP distributed on each chromosome were plotted as manhattan plots by using –log(p) values. Genome wide LD was estimated at 250 kb and $p < 3.35 \times 10^{-5}$ was calculated as GWAS threshold, which was used to identify significant associations. On the basis of April disease severity index data when significant rate of stunting was observed, significant associations could be observed on 4, 6 and 8 chromosomes, based on lower threshold limit. On the basis of disease severity index data of July when

significant elongation was observed, QTLs on chromosomes 1, 7 and 8 were found to be highly significant. On the basis of combined data of disease severity index (April and July), significant QTLs were identified on chromosome 6 and 9.Two QTLs were mapped on chromosome 4 have positions 31,669,212 (April) and 31,263,991 (July) resembled to the positions of previously identified QTL *qBK4_31750955* (31,162,467 bp to 31,757,436 bp). The QTL identified on chromosome 1 (position 26,171,963 bp) in present study could not relate to locus of *sd1* allele as it was it thought to be associated with foot rot disease. The QTLs on chromosomes 1, 6, 7, 8 and 9 indicated the new identified regions associated with the disease.

To study the expression analysis of GA regulated/synthesis genes in relation to bakanae disease, seven genes were selected. RNA extracted from three selected cultivars viz: PR127 (resistant), Pusa Basmati 1121 and Pusa Basmati 1509 (susceptible) at 10, 17, 24, 31 days after sowing was normalized, converted to cDNA and qRT-PCR was performed for selected genes. It was expected that expression of *sd1* gene should be high in susceptible varieties and no change in resistant variety was expected. However, in resistant cultivar high expression was observed at all stages except 10 DAS. And expression was very low in both susceptible varieties. So, the susceptible plant may be responding only to the GA which is externally provided by fungus after infection. The expression of *OsXTH8* gene (associated with cell wall formation and cell wall organisation) was very low in all cultivars except at 24DAS in PR127. Similarly in case of *OsGAE1* (controlling cell wall breakdown), high expression was observed only in PR127 at 17 DAS and 24 DAS. The expression of *EUI*, *GID*1, *D1 (G alpha)* genes was reduced in all the stages except at 24 DAS where both PB 1121 as well as PR127 showed high expression.

In this experiment the GA synthesis/regulated genes were chosen for studies with a view that *sd1* gene controls GA synthesis and germplasm carrying *sd1* allele is insensitive to Gibberellic acid. Earlier reports (Pannu *et al* 2013) from PAU had found that PAU released non basmati varieties were tolerant to foot rot and being semi dwarf they are expected to carry *sd1* allele. So, the association of *sd1* and other GA synthesis/regulated pathway genes were studied. However, none of the genes studied could reveal any association with the foot rot resistance/susceptibility during the expression analysis. So, there is need to study the expression of other GA regulator/inhibitor genes for further analysis.

In the present studies all the genes showed significant change in expression either in susceptible or resistant variety at 24DAS. So, it is suggested that 24DAS is the most important stage to study further association of these type of genes with the bakanae disease.

Overall, the study has led to the identification of diverse sources of foot rot resistance for use in varietal development program. It is suggested that more research efforts aimed at the identification major QTL's associated with foot rot resistance are needed. Also, studies on the epidemiology of foot rot disease are needed so that modern genome editing techniques of molecular biology can be employed to develop foot rot resistant varieties.

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APPENDIX I

List of 159 germplasms derived from Rice 2k diversity panel

S. No.	Irgc No.	Designation	Country
1	121310	CHIGYUNGDO::IRGC 55466-1	Korea
2	121549	YAKUMO::IRGC 5320-1	Japan
3	117280	ZHENSHAN 97 B	China
4	121551	YAN ZHAO 9::IRGC 63062-1	China
5	117454	CO 39::IRGC 51231-1	India
6	117593	YANGKUM (RED)::IRGC 32406-1	Bhutan
7	117420	AEDAL::IRGC 55441-1	Republic of Korea
8	121344	GHASELU MAP::IRGC 72527-1	Bhutan
9	117274	NIPPONBARE	Japan
10	121377	KAM MRA::IRGC 62172-1	Bhutan
11	117276	SADU CHO	Republic of Korea
12	120903	BR IRGA 409::IRGC 116960-1	Brazil
13	121781	KUROKA::IRGC 74556-C1	Japan
14	121762	IRAT 144::IRGC 55685-C1	Ghana
15	121230	WARABEHATOMOCHI::IRGC 14779-1	Japan
16	121741	GOGO LEMPAK::IRGC 43392-C1	Indonesia
17	121486	RIZZOTTO 264::IRGC 65727-1	Italy
18	121537	WAB 368-B-1-H1-HB::IRGC 117359-1	Cote d'Ivoire
19	117462	DAWASAM (RED)::IRGC 32389-1	Bhutan
20	121580	RIENALDO BERZANO::IRGC 3230-1	Turkey
21	117269	LI JIANG XIN TUAN HEI GU	China
22	117266	DULAR	India
23	121333	EDOGAWA::IRGC 74468-1	Japan
24	121914	INDIO::IRGC 116998-1	France
25	117492	INDANE::IRGC 33130-1	Myanmar
26	121267	ARGO::IRGC 82418-1	Italy
27	121296	BREVIARISTATA (RAIA)::IRGC 3189-1	Portugal
28	121389	KHUDWANI ACC 409::IRGC 34216-1	India
29	121076	NSICRC 106::IRGC 117370-1 (17370-1 ?)	Philippines
30	121085	PICONEGRO::IRGC 117022-1	Ecuador
31	121368	ITA 235::IRGC 64854-1	Cote d'Ivoire
32	117459	DA 8::IRGC 6422-1	Bangladesh
33	121295	BRAZOS::IRGC 24273-1	USA
34	120918	CR 5272::IRGC 116971-1	Costa Rica

S. No.	Irgc No.	Designation	Country
35	121625	LATSIKA::IRGC 69367-1	Madagascar
36	121772	JIMBRUK JOLOWORO::IRGC 43420-C1	Indonesia
37	117434	BARAN BORO::IRGC 27509-1	Bangladesh
38	117512	KEN CHIAO JU HSIAO LI::IRGC 1217-1	China
39	121182	1-52-6::IRGC 39111-1	Brazil
40	121290	BOSSA::IRGC 57781-1	Guinea
41	121787	LUDAN::IRGC 64189-C1	Philippines
42	121315	CIRAD 409::IRGC 116969-1	Colombia
43	117271	MINGHUI 63	China
44	120916	CIMARRON::IRGC 116967-1	Venezuela
45	121676	PALMIRA::IRGC 5097-1	Costa Rica
46	121359	IAC 164::IRGC 117251-1	Brazil
47	121327	DOONGARA::IRGC 78392-1	Australia
48	121775	KETAN KONIR::IRGC 43444-C1	Indonesia
49	122270	TRES MESES::IRGC 6464-C1	Brazil
50	124469	UCHUTI::IRGC 14694-1	Kenya
51	121483	RHS 107-2-1-2TB-1JM::IRGC 117025-1	Mexico
52	117264	AZUCENA	Philippines
53	121346	GUATEMALA 1021::IRGC 3388-1	Guatemala
54	121665	IAC 120::IRGC 22712-1	Brazil
55	121613	GUAYQUIRARO P A::IRGC 116987-1	Argentina
56	121524	TOS 10483::IRGC 56723-1	Guinea-Bissau
57	121525	TOX 1011-4-1::IRGC 117033-1	Nigeria
58	121529	TX 10438::IRGC 117035-1	USA
59	124456	BAKILIKINDA::IRGC 63121-1	Zimbabwe
60	121694	PACHOLINHA::IRGC 50531-1	Brazil
61	121461	ORIENTE 10::IRGC 55808-1	Ecuador
62	121427	MATAHAMBRE::IRGC 53200-1	Cuba
63	121302	CAIAPO::IRGC 116962-1	Brazil
64	121205	IC 27525::IRGC 53989-1	India
65	117475	GHARIB::IRGC 32303-1	Iran
66	117429	AUS JOTA::IRGC 66767-1	Bangladesh
67	121193	BAKUNG (H)::IRGC 60220-1	Malaysia
68	121595	ARC 12701::IRGC 22267-1	India
69	121672	MUT IAC 25-44-807::IRGC 68799-1	Guyana
70	121373	JI BO YA::IRGC 77446-1	Congo

S. No.	Irgc No.	Designation	Country
71	117282	CYPRESS	United States
72	117440	CANA ROXA::IRGC 25966-1	Brazil
73	117505	KALAMKATI::IRGC 45975-1	India
74	121211	MIKHUDEB::IRGC 25892-1	Bangladesh
75	121259	ARC 12451::IRGC 41052-1	India
76	121391	KIKILONG::IRGC 71539-1	Malaysia
77	121583	TOS 724::IRGC 11108-1	USA
78	117484	HAWM OM::IRGC 23729-1	Thailand
79	124476	IAC 1111::IRGC 39050-1	Brazil
80	121385	KHAO KAP SANG::IRGC 23423-1	Laos
81	121612	EL PASO L 227::IRGC 116979-1	Uruguay
82	124464	NATO::IRGC 1819-1	USA
83	117513	KHAO DAM::IRGC 23385-1	Laos
84	117517	KINANDANG PATONG::IRGC 23364-1	Philippines
85	117571	RT 1031-69::IRGC 15092-1	Congo
86	117585	TCHAMPA::IRGC 32362-1	Indonesia
87	121690	HEI CHIAO CHUI LI HSIANG KENG::IRGC 1112-1	China
88	121496	SAYLLEBON::IRGC 32509-1	Liberia
89	121791	MAINTIMOLOTSY 1226::IRGC 11010-C1	Madagascar
90	117279	TAINUNG 67	Taiwan
91	121195	DINOLORES::IRGC 67431-1	Philippines
92	117268	IR 64-21	Philippines
93	120892	BG 301::IRGC 117315-1	Sri Lanka
94	121241	62-667::IRGC 15147-1	Cote d'Ivoire
95	121488	RXAR RGUE::IRGC 1943-1	USA
96	121700	63-104::IRGC 15100-C1	Cote d'Ivoire
97	121685	VARY LAVA::IRGC 386-1	Madagascar
98	124468	TORO 2::IRGC 66761-1	USA
99	121423	MALAGKIT PUTI::IRGC 19451-1	Philippines
100	122063	GOGO::IRGC 43390-C1	Indonesia
101	121257	ARC 11495::IRGC 21431-1	India
102	121361	IAC 47::IRGC 116992-1	Brazil
103	121523	TOANG::IRGC 19144-1	Indonesia
104	121644	TAINUNG 29::IRGC 65309-1	Taiwan
105	121646	TOS 5790::IRGC 117256-1	Nigeria

S. No.	Irgc No.	Designation	Country
106	117502	JHONA 26::IRGC 27967-1	Pakistan
107	121208	KHAO DO NGOI::IRGC 29772-1	Laos
108	121810	PULU LAPA::IRGC 48857-C1	Indonesia
109	124495	SILADON::IRGC 71620-1	Malaysia
110	121081	PERLA::IRGC 117021-1	Cuba
111	122052	GANIGI::IRGC 48698-C1	Indonesia
112	124490	PADI KOMPAI::IRGC 25510-1	Indonesia
113	122185	OS 4::IRGC 11335-C1	Nigeria
114	121212	MIMIDAM::IRGC 25897-1	Bangladesh
115	121320	CT 6946-9-1-2-M-1P::IRGC 117329-1	Colombia
116	121301	C 8434::IRGC 13496-1	Papua New Guinea
117	120977	ICTA POLOCHIC::IRGC 116997-1	Guatemala
118	120979	INIAP 415::IRGC 117001-1	Ecuador
119	117596	IR 60080-46 A::IRGC 117396-1	Philippines
120	121600	BENGIZA::IRGC 69845-1	Madagascar
121	121649	VILLAGUAY P A::IRGC 117259-1	Argentina
122	121456	NS 1288::IRGC 68930-1	Madagascar
123	121088	PITIPO::IRGC 117023-1	Peru
124	124484	MAHAPLEU (504)::IRGC 50865-1	Cote d'Ivoire
125	121906	CT 9993-5-10-1-M::IRGC 116974-1	Colombia
126	121876	D 4-136::IRGC 31051-1	Liberia
127	121635	RANAU KADAI::IRGC 71604-1	Malaysia
128	121658	ARC 11294::IRGC 21296-1	India
129	121563	DAKPA::IRGC 64888-1	Bhutan
130	121877	FOSSA HV::IRGC 16069-1	Burkina Faso
131	124471	VASSE NANAN::IRGC 56812-1	Cote d'Ivoire
132	117272	MOROBEREKAN	Guinea
133	121675	NILO 3 B::IRGC 10283-1	El Salvador
134	121859	YANCAOUSSA::IRGC 16071-C1	Cote d'Ivoire
135	121341	GBANTE::IRGC 16081-1	Cote d'Ivoire
136	121340	GAO GAN DA NUO::IRGC 73974-1	China
137	122128	KAKANI 2::IRGC 13373-C1	Nepal
138	121002	JUMA 51::IRGC 117009-1	Dominican Republic
139	121224	TAK SIAH::IRGC 73126-1	Pakistan
140	121463	PAE URA::IRGC 27321-1	Indonesia
141	121460	ONDEYKAM::IRGC 67846-1	Bhutan

S. No.	Irgc No.	Designation	Country
142	120983	IR 43::IRGC 117005-1	Philippines
143	120988	IR 77384-12-35-3-12-1-B::IRGC 117299-1	Philippines
144	117539	NHTA 5::IRGC 186-1	India
145	120976	ICTA PAZOS::IRGC 116996-1	Guatemala
146	121739	GEMJYA JYANAM::IRGC 32411-C1	Bhutan
147	121905	CINA::IRGC 27116-1	Indonesia
148	122031	DAVAO::IRGC 8244-C1	Philippines
149	117568	REXORO::IRGC 1715-1	USA
150	121940	WALANGA::IRGC 27502-1	Indonesia
151	121450	NENG NAH::IRGC 78275-1	Thailand
152	117542	OIRAN::IRGC 8257-1	Japan
153	121669	KHAU MEO::IRGC 78330-1	Viet Nam
154	121923	NEP ME HOA BINH::IRGC 78366-1	Viet Nam
155	121773	JUMALI::IRGC 9542-C1	Nepal
156	121386	KHAO' MUM::IRGC 78259-1	Thailand
157	120919	CR 8334::IRGC 116972-1	Costa Rica
158	121639	SAO::IRGC 61467-1	Liberia
159	117425	ARC 10497::IRGC 12485-1	India

List of 10 *O. glaberrima* accessions:

S. No.	Irgc No.	Country Of Origin
1	*O. glaberrima (IR101800)*	Nigeria
2	*O. glaberrima (IR102277)*	Liberia
3	*O. glaberrima (IR102356)*	Liberia
4	*O. glaberrima (IR102512)*	Liberia
5	*O. glaberrima (IR102526)*	Liberia
6	*O. glaberrima (IR102538)*	Liberia
7	*O. glaberrima (IR102615)*	Liberia
8	*O. glaberrima (IR103292)*	Senegal
9	*O. glaberrima (IR103545)*	Mali
10	*O. glaberrima (IR104033)*	Guinea

List of ANP cultivars

S. No.	Designation
1	Athma Shital
2	Amrit Bhog
3	Jira Phool
4	RAU 3043
5	Jalaka
6	Chhabiswa
7	Kalanamak 1
8	Raju Bhog
9	Kamod
10	Vallabh Basmati 24
11	Bindali
12	Basmati 385
13	Karigilas
14	Sukala Phool
15	Jai Gundi
16	Kubrimohor
17	Bhainsa Punchii
18	Fundri
19	Tedesi
20	Godawari Isukala
21	Pankhali 263
22	GR-101
23	Narmada
24	Bas Kamon
25	Basmati 397
26	Bas Durkh 189-5
27	Basmati 334
28	Jasmine Scented
29	HBC45
30	HBC48

S. No.	Designation
31	Basmati Jamuna
32	Basmati 5888
33	RAU 3041
34	Shabanmasi
35	Chilti Mutyalu
36	Khasakani
37	R1432-261-105-2-1-2
38	NDR 8497-2
39	Akitikari
40	Pusa Basmati 6
41	Basmati Narot 439
42	Basmati 372
43	Basmati Mohan 381
44	Basmati 377
45	Basmati 1-1-A
46	Basmati 106-12
47	Basmati 140
48	Basmati 6131
49	Basmati 6141
50	Basmati 127A
51	D-66
52	Du Thom Thai Binhho
53	415
54	Kaojao Hawn
55	Khai Mali
56	Lua Nhe
57	Milfor 6
58	Niaw Ping
59	Xiang Genghi
60	Calrose 76
61	HBC85

S. No.	Designation
62	HBC 98
63	Chanan
64	Ganga Balli
65	IR 74720-13-1-2-2
66	IET 15834
67	IET 22788

List of RYT lines

S. No.	Designation
1	PAU6886-6285-2-1-B
2	PAU6886-6362-2-1-B
3	Pusa 1718-14-2-150
4	Pusa 1718-19-8-152
5	1316
6	1515
7	1294
8	1406
9	1508
10	1633
11	PAU 5635-6-2-1-1
12	PAU 5673-22-3-2-1
13	PAU 5366-47-3-2-2-3
14	PAU 5089-3-7-2-1-1-2
15	Kal-Kamod-A
16	PAU 6141-14-1-1-1
17	PAU 6141-14-1-1-2

List of 22 released varieties along with three checks used for screening

S. No.	Designation
1	PR113
2	PR114
3	PR115
4	PR120
5	PR121
6	PR122
7	PR123
8	PR124
9	PR126
10	PR127
11	Pusa Basmati 1
12	Pusa Basmati 2
13	Pusa Basmati 3
14	Pusa Basmati 4
15	Pusa Basmati 5
16	Basmati370
17	Basmati386
18	Pusa1718
19	BPT 5204
20	Sharbati
21	Pusa 1637
22	Improved Pusa Basmati 1
23	Pusa basmati 1121 (Susceptible check)
24	Basmati 1509 (Susceptible check)
25	CSR30 (Control check)

APPENDIX II

(Disease reaction of ANP and RYT lines, April 2019)

S. No.	Designation	G (%)	SL	t value	DSI	SM	DI	DR
1	Athma Shital	65	12.13	3.758^{S}	2.429	29.762	45.238	S
2	Amrit Bhog	75	14.80	1.165^{NS}	0.667	13.889	13.889	S
3	Jira Phool	0						
4	RAU 3043	20	13.70	2.024^{NS}	1.667	16.667	16.667	S
5	Jalaka	95	10.25	4.933^{S}	1.356	15.556	31.667	S
6	Chhabiswa	85	12.28	4.728^{S}	1.083	6.250	6.250	S
7	Kalanamak 1	70	10.59	3.017^{S}	3.042	50.000	50.000	S
8	Raju Bhog	15	9.50	6.133^{S}	3.500	50.000	50.000	S
9	Kamod	20	12.40	1.338^{NS}	2.000	50.000	50.000	S
10	Vallabh Basmati 24	50	11.00	1.424^{NS}	3.417	75.000	75.000	S
11	Bindali	0						
12	Basmati 385	95	10.60	1.158^{NS}	3.000	72.222	77.778	S
13	Karigilas	95	9.32	2.347^{S}	1.811	21.667	42.778	S
14	Sukala Phool	50	16.58	0.192^{NS}	3.000	0.000	0.000	R
15	Jai Gundi	60	11.24	4.065^{S}	1.629	10.000	27.143	S
16	Kubrimohor	95	8.65	1.485^{NS}	2.044	25.000	56.667	S
17	Bhainsa Punchii	10	10.76	1.050^{NS}	2.000	50.000	50.000	S
18	Fundri	15	13.67	0.526^{NS}	0.000	0.000	0.000	R
19	Tedesi	0						
20	Godawari Isukala	85	8.80	7.778^{S}	1.714	27.143	27.143	S
21	Pankhali 263	65	5.50	6.177^{S}	1.875	22.500	45.000	S
22	GR-101	40	13.00	2.893^{S}	2.167	25.000	25.000	S
23	Narmada	95	20.36	2.402^{NS}	0.633	0.000	31.667	S
24	Bas Kamon	65	12.67	1.912^{NS}	2.025	26.250	52.500	S
25	Basmati 397	55	9.38	4.139^{S}	3.429	66.071	66.071	S
26	Bas Durkh 189-5	40	9.65	3.795^{S}	2.533	50.000	50.000	S
27	Basmati 334	35	10.47	4.354^{S}	3.167	58.333	58.333	S
28	Jasmine Scented	55	9.80	1.899^{NS}	3.107	67.857	67.857	S
29	HBC45	45	16.93	0.418^{NS}	3.333	58.333	91.667	S

S. No.	Designation	G (%)	SL	t value	DSI	SM	DI	DR
30	HBC48	40	10.00	1.213^{NS}	3.833	91.667	91.667	S
31	Basmati Jamuna	65	6.33	2.331^{S}	2.643	46.429	61.905	S
32	Basmati 5888	90	11.23	2.404^{S}	1.575	22.500	33.750	S
33	RAU 3041	0						
34	Shabanmasi	0						
35	Chilti Mutyalu	55	9.70	4.215^{S}	2.900	45.000	81.667	S
36	Khasakani	70	13.14	3.954^{S}	0.958	12.500	12.500	S
37	R1432-261-105-2-1-2	75	17.67	4.394^{S}	0.929	13.393	13.393	S
38	NDR 8497-2	20	23.43	0.218^{NS}	0.000	0.000	0.000	R
39	Akitikari	70	17.50	2.893^{S}	1.125	20.833	20.833	S
40	Pusa Basmati 6	90	16.05	4.441^{S}	3.250	100.000	100.000	S
41	Basmati Narot 439	60	11.00	7.680^{S}	3.200	71.429	71.429	S
42	Basmati 372	40	8.65	1.679^{NS}	4.000	75.000	100.000	S
43	Basmati Mohan 381	60	9.19	4.753^{S}	2.125	31.250	31.250	S
44	Basmati 377	75	8.40	5.066^{S}	1.889	27.778	33.333	S
45	Basmati 1-1-A	80	15.02	4.053^{S}	2.000	31.250	50.000	S
46	Basmati 106-12	90	11.57	3.428^{S}	1.825	22.500	28.750	S
47	Basmati 140	55	6.00	2.523^{S}	1.357	14.286	21.429	S
48	Basmati 6131	75	8.07	6.464^{S}	2.554	53.571	53.571	S
49	Basmati 6141	70	9.67	2.628^{S}	2.375	22.917	66.667	S
50	Basmati 127A	85	8.00	2.782^{S}	1.556	24.306	41.667	S
51	D-66	15	12.68	2.374^{NS}	3.500	75.000	75.000	S
52	Du Thom Thai Binhho	20	14.90	19.000^{S}	4.000	75.000	75.000	S
53	415	90	15.50	1.396^{NS}	1.400	23.750	46.250	S
54	Kaojao Hawn	90	15.00	2.488^{S}	1.333	16.667	38.889	S
55	Khai Mali	55	17.67	2.392^{S}	1.036	12.500	12.500	S
56	Lua Nhe	65	11.33	1.792^{NS}	1.425	22.500	22.500	S
57	Milfor 6	70	7.73	2.596^{S}	2.708	41.667	64.583	S
58	Niaw Ping	50	13.45	0.391^{NS}	2.000	40.000	60.000	S
59	Xiang Genghi	65	10.00	3.830^{S}	0.881	14.286	14.286	S
60	Calrose 76	85	10.00	2.763^{S}	1.500	22.917	34.722	S

S. No.	Designation	G (%)	SL	t value	DSI	SM	DI	DR
61	HBC85	95	16.83	0.677^{NS}	0.733	10.556	26.111	S
62	HBC 98	95	10.00	3.826^{S}	1.411	16.667	43.333	S
63	Chanan	70	10.75	3.860^{S}	2.857	50.000	64.286	S
64	Ganga Balli	90	16.56	1.018^{NS}	0.225	0.000	11.250	R
65	IR 74720-13-1-2-2	80	29.36	2.188^{NS}	0.800	0.000	40.000	S
66	IET 15834	80	12.60	3.091^{S}	0.750	0.000	18.750	S
67	IET 22788	90	13.80	2.145^{NS}	1.100	16.250	27.500	S
68	RYT 3426 (PAU 6886-6285-2-1-8)	45	8.93	0.311^{NS}	1.667	25.000	50.000	S
69	RYT3433 (PAU 6886-6362-2-1-B)	55	10.30	0.622^{NS}	1.667	28.333	55.000	S
70	RYT3517 (PUSA 1718-14-2-150)	60	22.64	3.837^{S}	1.143	20.000	37.143	S
71	RYT3518 (Pusa 1718-19-8-152)	95	12.87	1.823^{NS}	1.011	20.000	30.556	S
72	RYT 3644 (1316)	15	14.30	2.167^{NS}	2.000	50.000	50.000	S
73	RYT 3648 (1515)	85	17.94	0.996^{NS}	0.903	16.667	28.472	S
74	RYT 3659 (1294)	85	9.00	9.453^{S}	1.694	30.556	42.361	S
75	RYT 3662 (1406)	80	9.10	3.473^{S}	1.333	14.286	26.984	S
76	RYT 3671 (1508)	40	9.00	0.989^{NS}	2.133	26.667	53.333	S
77	RYT 3676 (1633)	70	14.93	2.325^{S}	1.143	7.143	21.429	S
78	RYT 3709 (PAU 5635-6-2-1-1)	70	9.37	8.590^{S}	3.222	62.222	62.222	S
79	RYT 3717 (PAU 5673-22-3-2-1)	85	21.58	0.274^{NS}	0.222	0.000	0.000	S
80	RYT 3722 (PAU 5366-47-3-2-2-3)	75	12.67	0.845^{NS}	1.089	20.536	33.929	S
81	RYT 3727 (PAU 5080-3-7-2-1-1-2)	90	12.50	3.000^{NS}	0.550	5.000	22.500	S
82	RYT 3737 (Kali Kamod A)	0						
83	RYT 3823 (PAU 6141-14-1-1-1)	75	19.52	2.466^{NS}	0.911	12.500	25.893	S
84	RYT 3824 (PAU 6141-14-1-1-2)	90	12.55	0.274^{NS}	0.525	5.000	16.250	S

APPENDIX III

(Disease reaction of rice germplasm, July 2019)

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
1	CHIGYUNGDO::IRGC55466-1	26.67	28.52	0.909^{NS}	0.286	7.143	7.143	87.00	1.933^{NS}	0.000	0.000	0.000	R
2	YAKUMO::IRGC5320-1	31.67	30.33	0.706^{NS}	0.000	0.000	0.000	75.33	1.868^{NS}	0.500	12.500	12.500	S
3	ZHENSHAN 97B	56.67	41.83	1.858^{NS}	0.174	5.618	0.000	93.00	2.397^{NS}	0.000	0.000	0.000	R
4	YAN ZHAO 9::IRGC63062-1	28.33	28.56	0.708^{NS}	0.597	6.472	6.250	88.25	1.073^{NS}	0.000	0.000	0.000	S
5	CO 39	66.67	33.83	0.549^{NS}	0.000	0.000	0.000	87.50	0.699^{NS}	0.000	0.000	0.000	R
6	YANGKUM (RED)	86.67	43.62	3.995^{S}	0.191	5.636	0.000	125.50	7.637^{S}	0.500	16.667	0.000	S
7	AEDAL	53.33	28.91	0.669^{NS}	0.384	6.784	9.608	92.75	0.089^{NS}	0.333	8.333	8.333	S
8	GHASELU MAP::IRGC72527-1	43.33	37.40	5.039^{S}	0.155	4.238	0.000	116.33	5.203^{S}	0.833	33.333	8.333	S
9	NIPPONBARE	60.00	30.90	1.219^{NS}	0.000	0.000	0.000	86.50	1.414^{NS}	1.000	25.000	25.000	S
10	KAM MRA::IRGC62172-1	10.00	46.45	9.249^{S}	0.750	25.250	0.000	116.00	9.000^{S}	1.000	50.000	0.000	S
11	SADU CHO	25.00	47.75	4.810^{S}	0.268	6.393	0.000	97.33	0.466^{NS}	0.000	0.000	0.000	S
12	BR IRGA 409::IRGC116960-1	95.00	47.42	6.593^{S}	0.278	8.444	0.000	80.00	0.615NS	0.000	0.000	0.000	S
13	KUROKA::IRGC74556-C1	13.33	40.30	4.663^{S}	0.533	16.867	0.000	78.50	0.307^{NS}	0.000	0.000	0.000	S
14	IRAT 144::IRGC55685-C1	35.00	30.79	0.262^{NS}	0.000	0.000	0.000	70.00	0.191^{NS}	0.500	12.500	12.500	S
15	WARABEHATOMOCHI::IRGC14779-1	6.67	41.57	1.811^{NS}	0.667	0.667	16.667			4.000	100.000	100.000	S
16	GOGO LEMPAK::IRGC43392-C1	70.00	35.00	0.300^{NS}	0.000	0.000	0.000	77.75	0.331^{NS}	0.000	0.000	0.000	R
17	RIZZOTTO 264::IRGC65727-1	13.33	35.56	0.790^{NS}	0.000	0.000	0.000	72.50	0.083^{NS}	0.667	0.000	0.000	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
18	WAB 368-B-1-H1-HB::IRGC117359-1	68.33	28.59	0.265^{NS}	0.000	0.000	0.000	79.67	0.521^{NS}	0.000	0.000	0.000	R
19	DAWASAM (RED)	46.67	34.51	5.069^{S}	0.364	11.672	0.000	90.00	0.575^{NS}	0.333	8.333	8.333	S
20	RIENALDO BERZANO::IRGC3230-1	11.67	46.10	4.208^{S}	0.583	16.917	0.000	104.00	1.342^{NS}	3.000	75.000	75.000	S
21	LI-JIANG-XIN-TUAN-HEI-GU	53.33	45.03	4.476^{S}	0.563	15.813	3.125	137.50	6.377^{S}	0.000	0.000	0.000	S
22	DULAR	81.67	45.50	5.892^{S}	0.082	2.042	0.000	100.00	0.512^{NS}	0.000	0.000	0.000	S
23	EDOGAWA::IRGC74468-1	70.00	28.80	0.565^{NS}	0.000	0.000	0.000	98.50	0.208^{NS}	0.333	0.000	0.000	S
24	INDIO::IRGC116998-1	28.33	44.30	5.933^{S}	1.431	25.556	29.861	77.33	1.025^{NS}	0.000	0.000	0.000	S
25	INDANE	58.33	32.75	0.263^{NS}	0.000	0.000	0.000	77.33	0.336^{NS}	0.500	8.333	8.333	S
26	ARGO::IRGC82418-1	15.00	30.94	0.310^{NS}	1.000	17.000	25.000	71.00	0.728^{NS}	2.000	50.000	50.000	S
27	BREVIARISTATA (RAIA)::IRGC3189-1	25.00	41.90	7.851^{S}	1.625	19.750	26.786	111.50	3.825^{S}	0.500	25.000	0.000	S
28	KHUDWANI ACC 409::IRGC34216-1	48.33	25.41	0.596^{NS}	0.000	0.000	0.000	76.33	0.235^{NS}	0.667	16.667	16.667	S
29	NSICRC 106::IRGC117370-1	31.67	30.36	0.769^{NS}	0.222	5.556	5.556	75.67	0.206^{NS}	0.000	0.000	0.000	R
30	PICONEGRO::IRGC117022-1	73.33	49.10	7.148^{S}	0.356	13.139	0.000	98.50	0.153^{NS}	0.000	0.000	0.000	S
31	ITA 235::IRGC64854-1	58.33	38.20	4.554^{S}	0.454	9.101	0.000	85.00	0.277^{NS}	0.285	0.000	0.000	S
32	DA 8	90.00	46.58	5.865^{S}	0.225	3.725	0.000	103.50	5.374^{S}	0.833	25.000	8.333	S
33	BRAZOS::IRGC24273-1	55.00	29.09	0.111^{NS}	0.000	0.000	0.000	105.00	4.409^{S}	0.667	25.000	8.333	S
34	CR 5272::IRGC116971-1	93.33	41.20	6.371^{S}	0.321	7.286	3.571	113.00	0.598^{NS}	0.167	8.333	0.000	S
35	LATSIKA::IRGC69367-1	16.67	30.52	0.079^{NS}	1.238	17.238	30.952	98.00	5.64^{S}	0.500	16.667	8.333	S
36	JIMBRUK JOLOWORO::IRGC43420-C1	78.33	34.81	1.420^{NS}	0.091	2.273	2.273	101.50	0.447^{NS}	1.000	25.000	25.000	S
37	BARAN BORO	31.67	38.17	0.861^{NS}	0.822	6.156	20.556	117.50	8.293^{S}	0.667	25.000	8.333	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
38	KEN CHIAO JU HSIAO LI	65.00	46.30	12.519^{S}	1.126	20.526	20.526	102.50	8.277^{S}	0.167	8.333	0.000	S
39	1-52-6::IRGC39111-1	68.33	32.37	0.629^{NS}	0.000	0.000	0.000	80.00	2.124^{NS}	0.500	0.000	0.000	S
40	BOSSA::IRGC57781-1	43.33	35.63	0.871^{NS}	0.143	3.571	3.571	88.00	0.189^{NS}	0.333	8.333	8.333	S
41	LUDAN::IRGC64189-C1	90.00	38.39	0.641^{NS}	0.000	0.000	0.000	84.50	22.63^{S}	0.333	16.667	0.000	S
42	CIRAD 409::IRGC116969-1	65.00	36.62	1.323^{NS}	0.000	0.000	0.000	81.50	1.897^{NS}	0.667	8.333	0.000	S
43	MINGHUI 63	78.33	40.03	0.538^{NS}	0.000	0.000	0.000	89.00	25^{S}	0.167	8.333	0.000	S
44	CIMARRON::IRGC116967-1	65.00	45.60	8.066^{S}	0.974	24.184	10.000	87.50	0.598^{NS}	0.000	0.000	0.000	S
45	PALMIRA::IRGC5097-1	51.67	36.39	0.219^{NS}	0.250	0.250	6.250	85.50	0.108^{NS}	1.167	25.000	25.000	S
46	IAC 164::IRGC117251-1	50.00	38.48	0.648^{NS}	0.000	0.000	0.000	105.00	31^{S}	1.333	33.333	25.000	S
47	DOONGARA::IRGC78392-1	53.33	32.42	0.618^{NS}	0.569	6.902	9.608	96.50	2.177^{NS}	0.500	25.000	0.000	S
48	KETAN KONIR::IRGC43444-C1	61.67	38.37	0.464^{NS}	0.000	0.000	0.000	83.00	20^{S}	0.333	16.667	0.000	S
49	TRES MESES::IRGC6464-C1	33.33	29.47	0.832^{NS}	0.000	0.000	0.000	84.50	0.343^{NS}	1.833	41.667	41.667	S
50	UCHUTI::IRGC14694-1	51.67	34.73	0.443^{NS}	0.000	0.000	0.000	86.00	0.175^{NS}	1.167	16.667	16.667	S
51	RHS 107-2-1-2TB-1JM::IRGC117025-1	58.33	31.43	0.130^{NS}	0.464	3.131	11.601	111.00	7.104^{S}	2.167	58.333	41.667	S
52	AZUCENA	43.33	33.47	0.815^{NS}	0.143	3.571	3.571	93.67	0.196^{NS}	0.333	8.333	8.333	S
53	GUATEMALA 1021::IRGC3388-1	58.33	40.55	5.190^{S}	0.742	9.212	11.438	85.00	0.308^{NS}	0.500	8.333	8.333	S
54	IAC 120::IRGC22712-1	46.67	33.20	2.981^{S}	0.000	0.000	0.000	96.00	9.241^{S}	1.000	16.667	8.333	S
55	GUAYQUIRARO P A::IRGC116987-1	58.33	34.64	0.919^{NS}	0.000	0.000	0.000	93.00	0.372^{NS}	0.000	0.000	0.000	R
56	TOS 10483::IRGC56723-1	55.00	35.33	0.383^{NS}	0.167	2.778	2.778	77.67	14.04^{S}	0.833	25.000	16.667	S
57	TOX 1011-4-1::IRGC117033-1	28.33	30.92	0.581^{NS}	0.333	0.333	0.000	64.67	0.18^{NS}	0.833	16.667	16.667	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
58	TX 10438::IRGC117035-1	70.00	29.81	1.009^{NS}	0.000	0.000	0.000	94.33	1.025^{NS}	0.667	16.667	16.667	S
59	NS 1288::IRGC68930-1	98.33	33.42	0.954^{NS}	0.000	0.000	0.000	83.00	0.612^{NS}	0.000	0.000	0.000	R
60	PACHOLINHA	75.00	42.03	2.757^{S}	0.354	7.036	2.174	74.00	0.212^{NS}	1.667	33.333	33.333	S
61	ORIENTE 10::IRGC55808-1	65.00	33.04	0.583^{NS}	0.511	5.563	12.763	90.50	7.225^{S}	0.333	16.667	0.000	S
62	MATAHAMBRE::IRGC53200-1	83.33	43.70	4.532^{S}	0.124	4.385	0.000	104.00	7.63^{S}	0.500	16.667	8.333	S
63	CAIAPO::IRGC116962-1	73.33	34.69	1.707^{NS}	0.267	2.667	6.667	95.00	0.28^{NS}	0.333	8.333	8.333	S
64	IC 27525::IRGC53989-1	70.00	39.38	8.367^{S}	1.293	21.661	21.854	131.00	23.33^{S}	1.778	44.444	33.333	S
65	GHARIB	91.67	30.51	1.134^{NS}	0.362	3.918	9.061	86.67	1.347^{NS}	0.000	0.000	0.000	S
66	AUS JOTA	96.67	44.63	9.447^{S}	0.314	7.243	3.571	99.00	0.824^{NS}	0.000	0.000	0.000	S
67	BAKUNG (H)::IRGC60220-1	63.33	35.55	1.066^{NS}	0.000	0.000	0.000	108.00	7.122^{S}	0.500	16.667	8.333	S
68	ARC 12701::IRGC22267-1	43.33	40.08	4.480^{S}	0.385	7.923	0.000	91.00	0.053^{NS}	0.000	0.000	0.000	S
69	MUT IAC 25-44-807::IRGC68799-1	46.67	32.40	2.093^{NS}	0.133	0.133	3.333	109.00	7.135^{S}	0.833	25.000	16.667	S
70	JI BO YA::IRGC77446-1	58.33	46.50	10.372^{S}	0.343	6.049	2.941	84.50	0.461^{NS}	1.000	12.500	12.500	S
71	CYPRESS	66.67	42.10	10.074^{S}	0.100	2.550	0.000	92.67	0.359^{NS}	0.250	0.000	0.000	S
72	CANA ROXA	83.33	28.47	1.594^{NS}	0.000	0.000	0.000	73.00	0.145^{NS}	0.222	0.000	0.000	R
73	KALAMKATI	65.00	35.62	1.146^{NS}	0.000	0.000	0.000	128.00	4.908^{S}	1.000	25.000	16.667	S
74	MIKHUDEB::IRGC25892-1	51.67	31.69	0.439^{NS}	0.000	0.000	0.000	83.00	1.473^{NS}	0.667	8.333	8.333	S
75	ARC 12451::IRGC41052-1	73.33	45.75	5.958^{S}	0.091	2.222	0.000	119.50	5.162^{S}	1.167	33.333	25.000	S
76	KIKILONG::IRGC71539-1	63.33	39.98	7.484^{S}	0.378	5.278	5.556	85.67	1.073^{NS}	0.667	16.667	16.667	S
77	TOS 724::IRGC11108-1	28.33	35.20	1.832^{NS}	0.000	0.000	0.000	103.00	8.002^{S}	1.500	41.667	33.333	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
78	HAWM OM	95.00	37.41	1.648^{NS}	0.176	1.855	1.786	91.00	0.457^{NS}	0.000	0.000	0.000	R
79	IAC 1111::IRGC39050-1	51.67	39.23	5.255^{S}	0.588	9.775	6.667	89.33	0.4^{NS}	0.333	8.333	8.333	S
80	KHAO KAP SANG::IRGC23423-1	43.33	36.07	0.400^{NS}	0.000	0.000	0.000	79.33	0.299^{NS}	0.667	16.667	16.667	S
81	EL PASO L 227::IRGC116979-1	53.33	37.03	0.304^{NS}	0.000	0.000	0.000	83.67	0.109^{NS}	1.333	25.000	25.000	S
82	NATO::IRGC1819-1	48.33	29.97	0.951^{NS}	0.529	9.529	13.221	104.00	4.237^{S}	0.667	25.000	8.333	S
83	KHAO DAM	65.00	46.95	4.372^{S}	0.103	2.682	0.000	91.67	10.84^{S}	1.500	50.000	25.000	S
84	KINANDANG PATONG	98.33	34.45	1.688^{NS}	0.000	0.000	0.000	85.00	3.649^{S}	0.167	8.333	0.000	S
85	RT 1031-69	66.67	35.73	0.374^{NS}	0.000	0.000	0.000	90.00	10.68^{S}	1.667	25.000	16.667	S
86	GOGO LEMPAK::IRGC43392-C1	53.33	46.38	6.999^{S}	0.369	11.898	0.000	114.00	4.596^{S}	0.333	16.667	0.000	S
87	HEI CHIAO CHUI LI HSIANG KENG	88.33	35.51	0.179^{NS}	0.000	0.000	0.000	80.00	5.434^{S}	0.167	8.333	0.000	S
88	SAYLLEBON::IRGC32509-1	61.67	36.24	0.624^{NS}	0.000	0.000	0.000	70.50	0.077^{NS}	1.000	16.667	16.667	S
89	MAINTIMOLOTSY 1226::IRGC11010-C1	100.00	44.40	4.978^{S}	0.067	1.700	0.000	86.00	11.13^{S}	0.167	8.333	0.000	S
90	TAINUNG 67	41.67	31.28	0.008^{NS}	0.000	0.000	0.000	92.50	4.405^{S}	0.286	14.286	0.000	S
91	DINOLORES::IRGC67431-1	93.33	34.14	0.229^{NS}	0.000	0.000	0.000	62.00	0.902^{NS}	0.333	8.333	8.333	S
92	IR64-21	98.33	40.87	1.158^{NS}	0.069	3.448	0.000	71.00	0.049^{NS}	0.000	0.000	0.000	R
93	BG 301::IRGC117315-1	76.67	37.17	0.224^{NS}	0.000	0.000	0.000	105.67	0.59^{NS}	0.833	33.333	8.333	S
94	62-667::IRGC15147-1	80.00	37.93	0.002^{NS}	0.000	0.000	0.000	109.05	6.392^{S}	2.500	58.333	50.000	S
95	RXAR RGUE::IRGC1943-1	81.67	32.86	0.198^{NS}	0.000	0.000	0.000	82.00	0.173^{NS}	1.000	25.000	25.000	S
96	63-104::IRGC15100-C1	48.33	31.62	0.311^{NS}	0.000	0.000	0.000	79.00	0.088^{NS}	0.167	0.000	0.000	R
97	VARY LAVA::IRGC386-1	40.00	33.28	0.098^{NS}	0.000	0.000	0.000	119.00	6.847^{S}	0.833	25.000	8.333	S

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		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
98	TORO 2::IRGC66761-1	85.00	31.93	0.653^{NS}	0.000	0.000	0.000	78.33	0.105^{NS}	1.000	25.000	25.000	S
99	MALAGKIT PUTI::IRGC19451-1	41.67	37.82	0.366^{NS}	0.000	0.000	0.000	96.00	22^{S}	0.500	16.667	8.333	S
100	GOGO::IRGC43390-C1	56.67	47.78	8.092^{S}	0.528	12.778	2.778	115.00	3.909^{S}	0.800	20.000	10.000	S
101	ARC 11495::IRGC21431-1	60.00	35.39	2.148^{NS}	0.000	0.000	0.000	89.00	0.365^{NS}	0.667	16.667	16.667	S
102	IAC 47::IRGC116992-1	61.67	26.02	0.409^{NS}	0.000	0.000	0.000	83.33	0.225^{NS}	1.333	25.000	25.000	S
103	TOANG::IRGC19144-1	71.67	37.69	0.585^{NS}	0.000	0.000	0.000	114.00	5.517^{S}	0.500	25.000	0.000	S
104	TAINUNG 29::IRGC65309-1	78.33	33.02	0.577^{NS}	0.000	0.000	0.000	98.00	3.189^{S}	0.333	16.667	0.000	S
105	TOS 5790::IRGC117256-1	75.00	36.93	0.076^{NS}	0.000	0.000	0.000	102.00	5.922^{S}	1.000	25.000	8.333	S
106	JHONA 26	48.33	47.33	5.402^{S}	0.293	11.601	0.000	132.00	12^{S}	0.333	16.667	0.000	S
107	KHAO DO NGOI::IRGC29772-1	11.67	38.37	1.389^{NS}	0.000	0.000	0.000	118.00	3.169^{S}	0.333	16.667	0.000	S
108	PULU LAPA::IRGC48857-C1	88.33	39.00	0.756^{NS}	0.000	0.000	0.000	86.67	0.406^{NS}	0.667	0.000	0.000	S
109	SILADON::IRGC71620-1	45.00	37.04	0.628^{NS}	0.302	3.918	3.846	101.50	3.413^{S}	1.000	50.000	0.000	S
110	PERLA::IRGC117021-1	68.33	41.55	0.966^{NS}	0.098	2.431	0.000	82.50	0.89^{NS}	0.000	0.000	0.000	R
111	GANIGI::IRGC48698-C1	78.33	34.66	0.193^{NS}	0.000	0.000	0.000	100.00	2.58^{NS}	0.167	8.333	0.000	R
112	PADI KOMPAI::IRGC25510-1	61.67	32.46	0.878^{NS}	0.000	0.000	0.000	112.00	4.18^{S}	1.667	33.333	25.000	S
113	OS 4::IRGC11335-C1	66.67	33.15	0.718^{NS}	0.000	0.000	0.000	73.67	0.487^{NS}	0.167	0.000	0.000	R
114	MIMIDAM::IRGC25897-1	60.00	45.48	8.007^{S}	0.170	5.935	0.000	137.50	5.288^{S}	1.000	41.667	8.333	S
115	CT 6946-9-1-2-M-1P::IRGC117329-1	28.33	45.47	3.490^{S}	1.403	25.778	17.361	105.50	2.965^{S}	1.000	37.500	12.500	S
116	C 8434::IRGC13496-1	40.00	45.20	6.360^{S}	1.846	37.210	25.175	103.00	5.171^{S}	0.600	30.000	0.000	S
117	ICTA POLOCHIC::IRGC116997-1	26.67	32.02	0.913^{NS}	0.250	6.250	6.250	85.00	0.389^{NS}	0.000	0.000	0.000	R

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
118	INIAP 415::IRGC117001-1	93.33	31.19	0.130^{NS}	0.000	0.000	0.000	76.67	0.349^{NS}	0.333	8.333	8.333	S
119	IR 60080-46 A	85.00	40.77	5.461^{S}	0.312	6.192	1.923	74.33	0.415^{NS}	0.000	0.000	0.000	S
120	BENGIZA::IRGC69845-1	25.00	29.82	0.544^{NS}	0.000	0.000	0.000	71.33	0.017^{NS}	0.400	0.000	0.000	S
121	VILLAGUAY P A::IRGC117259-1	50.00	37.02	0.695^{NS}	0.000	0.000	0.000	99.50	7.034^{S}	1.000	25.000	8.333	S
122	BAKILIKINDA::IRGC63121-1	93.33	41.38	8.242^{S}	0.421	5.866	6.897	94.50	1.943	1.000	25.000	8.333	S
123	PITIPO::IRGC117023-1	96.67	35.20	1.311^{NS}	0.000	0.000	0.000	85.00	6.085^{S}	0.667	16.667	8.333	S
124	MAHAPLEU (504)::IRGC50865-1	86.67	36.46	0.889^{NS}	0.000	0.000	0.000	102.50	3.638^{S}	0.500	25.000	0.000	S
125	CT 9993-5-10-1-M::IRGC116974-1	31.67	27.40	0.295^{NS}	0.000	0.000	0.000	51.50	1.274^{NS}	0.000	0.000	0.000	R
126	D 4-136::IRGC31051-1	95.00	32.65	1.174^{NS}	0.000	0.000	0.000	89.33	0.458^{NS}	1.000	25.000	25.000	S
127	RANAU KADAI::IRGC71604-1	83.33	36.45	0.062^{NS}	0.000	0.000	0.000	110.00	4.788^{S}	0.833	25.000	16.667	S
128	ARC 11294::IRGC21296-1	48.33	44.03	3.133^{S}	0.327	4.096	0.000	115.50	5.012^{S}	1.000	25.000	8.333	S
129	DAKPA::IRGC64888-1	75.00	29.54	1.123^{NS}	0.000	0.000	0.000	91.67	1.259^{NS}	0.500	12.500	12.500	S
130	FOSSA HV::IRGC16069-1	51.67	37.27	1.336^{NS}	0.118	0.118	2.941	93.25	0.631^{NS}	0.667	16.667	16.667	S
131	VASSE NANAN::IRGC56812-1	53.33	31.28	2.152^{NS}	0.000	0.000	0.000	95.00	2.012^{NS}	0.333	16.667	0.000	S
132	MOROBEREKAN	91.67	26.42	0.693^{NS}	0.000	0.000	0.000	78.25	0.686^{NS}	1.200	30.000	30.000	S
133	NILO 3 B::IRGC10283-1	85.00	31.38	0.144^{NS}	0.080	2.000	2.000	96.00	3.544^{S}	0.833	16.667	8.333	S
134	YANCAOUSSA::IRGC16071-C1	58.33	32.51	2.587^{S}	0.503	3.441	8.388	96.50	10.71^{S}	0.667	25.000	8.333	S
135	GBANTE::IRGC16081-1	31.67	32.56	0.756^{NS}	0.619	7.476	15.476	95.00	0.149^{NS}	2.000	33.333	33.333	S
136	GAO GAN DA NUO::IRGC73974-1	60.00	30.71	0.754^{NS}	0.000	0.000	0.000	85.67	0.149^{NS}	0.667	16.667	16.667	S
137	KAKANI 2::IRGC13373-C1	50.00	30.45	0.525^{NS}	0.000	0.000	0.000	85.33	0.422^{NS}	0.000	0.000	0.000	R

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
138	JUMA 51::IRGC117009-1	48.33	40.25	9.960^{S}	0.760	11.913	10.096	78.00	0.055^{NS}	0.000	0.000	0.000	S
139	TAK SIAH::IRGC73126-1	80.00	37.64	0.032^{NS}	0.000	0.000	0.000	103.50	3.702^{S}	0.333	16.667	0.000	S
140	PAE URA::IRGC27321-1	63.33	40.20	2.370^{S}	0.106	2.828	0.000	78.50	0.568^{NS}	0.667	16.667	16.667	S
141	ONDEYKAM::IRGC67846-1	6.67			3.333	51.333	83.333						S
142	IR 43::IRGC117005-1	53.33	29.67	1.149^{NS}	0.000	0.000	0.000	88.75	0.379^{NS}	0.333	8.333	8.333	S
143	IR 77384-12-35-3-12-1-B::IRGC117299-1	50.00	37.04	1.214^{NS}	0.154	3.846	3.846	104.00	5.011^{S}	1.400	40.000	30.000	S
144	NHTA 5	65.00	36.36	0.879^{NS}	0.416	5.363	7.763	76.50	1.151^{NS}	0.400	10.000	10.000	S
145	ICTA PAZOS::IRGC116996-1	96.67	46.05	4.700^{S}	0.140	1.774	0.000	69.75	0.015^{NS}	1.000	25.000	25.000	S
146	GEMJYA JYANAM::IRGC32411-C1	16.67	30.67	0.194^{NS}	0.000	0.000	0.000	97.00	4.326^{S}	2.000	75.000	25.000	S
147	CINA::IRGC27116-1	51.67	28.53	1.099^{NS}	0.000	0.000	0.000	83.00	0.322^{NS}	0.667	16.667	16.667	S
148	DAVAO::IRGC8244-C1	96.67	30.70	1.219^{NS}	0.200	5.000	5.000	82.75	0.238^{NS}	0.333	8.333	8.333	S
149	REXORO	81.67	27.58	0.122^{NS}	0.087	2.174	2.174	94.00	19.36^{S}	0.500	16.667	8.333	S
150	WALANGA::IRGC27502-1	88.33	43.58	6.327^{S}	0.342	7.803	3.846	104.00	3.593^{S}	0.167	8.333	0.000	S
151	NENG NAH::IRGC78275-1	60.00	30.64	1.188^{NS}	0.125	3.125	3.125	94.33	0.96^{NS}	0.571	14.286	14.286	S
152	OIRAN	23.33	38.99	7.963^{S}	0.000	0.000	0.000	65.67	0.686^{NS}	1.333	33.333	33.333	S
153	KHAU MEO::IRGC78330-1	65.00	36.10	0.386^{NS}	0.211	5.263	5.263	123.00	4.814^{S}	1.500	33.333	33.333	S
154	NEP ME HOA BINH::IRGC78366-1	15.00	32.70	0.344^{NS}	0.000	0.000	0.000	77.33	1.163^{NS}	0.800	20.000	20.000	S
155	JUMALI::IRGC9542-C1	43.33	32.98	0.405^{NS}	0.310	4.310	7.738	92.75	0.648^{NS}	0.667	16.667	16.667	S
156	KHAO' MUM::IRGC78259-1	50.00	35.75	8.390^{S}	0.929	12.929	23.214	99.00	0.048^{NS}	0.500	12.500	12.500	S
157	CR 8334::IRGC116972-1	66.67	26.27	1.177^{NS}	0.000	0.000	0.000	96.33	0.805^{NS}	0.667	16.667	16.667	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
158	SAO::IRGC61467-1	88.33	26.92	1.674^{NS}	0.151	1.866	3.786	99.00	0.157^{NS}	0.400	10.000	10.000	S
159	ARC 10497	6.67			3.000	52.000	16.667						S
160	*O. glaberrima (IR101800)*	50.00	52.11	2.833^{S}	0.679	15.679	3.846	114.50	2.582^{S}	0.333	16.667	0.000	S
161	*O. glaberrima (IR102277)*	68.33	33.32	0.389^{NS}	0.000	0.000	0.000	81.33	0.179^{NS}	0.667	16.667	16.667	S
162	*O. glaberrima (IR102356)*	85.00	37.39	0.213^{NS}	0.000	0.000	0.000	88.00	2.107^{NS}	0.333	8.333	8.333	S
163	*O. glaberrima (IR102512)*	95.00	32.61	0.780^{NS}	0.000	0.000	0.000	79.00	0.639^{NS}	0.000	0.000	0.000	R
164	*O. glaberrima (IR102526)*	86.67	34.09	1.740^{NS}	0.000	0.000	0.000	85.67	0.392^{NS}	0.000	0.000	0.000	R
165	*O. glaberrima (IR102538)*	73.33	37.38	4.295^{S}	0.182	4.849	0.000	88.00	0.264^{NS}	0.000	0.000	0.000	S
166	*O. glaberrima (IR102615)*	81.67	28.75	0.484^{NS}	0.000	0.000	0.000	86.75	0.202^{NS}	0.000	0.000	0.000	R
167	*O. glaberrima (IR103292)*	93.33	26.67	2.011^{NS}	0.000	0.000	0.000	80.00	0.055^{NS}	0.000	0.000	0.000	R
168	*O. glaberrima (IR103545)*	10.00	29.08	0.026^{NS}	0.500	12.500	12.500	79.33	0.265^{NS}	0.000	0.000	0.000	R
169	*O. glaberrima (IR104033)*	25.00	32.03	2.388^{S}	0.571	14.286	14.286	86.00	0.574^{NS}	1.200	30.000	30.000	S
170	PR113	100.00	30.56	0.151^{NS}	0.000	0.000	0.000	74.67	0.5^{NS}	0.000	0.000	0.000	R
171	PR114	71.67	30.84	0.911^{NS}	0.000	0.000	0.000	86.00	2.107^{NS}	0.167	0.000	0.000	R
172	PR115	75.00	30.85	1.586^{NS}	0.000	0.000	0.000	80.00	1.408^{NS}	0.167	0.000	0.000	R
173	PR120	61.67	40.94	2.737^{S}	1.345	23.012	18.713	102.00	5.397^{S}	1.167	33.333	25.000	S
174	PR121	75.00	29.72	0.759^{NS}	0.000	0.000	0.000	83.50	1.821^{NS}	0.000	0.000	0.000	R
175	PR122	93.33	29.11	1.618^{NS}	0.000	0.000	0.000	85.00	1.054^{NS}	0.000	0.000	0.000	R
176	PR123	65.00	28.98	1.511^{NS}	0.000	0.000	0.000	86.33	0.611^{NS}	0.000	0.000	0.000	R
177	PR124	68.33	30.59	0.665^{NS}	0.000	0.000	0.000	83.50	0.607^{NS}	0.000	0.000	0.000	R

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
178	PR126	85.00	30.74	0.322^{NS}	0.000	0.000	0.000	72.75	1.007 ^{NS}	0.000	0.000	0.000	R
179	PR127	83.33	28.11	0.737^{NS}	0.000	0.000	0.000	83.67	0.019 ^{NS}	0.000	0.000	0.000	R
180	Pusa Basmati 1	50.00	35.08	1.467^{NS}	0.166	0.000	4.166						R
181	Punjab Basmati 2	96.67	45.93	3.923^{S}	0.583	9.262	8.571	95.50	0.359 ^{NS}	0.333	8.333	8.333	S
182	Punjab Basmati 3	80.00	46.40	5.079^{S}	0.483	7.164	0.000	112.00	2.253^{NS}	0.500	16.667	8.333	S
183	Punjab Basmati 4	88.33	40.60	6.863^{S}	0.570	4.088	1.852	112.00	2.859^{S}	1.000	33.333	16.667	S
184	Punjab Basmati 5	58.33	53.31	5.550^{S}	1.758	42.314	8.660	115.00	3.71^{S}	0.667	33.333	0.000	S
185	Basmati370	76.67	48.05	6.093^{S}	1.140	20.913	9.091	120.00	8.212^{S}	0.167	8.333	0.000	S
186	Basmati386	71.67	47.55	12.477^{S}	1.828	30.181	18.552	103.50	4.506^{S}	1.333	41.667	25.000	S
187	Pusa1718	68.33	49.38	13.389^{S}	2.182	39.636	13.995	81.00	0.071 ^{NS}	0.000	0.000	0.000	S
188	BPT 5204	11.67	29.41	4.314^{S}	0.000	0.000	0.000	72.33	0.607 ^{NS}	2.000	50.000	50.000	S
189	Pusa 1637	46.67	38.83	2.576^{S}	1.374	18.829	29.947	67.67	2.123 ^{NS}	0.000	0.000	0.000	S
190	Athma Shital	61.67	36.93	5.129^{S}	0.161	2.883	0.000	94.50	4.992^{S}	0.333	16.667	0.000	S
191	Raju Bhog	13.33	37.47	0.581^{NS}	0.250	12.500	0.000	84.67	0.587^{NS}	0.000	0.000	0.000	S
192	Kamod	46.67	42.18	6.360^{S}	0.333	4.417	0.000	100.00	4.906^{S}	0.333	16.667	0.000	S
193	Vallabh Basmati 24	43.33	32.69	0.754^{NS}	0.564	9.291	12.424	80.33	0.552^{NS}	1.500	37.500	37.500	S
194	Bindali	21.67	33.19	0.918^{NS}	0.167	8.333	0.000	99.00	5.699^{S}	0.750	25.000	12.500	S
195	Basmati 385	88.33	36.35	0.299^{NS}	0.080	2.000	2.000	112.00	2.893^{S}	0.500	16.667	8.333	S
196	Sukala Phool	71.67	36.85	0.452^{NS}	0.105	2.632	2.632	87.67	0.333^{NS}	0.000	0.000	0.000	R
197	Kubrimohor	83.33	40.65	6.662^{S}	0.366	6.670	4.348	104.50	4.455^{S}	0.667	25.000	8.333	S

S. No.	Designation	Nursery						Transplanted					
		G (%)	SL	t value	DSI	DI	SM	PH	t value	DSI	DI	SM	DR
198	Bhainsa Punchii	18.33	33.53	1.661^{NS}	0.000	0.000	0.000	110.00	3.65^{S}	1.000	50.000	0.000	S
199	GR-101	18.33	44.20	10.149^{S}	3.100	31.500	45.000	75.00	2.108^{NS}	0.000	0.000	0.000	S
200	HBC-45	85.00	34.62	0.517^{NS}	0.000	0.000	0.000	110.00	5.952^{S}	0.167	8.333	0.000	S
201	NDR8497-2	8.33	32.00	0.504^{NS}	1.250	50.250	0.000	92.00	0.832^{NS}	1.000	25.00	0.000	R
202	Pusa Basmati 6	41.67	41.03	0.911^{NS}	0.333	10.133	3.333	121.00	5.11^{S}	0.500	25.000	0.000	S
203	Basmati Narot 439	60.00	38.23	4.662^{S}	0.393	5.498	5.573	113.00	3.845^{S}	1.167	33.333	25.000	S
204	Basmati 1-1-A	41.67	29.98	0.854^{NS}	0.167	4.167	4.167	115.00	5.843^{S}	1.167	33.333	25.000	S
205	D-66	15.00	34.60	5.521^{S}	0.450	12.700	0.000	90.00	3.462^{S}	1.000	33.333	16.667	S
206	Du Thom Thai Binhtia	45.00	36.68	4.692^{S}	0.583	12.833	0.000	81.33	2.108^{NS}	1.500	37.500	37.500	S
207	Milfor 6	50.00	31.40	1.061^{NS}	0.000	0.000	0.000	83.67	0.021^{NS}	0.333	8.333	8.333	S
208	IR 74720-13-1-2-2	28.33	38.58	6.005^{S}	1.653	25.778	6.250	103.00	2.623^{NS}	0.333	16.667	0.000	S
209	1294	10.00	29.91	0.487^{NS}	0.667	16.667	16.667	102.50	3.771^{S}	0.333	16.667	0.000	S
210	PAU 5673-22-3-2-1	10.00	26.42	0.610^{NS}	0.500	0.500	12.500						S
211	Pusa basmati 1121	60.00	56.97	5.758^{S}	2.338	35.525	28.125	125.00	3.417^{S}	2.500	75.000	50.000	S
212	Basmati 1509	78.33	64.09	13.385^{S}	2.245	39.836	21.091	108.00	6.843^{S}	1.833	50.000	41.667	S
213	CSR30	100.00	36.90	1.767^{NS}	0.000	0.000	0.000	88.00	0.345^{NS}	0.000	0.000	0.000	R

G (%) = Germination percent, SL = Seedling length (cm), t = t test, DSI = Disease severity index, DI = Disease incidence (percent), SM = Seedling mortality (percent), PH = Plant height (cm), DR = Disease reaction (S= Susceptible, R = Resistant), S = Significant and NS = Non-significant at 5% level of significance

VITA

Name of the student	**:**	Baljeet Kaur
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EDUCATIONAL QUALIFICATION

Bachelor's Degree	**:**	B.Sc. (Agri.)
University and Year of Award	**:**	Punjabi University, Patiala (2017)
Percentage of marks	**:**	81.70
Master's Degree	**:**	M.Sc. (Plant Breeding and Genetics)
University and Year of Award	**:**	Punjab Agricultural University, Ludhiana (2020)
OCPA	**:**	8.63/10.00
Title of Master's Thesis	**:**	Identification of Donors/QTLs for foot rot resistance in rice and studies on association of gibberellic acid regulated/synthesis genes with the disease