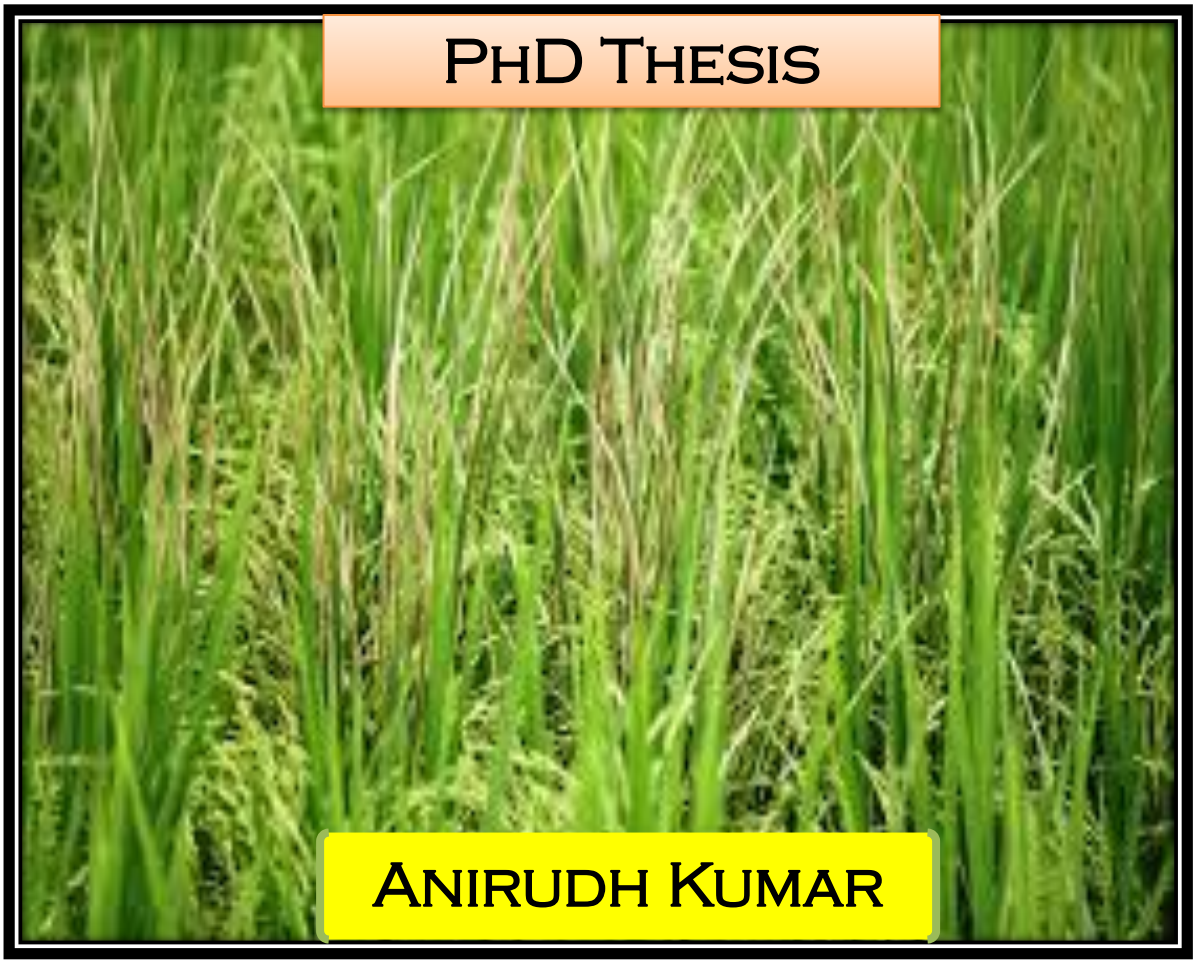
# Molecular cloning and functional validation of Bacterial Blight resistance genes in rice




Thesis Submitted to the University of Hyderabad

for the Award of the Degree of

**Doctor of Philosophy**

In Plant Sciences

July, 2013

# Molecular cloning and functional validation of Bacterial Blight resistance genes in rice

THESIS SUBMITTED TO THE
UNIVERSITY OF HYDERABAD FOR THE AWARD OF

**DOCTOR OF PHILOSOPHY**

**By**


**Anirudh Kumar**

(Enrolment No. 07LPPH03)




Department of Plant Sciences

School of Life Sciences

University of Hyderabad

Hyderabad, 500046

India


July, 2013

UNIVERSITY OF HYDERABAD

हैदराबाद विश्वविद्यालय

(A Central University established in 1974 by act of parliament)

HYDERABAD – 500046, INDIA

## "DECLARATION"

I, **Anirudh Kumar** hereby declare that this thesis entitled **"Molecular cloning and functional validation of Bacterial Blight resistance genes in rice"** submitted by me under the guidance and supervision of **Dr. Irfan Ahmad Ghazi** is an original and independent research work. I also declare that it has not been submitted previously in part or in full to this University or any other University or Institution for the award of any degree or diploma.

**Anirudh Kumar**

**(Research Scholar)**


UNIVERSITY OF HYDERABAD
हैदराबाद विश्वविद्यालय

(A Central University established in 1974 by act of parliament)
HYDERABAD – 500046, INDIA

## "CERTIFICATE"

This is to certify that this thesis entitled **"Molecular cloning and functional validation of Bacterial Blight resistance genes in rice"** is a record of bonafide work done by **Mr. Anirudh Kumar,** a research scholar for Ph.D programme in the Department of Plant Sciences, School of Life Sciences, University of Hyderabad, under my guidance and supervision.

**Prof. Ch. Venkata Ramana (HEAD)**
**(Dept. of Plant Sciences)**

**Dr. Irfan Ahmad Ghazi**
**(Research Supervisor)**

**Prof. R. P. Sharma (DEAN)**
**(School of Life Sciences)**

**ACKNOWLEDGEMENTS**

I feel extremely privileged to express my veneration for my supervisor Dr. Irfan Ahmad Ghazi, Assistant Professor, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad. It is a great honor for me to be his first Ph.D student. His whole-hearted co-operation, support and encouragement rendered throughout made this thesis possible. I am extremely lucky to prosecute this investigation under his exceptional guidance. Working under him gave me a true sense of freedom, broadened my scientific outlook and inculcated a spirit of positive attitude in me. Without his unflinching support and active guidance, the research work presented in the thesis would not have been possible. As I wrap up this work, I really do not know how far I have met his expectations but I can't forget his care and help showed on me, especially when the time was becoming difficult.

I express my heartfelt gratitude to Dr. Insaf Ahmad Qureshi, Assistant Professor, Dept. of Biotechnology, Dr. Ragiba Makandar, Associate Professor Dept. of Plant Sciences, University of Hyderabad, Dr. R.M. Sundaram and Dr. G.S. Laha, Senior Scientists, Directorate of Rice Research, Hyderabad, for guiding me in all the research activities throughout my work. Their suggestions while planning and executing the experiments have been helpful in bringing a sense of direction to the whole investigation and I would also like to thank profusely for their kind help and support while writing the thesis.

The members of doctoral committee Prof. P.B. Kirti and Prof. Appa Rao Podile, Dept. of Plant Sciences, have contributed immensely; I express my deepest sense of gratitude to them. Their timely support, suggestions and inspiring words are simply unforgettable. I also express my loyal thanks and heartfelt gratitude to both of them for allowing me to use their facility with full freedom.

I take this opportunity to acknowledge Prof. R.P. Sharma (Dean), Prof. A.S. Raghavendra and Prof. M .Ramanadham (former Deans) of the school of Life Sciences for all the help and necessary infrastructure.

I am equally grateful to Prof. A.R. Reddy (Head), Prof. A.R. Podile (Former Head), Department of Plant Sciences, School of Life Sciences, University of Hyderabad for providing high impact common infrastructure facilities to the department.

I am especially thankful to Prof. A.R. Reddy (Head) for his support and encouragement and the time he had spent for editing one of my article, which got published in Australian Journal of Crop Sciences.

I am really grateful to Prof. Arjula R. Reddy, distinguished biologist and Hon'ble Vice chancellor, Yogi Vemana University, Kadapa for his elderly advice, support and guidance which helped me in completing this task through various stages.

I express my deepest sense of gratitude to the entire Faculty in the school of Life Sciences for inspiring me and helping me directly or indirectly.

PhD students often talk about loneliness during the course of their study but this is something which I never experienced at University of Hyderabad. A heartfelt thanks to the really supportive and active student community of the School and all my friends who made me experience something special, in particular Naveen, Pawan, Israr, Deepankar, Abhay Kumar, Abhay Pratap, Das, Manish, Ahan, Rakesh, Kamal, Kapil, Suresh, Prateek, Debashree, Sumit, Mahesh, Malthi, Abin, Akanksha, Moein, and many more.....

Special thanks to Anirban Guha and Ritambhara for constructive suggestions during final draft preparation, Pawn Shukla and Moein for helping me in transgenic works, Dilip Verma, Monica Kannan and Kalpana for 2-DE/MALDI-TOF work .

I am appreciative of my fellow graduate students of my laboratory W. Bimolta, Mr. Zahoor Gul, and Yasin Bhatt. I give my sincere thanks to all lab members of Prof. P.B. Kirti and Prof. A.R. Podile for their timely help.

I gratefully acknowledge the funding sources that made my Ph.D. work possible. I was funded from DBT project as JRF and SRF for 3 years and rest by BBL fellowships of the University of Hyderabad. I also acknowledge PURSE grant for financing me to attend the international conference at Bangkok. The financial support given to the Department of Plant Science from the DST, UoH DBT-CREBB, DST-FIST, UGC-SAP- CAS are gratefully acknowledged. DBT, DST and CSIR are duly acknowledged for providing generous grant to our lab.

I would like to thank Mr. Raju, Field Assistant for carrying out the field work and mentioning the plant in healthy condition.

I would also like to thank my friends from other institutes Dr. Manish Pandey and Dr. Pawan Khera (ICRISAT) Dr. Arun Kumar Pandey (DSR), Dr. Laxminarayan Mishra (JNCASR), Dr. Akshya Biswal (IRRI), Dipesh Singh (CCMB), Rahul Priyadarshi and Akhilesh Singh (Barwale Foundation) and Amit Kumar (CDRI) for their moral support, timely help and encouragement.

I am obliged to non-teaching staff members of the Department, School and Administration for the valuable information provided by them time to time. I am grateful for their cooperation during the complete period of my assignment.

Although there may be many who remain unacknowledged in this humble note of gratitude there are none who remain unappreciated.

I would like to extend my deepest gratitude to my family: my parents and my brothers and sister. I would also like to remember my wonderful and jovial nephew and niece. They always have provided unwavering love, affection and encouragement. Thank you for believing in me.

It gives me great pleasure in acknowledging Mr. Rajesh Ranjan and Mrs. Swati, my local guardian and well-wisher who cheers me up and stood by me through the good times and bad. I really appreciate their unending encouragement and support.

I thank the almighty for the great opportunity he has given me to be, and to be in this beautiful world with all those loving and encouraging people around, for crafting my strength with patience, tolerance and hard work to face and overcome the difficulties.

ANIRUDH KUMAR

I dedicate this dissertation to my family and friends, whose love and patience has encouraged me during my struggle with this research

**TABLE OF CONTENTS** **Page No.**

Abbreviations……i–iii

List of Figures……...iv–v

List of Tables and Annexures…...……vi

**Chapter 1: Introduction**……...1–5

**Chapter 2: Review of Literature**……6–26

2.1 Taxonomy and Origin of Cultivated Rice
2.2 Biotic and Abiotic stresses
2.3 Bacterial pathogen and mode of infection
2.4 History of occurrence of BB menace and extent of damage
2.5 Effect of BB on leaf gas exchange physiology
2.6 Different mechanism of BB control
2.7 Classification and function of plant disease resistance genes
2.8 Structural organization of protein kinase genes
2.9 Resistance gene analogues
2.10 Expression studies of disease resistance genes
2.11 Proteomic studies of disease resistance proteins
2.12 Comparative genomics
2.13 Comparative genomics of disease resistance genes

**Chapter 3: Materials and Methods**……..27–47

**3.1 Screening for Bacterial Blight resistance and characterization of photosynthetic and yield responses in rice genotypes**
3.1.1 Plant growth and experimental set up
3.1.2 Inoculum preparation, infestation and disease scoring
3.1.3 Leaf gas exchange analysis
3.1.4 Measurements of yield and yield attributing components

**3.2 Identification, mapping and synteny analysis of genes associated with Bacterial Blight resistance from chromosome 11 of rice genome sequence**
3.2.1 *Ab initio* gene prediction

3.2.2 Construction of physical map of Receptor kinases (Class 5 disease resistance gene)
3.2.3 Sub-cellular location of genes
3.2.4 GC content and domain determination
3.2.5 Comparison of *Xa21* and *Xa26* with known R genes using BL2SEQ algorithm
3.2.6 Synteny analysis of class-5 resistance genes
3.2.7 Comparative analysis of the genomic regions flanking *Xa21* locus in *indica* and *japonica* ssp. of rice

**3.3 Molecular cloning and expression analysis of Bacterial Blight resistance genes**
3.3.1 Plant materials
3.3.2 Preparation of competent cells of *Escherichia coli*
3.3.3 Genomic DNA isolation and quantification
3.3.4 Primer designing
3.3.5 PCR Analysis and gel elution
3.3.6 Ligation and Cloning of polymorphic PCR product
3.3.7 Sequencing and Sequence analysis
3.3.8 Total RNA isolation for expression analysis of resistance genes
3.3.9 Determination of RNA quantity, quality and cDNA synthesis
3.3.10 Real time PCR
3.3.11 Amplification of full length ORF and its cloning
3.3.12 Preparation of expression cassette
3.3.13 Preparation of *Agrobacterium* culture
3.3.14 Tobacco transformation
3.3.15 Rice transformation
3.3.16 Acclimatization and Green house transfer

**3.4 Proteomic analysis to identify genes potentially involved in Bacterial Blight resistance**
3.4.1 Plant materials and infection treatment
3.4.2 Total protein extraction
3.4.3 Bradford method of protein quantification
3.4.4 2-DE separation, gel staining and image analysis
3.4.5 Enzymatic digestion of protein in-gel, mass spectrometry and protein identification
3.4.6 Statistical analysis

**Chapter 4: Results**.....48-101

**4.1 Analysis of genotypic variability, photosynthetic and yield responses against BB infection**

4.1.1 Reaction of rice genotypes against *Xoo* strain DX133

4.1.2 Photosynthetic leaf gas exchange responses

4.1.3 Yield and yield components

**4.2 *In silico* analysis of chromosome 11 of rice**

4.2.1 Annotation of BAC/PAC Sequences from chromosome 11 of rice

4.2.2 Identification of protein sub-cellular localization using neural net program

4.2.3 Determination of GC content and domains of resistance genes

4.2.4 Comparison of *Xa21* and *Xa26* with known R genes using BL2SEQ algorithm

4.2.5 Synteny analysis of class 5 resistance genes sequence to other plant genomes

**4.3 Comparative analysis of *Xa21* locus in *indica* and *japonica* ssp. of rice**

4.3.1 Gene prediction, annotation and classification

4.3.2 GC content in the 100 kb region

4.3.3 Distribution and identification of SSRs in the 100 kb region of *Xa21* locus

4.3.4 Microsynteny analysis for physical mapping of predicted genes

4.3.5 Sub-cellular localization of predicted gene products

4.3.6 Phylogenetic analysis

**4.4 Cloning and sequencing of receptor kinases**

4.4.1 PCR amplification resistance genes and polymorphism analysis

4.4.2 Real-time quantitative polymerase chain reaction (RT-qPCR)

4.3.4 Generation of transgenic rice and tobacco

**4.5 Proteomic analysis of bacterial blight susceptible and resistant rice genotypes**

4.5.1 2D-GE and protein expression profiling

4.5.2 Identification of differentially regulated proteins

**Chapter 5: Discussions**.....102-120

5.1 Effect of bacterial blight in the photosynthetic efficiency and in the yield

5.2 Sequence analysis of BAC clones from chromosome 11 of rice

5.3 Comparative analysis of *Xa21* gene in *indica* and *japonica* ssp.

5.4 Polymorphism analysis and preparation of expression cassettes

5.5 Proteomic approach to understand BB induced genes

**Chapter 6: Summary and conclusions**……121-125

**Chapter 7: References**……..126-147

**Annexures**…….148-162

**Appendix**……..163-167

**Publications**……..168-170

**ABBREVIATIONS**

| | |
|---|---|
| µg | : Microgram |
| µM | : Micromolar |
| °C | : Degree celcius |
| cM | : Centimorgan |
| cm | : Centimeter |
| ACN | : Acetonitrile |
| ATP | : Adenosine Tri Phosphate |
| Avr | : Avirulence |
| bp | : Base pair |
| BB | : Bacterial Blight |
| cDNA | : Complementary DNA |
| CRD | : Completely Randomized Design |
| CTAB | : Cetyltrimethylammonium Bromide |
| DAI | : Day After Infection |
| DEPC | : Diethylpyrocarbonate |
| DMSO | : Dimethyl sulfoxide |
| DNA | : Deoxy ribonucleic acid |
| dNTPs | : Deoxy Nucleotide Triphosphates |
| DTT | : Dithiothreitol |
| EDTA | : Ethylene diamine tetra acetic acid |
| EtBr | : Ethidium Bromide |
| EPS | : Extracellular polysaccharides |
| ER | : Endoplasmic reticulum |
| EST | : Expressed Sequence Tag |
| GLP | : Germin like protein |
| GST | : Glutathione S-transferases |
| h | : Hour(s) |
| HR | : Hypersensitive Response |
| IEF | : Isoelectric Focusing |
| IPTG | : Isopropyl β-D-thiogalactoside |
| IRGSP | : International Rice Genome Sequencing Project |
| IIRGS | : Indian Initiative for Rice Genome Sequencing |
| IPG | : Immobiline DryStrip gels |
| ISR | : Induced Systemic Resistance |

| | |
|---|---|
| kb | : Kilobase |
| kDa | : Kilodalton |
| L | : Litre |
| LL | : Lesion Length |
| LRR | : Leucine Rich Repeat |
| LB | : Luria-Bertani |
| M | : Molar |
| MALDI | : Matrix-assisted laser desorption/ionization |
| Mb | : Megabase |
| MB | : Membrane bound |
| MR | : Moderate Resistance |
| mg | : Milligram |
| min | : Minute |
| ml | : Milliliter |
| mM | : Millimolar |
| MMLV | : Moloney Murine Leukemia Virus |
| MS | : Mass spectrometry |
| MYA | : Million Years Ago |
| nm | : Nanometers |
| NILs | : Near Isogenic Lines |
| NBS | : Nucleotide-binding site |
| OD | : Optical Density |
| ORF | : Open Reading Frame |
| PAGE | : Polyacrylamide Gel Electrophoresis |
| PAR | : Photosynthetically active radiation |
| PCD | : Programmed cell death |
| PCR | : Polymerase Chain Reaction |
| PMSF | : Phenylmethylsulfonylfluoride |
| PAC | : P1 Artificial Chromosome |
| PAMP | : Pathogen Associated Molecular Pattern |
| R | : Resistance |
| pv | : Pathogen Variety |
| PR proteins | : Pathogenesis-related proteins |
| QTL | : Quantitative Trait Loci |

| | |
|---|---|
| qRT-PCR | : Quantitative reverse transcriptase PCR |
| RNA | : Ribonucleic acid |
| RGA | : Resistance gene analogues |
| RNase | : Ribonuclease |
| ROS | : Reactive Oxygen Species |
| rpm | : Revolutions Per Minute |
| RT-PCR | : Real Time PCR |
| RGCs | : Resistance-gene candidates |
| S | : Susceptible |
| SNP | : Single Nucleotide Polymporphisms |
| SSR | : Simple Sequence Repeat |
| SAR | : Systemic Acquired Resistance |
| SDS | : Sodium dodecyl sulphate |
| sec | : Seconds |
| TE | : Tris-EDTA |
| TAE | : Tris acetate EDTA |
| TBE | : Tris Borate EDTA |
| Tm | : Melting temperature |
| TOF | : Time of flight |
| TTSS | : Type Three Secretion System |
| V | : Volts |
| X-gal | : 5-bromo-4-chloro-3-indolyl β-D- galactoside |
| *Xoo* | : *Xanthomonas oryzae* pv. *oryzae* |

## LIST OF FIGURES

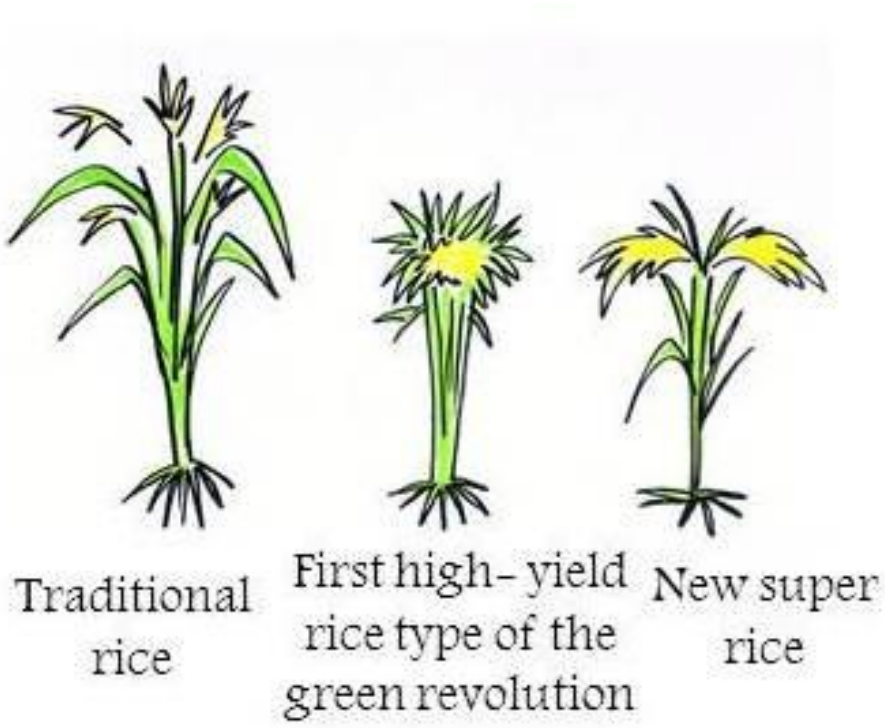

| Fig. No. | Description | Page No. |
|---|---|---|
| 2.1 | Evolutionary pathways for two cultivated species of rice. | 8 |
| 2.2 | Role of phytohormone in regulating the crosstalk during biotic and abiotic stresses concurrently. | 10 |
| 2.3 | Characteristics of *Xanthomonas oryzae* pv. *oryzae* (A) leaf blight symptom (B) *Xoo* colonies on glucose yeast extract agar (C) ) scanning electron micrograph of a single *Xoo* cell. | 12 |
| 2.4 | The Bacterial Blight disease progression under various stages of infection on leaves, stems and panicles leading to unfilled grains. | 14 |
| 2.5 | Representation of the location and structure of the five main classes of plant disease resistance proteins. | 18 |
| 2.6 | Genome organization of the seven *Xa21* family members and location of 15 transposon-like elements. | 20 |
| 4.1 | The effect of *Xoo* inoculation on the leaf blades of three rice genotypes (PB1, IRBB21 and *O. longistaminata*). | 52 |
| 4.2 | Net photosynthetic rate ($P_N$) and stomatal conductance to $CO_2$ (gs) in PB1, IRBB21 and *O. longistaminata* from 0 to 216 h after inoculation with *Xoo*. | 53 |
| 4.3 | Leaf Transpiration rate (*E*) and intracellular $CO_2$ concentration (*Ci*) in PB1, IRBB21 and *O. longistaminata* from 0 to 216 h after inoculation with *Xoo*. | 54 |
| 4.4 | The influence of *Xoo* infection on instantaneous water use efficiency ($WUE_i$) in PB1, IRBB21 and *O. longistaminata* from 0 to 216 h. | 55 |
| 4.5 | Relationships between net photosynthetic rate ($P_N$) versus stomatal conductance to $CO_2$ (*gs*) and net photosynthetic rate ($P_N$) versus internal $CO_2$ concentration (*Ci*) in the BB infected leaves of three tested rice genotypes (PB1, IRBB21 and *O. longistaminata*). | 55 |
| 4.6 | Display of NBS-LRR and LRR-PK/PK present on chromosome 11 of rice. | 59 |
| 4.7 | Determination of sub-cellular location of resistance genes through neural net program (ProtCompV 8.0) using amino acid sequences. | 59 |
| 4.8 | Determination of GC content and domains of resistance genes through online tools. | 62 |
| 4.9 | Comparative analysis of *Xa21* and *Xa26* with reported disease resistance gene of different plant origin. | 62 |
| 4.10 | Homology study of *Xa21* and *Xa26* with predicted class -5 resistance genes. | 64 |
| 4.11 | Synteny analysis of class-5 disease resistance genes with other chromosomes of rice and with EST database of cereals. | 64 |
| 4.12 | Alignment position of *Osin06* and *Osin08* with resistant (reference) *Xa21* allele. | 69 |
| 4.13 | Gene wise distribution of GC content in *japonica* and *indica* covering 100 kb region of *Xa21* locus. | 69 |

| | | |
|---|---|---|
| 4.14 | Distribution of different types of SSR markers in the 100 kb region of *Xa21* locus. | 72 |
| 4.15 | Physical map of *japonica* and *indica* predicted genes in the 100 kb regions of *Xa21* locus. | 73 |
| 4.16 | Phylogenetic analyses of the predicted genes of *japonica* and *indica* subspecies. | 77 |
| 4.17 | PCR amplification of eight different genotypes with IAG-6 exon based primer. | 80 |
| 4.18 | PCR amplification showing polymorphism in *O. longistaminata* with IAG-7 primer. | 81 |
| 4.19 | PCR amplification showing polymorphism with IAG-16 primer in *O. rufipogon* and *O. nivara*. | 82 |
| 4.20 | PCR amplification showing polymorphic bands in IRBB3 and *O. rufipogon* with IAG-20 primer. | 83 |
| 4.21 | Quantitative real time PCR analysis of RGIA gene in IRBB-3 near isogenic line and RK1 gene in *O. longistaminata* in response to bacterial blight stress. | 86 |
| 4.22 | PCR amplification of full length RGIA gene ORF and cloning into plant expression vector. | 86 |
| 4.23 | PCR amplification of full length RK1 gene ORF and cloning into plant expression vector. | 87 |
| 4.24 | Partial maps of vectors pCAMBIA2300 harbouring PK1 gene and pCAMBIA1300 harbouring PK1 and RGIA genes. | 87 |
| 4.25 | *Agrobacterium* mediated transformation of rice (BPT5204) and tobacco (*N. tabacum* L. cv. samsun). | 88 |
| 4.26 | PCR amplification of the putative transgenic plants of tobacco with RK1 and *npt* II specific primers respectively. | 88 |
| 4.27 | 2-DE patterns of proteins from rice leaf blades of PB1 inoculated with *Xoo*. | 92 |
| 4.28 | 2-DE patterns of proteins from rice leaf blades of *O. longistaminata* inoculated with *Xoo*. | 83 |
| 4.29 | Expression patterns (fold change) of total proteins in rice leaf blades after inoculation with *Xoo* in highly resistant *O. longistaminata* and susceptible PB1. | 94 |
| 4.30 | Relative abundance of the 22 protein spots in comparison with the controls. | 95 |
| 4.31 | Identification of protein spot 14 by MS/MS, PMF, Spectral peaks and Amino acid sequence s of peptide fragments. | 96 |
| 4.32 | Grouping of differentially expressed protein from both susceptible PB1 and highly resistant *O. longistaminata* based on their functional role. | 97 |
| 4.33 | Schematic representation of the inter-relationship and networking among the identified group of proteins and overall plant responses against BB stress based on current as well as previous reports. | 98 |

## LIST OF TABLES & ANNEXURES


| Table No. | Description | Page No. |
|---|---|---|
| 2.1 | Genes conferring resistance to the bacterial blight pathogen | 16 |
| 2.2 | The five classes of cloned plant disease resistance genes | 18 |
| 2.3 | Inter and intra specific microcolinearity studies in cereals | 25 |
| 4.1 | Disease score and percentage of disease infection in 14 tested rice genotypes. The genotypes were classified into three groups depending upon their level of resistance against BB *viz*., resistant (R), moderately resistant (MR) and susceptible (S) | 52 |
| 4.2 | Effect of BB on various yield characteristics of moderately tolerant (IRBB21) and susceptible (Pusa Basmati 1) rice genotypes | 56 |
| 4.3 | Predicted genes of *japonica* in the 100 kb region | 67 |
| 4.4 | Predicted genes of *indica* in the 100 kb region | 68 |
| 4.5 | Frequency of dimeric and trimeric repeats or SSRs in the 100kb *Xa21* locus (*japonica*) | 72 |
| 4.6 | Frequency of dimeric and trimeric repeats or SSRs in the 100kb *Xa21* locus (*indica*) | 73 |
| 4.7 | Microsynteny analysis of the predicted genes in the 100 kb region of the *japonica* cultivar group | 74 |
| 4.8 | Microsynteny analysis of the predicted genes in the 100 kb region of the *indica* cultivar group | 75 |
| 4.9 | Subcellular localization of *indic*a and *japonica* genes in the 100 kb region of *Xa21* locus | 76 |
| 4.10 | The accession number of R genes submitted to NCBI with putative function | 84 |
| 4.11 | Identification of major differentially expressed foliar proteins from susceptible rice genotype PB1 and highly resistant *O. longistaminata* during infection | 99-101 |
| Annexure No. | Description | Page No. |
| 3.1 | Predicted R genes from 130 ordered BAC/PAC clones present in the minimum tilling path of chromosome 11 of rice. | 149-155 |
| 3.2 | GC content of R genes determined through online software sciencebuddies.org. | 156-158 |
| 3.3 | Homology search taking *Xa21/Xa26* as subject sequence and predicted protein kinase as query sequence. | 159-161 |
| 3.4 | List of specific primers designed from the exonic region of R genes predicted in the BAC/PACs clones of chromosome of rice . | 162 |

# CHAPTER-1
# INTRODUCTION



Rice (O*ryza sativa* L.) is the staple food for about half of the world's population (Khush, 2005). It is a nutritious cereal crop, which provides 27 percent of the calories and 20 percent of proteins consumed by developing world's population (FAO, 2004). Besides being the chief source of carbohydrate in Asia, it also provides proteins, minerals and fibers. Rice straw and bran are important animal feed in many countries. Rice is a main food for about 65% of the people living in India (FAO, 2004). Rice is a supreme commodity to mankind, because rice is truly life, culture, tradition and a means of livelihood to millions. India is the largest rice growing country accounting for about one-third of the world acreage under the crop. It is grown in almost all the states of India which covers more than 30 percent of the total cultivated area. This grain yielding crop has its origins going back to about 3000 B.C. in the foothills of Himalayas in the north, and north-east of India stretching to the mountain ranges of south-east Asia and south-west China (as the primary center of origin of *Oryza sativa*). The delta of river Niger in Africa is considered to be the origin of *O. glaberrima*, the African rice, which was cultivated by the ancient civilizations of Mesopotamia. Rice gene pool exists worldwide. There are more than 8,000 indigenous varieties found in India alone and 3,500 in Philippines. Rice is grown in tropical, semi tropical and temperate regions; mostly in coastal plains, tidal deltas, and river basins. In India, rice is grown under widely varying conditions of altitude and climate. Therefore, the rice growing seasons vary in different parts of the country, depending upon temperature, rainfall, soil types, water availability and other climatic conditions. In about 80 to 120 days from planting, the fields are drained and rice is harvested. Based on a number of morphological, physiological, biochemical and molecular traits, Asian cultivated rices are organized in two major subspecies, i.e. *Oryza sativa japonica* and *Oryza sativa indica* (Oka, 1988). These two subspecies are commonly associated with differences in growth habitat (Khush, 1997) and are the products of independent domestication events from ancestral *Oryza rufipogon* populations in different locations and at different times thousands of years ago (Sang and Ge, 2007a).

The current production (480.1 million tonnes in 2011-12, FAO-USA) of rice will not be sufficient to meet the growing demand due to increasing population. The world rice production must increase by 30% to keep pace with the growing population (Peng et al. 1999). However, decrease in farming land, reduction in the level of ground water, different kind of biotic and abiotic stresses, change in climate and global warming are resulting in the decreased yield of rice. Therefore, continuous efforts towards innovative

research should be encouraged in order to address the vital aspects related to increasing rice productivity in the midst of all the obstacles associated with rice cultivation. Rice has the smallest genome size amongst all cereals, of around 389 million base pairs of DNA (IRGSP, 2005). Hence, it is considered as model system in plant biology research due to its compact genome and evolutionary relationships with other large-genome cereals including sorghum (750 Mb), maize (2,500 Mb), barley (5,000 Mb) and wheat (15,000 Mb) (Goff et al. 2002). It is also a model species to study crop yield and hybrid vigor, as well as single and multi-genic disease resistance of monocots. Complete knowledge on rice genome will be crucial in developing new, improved varieties aiming towards greater yield, better resistance against pest and disease and wider adaptability in diverse agroclimatic conditions.

Characterization and sequencing of the genome has been carried out in many organisms including plants as well as many scientifically and economically important eukaryotes. In the case of major cereal crops such as rice and maize, large-scale genome analyses have been carried out since the early 1990s with the aim of identifying biologically important genes. These includes, genes related to yield potential, biotic and abiotic stress tolerance, heading date, and other agronomically important traits which are either orthologous among cereal crops or unique to each crop. Rice is the first cereal crop to be completely decoded (IRGSP, 2005) and the high-quality map-based sequence of the entire rice genome is now available in the public domain. With the completion of sequencing, the next challenge to the scientific community would be to determine the function of about 37,544 predicted genes in rice and to utilize this information in identifying agronomically important genes not only in cultivated rice species, but also in wild relatives of rice. The *japonica* rice variety "Nipponbare" was chosen as a common template for complete and accurate decoding of the *Oryza sativa* genome through clone-by-clone approach. This is because; Nipponbare was already being investigated for the analysis of high density genetic map and EST library construction (Harushima et al. 1998). Additionally, Nipponbare can be easily regenerated from callus, an important advantage for genetic transformation.

Plants being sessile in nature are constantly challenged by a wide spectrum of disease caused by various bacteria, fungi, viruses, nematodes, insects and even other plants where disease resistance genes play an important role in providing resistance. Bacterial blight (BB), leaf blast and sheath blight are three most serious diseases of rice (Ou, 1985). Till date, over 70 resistance (R) genes have been isolated from various plant

species (Liu, 2007). Sequence analysis of cloned disease resistance genes reveals that common motifs occurs in resistance (R) genes of diverse origin and pathogen specificity which are highly conserved like: nucleotide binding site (NBS), leucine-rich repeat (LRR), protein kinase (PK) domain, toll/interleukin receptor (TIR) domain etc. (Dangl et al. 2001; Jones 2001; McDowell et al. 2003). The presence of highly conserved domains of plant R-genes provides a convenient means for cloning of additional R-genes through PCR-based approach. The *Xa21* gene encodes receptor kinase, which confers resistance to various strain of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a representative member of class 5 R-genes (Song et al. 1995). It has three domains, an LRR extracellular ligand binding domain, a transmembrane domain (TM) and a kinase domain, which is a landmark feature of any typical receptor kinase.

The sequence analysis of chromosome 11 revealed that there are at least 4.5% of the total numbers of gene models predicted on this particular chromosome are disease resistance and most of these are present in large clusters of tandem arrays indicating their origin by duplication from a few ancestral genes (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005). It has been demonstrated that the resistivity of many plants is arbitrated by receptor kinase genes (Class 5 R-gene) clustered in a complex locus, which suggest the existence of multiple signaling pathways (Ayliffe and Lagudah, 2004). Therefore, cloning of receptor kinase like gene of rice will be worthwhile. The physical map generated out of this work would be an important resource for the engineering of candidate gene as well. Computational gene prediction and its annotation is the first step after sequencing. The sub-cellular location and domain determination would provide the additional information which could be taken into consideration during the selection of resistance genes for expression analysis during infection as well as during expression cassette preparation.

The plant response to pathogen attack or microbial signals has been a longtime interest of plant biologists and pathologists. In this context proteomics technique can potentially provide a direct evidence for target proteins involved in defense responses. Until now, there are scanty reports which focus on proteome-based expression profiling to study plant-bacterial pathogen interactions. Such study if undertaken would provide important information on bacterial infection-induced proteomic responses and defense mechanisms in rice. The qualitative and quantitative expression patterns of the proteins identified will provide integral information for better characterization of susceptible and tolerant genotypes against *Xoo.*

The work presented in this thesis is relevant for the scientific community who work with foliar pathogens. It provides an important outline of the theoretical background of BB disease as well as practical aspect of genomic and proteomic analysis of the same. It also underlines the effect of BB on various leaf gas exchange physiology. Hence, information generated by these lines of study would provide a basis for future research projects aiming to develop transgenic rice plants with enhanced disease resistance as well as plant pathogen interaction.

The specific aim of this study was achieved through the following objectives

**Objectives:**

1. Screening for bacterial blight resistance and characterization of photosynthetic and yield responses in rice genotypes.
2. Identification, mapping and synteny analysis of genes associated with bacterial blight resistance from chromosome 11 of rice.
3. Molecular cloning and expression analysis of bacterial blight resistance genes.
4. Proteomic analysis to identify genes potentially involved in bacterial blight resistance.

These objectives were pursued via accumulation of literature on the subject. At first, plants were evaluated on the basis of their performance against *Xoo* infection in terms of photosynthetic responses and resistivity. A detailed bioinformatics study of BAC clones was carried out to investigate the presence of potential resistance genes. Cloning, expression study and proteomic analysis exploring the differentially expressed protein during the bacterial infection was conceded using 2-DE and MALDI TOF-TOF analysis.

# CHAPTER-2
# REVIEW OF LITERATURE

Rice is the world's single most important crop that serves as the primary food source for more than half of the population. About 49% of the calories consumed by the human population are contributed by rice, wheat and maize, out of which 23% provided by rice, 17% by wheat and 9% by maize (Brar and Khush, 2002). Thus, almost one fourth of the calories consumed by the entire world population come from rice. Moreover, 90% of the total rice cultivation is done in Asia where more than 60% of the world's population lives. It takes a humongous land of about 154 million hectares for rice cultivation annually, which is about 11% of the world's cultivated land. The latest achievement of rice production was 721 million tons in 2011 (FAO, 2011). Presently, India has the largest area under rice cultivation (44.6 million hectares), and is second only to China in rice production (104.32 million tons in 2011). Other major rice-producing countries include Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, and the Philippines. Till date, global rice production has been able to meet population demands. However, the continuation of this scenario in future is a big question mark which requires to be pondered upon. The UN/FAO has estimated that global food production will need to increase by over 40% by 2030 and 70% by 2050 to achieve food security (OECD-FAO, 2009). The escalating population pressure in our country necessitates not only an increase in the current rice production but also its sustainability. On account of the population growth rate of 1.5% and the per capita consumption of about 230 g per person, estimation says that by 2025, India will need 140 million tons of rice in order to feed its gigantic population which is quite a big deal. When we look for the constraints of rice production, great variations are found across states and areas. So, in order to ensure food security, our major task is to check out the variety of factors causing yield loss and hampering productivity. Progress has been rapid in case of a limited number of abiotic (drought, submergence and salinity) and biotic stresses (gall midge, brown plant hopper, bacterial blight, blast etc.).

## 2.1 Taxonomy and origin of cultivated rice

Rice belongs to the grass family Gramineae. There are two cultivated species of rice. The Asian cultivated rice, *Oryza sativa*, is grown worldwide, while *Oryza glaberrima*, the African, is grown on a limited scale in West Africa. The cultivated rice (genus *Oryza*) probably originated at least 130 million years ago and spread as a wild grass in

Gondwanaland (Chang, 1976). There are 22 wild species of genus *Oryza* has been reported. Nine of the wild species are tetraploid and the remaining wild species and the two cultivated species are diploid. The common rice, *Oryza sativa*, and the African rice, *O. glaberrima*, are thought to be examples of parallel evolution in crop plants. Asian wild rice *O. rufipogon* is considered as the progenitor of *O. sativa*, which ranges from perennial to annual types. Annual type of *O. rufipogon* was named as *O. nivara* which were domesticated as *O. sativa* (Khush, 1997). Parallely, *O. glaberrima* was domesticated from annual *O. breviligulata*, which in turn evolved from perennial *O. longistaminata* (Fig 2.1). Domestication of wild rices probably started about 9,000 years ago (Whyte, 1972). Domestication in Asia occurred from the plains below the eastern foothills of the Himalayas (Roschevitz, 1931; Chang, 1976). Rice was introduced from India to Madagascar, to east Africa, and then to countries of west Africa. Indica rice varieties also spread eastward to south east Asia and north to China.

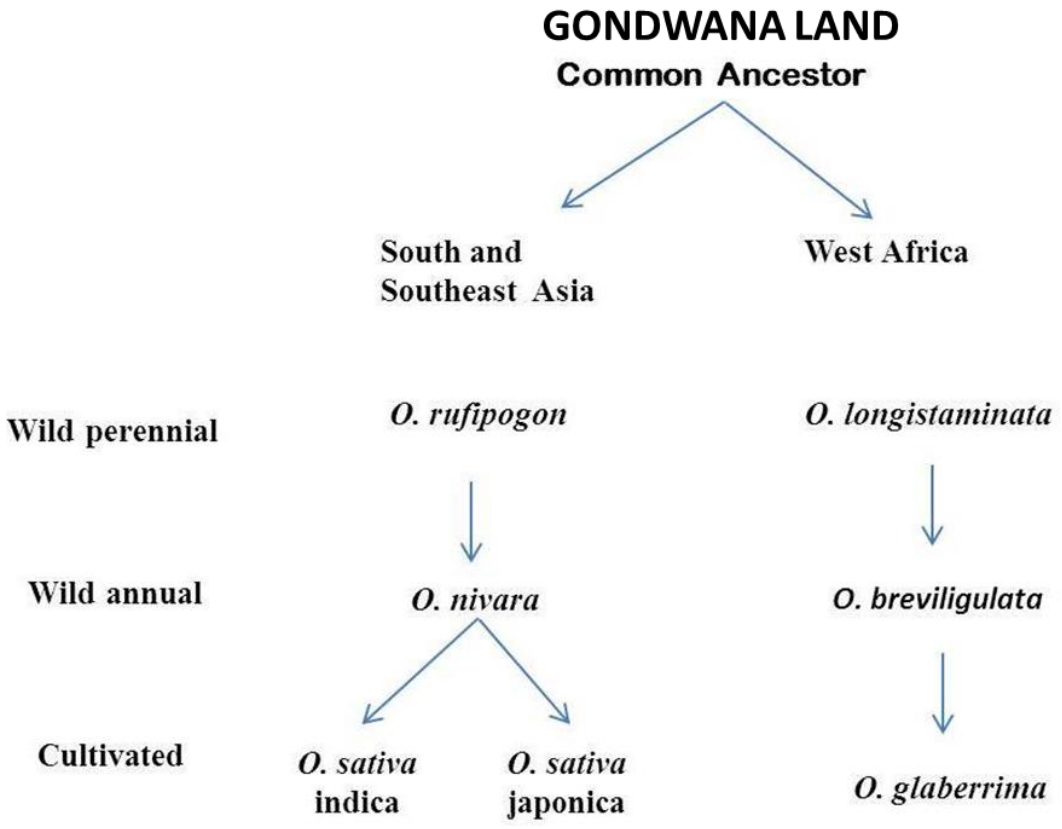
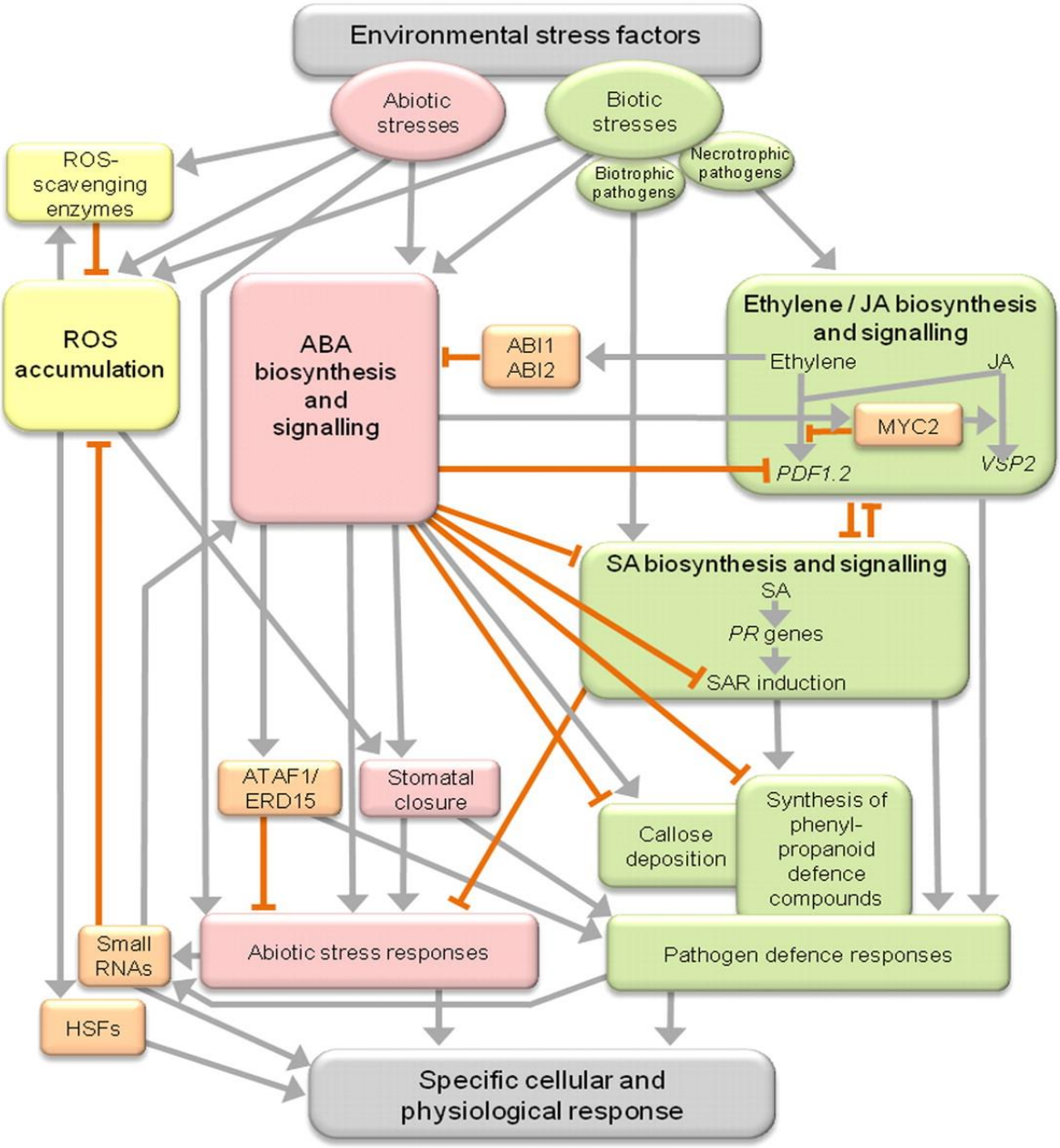



Fig 2.1 Evolutionary pathways for two cultivated species of rice

## 2.2 Biotic and abiotic stresses

Rice plants are constantly exposed to a variety of biotic and abiotic stresses throughout the year. Being sessile organism, it has developed specific mechanisms to detect the environmental changes and adjust their metabolism in response to biotic and abiotic stresses in order to minimize the damage and ensure survival. Each plant species activate a specific and unique stress response at the transcriptome, cellular, and physiological levels when subjected to one or combination of stresses (Rizhsky et al. 2004). When one plant encounters an abiotic stress, it can have the effect of reducing or enhancing susceptibility to a pathogen, and vice versa (Atkinson and Urwin, 2012). The interactions between biotic and abiotic stresses are mediated by hormone signalling pathways that may induce or antagonize one another (Fig 2.2). The host perception of pathogen infection is mediated by disease resistance (R) genes which trigger a cascade of signal transduction pathway that involves protein phosphorylation, ion fluxes, reactive oxygen species, and other signaling events (Yang et al. 1997). Further, transcriptional and/or post-translational activation leads to induction of diverse array of plant defense genes. Abiotic stress factors such as heat, cold, drought, salinity, and nutrient stress have a huge impact on world agriculture, and it has been suggested that they reduce average yields by >50–60% for most major crop plants (Wang et al. 2003; Seo et al. 2011). In India, rice production is basically affected by two important diseases. One is the 'leaf blight' caused by the bacteria *Xanthomonas oryzae* and the other 'rice blast' caused by the fungi *Magnaporthe grisea*. These diseases are responsible for reducing the crop yield and quality of the rice to a greater extent. Bacterial blight (BB) leads to yield loss up to 50 % (Khush et al. 1989) and in extreme cases even upto 81% (Srinivasan and Gnanamanickam, 2005). The use of resistant varieties has remained the most effective control measure against this disease, which requires the identification and characterization of resistance novel genes. Twenty one BB resistant genes (*Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, Xa12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22,* and *Xa27* as dominant and *xa5, xa8, xa13, xa15, xa19,* and *xa20* as recessive) have been found in rice (Khush et al. 1991; Kinoshita, 1995; Lin et al. 1996; Kaur et al. 2006). Combinations of these genes (pyramiding) result in a durable resistance against the disease (Rajpurohit et al. 2010; Dokku et al. 2013). In this context, we have taken class-5 resistance genes (receptor like kinases) for the preparation of expression cassette for rice transformation.




Source |J. Exp. Bot. (2012) 63 (10)| 3523-3543

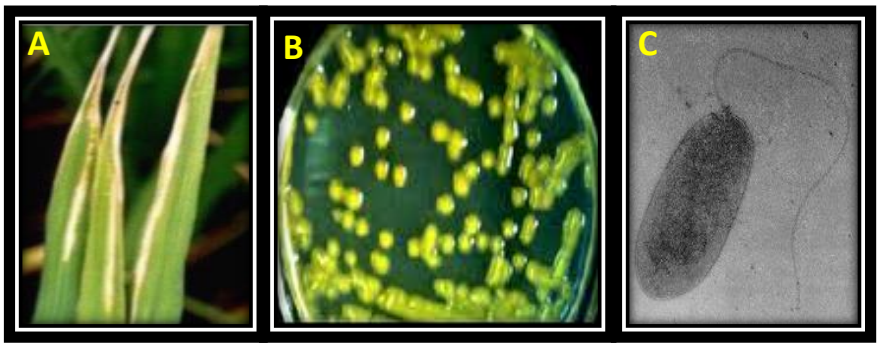
Fig 2.2 Role of phytohormone in regulating the crosstalk during biotic and abiotic stresses concurrently. Grey arrows show induction or positive regulation, while orange bars show inhibition or repression. Events characteristic of abiotic stress responses are shown in pink, while those characteristic of biotic stress responses are shown in green. Transcription factors and other regulatory genes are represented by orange boxes. ROS, reactive oxygen species; ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid; PR, pathogenesis-related; SAR, systemic acquired resistance; HSF, heat shock factor.

## 2.3 Bacterial pathogen and mode of BB infection

*Xanthomonas oryzae* pv. *oryzae (Xoo)* is gram negative, non-spore forming, rod shaped, round-ended bacterium which is motile with a single polar flagellum. Individual cells vary from approximately 0.7 µm to 2.0 µm in length and from 0.4 µm to 0.7 µm in width. Colonies on solid media containing glucose are round, convex, mucoid and yellow in colour due to the production of the pigment xanthomonadin (Fig 2.3), characteristic of the genus (Bradbury, 1984). Optimal temperature for growth is between 25°C and 30°C. Unlike mammals, plants have a complex cell wall and bacteria need to get through it to gain access to nutrients and water. This is achieved by the bacteria through destruction of the cell wall barrier by means of secreting cell wall degrading enzymes and cell wall permeable toxins, which is one of the most effective virulence strategy adapted by bacterial pathogens (Agrios, 1997). *Xoo* enters the rice leaf tissues either by wounds or through natural openings such as hydathodes, lenticels or stomata (Ou, 1985). Cells on the leaf surface may become suspended in guttation fluid as it exudes at night and enter the plant by swimming, or passively as the fluid is withdrawn into the leaf in the morning (Curtis, 1943). Bacteria multiply in the intercellular spaces (apoplast) of the underlying epithem [tissue connecting the water pores (hydathodes) with the xylem], then enter and spread into the plant through the xylem (Noda and Kaku, 1999). *Xoo* may also gain access to the xylem through wounds or openings caused by emerging roots at the base of the leaf sheath (Ou, 1985). Within a few days, bacterial cells and extra cellular polysaccharide (EPS) fill the xylem vessels and ooze out from hydathodes, forming beads or strands of exudate on the leaf surface, a characteristic sign of the disease and a source of secondary inoculum (Mew et al. 1993). When infection occurs during seedling stages or early tillering stage, rice plants become wilted but if infection occurs in later stages, lesions of leaf blight enlarge in length and width and turn gradually grayish green to chlorotic (Mew et al. 1993).

Plant pathogenic bacteria also use type III secretion system, an elaborate protein delivery system to transport toxin proteins and enzymes to disrupt host signaling and hijacks host metabolism for their own benefit. This work revealed that bacterial pathogens manipulate host processes to promote virulence (Mudgett, 2005). It has been also found that plants down regulate auxin levels in response to pathogen attack in order to enhance disease resistance. Therefore, bacteria produce auxin to counter this plant response and suppress

plant defense (Navarro et al. 2006). Bacteria also produce autoinducers (hormone like molecule) to detect the local population density (quorum sensing), which enable bacteria to regulate their gene expression in such a way that they induce only the expression of virulence factors when their population have reached high enough to effectively parasitize the plant cells. *Xanthomonas* secretes large amount of EPS (Extracellular Polysaccharide; high molecular-mass sugar molecules), which clog the xylem and cause characteristic wilting symptoms. EPS enhances pathogenicity by protecting the bacteria from antimicrobial compounds of the host plants. In many cases, all of the above diverse mechanism work together to promote pathogenesis (Leigh and Coplin, 1992).




Source**:** www.ceniap.gov.ve; www.apsnet.org

Fig 2.3 Characteristics of *Xoo* (A) leaf blight symptom (B) *Xoo* colonies on glucose yeast extract agar (C) Scanning electron micrograph of a single *Xoo* cell.

## 2.4 History of occurrence of BB menace and extent of damage

Though the disease has been known in Japan since 1884, its bacterial nature was established in 1922. In India, the first authentic report of typical BB broke out in epidemic form during 1960 in Shahabad district of Bihar (now split up into a northern district called Bhojpur and a southern one called Rohtas) and since then it has spread fast to other rice growing areas of the country often causing considerable yield loss especially in high yielding varieties during rainy season. This pathogen has also been listed as bioterrorism agent by U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) necessitating strict biosafety measures to combat the spread of the disease. Outbreaks of BB are more likely to occur during the monsoon season in the countries which are in south-east Asia (from June to September) than at other times of the year (Mew et al. 1993). Wind and rain disseminate

bacteria from infected rice plants and other hosts, as well as contaminated rice stubble from previous crop seasons are the most important sources of primary inoculum. Severe epidemics often occur following typhoons, the fierce winds, wind-blown rain and hail of which both wound rice plants and disperse bacteria. Bacteria may also be disseminated in irrigation water, as well as by humans, insects and birds (Nyvall, 1999; Ou, 1985).

In Punjab and Haryana, major BB epidemic occurred in 1979 and 1980 where total crop failure was reported (Mew, 1987). The extent of yield loss depends on the growth stages of the crop at which it is infected. The level of susceptibility of the cultivar, season, climatic condition and level of applied nitrogen fertilizer has a role to play in rapid buildup of the disease. Damage may be due to partial or total blighting of the leaves (leaf blight phase) or due to complete wilting of the affected tillers (Kresek phase) leading to unfilled grains. This disease is a problem of rainy season (Kharif season). In general, late infection results in only slight reduction in yield, but early infection leads to "Kresek" (wilt) causing heavier losses and sometimes the entire crop may be lost.

Generally, the stage between maximum tillering and booting is highly sensitive to *Xoo* infection, as it affects the yield significantly in terms of filled grain weight per hill and total yield. Some workers have reported yield loss upto 81% depending on the variety, severity and stage of infection (Srinivasan and Gnanamanickam, 2005). In addition to yield reduction, BB may also affect the grain quality by interfering with maturity, therefore BB can have serious economic, social and even political consequences (Strange and Scott, 2005). Grains from diseased crop were poor in water absorption, volume expansion and kernel elongation (Laha et al. 2009).

## 2.5 Effect of BB on leaf gas exchange physiology

Reduction in photosynthetic rate is considered to be one of the most important factors causing yield loss in BB infected rice (Rajarajeswari and Muralidharan, 2006). BB reduces photosynthesis in rice by causing necrosis of leaf tissue and down-regulation of $CO_2$ fixation rates in the existing green leaf tissues nearby the necrotic lesion (Bastiaans, 1991). The necrotic regions retained on the leaves may capture some light, however, do not contribute to photosynthesis (Bingham et al. 2009). Reductions in photosynthetic efficiency have been attributed to numerous mechanisms, such as self-shading, stomatal limitation and other

metabolic impairments (Bassanezi et al. 2002). Reductions in plant growth and yield under diseased conditions have also been correlated with loss of photosynthetic capacity (Jesus et al. 2001; Kumar et al. 2013). Previous studies demonstrated that BB infected rice showed an increase rate of respiration and loss of light use efficiency concomitantly with photosynthetic down-regulation. Under bacterial infection, photosynthesis/chloroplast synthesis genes were found to change their transcriptional activity more or less in one direction and majority of such genes were down-regulated (Bozso, 2009). BB drastically reduces the mean weight of well-filled grains/hill, which resulted in 17-31% loss as estimated by Cramer method (Cramer, 1967). The reduced nutrient availability in diseased tissue and the consumption of nutrients by the pathogen in the epidemics depressed grain filling and grain weight in infected rice plants (Savary et al. 2000). All these studies emphasize the immense need to investigate detailed photosynthetic functions for better understanding of photosynthetic physiology in BB infected rice. The present state of knowledge on photosynthetic leaf gas exchange traits in BB infected rice is preliminary and the responses of some of the important gas exchange characteristics including stomatal conductance to $CO_2$, leaf transpiration rate and internal $CO_2$ concentration have not been yet investigated in detail during progressive stages of BB infection in rice. A comprehensive study that incorporates detailed photosynthetic leaf gas exchange analysis under various stages of BB disease progression can be helpful in order to understand $CO_2$ assimilation physiology in infected rice leaves which can also be linked further to plant growth and grain yield related responses (Fig 2.4).

Source**:** www.agriskmanagementforum.org

Fig 2.4 The bacterial blight disease progression under various stages of infection on leaves stems and panicles leading to unfilled grains.

## 2.6 Different mechanisms of BB control

Practicing field sanitation such as removing weed hosts, rice straws, ratoons, maintaining shallow water in nursery and providing good drainage during severe flooding are important measures to avoid infection caused by this disease. Similarly proper application of nitrogen fertilizer and plant spacing are also important for BB management. Chemical control of BB in rice fields began in the 1950s with the preventative application of bordeaux mixture. Seed treatment with bleaching powder (100µg/ml) and zinc sulfate (2%) reduce BB to some extent. Other chemicals such as tecloftalam, phenazine oxide and nickel dimethyl dithio carbamate were also tried but has not proven highly effective and therefore were unreliable. Along with cultural practices, disease forecasting and other measures were also taken but none of them were promising. The use of resistant varieties is the most effective and the most common management practices adopted by farmers in most growing countries in Asia. When different strains of bacteria are present, it is recommended to grow resistant varieties possessing field resistant genes. So far, 38 R-genes conferring resistance to different *Xoo* races have been identified, mostly from *O. sativa* ssp. *indica* cultivars, but some also from *japonica* varieties and from related wild species including *O. longistaminata*, *O. rufipogon*, *O. minuta* and *O. officinalis* (Bhasin et al. 2012; Natrajkumar et al. 2012). The exploitation of many resistant genes by marker-assisted breeding (MAB) or conventional back cross-breeding has resulted in the development of many resistant varieties that have played an important role in protecting rice from *Xoo* (Sundaram et al. 2009; Perumalsamy et al. 2010; Pandey et al. 2013). As we know that some R-genes are effective only in adult plants (e.g. *Xa21*), indicating that there are other gene(s) epistatically regulating the defense pathway (Century et al. 1999), whereas most do not seem to be developmentally regulated (e.g. *Xa23, Xa26*). Curiously, *Xa3* is typically effective only in adult plants, but against one race it is effective at all stages of growth. Unlike any previously studied R-genes, *Xa1* gene is induced by pathogen infection and wound. Most of the BB resistant genes are dominant (*Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, Xa12, Xa14, Xa16, Xa17, Xa18, Xa21*and *Xa22*), and some are recessive (e.g. *xa5, xa8, xa13, xa15, xa19 and xa20*), and some display semi-dominance (e.g. *Xa27*) (Table 2.1). Therefore, research on bacterial diseases of plants will help to elucidate fundamental aspects of bacterial pathogenesis and associated host responses and also to develop more effective and sustainable disease-control methods.

Table 2.1 Genes conferring resistance to the BB pathogens

| Gene | Chr. No. | Source | Resistant to races | Reference |
|---|---|---|---|---|
| *Xa1* | 4 | Kogyoku | JR I | Yoshimura et al. 1998 |
| *Xa2* | 4 | Tetep | JR II | He et al. 2006 |
| *Xa3* | 11 | Wase Aikoku3, Java-14,Chugoku-45, Cempocelek | PR 1,2,4,5 and All JR | Kaku and Ogawa, 2000; Sun et al. 2004 |
| *Xa4* | 11 | TKM 6, IR 20, IR 22 | PR 1, 4, 5, 7, 8 and 10 | Wang et al. 2001 |
| *xa5* | 5 | Aus boro lines(e.g. DZ192), DV85, DV86, DZ78 | PR 1, 2, 3, 5,7, 8, 9,10 | Iyer and McCouch, 2004 |
| *Xa7* | 6 | DV85, DV86, DZ78 | PR 1, 2, 3, 5, 7, 8,10 | Lee and Khush, 2000; Porter et al. 2001 |
| *xa8* | 7 | PI231129 | PR 5, 8 | Sidhu et al. 1978; Singh et al. 2002 |
| *Xa10* | 11 | Cas 209 | PR 2, 5, 7 | Yoshimura et al. 1983 |
| *Xa11* | ND | IR 8, IR 944 | JR IB, II, IIA, V | Mew, 1987 |
| *Xa12* | 4 | Kogyoku,Tetep, Java-14 | JR V | Mew, 1987 |
| *xa13* | 8 | BJ-1,Chinsurah Boro II | PR 6 | Chu et al. 2006 |
| *Xa14* | 4 | TN-1 | PR 5, 8 | Oryzabase, 2006 |
| *xa15* | ND | M41 | All JR | Gnanamanickam et al. 1999; Nakai et al. 1988 |
| *Xa16* | ND | Tetep | JI H8581and H8584 | Oryzabase, 2006 |
| *Xa17* | ND | Asominori | JI H8513 | Oryzabase, 2006 |
| *Xa18* | ND | IR 24,Toyonishiki | BI-BM8417and BM8429 | Liu et al. 2004; Oryzabase 2006 |
| *xa19* | ND | XM 5 | PR 1, 2, 3, 4, 5 and 6 | Lee et al. 2003; Oryzabase, 2006 |
| *xa20* | ND | XM 6 | | |
| *Xa21* | 11 | *O. longistaminata* | PR 1, 2, 3, 4,5, 6, 7, 8 and 9 | Song et al. 1995 |
| *Xa22* | 11 | Zhachanglong | BSR | Sun et al 2004; Wang et al. 2003 |
| *Xa23* | 11 | *O. rufipogon* | All PR, most JR and CR | Zhang et al. 1998, 2001 |
| *Xa24* | ND | DV85, DV86, Aus 295 | PR 6 | Khush and Angeles, 1999; Lee et al. 2000 |
| *Xa25a* | 4 | HX-3 | PR 1,3 4, and to CR | Gao et al. 2001, 2005 |
| *Xa25b* | 12 | Minghui 63 | PR 9 | Chen et al. 2002 |
| *Xa26* | 11 | Minghui 63 | BSR | Sun et al. 2004; Yang et al. 2003 |
| *Xa27* | 6 | *O. minuta* | PR 2, 5 | Gu et al. 2004, 2005 and Lee et al. 2003 |
| *xa28* | ND | Lota Sail | PR 2, 5 | Lee et al. 2003 |
| *Xa29(t)* | 1 | *O. officinalis* | Not fully characterized | Tan et al. 2004 |
| *Xa30(t)* | | *O. nivara* | Not fully characterized | Tan et al. 2004 |
| *Xa31(t)* | | *Zhachanglong* | Not fully characterized | Wang et al. 2008 |

Source: Nino-Liu et al 2006, Mol. Pl. Path; 7(5:303); ND: Not determined, JR-Japanese race, PR-Philippines race, CR-Chinese race, JI- Japanese isolate, BI-Burmese isolate, BSR-Broad spectrum resistance

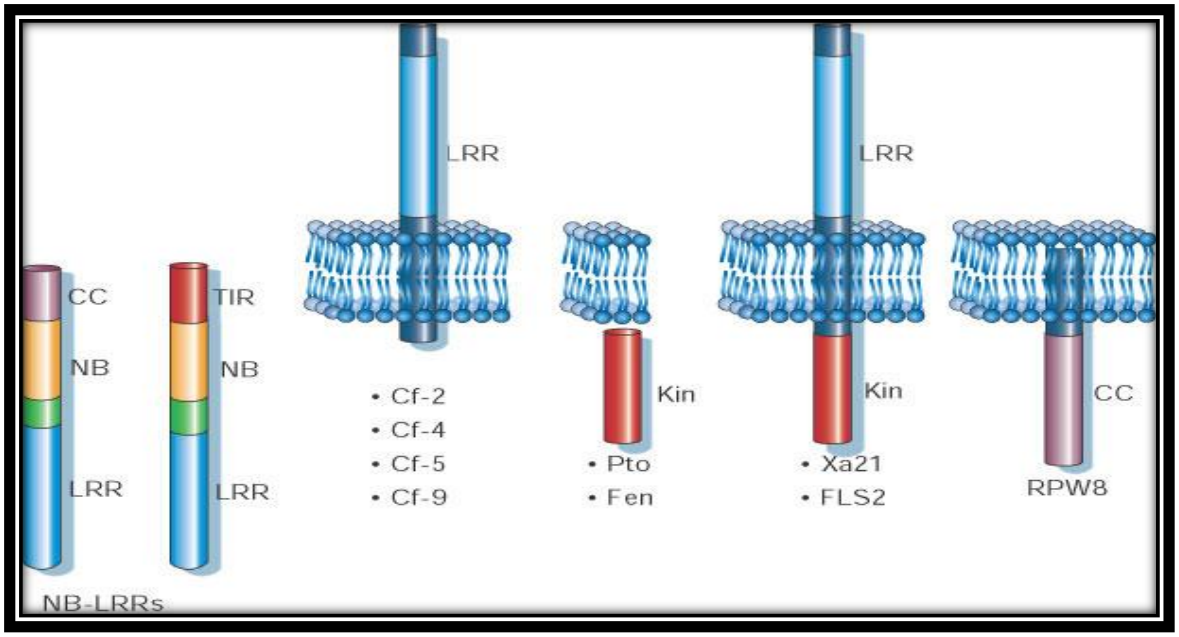
## 2.7 Classification and function of plant disease resistance genes

Over the past 2 decades, numerous R-genes have been cloned from several plant species and their structures have been studied. High degree of similarity among these R-genes has been observed. Based on predicted protein structure, cloned R-genes can be grouped into five classes (Table 2.2; Fig 2.5). The first class is represented by the maize *Hm1* gene, the first cloned plant R-gene, which encodes a reductase that inactivates the HC toxin from *C. carbonum* race 1 (Johal and Briggs, 1992). The second class of genes confers resistance to the bacterial pathogen *P. syringae* pv. *tomato. Pto* gene belong to the second class which encode membrane-associated serine-threonine kinase. The third and most abundant class of R-genes encodes a cytoplasmic receptor-like protein with a nucleotide binding site (NBS) and a leucine-rich repeat (LRR) domain. *Arabidopsis* (*Rps2* and *Rpm1*), tobacco (*N*), flax (*L6*), and tomato (*Prf*) are members of this class (Salmeron et al. 1996). *Xa1* gene of rice also belongs to class III resistance gene encoding the cytoplasmic receptor-like protein with NBS and LRR domains (Yoshimura et al. 1998). It deliberate high level of specific resistance to race 1 strains of *Xoo* in Japan and was mapped on rice chromosome 4 through map-based cloning strategy (Yamada et al.1993). The *Xa1* gene encodes a protein similar to the deduced polypeptide domains of other disease-resistance genes such as *Arabidopsis RPS2* and *RPM*, tobacco *N* and flax *L6*. The fourth class is represented by the tomato *Cf* genes. This class of gene encodes LRR motifs in the presumed extracellular domain and a short C-terminal tail in the intracellular domain (Dixon et al. 1996). It shows homology to the receptor domain of several receptor-like protein kinases and to members of the LRR family of proteins (Jones et al.1994). The fifth class is represented by the rice gene *Xa21*, conferring resistance to *Xoo* (Song et al. 1995). It is a member of a small multigene family containing seven members. *Xa21* encodes a putative trans-membrane receptor with an extracellular LRR domain similar to that of the tomato Cf-9 protein and an intracellular serine/threonine kinase domain similar to that of the *Pto* kinase. Therefore, the structure of *Xa21* implies an evolutionary link between two different classes of plant resistance genes. In the present study, we investigated all kind of R-genes which were physically present on chromosome 11 of rice through *in silico* method using rice genome database. We emphasized on class-5 disease resistance gene (receptor like kinases) in our study.

Table 2.2 Five classes of cloned plant disease resistance genes

| Class | R gene | Plant | Pathogen | *Avr* gene | Structure |
|---|---|---|---|---|---|
| 1 | *Hm1* | Maize | *C. carbonum* (race 1) | None | HCTR |
| 2 | *Pto* | Tomato | *P. syringae* pv. *tomato* | *avrPto* | Ser/Thr PK |
| 3a | *RPS2* | Arabidopsis | *P. syringae* pv. *tomato* | *avrRpt2* | LZ-NBS-LRR |
| | *RPM1* | Arabidopsis | *P. syringae* pv. *maculicola* | *avrRpm1/avrB* | LZ-NBS-LRR |
| | *Prf* | Tomato | *P. syringae* pv. *tomato* | *avrPto* | LZ-NBS-LRR |
| | *I2* | Tomato | *F. oxysporum* f. sp. lycopersici | Unknown | LZ-NBS-LRR |
| 3b | *N* | Tobacco | Tobacco mosaic virus | *TMV* replicase? | TIR-NBS-LRR |
| | *L6* | Flax | *Melampsora lini* | AL | TIR-NBS-LRR |
| | *RPP5* | Arabidopsis | *Peronospora parasitica* | *avrPp5* | TIR-NBS-LRR |
| 3c | *Xa1* | Rice | *X. oryzae* pv. *oryzae* | *AvrXa1* | NBS-LRR |
| 4 | *Cf-9* | Tomato | *Cladosporium fulvum* | *Avr9* | LRR-TM |
| | *Cf-2* | Tomato | *C. fulvum* | *Avr2* | LRR-TM |
| 5 | *Xa21* | Rice | *X. oryzae* pv. *oryzae* | *AvrXa21* | LRR-PK |

Source: Lehmann, 2002, J. Appl. Genet. 43, 403-414. LZ: Leucine zipper, NBS: Nucleotide binding site, LRR: Leucine rich repeat, TIR: Toll/interleukin-1 receptor, TM: Transmembrane domain, PK: Protein kinase




Source: Nature (2001)| 411 |6839|843-847

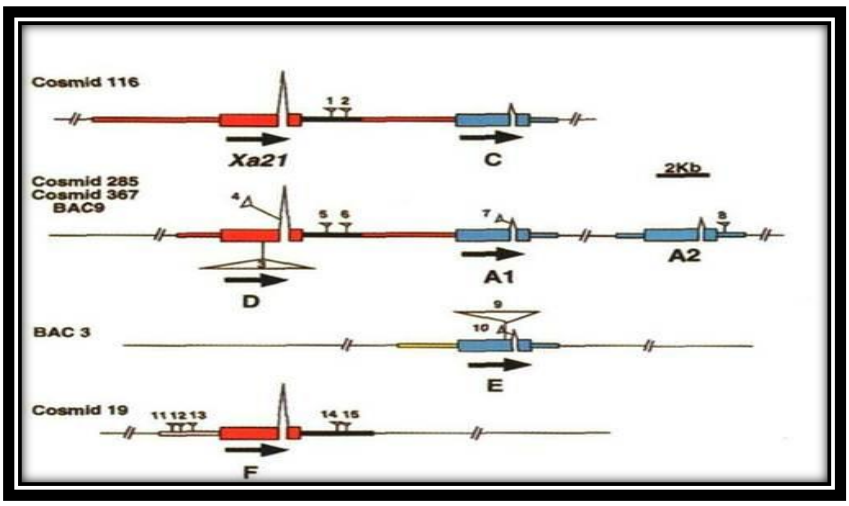
Fig 2.5 Location and structure of the five main classes of plant disease resistance proteins.

## 2.8 Structural organization of protein kinase genes

The rice *Xa21* gene encode protein kinase/receptor kinase gene and confers immunity to diverse strains of the gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). *Xa21* encodes a 1025-amino acid containing a signal peptide of 23 hydrophobic residues, 23 imperfect copies of 24 amino acid juxtamembrane LRRs with numerous potential glycosylation sites, a single transmembrane domain of a 26-amino acid hydrophobic stretch, and an intracellular serine-threonine protein kinase catalytic domain of 297 amino acids (Song et al. 1995). Tomato *Pto* gene is also an example of protein kinase gene and its catalytic region carries 11 subdomains and all 15 invariant amino acids.

Gel blot analysis revealed that *Xa21* gene is a member of a multigene family containing at least seven members (Song et al. 1995). *Xa21* gene family members from the resistant rice line (IRBB21) designated as A1, A2, B (*Xa21*), C, D, E, and F (Fig. 2.6) (Song et al. 1995; Wang et al. 1995). But only the B (*Xa21*) and A1 ORFs encode receptor kinase-like proteins while rest other members produces truncated ORF of receptor kinase. Pulsed-field gel electrophoresis analysis demonstrated that most of these members can be mapped to a single locus on chromosome 11 that is linked to at least nine major resistance genes and one quantitative trait locus for resistance (Ronald et al. 1992). Restriction enzyme and sequence analysis of BAC and cosmid clones proved that all the seven members of *Xa21* gene are distributed on four contig. There are at least 15 transposable elements like sequence in the vicinity of (both in coding and non-coding region) of *Xa21* gene family members. Sequence comparison of *Xa21* gene family members revealed that downstream of start codon upto 233-bp is highly conserved. The average GC content of the rice genome is 40.9% whereas this highly conserved region of *Xa21* gene family member contains 61.8%.

Cell surface pattern recognition receptors (PRRs) are key components of the innate immune response across the animal and plant kingdom. The rice PRR, *Xa21* (Song et al. 1995), recognizes the pathogen associated molecular pattern (PAMP), Ax21 (Activator of *Xa21*-mediated immunity) which is highly conserved in all tested species of the genus *Xanthomonas* as well as in *Xylella* species (Lee et al. 2009). The PRRs and NBS-LRR type proteins have been reported to be involved in recognition and surveillance of pathogens molecules, are largely encoded by R-genes (Park et al. 2010).




Source: Plant Cell | (1997) 9(8) |1279 - 1287

Fig 2.6 Genome organization of the seven *Xa21* family members and location of 15 transposon-like elements.

## 2.9 Resistance gene analogues

It has been observed that most of the above mentioned R-genes possess conserved amino acid motifs such as *Xa21* which consist of LRR domain (extracellular), a TM domain (transmembrane) and a kinase domain (intracellular). LRR domains mediate protein-protein interactions, and are the major determinants of recognition specificity whereas kinases mediate the signal transduction activity (Fluhr, 2001). Various investigators have used conserve domains of different R-genes to design the primer for amplifying similar regions from the genomes of diverse plant species by the polymerase chain reaction (PCR) (Aarts et al. 1998; Collins et al.1998; Feuillet et al. 1997; Kanazin et al. 1996; Leister et al. 1996; Shen et al. 1998). These sequences have been called resistance-gene analogs (RGAs) (Kanazin et al. 1996) or resistance-gene candidates (RGCs) (Shen et al. 1998). RGAs can also be cloned by PCR-based approaches using genomic information and genetic map. RGAs may or may not be the integral part of the R-genes, but they may reside in proximity of known functional R-genes, suggesting the evolutionary relationship with the functional R-genes (Dangl and Jones, 2001; Backes et al. 2003). Therefore, RGAs can be explored to study the evolutionary dynamics of R-genes. Leister et al. (1998) studied a large number of RGAs from different species of Gramineae and documented that RGAs are evolving rapidly when compared to other components of the genome. This evolution process has been

reported very slow and gradual in case of non-grass plants (Noir et al. 2001; Lee et al. 2003). Genetic analysis has revealed that these RGA sequences confer resistance to viruses, bacteria, fungi and nematodes. Mapping studies of fungal RGAs have provided strong evidence that they co-segregate with disease resistance markers (Aarts et al. 1998; Meyers et al. 1999). Consequently, this approach provide an alternative strategy to isolate disease resistance candidate genes that can be used to develop molecular marker which can be deployed in marker assisted selection (MAS) or this may even lead to identification of unknown disease resistance genes (Deng et al. 2000). Using a similar approach, we have amplified resistance gene analogues from rice. The different RGAs were subsequently cloned, sequenced and mapped onto a rice physical map. RGAs are distributed in many small and large clusters (Botella et al. 1997; Ghazi et al. 2009). The presence of RGAs in clusters indicates that they might be involved in multiple signalling pathways to confer protection against a pathogen. At least six *Xa21* like RGAs were reported on the long arm of chromosome 11. A cluster of NBS-LRR was also predicted at the distance of 0.6Mb which indicated the possibility of additional NBS-LRR-type genes for activation and expression of the *Xa21* gene (Ghazi et al. 2009). The same theory was postulated in tomato against the pathogen *P. syringae* where *Pto* gene, encoding a serine/threonine protein kinase, required additional NBS-LRR gene, *Prf*, for defense activation (Salmeron et al. 1996).

## 2.10 Expression studies of disease resistance genes

As many as 70 different R-genes which show resistance to major plant pathogens has been isolated, cloned, and characterized in different plant species in the last 15 years (Sharma et al. 2009). Pathogen infestation can cause accumulations or reductions in transcript levels of host plant tissues (Iqbal et al. 2005). Probenazole (PBZ) is a bactericide and fungicide that acts by inducing plant defense systems. It has been shown to induce the expression of RPR1 (rice probenazole responsive) gene and activate systemic acquired resistance in rice. Two maize (*Zea mays* L.) genes *Zmnbslrr*1 and *Zmgc*1, which are associated to *Fusarium graminearum* QTL (quantitative resistance/trait loci), behave differently to PBZ inducer. The transcript level of *Zmnbslrr*1 gene was significantly reduced whereas the transcript level of *Zmgc*1 gene increased more than 10-fold in ear rot resistant genotype after PBZ treatment (Yuan et al. 2008). *Xa1* represents the second major class of R-genes whose expression is

elevated upon bacterial infection (Zhang and Gassmann, 2007). It is effective against some *Xoo* isolates in Japan but not against most strains from the Philippines. *Xa27* confers broad resistance which contains identical open reading frames in resistant and susceptible cultivars but is only expressed in resistant cultivars upon bacterial infection. No function or conditional expression for the susceptible allele of *Xa27* has been determined. *Xa27* is only expressed upon inoculation with *Xoo* strains harboring the type III effector gene *avrXa27* (Gu et al. 2005). The expression of *Xa3/Xa26* is developmentally regulated. It has lower level of expression at the early developmental stage and higher level of expression at the late developmental stage, which results in rice plants carrying *Xa3/Xa26* being susceptible to some *Xoo* strains at the seedling stage but the resistance is enhanced to the same *Xoo* strains at the adult stages (Cao et al. 2007).

In the present investigation, Real time PCR analysis was performed to see the changes in transcript level in susceptible and resistant genotypes. Gene specific primer was used and expression of protein kinase gene at different point of time is reported.

## 2.11 Proteomic studies of disease resistance proteins

The completion of the rice and *Xoo* genome sequence are important accomplishments in the field of agriculture. Although genomes can change due to movement of transposable elements or from epigenetic changes, they are generally considered more static than the highly dynamic proteome. Therefore, the main target ahead is to elucidate the function of protein involve in plant-pathogen interaction. Comparative proteomics is very promising approach for global analysis of protein expression under various conditions (Agrawal and Rakwal, 2011; Ding et al. 2012). Hence, different biological processes including protein–protein interaction, post translational modification, protein expression could be successfully analyzed during plant development and stress condition (Hashiguchi et al. 2010). For example, proteomic studies of the embryo and endosperm development (Komatsu et al. 1993) metabolic pathway in leaf, root and seed (Koller et al. 2002), the basal region of developing seedling (Tanaka et al. 2005) have provided rice protein profiles. Proteomics analysis was also employed to study rice responses to different environmental factor, such as hormones including gibberellin, jasmonic acid and auxin (Casimaro et al. 2001; Yang et al. 2004; Fan et al. 2011), abiotic stresses such as cold (Yan et al. 2006), high temperature (Lin

et al. 2005), drought (Salekdeh et al. 2002), salinity (Yan et al. 2005; Zhang et al. 2009), wound (Shen et al. 2003) and ozone (Agrawal et al. 2002). Proteomics provides direct evidence for target differentially expressed proteins involved in defense responses in rice (Fan et al. 2011; Ding et al. 2012). The analysis of infected rice leaf with blast fungus (*M. grisea*) discovered 14 pathogenesis related (PR) proteins that might be involved in the rice incompatible interaction with the pathogen (Konishi et al. 2001). PR10 and probenazole-inducible protein 1 (PBZ1) protein expression were upregulated after inoculating rice with blast fungus, *M. grisea* or treating it with JA (Kim et al. 2003). PR5 was induced when rice was inoculated with *M. grisea*, followed by application of nitrogen fertilizer (Konishi et al. 2001). Protein related to stress (Superoxide dismutase), metabolism (heat shock proteins) and translocation (dehydrins), were induced by rice yellow mottle virus (RYMV) in the host after inoculation with the virus (Ventelon-Debout et al. 2004). Proteomic analysis of rice subcellular compartments has been carried out, including the plasma membrane, vacuolar membrane, mitochondria and chloroplast. PR5, PBZ1, SOD and peroxiredoxin like protein were induced in rice leaf blade after *Xoo* inoculation (Mahmood et al. 2006). Rice plasma membrane proteomics study revealed 20 protein spots regulated by the *Xoo* challenge, of which, at least 8 were PM-associated with potential function in BB defense (Chen et al. 2007). In this study, quantitative changes in protein expression characteristic to *Xoo* reaction were analyzed in susceptible and resistant rice genotypes.

## 2.12 Comparative genomics

Cereals such as wheat, rice, maize, barley, oat, sorghum constitute over 50% of the total crop production worldwide (http://www.fao.org/). All cereal crop species are members of the grass (Poaceae) family which is the fourth largest family of flowering plants. In terms of genome organisation they represent a very diverse family with basic chromosome numbers ranging from 4 to 266 and genome sizes ranging from 400 Mb to 17 Gb (Feuillet and Keller, 2002). Early comparative studies were performed using isozymes but after genomic revolution in 1980's DNA markers are available to compare different genomes. A comparative genomics study is the study of similarity/differences and relationships between genomes of different and related species. It enables the identification of the portion of genomes that are conserved and those that are unique, thereby allowing one to relate specific

changes in genome structure and content to differences in the biology of the different species. It gives insight into the mechanisms of genome evolution and speciation as well as provides tools for a variety of studies and applications that range from the densification of DNA markers on genetic maps to the identification of conserved genes and regulatory sequences. Early results indicated a good level of conservation of marker order at the genetic map level (macrocolinearity) and thus with its small genome size and well-studied genetics, rice was promoted as a reference genome for grasses. Initial comparisons between the genomes of all important grass species were performed with restriction fragment length polymorphisms (RFLP) markers, but this technique was limiting the detection of whole or partial genome duplication events and it was difficult to assess orthologous and paralogous relationships of gene families (Moore et al. 1995).

The release of the rice genome sequences and the development of large EST collections from other cereals have provided new insights into the level of colinearity among the cereal genomes. With these data, it became possible to compare the sequences of EST markers mapped in different cereal species to each other and to the rice genome sequence and thus, to study macrocolinearity at a higher resolution (Table 2.3). These studies greatly enhanced the resolution of comparative mapping and revealed additional features of the conservation among cereal genomes. In maize, more than 2,600 mapped sequence markers identified 656 (46%) putative orthologous genes in the rice genome (Salse et al. 2004). In addition to ESTs, low-pass BAC sequences also can be used for sequence based comparative studies. Klein et al. (2003) have used them successfully in sorghum to align chromosome 3 BAC sequences against the rice chromosome 1 sequence and to identify a previously undetected inversion between the two chromosomes. Complete sequences from the two major rice subspecies *japonica* and *indica* can be used for comparative studies between genomes that diverged less than 0.5 MYA (Ma and Bennetzen, 2004). More than 9,000 Single Nucleotide Polymporphisms (SNPs) as well as 63 and 138 Insertion/Deletions (InDels) were observed for the *indica* and *japonica* sequences, respectively. Monna et al. (2006) identified 7,805 polymorphic loci (SNP, Indels) within 1,117 predicted intergenic sequences that were obtained from eight rice cultivars and a wild *O. rufipogon* accession and demonstrated the potential of intraspecific comparisons for association studies in rice. Beyond the information that these comparisons provide on the divergence between the

subspecies, they represent an extremely useful source of markers for genetic mapping and map-based cloning in populations derived from crosses between the two subspecies since the InDels are conserved beyond the sequenced cultivars within each subspecies (Shen et al. 2005). Therefore, the comparative analysis of 100 Kb region flanking *Xa21* gene homologue was carried out from the two subspecies of rice *Oryza sativa* L. ssp. *japonica* cv. Nipponbare and *Oryza sativa* L. ssp. *indica* cv. 93-11 in order to understand the evolution and divergence of *Xa21* locus.

As genes are the most conserved features between genomes, the availability of a genome sequence can help to predict genes in other genomes. Even between distantly related genomes such as the one of rice and *A. thaliana* the ancestors of which diverged 200 million years ago and do not show extensive macrocolinearity, a large number of genes have been conserved (Salse et al. 2002). Thus, the rice genome sequence represents a unique tool to support gene annotation in other cereals. The alignment of ESTs from other cereal species with the rice genome sequence can help to predict new genes from rice.

Table 2.3 Inter and intra specific micro-colinearity studies in cereals: List of the various loci that have been compared at the sequence level through BAC sequencing among different cereal species. The asterix (*) indicates comparisons that have also been performed at the intraspecific level

| Locus | Compared plant species | Reference |
|---|---|---|
| ***Lrk*** | Wheat, barley, maize, rice | Feuillet and Keller 1999 |
| ***Rph7*** | Barley*, rice | Scherrer et al. 2005 |
| ***adh1/adh2*** | Maize, sorghum, rice | Ilic et al. 2003 |
| ***Vrn1*** | Wheat, barley, sorghum, rice | Ramakrishna et al. 2002a |
| ***lg2/lrs1*** | Maize, rice | Langham et al. 2004 |
| ***sh2/a1*** | Maize, sorghum, rice, wheat | Bennetzen and Ma 2003 |
| ***Zein*** | Maize*, sorghum, rice | Song and Messing, 2003 |
| ***Ha*** | Barley, rice, wheat* | Chantret et al. 2005 |
| ***r1/b1*** | Maize, sorghum, rice | Swigonova et al. 2005 |
| ***Orp1/Orp2*** | Rice, sorghum | Ma et al. 2005 |
| ***Rp1*** | Maize, sorghum | Ramakrishna et al. 2002b |
| ***Phd-H1*** | Barley , rice | Dunford et al. 2002 |
| ***Glutenin*** | Rice, wheat* | Gu et al. 2004 |
| ***Bz*** | Maize*, rice | Lai et al. 2005 |

Cooke et al. (2007). From Rice to Other Cereals: Comparative Genomics. Rice Functional Genomics, 429-479.

## 2.13 Comparative genomics of disease resistance genes

Disease resistance has been comprehensively studied in rice because it is one of the most important traits for rice breeding. Full genome sequences of *indica* and *japonica* offer unprecedented resources to study the evolution of sequence and function of homologue genes and to understand diversification and adaptation. The chromosome 11 of rice is a particularly interesting genomic region to investigate the evolution of R loci in a comparative genomics perspective. Comparative analysis of DNA sequences have revealed that disease resistance loci evolve faster than the rest of the genome (Paterson et al. 2003). Genomic sequencing of *Arabidopsis* and rice has already yielded interesting insights into the numbers and organization of disease resistance genes. *Arabidopsis* genome analysis suggested that ~200 NBS-encoding genes are present in *Arabidopsis* (~150 encoding NBS of the TIR-type and ~50 of the non-TIR type). This would represent close to 1% of all *Arabidopsis* genes. All NBS-encoding genes in rice encode non-TIR-type NBS; TIR-type genes have not been detected in genomic or expressed sequence tag (EST) sequences from any grass species (Meyers et al. 1999, Pan et al. 2000). The complete sequencing of the *RPP5* (encoding NBS-LRR-type proteins) cluster in *Arabidopsis*, the *Cf-4/9* (encoding LRR-transmembrane-type proteins) and *Pto* (encoding protein kinases) clusters in tomato, and partial sequencing of the *Dm3* (encoding NBS-LRR-type proteins) cluster in lettuce revealed highly duplicated regions containing little more than resistance-gene homologs. *Xa21* encodes a receptor kinase protein carrying leucine-rich repeats (LRR) and the FLS2 of *Arabidopsis*, which is thought to be a receptor for the flagellin molecule has been shown to have a similar structure (Song et al.1995). In both proteins, LRR functions as a receptor and the kinase domain as a signal transducer (Gomez-Gomez et al. 2001). Another cloned rice R-gene for BB is *Xa1*, which encodes a protein carrying a nucleotide-binding site (NBS) and LRR (Yoshimura et al. 1998); the encoded protein is structurally similar to the most typical group of R-genes: *RPS2*, *RPM1*, and *RPP5* of *Arabidopsis*, *N* of tobacco, and *L6* and *M* of flax. Two blast resistance genes of rice, *Pi-b* (Wang et al. 1999) and *Pi-ta* (Bryan et al. 2000), have been isolated, and both of them were shown to encode proteins of the NBS-LRR class. Therefore, it would be worth comparing the *Xa21* loci in both ssp. of rice.

# CHAPTER-3
# MATERIALS AND METHODS


The present study was conducted at University of Hyderabad, Hyderabad, India. All the rice genotypes were provided by Dr. R. M. Sundaram and *Xanthomonas* strain by Dr. G. S. Laha, Directorate of Rice Research (DRR), Hyderabad. The methods used in this study are described below.

## 3.1 Screening for Bacterial Blight resistance and characterization of photosynthetic and yield responses in rice genotypes

### 3.1.1 Plant growth and experimental set up

The experiment was carried out at the glasshouse complex of University of Hyderabad, Hyderabad, India (17.20 N latitude, 37.30 E longitude, and 536 m above sea level). Seeds of all genotypes were germinated on separate seed beds maintaining the temperature of the growth chamber at 25/17$^{o}$C (day/night). Seed beds were uniformly watered and fertilized with a half-strength Hoagland nutrient solution. On 30$^{th}$ day after sowing, the healthy uniform seedlings were selected and used for transplantation in 20 liters' earthen pots (four pots for each genotype with three plants in each pot) filled with a mixture of clay and peat (1:1, v/v). Single seedling was transplanted in each hill. The experiment was conducted in three replications (n=3) following a completely randomized design (CRD). Seedlings were uniformly irrigated and fertilised using Hoagland nutrient solution as per requirement. Soil water content (SWC) was kept at 100% pot water holding capacity (PWC) and periodically measured (gravimetrically) at different points of the pot to check the homogeneity of moisture content in soil. Inside the glasshouse, photosynthetically active radiation (PAR) ranged from 900 to 1200 $\mu molm^{-2}s^{-1}$ between 10:00–11.00 AM, air temperature was 25/17$^{o}$C (day/night), relative humidity was 60 ± 5% and ambient $CO_2$ concentration was 360 to 370 $\mu molmol^{-1}$.

### 3.1.2 Inoculum preparation, infestation and disease scoring

Bacterial pathogen (*Xanthomonas oryzae* pv. *oryzae*) was cultured on modified Wakimoto's agar medium at room temperature for 72 hours, and then suspended in distilled water into approximately $10^9$ cfu/ml. Fully developed leaves of each plant were clip-inoculated when the plants were in maximum tillering to booting stage, as described by Kauffman et al. (1973). The control treatment consisted of clipping with scissors dipped in sterile water.

Disease scoring was done after 15 days of inoculation according to the IRRI standard evaluation system for rice (IRRI, 2002). Percent leaf area infected on inoculated leaves was measured manually with scale and graph paper. Disease scores of the genotypes to bacterial infection was determined based on percentage of lesion length according to the standard evaluation system for rice (IRRI, 2002) (score 0=immune; score 1=1–5%; score 3=6–12%; score 5=13–25%; score 7=26–50% and score 9=51–100%). Genotypes were categorized as resistant (R: score 0–3), moderately resistant (MR: score 5–7) and susceptible (S: score > 7).

### 3.1.3 Leaf gas exchange analysis

One highly resistant (*O. longistaminata*), one resistant (IRBB 21) and one susceptible (PB1) genotype was selected for leaf gas exchange analysis.

To study plant $CO_2$ assimilation physiology, leaf gas exchange and microclimatic data were measured using a portable infrared $CO_2/H_2O$ gas analyzer (IRGA) (LCpro-32070, ADC Bioscientific Ltd., UK) equipped with a detachable leaf chamber with photosynthetically active radiation (PAR) sensor (silicon based sensor, LCpro-32070) and leaf thermistor probe (ADC-M.PLC-011, LICOR ) attached to it. The gas analyzer was used to measure instantaneous net photosynthetic rate ($P_N$), stomatal conductance to $CO_2$ ($g_s$), and leaf transpiration rate ($E$) periodically during the study period (0 to 216 h, between 10:00 to 11:00 AM). Instantaneous water use efficiency (*WUEi*) was calculated (*WUEi*=$P_N$/E) and plants were also analyzed for internal $CO_2$ concentration ($C_i$). Microclimatic parameters such as irradiance (PAR: 900–200 $\mu molm^{-2}s^{-1}$), relative humidity (RH: 40%), air temperature (Ta: 24–25%), $CO_2$ concentration (360–370 $\mu molmol^{-1}$) and flow rate (~500 $\mu mols^{-1}$) were recorded by the instrument. Each measurement was made when $P_N$ and $g_s$ readings were stabilized; this process took 1–2 min. Single intact leaf were kept inside the leaf chamber and an incubation period of 2 min was given for adaptation of leaf to the microclimate of the leaf chamber and readings were taken thereafter. All photosynthetic measurements were performed on well-expanded leaves ($3^{rd}$ to $4^{th}$ from apex). In case of infected plants, measurements were performed just below the visible necrotic leaf tissue region.

### 3.1.4 Measurements of yield and yield attributing components

Three plants each from three replications were randomly selected for measuring rice yield and different yield components including; stem length, panicle length, plant height, total tillers per plant and productive tillers per plant at appropriate growth stages. Post-harvest data on number of filled, semi-filled, unfilled grains per panicle, percentage of ripened grains, panicle weight and 1000 grain weight were measured (Yoshida et al. 1976). *O. longistaminata* could not be studied for agronomic trait as sufficient seeds were not obtained due to its sterile nature.

The detailed method of recording the observations is presented character-wise as follows.

**Stem length:** Length of stem from each plant was measured in centimetres from base to the start of panicle and the mean was computed.

**Panicle length:** Length of main panicles from each plant was measured in centimetres from panicle base to the tip of panicle (excluding awn) and the mean was computed.

**Plant height:** The plant height was measured in centimetres from the ground level to the tip of the panicle (excluding awns) of mother tiller at dough stage.

**Total tillers/plant:** Total number of tillers was counted just before harvesting.

**Productive tillers:** The percentage of productive tillers was calculated as panicle number divided by the maximum tiller number per plant.

**Filled, semi-filled, unfilled grains per panicle:** Filled, unfilled and semi-filled grains from each panicle were selected and segregated and their percentage was calculated.

**Percentage of ripened grains:** The percentage of ripened grains was calculated by dividing the number of filled grains by the total number of grains.

**Panicle weight:** It was measured for each panicle individually from each replication.

**1000 grain weight:** Thousand (1000) whole grains were counted and weighed.

## 3.2 Identification, mapping and synteny analysis of genes associated with BB resistance from chromosome 11 of rice genome sequence

### 3.2.1 *Ab initio* gene prediction

Gene finding, locating the positions of genes and determining their structure is one of the first and most important steps towards understanding the sequenced genome. There are two approaches available for genome annotation. The first one is based on sequence homology

and the second one is *ab initio* gene prediction program. *Ab initio* gene prediction uses statistical and computational methods to detect coding regions, splice sites and start and stop codons in genomic sequences. This approach does not depend on sequence similarity and is therefore not limited by the availability of sequence data. In the present study, *ab initio* gene prediction approach was used to predict the genes using Fgenesh software (www.softberry.com; Salamov and Solovyev, 2000) trained for monocot genome annotation. We have performed gene prediction of 130 ordered BAC/PAC clones present in the minimum tilling path of chromosome 11 of rice (IRGSP Build5 Pseudomolecules). The BAC clone sequences of *O. sativa* L. ssp. *japonica* cv. Nipponbare were downloaded from the database of International Rice Genome Sequencing Project (IRGSP,http://rgp.dna.affrc.go.jp/cgibin/statusdb/Build5/ uild5 clonelist. cgi? chr=11/). The BAC clone sequences were converted into FASTA format and saved as a new text file. The monocotyledon parameter was taken into consideration before submission of the sequences in the dialogue box of the Fgenesh website. The output file of program was then grouped and tabulated for statistical analysis (Annexure 3.1).

Once the genes were predicted through Fgenesh, the coding sequences of predicted genes were taken as query sequence and BLASTN (nucl query vs nucl db) (basic local alignment search tool) program was run on the server (http://www.genome.ad.jp). The parameter for BLASTN was set as, Scoring matrix: BLOSUM62, Filter: DUST, Alignment views: Pairwise, maximum number of alignments to be displayed. Finally, significant coding sequences of R-genes were picked (bit score of 1000 or above and $0 \leq$ e-value) from the BLAST results and statistical analysis was made out of them. The significant resistance genes sequences were downloaded and were used for primer designing.

### 3.2.2 Construction of physical map of receptor like kinases (Class-5 disease resistance gene)

After gene prediction from the BAC/PAC sequence of chromosome 11 pseudomolecule, the sequence of each resistance gene was taken and their exact position on chromosome 11 was determined through gramene BLASTN tool (http://www.gramene.org/Multi/blastview). The output data of BLASTN analysis were noted down and classified into different categories of resistance genes. The positions of all predicted LRR-protein kinase and protein kinase (receptor like kinases) were seen on the finished quality sequence of rice to understand their

distribution on chromosome 11 pseudomolecules. A physical map was drawn and the position of each LRR-protein kinase and protein kinase was shown on a centimorgan scale.

### 3.2.3 Sub-cellular location of genes

ProtCompV8.0 (www.softberry.com) software tool was used to predict the sub-cellular localization of the gene products using protein sequence of the predicted genes. Protein sequences of all predicted resistance genes were taken one-by-one in FASTA format and were submitted in the dialogue box of the ProtCompV8.0 for the determination of their sub-cellular location. The outputs of the search result were categorized based on their sub-cellular location which showed highest score. ProtCompV program recognizes animal/fungal and plant proteins separately. Its accuracy rate of protein localization prediction in the cell is 80-90% (Annexure 3.1).

### 3.2.4 GC content and domain determination

The GC content of resistance genes was determined using online tool of science buddies (www.sciencebuddies.org/science-fair-projects/projectideas/GenomGCCalculator.html). It calculates the total number of adenine, guanine, cytosine, thymine and GC% (Annexure 3.2). Sequence based (protein query sequences) prediction of resistance genes domain was carried out using NCBI CD-search online tool (http://www.ncbi.nlm.nih.gov/ structure/cdd/wrpsb.cgi). The input protein query sequence was submitted in FASTA format with default options setting. The output of CD-Search was presented as an annotation of protein domains on the user query sequence and can be visualized as domain multiple sequence alignments with embedded user queries along with graphical summery of domain with score and e-value.

### 3.2.5 Comparison of *Xa21* and *Xa26* with known R genes using BL2SEQ algorithm

The cloned *Xa21* and *Xa26*, which encodes protein kinase were compared with other reported R-genes viz., *Xa1* and *BWMK1* of rice, *RPS2*, *RPP5* and *RPM1* of *A. thaliana*, *N* gene of tobacco, *L6* of flax, *Cf2*, *Cf9*, *Prf* and *Pto* of tomato, rust resistant *Lr21* of bread wheat, blast resistant *Pib*, *Pi-9*, *Pi-ta*, *Pid2* and *Piz-t* of rice through BL2SEQ algorithm (Tatusova and Madden, 1999). The comparison of *Xa21* and *Xa26* was also performed with all predicted class-5 resistance genes (LRR protein kinase + protein kinase) in order to

understand the homology between cloned receptor kinase and predicted class 5 resistance genes through BL2SEQ algorithm (Annexure 3.3).

### 3.2.6 Synteny analysis of class-5 resistance genes

All the chromosome 11 predicted protein kinase/LRR protein kinase sequences were analyzed for synteny with rest of the chromosomes of rice and other plant species including monocots (wheat, maize, barley, sorghum) and dicots (*arabidopsis*, tomato). In order to map the protein kinase/LRR protein kinase genes on other chromosomes, the cDNA sequence of predicted protein kinase/LRR protein kinase were submitted to NCBI blast (http://blast.ncbi.nlm.nih.gov/) against *O. sativa* (*japonica* cultivar- Group) HTGS with default parameters. Sequences producing significant alignment with other chromosomes were tabulated with accession number and megabase/centimorgan position. The same sequence of protein kinases were submitted to NCBI map viewer (www.ncbi.nih.gov/mapview) to see the synteny with wheat, maize, barley, sorghum, arabidopsis and tomato EST. At first we have selected the organism for which analysis to be performed followed by sequence submission. The best feedback provided by the software in terms of query coverage (>10 %) and sequence identity (>65%) were extracted for further analysis. Finally synteny map was generated manually based on homology obtained from the above analysis.

### 3.2.7 Comparative analysis of the genomic regions flanking *Xa21* locus in *indica* and *japonica* ssp. of rice

The genome sequences of chromosome 11 of *japonica* and *indica* were downloaded from the International Rice Genome Sequencing Project (IRGSP) database (http://rgp.dna.affrc. go.jp/irgsp) and Beijing Rice Genome Sequence database (http://rice.genomics.org.in/ rice/ index2.jsp), respectively. The IRGSP gene Os11g0559200, which encodes *Xa21*, was downloaded from gramene database (www.gramene.org). The position of *Xa21* on chromosome 11 of *japonica* and *indica* was located through BLASTN search tool (Altschul et al. 1990). The resistant homologues allele of *Xa21* is 3.57 kb long and encodes a receptor kinase. A 100 kb sequence flanking *Xa21* locus (with ~ 50 kb upstream and ~ 50 kb downstream of the locus) was located with the help of BioEdit software (Hall, 1999). All the analysis was performed including *Xa21* locus (3.57 kb).

Gene prediction from the 100 kb region flanking to *Xa21* locus of chromosome 11 in *japonica* and *indica* rice was carried out using HMM based gene structure prediction software FGENESH tool. The nomenclature of predicted genes in this region were given as *Osjp01, Osjp02* etc. (*O. sativa* ssp. *japonica* gene number 1 and 2, respectively) and *Osin01*, *Osin02* etc. (*O. sativa* ssp. *indica* gene number 1 and 2, respectively).

FastPCR (Kalendar et al. 2009) was used to identify monomers. SSRIT (simple sequence repeat identification tool; Temnykh et al. 2001) available at gramene database was used to find out other types of SSR markers present in the 100 kb region. The tool SSRIT uses pearl regular expressions to find perfect SSR repeats within a particular sequence. It can detect repeats between 2 to 10 bases in length, but eliminates mononucleotides repeats (http://www.gramene.org/db/searchers/ssrtool).

Physical positioning, sub-cellular location and GC content of each gene in this region was carried out through methods described in the section 3.2.2, 3.2.3 and 3.2.4 respectively. To study the phylogenetic relationship among the predicted genes of both the rice subspecies, a phylogenetic tree was drawn using clustalW program (http://www.ebi.ac.uk/tools/msa/clustalw2/; Thompson et al. 1994). This was visualized using programs like treeviewX and Neighbor Joining (NJ) plot (Saitou and Nei, 1987).

## 3.3 Molecular cloning and expression analysis of BB resistance genes

### 3.3.1 Plant materials

Eight rice genotypes were used in the experiment which include three near isogenic lines (NILs) *viz*., IBBB3, IRBB4 and IRBB21 carrying *Xa-3*, *Xa-4* and *Xa-21* genes respectively); three wild genotypes (*O. longistaminata*, *O. rufipogon* and *O. nivara* ) and two high yielding popular cultivars i.e. Pusa Basmati 1 and Taichung Native 1 (TN1).

### 3.3.2 Preparation of competent cells of *Escherichia coli*

*Escherichia coli* DH5-α was streak out onto an LB agar plate from the glycerol stock stored in the –80°C freezer and was incubated overnight at 37°C. Single colony from the plate was inoculated in 5 ml of LB (Luria Broth, Himedia, India) and was incubated overnight at 37°C on a rotary shaker at 200 rpm. From the overnight culture, 1 ml of culture was taken and inoculated in 50 ml of LB and incubated again until the OD reached 0.2 (log phase growth)

at 600 nm. From here onwards all the remainder procedure were performed on ice. Sterile micro tubes were cooled on ice and 1.5 ml of the chilled bacterial suspension was transferred to each one of them. The cells were centrifuged in a cooling centrifuge at 5000 rpm for 10 minutes at 4°C. Supernatant was removed immediately and the pellet was resuspended thoroughly in 0.5 ml of ice-cold 0.1M $CaCl_2$ solution and was stored on ice for 30 min. The cells were centrifuged at 5000 rpm for 10 min at 4°C, and the pellet was resuspended in 0.5 ml of ice-cold 0.1M $CaCl_2$ solution in 20% (w/v) glycerol. Dispensed in 200 µl aliquots and freeze cells were stored at –80°C freezer for further use.

### 3.3.3 Genomic DNA isolation and quantification

Genomic DNA was isolated from young and freshly collected leaves using the CTAB method as described by Murray and Thomson (1980) with slight modifications. The composition of stock solution is mentioned in Appendix 3.1. Fresh-leaf tissue (0.5 g) was ground to fine powder in liquid nitrogen by using pre-chilled mortar and pestle. Leaf powder was distributed into 1.5 ml eppendorf tube and 600 µl of preheated freshly prepared extraction buffer (CTAB) was immediately added to the tube and mixed smoothly. Now the tube was then incubated at 60ºC for 35–45 min with inversion. An equal volume of chloroform: isoamyl alcohol (24:1, v/v) was added and then samples were mixed by gentle inversion for 5 min. All tubes were centrifuged at 12,000 rpm for 15 min at room temperature. Supernatant (400µl) was placed in a new eppendorf tube and equal volume of ice cold isopropanol was added and kept undisturbed for 45 min at 4ºC in fridge. Tubes were then centrifuged at 12,000 rpm for 10 min. Supernatant was discarded and the pellet was washed with 70% (v/v) ethanol by brief centrifugation. DNA pellet was air-dried for 1 h at room temperature and then dissolved in 100 µl 1X TE buffer (pH 8.0). To remove RNA and protein from crude DNA sample, it was treated with RNaseA [2 µl (1 µg/µl)] and the tubes were incubated at 37°C for 1 h. For further purification, extraction with an equal volume of chloroform: isoamyl alcohol (24:1, v/v) was performed. DNA was precipitated by adding 1/10th volume of 3 M sodium acetate (pH 5.2) and 2.5 volume of ice-cold ethanol. Tube was inverted gently and kept for 30 min at –20°C. Tube was centrifuged at 12,000 rpm for 10 min, supernatant was discarded and the pellet was washed with 70% (v/v) ethanol. The pellet was dried and dissolved in 100 µl of TE buffer. Quantification was made through

NanoDrop spectrophotometer and its integrity and quality was examined through running the dissolved DNA in 0.8% agarose gel. The DNA was diluted to 20 ng per µl for PCR analysis.

### 3.3.4 Primer designing

The coding sequence of receptor kinase genes were taken one by one (output of Fgenesh, annotated through BLASTN) and primer designing was performed using online primer 3 software (http://frodo.wi.mit.edu/primer3/). While selecting small oligos for primers, primer 3 considers many factors including oligo melting temperature, length, GC content, 3′-stability, estimated secondary structure, primer–dimer formation and the product size. The Primer3 parameters in this study were: primer size = 18–24, Tm = 56–62ºC, GC% = 50–60%, CG Clamp = 1–2, product size = 300–800 bp and rest of the parameters were left as default. To ensure the unique binding site of primers, all forward and reverse primers were taken one-by-one and BLASTN analysis of NCBI was performed. Primers whose sequences were not unique in the rice genome were discarded. Primers were synthesized and procured from Sigma-Aldrich Chemicals Pvt. Ltd (Annexure 3.4).

### 3.3.5 PCR analysis and gel elution

PCR amplification reactions were carried out using 150 ng of genomic DNA for 50 µl reactions, containing 5 µl of 10X PCR buffer (10 mM Tris-HCl, pH 8.0, 2.5 mM $MgCl_2$), 50 pmol of each primer, 200 mM of each dATP, dCTP, dGTP and dTTP, 2.5 units (0.05unit/µl) of *Taq* DNA polymerase (Sigma Aldrich, USA). Annealing temperature was different for different individual set of primers. The PCR was carried out in a thermal cycler (Eppendorf vapo.protect™). The first cycle consisted of denaturation of template DNA at 94$^{o}$C for 5 min, primer annealing at 55–57$^{o}$C for 1 min and primer extension at 72$^{o}$C for 1 min. In the subsequent 32 cycles the period of denaturation was reduced to 1 min while the primer annealing and primer extension time was maintained same as in the first cycle. The last cycle consisted of only primer extension at 72$^{o}$C for 10 min.

For recovering PCR product, 1.2% agarose gel was prepared by melting 0.72 g agarose (SeaKem® LE Agarose, Lonza) in 60 ml of 1XTAE buffer (10mM Tris-HCl, 40 mM acetic acid and 1mM EDTA pH 8.0). The melted agarose was cooled to 50$^{o}$C and 2µl of EtBr from a stock of 10 mg ml$^{-1}$ was added. This was poured into a gel-casting tray with a

comb placed properly in the grooves provided. Once the gel got polymerized, it was placed inside the electrophoresis tank containing 1XTAE buffer so as to cover the gel. The PCR product mixed with 6X loading dye to a concentration of 1X and was loaded in the wells made by comb. The gel was electrophoresed at 60 V for 1 h or till the dye front reached almost 3/4$^{th}$ part of the gel. The sizes of the amplicon were determined using standard size marker (100 bp ladder, MBI Fermentas). DNA fragments were visualized under UV transilluminator and photographed. The major polymorphic amplicon of expected size were excised from gels and eluted using sigma GenElute$^{TM}$ Gel Extraction Kit (Saint Louis, Missouri 63103 USA) following manufacturer's instructions. The DNA was eluted in 30 µl of gel elution buffer. A small amount (2.5µl) of the eluted DNA was electrophoresed on agarose gel to check the concentration and quality and rest amount was stored at -20°C for further experiments.

### 3.3.6 Ligation and cloning of polymorphic PCR product

The polymorphic eluted fragments were ligated into plasmid vector pTZ57R/T. The ligation mixture was prepared as given below to a final volume of 15 µl.

| | |
|---|---|
| 5X ligation buffer | : 3.0 µl (1x final concentration) |
| Vector DNA | : 30 pmol |
| Insert DNA | : 90 pmol |
| $T_4$ DNA ligase | : 1.0 unit (MBI Fermentas) |
| Sterile DDW | : to make up to15 µl |

The reaction mixture was mixed gently and centrifuged briefly to bring the contents at the bottom of the tube. Reaction mixture was incubated at 16°C for almost 16 h and then used for transforming *E. coli* competent cells. For each transformation reaction 5 µl of this reaction mixture was added in eppendorf tube containing 200 µl of competent cells. Components were mixed gently by flicking the bottom of the tube with finger for a few times and tubes were placed on ice for 30 min. Subsequently the tubes were kept at 42$^{o}$C for exactly 90 seconds in water bath for heat shock. After giving heat shock, the tubes were kept back on ice for 2 min. Then, 1ml of Luria Broth (LB) medium was added to the tubes and the tubes were incubated at 37$^{o}$C for 1 h on shaking incubator. The tubes were centrifuged at 4,000 rpm for 2 min. Around 100 µl of supernatant was left in the tube and rest all supernatant was discarded. Pellet was resuspended and spread on LB agar plate containing

X-gal, IPTG and 100 µg/ml ampicillin. Then the plates were incubated at 37$^{o}$C for overnight. The successful ligation of gene with vector was confirmed through blue-white screening method on LB agar plate. White (positive) colonies were picked and grown in 5ml of LB with ampicillin for overnight in shaking incubator shaker. Plasmid isolation was done by plasmid isolation kit (Promega) following manufacturer's instructions and DNA concentration was determined through NanoDrop Spectrophotometer (ND 1000). Insertion of gene of interest was initially confirmed by PCR followed by double digestion of plasmid DNA with *Eco*RI and *Bam*HI restriction enzymes (Fermentas Life Sciences, Canada). The digested plasmid DNA was electrophoresed on 1.0% agarose gel stained with EtBr (Ethidium Bromide) and was visualized under a UV-transilluminator. The restriction digestion reaction was set up as follows:

| | |
|---|---|
| Plasmid | : 0.5 µg |
| 10x Restriction buffer | : 2.0 µl (1x final concentration) |
| Restriction enzyme | : 0.5 µl BamH1+ 0.5 µl EcoR1 |
| Sterile DDW | : to make up to 20µl |

The plasmid harbouring the desired gene was used for sequencing.

### 3.3.7 Sequencing and sequence analysis

Positive clones having desired inserts were sent for sequencing to Vimta Labs Pvt. Ltd., Hyderabad, India. M13 universal primers were used for sequencing and it was based on Sanger's sequencing method i.e. dideoxy chain termination method.

Initially, vector sequences were identified by pair wise alignment with the pTZ57R/T sequences and were trimmed to generate consensus sequence. Consensus sequence of the cloned PCR products was used to search the GenBank databases by the BLASTX algorithm at NCBI (http://blast.ncbi.nlm.nih.gov). Primer binding sequences were also detected to ascertain the completeness of the sequence. The resistance gene sequences were converted into fasta format and submitted to BankIt, a sequence submission tool of NCBI (http://www.ncbi.nlm.nih.gov/BankIt/).

### 3.3.8 Total RNA isolation for expression analysis of resistance genes

Prior to RNA isolation, distilled water, mortars, pestles, eppendorf tubes, tips and other equipments were washed with DEPC-treated water (0.1% w/v) and placed in oven for overnight to evaporate the excess DEPC. All the solutions used in the RNA isolation were prepared with DEPC-treated water and autoclaved before use. Mortar, pestle and eppendorf tubes were chilled with liquid nitrogen before usage. Quickly, 0.5 gram of leaf samples which were collected from control and treated plant at different time interval (0 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h) were ground in liquid nitrogen with mortar and pestle and fine powder was collected in 1.5 ml eppendorf tube. One ml TRI reagent (Sigma Aldrich, USA) was added and the eppendorf tubes were mixed by vortexing for 10 minutes. To these, 200 µl of chloroform was added and the contents were mixed for 15 seconds with vigorous shaking and incubated at room temperature for 15 min. After incubation, centrifugation was done for 15 minutes at 4°C with 12,000 rpm. After centrifugation, supernatants were transferred to a new tube without disturbing the pellet and to this 500 µl of isopropanol were added. The tubes were shaken vigorously for 15 seconds, incubated at room temperature for 5 minutes, centrifuged for 10 min at 4°C with 12,000 rpm. RNA was obtained in the form of pellet on the side and bottom of the tube. The supernatants were discarded and pellets were washed with 1 ml of 70% ethanol with 3 minutes incubation at room temperature. After a centrifugation for 5 minutes at room temperature and maximum speed, supernatants were discarded again. The pellets were air dried for 10 minutes. Finally, 40–50 µl DEPC-treated water was added to each one and the tubes were incubated for 15 minutes at 65ºC to dissolve pellet, and vortex occasionally until it dissolves. RNA can be immediately used after determination of its quantity and quality or kept at –80ºC freezer for further use.

### 3.3.9 Determination of RNA quantity, quality and cDNA synthesis

The RNA concentration was determined by NanoDrop spectrophotometer. The A260/A280 ratio should be approximately 2.0, but figures between 1.8 and 2.1 are considered acceptable. The quality of RNA was determined by EtBr-stained agarose gel electrophoresis (1.0%) in 0.5X TBE buffer at 60V for 1 h. Approximately 2 µg RNA was loaded from each sample.

First strand cDNA was synthesized from 2 µg of total RNA, after DNAse-I (Sigma Aldrich, USA) treatment in the presence of Oligo dT, dNTPs and MMLV reverse transcriptase enzyme (Sigma Aldrich, USA). Obtained cDNA was used to analyze the transcript levels of protein kinase gene through gene specific primers designed from exonic region of the candidate gene.

### 3.3.10 Real time PCR

Primers were designed using Primer3 software, criteria for generation of amplicons were ranging from 120 to 200 bp with Tm of 58ºC±2ºC. Both candidate and housekeeping gene were amplified in a one-step protocol. As housekeeping gene 18S rRNA was used for normalization of target gene expression. The primer pairs were as follows: 18S rRNA forward primer (5'-ATGATAACTCGACGGATCGC-3') and reverse primer (5'-CTTGGAT GTGGTAGCCGTTT-3'); RGIA forward primer (5'-CTGGGAAATGCTCTACCAACT GGA-3') and reverse primer (5'-GAAGCGTGTGGCAATAATGGTGG-3') & RK1 forward primer (5'-TGGCATTGCTATAGGGTGTG-3') and reverse primer (5'-GAGAGCCCAAAA TCTGCAC-3'). The qPCR was carried out using SYBR Green I technology on a 7,500 Real Time PCR System (Applied Biosystems, Foster City, USA). Experiments were performed in 96-well optical reaction plate with MicroAmp optical adhesive film from Applied Biosystems. A master mix with a total volume of 25 µl containing 2 µl of cDNA (40 ng), 12.5 µl of iQ SYBR Green Supermix (2X) (Bio-Rad) and a primer pair with 200 nM concentration was prepared for each PCR run. Reaction mixture were incubated for 95ºC for 2 min followed by 40 cycles at 95°C for 20 sec, 57°C for 20 sec and 72°C for 15 sec. The final elongation step was carried out at 72°C for 10 min. Each sample was analyzed in biological triplicate and mean threshold cycle ($C_t$) values were calculated for normalization.

### 3.3.11 Amplification of full length ORF and its cloning

The full length cDNA of RGIA (954 bp) and RK1 (1926 bp) were amplified using gene specific primers RGIA-F (5'-GGTACCATGCGGAGAGGGAGCAT-3'), RGIA-R (5'-CGG ATCCCTAAAGTCTAAAAAGCAACCG-3') and RK-F (5'-TAGGGCCCATGACAATG CGTACGG-3'), RK-R (5'-CGAGCTCTCAGTCAACAAATTGTGAGCG-3') respectively. PCR amplified product of resistance genes were electrophoresed, gel eluted (Gel elution kit,

Sigma-Aldrich, USA), cloned into pTZ57R vector (Insta clone T/A cloning kit, Fermentas, Germany) and then sequenced commercially (Vimta Labs Pvt. Ltd.).

### 3.3.12 Preparation of expression cassette

*Kpn*1 and *Bam*H1 (for RGIA), *Apa*1 and *Sac*1 (for RK1) restriction sites were incorporated in the primers at 5' and 3' ends to facilitate cloning into plant expression vector pRT100 by digesting it with the same set of the enzymes such that the coding region would be flanked by 35S promoter and poly-A signal in the sense orientation. All-inclusive cassette with RGIA and RK1 coding region flanked by 35S promoter and poly-A signal was released from pRT100 by digesting with *Hind*III and *Pst*l respectively and cloned into the binary vector pCAMBIA1300 and pCAMBIA2300 for rice and tobacco transformation respectively (Figure 3.1). The recombinant binary vector was mobilized into *Agrobacterium tumefaciens* strain *EHA105* using freeze thaw method.

### 3.3.13 Preparation of *Agrobacterium* culture

Primary culture of *Agrobacterium* was prepared by inoculating 5ml of LB medium (with 50 µg/ml each of ampicillin and rifampicin) with small loop of glycerol stock of *Agrobacterium tumefaciens* strain *EHA105* incubated at 170 rpm for 24 h at 28°C**.** The secondary culture was initiated by inoculating 200 µl of primary culture in 50 ml of LB (with 50 µg/ml each of ampicillin and rifampicin) grown at 28°C till O.D.600 nm reached to 0.2–0.3 and centrifuged at 5000 rpm for 15 min. The pellet was dissolved in the *Agrobacterium* suspension medium (MS+sucrose-30g/casein hydrolyte-0.3g/l) supplemented with 100 mM acetosyringone. This *Agrobacterium* suspension was used for the infection of rice embryonic region (in planta transformation) and tobacco leaf discs.

### 3.3.14 Tobacco transformation

Leaf samples of *N. tabacum* L. cv. samsun from *in vitro* one-month-old plantlets were collected and washed 4–5 times with sterile water. Square sections of leaf tissue (approximately 0.5 $cm^2$) were made using a scalpel and were carefully punched 4-5 times with scalpel blade. Thereafter, explants were soaked in 20 ml of suspension medium (MS + sucrose-30g/l + Casein Hydrolysate-0.3g/l) containing $10^8$ cells/ml of *Agrobacterium* EHA105 for 30 minutes. Subsequently, the explants were blot dry with sterile tissue paper,

and incubated for 3 days on co-cultivation media (MS + 0.1 mg/l NAA + 2 mg/l BAP) under dark condition (Appendix 3.2). After the co-cultivation, the explants was washed three times with sterile water and finally rinsed with 500 mg/l of cefotaxime. Then the explants were blot dry with sterile tissue paper and placed upside down onto kanamycin selection medium (MS + 0.1 mg/l NAA + 2 mg/l BAP + 125 mg/l kanamycin) for 20 days and on regeneration medium (MS + 0.1 mg/l NAA + 2 mg/l BAP + 125 mg/l kanamycin) for 30 days. Callus formation was observed after 3–4 weeks. Regenerated shoots formed at the cut end of the leaf discs were separated from the rest of the explants and transferred onto fresh rooting medium (MS+ 125 mg/l kanamycin) to induce root formation and further development for about 30 days. Kanamycin resistant shoots were separated (3–4 cm high) and placed in a rooting medium supplemented with 125 mg/l kanamycin for 20 days. All the plantlets raised *in vitro* were subsequently transferred to greenhouse once roots were grown. All plant tissue cultures were incubated at 24 ± 2ºC under fluorescent light at 100 µmol $m^{-2}s^{-1}$ with a 16/8 hour (light/dark) photoperiod.

### 3.3.15 Rice transformation

Mature seeds of indica rice genotype BPT5204 were dehusked and sterilized in 70% (v/v) ethanol for 4–5 min and then transferred into 0.1% $HgCl_2$ for 5–6 min with gentle shaking. The seeds were rinsed 5-6 times with sterile water and then soaked in sterile water at 20-25ºC for 24 h. After 24 h soak, the embryo region of the seed turned white and bulge, but neither shoots nor roots had appeared. In order to inoculate *A. tumefaciens*, the embryonic region (embryonic apical meristem) was pierced 2–3 times to a depth of 1–1.5 mm with a needle that had been dipped in the *A. tumefaciens* inoculum. The inoculated seeds were placed on co-cultivation media (MS + 0.1M Acetosyringone) and incubated in dark at 20-25ºC for 5 days, during which inoculated seeds germinated to seedlings. Then, the seedlings were placed on selection media (MS+ 50mg/l Hygromycin) for 25 days. Seedlings survived on selection media were transferred in glass tubes containing water for proper root development. For control plants, sterile water was used as inoculum. All the plantlets raised *in vitro* were transferred to greenhouse for further development and analysis.

### 3.3.16 Acclimatization and green house transfer

Rooted shoots were cleaned properly under running tap water and were transferred to sterile vermiculite soil mixture (1:1) and were kept under conditions similar to culture for 10-15 days covered with polythene bags to retain humidity. They were kept for another 5-10 days in green house before their transfer to the pot containing sand soil mixture (1:1) with added organic manure. The plantlets were covered with polythene bags till they developed new leaves. Once new leaves started appearing, polythene bags were removed and plants were watered regularly and when required. The genomic DNA for PCR was isolated from young leaves following CTAB method (Murray and Thomson, 1980) and PCR analysis was done for primary confirmation of putative transgenics.

## 3.4 Proteomic analysis to identify genes potentially involved in BB resistance

### 3.4.1 Plant materials and infection treatment

Two rice genotypes, BB susceptible (Pusa Basmati 1) and BB highly resistant (*O. longistaminata*) were used in this study. Rice plants growth and bacterial inoculum preparation were carried out as discussed previously in section 3.1.1 and 3.1.2 respectively. The uppermost fully opened leaves were inoculated with bacterial suspension as treatment and infiltrated with sterilized distilled water as control, by needles pricking methods (Mahmood et al. 2006). The midribs of rice leaf were pricked from top to bottom with needle from abaxial side. Leaf samples were collected at 72 h post inoculation in liquid $N_2$ and were stored in –80ºC for further analysis.

### 3.4.2 Total protein extraction

Rice leaf blade from both control and treated plants were collected and immediately frozen in liquid nitrogen and stored at –80ºC for further experiments. Total leaf proteins were extracted as described by Parker et al. (2006) with minor modifications. One gram of leaf tissue was homogenized to fine powder in liquid nitrogen with a mortar and pestle and suspended in 5 ml of ice cold protein extraction buffer (0.5 M Tris-HCl pH 7.5, 0.7 M sucrose, 0.1 M KCl, 50 mM EDTA, 2% β mercaptoethanol and 1 mM PMSF; Appendix 3.3). Equal volume of phenol saturated with Tris- HCl (pH 7.5) was added and mixed at 4ºC for 30 minutes followed by centrifugation at 6,000g for 20 minutes. The upper phenolic

phase was collected and an equal volume of extraction buffer was added to it. The above step was repeated and upper phenolic phase was re-extracted in fresh tube. Four volume of 0.1M ammonium acetate in methanol was added to it and kept for 4 hours at –20ºC for protein precipitation. After centrifugation at 10,000g for 30 minutes at 4ºC, the supernatant were discarded. Pellet was washed three times with ice cold methanol and three times with ice cold acetone for 3 minutes each time at 4ºC and 7,000g. Finally, pellet was air dried for few minutes, solubilized in 250 µL of the rehydration solution [(8 M (w/v) urea, 2 M (w/v) thiourea, 4% (w/v) CHAPS, 30 mM DTT, 0.8% (v/v) immobilized pH gradient (IPG) buffer pH range 4–7 (GE Healthcare, Uppsala, Sweden)] and concentration of protein was determined by Bradford method (Bradford, 1976).

### 3.4.3 Bradford method of protein quantification

Bradford protein assay is a simple procedure for determination of protein concentrations in solutions that depends upon the change in absorbance in coomassie Brilliant Blue G-250 upon binding of protein. Bradford reagent was prepared by dissolving 100 mg coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol. To this, 100 ml phosphoric acid was added and volume was made upto 200 ml with $H_2O$. The Bradford reagent is stable for 6 months at 4ºC. Samples of BSA were prepared with 10, 20, 30, 40, 50,…..and 100µg/100µl of BSA in the same buffer solution in which the protein samples were extracted. The concentrate Bradford dye was diluted to 5X with double distilled water and 1 ml was added to each sample. The red dye turned blue after binding to the protein and then samples were allowed to develop colour for 5 minutes. Absorbance was taken at 595 nm and a linear standard curve was prepared to calculate the concentration of protein.

### 3.4.4 Two Dimensional gel electrophoresis (2-DE), gel staining and image analysis

For 2-DE, 1 mg protein was mixed with rehydration solution (8 M urea, 2 M thiourea, 4% CHAPS, 30 mM DTT, 0.8% IPG buffer pH range 4–7 and 0.004% bromophenol blue) to a final volume of 320 µl. Active rehydration of protein was carried out on IPG strip (18 cm, 4–7 pH linear gradient; GE Healthcare) for 12 h at 50 V. Rehydration and focusing was carried out in Ettan IPG phor II (GE Healthcare) at 20ºC using the following program: 30 min at 500 V, 3 h to increase from 500 to 10,000 V and 6 h at 10,000 V (a total of 60,000

V/h). Prior to the second dimension separation, the gel strips were equilibrated twice in equilibration buffer [6 M urea, 30% (w/v) glycerol, 2% (w/v) SDS, 50mM Tris-HCl pH 8.8], first with 2% DTT and then with 2.5 % iodoacetamide each for 20 minutes. The strips were then transferred to 12% vertical SDS-PAGE gels for the second dimension electrophoresis using an EttanDalt6 chamber (GE Healthcare). SDS-PAGE was run at 10 mA $gel^{-1}$ for 1 h and then 38 mA $gel^{-1}$ for 6 -8 h until the bromophenol blue dye front reaches the gel end.

After electrophoresis, gels were stained with Brilliant Blue R-250 for overnight at orbital shaker and then distained with coomassie blue destaining solution on the very next day. The gels were scanned using calibrated densitometric scanner (GE Healthcare) at a 300 dpi resolution. The comparison between the gels of control and treated (normalization, spot matching, expression analysis and statistics) was performed with Image Master 2-D Platinum version 6 image analysis software (GE Healthcare) as described in the user manual. Background subtraction and normalization were done fully automatically but some manual editing was performed to correct the mismatched and unmatched spots between gels.

### 3.4.5 Enzymatic digestion of protein in-gel, mass spectrometry and protein identification

Protein spots of interest which showed significant changes after treatment were excised from two or three coomassie-blue stained replicated gels. Each excised protein spots were destained with 200 µL of 50% acetonitrile (ACN) in 50 mM of ammonium bicarbonate ($NH_4HCO_3$) until the gel was destained. Thereafter, the gel pieces were treated with 10 mM DTT in 50 mM $NH_4HCO_3$ and incubated at 56ºC for 1 h. This was followed by treatment with 55 mM iodoacetamide in 50 mM $NH_4HCO_3$ for 45 min in dark at room temperature (25 ± 2ºC). The gel pieces were then washed with 25 mM $NH_4HCO_3$ and ACN, dried in speed vac at ambient temperature and rehydrated in 15 µl of 25 mM $NH_4HCO_3$ solution containing 25 ng $\mu l^{-1}$ trypsin at 4ºC for 10 minutes and then digested at 37ºC for overnight (sequencing grade, Promega, Wisconsin, USA). After incubation, a short spin was given and the supernatant was collected in a fresh eppendorf tube. The left gel pieces were further sonicated for 10 minutes followed by frequent vortexing for 5 min in 10 µl of 0.1% trifluoroacetic acid (TFA) and 100% ACN (1:1) to extract the remaining peptides. This extraction step was repeated twice to improve the extraction yield. The supernatants were pooled together and dried using speed vac and were reconstituted in 5 µl of 100% ACN and

0.1% TFA (1:1 v/v). The above sample (1 µl) was mixed with 1 µl of a-cyano-4-hydroxycinnamic acid (CHCA) matrix in 50% ACN and 1% TFA (1:1) and 2 µl of samples were spotted onto a MALDI plate and dried at room temperature for mass spectrophotometry.

Matrix-assisted laser desorption/ionization time of flight mass spectrometric (MALDI-TOF MS) analysis was carried out using MALDI-TOF/TOF mass spectrometer (Bruker Autoflex III smartbeam, Bruker Daltonics, Bremen, Germany) according to the protocol of Shevchenko et al. (1996) with minor modifications. Mass data acquisitions were piloted by FlexControl 3.0 (Build 100) software using batched-processing and automatic switching between MS and MS/MS modes. All MS survey scans were acquired over the mass range of 800 –3500 m/z in the reflectron positive-ion mode and accumulated from an average of 2500 laser shots with acceleration of 19 kV. Peptide precursor ions corresponding to contaminants including keratins and trypsins autolytic products were excluded in a mass tolerance of ± 0.5 Da. The filtered precursor ions with a user defined threshold were selected for the MS/MS scan. Fragmentation of precursor ions was performed using MS-MS 1Kv positive mode.

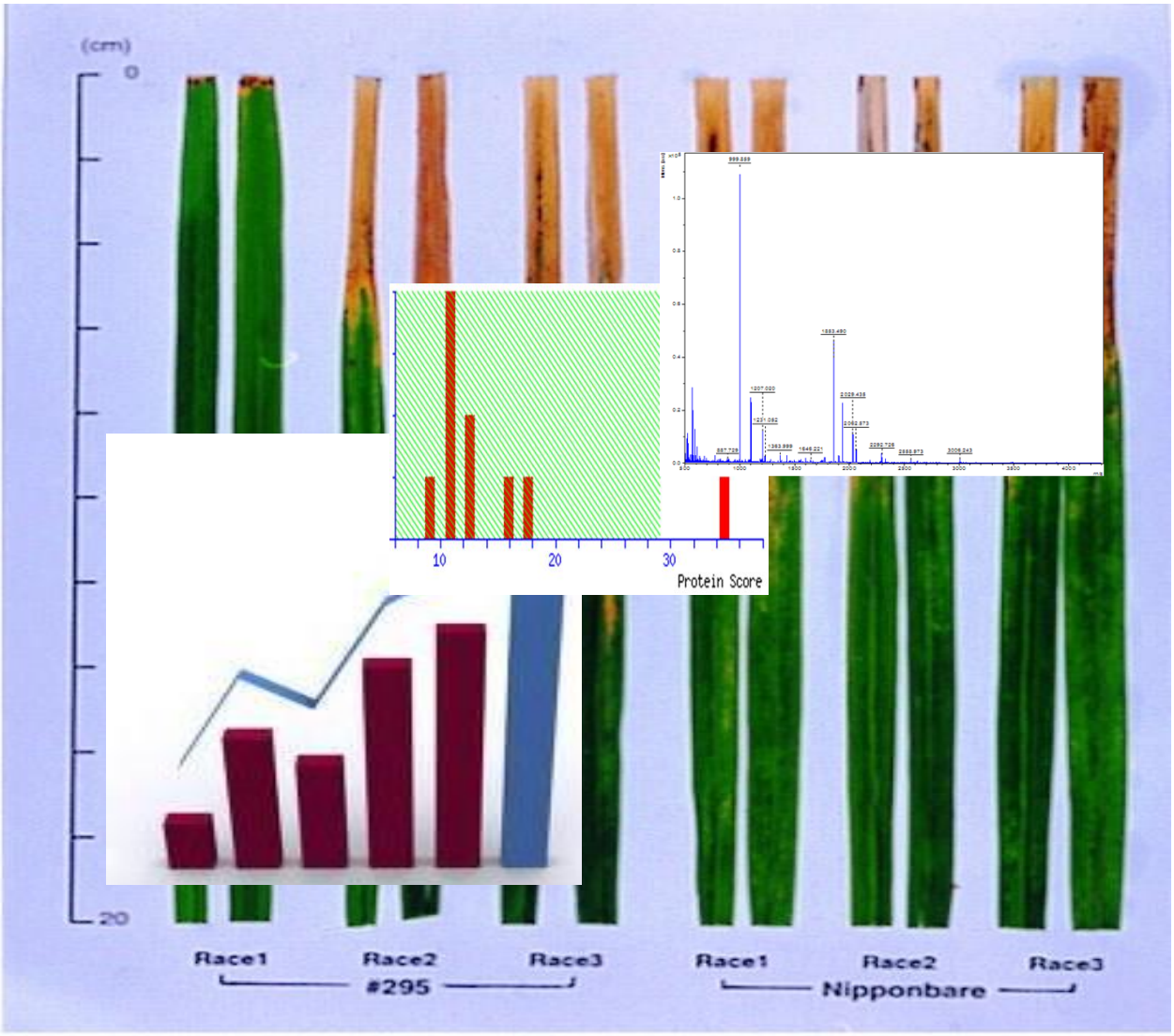
The MALDI-TOF/TOF data were loaded into the MASCOT program (http://www.matrixscience.com) employing Biotools software (Bruker Daltonics) and protein identification was performed against the NCBInr and Swiss Prot database using combine MS (peptide mass fingerprint approach) with MS/MS. The taxonomic category was set to *Oryza sativa* L. The other search parameters were: monoisotopic peptide mass ($MH^+$); one missed cleavage per peptide; enzyme, trypsin; precursor-ion mass tolerance on an average100 ppm; MS/MS fragment-ion mass tolerance, 0.1 Da; variable modifications, carbamidomethylation (C) for cysteine and oxidation for methionine (M) were allowed. Contaminating ions like trypsin and keratins were excluded from the peak lists before database searching. Top hit for each protein search were reported. Only proteins with a minimum of two matched peptides were considered to be positively identified. If a protein spot matched multiple protein under different accession numbers, the candidate protein with the maximum Mascot score with highest peptides matched were selected. The nearest experimental MW (molecular weight) and PI values to the theoretical values if having the same Mascot score were given equal weightage in spot selection. The identified proteins

were named according to the corresponding annotations in NCBI. Proteins which do not have functional annotation in the database, homologous proteins were taken and search against NCBI database (BLASTP: http://blast.ncbi.nlm.nih.gov/) to annotate the function of such proteins.

### 3.4.6 Statistical analysis

Three independent experiments with three replications of both control (untreated) and treated samples of each time point with each replication comprising of around 10 pooled plants were considered and spots were analyzed using Image Master 2-D Platinum image analysis software (GE Healthcare) and statistical one-way factor ANOVA ($p < 0.05$) was performed of the best match replicate gels. The normalised volume (% volume) of each spot was automatically calculated by the software as a ratio of a particular spot to the total volume of all the spots present on the gel. GraphPad software version 5.01 was used to make the bar diagram.

# CHAPTER-4
# RESULTS



The results obtained during present study have been described under following headings:

## 4.1 Analysis of genotypic variability, photosynthetic and yield responses against BB infection

### 4.1.1 Reaction of rice genotypes against *Xoo* strain DX133

In the present study 14 rice genotypes were clip-inoculated at maximum tillering stage as described by Kauffman et al. (1973) and were examined for their resistance/susceptibility based on IRRI standard evaluation system of rice (IRRI, 2002). Two accessions of the wild species, *O. longistaminata* and *O. rufipogon* were found to be highly resistant and one NIL IRBB21, resistant to the disease, with minimum disease infection intensity of 4.66% (score-1) and 7.69% (score-3) and 12% (score-3), respectively, whereas, IRBB13 and *O. nivara* were moderately resistant with the disease score of 5 (Table 4.1). The remaining genotypes, including IRBB1, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, PB1 and TN1 were highly susceptible with infection intensity ranging from 31.25% to 95.2%. PB1 exhibited highest susceptibility and showed 95.2% infection intensity (score-9). Out of 14 screened rice genotypes, eight were selected for polymorphism analysis based on their contrasting BB tolerance which includes three NILs (IRBB3, IRBB4, IRBB21), three wild rice (*O. longistaminata, O. rufipogon, O. nivara*) and two cultivars (PB1, TN1).

### 4.1.2 Photosynthetic leaf gas exchange responses

Photosynthetic $CO_2$ fixation characteristics were analyzed using three contrasting genotypes, viz., *O. longistaminata* (highly resistant), IRBB21 (resistant) and PB1 (susceptible) (Fig 4.1) based on resistance reaction.

Effects of BB infestation depicted contrasting variability in $P_N$, $g_s$ and $E$ among the tested genotypes. Initially an elevation in $P_N$ was observed (24 h, post infection) in all the three genotypes, consistent with previous report of Johnson (1984) where the same pattern was observed due to elevation in phytohormone, stomatal opening and ion transport in vesicular arbuscular mycorrhiza (VAM) infected *Citrus aurantium* plants.

$P_N$ of the healthy leaves was significantly higher than that of the infected leaves in each of the three genotypes, when measured periodically with disease progression (Fig 4.2). The control plants of *O. longistaminata* showed maximum $P_N$ of ~19.26 µmol $CO_2$ $m^{-2}s^{-1}$ on

216 h, whereas IRBB21 and PB1 showed a maximum $P_N$ of 12.5 µmol $CO_2$ $m^{-2}s^{-1}$ on 216 h and 48 h (11.83 µmol $CO_2$ $m^{-2}s^{-1}$ ) respectively. The $P_N$ values in infected plants of each genotype declined progressively during the course of experiment. PB1 and IRBB21 showed rapid and substantial reduction in $P_N$ compared to *O. longistaminata*. After 216 h of infection, the leaves of *O. longistaminata* showed $P_N$ of 7.18 µmol $CO_2$ $m^{-2}s^{-1}$, whereas in IRBB21 $P_N$ was 4.35 µmol $CO_2m^{-2}s^{-1}$ followed by PB1 (0.74 µmol $CO_2$ $m^{-2}s^{-1}$).

With the down-regulation of $P_N$ in infected leaves, we observed that $g_s$ and $E$ were also concomitantly reduced in all tested genotype when compared to their control counterparts. The healthy leaves of *O. longistaminata* showed highest $g_s$ (0.61 $mmolm^{-2}$ $s^{-1}$) on 144 h followed by IRBB21 and PB1 (0.32 mmol $m^{-2}$ $s^{-1}$on 24 h). During infection, $g_s$ was strongly reduced to 0.046 mmol $m^{-2}$ $s^{-1}$ on 216 h in PB1 followed by IRBB21 (0.16 mmol $m^{-2}$ $s^{-1}$ on 216 h), whereas *O. longistaminata* maintained highest $g_s$ of 0.22 mmol $m^{-2}$ $s^{-1}$ on 216 h. In comparison to the early stage of infection, the $g_s$ of infected leaves decreased more drastically at later stage, i.e. from 48 h of infection till the end of experiment (Fig 4.2).

As $g_s$ and $E$ are strongly coupled, we observed the same degree of down-regulation in $E$ along with $g_s$. The lowest $E$ was recorded in infected PB1 (0.69 mmol $m^{-2}$ $s^{-1}$ on 216 h) followed by IRBB21 (2.27 $mmolm^{-2}$ $s^{-1}$ on 216 h). However, *O. longistaminata* exhibited relatively higher $E$ of 2.31 $mmolm^{-2}$ $s^{-1}$ on 216 h when compared to other genotypes (Fig 4.3). Moreover, with the progression in disease severity, *WUEi* was significantly reduced in PB1 (1.07 µmol $CO_2$ /mmol $H_2O$ on 216 h), followed by IRBB21 (1.91 µmol $CO_2$/mmol $H_2O$ on 216 h) when compared to their control counterparts (Fig 4.4). In infected *O. longistaminata*, as the reduction in $P_N$ was comparatively less when compared to $E$, hence the photosynthetic *WUEi* was significantly enhanced during the later stage of disease progression.

The lowest $C_i$ value (263 µmol $mol^{-1}$) was detected in resistant IRBB21 on 216 h, whereas in susceptible genotype PB1 (277.16 $\mu molmol^{-1}$) and highly resistant genotype *O. longistaminata* (283.66 $\mu molmol^{-1}$) the values were comparatively higher on 216 h (Fig 4.3). Across the time period from 24 h to 216 h, IRBB21 has lowest $C_i$ value (206.16 $\mu molmol^{-1}$) on 72 h. To understand the association of $P_N$ with $g_s$ and $C_i$ in the BB-infected symptomatic rice leaves, we performed linear regression analysis using the variables obtained from infected plants of all three genotypes. In BB-infected condition, the regulation of $P_N$ by $g_s$

followed a linear function and resulted in a significantly positive correlation between $P_N$ and $g_s$ for all the rice genotypes (Fig 4.5). The regression slopes were steeper for *O. longistaminata* ($r^2$ = 0.81, p < 0.001) and PB1 ($r^2$ = 0.87, p < 0.001) when compared to IRBB21 ($r^2$ = 0.75, p < 0.001). The relationships between $P_N$ vs $C_i$ were linear and positively correlated for all the tested rice genotypes (Fig 4.5). The correlation was strong in IRBB21 ($r^2$=0.4, < 0.001), and *O. longistaminata* ($r^2$=0.3, p < 0.001) however, it was comparatively weak though significant in PB1 ($r^2$=0.2, p < 0.001).

### 4.1.3 Yield and yield components

There was no significant effect of stress caused by BB pathogen on stem length, panicle length, plant height and total tillers in control as well as in treated plants of PB1 and IRBB21. These growth features remained unaffected because the infection was done at maximum tillering stage. However, upon infection, productive tillers (–29.3%), seeds per panicle (–18.4%), panicle weight (–39.3%), number of filled grains (–34%) and yield per plant (–61.75%) were significantly reduced, while semi-filled (+12.0%) and unfilled grains (+63.9%) increased significantly in susceptible genotype PB1 (Table 4.2). Comparatively less reduction in the above mentioned yield traits was recorded in the resistant genotype, IRBB21 under infected condition. Reduction in productive tillers per plant in infected PB1 can be linked to the fact that plant under stress condition could not produce required assimilates due to low photosynthesis and loss in photosynthetically active tissues (Savary et al. 2000). Since, grain-filling is also closely related to whole plant senescence (Mi et al. 2002; Yang et al. 2006a), the control plants of both the genotypes PB1 and IRBB21 showed high 1000-grain weight as compared to infected plants. This difference was significantly high in PB1. IRBB21 showed less yield loss (18.45%) when compared to PB1 (61.75%), and the presence of *Xa21* in IRBB21 might have redeemed/rescued 43.3% of the yield loss.

Table 4.1 Disease score and percentage of disease infection in 14 tested rice genotypes. The genotypes were classified into three groups depending upon their level of resistance against BB *viz*., resistant (R), moderately resistant (MR) and susceptible (S)

| Rice genotypes (R genes present) | Disease score | Disease infection (%) | Level of resistance |
|---|---|---|---|
| **IRBB1 (*Xa1*)** | 9 | 94.00 | S |
| **IRBB3 (*Xa3*)** | 9 | 58.82 | S |
| **IRBB4 (*Xa4*)** | 7 | 31.25 | S |
| **IRBB5 (*xa5*)** | 9 | 90.50 | S |
| **IRBB7 (*Xa7*)** | 9 | 83.60 | S |
| **IRBB10 (*Xa10*)** | 9 | 90.46 | S |
| **IRBB11 (*Xa11*)** | 9 | 78.90 | S |
| **IRBB13 (*xa13*)** | 5 | 18.60 | MR |
| **IRBB21 (*Xa21*)** | 3 | 12.0 | R |
| ***O. longistaminata*[1]** | 1 | 4.66 | R |
| ***O. rufipogon*** | 3 | 7.69 | R |
| ***O. nivara*** | 5 | 14.86 | MR |
| **Pusa Basmati 1(PB1)[2]** | 9 | 95.20 | S |
| **Taichung Native1(TN1)** | 9 | 88.80 | S |

1: Resistant check, 2: Susceptible check

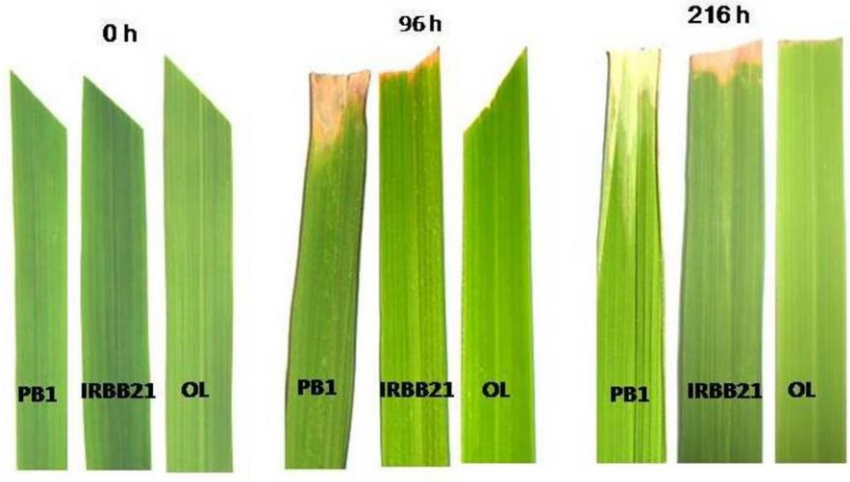



Fig 4.1 The effect of *Xoo* inoculation on the leaf blades of three rice genotypes (PB1, IRBB21 and *O. longistaminata*). Rice seedlings were inoculated with isolate *DX133* of *Xoo* by leaf clipping method. The progress in disease infestation as observed on the infected leaf blades was photographed on 0 h, 96 h and 216 h of post inoculation. No necrotic symptoms and senescence were detected in the leaf blade of BB highly resistant *O. longistaminata* when compared to resistant IRBB21 and susceptible PB1.

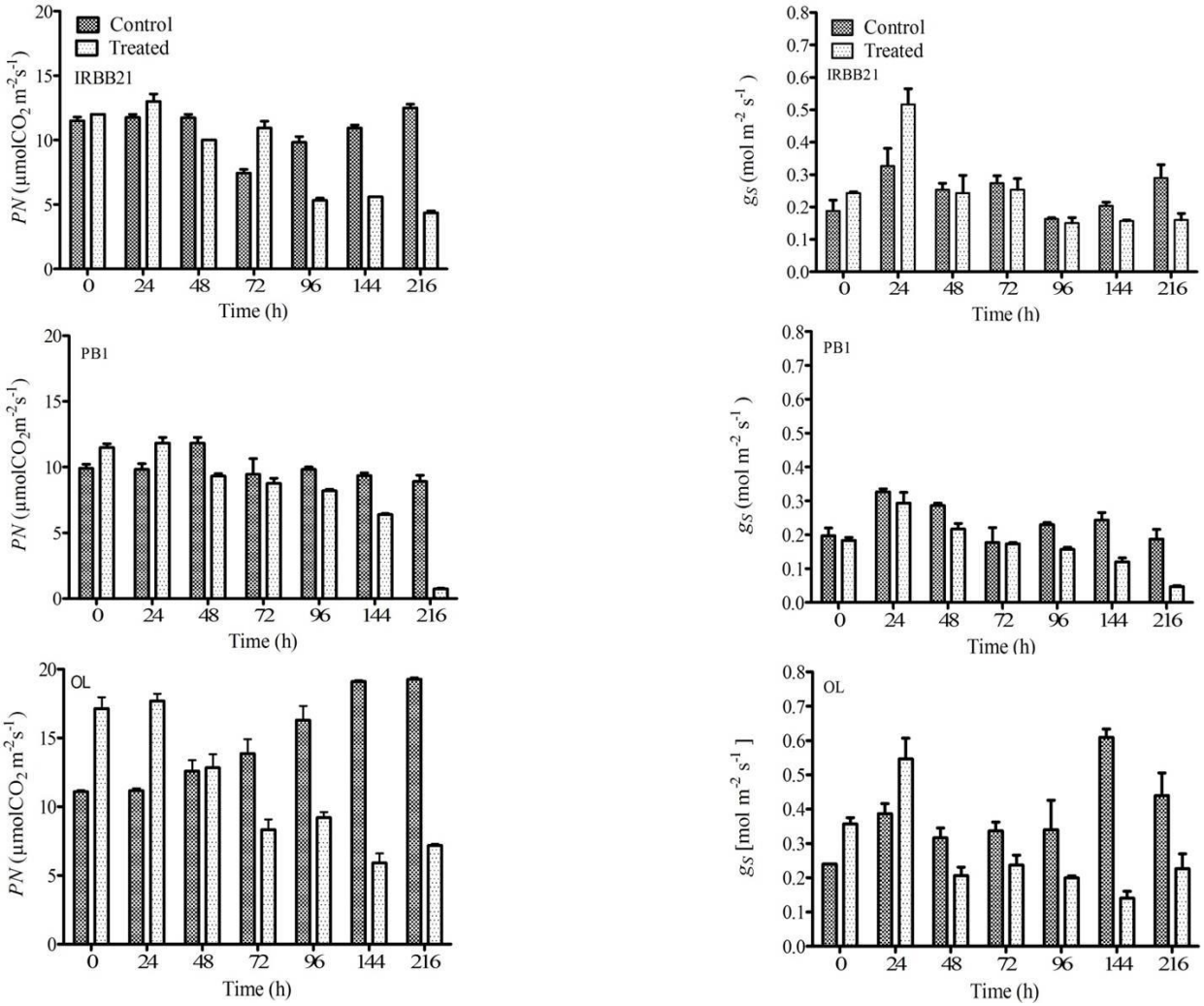



Fig 4.2 Net photosynthetic rate ($P_N$) and stomatal conductance to $CO_2$ (*gs*) in IRBB21, PB1 and *O. longistaminata* from 0 to 216 h after inoculation with *Xoo*. The data were measured using a portable infrared $CO_2/H_2O$ gas analyzer (IRGA) equipped with a detachable leaf chamber with PAR sensor. Each measurement was made when $P_N$ and $g_s$ readings were stabilized. All photosynthetic measurements were performed on well-expanded leaves (3rd to 4th from apex). In case of infected plants, measurements were performed just below the visible necrotic leaf tissue region. Data are mean ± SD.

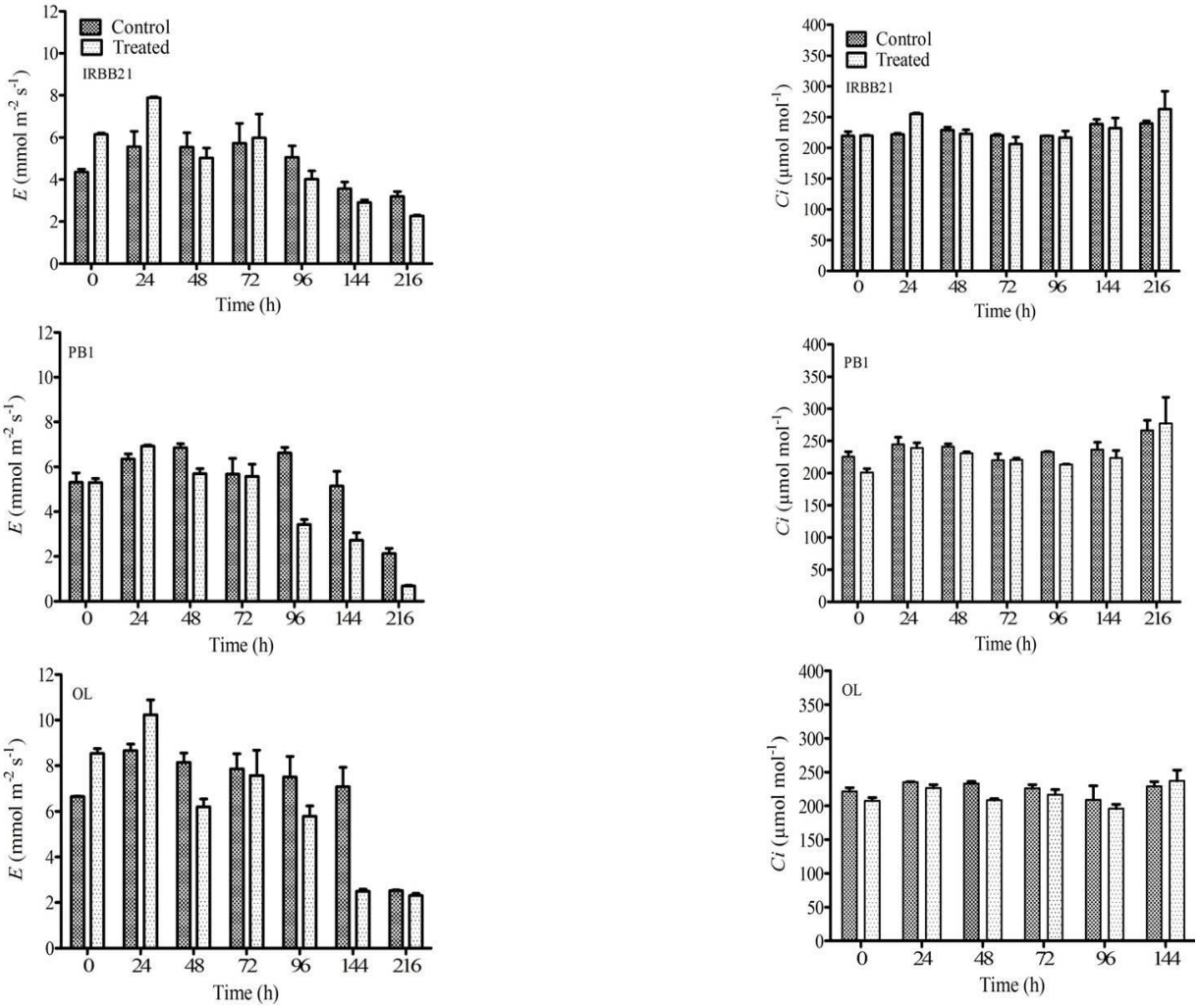



Fig 4.3 Leaf transpiration rate (*E*) and intracellular $CO_2$ concentration (*Ci*) in IRBB21, PB1 and *O. longistaminata* from 0 to 216 h after inoculation with *Xoo*. All photosynthetic measurements were performed on well-expanded leaves where each single intact leaf were kept inside the leaf chamber and an incubation period of 2 min was given for adaptation of leaf to the microclimate of the leaf chamber and readings were taken thereafter. In case of infected plants, measurements were performed just below the visible necrotic leaf tissue region. Data are mean ± SD.

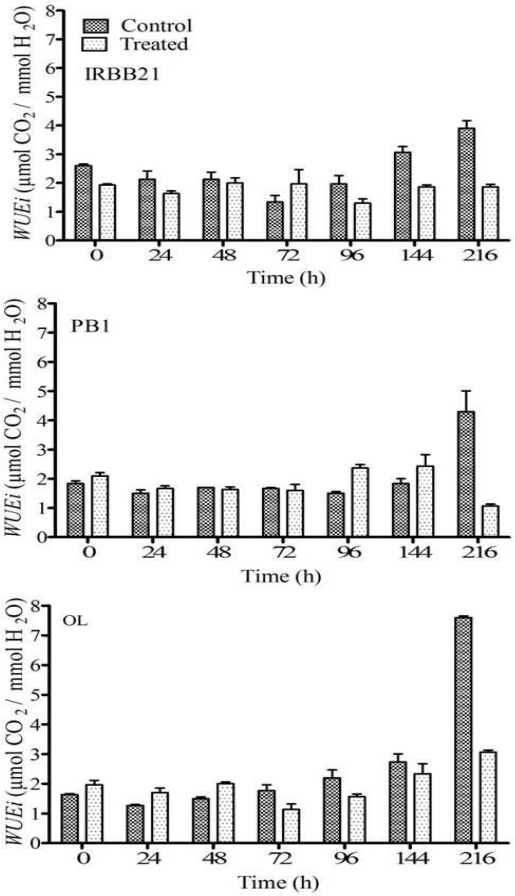



Fig 4.4 The influence of *Xoo* infection on instantaneous water use efficiency ($WUE_i$) in IRBB21, PB1, and *O. longistaminata* from 0 to 216 h. Data are means ± SD.

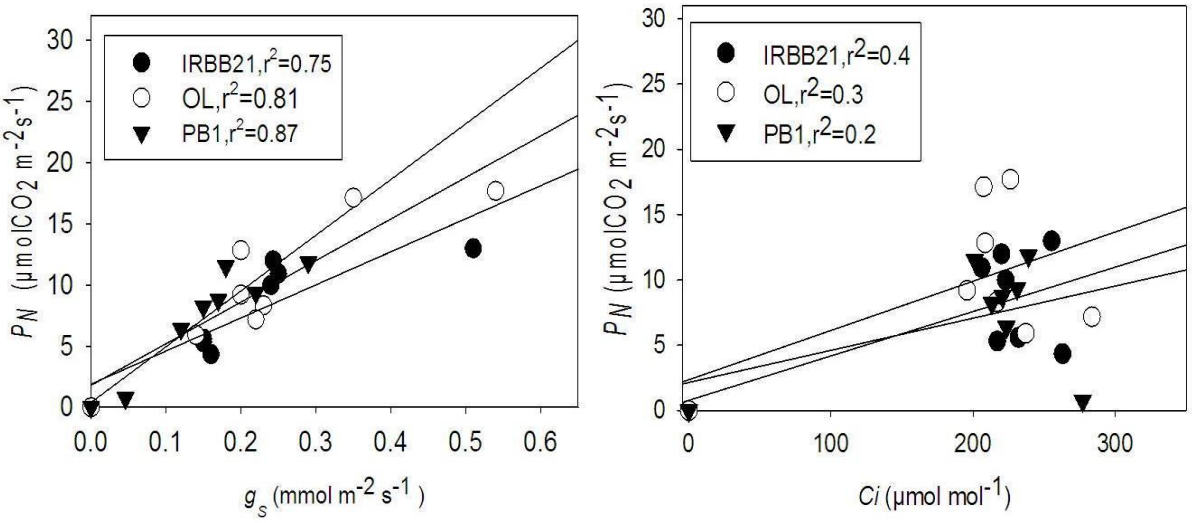



Fig 4.5 Relationships between net photosynthetic rate ($P_N$) versus stomatal conductance to $CO_2$ (*gs*) and net photosynthetic rate ($P_N$) versus internal $CO_2$ concentration (*Ci*) in the BB infected leaves of three tested rice genotypes (PB1, IRBB21 and *O. longistaminata*).

Table 4.2 Effect of BB on various yield characteristics of moderately tolerant (IRBB21) and susceptible (Pusa Basmati 1) rice genotypes. Values are expressed as mean ± SD. *, ** and ***: Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: Not significant

| Parameters | Pusa Basmati 1 | | | IRBB21 | | |
|---|---|---|---|---|---|---|
| | Control | BB Treated | %increase(+)/ decrease (–) | Control | BB Treated | %increase(+)/ decrease (–) |
| **Plant height(cm)** | 92.75±4.68 | 90±4.37 | – 2.96 ns | 68±4.28 | 65±3.84 | –4.41 ns |
| **Tillers/plant** | 10.66±2.16 | 9.16±1.32 | – 15.52* | 10.5±1.37 | 9.5±1.97 | – 9.52 ns |
| **Productive tillers/plant** | 9.66±1.63 | 6.83±0.75 | – 29.29** | 10.5±1.37 | 9.16±1.60 | – 12.76 ns |
| **Panicle length(cm)** | 24.75±0.93 | 23.91±1.06 | –3.39 ns | 17.91±0.62 | 17.65±0.62 | – 1.45 ns |
| **seeds/panicle** | 155.16±21.12 | 126.5±19.58 | –18.47* | 117.5±8.66 | 114±6.16 | –2.97 ns |
| **Filled grains/panicle** | 126±17.07 | 83±25.07 | –34.12** | 80.66±8.06 | 76±4.28 | –5.77 ns |
| **semi-filled grains/panicle** | 8.33±3.14 | 9.33±2.73 | +10.71 ns | 9.16±2.78 | 11.16±1.16 | +17.92* |
| **Unfilled grains/ panicle** | 20.83±7.05 | 34.16±7.35 | +39.02** | 23.33±5.20 | 26.16±3.97 | +10.81 ns |
| **Panicle weight with seed (g)** | 3.33±0.59 | 2.02±0.61 | – 39.33** | 3.04±0.31 | 2.80±0.19 | –7.89 ns |
| **1000 -grain weight (g)** | 28.69±3.24 | 24.16±2.62 | – 15.78* | 24.3±3.01 | 22.52±2.11 | –7.72 ns |
| **Yield per plant** | 26.33±4.5 | 10.19±3.7 | – 61.29*** | 26.49±4.2 | 21.6±3.1 | –18.45* |

## 4.2 *In silico* analysis of chromosome 11 of rice

### 4.2.1 Annotation of BAC/PAC sequences from chromosome 11

There were 255 BACs/PACs clones present on chromosome 11 of rice (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005). Chromosome 11 is 28.4 Mb in size and there were six physical gaps remain on chromosome 11 pseudomolecules. We have downloaded the sequences of 130 BACs/PACs clones one-by-one and gene prediction was carried out using Fgenesh software for protein-coding genes. Subsequently, BLASTP analysis was performed in order to know the function of each predicted genes. The genes which have shown homology with disease resistance gene were extracted and tabulated for further analysis. A total of 213 R-genes were identified out of 130 BAC clones with bit score ≥ 1000.0 and zero e-value (Annexure 3.1). The position of each R-gene was determined through BLASTN and a map was drawn to indicate the position of each R genes as well as to know the nature of distribution of R-genes (Fig 4.6). Most of the R genes were present in small and large clusters indicating the uneven distribution of R genes on chromosome 11 of rice. These R genes belonged to different categories of disease resistance genes such as NBS-LRR, LRR protein kinase, serine/threonine protein kinase, tyrosine protein kinase, NB-ARC domain containing protein etc. Most of the R-genes present on chromosome 11 pseudomolecules are NBS-LRR type which belongs to class three of disease resistance gene. There were 80 putative NBS-LRR type R-genes, 48 LRR-protein kinase, 33 protein kinase, 24 NB-ARC domain containing protein, 13 tyrosine protein kinase, 11 NBARC-NACHT and 3 serine/threonine protein kinase. There were no class 1 and class 4 resistance gene analogues present on chromosome 11 pseudomolecules. Four large clusters of R-genes (LRR-protein kinase + protein kinase + tyrosine protein kinase) were found between 80.5–85.7 cM (25 R-genes), 85.7–90.1cM (18 R-genes; NBS-LRR), 19 -19.8 cM (16 R genes; LRR-protein kinase + protein kinase) and 116.2-117cM (15 R genes; LRR-protein kinase + protein kinase). Several small clusters of R-genes were also found between positions 115.1–116.2 cM (8 R-genes; NBS-LRR), 80.2–80.5 cM (6 genes; NBS-LRR), 97.3-99.2 cM (6 R-genes; LRR-protein kinase) and 112.9–114.4 cM (6 R-genes; NBS-LRR).

### 4.2.2 Identification of protein sub-cellular localization using neural net program

In this study, sub-cellular localization has been predicted through neural net program (ProtCompV 8.0) using amino acid sequence. The amino acid sequence might carry a signal which locates the sub-cellular position of the protein within the cell. To make the protein databank easily accessible, proteins are classified into twelve groups (1) chloroplast (2) cytoplasm (3) cytoskeleton (4) endoplasmic reticulum (5) extracellular (6) golgi apparatus (7) lysosome (8) mitochondria (9) nucleus (10) peroxisome (11) plasma membrane and (12) vacuole (Chou and Elrod, 1999). The above classification has covered almost all the organelles and sub-cellular compartments. There were 75 R-genes which belonged to group 11 (PM), 65 belonged to group-1 (chloroplast), 25 R-genes group 9 (nucleus), 16 R-genes group 8 (mitochondria), 13 R-genes group 2 (cytoplasm), 6 R-genes group 6 (golgi apparatus) and 4 R-genes belonged to group 5 (extracellular). Out of 7 miscellaneous R-genes, 4 R-genes belonged to group 12 (vacuole), 1 belonged to group 10 (peroxisome) and 2 R-genes group 4 (ER). There were no R-genes which belonged to either group 3 (cytoskeleton) or group 7 (lysosome). We observed that most of the LRR-protein kinase genes were localized in the plasma membrane (77%) which indicates that LRR-protein might be involved in recognition of pathogen's elicitors, which is in support of the already established hypothesis of LRR protein function (Song et al. 1995). Most of the NBS-LRR R-genes were localized in chloroplast (42.5%) followed by nucleus (15%) (Fig 4.7). Tyrosine protein kinases, serine/threonine protein kinases and protein kinases were predominantly localized in plasma membrane. The sub-cellular localization of NB-ARC and NB-ARC-NACHT was very random (Annexure 3.1).

### 4.2.3 Determination of GC content and domains of resistance genes

The average GC content of the predicted R genes was 45.97%. The highest GC content was 49.37% in protein kinase among all classes of predicted R-genes (Fig 4.8A). The GC content of LRR-protein kinase, NBARC-NACHT and miscellaneous R-genes (β-lectin protein kinase, dirrigent protein kinase, TPR-LRR and PP2C protein kinase) was 46.8, 46.6 and 46.5% respectively. NBS-LRR and Tyrosine protein kinase gene contain 45.56 and 45.02 % GC content. The GC content of ser/thr protein kinase and NB-ARC gene was 44.8 and 43.15% which was in congruence with rice genome (43–44%).

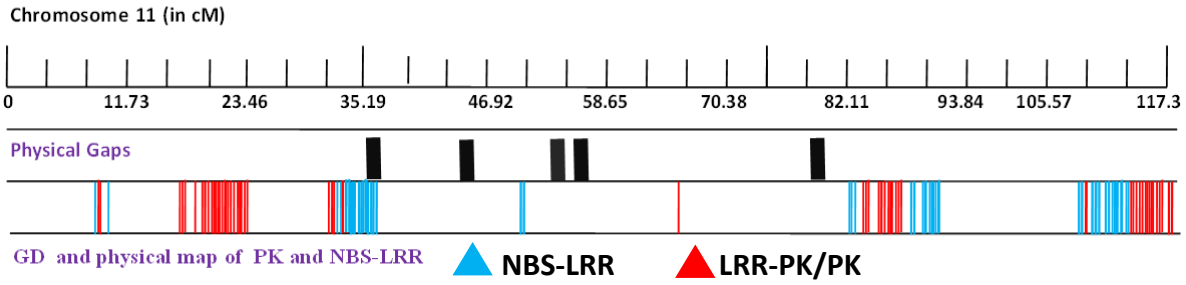



Fig 4.6 Display of NBS-LRR and LRR-PK/PK present on chromosome 11 of rice. Five physical gaps are also represented in black colour at their respective cM positions. Gene density and physical map are shown in red and light blue for LRR-PK/PK and NBS-LRR respectively.

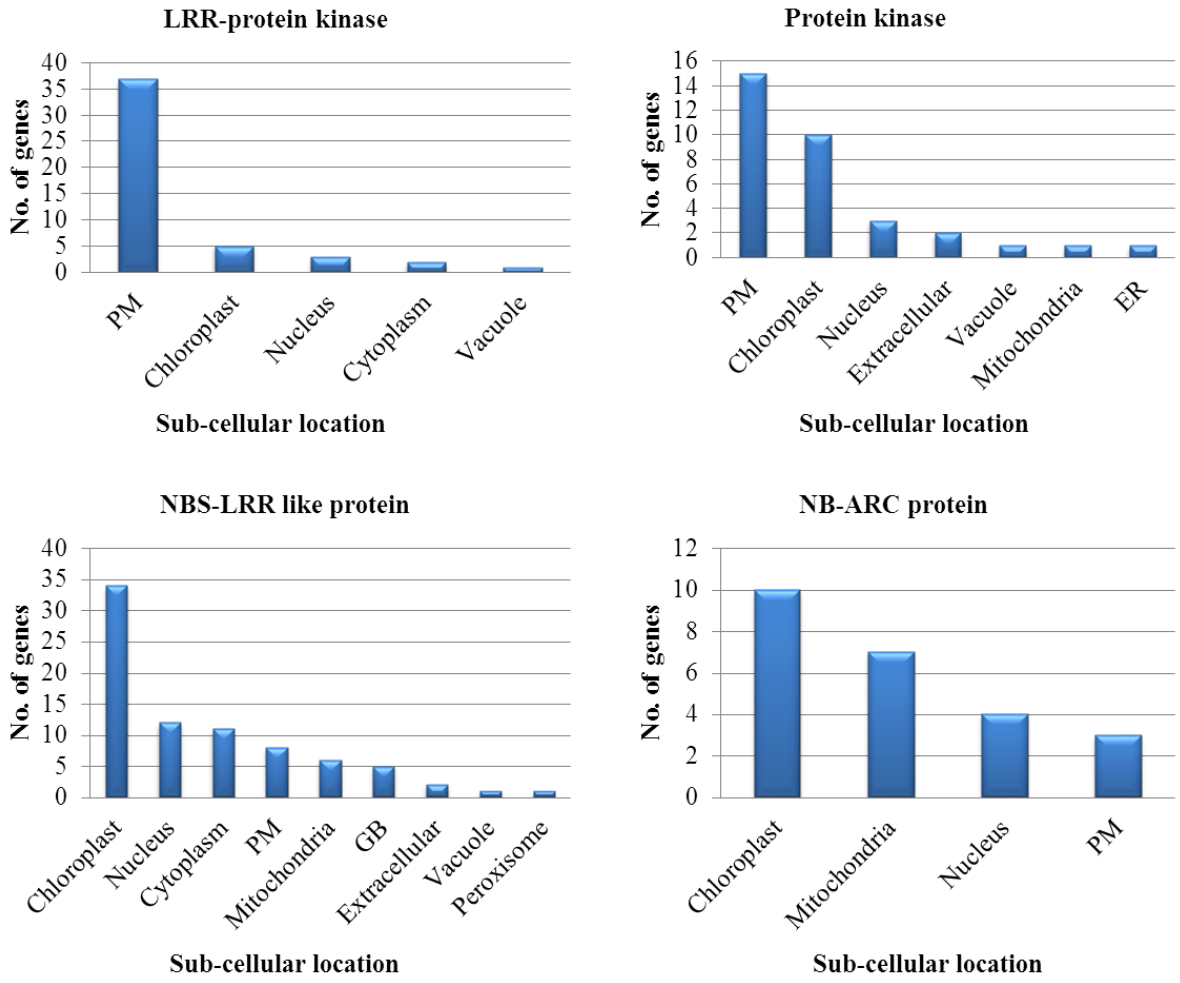



Fig 4.7 Determination of sub-cellular location of resistance genes through neural net program (ProtCompV 8.0) using amino acid sequences.

There were five genes (AC120307-21, AC123520-7, AC137753-19, AF161269-7 and AC122143-17) which contain more than 70% GC content, whereas, there were three genes which contain less than 35% GC content (AC119073-2, AC119073-5 and AC119670-16). The higher GC content in monocot genes in comparison to eudicot has been reported (Carels and Bernardi, 2000). The average GC content of *Oryza sativa* (monocot) was 43-44% whereas *Arabidopsis thaliana* (dicot) contain only 36% (Eguiarte et al. 2003; Saccone and Pesole, 2003). It has been shown that those gene copies which belong to subfamilies of very similar sequences (presumably undergoing gene conversion) have a higher GC content than unique gene copies (presumably not undergoing gene conversion) (Galtier, 2003). Interestingly, *Xa21* family members region was found to be GC rich and seemingly significant in influencing specific recombination in the domain (Dixon et al. 1998).

More than 100 genes controlling disease resistance have been cloned from different plant species (Liu et al. 2007). Most of which encode nucleotide-binding-site (NBS) and leucine-rich-repeat (LRR) domains. A large number of proteins contain multiple structural domains but the information about the structural domain is reliable only for proteins of known structure. Even for these the numbers of domains assigned by different methods and experts differed significantly. We have performed the domain prediction of only protein kinase and LRR protein kinase genes (class-5 disease resistance gene) and we found that most of the genes contained multiple domains. Single and two domains gene were also found. AC123897.17 showed maximum number of domains and LRR repeats whereas there were no domains predicted in gene AC114828-7. There were only 4 genes which were showing single conserve domains (AC104844-18, AC126057-15, AC133216-13 and AC122143-8). One gene (AC109365-12) contain salt stress response/antifungal conserve domain along with protein kinase catalytic domain. There was 5% class-5 resistance genes correctly assigned as single domain gene, 18% assigned as two-domain gene and 77% were assigned as three or multiple domain gene. We found that 25% of class-5 resistance genes have conserved protein kinase domain and P-loop NTPase domain each (Fig 4.8B).

### 4.2.4 Comparison of *Xa21* and *Xa26* with known R-genes using BL2SEQ algorithm

This *in silico* analysis was performed using BL2SEQ algorithm where query and subject sequence were amino acids. When *Xa21* gene was taken as subject sequence against all cloned disease resistance genes as query sequence, *Cf-2* was found to have maximum query coverage of 58% with 3e–89 e-value and 38% identity (Fig 4.9). In case of *L6*, the query coverage was only 10% but the identity was 42% with 0.12 e-value. The average query coverage and identity was 38.76% and 28.8% respectively. Whereas, *Cf-2* has shown maximum query coverage of 67%, identity 36% and e-value 2e–97, when *Xa26* gene was taken as subject sequence. *Lr21* has shown minimum query coverage of 17% with 30% identity and 0.27 e-value. The average query coverage was 38.52% and identity was 30.25%.

We have also studied the homology of *Xa21* and *Xa26* gene with the protein kinase and LRR-protein kinase (Class 5 R-genes). For this study, optimal query coverage and identity was ≥ 95% and ≥ 50% respectively with 0.0 e-value. There were only 12 R-genes which showed ≥ 50% homology with *Xa21* and 7 R-genes with *Xa26* (Fig 4.10) AC109644-2 (LRR-PK) was showing maximum homology with *Xa21* (QC=97%; identity=96%; e-value = 0.0) whereas AC146937-16 (PK) showed maximum homology with *Xa26* (QC=97%; identity=96%; e-value = 0.0).

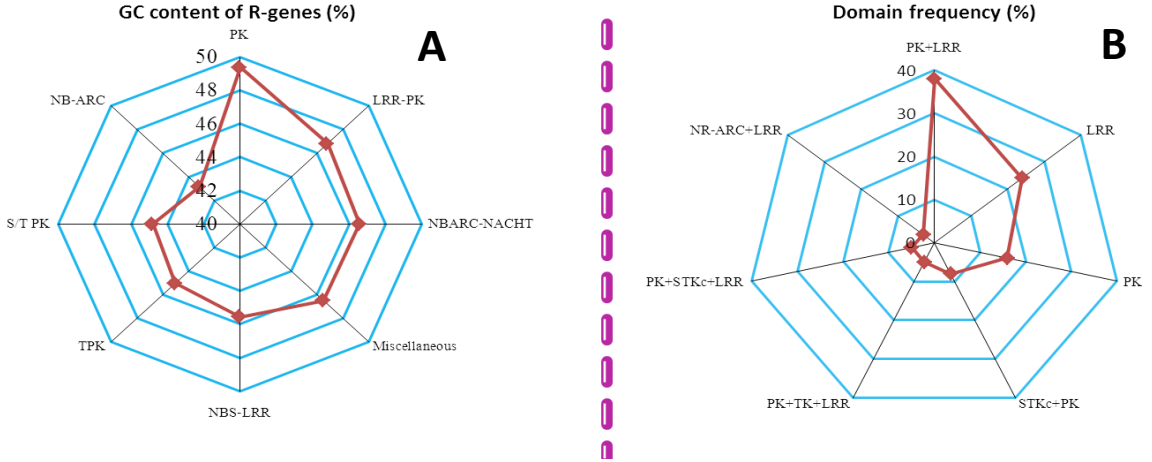



Fig 4.8 (A) Determination of GC content of all resistance genes through online tool of science buddies (Genomics %G~C Content Calculator) and (B) domain numbers (presented in terms of %) using amino acid sequence of receptor kinase like protein using NCBI conserved domain database.

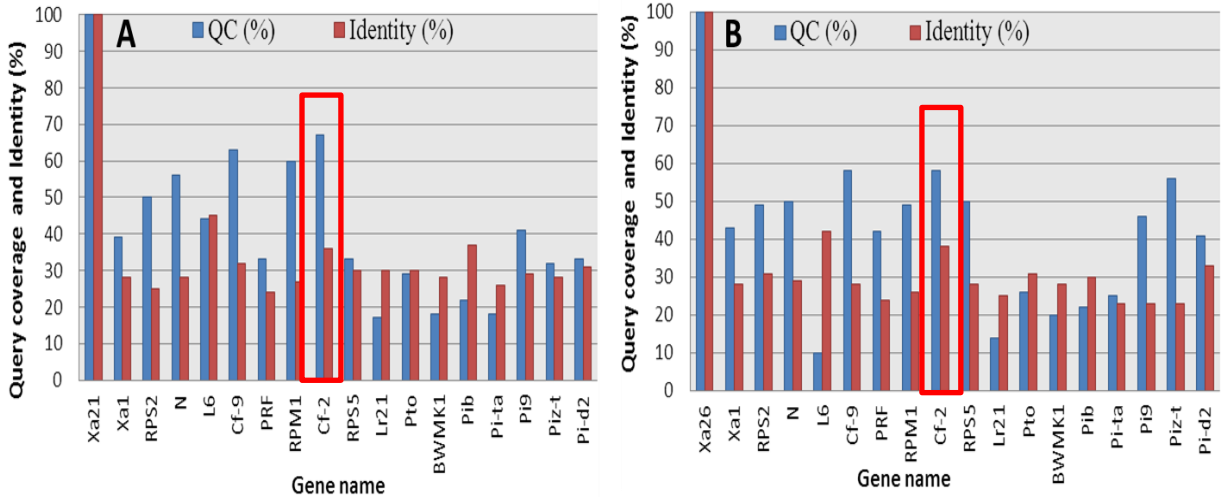



Fig 4.9 Comparative analysis of (A) *Xa21* and (B) *Xa26* with reported disease resistance gene of different plant origin. *Cf-2* was found to have maximum query coverage and identity with both *Xa21* and *Xa26*.

### 4.2.5 Synteny analysis of class-5 R-genes sequences to other plant genomes

The aim of this objective was to investigate the extent of conservation of genes in terms of sequence homology with other chromosomes of rice as well as with some monocots and dicots. Out of the 130 BAC/PAC clones analyzed in the present study, a total of 213 R-genes were predicted in the non-overlapping regions of the clones in which 81 were class-5 R-genes. Hit with query coverage ≥ 10% and sequence identity ≥ 65% were treated as significant. Identification of significant sequence homology is usually carried out using BLAST probability score and e-value but it does not distinguish between proteins showing similarity with total length of the protein or with only short conserved regions. Therefore, we decided to consider similarity criteria based on length (query coverage) and percent identity. Using our optimized cut-off-score, out of 81 predicted class 5 R-genes, 25.25% showed significant homology with chromosome 4, which was maximum among all chromosomes of rice whereas chromosome 8 and 9 showed minimum homology of 1.2%. There was no significant homology with chromosome 2. Chromosome 1 and 6 each showed equal homology of 4.93% with chromosome 11 similar to chromosome 8 and 9 which showed 1.2% significant homology (Fig 4.11A). Furthermore, homology search of 81 class-5 resistance genes was also executed with the EST database of wheat, maize, barley, sorghum, *Arabidopsis* and tomato. Overall, 81 of the predicted class 5 resistance genes (27.16%) showed significant homology with wheat ESTs which was highest among cereals under study followed by *Arabidopsis* (13.58%). There was no homology found between class-5 resistance genes and barley ESTs (Fig 4.11B). Among monocots and dicots, class-5 resistance genes showed minimum homology with tomato ESTs (2.46%). AC123515-5 and AC123515-10 has shown 100% homology with wheat ESTs and AC123897.15 with maize EST. The maximum homology in *Arabidopsis*, sorghum and tomato was 82% (AC136998-16), 75% (AC109832-13 and AC120527-2) and 71% (AC128643-10), respectively.

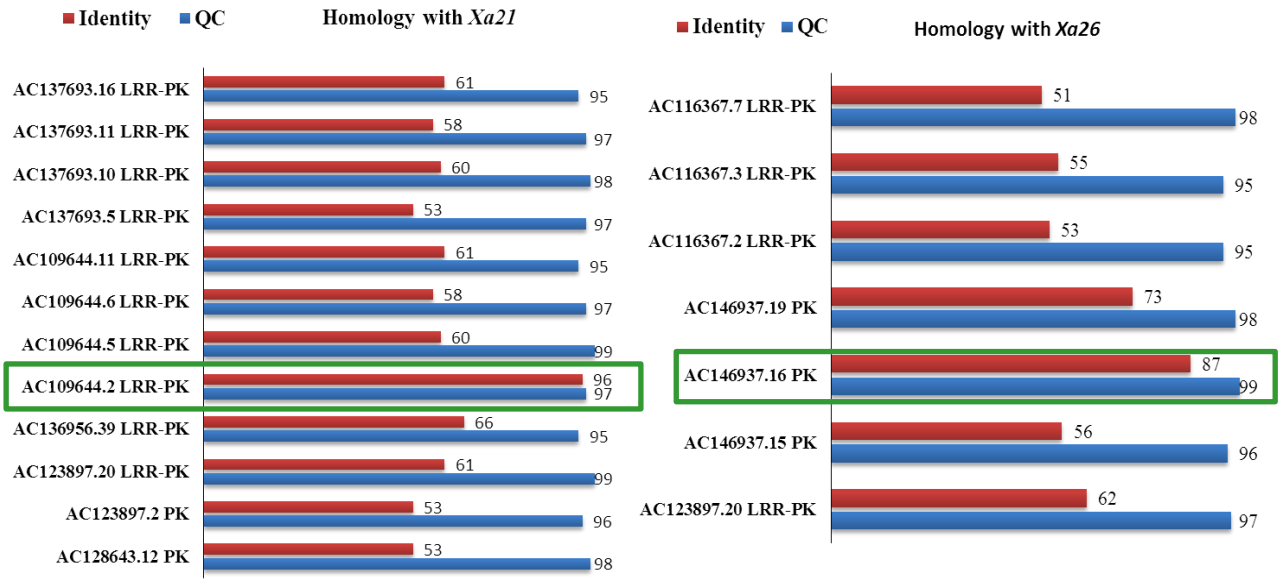



Fig 4.10 Homology study of *Xa21* and *Xa26* with predicted class-5 resistance genes. AC109644.2 (LRR-PK) showed maximum homology with *Xa21* whereas AC146937.16 (PK) showed maximum homology with *Xa26*.

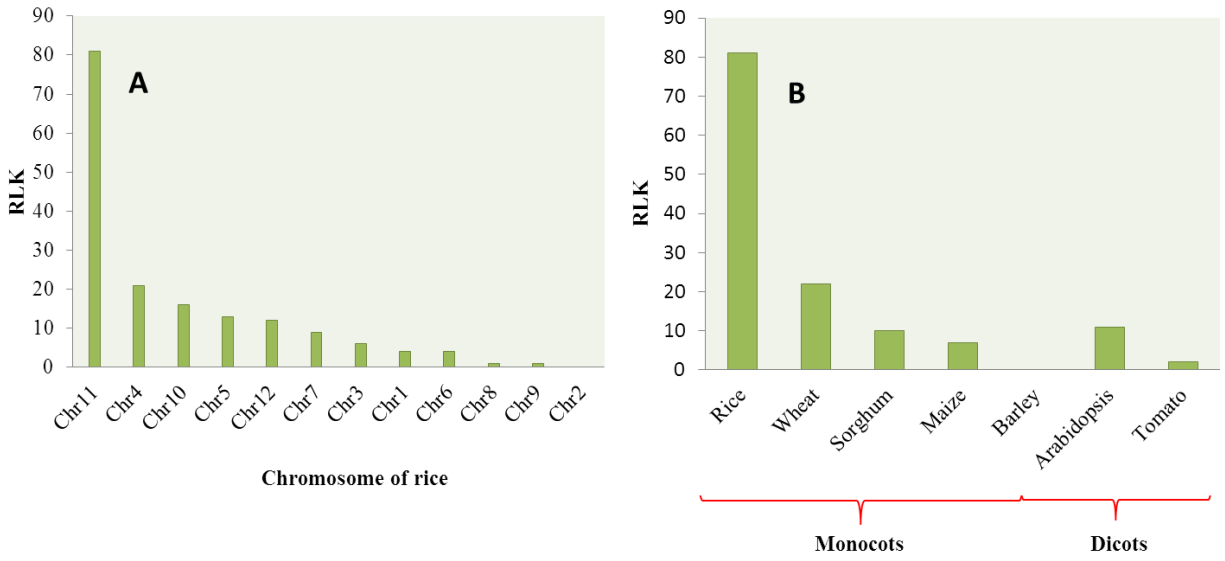



Fig 4.11 Synteny analysis of class-5 disease resistance genes (A) with other chromosomes of rice and (B) with EST database of wheat, maize, barley, sorghum, *Arabidopsis* and tomato.

## 4.3 Comparative analysis of *Xa21* locus in *indica and japonica* ssp. of rice

### 4.3.1 Gene prediction, annotation and classification

In the vicinity of the 100 kb region flanking *Xa21* locus, a total of 12 genes were predicted in *japonica* and 14 in *indica*. Equal numbers of genes were distributed both on the negative and the positive strand of both the subspecies. A *japonica* gene *Osjp12*, which coded for retrotransposon protein was observed to possess the longest coding sequence with a cDNA length of 4849 bp followed by *Osjp10* (4829 bp) and *Osjp08* (4536 bp). *Osjp12* had the maximum number of exons (11) in *japonica* (Table 4.3). In case of *indica*, *Osin09* possessed the longest cDNA length with 11,939 bp followed by *Osin04* (3108 bp) and *Osin14* (2932 bp). *Osin01* possessed the maximum number of exons (12) with a cDNA length of 2736 bp (Table 4.4). The gene density in *indica* was one gene per 7.85 kb while it was one gene per 8.62 kb in *japonica*. The annotation of the predicted genes revealed that *Osjp03*, *Osjp07* and *Osjp11* code for proteins possessing LRR domain while *Osjp08* code for LRR protein kinase. There were four genes namely *Osjp03*, *Osjp07*, *Osjp08* and *Osjp11* which could be putative disease resistance genes in *japonica* in the 100 kb region due to presence of leucine rich repeat domain, whereas there were seven such genes in *indica*. These genes were *Osin01*, *Osin03*, *Osin04*, *Osin05*, *Osin06*, *Osin08* and *Osin12*. An interesting observation was found in case of *Osin14*, which encodes for putative retrotrasposon but had leucine rich repeats in its conserved domain suggesting that a recombination event might have occurred in this region. The repetitive structure of LRR coding region favors recombination within this region (Ronald, 1998). *Osin01* possessed a conserved domain for ABC transporter, an ATP binding site and a p-loop. Hence, it may belong to class 3 families of R-genes. P-loop NTPase was found as a conserved domain in *Osin03*. So, this gene could be of NB-ARC NACHT (nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4 domain) type. *Osin06* and *Osin08* were observed to code for LRR protein kinase but interestingly, these two genes were found to be part of a single gene which had been separated by transposon related gene *Osin07*, probably, by the process of transposition. Overall there were six predicted genes coding for transposon protein each in *japonica* and *indica*. Sequence identity between *Xa21* gene (Os11g0559200) and *Osin08* was 100% while it was 99% between *Xa21* and *Osin06* (Fig.4.12).

### 4.3.2 GC content in the 100 kb region

The overall GC content of the 100 kb region was 42.63% in *indica* and 43.52% in *japonica*. When gene-by-gene comparison was carried out, seven genes of *indica* (excluding *Osin04, Osin 05, Osin06* and *Osin07*) had a higher GC content than that of japonica, whereas 4 genes of *japonica* i.e. *Osjp04, Osjp05, Osjp06* and *Osjp07* had slightly higher GC content in comparison to *indica* (Fig 4.13). Gene number 10 of both *indica* and *japonica* contain equal GC content (51%). The average GC content of *indica* was higher due to presence of two extra genes explicitly, viz., *Osin13* and *Osin14*.

### 4.3.3 Distribution and identification of SSRs in the 100 kb region of *Xa21* locus

SSR or simple sequence repeats are short stretches of tandem nucleotide repeats (Jarne and Lagoda, 1996). In our study, we have considered a minimum number of repeats as 7 for monomers (MNRs), 5 for dinucleotide repeats (DNRs), 4 for tri-nucleotide repeats (TNRs) and 3 for tetra-nucleotide repeats (TTRs), penta-nucleotide repeats (PNRs), hexa-nucleotide and hepta-nucleotide repeats (HNRs), respectively. SSRs were classified into class I and class II. Class I SSRs include the commonly used DNRs, TNRs and TTRs whereas MNRs, PNRs and HNRs were included in class II SSRs. During the sequence analysis of the 100 kb region of *Xa21* locus, we identified a total of 109 SSRs each in both *indica* and *japonica*. Monomeric repeats were highest in number in both *japonica* and *indica* followed by trimeric and dimeric repeats. Monomeric repeats constitute for 42.2% and 34.8% of the total SSRs identified in *indica* and *japonica*, respectively. The frequency of A/T/G/C repeats was almost similar in both the subspecies. However, when we checked the presence of monomeric repeats in the exonic region, we found that only one monomer i.e. 'G' repeat was present in the indica gene *Osin02*. 'A' repeat was found to be the most abundant among the four MNRs. The number of tetrameric repeats was 15 in *japonica* and 17 in *indica*, PNRs and HNRs were present in very less percentage while only one heptameric repeat was present in *japonica* and completely absent in *indica* (Fig 4.14). The frequencies of SSRs were more in *japonica* except the monomeric type. Analysis of different repeat motifs revealed that the AT/TA repeat motif was the most common one in *japonica* accounting for 13.76% of the SSRs identified (Table 4.5).

Table 4.3 Predicted genes of *japonica* in the 100 kb region of *Xa21* locus

| Gene | No. of exons | Start | End | cDNA length bp | strand | score | E-value | Function | GC content (%) |
|---|---|---|---|---|---|---|---|---|---|
| ***Osjp01*** | 5 | 2228 | 6029 | 832 | + | 42 | 0.78 | gatD; glutamyl-tRNA(Gln) amidotransferase subunit D Pfam: Asparaginase,PROSITE: ASN_GLN_ASE_ | 45.0 |
| ***Osjp02*** | 3 | 8983 | 10883 | 1005 | – | 44 | 0.24 | ypc00013; flavoprotein oxidoreductase protein Pfam: Flavin_Reduct, PROSITE:PROKAR_LIPOPROTEIN | 54.7 |
| ***Osjp03*** | 4 | 17196 | 24801 | 4088 | + | 5549 | 0.0 | Os11g0558900; hypothetical protein Pfam: LRRNT_2 LRR_1 | 43.3 |
| ***Osjp04*** | 5 | 32976 | 33970 | 631 | – | 1104 | 0.0 | Os10g0164800; hypothetical protein Pfam: p450 Peptidase_C48,PROSITE: SER_RICH ULP_PROTEASE | 45.45 |
| ***Osjp05*** | 2 | 34155 | 36673 | 2443 | – | 4559 | 0. 0 | Os07g0525000; hypothetical protein Pfam: Peptidase_C48 PROSITE: ARG_RICH PRO_RICH NLS_BP ULP_PROTEAS | 52.47 |
| ***Osjp06*** | 1 | 39431 | 42736 | 3305 | – | 5727 | 0.0 | Os07g0525900; hypothetical protein Pfam: Whi5 Transposase_21 Cps15 Peptidase_C48 PROSITE: PRO_RICH NLS_BP ULP_PROTEASE | 46.1 |
| ***Osjp07*** | 1 | 43751 | 46516 | 2765 | + | 587 | e-164 | Os11g0558900; hypothetical protein Pfam: LRRNT_2 LRR_1 | 42.4 |
| ***Osjp08*** | 6 | 50055 | 59214 | 4536 | + | 2159 | 0.0 | Os11g0569600; hypothetical protein Pfam: LRRNT_2 LRR_1 Myco_arth_vir_N Pkinase PROSITE: PROTEIN_KINASE_ST PROTEIN_KINASE | 46.5 |
| ***Osjp09*** | 6 | 69359 | 73391 | 2655 | + | 468 | e-128 | Os10g0157900; hypothetical protein Pfam: Whi5, PROSITE: IG_MHC | 43.3 |
| ***Osjp10*** | 4 | 76194 | 78607 | 4829 | + | 240 | 4e-60 | Os10g0345200; hypothetical protein | 51.0 |
| ***Osjp11*** | 4 | 84226 | 86528 | 1148 | – | 58 | 2e-05 | Os11g0558900; hypothetical protein Pfam: LRRNT_2 LRR_1 | 50.0 |
| ***Osjp12*** | 11 | 91960 | 102669 | 4849 | – | 1235 | 0.0 | Os11g0305400; hypothetical protein Pfam: Dimerisation Methyltransf_2 RVT_1 RnaseH PROSITE: ASN_RICH | 44.2 |

Table 4.4 Predicted genes of *indica* in the 100 kb region of *Xa21* locus

| Gene | No. of exons | Start | End | cDNA length | strand | Score bits | e-value | Functions | GC content % |
|---|---|---|---|---|---|---|---|---|---|
| ***Osin01*** | 12 | 1371 | 6560 | 2736 | - | 1929 | 0.0 | Os11g0416900; hypothetical protein<br>Pfam: ABC_tran MMR_HSR1 ABC2_membrane | 53.28 |
| ***Osin02*** | 1 | 7296 | 7922 | 626 | - | 234 | 8e-59 | Os04g0385600; hypothetical protein<br>Pfam: F-box Sel1 zf-MYND,PROSITE: ZF_MYND_1 ALA | 64.3 |
| ***Osin03*** | 1 | 19947 | 20870 | 923 | - | 64 | 2e-07 | Os10g0124300; hypothetical protein<br>Pfam: NB-ARC NACHT | 45.16 |
| ***Osin04*** | 3 | 34136 | 38214 | 3108 | + | 3872 | 0.0 | Os11g0558900; hypothetical protein<br>Pfam: LRRNT_2 LRR_1 | 42.8 |
| ***Osin05*** | 2 | 43747 | 46567 | 2758 | + | 504 | e-139 | Os11g0565000; hypothetical protein<br>Pfam: LRRNT_2 LRR_1, PROSITE: PROKAR LIPOPROTEIN | 42.76 |
| ***Osin06*** | 2 | 50055 | 52531 | 2026 | + | 831 | 0.0 | Os11g0569600; hypothetical protein<br>Pfam: LRRNT_2 LRR_1 Myco_arth_vir_N Pkinase Pkinase | 44.72 |
| ***Osin07*** | 1 | 55447 | 56688 | 1241 | - | 270 | 3e-69 | Os11g0305400; hypothetical protein<br>Pfam: Dimerisation Methyltransf_2 RVT_1 RnaseH zf-H2C2<br>PROSITE: ASN_RICH | 41.9 |
| ***Osin08*** | 3 | 58647 | 60499 | 747 | + | 581 | e-163 | Os11g0569600; hypothetical protein<br>Pfam: LRRNT_2 LRR_1 Myco_N Pkinase Pkinase | 53.59 |
| ***Osin09*** | 5 | 79452 | 83481 | 11939 | + | 492 | e-136 | Os10g0157900; hypothetical protein<br>Pfam: Whi5, PROSITE: IG_MHC | 45.18 |
| ***Osin10*** | 3 | 86330 | 87328 | 792 | + | 226 | 2e-56 | Os10g0345200; hypothetical protein | 50.91 |
| ***Osin11*** | 4 | 87422 | 89749 | 986 | + | 58 | 2e-05 | Os10g0157900; hypothetical protein<br>Pfam: Whi5, PROSITE: IG_MHC | 50.87 |
| ***Osin12*** | 6 | 95391 | 98017 | 1317 | - | 58 | 2e-05 | Os11g0558900; hypothetical protein<br>Pfam: LRRNT_2 LRR_1 | 50.47 |
| ***Osin13*** | 3 | 103413 | 104882 | 1086 | - | 90 | 5e-15 | Os09g0441600; hypothetical protein<br>Pfam: p450 Class_IIIsignal<br>PROSITE: CYTOCHROME_P450 GLY_RICH | 38.7 |
| ***Osin14*** | 5 | 106326 | 110450 | 2932 | - | 1697 | 0.0 | Pfam: Dimerisation Methyltransf_2 RVT_1 RnaseH zf-H2C2<br>PROSITE: ASN_RICH | 45.11 |


Fig 4.12 Alignment position of *Osin06* and *Osin08* with resistant (reference) *Xa21* allele. Position of *Osin07* indicates its insertion between *Osin06* and *Osin08* at *Xa21* locus in the long arm of chromosome 11 of *Oryza sativa* ssp. *indica*.

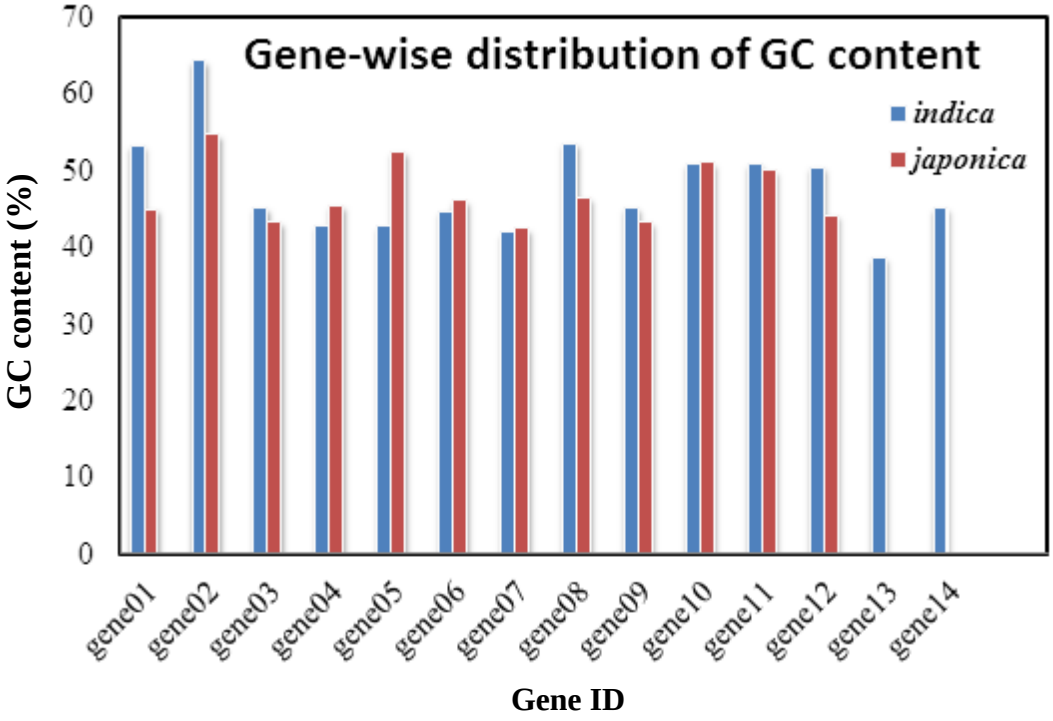



Fig 4.13 Gene-wise distribution of GC content in *japonica* and *indica* covering 100 kb region of *Xa21* locus. *Indica* and *japonica* genes are represented by blue and red colored bars, respectively. X-axis represents the predicted genes in order and Y-axis represents the percentage of GC content. *Gene01* represents gene no. 1 of *indica* and *japonica* (*Osin01* and *Osjp01*, respectively) and *gene02* represents gene no. 2 of *indica* and *japonica* (*Osin02* and *Osjp02*, respectively) and so on.

On the contrary AT/TA and AG/GA/CT/TC repeat motifs were equally present in *indica* representing 6.4% each of the total SSRs identified (Table 4.6). The abundance of AT repeat motif in different plant genomes followed by AG/CT and GT/CA has been shown by previous studies (Condit et al. 1991; Lagercrantz et al. 1993; Morgante and Olivieri, 1993; Wang et al. 1994; Powell et al. 1996). The same trend was observed in both the rice subspecies. However for trimeric repeats, GC rich motifs GGC/CCG/GCG/CGC showed the highest frequency of 17 and 21 in *japonica* and *indica*, respectively. GC rich trimeric repeats accounted for 15.5% in *japonica* and 19.26% in *indica*. In legumes such as soybean and chickpea, ATT repeat motif was abundantly found (Akkaya et al. 1992; Huttel et al. 1999), however, ATT repeat motif was completely absent in the 100 kb region of *Xa21* locus in both *indica* and *japonica*. Microsatellite studies on *Arachis hypogea* also showed less frequency of ATT repeat motifs (Cuc et al. 2008). The number of repeat motif for monomeric repeats ranged from 7–13, dimeric repeats 5–32 and for trimeric repeats, it was 4–6 in *japonica*. In *indica*, the number of repeat motif was comparatively less ranging from 7–17 for monomers, 5–14 for dimers and 4–8 for trimers. A total of seven trimeric repeats were identified each from *indica* and *japonica* and one monomeric repeat from *indica* in the coding regions of the predicted genes. Other types of repeats included in our study were completely absent in the genic regions. The percentage of SSRs identified in the genic region was comparatively less than that of the intergenic region. The percentage frequency of SSRs present in the exonic region was only 7.33% and 6.42% of the SSRs identified, respectively in *indica* and *japonica*.

### 4.3.4 Microsynteny analysis for physical mapping of predicted genes

As a part of the comparative genomic study, microsynteny analysis between *japonica* and *indica* subspecies for the predicted genes of *Xa21* locus was carried out against the available database for chromosome 11 of *japonica* and *indica* in NCBI. The predicted genes were grouped according to their function. Microsynteny analysis showed 100% homology for eleven genes and 99% homology for only one gene (i.e. *Osjp12*) with chromosome 11 database of *japonica* (Table 4.7). In case of *indica*, three genes namely *Osin04*, *Osin09* and *Osin14* showed 99% homology and the remaining eleven predicted genes showed 100% homology with chromosome 11 database of *indica* (Table 4.8). Based on the above study,

physical maps of the two rice subspecies for the 100 kb region were generated which covered all of the predicted genes. From gene prediction analysis, we have identified those genes, *Osjp08* and *Osin06* of *japonica* and *indica*, respectively codes for protein receptor kinase. Alignment of these two predicted genes with the reference *Xa21* sequence also showed 100% identity. In both the rice subspecies, *Xa21* was flanked by LRR-encoding gene in the upstream and transposon related gene in the downstream region. In *japonica*, six predicted genes were located in the sense strand and remaining genes were in the antisense strand. Even in *indica*, equal numbers of the predicted genes (7) were present in sense and antisense strand (Fig 4.15).

### 4.3.5 Sub-cellular localization of predicted gene products

The predicted gene products were classified as per Chou and Elrod (1999). In *indica*, gene *Osin09* belongs to group 2; *Osin01*, *Osin12* and *Osin14* belong to group 5; *Osin03*, *Osin10* and *Osin13* belong to group 8; *Osin02*, *Osin07*, *Osin08* and *Osin11* belong to group 9; *Osin04*, *Osin05* and *Osin06* belong to group 11 which has been predicted through neural net. For *japonica*, *Osjp01* belongs to group1, *Osjp02* and *Osjp04* belong to group 2, *Osjp11* belong to group 5, *Osjp05*, *Osjp06*, *Osjp09*, *Osjp10* and *Osjp12* belong to group 9, then *Osjp03*, *Osjp07* and *Osjp08* belong to group 11 (Table 4.9). We observed that most of the LRR coding gene products were localized in the plasma membrane and the transposon proteins in the nucleus.

### 4.3.6 Phylogenetic analysis

A phylogenetic tree was constructed for the predicted genes of *indica* and *japonica* to illustrate the relationship of the genes (Fig 4.16). All the 12 genes of *japonica* and 14 genes of *indica* were observed to fall into two large and two small clusters (Cluster I, II, III and IV). Cluster I was the largest one containing 10 genes. Out of 10 genes, 7 were encoding for LRR proteins and 3 for transposons related proteins. In cluster II, all the 7 genes were transposons related. Cluster III held 3 genes and it included 2 hypothetical proteins and one NB-ARC protein whereas cluster IV was composed of 4 genes in which 3 were encoding for kinases and one was encoding for NB-ARC.

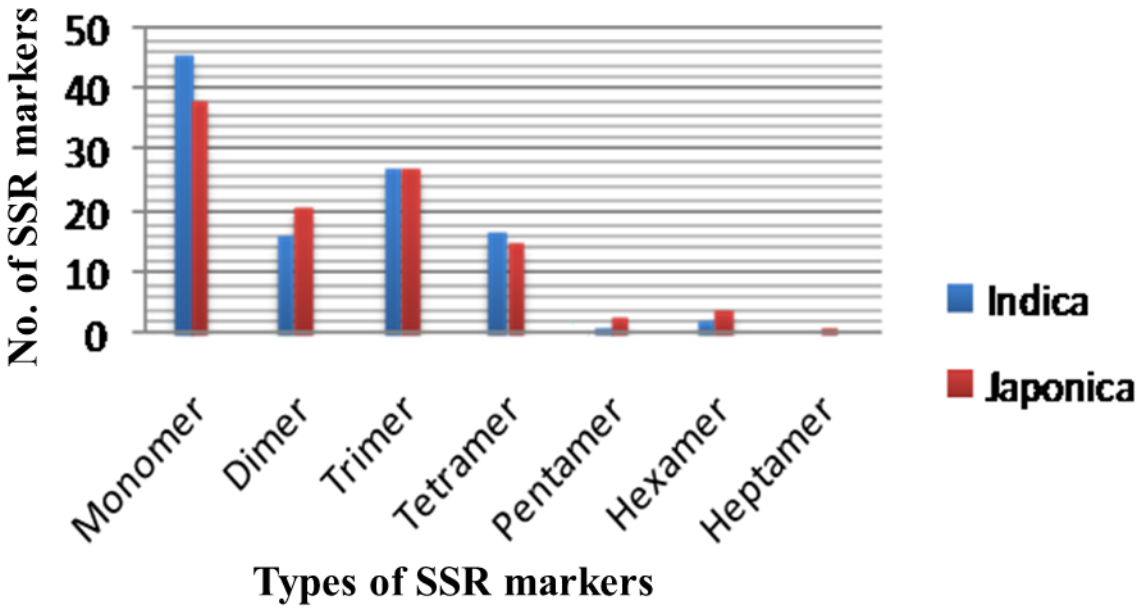



Fig 4.14 Distribution of different types of SSR markers in the 100 kb region of *Xa21* locus. FastPCR was used to identify monomers. SSRIT available at gramene database was used to find out other types of SSR markers present in the 100 kb region.

Table 4.5 Frequency of dimeric and trimeric repeats or SSRs in the 100kb of *Xa21* locus (*japonica*)

| Motif | Frequency | % Frequency |
|---|---|---|
| AT/TA | 15 | 13.76 |
| GC/CG | 0 | 0.0 |
| AG/GA/CT/TC | 5 | 4.5 |
| AC/CA/TG/GT | 1 | 0.917 |
| AAT/ATA/TAA/ATT/TTA/TAT | 0 | 0.0 |
| AAG/AGA/GAA/CTT/TTC/TCT | 1 | 0.917 |
| AAC/ACA/CAA/GTT/TTG/TGT | 1 | 0.917 |
| ATG/TGA/GAT/CAT/ATC/TCA | 1 | 0.917 |
| AGG/GGA/GAG/CCT/CTC/TCC | 3 | 2.75 |
| AGC/GCA/CAG/GCT/CTG/TGC | 0 | 0.0 |
| ACG/CGA/GAC/CGT/GTC/TCG | 3 | 2.75 |
| ACC/CCA/CAC/GGT/GTG/TGG | 1 | 0.917 |
| GGC/GCG/CGG/GCC/CCG/CGC | 17 | 15.5 |

Table 4.6 Frequency of dimeric and trimeric repeats or SSRs in the 100kb of *Xa21* locus (*indica*)

| Motif | Frequency | % Frequency |
|---|---|---|
| AT/TA | 7 | 6.4 |
| GC/CG | 2 | 1.83 |
| AG/GA/CT/TC | 7 | 6.4 |
| AC/CA/TG/GT | 0 | 0.0 |
| AAT/ATA/TAA/ATT/TTA/TAT | 0 | 0.0 |
| AAG/AGA/GAA/CTT/TTC/TCT | 0 | 0.0 |
| AAC/ACA/CAA/GTT/TTG/TGT | 0 | 0.0 |
| ATG/TGA/GAT/CAT/ATC/TCA | 1 | 0.917 |
| AGG/GGA/GAG/CCT/CTC/TCC | 2 | 1.83 |
| AGC/GCA/CAG/GCT/CTG/TGC | 0 | 0.0 |
| ACG/CGA/GAC/CGT/GTC/TCG | 1 | 0.917 |
| ACC/CCA/CAC/GGT/GTG/TGG | 2 | 1.83 |
| GGC/GCG/CGG/GCC/CCG/CGC | 21 | 19.26 |

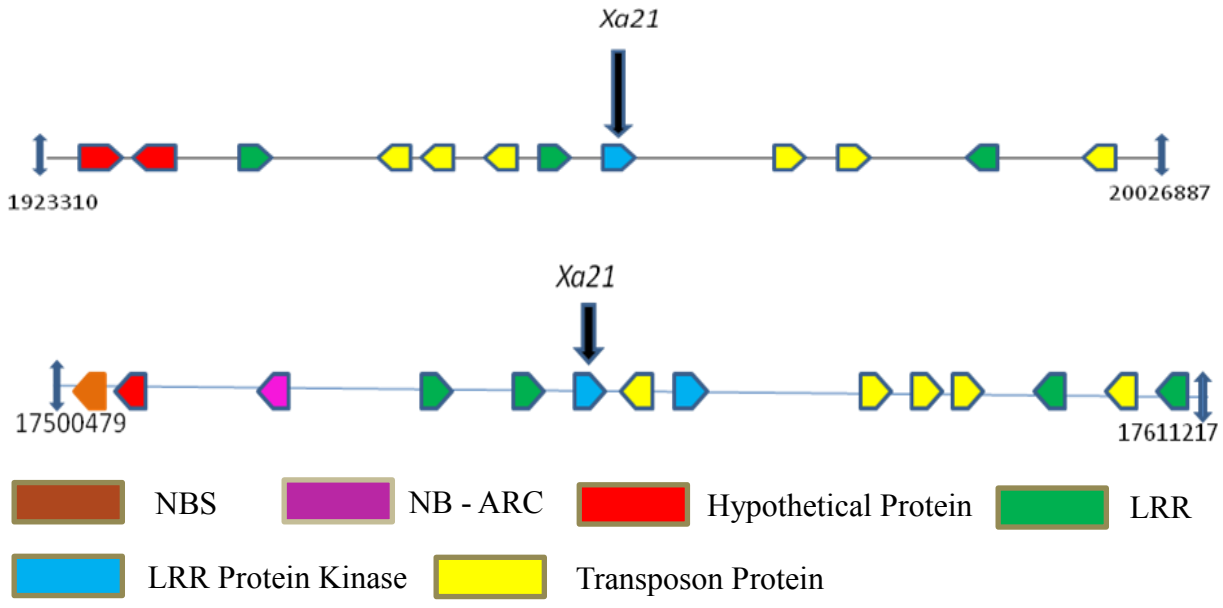



Fig 4.15 Physical map of *japonica* and *indica* predicted genes in the 100 kb regions of *Xa21* locus. Vertical arrow indicates the position of *Xa21* allele in *japonica* and *indica*. Position of arrow heads indicates the direction of the predicted genes.

Table 4.7 Microsynteny analysis of the predicted genes in the 100 kb region of *Xa21* locus of *japonica* cultivar group

| Gene ID | Blast hit | Bit score | E-value | Homology | Start (bp) | End (bp) |
|---|---|---|---|---|---|---|
| ***Osjp01*** | Chr 11_japonica | 499 | 1e-139 | 270/270 (100%) | 19925537 | 19929338 |
| ***Osjp02*** | Chr 11_japonica | 1146 | 0.0 | 620/620 (100%) | 19934192 | 19932292 |
| ***Osjp03*** | Chr 11_japonica | 5169 | 0.0 | 2799/2799 (100%) | 19940553 | 19943312 |
| ***Osjp04*** | Chr 11_japonica | 344 | 4e-93 | 186/186 (100%) | 19956470 | 19956285 |
| ***Osjp05*** | Chr 11_ japonica | 2630 | 0.0 | 1424/1424 (100%) | 19959982 | 19958559 |
| ***Osjp06*** | Chr 11_ japonica | 6080 | 0.0 | 3306/3306 (100%) | 19966045 | 19962740 |
| ***Osjp07*** | Chr 11_ japonica | 5083 | 0.0 | 2766/2766 (100%) | 19967060 | 19969825 |
| ***Osjp08*** | Chr 11_ japonica | 4839 | 0.0 | 2620/2620 (100%) | 19973364 | 19975983 |
| ***Osjp09*** | Chr 11_ japonica | 1971 | 0.0 | 1067/1067 (100%) | 19995634 | 19996700 |
| ***Osjp10*** | Chr 11_ japonica | 1561 | 0.0 | 845/845 (100%) | 20000828 | 20001672 |
| ***Osjp11*** | Chr 11_ japonica | 1138 | 0.0 | 652/652 (100%) | 20008369 | 20007759 |
| ***Osjp12*** | Chr 11_ japonica | 2902 | 0.0 | 1574/1575(99%) | 20021203 | 20019630 |

Table 4.8 Microsynteny analysis of the predicted genes in the 100 kb region of *Xa21* locus of *indica* cultivar group

| Gene ID | Blast hit | Bit score | E-value | Homology | Start (bp) | End (bp) |
|---|---|---|---|---|---|---|
| ***Osin01*** | Chr 11_indica | 1781 | 0.0 | 964/964 (100%) | 17502813 | 17501850 |
| ***Osin02*** | Chr 11_indica | 1158 | 0.0 | 627/627 (100%) | 17508401 | 17507775 |
| ***Osin03*** | Chr 11_indica | 1707 | 0.0 | 924/924 (100%) | 17521349 | 17520426 |
| ***Osin04*** | Chr 11_indica | 3683 | 0.0 | 1996/1997 (99%) | 17535468 | 17537464 |
| ***Osin05*** | Chr 11_indica | 3847 | 0.0 | 2083/2083 (100%) | 17544226 | 17546308 |
| ***Osin06*** | Chr 11_indica | 3057 | 0.0 | 1655/1655 (100%) | 17550534 | 17552188 |
| ***Osin07*** | Chr 11_indica | 2294 | 0.0 | 1242/1242 (100%) | 17557167 | 17555926 |
| ***Osin08*** | Chr 11_indica | 702 | 0.0 | 380/380 (100%) | 17560599 | 17560978 |
| ***Osin09*** | Chr 11_indica | 2084 | 0.0 | 1130/1131 (99%) | 17582830 | 17583960 |
| ***Osin10*** | Chr 11_indica | 704 | 0.0 | 381/381 (100%) | 17587158 | 17587538 |
| ***Osin11*** | Chr 11_indica | 1234 | 0.0 | 668/668 (100%) | 17588269 | 17588936 |
| ***Osin12*** | Chr 11_indica | 848 | 0.0 | 459/459 (100 %) | 17596552 | 17596194 |
| ***Osin13*** | Chr 11_indica | 887 | 0.0 | 480/480 (100%) | 17604371 | 17603892 |
| ***Osin14*** | Chr 11_indica | 2809 | 0.0 | 1524/1525 (99%) | 17608748 | 17607225 |

Table 4. 9 Subcellular localization of *indic*a and *japonica* genes in the 100 kb region of *Xa21* locus

| Gene | Neural net prediction | | Gene | Neural net prediction ▲ | |
|---|---|---|---|---|---|
| | Location | Score | | Location | Score |
| ***Osjp01*** | Chloroplast | 2.4 | *Osin01* | Extracellular (secreted) | 1.6 |
| ***Osjp02*** | Cytoplasmic | 1.9 | *Osin02* | Nuclear | 2.5 |
| ***Osjp03*** | Plasma membrane | 2.9 | *Osin03* | Mitochondrial | 2.2 |
| ***Osjp04*** | Cytoplasmic | 1.8 | *Osin04* | Plasma membrane | 3.0 |
| ***Osjp05*** | Nuclear | 3.0 | *Osin05* | Plasma membrane | 2.9 |
| ***Osjp06*** | Nuclear | 2.7 | *Osin06* | Plasma membrane | 2.3 |
| ***Osjp07*** | Plasma membrane | 2.7 | *Osin07* | Nuclear | 2.1 |
| ***Osjp08*** | Plasma membrane | 3.0 | *Osin08* | Nuclear | 2.9 |
| ***Osjp09*** | Nuclear | 2.2 | *Osin09* | Cytoplasmic | 3.0 |
| ***Osjp10*** | Nuclear | 2.7 | *Osin10* | Mitochondrial | 1.6 |
| ***Osjp11*** | Extracellular (secreted) | 3.0 | *Osin11* | Nuclear | 2.9 |
| ***Osjp12*** | Nuclear | 1.7 | *Osin12* | Extracellular | 3.0 |
| | | | *Osin13* | Mitochondrial | 1.3 |
| | | | *Osin14* | Extracellular (secreted) | 3.0 |

Footnote: ▲ Neural net is a program combined with ProtComp V software which predicts the localization of a particular protein in the cell.

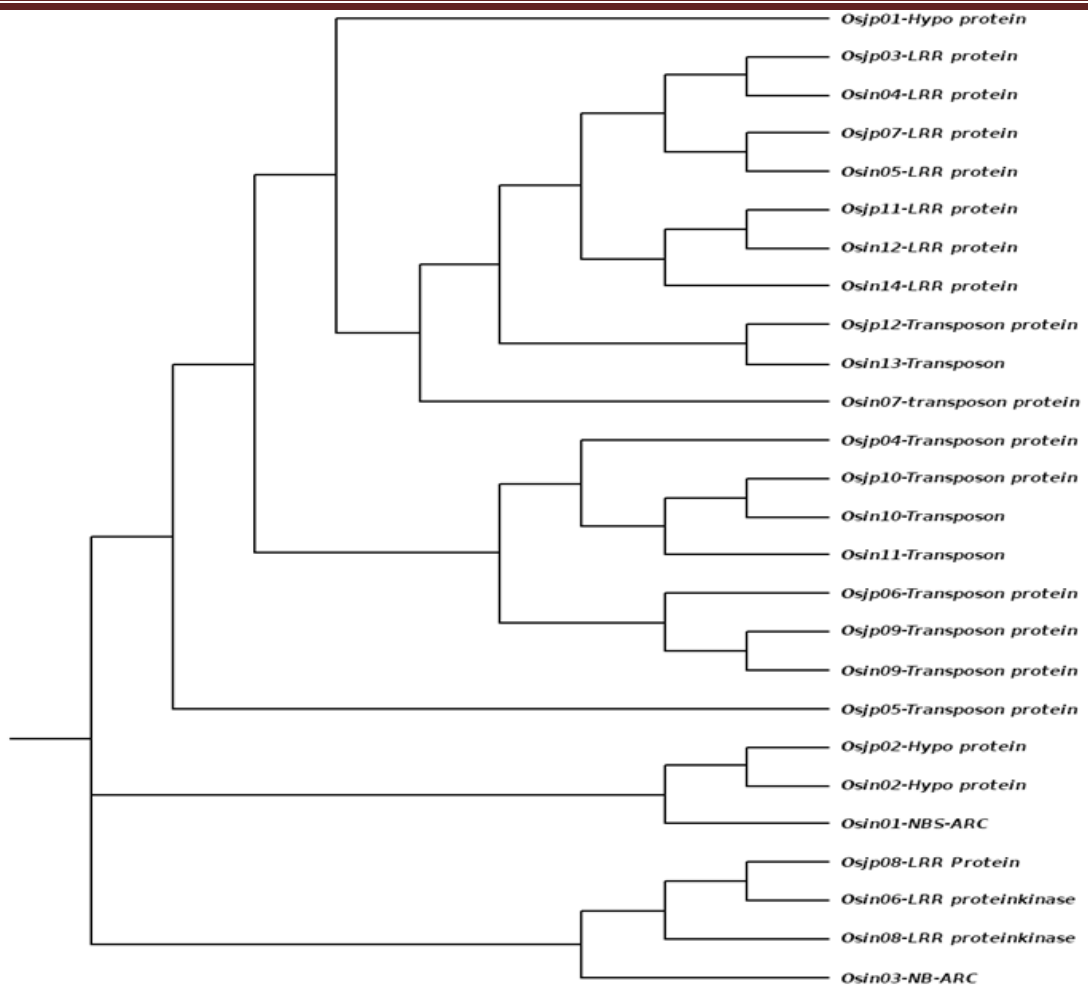



Fig 4.16 Phylogenetic analysis of the predicted genes of *japonica* and *indica* subspecies. All the genes of *japonica* and *indica* were clustered into two large (I and II) and two small (III and IV) clusters. Cluster I is the largest one with 10 genes followed by cluster II with 7 genes. Cluster III and IV held 3 and 4 genes, respectively.

## 4.4 Cloning and sequencing of receptor kinases

Out of 14 screened rice genotypes, eight were selected for polymorphism analysis which includes three NILs (IRBB3, IRBB4, IRBB21), three wild rice (*O. longistaminata*, *O. rufipogon*, *O. nivara*) and two cultivars (PB1, TN1).

### 4.4.1 PCR amplification of resistance genes and polymorphism analysis

Twenty five pairs of gene specific primers were used which were designed from exonic regions of class-5 disease resistance genes. These primers (Annexure 3.4) were used for PCR amplification of R-genes using genomic DNA of eight selected rice genotypes. Selection of rice genotype was done based on their contrasting behaviour to *Xoo* infection. Out of 25 exons based primers, only 21 gave amplification product in which 17 produced monomorphic and rest 4 furnished polymorphic amplicons. Primer IAG-6 successfully produced polymorphic amplicon (Fig 4.17A) in IRBB3 (Lane-1), IRBB4 (Lane-2), IRBB21 (Lane-3) and *O. longistaminata* (Lane-4), but the polymorphic amplicons of IRBB3, IRBB4 and IRBB21 were very faint. Therefore, we selected the polymorphic amplicon of *O. longistaminata* which was cloned into *pTZ57R/T* vector and transformed into *E. coli DH5α*. The cloned amplicon was confirmed through PCR and restriction digestion before sequencing (Fig 4.17B and 4.17C). The sequence of polymorphic amplicon is mentioned in Fig 4.17D. The product size of polymorphic amplicon was 466 bp. *O. rufipogon* (Lane-5) and *O. nivara* (Lane-6) did not produce amplification with IAG-6.

In case of primer IAG-7, *O. nivara* (Lane-5) has not produced any amplification whereas rest all selected genotype have shown amplification (Fig 4.18A). *O. longistaminata* (Lane-6) has shown a prominent polymorphic band of size ~252 bp. IRBB3 (Lane -1) has also produced single prominent band but that was common to IRBB4 (Lane-2), IRBB21 (Lane-3), *O. rufipogon* (Lane-4), Pusa Basmati 1 (Lane-7), and Taichung Native 1 (Lane-8). *O. rufipogon* (Lane-4) has produced 2 faint bands when compared to other genotypes. Therefore, IBBB3 amplification product was not considered for cloning and sequencing. The only conspicuous polymorphic band of *O. longistaminata* (Lane-6) was cloned and confirmed through PCR & restriction digestion (Fig 4.18B and Fig 4.18C). The restriction digestion was performed using *Eco*RI and *Bam*HI. The sequence length of this polymorphic amplicon was 252 bp (Fig 4.18D) and sequencing was done by Vimta Labs.

From primer no. IAG-8 to IAG-15, polymorphic amplification was not detected in any genotype under study. The polymorphic amplification was observed in *O. rufipogon* and *O. nivara* with IAG-16. All the genotypes have produced single band of around 400 bp (Fig 4.19A) except *O. rufipogon* (Lane-5) and *O. nivara* (Lane-6) which have produced the polymorphic band of 316 bp and 427 bp respectively. Both polymorphic bands were recovered from the agarose gel using Sigma Aldrich miniprep kit, cloned and sequenced after confirmation through PCR and restriction digestion (Fig 4.19B and 4.19C). The sequencing products of polymorphic amplicons are mentioned in Fig 4.19D and 4.19E.

PCR amplification with primer IAG-20 has produced polymorphic band (Fig 4.20A) in IRBB3 (Lane-1) and *O. rufipogon* (Lane-5). Rest all genotypes did not produce any amplification. The polymorphic fragments were gel-purified and used for PCR analysis (Fig 4.20B) and restriction digestion (Fig 4.20C) using 6-bp-recognizing restriction enzymes (*Eco*RI and *Bam*HI). Both of the polymorphic bands were sequenced and result is mentioned in Fig 4.20D and Fig 4.20E. All the sequences obtained from polymorphism analysis were finally submitted to NCBI database: (http://www.ncbi.nlm.nih.gov/BankIt/). Most of the polymorphic amplicons were obtained from wild rice and the amplicon range was in between 250-500 bp. The only one primer (IAG-20) has produced polymorphic amplification in IRBB3 near isogenic line. Bacterial Blight susceptible genotypes Pusa Basmati 1 and Taichung Native 1 have not displayed any polymorphism with any primers under study.

All the six major polymorphic amplicons were produced by four pairs of primers (IAG-6, IAG-7, IAG-16 and IAG-20). All polymorphic amplicons were gel eluted and purified using GenElute$^{TM}$ gel extraction kit (Sigma Aldrich, USA) and were cloned into plasmid vector *pTZ57R/T* (Insta clone T/A cloning kit, Fermentas, Germany). Functional classification revealed that there were two R genes encoding LRR-protein kinase (with IAG-6 and IAG-7), one collagen protein kinase (with IAG-16), protein kinase (with IAG-16), NB-ARC domain containing protein (with IAG-20) and one rust resistance Yr10 (with IAG-20). All the R genes have shown more than 80% similarity with the available database. Finally these R gene sequences were submitted to NCBI database: (http://www.ncbi.nlm. nih.gov/BankIt/). Accession no., sequence length and putative function of polymorphic R genes are given in Table 4.10

The expected size of the product was 500 bp but the polymorphic band was 466 bp with IAG-6 primer, which sequence identity of 90% with clone OSJNBb0035N09.

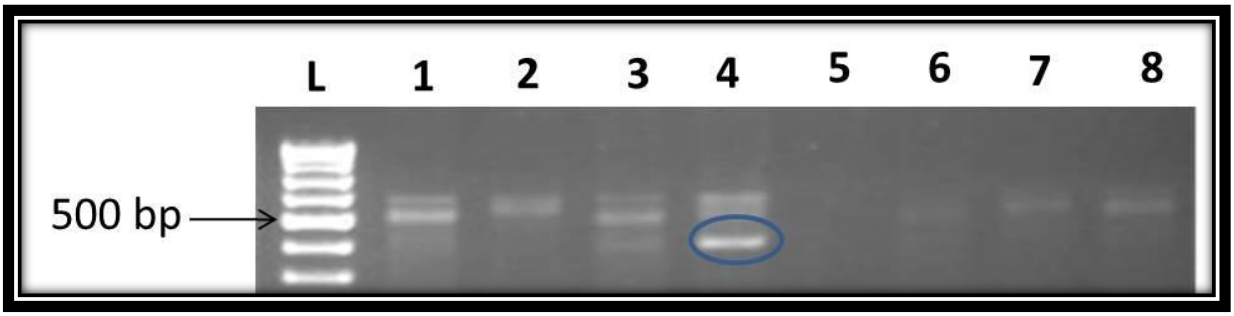
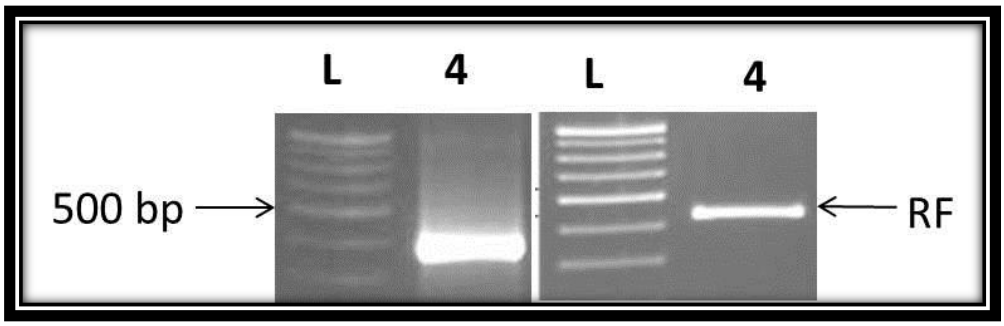



Fig 4.17(A)

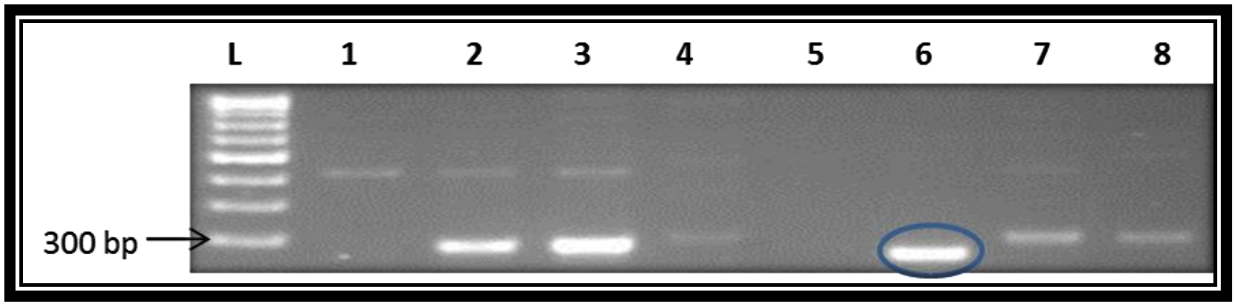


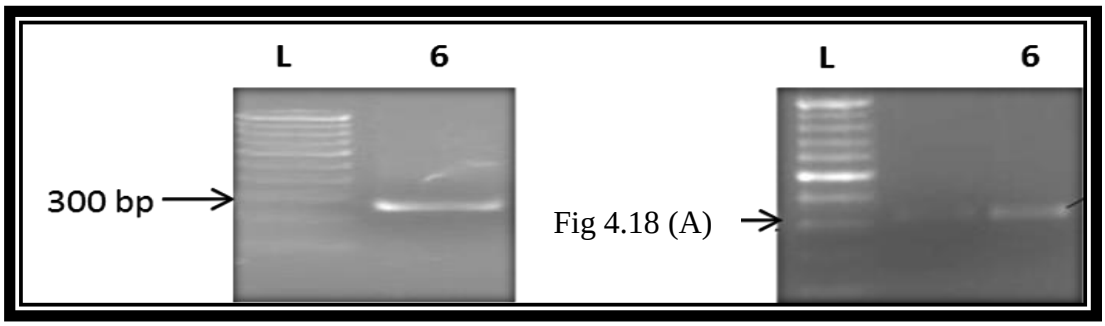

(B) (C)

acatcatctccgagttcatgccacgtggcagcctcctctacgtcctccacggtaataataatcacagcaaatactagtagttcttaat
caactactaatctcgattatagggcctgtttagattcatttggaatggcaaatggcaaatggaaaaagttttggtagcaaaagttttg
gtgacaaaagttttgacattgcaaaaatactagttgttgtttagatgcatgttaaagttttgccattctagcactaaaggagagagaga
aagagagagggagtggtcccaaagcactatttggcataatttgctactatggactagcaaatgccaaatgccaaattgacaacttt
tgctactagtgtttagatccaaaatggcaaaatgtgcttcaaaatggcaaaacttttactattttgaaggttctaaacagtgccatagtt
tgctaattacgatgtgtttgtttg

(D)

Fig 4.17(A) PCR amplification of 8 different genotypes with IAG-6 exon based primer. Primer IAG-6 produces one polymorphic band in wild genotype *O. longistaminata* (Lane-4). All PCR products were resolved on 1% agarose gel in 1X TAE buffer system. L= 100 bp ladder, 1= IRBB3, 2= IRBB4, 3= IRBB21, 4= *O. longistaminata*, 5= *O. rufipogon*, 6= *O. nivara*, 7= Pusa Basmati1, 8= Taichung Native 1. (B) PCR confirmation of the insert (C) Double digestion of plasmid DNA with *Eco*RI and *Bam*HI restriction enzymes. (D) Sequence product (466 bp) of polymorphic amplicon from *O. longistaminata* (Lane-4). It showed similarity with *O. sativa* cv. *japonica* Group chromosome 11 clone OSJNBb0035N09. Marking as 4 in Fig 4.17 (B) and (C) correspond the lane-4 of Fig 4.17 (A). RF= Released fragment.




(B) (C)

| |
|---|
| gaagccgtagtccacgatgcggggctccaggtgggcgtcgagcaggatgttgccggacttgaggttgccgtgcggcggcggcgg<br>cggcggcgcgtcgaagtcggcgccggtcatgctcaccagcctcatcgccgggatgcccagcttctcgtggaggtacgacaagccg<br>cgcaccacgccgacggcgatcctcatcctcgccggccaatccagcaccaccctctccgggctctggtcacctgaaccaaacaa |

(D)

Fig 4.18 (A) PCR amplification showing polymorphism in *O. longistaminata* with IAG-7 primer. It showed double band in almost all other genotypes but none of them was polymorphic except lane-6. All PCR products were resolved on 1% agarose gel in 1X TAE buffer system. L= 100 bp ladder, 1= IRBB3, 2= IRBB4, 3= IRBB21, 4= *O. rufipogon*, 5= *O. nivara*, 6= *O. longistaminata*, 7= Pusa Basmati1, 8= Taichung Native. (B) PCR confirmation of the insert (C) Double digestion of plasmid DNA with *Eco*RI and *Bam*HI restriction enzymes. (D) Sequence of cloned R-gene (252 bp) from *O. longistaminata* using M13 forward primer, which has shown polymorphic band by IAG-7 primer. Marking as 6 in Fig 4.18 (B) and (C) corresponds the lane-6 of Fig 4.18 (A).

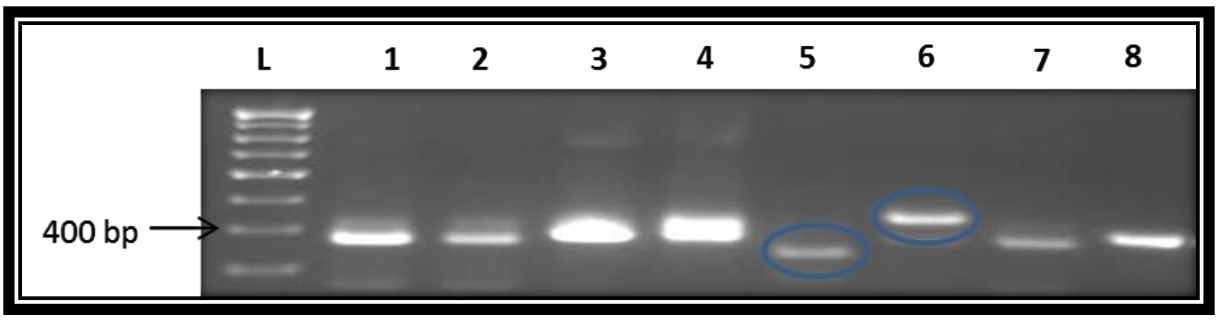
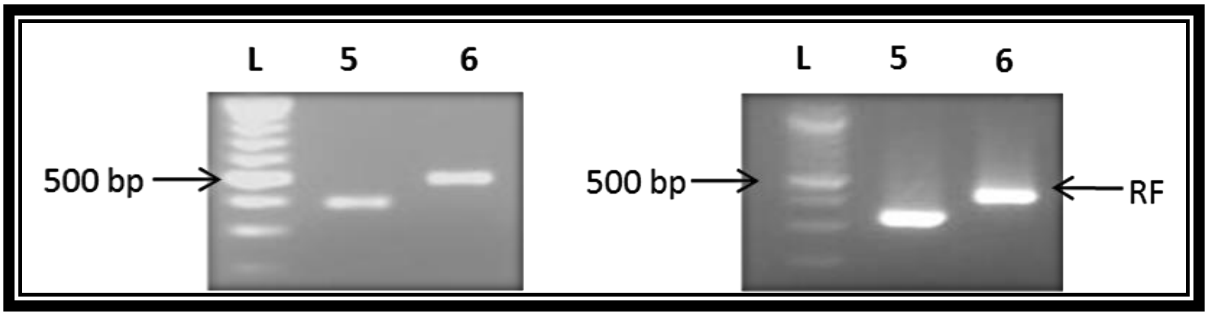



Fig 4.19 (A)




(B) (C)

ttgaggtattgacggggaagaaacgacttttcattaaagaacaacaggtggagaagaagagagaagagtgtgaggaagaggtgca
gaaaagagaagaatgtgagcaagagggcacgaaacgaaaattccaggacttggtgtccttggcgctgccactgattgaggagggg
aatctacggaaggtgctggacaggcggccatcactggagccaacgccgaggcagctggaagcggcggaactggtggcgcagac
ggcagctcgctgcctgcggctgcagtgggaggagcggccgaccatttcggaagtcgtggccaa

(D)

tggaggtattgacggggaagaaaccatatttctctaaagaggaatgggaggagatggagaagacagaagaatgtgtggaggagga
gaagagagaagaacgtgagcaagaggggaagaacacaggagaggacaaggaggagagtgaacgagaagaagaggagaaga
caacagaagaatcgccgcctgagtggctaaagctaaatcagtacgatttttgggggccacctacggatttgttgttcttccgcgctgcc
acttaatcgaaggcggggaaagctgttggaaaggtgctgggaacaggcggccggcaccggaaccaacggcgaagcagcctgga
agccggcgaacttggtgggcgcaaaacggaactcgctgcttcggcttgcggtgggagggagcggcccacccttttggaagtcgtg
ggcca

(E)

Fig 4.19 (A) PCR amplification showing polymorphism with IAG-16 primer in *O. rufipogon and O. nivara*. It is showing prominent band in all genotypes but polymorphic band is observed in *O. rufipogon and O. nivara*. All PCR products were resolved on 1% agarose gel in 1X TAE buffer system. L= 100 bp ladder, 1= IRBB3, 2= IRBB4, 3= IRBB21, 4= *O. longistaminata*, 5= *O. rufipogon*, 6= *O. nivara* 7= Pusa Basmati1, 8= Taichung Native. (B) PCR confirmation of the insert (C) Restriction digestion of plasmid DNA (D) Sequence of cloned R-genes from *O. rufipogon* (316 bp) and *O. nivara* (427 bp). Marking as 5 and 6 in Fig 4.19 (B) and (C) corresponds the lane-5 & 6 of Fig 4.19 (A). RF= Released fragment.

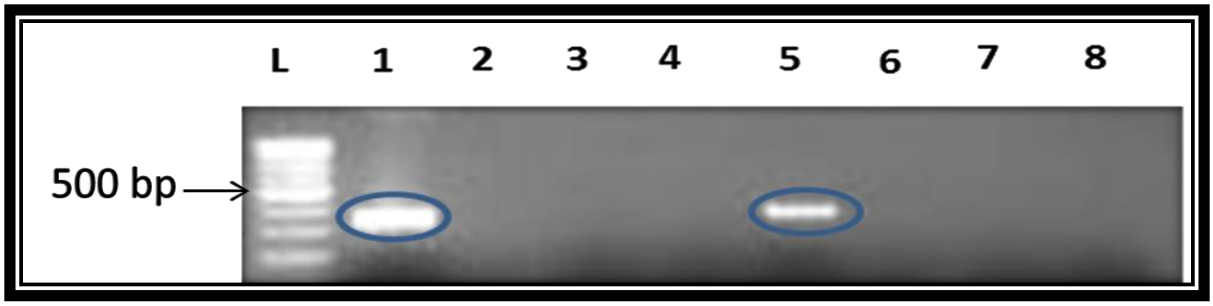
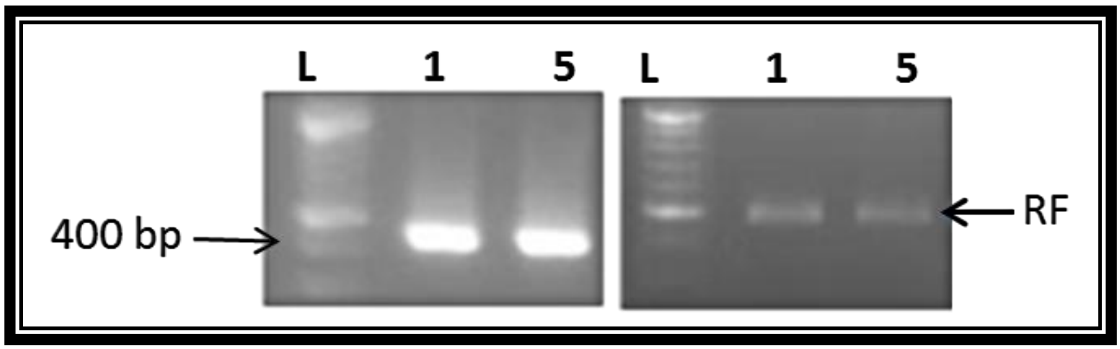



Fig 4.20 (A)




(B) (C)

tggtcaatcccaactaggctgtgctggtcgacgtacagacgatcaggtaggcttggagggttgtagatagtagaaacttgaggcttat
gtagactgtcatctatcctgatcatgtactgatcacgcatttcgcatacctccctgacgcgtgacttgatgtctttgatctcgtcaaagagc
ttatgggttgttgccgccctgagcctcgatctcgtgctgcttgcttcggtggactcaaggcccttgaggcgtaccaggatgttgtcgact
gcatcctccacgtcgtacgagagctcctggacatccagtgcccacagcttcatcatctgccccttctgctcctccgcgaagtcgcggc
gggaggactgtgcagatcatctttagttccatcagagtaagtggatgtcatgccttgcgc

(D)

tggtcatcccaactaggctgtgctggtcgacgtacagacgatcaggtaggcttggagggttgtagatagtagaaacttgaggcttatgt
agactgtcatctatcctgatcatgtactgatcacgcatttcgcatacctccctgacgcgtgacttgatgtctttgatctcgtcaaagagctt
atgggttgttgccgccctgagcctcgatctcgtgctgcttgcttcggtggactcaaggcccttgaggcgtaccaggatgttgtcgactg
catcctccacgtcgtacgagagctcctggacatccagtgcccacagcttcatcatctgccccttctgctcctccgcgaagtcgcggcg
gaggactgtgcagatcatctttagttccatcaagagtaagtggatgtcatgccttgc

(E)

Fig 4.20 (A) PCR amplification showing polymorphic bands in IRBB3 and *O. rufipogon* with IAG-20 primer. Primer produced amplicon in two genotypes and did not produce any amplicon in the rest of the genotypes. All PCR products were resolved on 1% agarose gel in 1X TAE buffer system. L= 100 bp ladder, 1= IRBB3, 2= IRBB4, 3= IRBB21, 4= *O. longistaminata*, 5= *O. rufipogon*, 6 = *O. nivara* 7= Pusa Basmati1, 8= Taichung Native 1. (B) PCR confirmation of the insert (C) Restriction digestion of plasmid DNA (D & E) Sequence of cloned R-gene from IRBB3 (419 bp) and *O. rufipogon* (415 bp). Marking as 1 and 5 in Fig 4.20 (B & C) corresponds the lane-1 & 5 of Fig 4.20 (A). RF= Released fragment.

Table 4.10 The accession number of R genes submitted to NCBI with putative function

| S. No. | Acc. No. | Gene Size (bp) | Species | Primer | Putative function |
|---|---|---|---|---|---|
| 1 | FJ938717 | 466 | *O. longistaminata* | IAG6-F&R | LRR-protein kinase |
| 2 | FJ827495 | 252 | *O. longistaminata* | IAG7-F&R | LRR-protein kinase |
| 3 | GQ202121 | 427 | *O. nivara* | IAG16-F&R | Collagen protein kinase |
| 4 | GQ367296 | 316 | *O. rufipogon* | IAG16-F&R | Protein kinase |
| 5 | GQ398491 | 415 | *O. rufipogon* | IAG20-F&R | NB-ARC containing protein |
| 6 | GQ381274 | 419 | IRBB3 | IAG20-F&R | Rust resistance gene,Yr10 |

### 4.4.2 Real-time quantitative polymerase chain reaction (RT-qPCR)

The expression pattern of two R-genes, one from polymorphic analysis [RGIA (GQ381274-Table 4.10); Sequence ID, AC133216.2) and one from NCBI (RK1; Sequence ID, AC136956.8) were investigated by qPCR in response to BB stress. The analysis was performed on cDNA sample from leaves of IRBB-3 (for RGIA) and *O. longistaminata* (for RK1) in order to study the possible relationship between the amount of R gene transcript formed with disease progression in the respective genotypes. The RGIA gene was induced progressively form 0 to 72 h post inoculation. It reached the highest expression level at 72 h where transcript level was increased by 4.37 fold. In case of RK1 gene the highest expression level was detected at 48 h post inoculation where 2.05 fold upturn was observed. It was interesting to note that the expression level of RK1 gene at 96 h was less (0.5 fold) when compared to 0 h (1.03 fold). Initially there was reduction in expression level from 0 to 6 h which continued till 12 h and then it increases progressively till 48 h (Fig 4.21). Nevertheless, these genes showed distinct expression dynamics and induction levels depending on BB stress and test conditions.

### 4.4.3 Generation of transgenic rice and tobacco

In an attempt to clone BB resistant genes, the complete open reading frame (ORF) of both the genes RGIA and RK1 from IRBB-3 and *O. longistaminata* respectively were cloned into pRT100 vector and cloned gene were confirmed through PCR/restriction digestion. Complete cassettes from both the confirmed clones were released and cloned separately in pCAMBIA1300 and pCAMBIA2300 binary vector (Fig 4.22 and Fig 4.23). Finally, clones were confirmed through restriction digestion and mobilized in *A. tumefaciens* strain EHA105 separately for plant transformation. Both constructs were driven by the CaMV 35S promoter (Fig 4.24). This construct was used for *Agrobacterium*-mediated plant transformation.

*Agrobacterium*-mediated inplanta transformation of the CaMV 35S::RK1 construct yielded 5 independent putative T0 plants of tobacco (Fig 4.25) which has shown PCR positive results with candidate RK1 and selection marker *npt*II genes (Fig 4.26). In case of rice, *Agrobacterium*-mediated transformation work has been initiated (Fig 4.25).

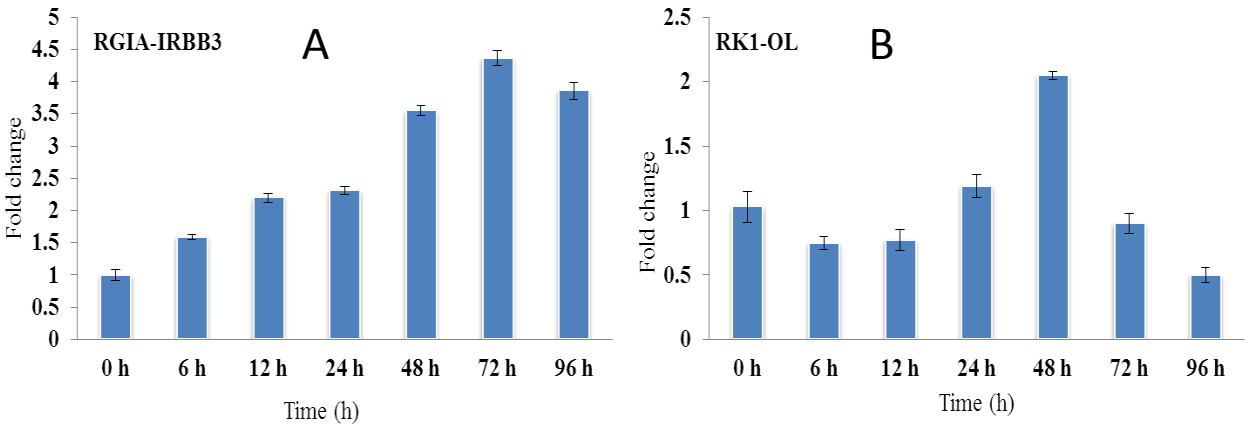



Fig 4.21 Quantitative real time PCR analysis of RGIA gene in IRBB-3 near isogenic line (A) and RK1 gene in *O. longistaminata* (B) in response to bacterial blight stress.

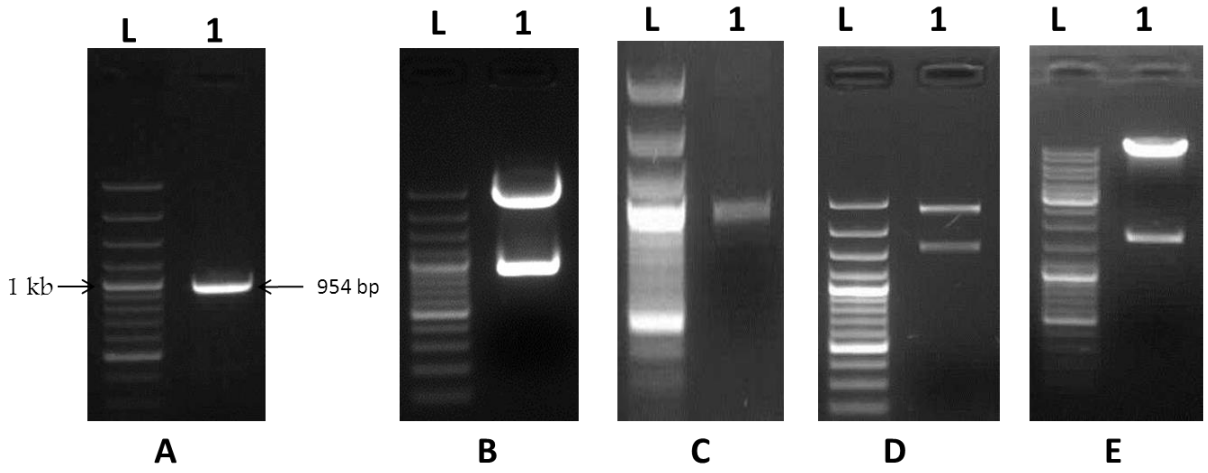



Fig 4.22 (A) PCR amplification of full length RGIA ORF (B) restriction digestion of gene cloned into TA cloning vector (C,D) PCR and restriction digestion confirmation of gene cloned into plant expression vector pRT100 (E) restriction digestion confirmation of gene cloned into pCAMBIA1300. [L=100 bp plus ladder in A, B, C, D gel; and L= ladder mix in E gel]

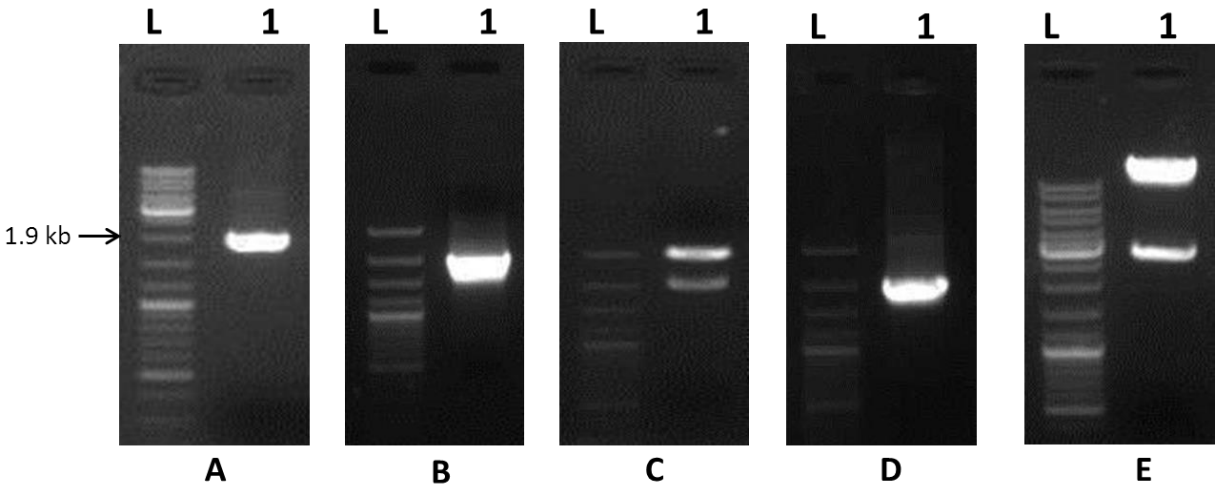



Fig 4.23 (A) PCR amplification of full length RK1 ORF (B,C) PCR confirmation and restriction digestion confirmation of gene cloned into TA cloning vector (D) PCR confirmation of gene cloned into plant expression vector pRT100 and (E) restriction digestion of gene cloned into pCAMBIA1300. [L= ladder mix in A & E); and L= 100 bp plus ladder in B, C & D gel]

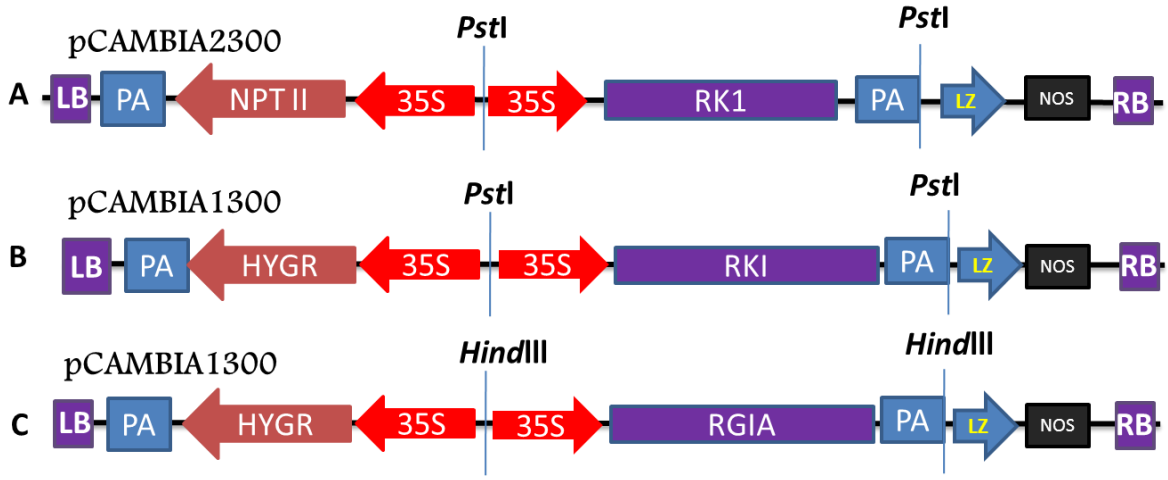



Fig 4.24 Partial map of constructs (A) Partial map of vector pCAMBIA2300 harbouring RK1 gene cloned at *Pst*I sites and contain nptII gene as plant selection marker (B) Partial map of vector pCAMBIA1300 harbouring RK1 gene cloned at *Pst*I sites and contain HYGR gene as plant selection marker (C) Partial map of construct pCAMBIA1300 harbouring RGIA gene cloned at *Hind*III sites and contain HYGR (Hygromycin) gene as plant selection marker.

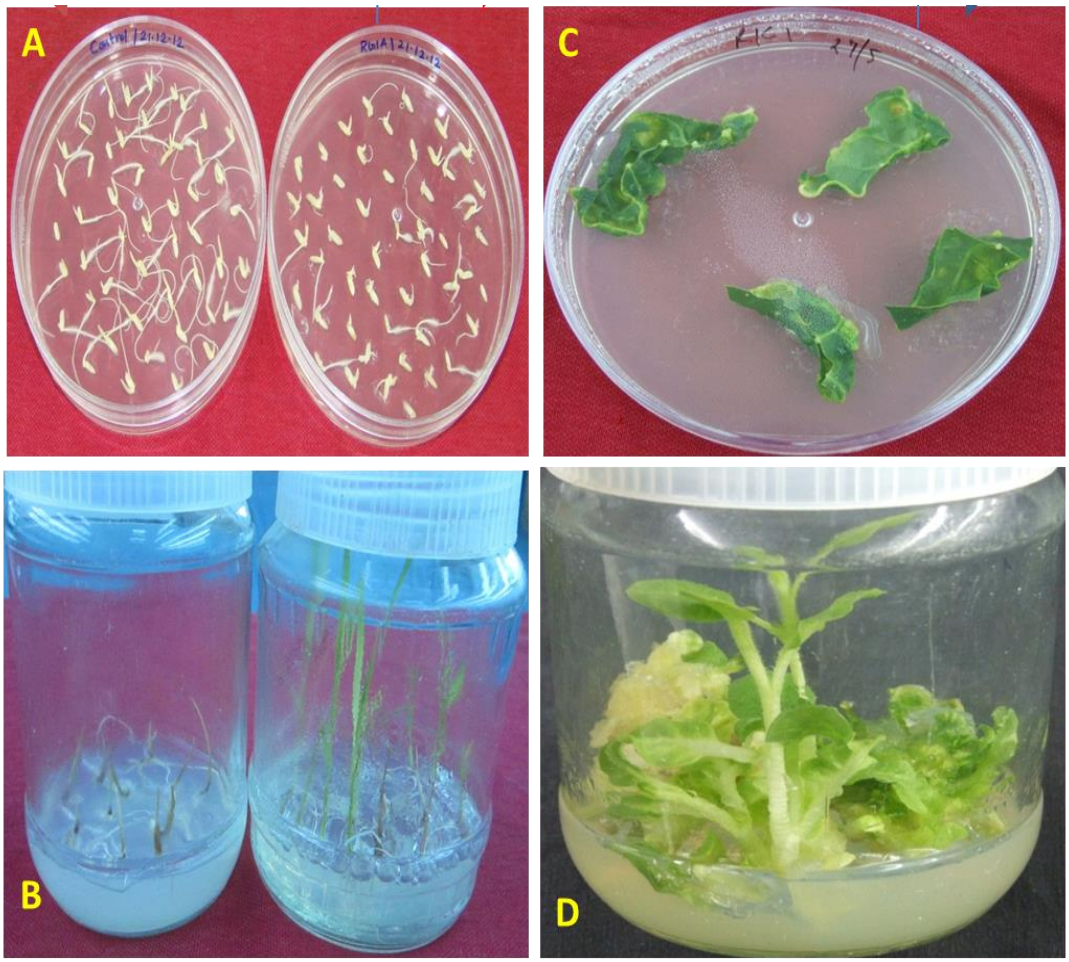



Fig 4.25 *Agrobacterium* mediated transformation of (A) rice (BPT5204) and (B) BPT5204 seedlings on hygromycin selection media (C) tobacco (*N. tabacum* L. cv. samsun) with RK1 gene cloned into plant transformation vector pCAMBIA1300 and pCAMBIA2300. (D) *N. tabacum* L. cv. Samsun on kanamycin selection media.

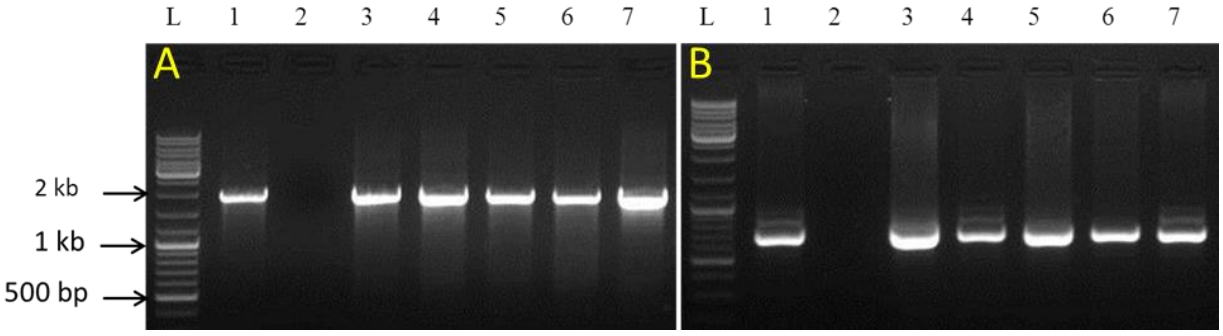



Fig 4.26 PCR amplification of the putative transgenic plants of tobacco (A) with gene specific RK1 primers (954 bp) (B) and *nptII* marker specific primers (~700 bp). Lane L: ladder mix; lane 1: positive control; lane 2: negative control (untransformed plant); lane 3-7: PCR amplicon from transformed plant.

## 4.5 Proteomic analysis of BB susceptible and resistant rice genotypes

### 4.5.1 2D gel electrophoresis and protein expression profiling

Disease occurrence or resistance depends on compatible or incompatible interactions of plants with pathogen, based on gene for gene theory (Flor, 1971). Incompatible interaction elicits HR in host plant which prevents disease development whereas compatible interaction does not induce HR and results in disease development. In order to understand this mechanism, a comprehensive analysis is required. In this context, a proteomic approach was applied to analyze the changes in protein profile during rice and *Xoo* interaction. For this, 45 days old PB1 (susceptible) and *O. longistaminata* (highly resistant) genotype were inoculated with DX133 isolate of *Xoo* by needle pricking method. BB symptoms were started appearing gradually three days after inoculation as very minute white spots on and around the pricked points on the leaves which was more prominent in susceptible PB1 compared to highly resistant *O. longistaminata*. Therefore, plants were selected for proteomic analysis 3 days after bacterial inoculation. Triplicate gels were obtained from three independent experiments and the representative gels of *O. longistaminata* and PB1 were illustrated in Fig 4.27 and Fig 4.28 respectively. We observed a total of nearly 250–300 protein spots on each 2-DE gel stained with coomassie brilliant blue dye. Thereafter, we systematically screened the protein spots that were differentially regulated in response to bacterial inoculation and were grouped according to their relative expression. Image Master 2-D platinum version 6 software was used to comprehend the differentially regulated spots on the gel. There was around 80–85% correlation between biological repeats, indicating reliable reproducibility of the experiments. There were 34% and 39% protein spots upregulated by ≥ 2 fold in PB1 and *O. longistaminata* at 3 DAI respectively. On an average there were about 34.5% protein spots where no change in expression level was observed.

Overall 2-DE gel analysis revealed that there were more proteins spots expressed in treated *O. longistaminata* when compared to its corresponding control and susceptible PB1. In treated *O. longistaminata* there were around 29 protein spots where more than 2 fold changes was observed. There were 23 and 11 protein spots depicted with more than 3 and 5 fold higher expression respectively in treated *O. longistaminata*. In susceptible PB1 (treated), 5 fold upregulated protein spots were only three. When we compare 2 and 3 fold

upregulated proteins of PB1 with *O. longistaminata*, an unambiguous difference can be perceived. The fold change profiling of proteins are illustrated in Fig 4.29. Regulated proteins were determined based on the two basic criterion (1) reproducibility (2) fold change (% volume) at least ≥ 1.5 times. Finally, total 40 protein spots (20 spots each from PB1 and *O. longistaminata*) including up-regulated and new spots were selected, which were distinct, and well separated with reliable expression pattern in rice *Xoo* interaction for MALDI TOF-TOF analysis. Out of 40 spots, only 29 proteins were successfully identified with putative function (Table 4.11) and the rest 11 did not show any significant hits either in the NCBI or Swiss-Prot database and hence were not considered. Out of 29 differentially expressed proteins, 22 were upregulated whereas 7 were newly expressed. We have studied the relative abundance of 22 differentially expressed proteins of PB1 (spot 1–15) and *O. longistaminata* (spot 16 – 22) spot-by-spot in comparison with the control (Fig 4.30). Rest seven protein spots were novel (not expressed in control) and hence were not included in this figure. For MALDI-TOF-TOF analysis, we have selected protein spots with one and half fold or above upregulated proteins. Interestingly, protein spots 13 and 14 were induced more than five folds, but both were detected as hypothetical protein in protein database. Protein spots 1, 12, 15 and 22 were expressed more than three folds whereas rests all were expressed below three folds. As an example, the PMF resulting from MS analysis, spectral peak with masses and histogram of a protein spot (14) representing the score of each ion of a protein and the amino acid sequences of each peptide fragments obtained during PMF search against protein database is mentioned in Fig 4.31.

### 4.5.2 Identification of differentially regulated proteins

In order to understand the functions of 29 differentially expressed proteins, they were categorized according to Bevan et al. (1998) with minor modification as related to (1) energy and metabolism-RuBisCO activase and RuBisCO large subunit, putative photosystem I reaction center subunit IV, enolase, triose phosphate isomerase (2) defense and stress - germin like protein, putative r40c1protein, cyclin-dependent kinase C, ent-isokaur-15-ene synthase, GSH-dependent dehydro ascorbate reductase 1 (3) hypothetical protein with disease resistance conserved domain - F-box, peroxiredoxin (PRX), $H^+$ATPase (4) protein synthesis - chloroplast translational elongation factor Tu (5) cell signalling -

putative ferredoxin-NADP(H) oxidoreductase (6) protein with unknown function/un-named protein, DUF-1 (Fig 4.32). Maximum numbers of protein (41.4%) were found to be hypothetical protein. Defense/stress and energy/metabolism related protein were 20.6% each. There were 10.34% hypothetical protein were having conserve domain related to defense and stress. Cell signaling and protein synthesis protein were 3.3% each (Fig 4.32).

The predicted molecular weight (MW) and pI of all these proteins were compared with their positions on the gel and were found consistent. The matched peptide sequence and its number, accession number, reference organism, sequence coverage, experimental and theoretical molecular weight and pI, MS/MS score of each protein were determined (Table 4.11). Hypothetical proteins were most common in our study. RuBisCO was identified at more than one spot which could be due to presence of different isoform of same protein or could be due to post translational modification.

The analysis of the expressed protein highlights their probable role in plants response against bacterial blight infection. We observed that an inter-relationship and rationality exist among the identified proteins. Therefore, based on previous reports and our results we tried to show the relationship between the biotic and abiotic stresses though schematic representation as depicted in Fig 4.33.

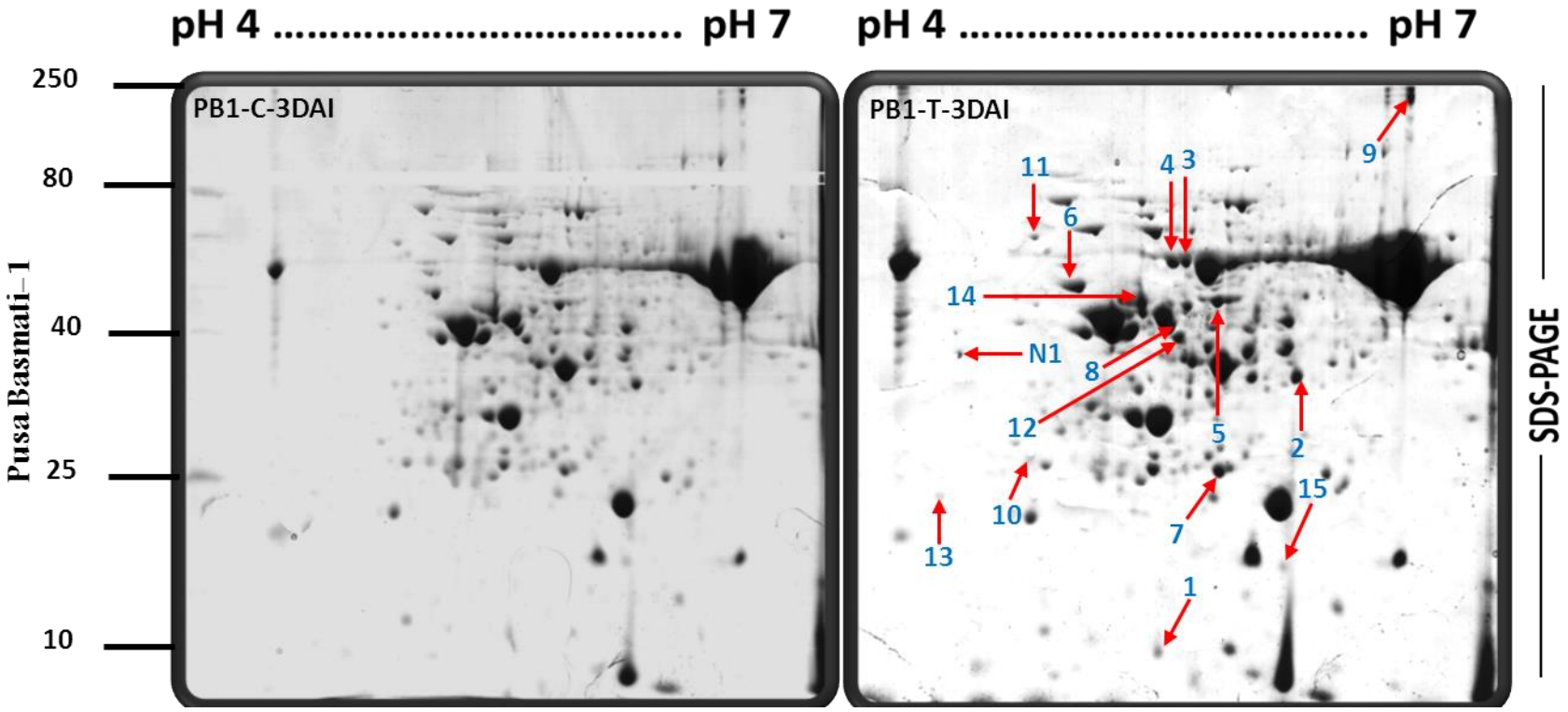



Fig 4.27 2-DE patterns of proteins from rice leaf blades of PB1 inoculated with *Xoo*. The uppermost fully opened leaves were inoculated with bacterial suspension as treatment and infiltrated with sterilized distilled water as control, by needles pricking method.

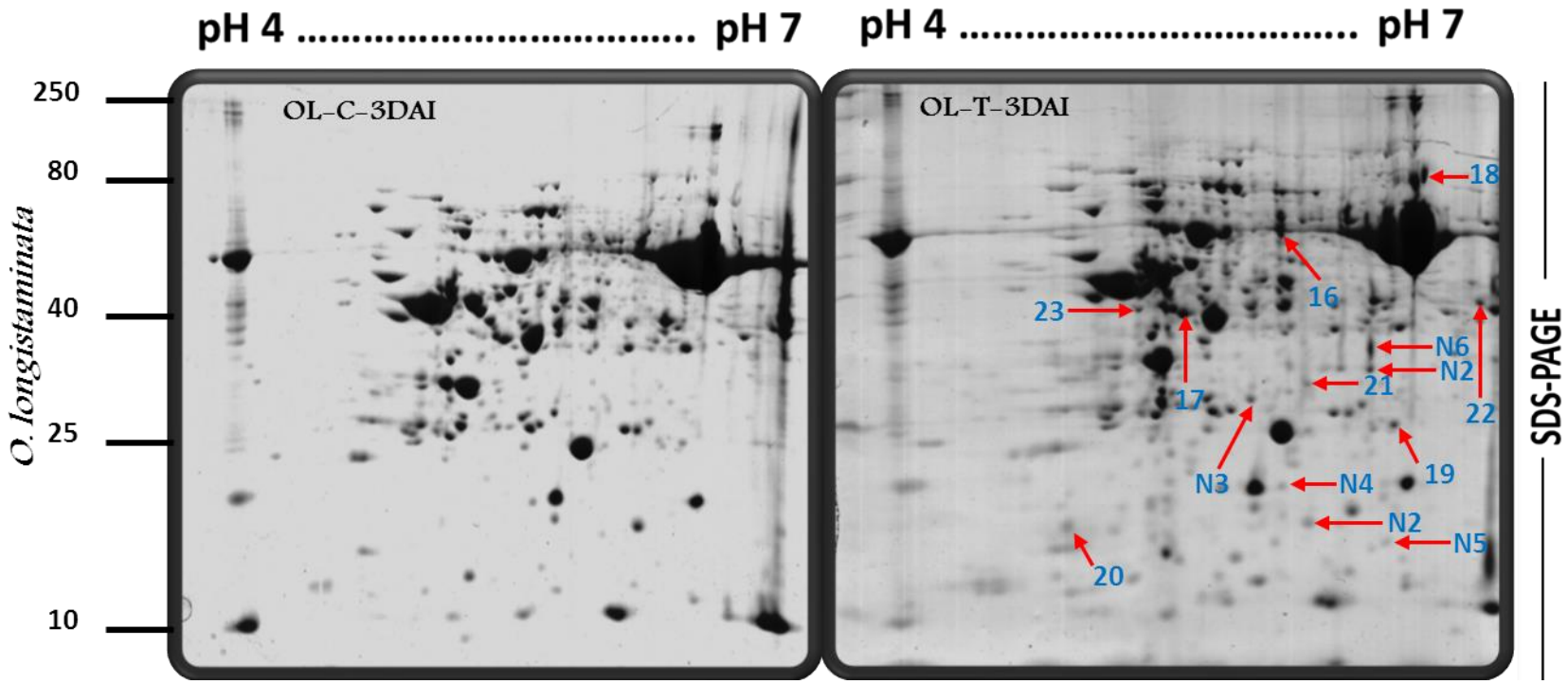



Fig 4.28 2-DE patterns of proteins from rice leaf blades of *O. longistaminata* inoculated with *Xoo*. The uppermost fully opened leaves were inoculated with bacterial suspension as treatment and infiltrated with sterilized distilled water as control, by needles pricking method. Leaf samples were collected on 3$^{rd}$ and 4$^{th}$ days after inoculation. Total foliar proteins were extracted and used for 2-DE.

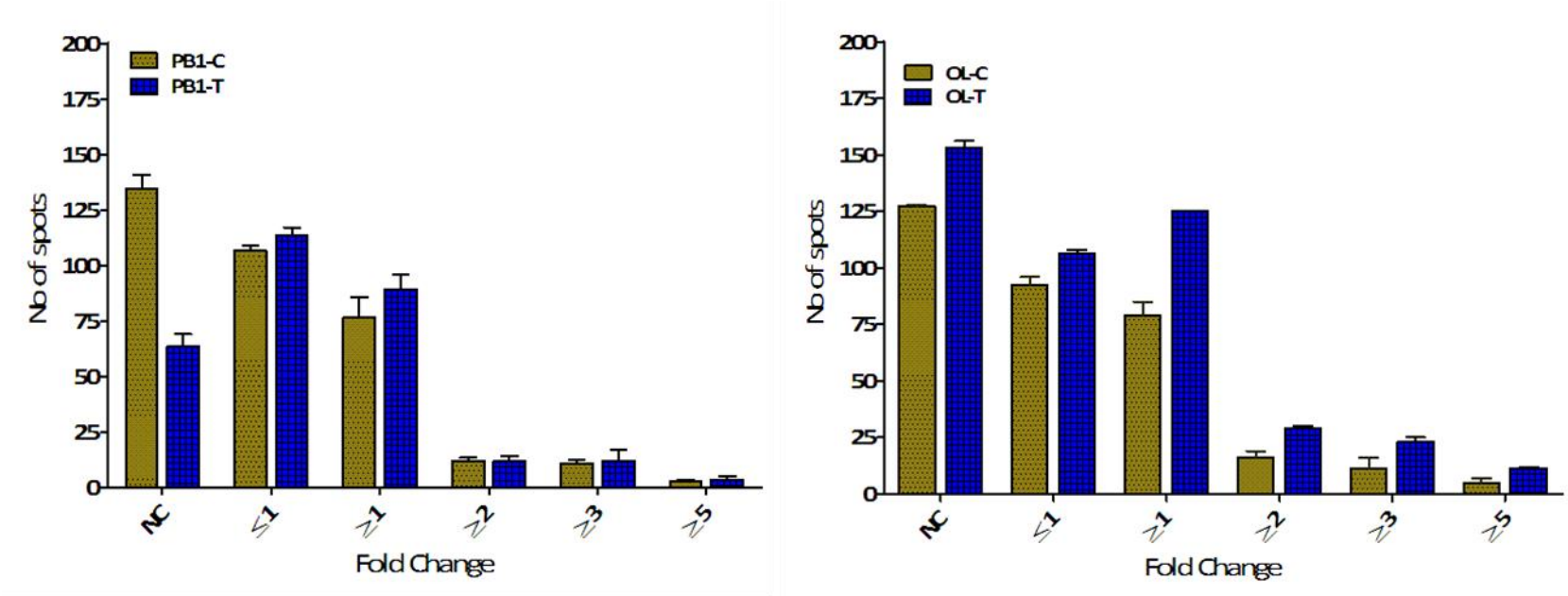



Fig 4.29 Expression patterns of total proteins in rice leaf blades after inoculation with *Xoo* in highly resistant *O. longistaminata* and susceptible PB1.The changed protein spots were calculated in terms of relative fold change with Image Master 2D Platinum software. Value are the mean s ± SE of protein volume of gels from three independent experiments. NC: no change in expression after *Xoo* inoculation.

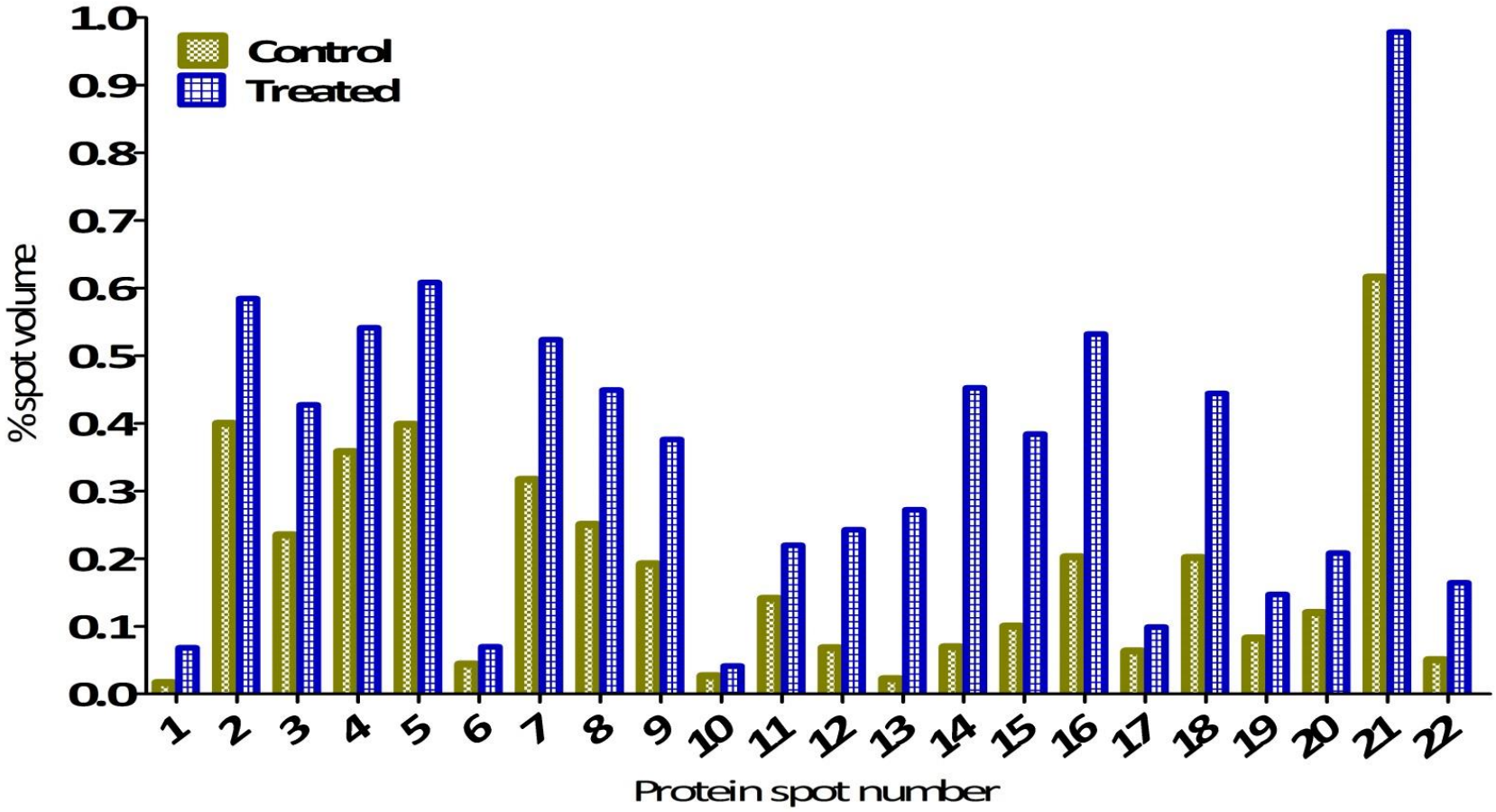
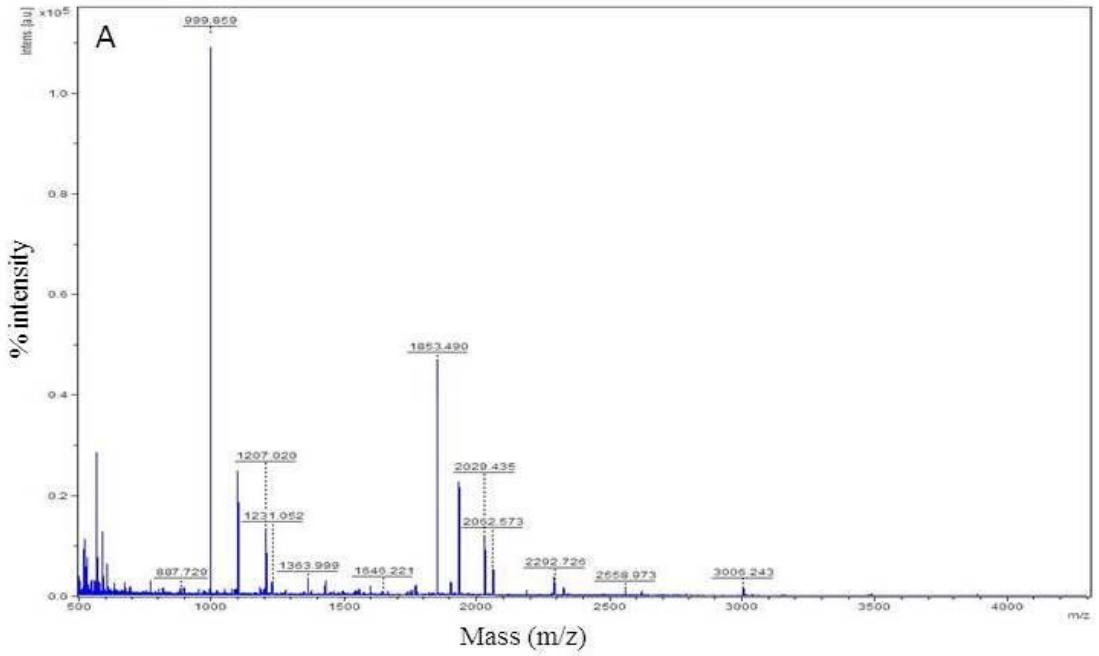



Fig 4.30 Relative abundance of the 22 protein spots in comparison with the controls. Spot number 1 to 15 are from Pusa Basmati 1while 16 to 22 are from *O. longistaminata*. Only those with 1.5 or above fold changes are shown. There spots were successfully identified by MS/MS.




C

| Calc. Mass | Obser. Mass | ppm | Start seq. | End seq. | Peptide Sequence |
|---|---|---|---|---|---|
| 999.8590 | 998.5913 | 261 | 74 | 82 | K.FSLAPLVPR.L |
| 1102.8990 | 1101.6295 | 238 | 191 | 201 | K.RPFAAIVGGSK.V |
| 1207.0200 | 1205.7244 | 239 | 58 | 68 | K.VILSSHLGRPK.G |
| 1853.4900 | 1851.0465 | 776 | 105 | 121 | K.LVSELPNGSVLLLENVR.F |
| 1934.4611 | 1932.9694 | 251 | 137 | 154 | K.LASLADLYVNDAFGTAHR.A |
| 3006.2429 | 3003.6128 | 540 | 94 | 121 | K.AEDVIGPDVEKLVSELPNGSVLLLENVR.F |

Fig 4.31 Identification of protein spot 14 by MS/MS (A) PMF resulting from MS analysis. Spectral peaks with masses of 999.85,1102.89,1207.02,1853.49,1934.46 and 3006.24 were most prevalent and were selected for MS/MS analysis (B) Mascot search histogram representing ion protein score (C) Amino acid sequence s of peptide fragments were obtained by PMF searching in database.

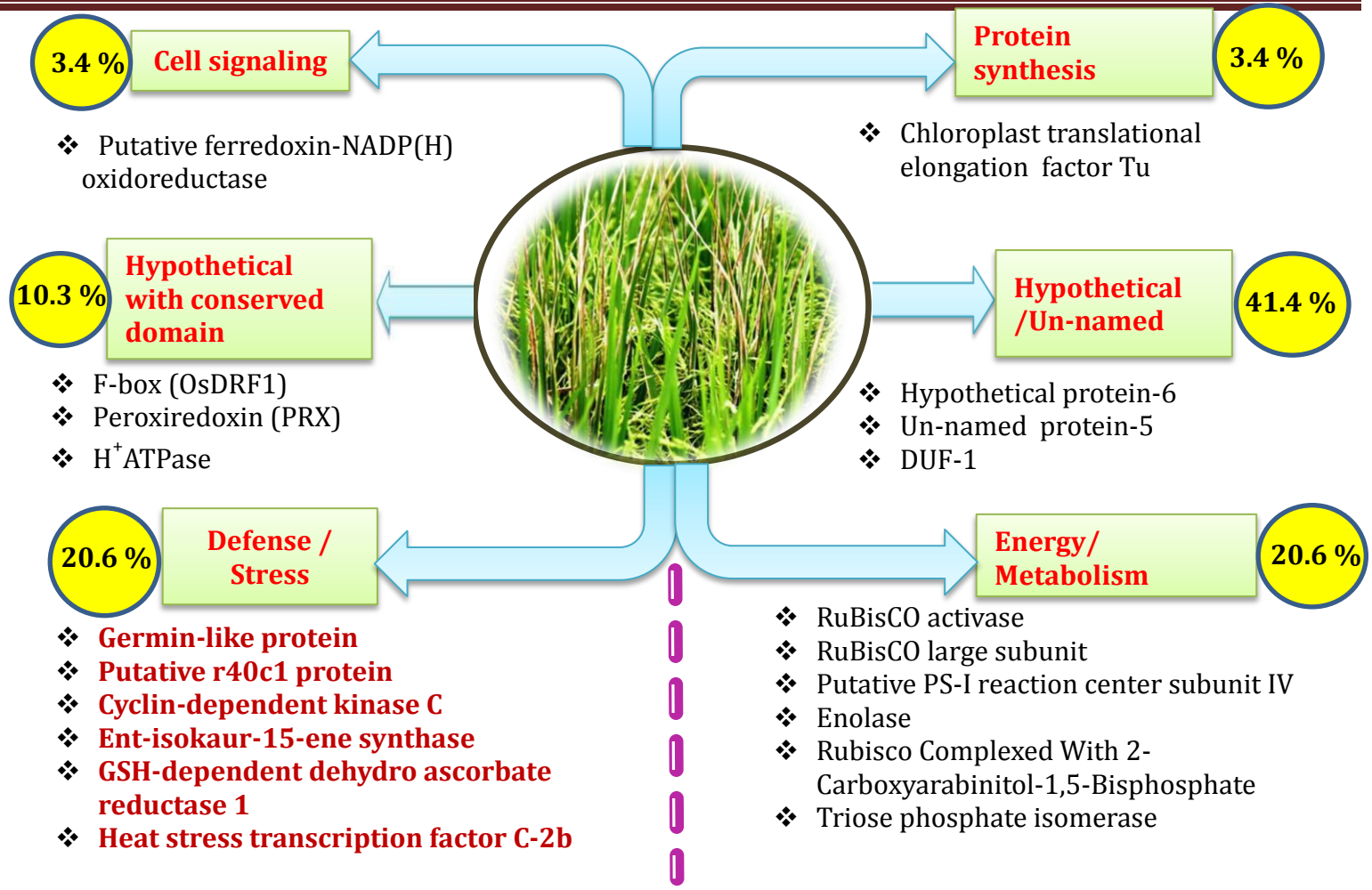



Fig 4.32 Grouping of differentially expressed protein from both susceptible PB1 and highly resistant *O. longistaminata* based on their functional role.

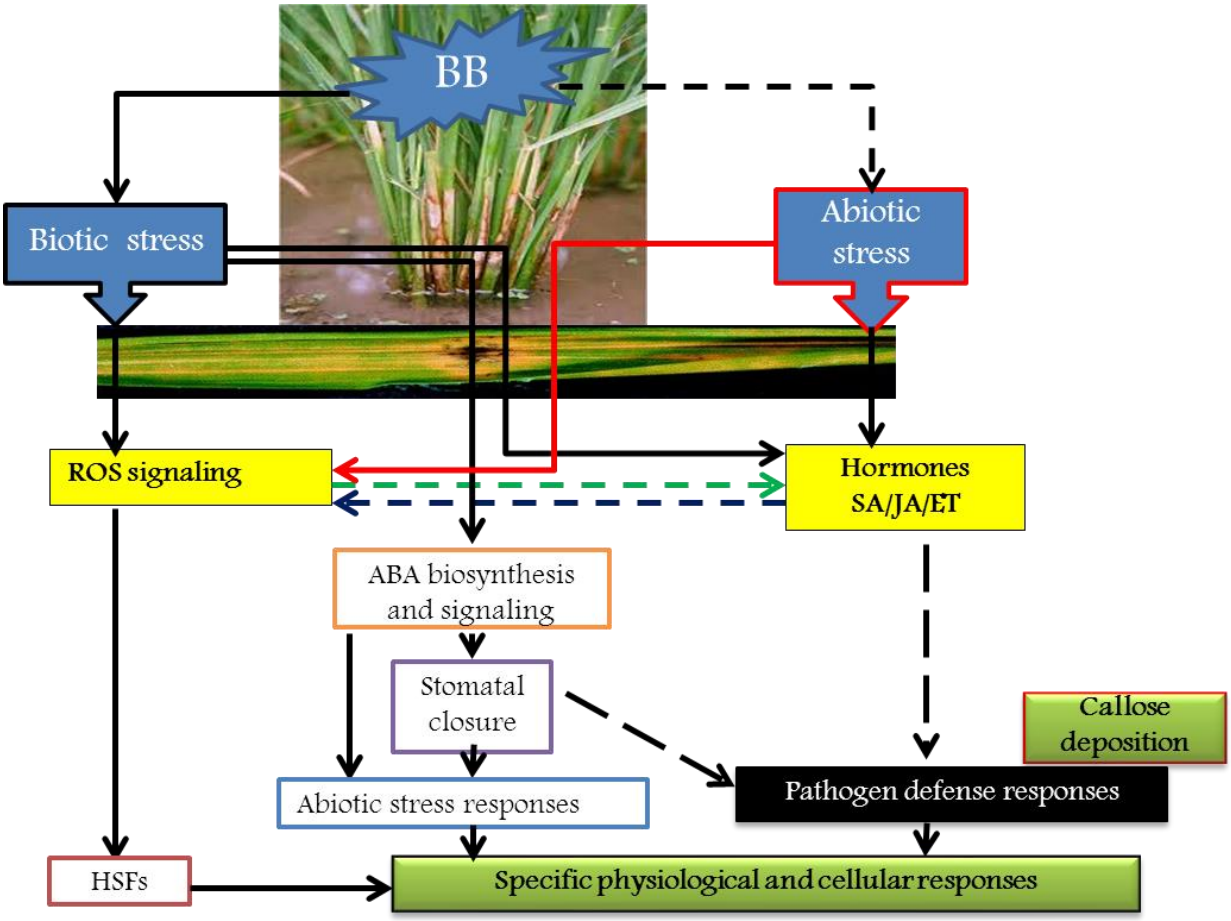



Fig 4.33 Schematic representation of the inter-relationship and networking among the identified group of proteins and overall plant responses against BB stress based on current as well as previous reports. During BB plant faces both biotic and abiotic stresses concurrently which imitate cross talk between biotic and abiotic stresses in plants.

Table 4.11 Identification of major differentially expressed foliar proteins from BB susceptible rice genotype PB1 and highly resistant *O. longistaminata* during BB infection.

| Group | Spot no. | Protein identified | Peptide sequences Matched (PSM) | Accession no. | No. of PSM / SC % | Reference organism | MS/MS score | CD |
|---|---|---|---|---|---|---|---|---|
| PB1-3 DAI | 1 | Os02g0192700 Hypothetical protein | HLPGFIEK;GVDAIACVSVNDAFV MR;ALGVEMDLSDKPMGLGVR | gi\|115444771 (NCBI) | 3/19 | *Oryza sativa* ssp.*japonica* | 174 | PRX |
| | 2 | Putative ferredoxin-NADP(H) oxidoreductase | MKEIAPER;YRPKEPYTGR;ITGD DAPGETWHMVFSTDGEIPYR | gi\|41052915 (NCBI) | 3/11 | *Oryza sativa* ssp. *japonica* | 128 | - |
| | 3 | Enolase | FRAPVEPY;GNPTVEVDVCCSD GTFAR;SFVSEYPIVSIEDPFD QDDWEHYAK | gi\|780372 ((NCBI) | 3/11 | *Oryza sativa* ssp. *japonica* | 43 | - |
| | 4 | Hypothetical protein | FDEGLPPILTALEVLDHNIR;DAE GQDVLLFIDNIFR;NLQDIIAILG MDELSEDDKLTVAR | gi\|115439241 (NCBI) | 3/10 | *Oryza sativa* ssp. *japonica* | 130 | atpB |
| | 5 | Chloroplast translational EF-Tu | KYDEIDAAPEER;MVVELIQPVAC EQGMR;HSPFFPGYRPQFYMR;DQ VDDEELLQLVELEVR;KDQVDDEE LLQLVELEVR | gi\|6525065 (NCBI) | 5/13 | *Oryza sativa* ssp. *japonica* | 281 | tufA |
| | 6 | Heat stress transcription factor C-2b | DASADGGGGGGDEDMTMVATEVVR | HFC2B ORYSJ (Swis-Prot) | 1/8 | *Oryza sativa* ssp. *japonica* | 15 | - |
| | 7 | Triose phosphate isomerase, partial | VIACVGETLEQR;WLAANVSAEVA ESTR;SLLGESNEFVGDKVAYALS QGLK | gi\|553107 (NCBI) | 3/19 | *Oryza sativa* ssp. *japonica* | 87 | TIM |
| | 8 | RuBisCO activase | IVDSFPGQSIDFFGALR; VPIIVTGNDFSTLYAPLIR | gi\|1778414 (NCBI) | 2/6 | *Oryza sativa* ssp. *japonica* | 112 | - |
| | 9 | RuBisCO large subunit | TFQGPPHGIQVER;AIKFEFEPVD KLDS;GGLDFTKDDENVNSQPFMR | gi\|11466795 (NCBI) | 3/9 | *Oryza sativa* ssp. *japonica* | 191 | - |
| PB1-4 DAI | 10 | Hypothetical protein | TTVPDNDGGGGSR;EGINLDLAR | gi\|15289845 (NCBI) | 2/20 | *Oryza sativa* ssp. *japonica* | 76 | - |
| | N1 | OSJNBa0088A01.12 | CGSWSAVRGSGGRGAR; | gi\|38346805 | 3/8 | *Oryza sativa* | 97 | F- |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Hypothetical protein | RGVCSTGR;WWSAAGGR | (NCBI) | | ssp. *japonica* | | box |
| | 11 | Methyl-CpG binding domain containing protein, expressed | AHPGGPASSEFDWGTGDTPR;AHPGGPASSEFDWGTGDTPRR | gi\|77557101 (NCBI) | 2/6 | *Oryza sativa* ssp. *japonica* | 24 | MBD |
| | 12 | Os01g0978100 Hypothetical protein | IQGIGAGFVPR;LVVVVFPSFGER;VDVFIGGIGTGGTISGAGR | gi\|115442595 (NCBI) | 3/10 | *Oryza sativa* ssp. *japonica* | 131 | CBS |
| | 13 | Hypothetical protein | IFAEEAIAEFFDNPTVPRDEK;IDPELIAGFTIQYGR;KQIEEITSEFEMPAVTLDV | gi\|115448701 (NCBI) | 3/22 | *Oryza sativa* ssp. *japonica* | 269 | F0F1 ATP-D |
| | 14 | Hypothetical protein | FSLAPLVPR;RPFAAIVGGSK;VILSSHLGRPK;LVSELPNGSVLLLENVR;LASLADLYVNDAFGTAHR;AEDVIGPDVEKLVSELPNGSVLLLENVR | gi\|125552851 (NCBI) | 6/27 | *Oryza sativa* ssp. *indica* | 429 | PGK |
| | 15 | Os08g0504500 Hypothetical protein | KDAAEYVYEVPEGWKER;DAAEYVYEVPEGWKER;GTNGTDSEFFNPR | gi\|115477166 (NCBI) | 3/12 | *Oryza sativa* ssp. *japonica* | 187 | PsbP |
| **OL-3 DAI** | 16 | Rubisco Complexed With 2-Carboxyarabinitol-1,5-Bisphosphate | GGLDFTEDDENVNSQPFMR;MSGGDHIHAGTVVGK | gi\|56966762 (NCBI) | 2/5 | *Oryza sativa* ssp. *japonica* | 65 | - |
| | 17 | Os01g0978100 Hypothetical protein | NLDSEVLDEVIEISSDEAVETAK; | gi\|115442595 (NCBI) | 1/5 | *Oryza sativa* ssp. *japonica* | 112 | CBS |
| | N2 | Putative PS- I reaction center subunit IV | VNYAGVSTNNYALDEIQEVK;FLLAGGNGGSGGGGASR | gi\|34394725 (NCBI) | 2/24 | *Oryza sativa* ssp. *japonica* | 127 | - |
| | 18 | RuBisCO large subunit | LTYYTPEYETKDTDILAAFR;DTDILAAFR;TFQGPPHGIQVER;GGLDFTKDDENVNSQPFMR;NHGMHFR;NEGRDLAREGNEIIR;AIKFEFEPVDKLDS | gi\|11466795 (NCBI) | 7/18 | *Oryza sativa* ssp. *japonica* | 483 | RbcL |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 19 | GSH-dependent dehydro ascorbate reductase 1 | AAVGHPDTLGDCPFSQR;WIPDSDVITQVIEEKYPTPSLVTPPEYASVGSK;ALLTELQALEEHLK;AHGPFINGQNISAADLSLAPK;LYHLQVALEHFK;IPEDLTNVHAYTEALFSR | gi\|6939839 (NCBI) | 6/53 | *Oryza sativa* ssp. *japonica* | 423 | - |
| | 20 | Os06g0597400 Hypothetical protein | NLESTLVR; | gi\|297725039 (NCBI) | 1/35 | *Oryza sativa* ssp. *japonica* | 1 | - |
| **OL-4 DAI** | N3 | Putative PS-I reaction center subunit IV | ESYWYNGTGSVVTVDQDPNTR;ESYWYNGTGSVVTVDQDPNTRYPVVVR;RESYWYNGTGSVVTVDQDPNTRYPVVVR;AEEEAAAPPPPPPPAAEEKPAEAEAAVATKEPAAAKPPPIGPK | gi\|34394725 (NCBI) | 4/47 | *Oryza sativa* ssp. *japonica* | 202 | - |
| | 21 | Germin-like protein | AAVTPAFVGQFPGVNGLGISAAR;VTFLDDAQVK | GL814 ORYSJ (Swiss-Prot) | 2/15 | *Oryza sativa* ssp. *japonica* | 205 | - |
| | N4 | Cyclin-dependent kinase C | DEQGKPIEGNK;VVLAINNSMETGNSTAVS;EEAMGVAHIHSK | gi\|31442141 (NCBI) | 3/8 | *Oryza sativa* ssp. *japonica* | 131 | S/T Kinase |
| | N5 | UPF0496 protein 1 | AISTLAVGVEVR;DLDSIRVLINR | U496A ORYSI (Swiss-Prot) | 2/6 | *Oryza sativa* ssp. *indica* | 42 | - |
| | N6 | unnamed protein product | GGLDFTKDDENVNSQPFMR;DRFVFCAEAIYK | gi\|11955 (NCBI) | 2/5 | *Oryza sativa* ssp. *japonica* | 86 | rbcL |
| | 22 | Putative r40c1 protein-rice | ILPWGDEAYAGGSANAPR;HSNSIKDEEGYPAFALVNR;LVPYNPGYQDESVLWTESR | gi\|24899397 (NCBI) | 3/15 | *Oryza sativa* ssp. *japonica* | 111 | lectin |
| | 23 | Ent-isokaur-15-ene synthase | MLPMSSACSGGQFPGASPHGIIPK;MIHISELPEVEYALK;TGLELPVMQTDIDGIIHIR | KSL6 ORYSJ (Swiss-Prot) | 3/3.5 | *Oryza sativa* ssp. *japonica* | 79 | DDXXD/E |

AtpB = F1 ATP synthase beta subunit; TIM = Triose phosphate isomerase; CBS = Cystathionine beta-synthase; PGK = Phosphoglycerate kinase; rbcL= ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit; DDXXD/E: Asp-Asp-Xaa-Xaa-Asp/Glu

# CHAPTER-5
# DISCUSSIONS

## 5.1 Effect of bacterial blight in the photosynthetic efficiency and in the yield

Bacterial blight (BB) caused by the rod-shaped, gram negative bacterium, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most shattering diseases in rice (*Oryza sativa* L.). Outbreaks of BB usually occur in irrigated and rainfed lowland ecologies throughout Asia and worldwide. BB symptom transpires at all stages of rice plants. Wilting and death are always found in seedling stage whereas higher numbers of sterile and undeveloped grains are found in reproduction stage. BB reduces photosynthesis through necrosis of leaf tissue and by down-regulation of $CO_2$ fixation rate (Bastiaans, 1991). In the present study 14 different rice genotypes were used, where *O. longistaminata* was highly resistant, IRBB21 resistant and PB1 was susceptible against *Xoo* strain DX133. It was demonstrated that wild rice tend to have a higher frequency of resistant genotypes than cultivars due to high selection pressure. Loss of resistance in certain genotypes in disease-free environments may be explained by assuming "fitness cost" in the host plants. A number of factors are responsible for host resistance as reviewed by Burdon (1987). It is difficult to explain the resistance/susceptibility of host plant based on phenotypic reactions but this is one of the easier ways to find out the characteristic response of plant against a particular bacterial strain.

Our preliminary study confirmed the earlier findings of disease reaction with most of the genotypes but the percentage of lesion length was varying. *O. longistaminata* was the most dominant one. The stable resistance to BB in *O. longistaminata* across locations indicates that it is resistant to more than one pathotype and consequently is invaluable for comparative photosynthetic physiology study against IRBB21(R) and PB1(S). Photosynthetic $CO_2$ fixation characteristics were analyzed using three contrasting genotypes, viz., *O. longistaminata* (highly resistant), IRBB21 (resistant) and PB1 (susceptible) based on resistance reaction. Effects of BB infestation depicted contrasting variability in $P_N$, *gs* and *E* among the tested genotypes. Initially an elevation in $P_N$ was observed (24 h of post infection) in all the three genotypes, consistent with previous report of Johnson (1984) where the same pattern was observed due to elevation in phytohormone, stomatal opening and ion transport in vesicular arbuscular mycorrhiza (VAM) infected *Citrus aurantium* plants. $P_N$ of the healthy leaves was significantly higher than that of the infected leaves in

each of the three genotypes, when measured periodically with disease progression. The $P_N$ values in infected plants of each genotype declined progressively during the course of experiment. PB1 and IRBB21 showed rapid and substantial reduction in $P_N$ compared to *O. longistaminata.* Photosynthetic down-regulation under bacterial infection as recorded in our present study could be attributed to several factors. Lower activity of Rubisco and carbonic anhydrase (Goodwin et al. 1988; Saeed et al. 1999), reduction in mesophyll conductance to $CO_2$ diffusion (Khairi and Hall, 1976), increase of respiratory and photorespiratory activities (Laisk et al. 1998) and biochemical damages (Ribeiro et al. 2004) were reported to cause loss in photosynthetic activities under BB infection. Some recent reports demonstrated that the reduction of $P_N$ in infected leaves is connected with the decrease of *gs* and also a result of transpiration rate inhibition (Chołuj et al. 2011).

With the down-regulation of $P_N$ in infected leaves, we observed that *gs* and *E* were also concomitantly reduced in all tested genotype when compared to their control counterparts. In comparison to the early stage of infection, the *gs* of infected leaves decreased more drastically at later stage of infection from 48 h of infection till the end of experiment. Our results with BB infected rice genotypes suggest that one of the most probable cause of reduction in $P_N$ of infected plants is loss in *gs*. Petit and co-workers (2006) reported that the simultaneous reduction of $P_N$ and *gs* in symptomatic leaves indicate a global water stress in plant. Pathogens like *Xoo* (as in our experiment) which can move through vascular tissues of plants, enters the xylem vessels and then proliferate within the vessels causing water stress in host plants body enhancing resistance to hydraulic conductance (Tyree et al. 1989). Reduced water conductance results in leaf moisture deficits and cause stomatal closure while down- regulation in *gs* concurrently leads to reduction in $P_N$ (Saeed et al. 1999). As *gs* and *E* are strongly coupled, we observed the same degree of down-regulation in *E* along with *gs*. Our findings are in agreement with the earlier studies where similar reduction in *E* was recorded following pathogen infection (Scholes and Rolfe, 1995; Fleischmann et al. 2005; Guo et al. 2005). Meyer et al. (2001) and Bassanezi et al. (2002) also exhibited that the reduction of *E* in other pathosystems (rust in bean leaves) was directly related to *gs* and therefore it was associated with stomatal closure. As $P_N$ was highly sensitive to disease severity in PB1 and IRBB21, a reduction in *E* could not improve photosynthetic *WUEi* in those genotypes. In infected *O. longistaminata*, as the reduction in

$P_N$ was comparatively less when compared to *E*, hence the photosynthetic *WUEi* was significantly enhanced during the later stage disease progression. Previous reports have suggested that the reduced rate of $P_N$ is also attributed to non-stomatal limiting factors including damage to primary photochemical (inhibited functional activity of PSII and reduced amount of photosynthetic pigments) and biochemical processes (Lawlor, 2002). Besides down-regulation in $P_N$, the concomitant decrease in *gs* and *E* values in our study indicates strong existence of stomatal limitations besides the non-stomatal factors (Shangguan et al. 1999) which was also reported in case of water-stressed in alfalfa and sorghum (Li et al. 2011). Since, infected plants were accompanied by lower *gs* and *E* at the later stage of infection, therefore, stomata in the symptomatic leaves might be largely closed. Unlike other bacterial infection, where high *Ci* values (mostly due to patchy stomata, cuticular transpiration etc) were recorded in plants (Ribeiro et al. 2004), our study clearly indicates the presence of stomatal effect on the reduction of $P_N$. To understand the association of $P_N$ with *gs* and *Ci* in the BB-infected symptomatic rice leaves, we performed linear regression analysis using the variables obtained from infected plants of all three genotypes. In BB-infected condition, the regulation of $P_N$ by *gs* followed a linear function and resulted in a significantly positive correlation between $P_N$ and *gs* for all the rice genotypes. The regression slopes were steeper for *O. longistaminata* when compared to IRBB21. The relationships between $P_N$ vs *Ci* were linear and positively correlated for all the tested rice genotypes. The correlation was strong in IRBB21, however, it was comparatively weak though significant in PB1. Thus from our results, it is evident that apart from existing non-stomatal limiting factors, significant stomatal limitations also coexist in BB-infected rice genotypes which can substantially limit the photosynthetic efficiency of the infected leaves with disease progression.

Comparatively less reduction in the yield traits was recorded in the resistant genotype (IRBB21) under infected condition. Reduction in productive tillers per plant in infected PB1 can be linked to the fact that plant under stress condition could not produce required assimilates due to low photosynthesis and loss in photosynthetically active tissues (Savary et al. 2000). *Xoo* blocks conductive vessels and restricts water conduction and nutrient supply from roots to leaves and shoots. Such function of *Xoo* in BB-infected rice might have contributed in lowering seeds per panicle, panicle weight and increased unfilled

and semi-filled grains as well (Tan et al. 2007). Few biochemical disturbances due to production of pathogen-induced enzymes, toxins and extracellular polysaccharides under stress conditions have also been reported (Tan, 1993). Photosynthesis in rice plants contributes 60–80% of the final carbon content during the grain filling period (Yoshida, 1981) and hence metabolic activity within the grain must coincide with maximum activity of source leaves during the grain-filling stage (Murchie et al. 1999). Since, grain-filling is also closely related to whole plant senescence (Mi et al. 2002; Yang et al. 2006), the control plants of both the genotypes PB1 and IRBB21 showed high 1000-grain weight as compared to infected plants. BB infection reduces the nutrient availability in the diseased tissue because pathogen uses the nutrient which is available for plant leading to reduced grain filling and grain weight in infected rice plants (Reddy et al. 1979). IRBB21 showed less yield loss (18.45%) when compared to PB1 61.75%), and the presence of *Xa21* in IRBB21 might have redeemed/rescued 43.3% of the yield loss. Since a single gene, *Xa21* can reduce yield significantly, pyramiding of multiple resistance genes with varied defense mechanism can even decrease the yield loss further and provide yield stability.

## 5.2 Sequence analysis of BAC clones from chromosome 11 of rice

Since 2005, BAC libraries of Nipponbare have become a vital resource to initiate genomics research in the areas of physical mapping, positional cloning, complex analysis of targeted genomic regions, and analysis of gene structure and function. Present study was carried out in order to investigate the BB resistance genes, their sub-cellular location, nature of distribution and physical position on chromosome 11 pseudomolecule. Predicted protein kinase sequences were also analyzed for their syntenic relationship with other chromosomes and with cereals. We found that there were 213 resistance genes of different classes and 81 were class 5 resistance genes (receptor like kinases) on chromosome 11 of rice. It is reported that most of the class 5 resistance genes were present in small or large clusters. Most BB and rice blast resistance genes are closely associated with clusters of NBS-LRR genes (Goto et al. 2009). Complex clusters of R-genes are common in plant genomes.

The *Xa21* gene family in rice comprises seven homologs within a 230 kb region on chromosome 11(Song et al. 1997). Interestingly, the *I2* gene family of tomato, *RPW8* of *Arabidopsis* and *Mla* of barley have also reported to be present within cluster (Simons et al.

1998; Xiao et al. 2001; Wei et al. 1999, 2002). Resistance gene analogues (RGAs) has also been used to demonstrate clustering of R-genes in a variety of species including soybean (Kanazin et al. 1996), potato (Leister et al. 1996), lettuce (Shen et al. 1998), *Arabidopsis* (Aarts et al. 1998) and rice (Ghazi et al. 2009). Genes within a single cluster can determine resistance to different pathogens. On the basis of clustered distribution and by extrapolation from other cell-cell recognition systems, R-genes were hypothesized to encode functionally and evolutionarily related members of recognition systems (Dangl et al. 1996). Receptor kinases were localized in plasma membrane presumably for recognition of PAMPs and most of the NBS-LRR was cytoplasmic for intracellular recognition of PAMPs. We know that NBS-LRR also stops bacterial pathogenesis through Avr protein recognition, which is secreted through type III secretion system. It has been observed that there was a lot of variation in LRR repeats in receptor kinases which might be providing recognition specificities according to new pathotype. During domain determination we found that some R-genes participate in conferring resistance to specific avirulent strains of *Xanthomonas* without containing kinase domain (Wang et al. 1998).

If R proteins are directly involved in recognition of Avr proteins, then it seems that they are localized to the plasma membrane to intercept the incoming pathogen protein. Unfortunately, there is little information available at present about the localization of most R proteins in the plant cell. Cloned receptor kinases were compared with other reported disease resistance genes of plant and with predicted class 5 R-genes. Tomato *Cf-2* gene has shown maximum identity with *Xa21* and *Xa26* because it also encode for LRR protein kinase. The tomato *Cf-9* gene confers resistance to *C. fulvum* which contain primarily LRR extra-cytoplasmic domain, and the predicted cytoplasmic domain of *Cf-9* contains only 25 amino acids (Jones et al. 1994b). The tomato *Pto* gene, conferring resistance to a bacterial pathogen, encodes a serine/threonine protein kinase, but lacks LRRs (Martin et al. 1993). The rice *Xa21* gene, also conferring resistance to a bacterial pathogen, carries features of both classes, encoding a transmembrane protein kinase with 23 extracellular LRRs domain (Song et al. 1995).

Out of 81 predicted class-5 R genes from chromosome 11 of rice, 25.25% showed significant homology with chromosome 4, which was maximum among all chromosomes of rice which might be due to gene duplication. Whole genome study indicated that gene

duplication has occurred throughout the genome and there are about 7.4% genes in rice which are result of gene duplication (Yu et al. 2005). Based on comparative mapping analysis of closely related grass species, large-scale duplications have been documented in the grass family, probably resulting from polyploidy (Levy and Feldman, 2002). Goff and colleagues (2002) studied the draft sequence of *japonica* rice genome and found that there are around 59% of cDNA markers which had two or more copies and proposed that whole-genome duplication occurred 40–50 MYA and also suggested another duplication involving chromosome 11 and 12 occurred c. 25 MYA. In 2003, Vandepoele group analyzed the *japonica* ssp. BAC sequences generated by IRGSP and claimed that approximately 15% of all rice genes are in duplicated segments, with a major fraction of the duplication associated with chromosome 2. On the basis of this study they proposed that the gene duplication event in rice has occurred 70 MYA. In the year of 2004 when rice sequencing was completed they further asserted the aneuploid hypothesis of rice genome duplication. However, Paterson et al. (2003, 2004) found that much more extensive duplicated regions than previous reports (approx. 61.9% of the rice transcriptome) in the rice genome, involving all rice chromosomes and suggested that an ancient polyploidy rather than aneuploidy occurred before the divergence of the major cereals (c. 70 MYA). On the basis of above inconsistency we can assume that the extent of the duplication, frequency of duplications and the subsequent gene losses after duplication in the rice genome has not been fully investigated. In the present study, we analyzed the most updated sequence data of the genome of the subspecies *japonica* of rice (IRGSP Build5 Pseudomolecules) to analyze the receptor kinase synteny with the monocots and dicots. Predicted protein kinase has shown maximum synteny with wheat EST (27.16%) followed by *Arabidopsis* EST (13.58%). Small rearrangements of genes were considered to be a key factor differentiating the grass genomes (Bennetzen and Ramakrishna, 2002). AC123515-5 and AC123515-10 has shown 100% homology with wheat ESTs while AC123897-15 with maize EST. This might be due to segmental duplication of genes among the cereals. All the above study will help us in identification and isolation of genes in rice as well as in related cereals leading to cloning of disease resistance genes.

## 5.3 Comparative analysis of *Xa21* gene in *indica* and *japonica* ssp.

Comparative analysis of *Xa21* locus revealed that the gene density in *indica* was one gene per 7.85 kb while it was one gene per 8.62 kb in *japonica*, higher than overall gene density of the rice genome which was one gene per 9.9 kb (IRGSP, 2005). The higher density of gene in the vicinity of *Xa21* could be explained in the light of co-localization and clustering of R-genes as reported by Hulbert et al. (2001) and Ghazi et al. (2009). The study of the genomic region encompassing gall midge resistance gene *Gm4* and *Gm5* also provide evidence that putative R-genes are often organize in clusters (Dubey and Chandel, 2010). Few reports suggested that the clustering of R-genes is a result of tandem duplications of paralogous sequences (Richly et al. 2002; Meyers et al. 2003). The annotation of the predicted genes have shown that there were at least four genes which could be putative disease resistance genes in *japonica* in the 100 kb region due to presence of leucine rich repeat domain, whereas there were seven such genes in *indica*. The repetitive structure of LRR coding region favors recombination within the region (Ronald, 1998). *Osin06* and *Osin08* were observed to code for LRR protein kinase but interestingly, these two genes were found to be part of a single gene which had been separated by transposon related gene *Osin07* (Fig.1), probably, by the process of transposition. Comparative sequence analysis showed that a recombination event must have occurred within the coding region of *indica Xa21* allele. Song et al. (1997) had reported the evidence for recombination in the intergenic regions of the *Xa21* gene family members.

Transposon related genes accounted for 50% of the predicted genes in the 100 kb region in *japonica* while it was 42.8% in *indica*. The presence of higher number of TE genes in this region in the two subspecies suggest that this part of the genome might be still actively evolving. We also observed that the co-linearity of genes between the two subspecies within the 100 kb region has also been affected by the presence of transposon related elements. *Xa21* locus is rich in TE and there are 14 transposon like elements in the noncoding region of *Xa21* family members. These *Xa21* associated transposon like elements may play a major role in variability among *Xa21* gene family members and its evolution (Song et al. 1997). ProtCompV 8.0 (http://linux1.softberry.com/berry.phtml) analysis for the sub-cellular location of proteins showed that 80% of the predicted TEs were nuclear in

localization while the remaining were cytoplasmic or mitochondrial in both the rice lines (Table 9). The average GC content of the predicted genes was 47.83% in *indica* and 47.03% in *japonica*. The overall GC content of the 100 kb region was 42.63% in *indica* and 43.52% in *japonica*. This study revealed that GC content varies between the ssp. of the same genus.

The importance of SSR markers has been well documented. They are being used popularly as genetic markers in the study of genetic diversity (Cho et al. 2000), positional cloning (Xiao et al. 1998 and Zou et al. 2000) and marker assisted backcrossing in rice (Mackill, 2007). During the sequence analysis of the 100 kb region of *Xa21* locus, we identified a total of 109 SSRs each in both *indica* and *japonica*. It was observed that the number of repeat motifs decreased as the number of nucleotide increased. An immense variation was observed in the number of SSRs present in the exonic and intronic regions. The percentage of SSRs identified in the genic region was comparatively less than that of the intergenic region. The percentage frequency of SSRs present in the exonic region was only 7.33% and 6.42% of the SSRs identified, respectively in *indica* and *japonica*. The differences between the two subspecies may be the result of species diversification followed by individual evolution of this region; however, the functional significance of these SSRs markers is yet to be determined. An assessment of genes that are associated with microsatellites may also help in deciphering the evolutionary processes of the *Oryza* genome. Based on microsynteny analysis between *japonica* and *indica* subspecies a physical map was generated which covered all of the predicted genes in the 100 kb regions. In both the rice subspecies, *Xa21* was flanked by LRR-encoding gene in the upstream and transposon related gene in the downstream region. In *japonica*, six predicted genes were located in the sense strand and remaining genes were in the antisense strand. Even in *indica*, equal numbers of the predicted genes (7) were present in sense and antisense strand (Fig. 4). Sub-cellular study indicated that most of the LRR coding gene products were localized in the plasma membrane and the transposon proteins in the nucleus as previous. The phylogenetic tree showed that the majority of predicted genes with similar function of both the subspecies were grouped together as per our expectation due to their common ancestry. In cluster I, all the genes encoding the putative LRR protein and transposons of both *indica* and *japonica* subspecies were observed to cluster together. However, *Osjp01* (hypothetical protein) and *Osjp05* (transposon protein) did not cluster with functionally similar protein.

## 5.4 Polymorphism analysis and preparation of expression cassettes

Plant receptor-like kinases (RLKs) are transmembrane proteins with putative amino-terminal extracellular domains and carboxyl-terminal intracellular serine/threonine protein kinase domains (Shiu and Bleecker, 2001). The RLK family is a superfamily in plants with at least 610 members in *Arabidopsis* and approximately 1132 members in rice (Shiu et al. 2004). Through a reiterative process of sequence analysis and re-annotation, we identified 81 receptor kinase genes from chromosome 11 of rice genome (Nipponbare). Eight genotypes were selected based on their varying degree of resistance and primers designed from the exonic regions of receptor kinase genes were used for this study. Amplification of the genomic DNA of rice with receptor kinase primers resulted in mostly one to two bands in 1% agarose gels. Polymorphism was not found within susceptible genotypes. However, all the three genotypes of wild have given polymorphic bands. Most of the wild rice is perennial grass which possesses numerous traits valuable to rice breeding, such as disease and insect resistance, cold and drought tolerance. In our study, we have seen the same pattern because out of 6 polymorphic genes, 5 were from wild genotypes. Moreover, many of the R-genes are species-specific or even cultivar-specific, i.e., they present in only some cultivars and absent from others within a species (Leister et al. 1998). The pattern of polymorphism of our study validates the study of Leister et al. (1998). The present study showed that six R-genes identified are exclusively specific to genotypes suggesting their occurrence after speciation of rice or, more likely, extensive divergence at the nucleotide level at these loci. In either case, it supports the notion that the grass R-genes have evolved at an exceptionally rapid rate compared with most of the other loci of the genomes. It has been observed that most of the R-genes under study possess LRR, NB-ARC and kinase conserved motifs. The homology of R-genes clones isolated in this study varied from 78 % (rust resistance gene, genotype-IRBB3) to 99% (LRR-protein kinase, genotype- *O. longistaminata*) amongst the different classes. Different classes of genes have been previously cloned from *Arabidopsis*, lettuce, maize, potato, rice and soybean using a similar strategy, and a number of these genes have also been mapped onto their respective genetic maps (Aarts et al. 1998; Collins et al. 1998; Kanazin et al. 1996; Leister et al.1998; Shen et al. 1998; Speulman et al. 1998; Yu et al. 1996). The high sequence homology of the rice

amplification products indicates that this strategy could be useful for isolating resistance genes.

Receptor-like kinases comprise a major gene family in plants and control a wide range of biological processes including development, disease resistance, hormone perception and self-incompatibility but the real time functions of most RLKs are unclear. So far, only a limited number of the RLKs have been characterized, such as *NtPK*1 and *NtPK*2 in tobacco (Kumara et al. 2004), *Pto* and *Pti*1 in tomato (Zhou et al. 1995), *CDG*1 in *Arabidopsis* (Muto et al. 2004), *MLPK* in *Brassica* (Murase et al. 2004), *GmPti*1 in soybean (Tian et al. 2004) and *ZmPti* kinases in maize (Herrmann et al. 2006).

We believe that real time analysis about resistance gene will be a good addition to facilitate engineering of genes conferring durable resistance to a broad spectrum of pathogens. Previous reports suggest that different bacterial blight genes have different nature of expression based on host genetic background and pathogen strain. Interestingly, mRNA *of Xa1* was detected in control as well as treated samples of rice leaves at 5 days after inoculation but was not detected in intact and healthy leaves, which suggest that *Xa1* gene might be induced by wounding during pathogen infection (Yoshimura et al. 1998). The RT-PCR analysis of *Xa5* (*TFIIAγ5*)/*xa5* revealed that both the genes are constitutively expressed in stem and roots at similar level from 12 to 96 h in Nipponbare and NBB5 (Jiang et al. 2006). The transgenic study of receptor like kinases (*Xa3/Xa26*) in the genetic backgrounds of Zhonghua 11, Mudanjiang 8 and 02428 showed 3, 242 and 44 fold more *Xa3* transcripts respectively (Cao et al. 2007). Study of another receptor like kinases (*Xa21*) response indicate that *Xa21*-mediated resistance to *Xoo* is not fully expressed in early stages of development, but the resistance also increases further as the plant matures (Century et al. 1999). The *Xa7* was reported to be expressed at higher level in the resistant lines than that of the susceptible ones as expected (Utami et al. 2013). In the present study, expression pattern of two R-genes RGIA (AC133216-2) and RK1 (AC136956-8) in response to BB stress was investigated by qPCR. Under pathogen inoculation both RGIA and RK1 exhibited higher level of transcripts comparing to their control counterpart. Further, gene expression analysis showed that RGIA gene was induced progressively from 0 to 72 h post inoculation followed by slight decline at 96 h post inoculation. It reached the highest expression level at 72 h where transcript level was increased by 4.37 fold. When protein prediction was performed,

we found that RGIA encode a putative stripe rust resistance gene Yr10 whereas RK1 gene encodes receptor kinase. In case of RK1, the maximum induction was observed at 48 h post inoculation. These results indicate that the expression of both genes have induced at transcriptional level. Our results suggest that both genes act as functional gene and may play a role during pathogenesis. Further studies at translational level and especially generation of the loss-of-function alleles, will shed light on the signal transduction during BB attack.

One of the major challenges in plant genetic engineering is to prepare a transformation-cassette that would enable the precise control of transgene activity. The choice of promoter to confer constitutive, spatial and/or temporal transgene expression is one of the key determinants used in plant biotechnology (Jones and Sparks, 2009). In recent years, a wide range of different promoters from plant, viral and bacterial origin have been characterized and used extensively in regulated transgene expression systems in plant cells (Yoshida and Shinmyo, 2000; Lessard et al. 2002). Several plant genetic engineering strategies have incorporated the use of strong constitutive promoters in the study of gene and transcription factor (TF) function as well as for conferring transgene expression for crop improvement and bio-pharmaceutical applications. The well-described cauliflower mosaic virus (CaMV) 35S promoter (Odell et al. 1985; Benfey et al. 1990) has been used most commonly in plants to drive genes and TFs imparting resistance to diseases. In our study, we have cloned RGIA and RK1 gene separately in pCAMBIA1300 and pCAMBIA2300 binary vector which contain CaMV 35S promoter and were mobilized into *Agrobacterium tumefaciens* strain EHA105 for plant transformation. *Agrobacterium tumefaciens* has the unique capability of transferring the T-DNA portion of its large tumor-inducing (Ti) plasmid into the nucleus of infected cells where it integrate into plant cells at infected wound sites, which results in the formation of crown gall phenotype (Chilton et al. 1977). The infection process involves a set of chromosome-encoded genes (*chv*) involved in attachment of bacteria to plant cells and Ti plasmid-encoded *vir* genes that function in trans, helping in the generation, transfer, and integration of T strands into the plant genome. Neomycin phosphotransferase (*npt*), phosphinothricin acetyltransferase (PAT), and hygromycin phosphotransferase is commonly used selection marker for *Agrobacterium*-mediated transformation. In our study hygromycin phosphotransferase (*hpt*) was used as selection marker. Using the above construct, plant transformation work was initiated and fortunately

we were able to get 5 tobacco putative transgenics which were positive with both gene specific and selection marker primers. The rice transformation work has not yet yielded positive transgenics and the work is under progress.

## 5.5 Proteomics approach to understand BB induced genes

With the completion of rice genome sequencing of two major cultivars (*japonica* cv. Nipponbare and *indica* cv. 93–11) rice proteomics has moved into the epoch of functional genomics of the post genome era. Although during the last decade an important but limited progress has been made in the field of rice proteomics, it is only recently the systematic analysis of the rice proteome at the cell, tissue, and whole plant level has progress rapidly. These studies mainly focused on two indices, one is developmental proteomics and other is environmental proteomics in responses to biotic and abiotic stresses (Peck et al. 2001; Agrawal et al. 2006; Hajheidari et al. 2005). Though we have observed contrasting variability in photosynthetic $CO_2$ fixation characteristics between PB1 and *O. longistaminata* during BB disease progression (Kumar et al. 2013), the question arises that what actually happens at the protein level in the infected foliar part? To answer this question, we have analyzed differentially regulated total leaf protein during BB infection. Our results depicted induction of both biotic and abiotic stress related protein during BB infection. Germin like protein (spot=21), Ent-isokaur-15-ene synthase (spot=23), GSH-dependent dehydro ascorbate reductase 1 (spot=19) and Cyclin-dependent kinase C (spot=N-4) were related to biotic stress, whereas putative r40c1 (spot=22) and heat stress transcription factor C-2b (spot=6) were related to abiotic stress tolerance.

Germin like proteins (GLP; spot=21): It is related to generation of active oxygen species and exhibits the activity of superoxide dismutase and increase the accumulation of $H_2O_2$. It belongs to cupin superfamily and has been identified in Thale cress (*Arabidopsis thaliana*), grapevine (*Vitis vinifera*), and many gramineae species (Godfrey et al. 2007; Dunwell et al. 2008). Previous studies outlines that GLPs are involved in common plant defense responses (Lane, 2002). The enhanced expression of GLPs after infection with pathogens, feeding insects along with application of some chemicals such as salicylic acid, H2O2, and ethylene has been reported (Zhang et al. 1995; Wei et al. 1998; Federico et al. 2006; Godfrey et al. 2007). The overexpression of barley GLP has resulted in enhanced

resistance to the powdery mildew whereas silencing augmented susceptibility to the pathogen and herbivore (Zimmermann et al. 2006; Lou and Baldwin, 2006). GLP mediates plant defense mechanism through generation of active oxygen species whereas some members of barley HvGER4 subfamily exhibit SOD activity (Godfrey et al. 2007). In response to pathogen attack, plant produces superoxide by NADPH oxidase or peroxidases. These enzymes are predicted to be dismutated to $H_2O_2$ by the GLP, leading the accumulation of $H_2O_2$ in plants (Bolwell and Wojtaszek, 1997). $H_2O_2$ is a vital component of plant defense which participate in oxidative cross-linking of cell wall proteins and lignin precursors along with papillae formation (Wei et al. 1998). $H_2O_2$ has also reported to be involved in hypersensitive cell death, signaling in systemic acquired resistance, and the induction of disease resistance gene expression (Alvarez et al. 1998). A cluster of 12 highly conserved GLP gene members were predicted within the rice chromosome 8 disease resistance QTL regions. Expression analysis of these genes and promoter analysis suggest that OsGLP family members contribute to defense responses in rice particularly against sheath blight and rice blast. Furthermore, orthologous GLP members in barley and grapevine are involved in basal defense responses (Zimmermann et al. 2006; Godfrey et al. 2007). In our study, germin like protein was induced in *O. longistaminata* by ~ 1.58 fold. Up-regulation of germin like protein was also reported in proteome analysis of rice defense response against *Xoo* induced by probenazole (Lin et al. 2008).

Ent-isokaur-15-ene synthase (spot=23): It catalyzes the conversion of ent-copalyl diphosphate to the phytoalexins precursor ent-isokaur-15-ene. Phytoalexins are diterpenoid secondary metabolites involved in the defense mechanism of the plant and produced in response to pathogens attack, elicitors and UV irradiation. Rice produces a number of phytoalexins in response to *M. grisea* infection (Peters 2006). Previous studies have reported that rice produces more than 10 labdane-related diterpenoids as phytoalexins including momilactones A & B (Cartwright et al. 1981), oryzalexins A-F (Kato et al. 1994), oryzalexin S (Kodama et al. 1992) and phytocassanes A-E (Koga et al. 1997). In addition, momilactone B is constitutively secreted from rice roots and acts as an allelochemical in suppressing germination in nearby seeds (Kato-Noguchi and Ino, 2003). Interestingly, it has been recently reported that barley and wheat similarly contain multiple copies of KS-like genes (Spielmeyer et al. 2004). Consistent with this hypothesis, maize (*Zea mays*) has been

shown to produce labdane-related diterpenes in response to fungal infection (Mellon and West, 1979).

GSH-dependent dehydro ascorbate reductase 1(spot=19): Glutathione transferases (GSTs), formerly known as glutathione S-transferases, are the enzymes involved in cellular detoxification. Different family members of GSTs have been reported in all the organisms, including plants, animals and microbes. Based on amino acid sequence dehydroascorbate reductase (DHAR) has been testified as plant specific GSTs (Edwards and Dixon, 2005). Plant GSTs have been a focus of attention because of its differential regulation by a variety of stimuli generated during abiotic and biotic stresses, such as auxins, cytokinins, ABA, heavy metals, GSH and $H_2O_2$ (Oztetik, 2008). Plant GSTs can also act as glutathione peroxidases and protect cells from oxygen toxicity and suppress apoptosis (Kampranis et al. 2000). Some of plant GSTs were originally identified as auxin and cytokinin binding proteins which have pointed their role in hormone signal transduction pathways as well (Gonneau et al. 1998). Furthermore, microarray analysis of *M. grisea* treated Nipponbare revealed that a large number (34) of GST genes were differentially regulated ($\geq$ 2 fold) (Jain et al. 2010). For ROS scavenging in cells the high ratio of reduced to oxidized ascorbate and GSH is essential. The reduced states of the antioxidants are maintained by glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) using NADPH as reducing power (Tsugane et al. 1999). Therefore, we can assume that DHAR might be involved in controlling the balance amount of antioxidant in the cell during BB stress, which has to be further validated for better understanding. In our study, GSH-dependent dehydro ascorbate reductase 1 was induced by 1.78 fold in highly resistant wild genotype *O. longistaminata*.

Putative r40c1 protein (spot=22): Up-regulation of putative r40c1 protein have been reported in salt stressed rice plant which indicate that this protein is involve in osmotic regulation and provide resistance to osmotic stress. The similar response has been observed in salt-stressed *A. lagopoides,* therefore putative r40c1 protein could be considered as another candidate for improving salt/desiccation tolerance in plants. Putative r40c1 protein enhances tolerance of rice under cadmium (Cd) stress too and it contain lectin domain. In *O. sativa, a* mannose-specific jacalin-related lectin was induced in root and sheaths in response to salt and drought stress (Claes et al. 1990). Lectins were also found to be expressed in

response to jasmonic acid, ABA and fungal infections (Zhang et al. 2000). In banana and maize proteome analysis, up-regulation of euonymus lectin domain was observed in response to osmotic stress (Carpentier et al. 2007). All these observations suggest that lectins play an important role in stress related physiological processes. BB is a typical vascular disease where *Xoo* multiply in the xylem vessels and spread into the other parts of the plant. *Xoo* increases resistance to hydraulic conductance resulting from accumulation of pathogen produced high molecular weight substances in water conducting elements. This leads to leaf moisture deficit and hence low leaf water potential. Therefore, foliar part of plant faces both desiccation and heat stress. The result observed in the present study demonstrates the regulation of both desiccation tolerance putative r40c1 protein (3.25 fold up-regulations) and heat stress transcription factor C-2b (1.58 fold up-regulation). Moreover, up-regulation of these two proteins during BB stress could possibly enhance abiotic tolerance; however, it needs further research for validation.

Cyclin dependent kinase C (spot= N-4): The cyclin-dependent kinases (Cdks) are family of serine/threonine protein kinases which ensure cell progression in an orderly fashion over the different stages of cell division. Plants have developed very well coordinated mechanisms that allow them to adjust their cell cycle in response to environmental cues. Both biotic and abiotic stress stimuli negatively affect plant growth and development through the inhibition of the cell cycle machinery. Salinity inhibits *Arabidopsis* root growth by reducing the pool of dividing cells in the meristem (West et al. 2004). Similarly, in leaves of wheat (*Triticum aestivum*) and maize (*Zea mays*), water stress induces a shortening of the meristem and prolongs cell cycle duration as a result of reduced CDK activity (Granier et al. 2000). On the biotic side, cell cycle activity is inhibited in parsley (*Petroselium crispum*) and tobacco (*Nicotiana tabacum*) cell cultures upon treatment with fungal elicitors (Kadota et al. 2004).

Protein synthesis is one of the major metabolic pathways to be affected by both biotic and abiotic stresses. We observed that a chloroplast translational elongation factor Tu (spot=5) expression level was enhanced after BB infection by 1.52 fold. EF-Tu expression is down-regulated in response to salinity and abscisic acid, but up-regulated in response to low temperature and salicylic acid treatment in pea chloroplasts (Singh et al. 2004). Interestingly, N-terminal part of EF-Tu has been known to comprise active inducer of

defense responses. It also impart roles in heat tolerance (Ristic et al. 1996). Therefore, there is the possibility for application of EF-Tu in developing crop varieties both tolerant to abiotic stress and resistant to disease.

There are evidences to support that photosynthetic pigments related genes and light-reaction genes are down-regulated in response to biotic attack. But there is an exception that ferredoxin (Fd) NADPH oxidoreductase (FNR) is upregulated following pathogen attack (Knaff and Hirasawa, 1991). In our study (spot=2) ferredoxin (Fd) NADPH oxidoreductase was upregulated by 1.5 fold in PB1. In photosynthesis, Fd accepts electrons from PSI and reduces $NADP^+$ via FNR. The transcriptional up-regulation of Fd may reflect its direct participation in pathogen defense. Dayakar et al. (2003) observed an interaction between a ferredoxin-like protein and harpin, where ferredoxin-like protein enhanced the ability of harpin to induce production of active oxygen species to mount a hypersensitive response. It was observed that over-expression of ferredoxin in tobacco conferred resistance to *P. syringae* and *Erwinia carotovora* (Huang et al. 2007). The up-regulation of Fd and FNR gene expression following biotic attack may be related to defense rather than a response of photosynthesis per se.

The RuBisCO large subunits were increased in both susceptible and resistant rice genotypes by 1.95 and 2.20 fold in our study (spot=9 and spot=18 respectively). Activation of RcbL and other photosynthetic related proteins was found to be quite common in stress induced plants which was observed both in susceptible/resistant host and compatible/incompatible interaction (Lin et al. 2008; Yu et al. 2008; Zimaro et al. 2011; Mahmood et al. 2006). RuBisCO activase (spot=8), enzyme catalyzes the activation of RuBisCO (Portis, 1992), was also induced in PB1. The RuBisCO enzyme found in the chloroplast, play an important role in photosynthesis, is known to be reduced in infected plant cells because attack of pathogens lead to degradation of chloroplasts (Agrios, 1997). However, a rice proteomic study showed an increase activity of RuBisCO proteins in leaf sheath by wounding stress (Shen et al. 2003). Moreover, the sampled leaf were mostly green in colour, hence it is quite possible that the chloroplasts of the sampled cells were not yet directly affected by the infection, and photosynthetic activity was increased possibly to compensate for loss in adjacent infected cells. Similarly, triosephosphate isomerase, an enzyme of glycolysis pathway, has also been reported to be involved in abiotic and biotic

stresses, and reduction in its expression has been observed (Riccardi et al. 1998; Morris and Djordjevic, 2001). Nevertheless, the interaction study of tomato and *R. solanacearum* demonstrated the up-regulation of triose phosphate isomerase in susceptible species (Coaker et al. 2004). Interestingly, we also observed that triose phosphate isomerase was upregulated in susceptible PB1 (spot=7) by 1.65 fold. The interaction study between rice and *R. solani* at the molecular level have shown that the glycolytic pathway enzyme triosephosphate isomerase was highly expressed in leaf sheaths of infected rice plants (Mutuku and Nose, 2010). Another glycolytic enzyme, enolase, which catalyze the conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP) was upregulated (spot=3) in PB1. This enzyme has been known to contain various isoforms and was induced by the plant-rhizospheric bacterial interaction (Farinati, et al. 2011). It was reported that enolase also expressed during chilling stress (Lee et al. 2002). Photosystem I complex is an important source of reactive oxygen species (Asada et al. 1974) and the stromal and thylakoid-bound scavenging systems around PSI play an important role in detoxification of ROS (Asada, 1999). The induction of PS1 complex (spot=N2 and N3) may be part of a general defensive physiological adaptation of the cells to BB stress. In susceptible PB1 none of the defense related proteins were induced.

There were some hypothetical proteins which contains conserved domain for disease resistance such as F-box, $H^+$ATPase and peroxiredoxin were significantly up-regulated. The F-box domain is about 50 amino acids that mediate protein–protein interaction. F-box proteins have also been reported to be expressed during panicle and seed developmental stage and therefore appear to be involved in regulating plant growth and development. Wang et al. (2004) have demonstrated the role of F-box during various stress responses like water deficit, salts, wounding, and elicitation. $H^+$-ATPase use energy derived from ATP hydrolysis to pump protons from the cytosol to the extracellular space for maintaining a negative membrane potential and a transmembrane pH gradient (acidic outside). $H^+$ATPase has been recognized as the proton pump to stimulate the hypersensitive response during plant defense responses (Finni et al. 2002). In rice, this protein has not been given much attention with regard to defense responses but Fang Chen group studied the plasma membrane protein of rice after *Xoo* treatment in 2007 and suggested that the rice $H^+$ATPase might play a similar function as a proton pump in defense (Chen et al. 2007).

Peroxiredoxin (Prxs) uses thioredoxin to detoxify hydrogen peroxide produced during stress condition. Prxs exert it protective antioxidant role in cells mainly through its peroxidase activity (Hofmann, 2002). It was reported that peroxiredoxin get up-regulated against *Xoo* in rice and play the role of balancing agent of oxidative stress to secure resistance (Mahmood et al. 2006). Apart from being the protective antioxidant, prxs also perform certain very dynamic function by mediating signaling cascades leading to cell proliferation, differentiation and apoptosis (Fujii et al. 2002).

To put it briefly, the proteomics based study provided an insight into differentially expressed foliar proteins in response to *Xoo* strain DX133 inoculation. Our result clearly demonstrate that both biotic and abiotic stress related proteins such as Germin like protein, Ent-isokaur-15-ene synthase, GSH-dependent dehydro ascorbate reductase 1, Cyclin-dependent kinase C, putative r40c1, heat stress transcription factor C-2b, chloroplast translational elongation factor Tu and ferredoxin (Fd) NADPH oxidoreductase were induced in leaf blades. Primarily, the foliar proteins involved in the stress response, ROS detoxification, energy metabolism, metabolic pathways and cell signaling were differentially expressed during BB infection. Defense, oxidative stress and energy metabolism mechanisms are in active states of functions that support each other to give resistance to rice against bacterial blight disease. This is the first proteomic study report regarding the induction of both biotic and abiotic proteins during *Xoo* infection which possibly explain the activation of common pathway during bacterial blight of rice. How plants regulate the photosynthetic apparatus and increases systemic pathogen resistance during defense however, remains unclear and further studies are required.

# CHAPTER-6
# SUMMARY AND CONCLUSION

Rice is one of the most indispensable food commodities upon which more than three billion people of this world depend (Khush, 2005). Due to the green revolution, a quantum leap in rice yield took place over the past three decades, although increased food production did not eliminate poverty and hunger completely. Worldwide a good number of diseases impede rice production. Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is found throughout the world and is especially endemic to much of Asia and parts of west Africa (Nino-Liu et al. 2006; Latif et al. 2011). Reduction in photosynthesis and down-regulation of $CO_2$ fixation are the most substantial negative impact of BB. Genomics and proteomics based strategies for gene discovery coupled with the validation of transgenes by genetic transformation have accelerated the identification of a functional profile of candidate genes. Cultivated rice has evolved for centuries through domestication, which preferentially narrowed the diversity of genetic resources. It is also important to explore wild rice species and characterize their genes for further use. The rice genome sequences developed by Monsanto, Syngenta, BGI and IRGSP have accelerated gene discovery and rice improvement.

In the present study, we have screened fourteen rice genotypes based on resistance against one of the most virulent strain of *Xoo*, DX133. *O. longistaminata* was highly resistant and PB1 was most susceptible, whereas IRBB21 was intermediate between these two genotypes. Further, three phenotypically different genotypes with differential resistance towards *Xoo* infections were selected for photosynthetic leaf gas exchange analysis. Our results indicated that the leaf gas exchange physiology is widely affected in BB-infected susceptible rice genotypes when compared to the resistant ones. Besides known non-stomatal limitations, stomatal restrictions to $CO_2$, low leaf transpiration rates and less intercellular $CO_2$ concentration clearly play significant roles in the intensity of photosynthetic down-regulation. The resistant genotype IRBB21 which maintained better gas exchange functions also exhibited greater seed filling and grain yield when compared to susceptible PB1. Thus photosynthetic down-regulation could lead to a deprivation of yield and plant vigour affecting crop productivity, demonstrating that the chronic form of bacterial blight infection will lead to carbon starvation and is deleterious for rice. To extend the utility of these results for a better comprehension of the host-pathogen physiological relationships, the quantification of the impact of BB disease should be done using further techniques like

photosynthetic light response curve studies along with chlorophyll fluorescence transients in conjugation with leaf gas exchange analysis. While screening genotypes, besides growth and yield parameters, physiological attributes like photosynthetic rate and associated leaf gas exchange characterization should also be given due consideration.

Once we have studied the resistance of individual genotypes towards *Xoo* which was also validated by photosynthetic leaf gas exchange physiology, the next task was to look for candidate receptor kinase gene(s) for polymorphism survey and expression cassette preparation. In order to do so, we studied 130 BAC/PAC clones out of 255 present on chromosome 11 pseudomolecule of rice. A total of 213 disease resistance genes were predicted, out of which 81 were receptor like kinases. Sub-cellular localization study revealed that most of the LRR-protein kinase genes were localized in the plasma membrane (77%) which indicates that LRR-protein might be involved in recognition of pathogen's elicitors, which is in support of the already established hypothesis of LRR protein function. Physical mapping of these genes suggests that they were distributed in clusters along the chromosome. The average GC content of the predicted R genes was 45.97%. The highest GC content was 49.37% in protein kinase among all classes of predicted R genes. We performed the domain prediction of only protein kinase and LRR protein kinase genes (class-5 disease resistance gene) and we found that the most of the genes contained multiple domains. One gene (AC109365-12; on long arm of chr11 at 90.1cM) contained salt stress response/antifungal conserve domain along with protein kinase catalytic domain. There was 5% class-5 resistance genes correctly assigned as single domain gene, 18% assigned as two-domain gene and 77% were assigned as three or multiple domain. Comparison of *Xa21* and *Xa26* with known R genes confirmed that *Cf-2* was found to have maximum query coverage and identity of 58% and 38% with *Xa21* and 67% and 36% with *Xa26* respectively. When we performed the homology study of predicted receptor kinase, we found that AC109644.2 (LRR-PK) showed maximum homology with *Xa21* (QC=97%; identity=96%; e-value = 0.0), whereas AC146937-16 (PK) showed maximum homology with *Xa26* (QC=97%; identity=96%; e-value = 0.0). Class-5 disease resistance genes exhibited significant homology with chromosome 4, which was maximum among all chromosomes, whereas, among cereals, wheat ESTs exhibited maximum homology. Therefore, R-genes described in this study can be used to investigate new gene in other cereals. Comparative analysis of

*Xa21* flanking region in *indica* and *japonica* is expected to facilitate cloning and expression analysis of candidate resistance genes present in the vicinity of *Xa21* gene and decipher their role in *Xa21* mediated resistance. One has to elucidate the co-expressed gene and its role in broad spectrum disease resistance. The disease resistance genes described in this study can be used as a probe to identify novel R gene or its homologues. The predicted chromosomal location of genes can be employed in the successive breeding program through deployment of functional markers. Sequence comparison of the disease resistance genes under study may give some clues regarding evolution of plant disease resistance. Through the prediction of protein sub-cellular localization, we found that 34.6% (9 genes) genes coding for transposon related elements and these genes were localized in the nucleus and 23% (6 genes) were localized in the plasma membrane and majority of those were coding for leucine rich repeats. *Xa21* belongs to receptor kinase class of disease resistance gene with great potential against bacterial blight; hence, further molecular effort is required for its complete elucidation. It is a significant gene from socio-economic point of view for sustainable management of bacterial blight and to combat hunger across the world.

Subsequently, gene specific primers were designed from the exonic region of receptor like kinases predicted from the 2$^{nd}$ part of present study and polymorphism analysis was performed with eight rice genotypes screened from the first part of the work. Six major polymorphic amplicons were identified and submitted to NCBI. Interestingly, most of the wild rice genotypes possess numerous traits valuable to rice breeding. In our study, we have the similar pattern of amplification in all genotypes. Out of six polymorphic genes, five were from wild genotypes and most of them were species-specific. The expression pattern of the two R genes (RGIA and RK1) in response to BB stress was induced progressively from 0 to 72 h post inoculation and exhibited highest expression level at 72 h (~ 4.37 fold) and at 48 h (~2.05 fold), respectively. Our results suggest that both of the genes were functional at transcripts level. Further studies are required for functional characterization at translational level. Both of these genes were further used for designing plant transformation cassette and *Agrobacterium tumefaciens* strain *EHA105* was employed to transform rice and tobacco. Now both kinds of transformation cassettes are available in our laboratory for further transgenic experiments.

To gain insights into the molecular mechanisms underlying activation of complex plant defense responses that occur in resistant and susceptible rice genotypes challenged with *Xoo*, a time-course protein profiling experiment was conducted. Our results revealed a clear differentiation between resistant and susceptible interactions and led to the identification of biotic and abiotic stress regulated proteins potentially involved during *Xoo* infection. Candidates conferring BB tolerance include germin-like protein, putative r40c1, Cyclin dependent kinase c, Ent-isokaur-15-ene synthase and GSH-DHAR1. Hypothetical with conserve domain for disease resistance were F-box (OsDRF1), peroxiredoxin (PRX) and $H^+$ATPase. Along with defense and stress, energy metabolism, ROS detoxification and cell signaling proteins were also differentially expressed. Determining the changes in protein expression patterns elicited by *Xoo* in a resistant and susceptible genotype of rice has not only led to identification of key molecular components of this specific host-pathogen interaction, but also has open the way for developing genetic markers that could be used to screen BB resistant rice genotypes. Such studies may also open novel avenues for engineering durable resistance to this pathogen. The present study is the first step in identifying the defense-related proteins that are required for mounting a successful defense response in rice against *Xoo*. Looking at functional relationships between different genes-of-interest using transgenic over-expression will probably be the next step towards characterization of these proteins.

# BIBLIOGRAPHY

Aarts MGM, Hekkert BL, Holub EB, Beynon JL, Stiekema WJ, Pereira A (1998) Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol Plant-Microbe Interact 11:251–258.

Agrawal GK, Rakwal R (2011) Rice proteomics: a move toward expanded proteome coverage to comparative and functional proteomics uncovers the mysteries of rice and plant biology. Proteomics 11:1630–1649.

Agrawal GK, Rakwal R, Yonekura M, Kubo A, Saji H (2002) Proteome analysis of differentially displayed proteins as a tool for investigating ozone stress in rice (*Oryza sativa* L.) seedlings. Proteomics 2:947–959.

Agrawal, GK, Rakwal R (2006) Mass Spectrom. Rev 25:1–53.

Agrios GN (1997) Plant Pathology. Academic Press; San Diego.

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphism of simple sequence repeat DNA in soybean. Genetics 132:1131–1139.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410.

Alvarez M, Pennell R, Meijer P, Ishikawa A, Dixon R, Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92:773–784.

Asada K, Kiso K, Yoshikawa K (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J Biol Chem 249:2175–2181.

Asada K (1999) The water-water cycle in chloroplast: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639.

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63: 3523–43.

Ayliffe MA, Lagudah ES (2004) Molecular genetics of disease resistance in cereals. Ann Bot 94:1–9.

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localisation of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet 106:353–362.

Bassanezi RB, Amorim L, Bergamin FA, Berger RD (2002) Gas exchange and emission of chlorophyll fluorescence during the monocycle of rust, angular leaf spot and anthracnose on bean leaves as a function of their trophic characteristics. J Phytopathol 150:37–47.

Bastiaans L (1991) Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. Phytopathol 81:611–615.

Bhasin H, Bhatia D, Raghuvanshi S, Lore JS, Sahi GK, Kaur B, Vikal Y, Singh K (2012) New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa38* of rice. Mol Breed 30:607–611.

Benfey PN, Chua NH (1990) Regulated genes in transgenic plants. Science 244:174–81.

Bennetzen JL, Ramakrishna W (2002) Numerous small rearrangements of gene content, order and orientation differentiate grass genomes. Plant Mol Biol 48:821–827.

Bevan M, Bancroft I, Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W, Van Staveren M, Stiekema W et al (1998) Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature 391:485–488.

Bingham IJ, Walters DR, Foulkes MJ, Paveley ND (2009) Crop traits and the tolerance of wheat and barley to foliar disease. Ann Applied Biol 154:159–173.

Bolwell G, Wojtaszek P (1997) Mechanism for the generation of reactive species of oxygen species in plant defence: a broad perspective. Physiol Mol Plant Pathol 51:347–366.

Botella MA, Coleman MJ, Hughes DE, Nishimura MT, Jones JDG, Somerville SC (1997) Map positions of 47 *Arabidopsis* sequences with sequence similarity to disease resistance genes. Plant J 12:1197–1211.

Bozso Z, Maunoury N, Szatmari A, Mergaert P, Ott PG, Zsiros LR, Szabo E, Kondorosi E, Klement Z (2009) Transcriptome analysis of a bacterially induced basal and hypersensitive response of *Medicago truncatula.* Plant Mol Biol 70:627–646.

Bradbury JF (1984) Genus II. *Xanthomonas* Dowson. In: Bergey's Manual of Systematic Bacteriology (Krieg, N.R. and Holt, J.G., eds),Baltimore: Williams & Wilkins, pp. 199–210.

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72**:**248–254.

Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35:35–47.

Brar DS, Khush GS (2002) Transferring gene from wild species into rice. Quantitative Genet, Genomic Plant Breed, (ed. M.S. Kang), 14:197–217.

Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP et al. (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12:2033–45.

Burdon JJ (1987) Diseases and Plant Population Biology. Cambridge University Press, Cambridge. pp. 208.

Cao Y, Ding X, Cai M, Zhao J, Lin Y, Li X, Xu C, Wang S (2007) The expression pattern of a rice disease resistance gene *Xa3/Xa26* is differentially regulated by the genetic backgrounds and developmental stages that influence its function. Genetics 177:523–533.

Carels, Bernardi G (2000) The compositional organization and the expression of the *Arabidopsis* genome. FEBS Lett 472:302–306.

Carpentier SC, Witters E, Laukens K, Van Onckelen H, Swennen R, Panis B (2007) Banana (*Musa* spp.) as a model to study the meristem proteome: acclimation to osmotic stress. Proteomics 7:92–105.

Cartwright DW, Langcake P, Pryce RJ, Leworthy DP, Ride JP (1981) Isolation and characterization of two phytoalexins from rice as momilactones A and B. Phytochemistry 20:535–537.

Casimaro I, Marchant A, Bhalerao RP, Beeckman T et al. (2001) Auxin transport promotes *Arabidopsis* lateral root initiation. Plant Cell 13:843–852.

Century KS, Lagman RA, Adkisson M, Morlan J, Tobias R, Schwartz K, Smith A, Love J, Ronald PC, Whalen MC (1999) Developmental control of *Xa21* mediated disease resistance in rice. Plant J 20:231–236.

Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rice. Euphytica 25:435–444.

Chen F, Yuan Y, Li Q, He Z (2007) Proteomic analysis of rice plasma membrane reveals proteins involved in early defense response to bacterial blight. Proteomics 7:1529–1539.

Chilton MD, Drummond MH, Merlo DJ, Sciaky D, Montoya AL, Gordon MP, Nester EW (1977) Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. Cell 11:263–271.

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:713–722.

Chołuj D, Moliszewska EB (2011) The influence of *Aphanomyces cochlioides* on selected physiological processes in sugar beet leaves and yield parameters. Eur J Plant Pathol 132:59–70.

Chou KC and Elrod DW (1999) Protein sub-cellular location prediction. Protein Eng 12:107–118.

Claes B, Dekeyser R, Villarroel R, Van Den Bulcke M and Bauw G et al. (1990) Characterization of rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell 2:19–27.

Coaker GL, Willard B, Kinter M, Stockinger EJ, Francis DM (2004) Proteomic analysis of resistance mediated by Rcm2.0 and Rcm5.1, two loci controlling resistance to bacterial canker of tomato. Mol Plant-Microbe Interact 17:1019–1028.

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance analogs in maize. Mol Plant-Microbe Interact 11:968–978.

Condit R, Hubbel SP (1991) Abundance and DNA sequence of two base repeat regions in tropical tree genomes. Genome 34:66-71.

Cramer HH (1967) Plant protection and world crop production. Pflanzenschutz Nachr. Bayer 20:1–524.

Cuc LM, Mace ES, Crouch JH, Quang VD, Long TD, Varshney RK (2008) Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol 8:55.

Curtis LC (1943) Deleterious effects of guttated fluid on foliage. Am J Bot 30:778–781.

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defense responses to infection. Nature 411:826–833.

Dangl JL, Dietrich RA, Richberg MH (1996) Death don't have no mercy: Cell death programs in plant-microbe interactions. Plant Cell 8:1793–1807.

Dayakar BV, Lin HJ, Chen CH et al. (2003) Ferredoxin from sweet pepper (*Capsicum annuum* L.) intensifying harpin (pss)-mediated hypersensitive response shows an enhanced production of active oxygen species (AOS). Plant Mol Biol 51:913–924.

Deng Z, Huang S, Ling P, Chen C, Yu C, Weber CA, Moore GA, Gmitter FG Jr. (2000) Cloning and characterization of the NBS-LRR class resistance-gene candidate sequence in citrus. Theor Appl Genet 101:814–822.

Ding C, You J, Wang S, Liu Z, Li G, Wang Q, Ding Y (2012) A proteomic approach to analyze nitrogen and cytokinin-responsive proteins in rice roots. Mol Biol Rep 39:1617–1626.

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD (1998) The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic in leucine-rich repeat copy number. Plant Cell 10:1915–1925.

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG (1996) The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459.

Dokku P, Das KM, Rao GJN (2013) Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. Euphytica 192:87–96.

Dubey M, Chande G (2010) *In silico* survey and characterization of Resistance Gene Analogues (RGAs) in the genomic regions encompassing gall midge resistance genes *Gm4* and *Gm5* in rice (*Oryza sativa* L.). Plant Omics J 3(5):140–148.

Dunwell JM, Gibbings JG, Mahmood T, Naqvi SMS (2008) Germin and germin-like proteins: evolution, structure, and function. Crit Rev Plant Sci 27:342–375.

Edwards R, Dixon DP (2005) Plant glutathione transferases. Methods Enzymol 401:169–186.

Eguiarte LE, Castillo A, Souza V (2003) Molecular and genomic evolution in angiosperms. Interciencia 28:141–147.

Fang Chen, Qun Li and Zuhua He (2007) Proteomic Analysis of Rice Plasma Membrane associated Proteins in Response to Chitooligosaccharide Elicitors. J Integr Plant Biol 49:863−870.

FAO (2004) International Year of Rice. Food and Agriculture Organization.

FAO (2009) The state of food and agriculture. Food and Agriculture Organization.

FAO (2011) Food and Agriculture Organization of the United Nations.

Fan W, Cui W, Li X, Chen S, Liu G, Shen S (2011) Proteomics analysis of rice seedling responses to ovine saliva. J Plant Physiol 168:500–509.

Farinati S, DalCorso G, Panigati M, Furini A (2011) Interaction between selected bacterial strains and *Arabidopsis halleri* modulates shoot proteome and cadmium and zinc accumulation. J Exp Bot 62:3433–3447.

Federico M, Iniguez-Luy F, Skadsen R, Kaeppler H (2006) Spatial and temporal divergence of expression in duplicated barley germin-like protein-encoding genes. Genetics 174:179–190.

Feuillet C, Keller B (2002) Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. Ann Bota 89:3–10.

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. Plant J 11:45–52.

Finni C, Andersen CH, Borch J, Gjetting S, Christensen AB, de Boer AH, Thordal-Christensen H, Collinge DB (2002) Do 14-3-3 proteins and plasma membrane H+-AtPases interact in the barley epidermis in response to the barley powdery mildew fungus? Plant Mol Biol 49:137–147.

Fleischmann F, Koehl J, Portz R, Beltrame AB, Osswald W (2005) Physiological changes of Fagus sylvatica seedlings infected with *Phytophthora citricola* and the contribution of its elicitin 'Citricolin' to pathogenesis. Plant Biol 7:650–658.

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296.

Fluhr R (2001) Sentinels of disease: Plant resistance genes. Plant Physiol 127:1367–1374.

Fujii J, Ikeda Y. (2002) Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. Redox Rep 7:123–130.

Galtier N (2003) Gene conversion drives GC content evolution in mammalian histones. Trends Genet 19:65–8.

Ghazi IA, Srivastava PS, Dalal V, Gaikwad K, Singh AK , Sharma TR, Singh NK and Mohapatra T (2009) Physical mapping, expression analysis and polymorphism survey of resistance gene analogues on chromosome 11 of rice. J Biosci 34:251–261.

Godfrey D, Able A, Dry I (2007) Induction of a grapevine germin-like protein (VvGLP3) gene is closely linked to the site of Erysiphe necator infection: a possible role in defense? Mol Plant Microbe Interact 20:1112–1125.

Goff SA, Ricke D, Lan TH, Presting G, Wang R et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100.

Gomez-Gomez L, Bauer Z, Boller T (2001) Both the extracellular leucine-rich repeat domain and the kinase activity of FSL2 are required for flagellin binding and signaling in *Arabidopsis*. Plant Cell 13:1155–63.

Gonneau M, Mornet R, Laloue M (1998) A Nicotiana plumbaginifolia protein labeled with an azido cytokinin agonist is a glutathione S-transferase. Physiol Plant 103:114–124.

Goodwin PH, De Vay JE, Meredith CP (1988) Physiological responses of *Vitis vinifera* cv. 'Chardonnay' to infection by the Pierce's disease bacterium. Physiol Mol Plant Pathol 32:17–32

Goto T, Matsumoto T, Furuya N,Tsuchiya K, Yoshimura A (2009) Mapping of Bacterial Blight Resistance Gene *Xa11* on Rice Chromosome 3. Jpn Agri Res Quart 43:221–225.

Granier C, Inze D, Tardieu F (2000) Spatial distribution of cell division rate can be deduced from that of p34(cdc2) kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. Plant Physiol 124:1393–1402.

Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, Yang F, Chu Z, Wang GL, White FF, et al. (2005) R gene expression induced by a type-III effector triggers disease resistance in rice. Nature 435:1122–1125.

Guo YP, Guo DP, Peng Y, Chen JS (2005) Photosynthetic responses of radish (*Raphanus sativus* var. *longipinnatus*) plants to infection by turnip mosaic virus. Photosynthetica 43: 457–462

Hajheidari M, Abdollahian-Noghabi M, Askari H, Heidari M, Sadeghian SY et al. (2005) Proteome analysis of sugar beet leaves under drought stress. Proteomics, 5:950–960.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Harushima Y, Yano M, Shomura A, Sato M, Shimano M et al. (1998) A high-density rice genetic linkage map with 2275 markers using a single $F_2$ population. Genetics 148:479–494.

Hashiguchi A, Ahsan N, Komatsu S (2010) Proteomics application of crops in the context of climate changes. Food Res Int 43:1803–1813.

Herrmann MM, Pinto S, Kluth J, Wienand U, Lorbiecke R (2006) The PTI1-like kinase ZmPti1a from maize (*Zea mays* L.) co-localizes with callose at the plasma membrane of pollen and facilitates a competitive advantage to the male gametophyte. BMC Plant Biol 6:22.

Hofmann B, Hecht H, Flohé L (2002) Peroxiredoxins. Biol Chem 383:347–364.

Huang HE, Ger MJ, Chen CY, Pandey AK, Yip MK, Chou HW, Feng TY (2007) Disease resistance to bacterial pathogens affected by the amount of ferredoxin-I protein in plants. Mol Plant Pathol 8:129–137.

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopatho l39:285–312.

Hüttel B, Winter P, Weising K, Choumane W, Weigand F, Kahl G (1999) Sequence-tagged microsatellite-site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217.

Iqbal MJ, Yaegashi S, Ahsan R, Shopinski KL, Lightfoot DA (2005) Root response to *Fusarium solani* f. sp . glycines: temporal accumulation of transcripts in partially resistant and susceptible soybean. Theor Appl Genet 110:1429–1438.

IRGSP (2005) The map-based sequence of the rice genome. Nature 436:793–800.

IRRI: International Rice Research Institute (2002) Standard Evaluation System for Rice.

Jain M, Challa G, Bhattacharjee A (2010) Comprehensive expression analysis suggests overlapping and specific roles of rice glutathione S-transferase genes during development and stress responses. BMC genomics, 11–73.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back review. Trends Ecol Evol 11:424–429.

Jesus de WC, Jr do Vale FXR, Coelho RR, Hau B, Zambolim L, Costa LC, Filho AB (2001) Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathol 91:1045–1053.

Jiang GH, Xia ZH, Zhou YL, Wan J, Li DY, Chen RS, Zhai WX, Zhu LH (2006) Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog TFIIAgamma1. Mol Genet Genomics 275:354–366.

Johal GS, Briggs SP (1992) Reductase activity encoded by the *HM1* disease resistance gene in maize. Science 258:985–987.

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG (1994a) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793.

Johnson CR (1984) Phosphorous nutrition on mycorrhizal colonization, photosynthesis, growth and nutrient composition of *Citrus aurantium*. Plant Soil 80:35–42.

Jones HD, Sparks CA (2009) Promoter sequences for defining transgene expression. In Transgenic Wheat, Barley and Oats: Production and Characterization Protocols.171–182.

Jones JDG (1994b). Paranoid plants have their genes examined. Curr Biol 4:749–751.

Jones JDG (2001) Putting knowledge of plant disease resistance genes to work. Curr Opin Plant Biol 4:281–287.

Kadota Y, Watanabe T, Fujii S, Higashi K, Sano T, Nagata T, Hasezawa S, Kuchitsu K (2004) Crosstalk between elicitor-induced cell death and cell cycle regulation in tobacco BY-2 cells. Plant J 40:131–142.

Kalendar R, Lee D, Schulman AH (2009) FastPCR Software for PCR Primer and Probe Design and Repeat Search. Genes, Genomes and Genomics 3:1–14.

Kampranis SC, Damianova R, Atallah M, Toby G, Kondi G, Tsichlis PN, Makris AM (2000) A novel plant glutathione S-transferase/peroxidase suppresses Bax lethality in yeast. J Biol Chem 275:29207–29216.

Kanazin V, Frederick ML, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11746–11750.

Kato H, Kodama O, Akatsuka T (1994) Oryzalexin F, a diterpenes phytoalexin from UV-irradiated rice leaves. Phytochemistry 36:299–301.

Kato-Noguchi H, Ino T (2003) Rice seedlings release momilactone B into the environment. Phytochemistry 63:551–554.

Kauffman HR, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 57:537–541.

Kaur R, Grewal N, Das A, vikal Y, Singh J, Bharaj TS, Sdhu JS, Singh K (2006) Inheritance of bacterial blight resistance in two accessions of wild rice, *Oryza nivara*. Rice Genet Newsl 22:78–82.

Khush GS, Mackill DJ, Sidhu GS (1989) Breeding rice for resistance to bacterial blight. Bacterial Blight of Rice. Proceedings of the International Workshop on Bacterial Blight Rice, IRRI, Manila, Philippines 207–17.

Khush GS, Kinoshita T (1991) Rice karyotype, marker genes and linkage groups. In: Rice biotechnology (Khush GS and Toenniessen GH, eds). Wallingford, England: CAB International 83–108.

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34.

Khush GS (2005) What it will take to Feed 5.0 Billion Rice consumers in 2030. Plant Mol Biol 59:1–6.

Khairi MMA, Hall AE (1976) Temperature and humidity effects on net photosynthesis and transpiration of Citrus. Physiol Plant 36:29–34.

Kim ST, Cho KS, Yu S, Kim SG, Hong JC, Han CD *et al.* (2003) Proteomic analysis of differentially expressed proteins induced by rice blast fungus and elicitor in suspension-cultured rice cells. Proteomics 3:2368–2378.

Kinoshita T (1995) Report of committee on gene symbolization, nomenclature and linkage group. Rice Genet Newsl 12:9–53.

Klein PE, Klein RR, Vrebalov J, Mullet JE (2003) Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 reveals extensive conservation of gene order and one major chromosomal rearrangement. Plant J 34:605–621.

Knaff DB, Hirasawa M (1991) Ferrodoxin-dependent chloroplast enzymes. Biochim Biophys Acta 1056:93–125.

Kodama O, Li WX, Tamogami S, Akatsuka T (1992) Oryzalexin-S, a novel stemarane-type diterpene rice phytoalexin. Biosci Biotechnol Biochem 56:1002–1003.

Koga J, Ogawa N, Yamauchi T, Kikuchi N, Ogasawara N, Shimura M (1997) Functional moiety for the antifungal activity of phytocassane E, a diterpene phytoalexin from rice. Phytochemistry 44:249–253.

Koller A, Washburn MP, Lange BM, Andon NL, Deciu C, Haynes PA, Hays L, Schieltz D, Ulaszek R, Wei J et al. (2002) Proteomic survey of metabolic pathways in rice. Proc Natl Acad Sci USA 99:11969–11974.

Komatsu S, Hirano H (1993) Protein kinase activity and protein phosphorylation in rice (*Oryza sativa* L.). Plant Sci 94:127–137.

Konishi H, Ishiguro K, Komatsu SA (2001) Proteomics approach towards understanding blast fungus infection of rice grown under different levels of nitrogen fertilization. Proteomics 1:1162–1171.

Kumara D, Carlos C, Takeshi T, Tetsu N, Naoko N, Shigemi N (2004) Molecular cloning, functional expression and characterization of two serine/threonine-specific protein kinases from *Nicotiana tabacum* pollen. Sex Plant Reprod 17:165–175.

Kumar A, Guha A, Bimolata W, Reddy AR, Laha GS, Sundaram RM, Pandey MK, Ghazi IA (2013) Leaf gas exchange physiology in rice genotypes infected with bacterial blight: An attempt to link photosynthesis with disease severity and rice yield. Aust J Crop Sci 7:32–39.

Laha S, Tansel B, Ussawarujikulchai A (2009) Surfactant-soil interactions during surfactant-amended remediation of contaminated soils by hydrophobic organic compounds: a review. J. Environ Manage 90:95–100.

Laisk A, Rasulov BH, Loreto F (1998) Thermo inhibition of photosynthesis as analyzed by gas exchange and chlorophyll fluorescence. Russ J of Plant Physiol 45:412–21.

Lane B (2002) Oxalate, germins, and higher-plant pathogens. IUBMB Life 53:67–75.

Largercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115.

Latif MA, Badsha MA, Tajul MI, Kabir MS, Rafii MY, Mia MAT (2011) Identification of genotypes resistant to blast, bacterial leaf blight, sheath blight and tungro and efficacy of seed treating fungicides against blast disease of rice. Sci Res Essays 6:2804–2811.

Lawlor D (2002) Limitation of photosynthesis in water stressed leaves. Stomatal metabolism and the role of ATP. Ann Bot 89:871–885.

Lee SW, Han SW, Sririyanum M, Park CJ, Seo YS, Ronald PC (2009) A type I-secreted, sulfated peptide triggers *XA21*-mediated innate immunity. Science 326:850–853.

Lee H, Guo Y, Ohta M, Xiong L, Stevenson B, Zhu JK (2002) LOS2, a genetic locus required for cold-responsive gene transcription encodes a bi-functional enolase. EMBO Journal 21:2692–2702.

Lee SY, Seo JS, Rodriguez-Lanetty M, Lee DH (2003) Comparative analysis of super families of NBS-encoding disease resistance gene analogs in cultivated and wild apple species. Mol Genet Genomics 269:101–108.

Leigh JA, Coplin DL (1992) Exopolysaccharides in plant–bacterial interactions. Annu Rev Microbiol 46:307–346.

Leister DJ, Kurth DA, Laurie M, Yano T, Sasaki K, Devos A, Graner, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA 95:370–375.

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14:421–429.

Lessard PA, Kulaveerasingam H, York GM, Strong A, Sinskey AJ (2002) Manipulating gene expression for the metabolic engineering of plants. Metab Eng 4:67–79.

Levy AA, Feldman M (2002) The impact of polyploidy on grass genome evolution. Plant Physiol 130:1587–1593.

Li WR, Zhang SQ, Shan L, Eneji AE (2011) Changes in root characteristics, gas exchange and water use efficiency following water stress and rehydration of alfalfa and sorghum. Aust J Crop Sci 5:1521–1532.

Lin YZ, Chen HY, Kao R, Chang SP, Chang SJ, Lai EM (2008) Proteomic analysis of rice defense response induced by probenazole. Phytochemistry 69:715–728.

Lin SK, Chang MC, Tsai YG, Lur HS (2005) Proteomic analysis of the expression of proteins related to rice quality during caryopsis development and the effect of high temperature on expression. Proteomics 5:2140–2156.

Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q (1996) Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathol 86:1156–1159.

Liu J, Liu X, Dai L, Wang G (2007) Recent Progress in Elucidating the Structure, Function and Evolution of Disease Resistance Genes in Plants. J of Genet Genomics 34:765–776.

Lou Y, Baldwin I (2006) Silencing of a germin-like gene in *Nicotiana attenuata* improves performance of native herbivores. Plant Physiol 140:1126–1136.

Ma J, Bennetzen JL (2004) Rapid recent growth and divergence of rice nuclear genomes. Proc Natl Acad Sci USA 101:12404–12410.

Mackill DJ (2007) Molecular markers and marker-assisted selection in rice. In: R K Varshney and R Tuberosa (ed) Genomics assisted crop improvement. Genomics Appl Crops 2:147–168.

Mahmood T, Jan A, Kakishima M, Komatsu S (2006) Proteomic analysis of bacterial blight defense-responsive proteins in rice leaf blades. Proteomics 6:6053–6065.

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley S (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436.

McDowell JM, Woffenden BJ (2003) Plant disease resistance genes: recent insights and potential applications. Trends Biotechnol 21:178–183.

Mellon JE, West, CA (1979) Diterpene biosynthesis in maize seedlings in response to fungal infection. Plant Physiol 64:406–410.

Mew TW (1987) Current status and future prospects of research on bacterial blight of rice. Ann Rev Phytopathol 25:359–382.

Mew TW, Alvarez AM, Leach JE, Swings J (1993) Focus on bacterial blight of rice. Plant Dis 77:5–12.

Meyer S, Saccardy-Adji K, Rizza F, Genty B (2001) Inhibition of photosynthesis by *Colletotrichum lindemuthianum* in bean leaves determined by chlorophyll fluorescence imaging. Plant, Cell and Environ 24:947–955.

Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J 20:317–332.

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell 15:809–834.

Mi G, Tang L, Zhang F, Zhang J (2002) Carbohydrate storage and utilization during grain filling as regulated by nitrogen application in two wheat cultivars. J Plant Nutr 25:213–229.

Mishra NP, Mishra RK, Singhal GS (1993) Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. Plant Physiol 102:903–910.

Monna L, Ohta R, Masuda H, Koike A, Minobe Y (2006) Genome-wide searching of single-nucleotide polymorphisms among eight distantly and closely related rice cultivars (*Oryza sativa* L.) and a wild accession (*Oryza rufipogon* Griff.). DNA Res 13:43–51.

Moore G, Devos KM, Wang Z, Gale MD (1995) Cereal genome evolution: grasses, line up and form a circle. Curr Biol 5:737–739.

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182.

Morris AC, Djordjevic MA (2001) Proteome analysis of cultivar-specific interactions between *Rhizobium leguminosarum* biovar trifolii and subterranean clover cultivar Woogenellup. Electrophoresis 22:586–598.

Mudgett MB (2005) New insights to the function of phytopathogenic bacterial type III effectors in plants. Annu Rev Plant Biol 56:509–531.

Murase K, Shiba H, Iwano M, Che FS, Watanabe M, Isogai A, Takayama S (2004) A membrane anchored protein kinase involved in Brassica self-incompatibility signaling. Science 303:1516–1519.

Murchie EH, Chen Y-Z, Hubbart S, Peng S, Horton P (1999) Interactions between senescence and leaf orientation determine in situ patterns of photosynthesis and photoinhibition in field-grown rice. Plant Physiol 119:553–64.

Murray HG, Thompson WF (1980) Rapid isolation of high molecular weight DNA. Nucleilc Acids Res 8:4321-4325.

Muto H, Yabe N, Asami T, Hasunuma K, Yamamoto KT (2004) Overexpression of constitutive differential growth 1 gene, which encodes a RLCKVII-subfamily protein kinase, causes abnormal differential and elongation growth after organ differentiation in *Arabidopsis*. Plant Physiol 136:3124–3133.

Mutuku M, Nose A (2010) Rhizoctonia solani infection in two rice lines increases mRNA expression of metabolic enzymes genes in glycolytic, oxidative pentose phosphate pathways and secondary metabolism. Trop Agric Dev 54:119–131.

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant MiRNA contributes to antibacterial resistance by repressing auxin signalling. Science 312:436–439.

Natrajkumar P, Sujatha K, Laha GS, Srinivasarao K, Mishra B et al. (2012) Identification and fine-mapping of *Xa33* a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology 102:222–228.

Nino-Liu DO, Ronald PC, Bogdanove AJ (2006) *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol Plant Pathol 7:303–324.

Noda T, Kaku H (1999) Growth of *Xanthomonas oryzae* pv. *oryzae* in planta and in guttation fluid of rice. Ann Phytopathol Soc Japan 65: 9–14.

Noir S, Combes MC, Anthony F, Lashermes P (2001) Origin, diversity and evolution of NBS-type disease-resistance gene homologues in coffee trees (*Coffea* L.). Mol Genet Genomics 265:654–662.

Nyvall RF (1999) Field crop diseases. Ames, Iowa: State University Press.

Odell JT, Nagy F, Chua NH (1985) Identification of DNA sequence required for activity of the cauliflower mosaic virus 35S promoter. Nature 313:810–2.

OECD-FAO (2009) Agricultural Outlook 2009-2018. Food and Agriculture Organization of the United Nations.

Oka HI (1988) Weedy forms of rice. In: Origin of cultivated rice. Elsevier, Amsterdam 107–114.

Ou SH (1985) Rice diseases. Great Britain (UK): Commonwealth Mycological Institute.

Oztetik E (2008): A tale of plant glutathione S-transferase:since 1970. Bot Rev 74:419–437.

Pan Q, Wendel J, Fluhr R (2000) Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. J Mol Evol 50:203–213.

Parker R, Flowers TJ, Moore AL, Harpham NVJ (2006) An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. J Exp Bot 57**:**1109–1118.

Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci, USA 101:9903–9908.

Paterson AH, Bowers JE, Peterson DG, Estill JC, Chapman BA (2003) Structure and evolution of cereal genomes. Curr Opin Genet Dev 13:644–650.

Pandey MK, Shobha Rani N, Sundaram RM, Laha GS, Madhav MS et al. (2013) Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. Mol Breed 31:239–246.

Park CJ, Han SW, Chen X, Ronald PC (2010) Elucidation of *Xa21*-mediated innate immunity. Cell Microbiol 12:1017–1025.

Peck SC, Nühse TS, Iglesias A, Hess D, Meins F, Boller T (2001) Directed proteomics identifies a plant-specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. Plant Cell 13:1467–1475.

Peng S, Cassman KG, Virmani SS, Sheehy J, Khush GS (1999) Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci 39:1552–1559.

Peters RJ (2006) Uncovering the complex metabolic network underlying diterpenoid phytoalexin biosynthesis in rice and other cereal crop plants. Phytochemistry 67:2307–2317.

Petit AN, Vaillant N, Boulay M, Clément C, Fontaine F (2006) Alteration of photosynthesis in grapevines affected by esca. Phytopathol 96:1060–1066.

Perumalsamy S, Bharani M, Sudha M, Nagarajan P, Arul L, Saraswathi R, Balasubramanian P, Ramalingam J (2010) Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). Plant Breed 129:400–406.

Portis AR Jr. (1992) Regulation of ribulose 1,5-bisphosphate carboxylase/oxygenase activity. Annu Rev Plant Physiol Plant Mol Biol 43:415–37.

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222.

Rajarajeshwari NVL, Muralidharan K (2006) Assessments of farm yield and district production loss from bacterial leaf blight epidemics in rice. Crop Protect 25:244–252.

Rajpurohit D, Kumar R, Kumar M, Paul P, et al. (2010) Pyramiding of two bacterial blight resistance and a semi-dwarfing gene in Type 3 Basmati using marker-assisted selection. Euphytica 178:111–126.

Reddy APK, Katyal JC, Rouse DI, Mackenzie DR (1979) Relationship between nitrogen fertilization, bacterial leaf blight severity and yield of rice. Phytopathol 69:970–973

Ribeiro RV, Machado EC, Oliveira RF (2004) Growth- and leaf-temperature effects on photosynthesis of sweet orange seedlings infected with *Xylella fastidiosa*. Plant physiol 53: 334–340

Riccardi F, Gazeau P, de Vienne D, Zivy M (1998) Protein changes in response to progressive water deficit in maize. Quantitative variation and polypeptide identification. Plant Physiol 117:1253–1263.

Richly E, Kurth J, Leister D (2002) Mode of amplification and reorganization of resistance genes during recent *Arabidopsis thaliana* evolution. Mol Biol Evol 19:76–84.

Ristic Z, Williams G, Yang G, Martin B, Fullerton S (1996) Dehydration, damage to cellular membranes, and heat-shock proteins in maize hybrids from different climates. J Plant Physiol 149:424–432.

Rizhsky L, Liang HJ, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. Plant Physiol 134:1683–1696.

Ronald PC (1998) Resistance gene evolution. Curr Opin Plant Bio 1:294–298.

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa27*. Gen Genet 236:113–120.

Roschevitz RJ (1931) A contribution to the knowledge of rice. Bull Appl Bot Genet Plant Breed (Leningrad) 27:1–133.

Saccone C, Pesole G (2003) Handbook of comparative genomics: Principles and Methodology. J Wiley & Sons NJ USA.

Saeed IAM, MacGuidwin AE, Rouse DI, Sharkey TD (1999) Limitation to photosynthesis in *Pratylenchus penetrans* and *Verticillium dahlia* infected potato. Crop Sci 39:1340–1346.

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Salamov AA, Solovyev VV (2000) *Ab initio* gene finding in Drosophila genomic DNA. Genome Res 10:516–522.

Salekdeh GH, Siopongco HJ, Wade LJ, Ghareyazie B, Bennett J (2002) A proteomics approach to analysing drought and salt responsiveness in rice. Field Crop Res 76:199–219.

Salmeron JM, Oldroyd GED, Rommens CMT, Scofied SR, Kim HS, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. Cell 86:123–133.

Salse J, Piegu B, Cooke R, Delseny M (2002) Synteny between *Arabidopsis thaliana* and rice at the genome level: a tool to identify conservation in the ongoing rice genome sequencing project. Nucleic Acids Res 30:2316–2328.

Salse J, Piegu B, Cooke R, Delseny M (2004) New *in silico* insight into the synteny between rice (*Oryza sativa* L.) and maize (*Zea mays* L.) highlights reshuffling and identifies new duplications in the rice genome. Plant J 38:396–409.

Sang T, Ge S (2007a) Genetics and phylogenetics of rice domestication. Curr Opin Genet Dev 17:1–6.

Savary S, Willocquet L, Elanzegui FA, Castilla NP, Teng PS (2000) Assessing the representativeness of data on yield losses due to rice disease in tropical Asia. Plant Dis 84:357–369.

Scholes JD, Rolfe SA (1995) How do biotrophic pathogens affect the photosynthetic metabolism of their hosts? In Physiological Responses of Plants to Pathogens. Aspects Appl Biol 42:91–99.

Seo YS, Chern M, Bartley LE, Han M, Jung KH, Lee I, Walia H, Richter T, Xu X, Cao P (2011) Towards establishment of a rice stress response interactome. PLoS Genet **7**(4)**:**e1002020.

Shangguan Z, Shao M, Dyckmans J (1999) Interaction of osmotic adjustment and photosynthesis in winter wheat under soil drought. J Plant Physiol 154:753–758

Sharma TR, Das A, Kumar SP, Lodha ML (2009) Resistance gene analogues as a tool for rapid identification and cloning of disease resistance genes in plants. A review, J Plant Biochem Biotechnol 18:01–11.

Shen B, Wang DM, McIntyre CL, Liu CJ (2005) A 'Chinese Spring' wheat (*Triticum aestivum* L.) bacterial artificial chromosome library and its use in the isolation of SSR markers for targeted genome regions. Theor Appl Genet 111:1489–1494.

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant-Microbe Interact 11:815–823.

Shen S, Jing Y, Kuang T (2003) Proteomics approach to identify wound-response related proteins from rice leaf sheath. Proteomics 3:527–535.

Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 68:850–858.

Shiu SH, Bleecker AB (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA 98:10763–10768.

Shiu SH, Karlowski MW, Pan R, Tzeng YH, Mayer KFX et al. (2004) Comparative analysis of the receptor-like Kinase family in *Arabidopsis* and rice. Plant Cell 16:1220–1234.

Simons G, Groenendijk J, Wijbrandi J, Reijans M, Groenen J et al. (1998) Dissection of the Fusarium *I2* gene cluster in tomato reveals six homologs and one active gene copy. Plant Cell 10:1055–1068.

Singh BN, Mishra RN, Agarwal PK et al. (2004) A pea chloroplast translation elongation factor that is regulated by abiotic factors. Biochem Biophys Res Commun 320:523–530.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY et al. (1995) A receptor-kinase–like protein encoded by the rice disease resistance gene *Xa21*. Science 270:1804–1806.

Song WY, Pi LY, Wang GL, Gardner AJ, HoIsten BT, Ronald PC (1997) Evolution of the Rice Xa2I Disease Resistance Gene Family. Plant Cell 9:1279–1287.

Speulman E, Bouchez D, Holub EB, Beynon JL (1998) Disease resistance gene homologs correlate with disease resistance loci of *Arabidopsis thaliana*. Plant J 14:467–474.

Spielmeyer W, Ellis M, Robertson M, Ali S, Lenton JR, Chandler PM (2004) Isolation of gibberellin metabolic pathway genes from barley and comparative mapping in barley, wheat and rice. Theor Appl Genet 109:847–855.

Srinivasan B, Gnanamanickam S (2005) Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*. Curr Sci 88:25 (Scientific Correspondence).

Sundaram RM, Vishnupriya MR, Laha GS, Shobha Rani N et al. (2009) Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety. Biotechnol J 4:400–407.

Strange RN, Scott PR (2005) Plant disease: a threat to global food security. Annu Rev Phytopathol 43:83–116.

Tan DX, Chen LD, Poeggeler B, Manchester L, Reiter RJ (1993) Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1:57–60

Tan L, Liu F, Xue W, Wang G, Ye S, Zhu Z, Fu Y, Wang X, Sun C (2007) Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci. J Integr Plant Biol 49:871–884

Tanaka N, Mitsui S, Nobori H, Yanagi K, Komatsu S (2005) Expression and function of proteins during development of the basal region in rice seedlings. Mol Cell Proteomics 4:796–808.

Tatusova TA, Madden TL (1999) Blast 2 sequences - a new tool for comparing protein and nucleotide sequences. FEMS Microbiology Lett 174:247–250.

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S and McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452.

The Rice Chromosomes 11 and 12 Sequencing Consortia (2005) The sequence of rice chromosomes 11 and 12, rich in disease resistance genes and recent gene duplications. BMC Biol 3:20.

Thompson JD, Higgins DG, Gibson TJ (1994) Improved sensitivity of profile searches through the use of sequence weights and gap excision. Comput Appl Biosci 10:19–29.

Tian AG, Luo GZ, Wang YJ, Zhang JS, Gai JY, Chen SY (2004) Isolation and characterization of a Pti1 homologue from soybean. J Exp Bot 55:535–537.

Tsugane K, Kobayashi K, Niwa Y, Ohba Y, Wada K, Kobayashi H (1999) A recessive Arabidopsis mutant that grows enhanced active oxygen detoxification. Plant Cel l11:1195–1206.

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. Annu Rev Plant Physiol Plant Mol Biol 40:19–38.

Utami DW, Lestari P, Koerniati S (2013) A Relative Expression of *Xa7* Gene Controlling Bacterial Leaf Blight Resistance in Indonesian Local Rice Population (*Oryza sativa* L.). J Crop Sci Biotech 16:1–7

Vandepoele K, Simillion C, van de Peer Y (2003) Evidence that rice and other cereals are ancient aneuploids. Plant Cell 15:2192–2202.

Ventelon-Debout M, Delalande F, Brizard JP, Diemer H, Van Dorsselaer A, Brugidou C (2004) Proteome analysis of cultivar-specific deregulations of *Oryza sativa* indica and *O. sativa* japonica cellular suspensions undergoing rice yellow mottle virus infection. Proteomics 4:216–25.

Wang L, Dong L, Zhang Y, Wu W, Deng X, Xue Y (2004) Genome-wide analysis of S-locus F-box-like genes in *Arabidopsis thaliana*. Plant Mol Biol 56:929–945.

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14.

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L et al. (1999) The *Pi-b* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J 19:55–64.

Wang GL, Ruan DL, Song WY, Sideris S, Chen L, Pi LY, Zhang S, Zhang Z, Fauquet C, Gaut BS et al.(1998) Xa21D encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10:765–779.

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6.

Wang GL, Holsten TE, Song WY**,** Wang HP, Ronald PC (1995) Construction of a rice bacterial artificial chromosome library and identification of clones linked to the *Xa21* disease resistance locus. Plant J 7:525–533.

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L et al. (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948.

Wei F, Wing RA, Wise RP (2002) Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. Plant Cell 14:1903–1917.

Wei Y, Zhang Z, Andersen C, Schmelzer E, Gregersen P, Collinge D, Smedegaard-Petersen V, Thordal-Christensen H (1998) An epidermis/papilla-specific oxalate oxidase-like protein in the defense response of barley attacked by the powdery mildew fungus. Plant Mol Biol 36:101–112.

West G, Inze D, Beemster GTS (2004) Cell Cycle Modulation in the Response of the Primary Root of Arabidopsis to Salt Stress. Plant Physiol 135:1050–1058.

Whyte RO (1972) The Gramineae: Wild and cultivated plants of monsoonal and equatorial Asia, I. Southeast Asia. Asian Perspect 15:127–151.

Xiao J, Li J, Grandillo S, Ahn SN, Yuan L, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909.

Xiao S, Ellwood S, Calis O, Patrick E, Li T et al. (2001) Broad-spectrum resistance in *Arabidopsis thaliana* mediated by RPW8. Science 291:118–120.

Yan S, Tang Z, Su W, Sun W (2005) Proteomic analysis of salt stress-responsive proteins in rice root. Proteomics 5:235–244.

Yan SP, Zhang QY, Tang ZC, Su WA, Sun WN (2006) Comparative proteomic analysis provides new insights into chilling stress responses in rice. Mol. Cell. Proteomics 5:484–496.

Yamada A, Shibuya N, Kodama O, Akatuka T (1993) Induction of phytoalexin formation in suspension cultured rice cells. Bios, Biotech and Biochem 57:405–409.

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169:223–236.

Yang G, Komatsu S (2004) Microarray and Proteomic Analysis of Brassinosteroid and Gibberellin-Regulated Gene and Protein Expression in Rice. Genomics proteomics bioinformatics 2:77–83.

Yang Y, Shah J, Klessig DF (1997) Signal perception and transduction in plant defense responses. Genes Dev 11:1621–39.

Yoshida K, Shinmyo A (2000) Transgene expression systems in plant, a natural bioreactor. J Biosci Bioeng 90:353–62.

Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory manual for physiological studies of rice. Philippins, IRRI 83

Yoshida S, Satake T, Mackil DS (1981) High temperature stress in rice. IRRI Research Paper Series, 67

Yoshimura K, Masuda A, Kuwano M, Yokota A, Akashi K (2008) Programmed proteome response for drought avoidance/tolerance in the root of a C-3 xerophyte (wild watermelon) under water deficits. Plant Cell Physiol 49:226–241.

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX et al. (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–68.

Yu QB, Li G, Wang G, Sun JC, Wang PC, Wang C, Mi HL, Ma WM, Cui J, Cui YL, et al (2008) Construction of a chloroplast protein interaction network and functional mining of photosynthetic proteins in *Arabidopsis thaliana*. Cell Res 18: 1007–1019.

Yu JJ, Wang W, Lin S, Li H, Li J, Zhou P, Ni W, Dong S, Hu C, Zeng J et al. (2005) The genomes of *Oryza sativa*: a history of duplication. PLoS Biol 3:266–281.

Yu YG, Buss GR, Saghai Maroof MA (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93:11751–11756.

Yuan J, Ali L, Taylor J, Liu J, Sun S, Liu W, Masilimany P, Gulati-Sakhuja A, Pauls KP (2008) A guanylyl cyclase-like gene is associated with Gibberella ear rot resistance in maize (*Zea mays* L.) Theor Appl Genet 116:465–479.

Zhang L, Tian LH, Zhao JF, Song Y, Zhang CJ, Guo Y (2009) Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis. Plant Physiol 149:916–928.

Zhang WL, Peumans WJ, Barre A, Astoul CH, Rovira P, Rouge P, Proost P, Truffa-Bachi P, Jalali AAH, van Damme EJM (2000) Isolation and characterization of a jacalin-related mannose-binding lectin from salt-stressed rice (*Oryza sativa*) plants. Planta 210:970–978.

Zhang XC, Gassmann W (2007) Alternative splicing and mRNA levels of the disease resistance gene *RPS4* are induced during defense responses. Plant Physiol 145:1577–1587.

Zhang Z, Collinge D, Thordal-Christensen H (1995) Germin-like oxalate oxidase, a $H_2O_2$-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. Plant J 8:139–145.

Zhou J, Loh YT, Bressan RA, Martin GB (1995) The tomato gene *Pti*1 encodes a serine/threonine kinase that is phosphorylated by *Pto* and is involved in the hypersensitive response. Cell 83:925–935.

Zimmermann G, Baumlein H, Mock H, Himmelbach A, Schweizer P (2006) The multigene family encoding germin-like proteins of barley: regulation and function in basal host resistance. Plant Physiol 142:181–192.

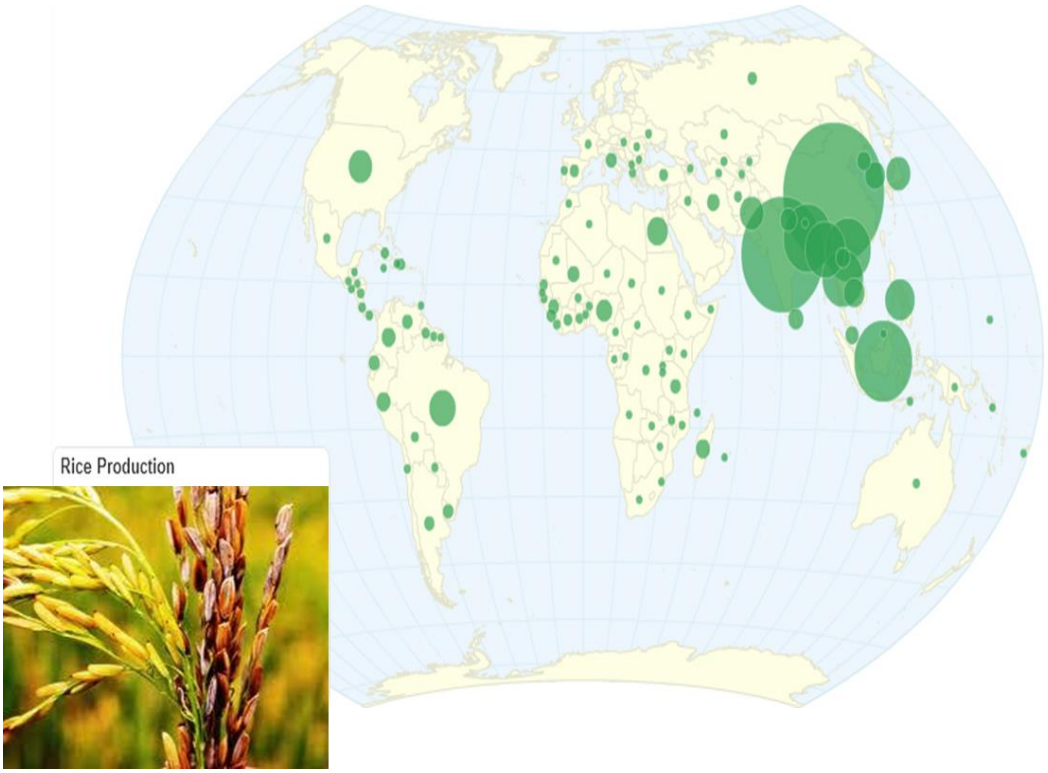
Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai W X , Zhu LH (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). Theor Appl Genet 101:569–573.

Zimaro T, Gottig N, Garavaglia BS, Gehring C, Ottado J (2011) Unraveling plant responses to bacterial pathogens through proteomics. J Biomed Biotechnol doi:10.1155/2011/354801.

# ANNEXURE

# &

# APPENDIX



Annexure 3.1: Predicted RGAs from 130 ordered BAC/PAC clones present in the minimum tilling path of chromosome 11 of rice.

| S.No. | Acc. No. | From | To | Exon length | strand | Function | Score | E-Value | Sub cellular Localization / Neural Net Score |
|---|---|---|---|---|---|---|---|---|---|
| 1 | AC123516-9 | 31186 | 41308 | 5484 | – | NB- ARC domain | 3634 | 0.0 | Plasma membrane – 9.7 |
| 2 | AC123528-11 | 48897 | 51176 | 1566 | + | Protein kinase | 2676 | 0.0 | Plasma membrane – 4.7 |
| 3 | AC120307-21 | 115240 | 119607 | 1377 | + | Protein kinase | 696 | 0.0 | Vacuolar (MB) – 3.8 |
| 4 | AC120533-2 | 5305 | 11701 | 3156 | + | NB-ARC domain | 1091 | 0.0 | Plasma membrane – 9.4 |
| 5 | AC120527-2 | 18599 | 20503 | 1167 | – | Protein Kinase | 1122 | 0.0 | Extracellular – 7.2 |
| 6 | AC128643-10 | 67617 | 73538 | 2901 | – | Protein kinase | 991 | 0.0 | Plasma Membrane – 9.5 |
| 7 | AC128643-12 | 93039 | 96344 | 3075 | – | Protein kinase | 1961 | 0.0 | Plasma Membrane – 5.1 |
| 8 | AC128643-16 | 111083 | 119692 | 5871 | – | LRR protein kinase | 1868 | 0.0 | Plasma membrane – 8.3 |
| 9 | AC123897-2 | 16221 | 19526 | 3075 | + | Protein kinase | 1927 | 0.0 | Plasma membrane – 5.0 |
| 10 | AC123897-6 | 34265 | 42874 | 5871 | + | LRR protein kinase | 1986 | 0.0 | Plasma membrane – 8.3 |
| 11 | AC123897-8 | 53299 | 55409 | 2001 | + | Protein kinase | 1257 | 0.0 | Plasma membrane – 5.1 |
| 12 | AC123897-10 | 63238 | 66375 | 3039 | + | Protein kinase | 1269 | 0.0 | Plasma membrane – 8.7 |
| 13 | AC123897-11 | 78627 | 81695 | 3012 | + | LRR protein kinase | 1936 | 0.0 | Plasma membrane – 9.8 |
| 14 | AC123897-12 | 86837 | 89984 | 3045 | + | LRR protein kinase | 1110 | 0.0 | Plasma membrane – 8.3 |
| 15 | AC123897-15 | 114705 | 117879 | 3075 | + | Protein kinase | 1233 | 0.0 | Plasma membrane – 3.9 |
| 16 | AC123897-17 | 125084 | 141538 | 9192 | + | Protein kinase | 1858 | 0.0 | Nuclear – 4.9 |
| 17 | AC123897-18 | 147111 | 150270 | 2994 | + | LRR protein kinase | 1771 | 0.0 | Plasma membrane – 6.3 |
| 18 | AC123897-19 | 155630 | 158790 | 3042 | + | Ser/thr protein kinase | 1911 | 0.0 | Plasma membrane – 7.4 |
| 19 | AC123897-20 | 162147 | 165305 | 3042 | + | LRR protein kinase | 1828 | 0.0 | Plasma membrane – 7.9 |
| 20 | AC120539-2 | 6165 | 9325 | 3042 | + | Ser/thr protein kinase | 1911 | 0.0 | Plasma membrane – 7.4 |
| 21 | AC120539-3 | 12682 | 15840 | 3042 | + | LRR protein kinase | 3042 | 0.0 | Plasma membrane – 7.9 |
| 22 | AC145322-15 | 122569 | 126109 | 2238 | + | Protein kinase | 1246 | 0.0 | Chloroplast (MB) – 7.8 |
| 23 | AC123523-3 | 43001 | 47354 | 2016 | + | Protein kinase | 960 | 0.0 | Nuclear – 7.7 |
| 24 | AC123522-6 | 30076 | 44286 | 1521 | – | Ser/thr protein kinase | 1268 | 0.0 | Plasma membrane – 8.4 |
| 25 | AC123522-8 | 47334 | 49805 | 2373 | – | B_lectin Pkinase | 1251 | 0.0 | Plasma membrane – 9.7 |
| 26 | AC123515-5 | 35319 | 45473 | 3882 | – | NBS-LRR like protein | 2438 | 0.0 | Golgi (MB) – 3.8 |
| 27 | AC123515-10 | 78587 | 84408 | 3468 | – | NBS-LRR like protein | 1986 | 0.0 | Plasma membrane – 9.0 |
| 28 | AC123520-7 | 35184 | 35624 | 441 | – | LLR protein kinase | 1701 | 0.0 | Nuclear – 7.1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 29 | AC123520-8 | 36428 | 39706 | 867 | – | NB-ARC domain | 797 | 0.0 | Mitochondrial | – 6.4 |
| 30 | AC123524-29 | 1288804 | 125764 | 2967 | – | NB-ARC NACHT | 1779 | 0.0 | Mitochondrial | – 5.0 |
| 31 | AC133005-3 | 16920 | 13880 | 2967 | – | NB-ARC domain | 1779 | 0.0 | Mitochondrial | – 5.0 |
| 32 | AC133005-32 | 130589 | 126132 | 3348 | + | NB-ARC domain | 1975 | 0.0 | Chloroplast (MB) | – 7.6 |
| 33 | AC119072-4 | 19038 | 25674 | 2178 | – | Protein kinase | 1357 | 0.0 | Plasma membrane | – 6.2 |
| 34 | AC119072-5 | 26884 | 30863 | 1623 | – | Protein kinase | 979 | 0.0 | Plasma membrane | – 4.9 |
| 35 | AC119072-11 | 60685 | 67487 | 3552 | – | NBS-LRR like protein | 1258 | 0.0 | Plasma membrane | – 4.7 |
| 36 | AC119072-13 | 76310 | 79748 | 2910 | + | NBS-LRR like protein | 1407 | 0.0 | Nuclear | – 5.1 |
| 37 | AC119072-15 | 86123 | 89188 | 3066 | + | NBS-LRR like protein | 1984 | 0.0 | Chloroplast (MB) | – 6.9 |
| 38 | AC119073-1 | 3116 | 7060 | 3944 | + | NBS-LRR like protein | 1357 | 0.0 | Nuclear | – 6.1 |
| 39 | AC119073-2 | 13350 | 22519 | 9169 | – | NBS-LRR like protein | 1232 | 0.0 | Mitochondrial | – 5.1 |
| 40 | AC119073-5 | 37368 | 45173 | 7805 | + | NBS-LRR like protein | 2725 | 0.0 | Nuclear | – 4.5 |
| 41 | AC119073-6 | 59172 | 61910 | 2738 | + | NBS-LRR like protein | 1785 | 0.0 | Mitochondrial | – 5.7 |
| 42 | AC119073-10 | 76869 | 79316 | 2447 | + | NBS-LRR like protein | 1447 | 0.0 | Plasma membrane | – 4.7 |
| 43 | AC119073-11 | 87171 | 89912 | 2741 | + | NBS-LRR like protein | 1758 | 0.0 | Cytoplasmic | – 7.8 |
| 44 | AC119071-42 | 131575 | 134790 | 453 | – | NB-ARC domain | 1684 | 0.0 | Mitochondrial | – 5.1 |
| 45 | AC119670-16 | 68678 | 6767 | 2922 | – | NB-ARC domain | 1743 | 0.0 | Mitochondrial | – 4.5 |
| 46 | AC119670-19 | 80111 | 82929 | 2718 | – | NB-ARC domain | 1763 | 0.0 | Mitochondrial | – 6.5 |
| 47 | AC145809-14 | 35194 | 35165 | 144 | – | NB-ARC NACHT | 1813 | 0.0 | Mitochondrial | – 6.8 |
| 48 | AC145809-30 | 88183 | 82947 | 2826 | – | NB-ARC NACHT | 1827 | 0.0 | Mitochondrial | – 8.7 |
| 49 | AC145809-32 | 102202 | 101885 | 2901 | – | NB-ARC NACHT | 1816 | 0.0 | Chloroplast (MB) | – 6.8 |
| 50 | AC135459-3 | 33215 | 40584 | 4302 | – | NBS-LRR | 1175 | 0.0 | Chloroplast (MB) | – 9.4 |
| 51 | AC135459-8 | 95264 | 100581 | 2694 | – | Tyrosine Pkinase | 1570 | 0.0 | Chloroplast (MB) | – 8.8 |
| 52 | AC138002-5 | 19420 | 23028 | 2808 | + | NB-ARC NACHT | 1793 | 0.0 | Chloroplast (MB) | – 8.2 |
| 53 | AC138002-13 | 55645 | 62676 | 3027 | + | NB-ARC domain | 1491 | 0.0 | Chloroplast (MB) | – 8.0 |
| 54 | AC128706-22 | 139485 | 143064 | 3012 | + | NBS-LRR | 5430 | 0.0 | Chloroplast (MB) | – 4.8 |
| 55 | AC134624-5 | 36660 | 40239 | 3012 | + | NBS-LRR | 5430 | 0.0 | Chloroplast (MB) | – 4.9 |
| 56 | AC134624-10 | 71555 | 75423 | 2604 | + | NBS-LRR | 4960 | 0.0 | Plasma membrane | – 7.9 |
| 57 | AC134624-14 | 88721 | 93319 | 2913 | + | NBS-LRR | 5658 | 0.0 | Mitochondrial | – 7.3 |
| 58 | AC150698-4 | 21640 | 25507 | 2709 | + | NBS-LRR | 3578 | 0.0 | Plasma membrane | – 6.6 |
| 59 | AC150698-8 | 38805 | 43403 | 2913 | + | NBS-LRR | 5658 | 0.0 | Mitochondrial | – 5.3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 60 | AC150698-20 | 114128 | 116167 | 2040 | – | Tyrosine Pkinase | 3931 | 0.0 | Chloroplast (MB) – 6.4 |
| 61 | AC137753-1 | 52 | 1788 | 1737 | – | Tyrosine Pkinase | 3330 | 0.0 | Chloroplast (MB) – 9.3 |
| 62 | AC137753-8 | 54092 | 58338 | 3075 | + | NB-ARC-NACHT | 6096 | 0.0 | Chloroplast (MB) – 9.0 |
| 63 | AC137753-14 | 83293 | 85221 | 1929 | – | Tyrosine Pkinase | 3663 | 0.0 | Plasma membrane – 5.3 |
| 64 | AC137753-19 | 127146 | 129200 | 2055 | – | Protein Kinase | 2468 | 0.0 | Mitochondrial – 6.5 |
| 65 | AC136956-8 | 20038 | 21966 | 1929 | – | Tyrosine Pkinase | 1281 | 0.0 | Plasma membrane – 5.3 |
| 66 | AC136956-26 | 96291 | 99371 | 3081 | – | LRR protein kinase | 1682 | 0.0 | Plasma membrane – 7.0 |
| 67 | AC136956-32 | 117572 | 123164 | 3717 | + | LRR protein kinase | 1719 | 0.0 | Plasma membrane – 6.9 |
| 68 | AC136956-37 | 144127 | 146892 | 2766 | + | LRR protein kinase | 1179 | 0.0 | Plasma membrane – 7.4 |
| 69 | AC136956-39 | 150431 | 153715 | 2997 | + | LRR protein kinase | 1267 | 0.0 | Plasma membrane – 9.6 |
| 70 | AC120889-18 | 121787 | 127131 | 4503 | + | LRR protein kinase | 1831 | 0.0 | Chloroplast (MB) – 9.3 |
| 71 | AC120889-19 | 131319 | 134168 | 2850 | + | LRR protein kinase | 1676 | 0.0 | Chloroplast (MB) – 9.0 |
| 72 | AC120889-20 | 136859 | 138859 | 1656 | – | Tyrosine Pkinase | 1014 | 0.0 | Chloroplast (MB) – 8.9 |
| 73 | AC133291-6 | 21831 | 27234 | 4503 | + | LRR protein kinase | 853 | 0.0 | Plasma membrane – 9.7 |
| 74 | AC133291-7 | 31422 | 34271 | 2850 | + | LRR protein kinase | 1688 | 0.0 | Plasma membrane – 7.2 |
| 75 | AC133291-8 | 36962 | 38962 | 1656 | – | Tyrosine Pkinase | 1023 | 0.0 | Plasma membrane – 7.1 |
| 76 | AC133291-11 | 69863 | 72820 | 2958 | + | LRR protein kinase | 1929 | 0.0 | Chloroplast – 3.9 |
| 77 | AC133291-23 | 166197 | 168134 | 1938 | + | LRR protein kinase | 1310 | 0.0 | Plasma membrane – 5.9 |
| 78 | AC109644-1 | 2449 | 5085 | 2376 | + | LRR protein kinase | 1446 | 0.0 | Plasma membrane – 9.4 |
| 79 | AC109644-2 | 12792 | 16702 | 3114 | + | LRR protein kinase | 1089 | 0.0 | Plasma membrane – 8.9 |
| 80 | AC109644-4 | 24436 | 27372 | 2937 | + | LRR protein kinase | 1594 | 0.0 | Plasma membrane – 7.1 |
| 81 | AC109644-5 | 35710 | 39028 | 3108 | + | LRR protein kinase | 1920 | 0.0 | Plasma membrane – 7.5 |
| 82 | AC109644-6 | 43420 | 46832 | 3207 | + | LRR protein kinase | 1941 | 0.0 | Plasma membrane – 9.9 |
| 83 | AC109644-11 | 73922 | 79524 | 3402 | + | LRR protein kinase | 2115 | 0.0 | Plasma membrane – 6.5 |
| 84 | AC109644 -16 | 112580 | 114517 | 1938 | – | LRR protein kinase | 1310 | 0.0 | Plasma membrane – 5.9 |
| 85 | AC137693-5 | 44084 | 47368 | 2616 | – | LRR protein kinase | 2486 | 0.0 | Plasma membrane – 8.7 |
| 86 | AC137693-6 | 57481 | 60117 | 2376 | + | Protein kinase | 4710 | 0.0 | Plasma membrane – 9.4 |
| 87 | AC137693-9 | 79468 | 82404 | 2937 | + | LRR protein kinase | 5812 | 0.0 | Plasma membrane – 7.2 |
| 88 | AC137693-10 | 90742 | 94060 | 3108 | + | LRR protein kinase | 6161 | 0.0 | Plasma membrane – 9.7 |
| 89 | AC137693-11 | 98452 | 101864 | 3207 | + | LRR protein kinase | 6238 | 0.0 | Plasma membrane – 10 |
| 90 | AC137693-16 | 128954 | 134556 | 3402 | + | LRR protein kinase | 6643 | 0.0 | Plasma membrane – 6.7 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 91 | AC136149-19 | 157838 | 160870 | 2757 | – | NBS-LRR like protein | 4157 | 0.0 | Extracellular | – 2.3 |
| 92 | AC108871-7 | 50052 | 53084 | 2757 | – | NBS-LRR like protein | 4157 | 0.0 | Extracellular | – 2.3 |
| 93 | AC133218-17 | 128271 | 131435 | 3165 | – | NBS-LRR like protein | 4401 | 0.0 | Cytoplasmic | – 4.3 |
| 94 | AC133218-18 | 139657 | 143919 | 4263 | + | NBS-LRR like protein | 5132 | 0.0 | Cytoplasmic | – 9.6 |
| 95 | AC137589-3 | 8968 | 12132 | 3165 | – | NBS-LRR like protein | 4401 | 0.0 | Cytoplasmic | – 4.3 |
| 96 | AC137589-4 | 20360 | 24622 | 4263 | + | NBS-LRR like protein | 5132 | 0.0 | Cytoplasmic | – 9.6 |
| 97 | AC104847-10 | 54254 | 58582 | 2661 | + | TPR-LRR like protein | 5103 | 0.0 | Mitochondrial | – 4.0 |
| 98 | AC104847-11 | 63007 | 76250 | 3258 | + | PP2C Protein Kinase | 1534 | 0.0 | Golgi (MB) | – 4.8 |
| 99 | AC136148-13 | 118924 | 128704 | 4110 | + | NBS-LRR like protein | 5424 | 0.0 | Vacuolar (MB) | – 3.9 |
| 100 | AC136148-14 | 133704 | 136604 | 2457 | + | NBS-LRR like protein | 2932 | 0.0 | Chloroplast (MB) | – 4.8 |
| 101 | AC146334-1 | 1 | 4095 | 2478 | + | NBS-LRR like protein | 1137 | 0.0 | Chloroplast (MB) | – 10 |
| 102 | AC146334-3 | 20767 | 25882 | 2994 | + | NBS-LRR like protein | 1379 | 0.0 | Golgi (MB) | – 4.9 |
| 103 | AC146334-5 | 51903 | 59260 | 1986 | + | NBS-LRR like protein | 1000 | 0.0 | Nuclear | – 3.4 |
| 104 | AC146334-10 | 88752 | 94352 | 2763 | + | NBS-LRR like protein | 1711 | 0.0 | Chloroplast (MB) | – 8.6 |
| 105 | AC146334-11 | 97899 | 104457 | 2904 | + | NBS-LRR like protein | 1649 | 0.0 | Chloroplast (MB) | – 4.8 |
| 106 | AC146334-12 | 111731 | 114877 | 2751 | + | NBS-LRR like protein | 1773 | 0.0 | Mitochondrial | – 9.7 |
| 107 | AC144700-24 | 120601 | 124488 | 3888 | + | NBS-LRR like protein | 2510 | 0.0 | Cytoplasmic | – 6.8 |
| 108 | AC114828-4 | 19772 | 23659 | 3888 | + | NBS-LRR like protein | 2510 | 0.0 | Cytoplasmic | – 6.8 |
| 109 | AC114828-7 | 55504 | 57347 | 1803 | + | LRR protein kinase | 2557 | 0.0 | Vacuolar | – 3.5 |
| 110 | AC114828-11 | 79053 | 83321 | 4269 | - | NBS-LRR like protein | 5212 | 0.0 | Nuclear | – 9.0 |
| 111 | AC114828-17 | 118217 | 122089 | 3873 | + | NBS-LRR like protein | 4551 | 0.0 | Cytoplasmic | – 6.9 |
| 112 | AC136842-2 | 7323 | 11591 | 4269 | – | NBS-LRR like protein | 5212 | 0.0 | Nuclear | – 9.4 |
| 113 | AC136842-8 | 46408 | 50280 | 3873 | – | NBS-LRR like protein | 4551 | 0.0 | Cytoplasmic | – 5.4 |
| 114 | AC109365-12 | 72140 | 75392 | 2031 | – | Protein kinase | 3324 | 0.0 | Plasma membrane | – 9.9 |
| 115 | AC121328-3 | 8925 | 16483 | 3561 | – | NBS-LRR like protein | 3223 | 0.0 | Plasma membrane | – 4.5 |
| 116 | AC121328-6 | 36884 | 40049 | 3054 | – | NB-ARC domain | 4647 | 0.0 | Chloroplast (MB) | – 6.4 |
| 117 | AC121328-14 | 93009 | 93779 | 771 | + | NB-ARC domain | 1528 | 0.0 | Chloroplast | – 5.2 |
| 118 | AC121328-15 | 97531 | 100640 | 2544 | + | LRR protein kinase | 3842 | 0.0 | Nuclear | – 7.8 |
| 119 | AC121328-17 | 106794 | 109917 | 2760 | – | NBS-LRR like protein | 3842 | 0.0 | Plasma membrane | – 6.9 |
| 120 | AC121328-18 | 111162 | 115878 | 3198 | + | NBS-LRR like protein | 5953 | 0.0 | Nuclear | – 2.5 |
| 121 | AC121328-19 | 118684 | 121697 | 2883 | – | NBS-LRR like protein | 1120 | 0.0 | Golgi (MB) | – 7.8 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 122 | AC121328-20 | 122500 | 128212 | 3276 | + | NBS-LRR like protein | 6494 | 0.0 | Nuclear | – 4.9 |
| 123 | AC121328-22 | 134761 | 135122 | 360 | – | Protein Kinase | 642 | 0.0 | Nuclear | – 4.0 |
| 124 | AC120884-3 | 8452 | 12432 | 1956 | – | Tyrosine Pkinase | 1048 | 0.0 | Plasma membrane | – 9.8 |
| 125 | AC120984-9 | 72000 | 82253 | 3204 | – | NBS-LRR like protein | 2357 | 0.0 | Nuclear | – 5.1 |
| 126 | AC120984-13 | 92389 | 102035 | 2616 | – | NBS-LRR like protein | 1927 | 0.0 | Chloroplast (MB) | – 3.8 |
| 127 | AC120887-1 | 18 | 953 | 876 | – | NBS-LRR like protein | 1737 | 0.0 | Chloroplast (MB) | – 6.4 |
| 128 | AC120885-18 | 126786 | 130805 | 891 | + | Dirigent protein kinase | 1606 | 0.0 | Vacuolar (MB) | – 6.8 |
| 129 | AC104844-18 | 82441 | 84060 | 1119 | – | Protein Kinase | 1176 | 0.0 | ER | – 0.9 |
| 130 | AC104844-21 | 99765 | 102891 | 903 | – | Tyrosine Pkinase | 1790 | 0.0 | Nuclear | – 8.4 |
| 131 | AF161269-7 | 46143 | 48850 | 2094 | – | Protein kinase | 1176 | 0.0 | Plasma membrane | – 7.3 |
| 132 | AC146766-11 | 123904 | 127068 | 3165 | – | NBS-LRR like protein | 6274 | 0.0 | Nuclear | – 6.4 |
| 133 | AC146766-14 | 158253 | 161152 | 2211 | – | LRR Protein kinase | 4105 | 0.0 | Plasma membrane | – 9.9 |
| 134 | AC144716-7 | 52622 | 56793 | 2382 | + | LRR Protein kinase | 2857 | 0.0 | Plasma membrane | – 9.1 |
| 135 | AC144716-11 | 95211 | 96464 | 1254 | + | LRR Protein kinase | 2284 | 0.0 | Plasma membrane | – 8.7 |
| 136 | AC144716-14 | 108960 | 111859 | 2211 | + | LRR Protein kinase | 4105 | 0.0 | Plasma membrane | – 9.9 |
| 137 | AC109832-3 | 14310 | 17741 | 3300 | – | LRR protein kinase | 5818 | 0.0 | Plasma membrane | – 8.8 |
| 138 | AC109832-13 | 96441 | 99906 | 3318 | + | LRR protein kinase | 6577 | 0.0 | Plasma membrane | – 6.6 |
| 139 | AC109832-20 | 144440 | 148026 | 2931 | + | NBS-LRR like protein | 783 | 0.0 | Golgi (MB) | – 7.3 |
| 140 | AC125780-20 | 125486 | 128263 | 2322 | + | NBS-LRR homologue | 1032 | 0.0 | Chloroplast (MB) | – 6.8 |
| 141 | AC145349-1 | 376 | 1445 | 858 | + | LRR protein kinase | 1606 | 0.0 | Cytoplasmic | – 7.5 |
| 142 | AC113249-3 | 15312 | 20445 | 1845 | + | LRR protein kinase | 1140 | 0.0 | Nuclear | – 5.8 |
| 143 | AC122143-8 | 56425 | 57412 | 462 | – | LRR protein kinase | 791 | 0.0 | Cytoplasmic | – 4.5 |
| 144 | AC122143-17 | 124841 | 126034 | 1194 | – | LRR protein kinase | 1534 | 0.0 | Plasma membrane | – 6.2 |
| 145 | AC125781-4 | 27650 | 32424 | 4326 | + | NBS-LRR like protein | 2659 | 0.0 | Nuclear | – 8.3 |
| 146 | AC125781-15 | 91322 | 95616 | 4149 | + | NBS-LRR like protein | 891 | 0.0 | Chloroplast (MB) | – 6.9 |
| 147 | AC108872-13 | 106157 | 110188 | 4032 | – | NBS-LRR like protein | 2572 | 0.0 | Cytoplasmic | – 5.8 |
| 148 | AC145775-7 | 35324 | 39355 | 1343 | + | NB-ARC domain | 2575 | 0.0 | Chloroplast | – 5.8 |
| 149 | AC145775-13 | 95641 | 98187 | 848 | + | NBS-LRR like protein | 1632 | 0.0 | Chloroplast | – 3.9 |
| 150 | AC145775-19 | 147337 | 149104 | 589 | – | NBS-LRR like protein | 1169 | 0.0 | Chloroplast (MB) | – 9.5 |
| 151 | AC129224-16 | 54141 | 56579 | 2439 | – | NB-ARC domain | 1511 | 0.0 | Chloroplast | – 6.4 |
| 152 | AC129224-23 | 76625 | 79543 | 2919 | + | NB-ARC domain | 1942 | 0.0 | Nuclear | – 5.5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 153 | AC120888-12 | 57294 | 60175 | 2841 | – | LRR protein kinase | 754 | 0.0 | Chloroplast | – 3.9 |
| 154 | AC120888-17 | 115935 | 119358 | 2931 | – | NBS-LRR like protein | 1347 | 0.0 | Peroxisomal (MB) | – 9.1 |
| 155 | AC108870-1 | 2111 | 2514 | 1722 | – | NB-ARC domain | 1118 | 0.0 | Chloroplast (MB) | – 6.8 |
| 156 | AC126057-15 | 101581 | 103350 | 1632 | + | Protein kinase | 1447 | 0.0 | Chloroplast (MB) | – 6.4 |
| 157 | AC129227-16 | 129418 | 131187 | 1632 | – | Protein kinase | 1447 | 0.0 | Chloroplast (MB) | – 6.4 |
| 158 | AC129226-4 | 28718 | 35310 | 3111 | – | Protein kinase | 1000 | 0.0 | Chloroplast (MB) | – 8.9 |
| 159 | AC133609-1 | 182 | 538 | 357 | – | NB-ARC domain | 696 | 0.0 | Chloroplast (MB) | – 9.8 |
| 160 | AC133609-16 | 118078 | 128847 | 8226 | + | Protein kinase | 985 | 0.0 | Plasma membrane | – 8.8 |
| 161 | AC134924-1 | 182 | 538 | 357 | – | NB-ARC domain | 696 | 0.0 | Chloroplast (MB) | – 7.3 |
| 162 | AC134924-16 | 118078 | 118847 | 8226 | + | Protein kinase | 985 | 0.0 | Plasma membrane | – 8.2 |
| 163 | AC134922-9 | 59879 | 62980 | 3102 | + | NBS-LRR like protein | 5786 | 0.0 | Nuclear | – 3.7 |
| 164 | AC134922-10 | 72223 | 75284 | 2898 | + | NBS-LRR like protein | 5299 | 0.0 | Cytoplasmic | – 5.8 |
| 165 | AC134922-12 | 80094 | 83185 | 2931 | + | NBS-LRR like protein | 3247 | 0.0 | Plasma membrane | – 6.2 |
| 166 | AC134922-17 | 99117 | 102218 | 3102 | + | NBS-LRR like protein | 6080 | 0.0 | Chloroplast (MB) | – 8.3 |
| 167 | AC134922-18 | 107610 | 111372 | 3312 | + | NBS-LRR like protein | 4571 | 0.0 | Chloroplast (MB) | – 6.0 |
| 168 | AC134922-20 | 120256 | 123069 | 2814 | + | NBS-LRR like protein | 5578 | 0.0 | Mitochondrial | – 5.4 |
| 169 | AC146582-1 | 274 | 1501 | 1227 | + | NB-ARC domain | 771 | 0.0 | Plasma membrane | – 2.8 |
| 170 | AC134923-3 | 15168 | 17952 | 2718 | + | NB-ARC domain | 1202 | 0.0 | Mitochondrial | – 6.9 |
| 171 | AC136998-16 | 106490 | 109520 | 1878 | – | Tyrosine Pkinase | 1234 | 0.0 | Plasma membrane | – 8.0 |
| 172 | AC135190-13 | 31186 | 35427 | 2016 | – | NB-ARC domain | 1218 | 0.0 | Chloroplast (MB) | – 7.4 |
| 173 | AC135190-29 | 112227 | 118997 | 3717 | – | NB-ARC domain | 1320 | 0.0 | Nuclear | – 4.0 |
| 174 | AC135190-31 | 120113 | 122263 | 2151 | + | NB-ARC NACHT | 1303 | 0.0 | Nuclear | – 6.9 |
| 175 | AC135190-33 | 131761 | 135058 | 2997 | – | NB-ARC NACHT | 1364 | 0.0 | Nuclear | – 6.0 |
| 176 | AC135644-1 | 15 | 2142 | 1335 | – | NBS-LRR homologue | 1007 | 0.0 | Chloroplast | – 5.0 |
| 177 | AC135644-2 | 4020 | 10200 | 3072 | + | NBS-LRR like protein | 2040 | 0.0 | Chloroplast (MB) | – 9.0 |
| 178 | AC135644-6 | 25074 | 28608 | 3288 | – | NBS-LRR homologue | 1675 | 0.0 | Chloroplast (MB) | – 7.7 |
| 179 | AC135644-7 | 29741 | 42838 | 8220 | + | NBS-LRR homologue | 1675 | 0.0 | Chloroplast (MB) | – 9.3 |
| 180 | AC135644-13 | 85879 | 87879 | 2001 | – | NB-ARC domain | 1025 | 0.0 | Chloroplast (MB) | – 9.0 |
| 181 | AC135644-17 | 115238 | 118535 | 2997 | + | NBS-LRR homologue | 1876 | 0.0 | Chloroplast (MB) | – 9.0 |
| 182 | AC135644-20 | 128033 | 131206 | 2853 | – | NBS-LRR homologue | 1753 | 0.0 | Chloroplast (MB) | – 8.9 |
| 183 | AC144734-1 | 98 | 2074 | 1977 | + | NBS-LRR like protein | 1338 | 0.0 | Chloroplast (MB) | – 5.4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 184 | AC133216-11 | 71244 | 77738 | 1917 | + | NB-ARC domain | 871 | 0.0 | Nuclear | – 7.0 |
| 185 | AC133216-13 | 84145 | 87410 | 1947 | + | LRR protein kinase | 973 | 0.0 | Chloroplast | – 3.9 |
| 186 | AC145395-7 | 60032 | 63323 | 3135 | + | NB-ARC domain | 2053 | 0.0 | Nuclear | – 6.3 |
| 187 | AC148822-7 | 31605 | 34244 | 1437 | + | Protein Kinase | 731 | 0.0 | Plasma membrane | – 9.0 |
| 188 | AC148822-8 | 54511 | 60656 | 2040 | + | Protein kinase | 1183 | 0.0 | Chloroplast | – 3.9 |
| 189 | AC148822-11 | 87300 | 92314 | 2121 | + | Protein kinase | 1329 | 0.0 | Extracellular | – 5.4 |
| 190 | AC146937-10 | 86571 | 90002 | 3321 | + | Protein kinase | 2087 | 0.0 | Chloroplast (MB) | – 9.4 |
| 191 | AC146937-13 | 95168 | 98506 | 1592 | – | Protein kinase | 2742 | 0.0 | Chloroplast (MB) | – 9.4 |
| 192 | AC146937-15 | 106374 | 109767 | 3294 | – | Protein kinase | 2025 | 0.0 | Chloroplast (MB) | – 9.4 |
| 193 | AC146937-16 | 113478 | 116868 | 3261 | – | Protein kinase | 1371 | 0.0 | Chloroplast (MB) | – 9.3 |
| 194 | AC146937-17 | 117677 | 121092 | 3093 | – | Protein kinase | 1354 | 0.0 | Chloroplast (MB) | – 9.2 |
| 195 | AC116367-2 | 14664 | 18089 | 3177 | + | LRR protein kinase | 1224 | 0.0 | Plasma membrane | – 9.6 |
| 196 | AC116367-3 | 21676 | 25109 | 3324 | – | LRR protein kinase | 2009 | 0.0 | Plasma membrane | – 10 |
| 197 | AC116367-4 | 31013 | 38183 | 3135 | + | LRR protein kinase | 1902 | 0.0 | Plasma membrane | – 8.7 |
| 198 | AC116367-7 | 58339 | 61765 | 3186 | – | LRR protein kinase | 1836 | 0.0 | Plasma membrane | – 9.7 |
| 199 | AC116367-10 | 66707 | 70120 | 2643 | – | Tyrosine protein kinase | 1281 | 0.0 | Plasma membrane | – 9.8 |
| 200 | AC116367-13 | 89749 | 93153 | 3309 | – | Tyrosine protein kinase | 1443 | 0.0 | Plasma membrane | –7.5 |
| 201 | AC116367-15 | 95495 | 98223 | 2529 | – | Tyrosine protein kinase | 1003 | 0.0 | Plasma membrane | – 9.9 |
| 202 | AC145319-10 | 73986 | 77933 | 2652 | + | NB-ARC_NACHT | 948 | 0.0 | ER | – 5.2 |
| 203 | AC134045-32 | 139424 | 143114 | 3408 | – | NBS-LRR like protein | 2085 | 0.0 | Golgi (MB) | – 3.9 |
| 204 | AC104846-5 | 35098 | 37905 | 2808 | – | NBS-LRR like protein | 1745 | 0.0 | Chloroplast (MB) | – 9.4 |
| 205 | AC104846-7 | 61482 | 63428 | 1947 | – | NBS-LRR like protein | 1292 | 0.0 | Chloroplast (MB) | – 9.1 |
| 206 | AC104846-8 | 67222 | 70578 | 3357 | + | NBS-LRR like protein | 2051 | 0.0 | Chloroplast (MB) | – 9.2 |
| 207 | AC104846-10 | 87486 | 90671 | 3186 | – | NBS-LRR like protein | 2018 | 0.0 | Chloroplast (MB) | – 9.4 |
| 208 | AC104846-11 | 103736 | 105598 | 1863 | + | NBS-LRR like protein | 1164 | 0.0 | Chloroplast | – 8.2 |
| 209 | AC135643-4 | 33974 | 36758 | 2718 | – | NBS-LRR like protein | 1452 | 0.0 | Chloroplast (MB) | – 9.1 |
| 210 | AC135643-16 | 111316 | 114414 | 3099 | – | NBS-LRR like protein | 1973 | 0.0 | Chloroplast (MB) | – 9.2 |
| 211 | AC135643-17 | 119411 | 122506 | 3096 | – | NBS-LRR like protein | 2004 | 0.0 | Chloroplast (MB) | – 9.1 |
| 212 | AC135643-18 | 124594 | 129320 | 2190 | – | NBS-LRR like protein | 1471 | 0.0 | Chloroplast (MB) | – 8.7 |
| 213 | AC135643-21 | 142494 | 144898 | 2094 | – | NBS-LRR like protein | 1380 | 0.0 | Chloroplast (MB) | – 9.4 |

Annexure 3.2: GC content of R genes determined using online software sciencebuddies.org

| S.No. | Accession number | GC% | S.No. | Accession number | GC% |
|---|---|---|---|---|---|
| 1 | AC123516-9 | 44.1 | 44 | AC119071-42 | 35.8 |
| 2 | AC123528-11 | 60.2 | 45 | AC119670-16 | 31.1 |
| 3 | AC120307-21 | 71.6 | 46 | AC119670-19 | 35.8 |
| 4 | AC120533-2 | 44.6 | 47 | AC145809-14 | 51.0 |
| 5 | AC120527-2 | 68.3 | 48 | AC145809-30 | 48.6 |
| 6 | AC128643-10 | 56.0 | 49 | AC145809-32 | 51.4 |
| 7 | AC128643-12 | 40.4 | 50 | AC135459-3 | 44.4 |
| 8 | AC128643-16 | 40.6 | 51 | AC135459-8 | 48.7 |
| 9 | AC123897-2 | 40.2 | 52 | AC138002-5 | 48.4 |
| 10 | AC123897-6 | 40.6 | 53 | AC138002-13 | 47.7 |
| 11 | AC123897-8 | 40.4 | 54 | AC128706-22 | 45.7 |
| 12 | AC123897-10 | 39.5 | 55 | AC134624-5 | 45.7 |
| 13 | AC123897-11 | 39.9 | 56 | AC134624-10 | 42.9 |
| 14 | AC123897-12 | 46.3 | 57 | AC134624-14 | 45.6 |
| 15 | AC123897-15 | 40.7 | 58 | AC150698-4 | 42.4 |
| 16 | AC123897-17 | 43.1 | 59 | AC150698-8 | 45.6 |
| 17 | AC123897-18 | 45.4 | 60 | AC150698-20 | 41.0 |
| 18 | AC123897-19 | 42.6 | 61 | AC137753-1 | 39.8 |
| 19 | AC123897-20 | 41.7 | 62 | AC137753-8 | 40.0 |
| 20 | AC120539-2 | 46.8 | 63 | AC137753-14 | 41.5 |
| 21 | AC120539-3 | 46.3 | 64 | AC137753-19 | 72.2 |
| 22 | AC145322-15 | 44.5 | 65 | AC136956-8 | 45.7 |
| 23 | AC123523-3 | 51.6 | 66 | AC136956-26 | 51.2 |
| 24 | AC123522-6 | 45.0 | 67 | AC136956-32 | 46.9 |
| 25 | AC123522-8 | 44.3 | 68 | AC136956-37 | 55.9 |
| 26 | AC123515-5 | 54.8 | 69 | AC136956-39 | 49.5 |
| 27 | AC123515-10 | 50.0 | 70 | AC120889-18 | 42.3 |
| 28 | AC123520-7 | 73.6 | 71 | AC120889-19 | 42.2 |
| 29 | AC123520-8 | 52.1 | 72 | AC120889-20 | 45.1 |
| 30 | AC123524-29 | 43.3 | 73 | AC133291-6 | 42.3 |
| 31 | AC133005-3 | 43.3 | 74 | AC133291-7 | 42.2 |
| 32 | AC133005-32 | 40.2 | 75 | AC133291-8 | 45.1 |
| 33 | AC119072-4 | 40.9 | 76 | AC133291-11 | 45.7 |
| 34 | AC119072-5 | 45.9 | 77 | AC133291-23 | 45.0 |
| 35 | AC119072-11 | 45.8 | 78 | AC109644-1 | 45.8 |
| 36 | AC119072-13 | 48.6 | 79 | AC109644-2 | 47.3 |
| 37 | AC119072-15 | 44.0 | 80 | AC109644-4 | 42.3 |
| 38 | AC119073-1 | 43.0 | 81 | AC109644-5 | 42.3 |
| 39 | AC119073-2 | 32.8 | 82 | AC109644-6 | 45.1 |
| 40 | AC119073-5 | 31.5 | 83 | AC109644-11 | 45.8 |
| 41 | AC119073-6 | 35.0 | 84 | AC109644-16 | 45.0 |
| 42 | AC119073-10 | 38.0 | 85 | AC137693-5 | 44.9 |
| 43 | AC119073-11 | 40.1 | 86 | AC137693-6 | 40.8 |
| 87 | AC137693-9 | 47.3 | 134 | AC144716-7 | 40.5 |

| | | | | | |
|---|---|---|---|---|---|
| 88 | AC137693-10 | 42.3 | 135 | AC144716-11 | 44.5 |
| 89 | AC137693-11 | 45.1 | 136 | AC144716-14 | 40.9 |
| 90 | AC137693-16 | 45.8 | 137 | AC109832-3 | 40.7 |
| 91 | AC136149-19 | 46.2 | 138 | AC109832-13 | 40.4 |
| 92 | AC108871-7 | 46.2 | 139 | AC109832-20 | 44.8 |
| 93 | AC133218-17 | 52.9 | 140 | AC125780-20 | 40.5 |
| 94 | AC133218-18 | 47.4 | 141 | AC145349-1 | 41.4 |
| 95 | AC137589-3 | 52.9 | 142 | AC113249-3 | 51.6 |
| 96 | AC137589-4 | 47.4 | 143 | AC122143-8 | 46.8 |
| 97 | AC104847-10 | 49.6 | 144 | AC122143-17 | 75.0 |
| 98 | AC104847-11 | 44.5 | 145 | AC125781-4 | 52.0 |
| 99 | AC136148-13 | 44.7 | 146 | AC125781-15 | 46.6 |
| 100 | AC136148-14 | 44.2 | 147 | AC108872-13 | 48.9 |
| 101 | AC146334-1 | 45.5 | 148 | AC145775-7 | 48.9 |
| 102 | AC146334-3 | 45.3 | 149 | AC145775-13 | 54.6 |
| 103 | AC146334-5 | 47.3 | 150 | AC145775-19 | 49.1 |
| 104 | AC146334-10 | 47.6 | 151 | AC129224-16 | 39.5 |
| 105 | AC146334-11 | 45.5 | 152 | AC129224-23 | 49.2 |
| 106 | AC146334-12 | 44.2 | 153 | AC120888-12 | 62.7 |
| 107 | AC144700-24 | 46.8 | 154 | AC120888-17 | 45.4 |
| 108 | AC114828-4 | 46.8 | 155 | AC108870-1 | 45.7 |
| 109 | AC114828-7 | 44.5 | 156 | AC126057-15 | 57.9 |
| 110 | AC114828-11 | 47.3 | 157 | AC129227-16 | 57.9 |
| 111 | AC114828-17 | 47.1 | 158 | AC129226-4 | 57.9 |
| 112 | AC136842-2 | 47.3 | 159 | AC133609-1 | 50.7 |
| 113 | AC136842-8 | 47.1 | 160 | AC133609-16 | 46.2 |
| 114 | AC109365-12 | 54.2 | 161 | AC134924-1 | WS |
| 115 | AC121328-3 | 48.9 | 162 | AC134924-16 | WS |
| 116 | AC121328-6 | 46.7 | 163 | AC134922-9 | 43.6 |
| 117 | AC121328-14 | 62.1 | 164 | AC134922-10 | 41.4 |
| 118 | AC121328-15 | 59.7 | 165 | AC134922-12 | 40.4 |
| 119 | AC121328-17 | 47.5 | 166 | AC134922-17 | 43.7 |
| 120 | AC121328-18 | 48.8 | 167 | AC134922-18 | 43.9 |
| 121 | AC121328-19 | 48.4 | 168 | AC134922-20 | 40.4 |
| 122 | AC121328-20 | 50.6 | 169 | AC146582-1 | 39.0 |
| 123 | AC121328-22 | 51.9 | 170 | AC134923-3 | 54.5 |
| 124 | AC120884-3 | 42.8 | 171 | AC136998-16 | 49.3 |
| 125 | AC120984-9 | 48.3 | 172 | AC135190-13 | 38.1 |
| 126 | AC120984-13 | 46.6 | 173 | AC135190-29 | 40.0 |
| 127 | AC120887-1 | 51.7 | 174 | AC135190-31 | 46.9 |
| 128 | AC120885-18 | 47.5 | 175 | AC135190-33 | 42.4 |
| 129 | AC104844-18 | 44.6 | 176 | AC135644-1 | 53.8 |
| 130 | AC104844-21 | 48.5 | 177 | AC135644-2 | 45.4 |
| 131 | AF161269-7 | 72.8 | 178 | AC135644-6 | 49.6 |
| 132 | AC146766-11 | 41.9 | 179 | AC135644-7 | 44.9 |
| 133 | AC146766-14 | 40.9 | 180 | AC135644-13 | 46.0 |
| 181 | AC135644-17 | 50.2 | 198 | AC116367-7 | 46.1 |

| | | | | | |
|---|---|---|---|---|---|
| 182 | AC135644-20 | 46.5 | 199 | AC116367-10 | 45.2 |
| 183 | AC144734-1 | 43.1 | 200 | AC116367-13 | 46.5 |
| 184 | AC133216-11 | 54.3 | 201 | AC116367-15 | 46.1 |
| 185 | AC133216-13 | 46.9 | 202 | AC145319-10 | 48.0 |
| 186 | AC145395-7 | 46.3 | 203 | AC134045-32 | 50.6 |
| 187 | AC148822-7 | 51.8 | 204 | AC104846-5 | 42.6 |
| 188 | AC148822-8 | 54.3 | 205 | AC104846-7 | 54.9 |
| 189 | AC148822-11 | 54.5 | 206 | AC104846-8 | 48.0 |
| 190 | AC146937-10 | 45.7 | 207 | AC104846-10 | 46.7 |
| 191 | AC146937-13 | 44.7 | 208 | AC104846-11 | 60.7 |
| 192 | AC146937-15 | 46.4 | 209 | AC135643-4 | 45.1 |
| 193 | AC146937-16 | 45.6 | 210 | AC135643-16 | 41.8 |
| 194 | AC146937-17 | 46.7 | 211 | AC135643-17 | 42.5 |
| 195 | AC116367-2 | 46.9 | 212 | AC135643-18 | 39.9 |
| 196 | AC116367-3 | 46.5 | 213 | AC135643-21 | 42.2 |
| 197 | AC116367-4 | 47.6 | | | |

Annexure 3.3: Homology search taking *Xa21/Xa26* as subject sequence and predicted protein kinase as query sequence

| Subject Seq *Xa21* | QC-(%) | E -value | Identities (%) | Subject Seq *Xa26* | QC- (%) | E- value | Identities (%) |
|---|---|---|---|---|---|---|---|
| AC123528.11 PK | 13 | 4e-04 | 38 | AC123528.11 PK | 12 | 3e-06 | 47 |
| AC120307.21 PK | 21 | 3e-06 | 71 | AC120307.21 PK | 26 | 5e-07 | 26 |
| AC128643.10 PK | 25 | 2e-04 | 32 | AC128643.10 PK | 21 | 3e-06 | 39 |
| AC120527.2 PK | 27 | 1e-37 | 35 | AC120527.2 PK | 27 | 1e-44 | 34 |
| AC128643.10 PK | 25 | 2e-04 | 32 | AC128643.10 PK | 21 | 3e-06 | 39 |
| AC128643.12 PK | 98 | 0.0 | 53 | AC128643.12 PK | 98 | 3e-145 | 35 |
| AC128643.16 LRR-PK | 98 | 0.0 | 40 | AC128643.16 LRR-PK | 98 | 3e-160 | 38 |
| AC123897.2 PK | 96 | 0.0 | 53 | AC123897.2 PK | 96 | 1e-144 | 35 |
| AC123897.6 LRR- PK | 95 | 0.0 | 41 | AC123897.6 LRR-PK | 95 | 2e-160 | 44 |
| AC123897.8 PK | 95 | 0.0 | 39 | AC123897.8 PK | 95 | 9e-143 | 35 |
| AC123897.10 PK | 98 | 6e-155 | 50 | AC123897.10 PK | 96 | 2e-135 | 38 |
| AC123897.11 LRR-PK | 97 | 0.0 | 40 | AC123897.11 LRR-PK | 96 | 4e-159 | 41 |
| AC123897.12 LRR-PK | 99 | 0.0 | 44 | AC123897.12 LRR-PK | 97 | 7e-152 | 35 |
| AC123897.15 PK | 91 | 6e-177 | 39 | AC123897.15 PK | 91 | 7e-124 | 31 |
| AC123897.17 PK | 96 | 0.0 | 44 | AC123897.17 PK | 97 | 1e-158 | 37 |
| AC123897.18 LRR-PK | 96 | 0.0 | 42 | AC123897.18 LRR-PK | 96 | 8e-152 | 37 |
| AC123897.20 LRR-PK | 99 | 0.0 | 61 | AC123897.20 LRR-PK | 97 | 0.0 | 62 |
| AC120539.3 LRR-PK | 96 | 0.0 | 42 | AC120539.4 LRR-PK | 96 | 8e-152 | 37 |
| AC145322.15 PK | 95 | 0.0 | 43 | AC145322.15 PK | 97 | 7e-152 | 34 |
| AC123523.3 PK | 21 | 3e-11 | 43 | AC123523.3 PK | 22 | 5e-13 | 50 |
| AC123520.7 LRR-PK | 92 | 0.0 | 46 | AC123520.7 LRR-PK | 92 | 1e-144 | 35 |
| AC123523.3 PK | 21 | 3e-11 | 43 | AC123523.3 PK | 22 | 5e-13 | 50 |
| AC119072.4 PK | 34 | 6e-35 | 37 | AC119072.4 PK | 25 | 4e-39 | 29 |
| AC119072.5 PK | 8 | 1.5 | 44 | AC119072.5 PK | 21 | 0.12 | 67 |
| AC137753.19 PK | 33 | 7e-31 | 41 | AC137753.19 PK | 19 | 2e-26 | 32 |
| AC136956.26 LRR-PK | 63 | 2e-50 | 32 | AC136956.26: LRR-PK | 65 | 3e-56 | 32 |
| AC136956.32 LRR-PK | 69 | 1e-47 | 30 | AC136956.32: LRR-PK | 65 | 3e-57 | 30 |
| AC136956.37 LRR-PK | 60 | 1e-46 | 27 | AC136956.37: LRR-PK | 62 | 3e-54 | 29 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AC136956.39 LRR-PK | 95 | 0.0 | 66 | AC136956.39: LRR-PK | 94 | 9e-144 | 35 |
| AC120889.18 LRR-PK | 63 | 2e-50 | 32 | AC120889.18 LRR-PK | 64 | 3e-56 | 32 |
| AC120889.19 LRR-PK | 91 | 0.0 | 46 | AC120889.19 LRR-PK | 63 | 3e-57 | 32 |
| AC133291.6 LRR-PK | 62 | 1e-55 | 31 | AC133291.6 LRR-PK | 67 | 8e-66 | 47 |
| AC133291.7 LRR-PK | 59 | 2e-42 | 38 | AC133291.7 LRR-PK | 69 | 8e-51 | 28 |
| AC133291.11 LRR-PK | 65 | 4e-49 | 44 | AC133291.21 LRR-PK | 65 | 2e-57 | 26 |
| AC133291.23 LRR-PK | 60 | 5e-40 | 40 | AC133291.23 LRR-PK | 67 | 8e-45 | 30 |
| AC109644.1 LRR-PK | 90 | 0.0 | 63 | AC109644.1 LRR-PK | 94 | 3e-137 | 36 |
| AC109644.2 LRR- PK | 97 | 0.0 | 96 | AC109644.2 LRR-PK | 89 | 6e-149 | 41 |
| AC109644.4 LRR- PK | 87 | 0.0 | 58 | AC109644.4 LRR-PK | 85 | 2e-133 | 33 |
| AC109644.5 LRR- PK | 99 | 0.0 | 60 | AC109644.5 LRR-PK | 95 | 5e-154 | 40 |
| AC109644.6 LRR-PK | 97 | 0.0 | 58 | AC109644.6 LRR-PK | 91 | 6e-153 | 36 |
| AC109644 .11 LRR-PK | 95 | 0.0 | 61 | AC109644 .11 LRR-PK | 95 | 1e-143 | 42 |
| AC109644-16 LRR-PK | 60 | 5e-40 | 40 | AC109644-16 LRR-PK | 67 | 8e-45 | 30 |
| AC137693.5 LRR-PK | 97 | 0.0 | 53 | AC137693.5 LRR-PK | 95 | 4e-142 | 36 |
| AC137693.6 PK | 90 | 0.0 | 63 | AC137693.6 PK | 94 | 1e-154 | 36 |
| AC137693.9 LRR-PK | 87 | 0.0 | 58 | AC137693.9 LRR-PK | 85 | 9e-148 | 33 |
| AC137693.10 LRR-PK | 98 | 0.0 | 60 | AC137693.10 LRR-PK | 95 | 6e-161 | 40 |
| AC137693.11 LRR-PK | 97 | 0.0 | 58 | AC137693.11 LRR-PK | 91 | 3e-171 | 36 |
| AC137693.16 LRR-PK | 95 | 0.0 | 61 | AC137693.16 LRR-PK | 95 | 4e-159 | 42 |
| AC114828.7 LRR-PK | 13 | 0.98 | 32 | AC114828.7 LRR-PK | 9 | 0.74 | 32 |
| AC109365.12 PK | 21 | 5e-40 | 37 | AC109365.12 PK | 29 | 8e-43 | 29 |
| AC121328.15 LRR-PK | 39 | 7e-06 | 38 | AC121328.15 LRR-PK | 43 | 6e-06 | 69 |
| AC121328.22 PK | 1 | 0.074 | 64 | AC121328.22 PK | 5 | 8e-04 | 33 |
| AC104844.18 PK | 29 | 4e-14 | 28 | AC104844.18 PK | 23 | 1e-11 | 50 |
| AF161269.7 PK | 79 | 2e-19 | 47 | AF161269.7 PK | 85 | 5e-20 | 50 |
| AC146766.14 LRR-PK | 91 | 4e-176 | 41 | AC146766.14 LRR-PK | 92 | 7e-139 | 38 |
| AC144716.7 LRR-PK | 91 | 1e-142 | 35 | AC144716.7 LRR-PK | 95 | 3e-124 | 33 |
| AC144716.11 LRR-PK | 66 | 2e-55 | 37 | AC144716.11LRR-PK | 66 | 3e-55 | 36 |
| AC144716.14 LRR-PK | 91 | 4e-176 | 41 | AC144716.14 LRR-PK | 92 | 7e-139 | 38 |
| AC109832.3 LRR-PK | 98 | 0.0 | 40 | AC109832.3 LRR-PK | 96 | 0.0 | 36 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AC109832.13 LRR-PK | 96 | 0.0 | 41 | AC109832.13 LRR-PK | 96 | 0.0 | 37 |
| AC145349.1 LRR-PK | 27 | 0.018 | 42 | AC145349.1 LRR-PK | 28 | 0.065 | 42 |
| AC113249.3 LRR-PK | 4 | 0.42 | 78 | AC113249.3 LRR-PK | 11 | 2.8 | 33 |
| AC122143.8 LRR-PK | NS | - | - | AC122143.8 LRR-PK | 7 | 0.64 | 46 |
| AC122143.17 LRR-PK | 61 | 4e-25 | 83 | AC122143.17 LRR-PK | 61 | 1e-25 | 31 |
| AC120888.12 LRR-PK | 55 | 2e-20 | 31 | AC120888.12 LRR-PK | 67 | 1e-18 | 55 |
| AC126057.15 PK | 22 | 2e-26 | 28 | AC126057.15 PK | 22 | 1e-29 | 32 |
| AC129227.16 PK | 22 | 2e-26 | 28 | AC129227.16 PK | 22 | 1e-29 | 32 |
| AC129226.4 PK | 25 | 1e-31 | 53 | AC129226.4 PK | 21 | 1e-30 | 31 |
| AC133609.16 PK | 37 | 6e-34 | 41 | AC133609.16 PK | 22 | 3e-30 | 33 |
| AC134924-16 PK | 37 | 6e-34 | 41 | AC134924-16 PK | 49 | 0.009 | 35 |
| AC133216.13 LRR-PK | 35 | 0.003 | 38 | AC133216.13 LRR-PK | 53 | 4e-04 | 75 |
| AC148822.7 PK | 34 | 3e-22 | 23 | AC148822.7 PK | 29 | 4e-25 | 83 |
| AC148822.8 PK | 35 | 8e-29 | 35 | AC148822.8 PK | 25 | 2e-30 | 56 |
| AC148822.11PK | 35 | 6e-27 | 38 | AC148822.11PK | 24 | 7e-30 | 28 |
| AC146937.13 PK | 94 | 2e-123 | 63 | AC146937.13 PK | 88 | 2e-98 | 65 |
| AC146937.15 PK | 96 | 1e-164 | 36 | AC146937.15 PK | 96 | 0.0 | 56 |
| AC146937.16 PK | 92 | 1e-146 | 36 | AC146937.16 PK | 99 | 0.0 | 87 |
| AC146937.19 PK | 97 | 4e-159 | 35 | AC146937.19 PK | 98 | 0.0 | 73 |
| AC116367.2 LRR-PK | 96 | 3e-161 | 38 | AC116367.2 LRR-PK | 95 | 0.0 | 53 |
| AC116367.3 LRR-PK | 95 | 9e-155 | 36 | AC116367.3 LRR-PK | 95 | 0.0 | 55 |
| AC116367.4 LRR-PK | 92 | 4e-128 | 36 | AC116367.4 LRR-PK | 92 | 4e-160 | 62 |
| AC116367.7 LRR-PK | 97 | 7e-168 | 46 | AC116367.7 LRR-PK | 98 | 0.0 | 51 |

Annexure 3.4 List of specific primers designed from the exonic region of R genes predicted in the BAC/PACs clones of chr-11 of rice

| **Primer Name** | BAC/PAC (In NCBI) | BAC/PAC | Forward primer | Reverse primer | Tm For/Rev | Expected product size |
|---|---|---|---|---|---|---|
| **IAG-1** | AF161269 | OSJNBa0034K24 | AGGGTGCAGTGCTACAATGG | GTGCGACACGTTGAACAGC | 62/60 | 411 |
| **IAG-2** | AF161269 | do | TCAACGTGTCGCACAACC | TGCTCATCAGGACGAACTCC | 56/62 | 498 |
| **IAG-3** | AF161269 | do | TTACCGCAAGGAAGAAAAGC | CTGAACCAAACAAACACATCG | 58/60 | 685 |
| **IAG-4** | AF161269 | do | AGGGTGCAGTGCTACAATGG | GTGCGACACGTTGAACAGC | 62/60 | 411 |
| **IAG-5** | AF161269 | do | TCAACGTGTCGCACAACC | TGCTCATCAGGACGAACTCC | 56/62 | 498 |
| **IAG-6** | AF161269 | do | TTACCGCAAGGAAGAAAAGC | CTGAACCAAACAAACACATCG | 54/58 | 685 |
| **IAG-7** | AF161269 | do | CGATGTGTTTGTTTGGTTCAG | AGAAGCCGTAGTCCACGATG | 60/62 | 505 |
| **IAG-8** | AC133609.2 | OSJNBa0002C14 | GACCCTGACATGTGGAAACC | GAAGTGTTGAGCAGGCAACC | 62/62 | 554 |
| **IAG-9** | AC133609.2 | do | CAAGTACCTGCTCTAGGGTTGC | AATGCTCTTCCAGGGATGC | 66/58 | 681 |
| **IAG-10** | AC133609.2 | do | CCTGGAAGAGCATTGCTACC | CCTCTTTCTCGTGCAATATCC | 62/62 | 602 |
| **IAG-11** | AC133609.2 | do | GGCAACATACTCCTGGATGC | TCCGTCTTTCCACAAGTTCC | 62/60 | 665 |
| **IAG-12** | AC123897 | OSJNBa0055G24 | TCCCTCTGGCATAGAACACC | GGATTTCGCCCTTAAGATGG | 62/60 | 650 |
| **IAG-13** | AC123897 | do | CCATCTTAAGGGCGAAATCC | ACAATGCTTGTCCTCTGAGC | 62/60 | 690 |
| **IAG-14** | AC123897 | do | CATTGGCTCAGAGGACAAGC | GGCAGTGTATGCCTTTCTCC | 62/62 | 589 |
| **IAG-15** | AC129224-11 | OSJNBa0029P13 | GTGGATGAACCAGGTCAAGG | CTGATCTGCCATCTCCTTCC | 62/62 | 434 |
| **IAG-16** | AC129227-2 | OSJNBb0099J16 | CAGTTTCGGTGTTGTGATGC | CTAGATTGGCCACGACTTCC | 60/62 | 395 |
| **IAG-17** | AC129225-11 | OSJNBa0051D10 | CAGCCAACCTGAAGAAGAGC | GGACAATCATCTGGGAAAGC | 62/60 | 406 |
| **IAG-18** | AC135190-9 | OSJNBa0064H09 | GAGAGGATGCACAGGAAAGC | GCCTGGACAAGCAACTAAGC | 62/62 | 379 |
| **IAG-19** | AC133291-3 | OSJNp0452B04 | GGGGCAGTTGACTAGTTTGG | AGCCATGCAGGAAACATAGG | 62/60 | 353 |
| **IAG-20** | AC133216-15 | OSJNBb0049B20 | AGCATAATCTGCCGAAGAGC | CTGGTCAATCCCAACTAGGC | 60/62 | 455 |
| **IAG-21** | AC120885-20 | OSJNBa0042J05 | GCCAAACAAGAACCAACAGG | TGCCTGGATTAGTCCTCACC | 60/62 | 374 |
| **IAG-22** | AC133609-12 | OSJNBa002C14 | GACCCTGACATGTGGAAACC | AAGCACCTACACCGATTTGG | 62/60 | 382 |
| **IAG-23** | AC129226-5 | OSJNBa0024E08 | CGTGTACAGGTTGGTGATGG | GAAGCCTTCCCATGTACACC | 62/62 | 713 |
| **IAG-24** | AC125783-3 | OSJNBb0039K08 | GAGACGGCCAGATGTACAGG | CGGTGTTGATCTCACTCTGC | 64/62 | 540 |
| **IAG-25** | AC136956.8 | OSJNBa0038B22 | TA CCATGACAATGCGTACGG | TCAGTCAACAAATTGTGAGCG | 63/61 | 548 |

**Appendix-3.1**

**1 M Tris HCl**: 30.275 g of 1 M Trizma base (Tris hydroxy methyl amino methane-MW 121.1) was dissolved in about 180 ml distilled water. Then, the pH was adjusted to 8 using 1 N HCl and finally the volume was made up to 250 ml. This stock solution was autoclaved and stored at room temperature.

**0.5 M EDTA**: 46.53 g of Ethyl diamine tetra acetic acid (EDTA-MW 372.24) was dissolved in the 180 ml of distilled water. Then, the pH of the solution was adjusted to 8.0 with NaOH and volume was made to 250 ml. This stock solution was autoclaved and stored at room temperature.

**TE buffer (pH 8.0):** The buffer was prepared with following concentrations.

| S.No. | Components | Stock Conc. | Final Conc. | Amount of stock/100 ml |
|---|---|---|---|---|
| 1 | Tris-Hcl (pH 8) | 1M | 10 mM | 1 ml |
| 2 | EDTA (pH 8) | 0.5 M | 1 mM | 0.2 ml |
| 3 | Distilled water | - | - | 98.8 ml |

**2.5 M NaCl:** 36.5 g of NaCl (MW 58.44) was dissolved in 250 ml of distilled water. The solution was autoclaved and stored at room temperature.

**5% SLS (Sodium Lauryl Sulphate):** 125 g of SLS was dissolved in 180 ml of distilled water, and then volume was made up to 250 ml.

**DNA extraction buffer:** It was prepared by mixing the following components.

| S.No. | Components | Stock Conc. | Amount of stock /100 ml |
|---|---|---|---|
| 1 | Tris-Hcl (pH 8) | 1.0 M | 10 ml |
| 2 | EDTA (pH 8) | 0.5 M | 4 ml |
| 3 | NaCl | 5.0 M | 28 ml |
| 4 | CTAB | 2 % | 2 g |
| 5 | PVP | 2 % | 2 g |
| 6 | Distilled water | - | 58 ml |

**Phenol : Chloroform : Isoamyl alcohol (25:24:1):** For preparation of 250 ml of stock solution, 125 ml phenol, 120 ml chloroform and 5 ml isoamyl alcohol were mixed well to get the required concentration.

**TAE Buffer preparation:** One litre of (50X) stock solution of TAE was prepared as follows: 242 g Tris base (FW = 121.14; Sigma, USA) was dissolved in approximately 750 ml deionized water. Carefully 57.1 ml of glacial acid and 100 ml of 0.5 M EDTA (pH 8.0) was added, mixed well by stirring. The volume was finally made up to 1000 ml using double distilled water. The solution was filter sterilized, thoroughly mixed, autoclaved and stored at room temperature. This stock solution can be stored at room temperature. The working solution of 1x TAE buffer was made by simply diluting the stock solution by 50X in deionized water. The buffer is now ready for running an agarose gel.

**10X TBE Buffer Preparation:** Mix following components to make 1 liter 10X TBE buffer

| S.No. | Components | Amount | 10X Stock Conc. | Final 1X Conc. |
|---|---|---|---|---|
| **1** | Tris Base | 108 g | 890 mM | 89 mM |
| **2** | Boric Acid | 55 g | 890 mM | 89 mM |
| **3** | EDTA (pH 8.0) | 40 ml | 20 mM | 2 mM |

**Appendix-3.2:**

**Preparation of stock solution of MS medium (Murashige and Skoog, 1962)**

**a) Macronutrient (Stock Solution I)-20X- 1L**

| S.No | Components | Amount |
|---|---|---|
| 1 | $NH_4NO_3$ | 33.0 g |
| 2 | $KNO_3$ | 38.0 g |
| 3 | $MgSO_4 \cdot 7H_2O$ | 7.4 g |
| 4 | $CaCl_2 \cdot 2H_2O$ | 8.8 g |
| 5 | $KH_2PO_4$ | 3.4 g |
| $DDH_2O$ to make up the final volume of | | 1000.0 ml |

**b) Micronutrients (Stock Solution II)-500X-100 ml**

| S.No | Components | Amount |
|---|---|---|
| 1 | $H_3BO_3$ | 0.31 g |
| 2 | $MnSO_4 \cdot H_2O$ | 1.11 g |
| 3 | $ZnSO_4 \cdot H_2O$ | 0.43 g |
| 4 | KI | 0.04 g |
| 5 | $NaMoO_4 \cdot 2\ H_2O$ | 0.01 g |
| 6 | $CuSO_4 \cdot 5H2O$ | 0.001g |
| 7 | $CoCl_2 \cdot 6H2O$ | 0.001 g |
| $DDH_2O$ to make up the final volume of | | 100.0ml |

**c) Iron source (Stock Solution III)-500X-100 ml**

| S.No | Components | Amount |
|---|---|---|
| 1 | $FeNa_2 \cdot EDTA$ | 1.83 g |
| $DDH_2O$ to make up the final volume of | | 100.0 ml |

**d) Vitamin (Stock Solution)-500X-100ml**

| S.No | Components | Amount |
|---|---|---|
| 1 | Thiamine·HCl | 5.0 mg |
| 2 | Pyridoxine·HCl | 25.0 mg |
| 3 | Niacin | 25.0 mg |
| $DDH_2O$ to make up the final volume of | | 100.0 ml |

**e) Stock Solution-500X-100ml**

| S.No | Components | Amount |
|---|---|---|
| 1 | Myo-inositol | 5 g |
| $DDH_2O$ to make up the final volume of | | 100.0 ml |

**Preparation of antibiotics**

| Reagent | Stock Conc. | Working Conc. | Dilution | Solvent |
|---|---|---|---|---|
| **Ampicillin** | 100 mg/ml | 100 µg/ml | 1,000× | $ddH_2O$ |
| **Kanamycin** | 50 mg/ml | 50 µg/ml | 1,000× | $ddH_2O$ |
| **Rifampicin** | 50 mg/ml | 100 mg/ml | 1000× | MeOH |
| **X-gal** | 40 mg/ml | 80 µg/ml | 500× | DMF |

**Appendix-3.3:** Preparation of protein extraction buffer

| S.No. | Components | Final concentration | Amount (For 100 ml) |
|---|---|---|---|
| 1 | 2MTris-HCl, pH 7.5 | 0.5 M | 25 ml |
| 2 | Sucrose | 0.7 M | 23.96 g |
| 3 | Kcl | 0.1M | 0.745 g |
| 4 | EDTA | 50 mM | 10 ml |
| 5 | β-mercaptoethanol | 2 % | * |
| 6 | PMSF | 1mM | * |

* β -mercaptoethanol and PMSF was added after grinding in Eppendorf tube directly.

**Rehydration buffer**

| S.No. | Components | Final concentration | Amount (For 10 ml) |
|---|---|---|---|
| 1 | Urea (MW-60) | 8 M | 4.8 g |
| 2 | Thiourea | 2 M | 1.52 g |
| 3 | CHAPS | 4 % | 0.4 g |
| 4 | DTT | 20 mM | 0.015 g |
| 5 | IPG Buffer | 0.8 % | 80 µl |

**SDS equilibration buffer**

(50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, bromophenol blue, 200 ml)

| S.No. | Components | Final concentration | Amount (for 200 ml) |
|---|---|---|---|
| 1 | Tris-HCl, pH 8.8 | 50 mM | 10.0 ml |
| 2 | Urea (FW 60.06) | 6 M | 72.07 g |
| 3 | Glycerol (87% v/v) | 30% (v/v) | 69 ml |
| 4 | SDS (FW 288.38) | 2% (w/v) | 4.0 g |
| 5 | Bromophenol blue | 0.002% (w/v) | 400 µl of 1% solution |
| 6 | Double distilled H2O | | to 200 ml |

**This is a stock solution. Prior to use DTT or iodoacetamide are added. Store at -20 °C.**

**12% SDS Polyacrylamide Gel:**

| S.No. | Components | Stock conc. | Amount (for 100 ml) |
|---|---|---|---|
| 1 | Acrylamide | 30 % | 40.0 ml |
| 2 | Tris, pH 8.8 | 1.5 M | 25 ml |
| 3 | SDS | 10% | 1 ml |
| 4 | APS | 10% | 1 ml * |
| 5 | TEMED | | 40 µl * |
| 6 | Double distilled H2O | | 33 ml |

***APS and TEMED are added just before poring.**

**Agarose Sealing Mixture:**

| S.No. | Components | Amount |
|---|---|---|
| 1 | 1X TAE Buffer | 50 ml |
| 2 | Agarose | 0.5 g |
| 3 | 1% Bromophenol Blue | 100 µl |

Microwave to boiling point to dissolve the Agarose.

**SDS PAGE Running Buffer (10x):**

| S.No. | Components | Amount (1 litre) |
|---|---|---|
| 1 | Glycine | 141.1 g |
| 2 | Tris base | 30.3 g |
| 3 | SDS | 10 g |
| 4 | Double distilled H2O | to 1000 ml |

# LIST OF PUBLICATIONS AND ACCESSIONS

## Research papers/proceeding publications

**Anirudh Kumar,** Waikhom Bimolata, Gouri Sankar Laha, R. Meenakshi Sundaram, Irfan Ahmad Ghazi (2011) Comparative analysis of the genomic regions flanking *Xa21* locus in *indica* and *japonica* ssp. of rice (*Oryza sativa* L.). Plant Omics Journal, 4(5):239–249.

**Anirudh Kumar**, Minu Marry John, Mir Zahoor Gul, Waikhom Bimolata and Irfan A. Ghazi (2011) Differential Responses of Non-enzymatic Antioxidative System under Water Deficit Condition in Rice (*Oryza sativa* L.); IPCBEE vol.9, IACSIT Press, Singapore.

**Anirudh Kumar,** Anirban Guha, Waikhom Bimolata, Attipalli R. Reddy, Gouri S. Laha, R. M. Sundaram, Manish K. Pandey, Irfan A. Ghazi (2013) Leaf gas exchange physiology in rice genotypes infected with bacterial blight: An attempt to link photosynthesis with disease severity and rice yield. Australian Journal of Crop Sciences, 7(1):32–39.

Waikhom Bimolata, **Anirudh Kumar,** Raman Meenakshi Sundaram, Gouri Shankar Laha, Insaf Ahmed Qureshi, Gajala Ashok Reddy, Irfan Ahmad Ghazi (2013) Analysis of nucleotide diversity of the major bacterial blight resistance gene *Xa27* in *Oryza sativa* and its wild relatives. Planta, 238(2):293–305.

**Anirudh Kumar**, Mir Zahoor Gul, Ayesha Zeeshan, Waikhom Bimolat, Insaf Ahmed Qureshi, Irfan Ahmad Ghazi (2013) Comparative analysis of antioxidative responses of rice genotypes under bacterial blight stress. Australian Journal of Crop Sciences, **Accepted.**

## Sequence submitted to NCBI (Genbank Accession No)

JF304301, JF304302, F304303, HQ888852, HQ888853, HQ888854, HQ888855, HQ888856, HQ888857, JN016505, JN016506, JN016507, JN016508, JN016509, JN016510, JN016511, JN016512, JN016513, JN016514, JN016515, JN016516, JN016517, JN016518, JN016519, JN016520, JN016521, FJ938717, FJ827495, GQ202121, GQ367296, GQ398491, GQ381274

## Seminars & Conferences

**Anirudh Kumar**, Mir Zahoor Gul, Ayesha Zeeshan, W. Bimolata, Rajiv Kumar, Insaf A. Qureshi and Irfan A. Ghazi (July 9-10, 2012). The International Dialogue on Designer Rice for Future: Perception & Prospects, held at ICRISAT, Hyderabad. Poster title: Comparative antioxidative responses of rice genotypes to bacterial blight stress.

**Anirudh Kumar**, Minu Marry John, Mir Zahoor Gul, W. Bimolata and Irfan A. Ghazi (My 7-9, 2011). International Conference on Food Engineering and Biotechnology, held at Bangkok, Thailand. Poster title: Differential Responses of Non-enzymatic Antioxidative System under Water Deficit Condition in Rice (*Oryza sativa* L.).

**Anirudh Kumar** and Irfan A. Ghazi (September 18$^{th}$, 2010). Plant Sciences Colloquium-2010, held at Dept. of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad (India). Presentation title: Comparative analysis of the genomic regions flanking *Xa21* locus in *indica* and *japonica* ssp. of rice (*Oryza sativa* L.) and screening of BAC clones for resistance gene analogues (RGAs).

**Anirudh Kumar**, W. Bimolata, Mir Zahoor Gul and Irfan A. Ghazi (February 11-13, 2010). 3$^{rd}$ Bihar Science Conference, held at Gaya College Gaya, Bihar (India). Presentation title: *Xa21* mediated disease resistance against *Xanthomonas oryzae* pv. *oryzae* in rice**.**

**Anirudh Kumar**, S. Fayaz Basha, W. Bimolata and Irfan A. Ghazi (October 01-03, 2010). International conference on frontiers in biological sciences (InCoFIBS-2010) held at NIT Rourkela, Odisha, India. Poster title: Screening of BAC clones for *NAM* [NO APICAL MERISTEM] genes on chromosome 11 of rice.