

**DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa*
L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO
BROWN PLANT HOPPER (BPH) BY MARKER-ASSISTED
SELECTION**

ARUN CHACKO

2019-21-057

**DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA**

2023

**DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa*
L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO
BROWN PLANT HOPPER (BPH) BY MARKER-ASSISTED
SELECTION**

by

ARUN CHACKO

(2019-21-057)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University



DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

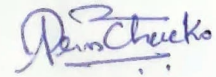
2023

DECLARATION

I, hereby declare that this thesis entitled “**DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa* L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO BROWN PLANT HOPPER (BPH) BY MARKER-ASSISTED SELECTION**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

Date: 21/07/2023

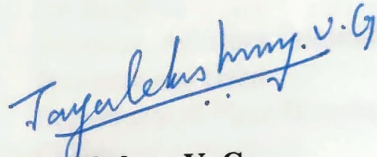


Arun Chacko.

(2019-21-057)

CERTIFICATE

Certified that this thesis entitled **"DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa* L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO BROWN PLANT HOPPER (BPH) BY MARKER-ASSISTED SELECTION"** is a record of research work done independently by **Mr. Arun Chacko** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to him.



Dr. Jayalekshmy V. G.

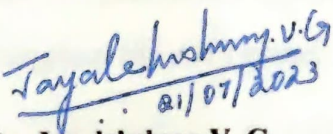
Vellayani

Date: 21/07/2023

(Major Advisor, Advisory Committee)
Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani
Trivandrum-695522

CERTIFICATE

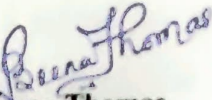
We, the undersigned members of the advisory committee of **Mr. Arun Chacko** a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Genetics and Plant Breeding, agree that the thesis entitled **"DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa* L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO BROWN PLANT HOPPER (BPH) BY MARKER ASSISTED SELECTION"** may be submitted by Mr. Arun Chacko in partial fulfillment of the requirement for the degree.


21/07/2023

Dr. Jayalekshmy V. G.
(Major Advisor, Advisory Committee)
Professor and Head
Department of Seed Science and
Technology
College of Agriculture, Vellayani



Dr. Seeja G.
(Member, Advisory Committee)
Professor and Head
Department of Plant Breeding and Genetics
College of Agriculture,
Vellayani



Dr. Beena Thomas
(Member, Advisory Committee)
Assistant Professor
Department of Plant Breeding and
Genetics
College of Agriculture, Vellayani



Dr. K. Karthikeyan
(Member, Advisory Committee)
Professor
Department of Agricultural Entomology
RARS, Pattambi, Palakkad



Dr. Shanas S.
(Member, Advisory Committee)
Assistant Professor,
Department of Agricultural Entomology,
IFSRS, Karamana, Trivandrum



External Examiner

Dr. S. JULIET HEPZIBA
Professor (PB&G.)
Dept. of Pl. Breeding and Genetics
Agri. College & Res. Instt.,
Kulikulam, Vallanadu - 628 252

Acknowledgement

First and foremost, praises and thanks to the Almighty, for everything that happens to me...

With immense pleasure, I would like to express my sincere gratitude to my major advisor Dr. Jayalekshmy V. G., Professor and Head, Department of Seed Science and Technology for the constructive guidance, constant inspiration, abundant encouragement, kind treatment, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for providing a stress-free situation with an open-minded approach and for the care and affection bestowed on me throughout the study period.

I convey my heartfelt gratitude to Dr. Seeja G., Professor and Head, Department of Plant Breeding and Genetics, for the unceasing encouragement, valuable advices, and wholehearted approach which helped me in the successful completion of the thesis.

I extend my sincere gratefulness to Dr. Beena Thomas, Assistant Professor, Department of Plant Breeding and Genetics, for the valuable suggestions, technical advices, and incessant motivation throughout the research work.

I am extremely thankful to Dr. Karthikeyan K., Professor, Department of Agricultural Entomology, RARS, Pattambi for the unstinting support, suggestions, and passionate approach rendered during the period of research work. Without his care, affection, assistance, and dedicated involvement in every step of the research, this work would have never been accomplished.

I express my sincere gratitude to Dr. Shanas S., Assistant Professor, Department of Agricultural Entomology, IFSRS, Karamana for his constant support, scientific advice, guidance, and motivation in all stages of the research work.

I am deeply grateful to all the teachers of Plant Breeding and Genetics, Dr. Lovely B, Dr. Gayathri, Ms. Adheena, Ms. Ninitha, and the retired faculties Dr. Mareen Abraham, and Dr. Lekha Rani C for their generous support, continuous and timely advice, constructive criticisms, and constant encouragement rendered to me during the course of my study and research work.

I wish to extend my sincere gratitude to beloved teachers Dr. Roy Stephen, Dr. Beena R., Dr. Sheeja K Raj, Ms. Nivedhitha, Dr. Smitha, S, Dr. Jyothi, Eldho Sir (AO), Dr. Biji, and Dr. Bhindhu J. S. for their advice, motivation, support, and encouragement throughout the research work.

It is my pleasure to express my special thanks to Dr. Raveendran M., Dr. Juliet Hepziba, Dr. Theradimani, Dr. Preethi, Dr. Sheela, Rohit Sir, Andrew Peter Sir, Sudhagar, Prabhu M., Baranidharan, Muniyappan and Williams from TNAU for their guidance and support throughout research programme.

Words are inadequate to express my wholehearted thanks to Shafi for standing beside me from my master's program onwards till now with unbound love and bighearted support. Without her efforts, care, and support this work would have never been accomplished.

I owe my deepest gratitude to my batch mates Anand S, Ankitha M.O., Bhaskar Reddy, and Swathy Sivan whose cooperation, love, support, and affection helped me through many hardships.

Words are inadequate to express my thanks to Nibinettan, Nadirsha, Govind, Christy, Kavya, Smera, Elzu, Namitha, and Akhila for their help and support throughout the journey.

I express my sincere thanks to all my seniors Nithishettan, Nyshanthettan, Dhaneshettan, Ananthu ettan, Jaslamikka, Athulettan, Ramling Sir, Hari Krishnan Chettan, Siddhesh Bhaiya, Vinay chettan, Manu chettan, Asoontha chechi, Namitha chechi, Thouseem chechi Reshma chechi, Chippy chechi, Rahana Chechi, Tejasree chechi, Praveena chechi, Darshan chettan, and lovely juniors Jibin, Abirami, Arya, Shazna, Bhalaji, Sethu, Murshi, Harish, Mythri, Jyothi, Mahesh, Vishak, Arya, Parvathy, Revathy, Anandhu, Boomiga, Dharshana, Reshma, Varsha, Rejith Bhai, Amruth Hari, Yedu, Sourav, Frankenstein, Syed, Arun Jose, and Ashik Sha for their help and support.

I express my sincere thanks to my batchmates Adarsh S., Karthik, Dhanu, Sreekanth, Sandy, Swathy, Dhorothy, Niji, Nikhil P.G., Arya Gopinath, Amrutha Unni, and Anju M Job for their help and support. A special mention of thanks to Arjun TP, Akhil Ajith, Fallu, Gokul, Abhinav, Fasseh, Ajmal, Surumani, Naveen, Guptha, Divya, Sruthy KT., Anaswara, Farhana, Amalnath, Ahalfith, Gautham, Hari chettan, Ajin, Amal Jose, Melvin, Alan, Savio, Gazal, Hari, Vipin, Prajish, Sumanya, and Anjana for their unbound love and bighearted support. A special thanks to Ajil M. S., Agricultural Officer, for his assistance in field visits, BPH collection, guidance, and motivation in thesis writing.

I owe thanks to my little ones and stressbusters Ponnus, Unnikuttan, Ponnu, and Aadhi for vanishing away my tiredness and uplifting me with happiness.

It is my pleasure to express my special thanks to the Professor and Head, Instructional Farm, Librarian, Academic staff, and all workers of CoA, Vellayani for their sincere cooperation and kind approach offered during the study period. I express my sincere thanks to Sthanulekshmi Chechi, Reji Chechi, Jisha Chechi, Sini Chechi, Radhamani Chechi, Ratheesh Chettan, and Divakaran Chettan for their immense support and cooperation during the study. I convey my special thanks to R G Sam Press for the timely support and service during thesis printing and binding, Chemical distributors Anoop, Sooraj, and Praveen for the timely delivery of chemicals for research work.

I wish to extend my sincere gratitude to beloved Rev. Fr. Francis Nellikunnel for his prayers, moral support, motivation, and encouragement in each and every stage of my life.

I am thankful to Kerala Agricultural University for the technical and financial support for carrying out my study and research work.

It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartfelt thanks to all those who helped me in completing this venture in time.

I am beholden beyond words to express my indebtedness to my loving Pappa, Mummy, Pinky, and Ammachi for their unconditional love, sacrifices, and support bestowed on me and for being the pillar of strength during challenging times.

ARUNCHACKO

CONTENTS

Sl. No.	CHAPTER	Page No.
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-58
3	MATERIALS AND METHODS	59-83
4	RESULTS	84-157
5	DISCUSSION	158-181
6	SUMMARY	182-186
7	REFERENCES	187-230
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	The reaction of genotypes of rice to the four biotypes of BPH	28-29
2.	List of major BPH resistance genes/QTLs have been reported in several <i>Oryza sativa</i> sp. <i>indica</i> cultivars and wild relatives	42-45
3.	List of major gene pyramiding studies in BPH resistance	54-57
4.	List of genes, chromosome location, SSR markers, and annealing temperature	73
5.	Features of recipient and donor parents used in the study	74
6.	Response of rice genotypes to BPH infestation	86
7	BPH resistance reaction in parent genotypes	87
8.	Morphological evaluation of parents	90
9.	Genetic parameters of fourteen characters in parent genotypes	91
10.	The biochemical parameters of recipient and donor parents before and after BPH infestation	96
11.	The genotypic correlation between biochemical parameters after infestation and phenotypic reactions to BPH	99
12.	The genotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents	100
13.	The phenotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents	103

14	The direct and indirect effects of morphological and biochemical characters in donor and recipient parents	106
15	Details of crossing in the development of F_1 and BC_1F_1	111
16	Genotyping of BC_1F_1 lines	112
17	The chi-square test for goodness of fit in BC_1F_1 generation	112
18	Phenotypic data of BC_1F_1 [(Jyothi x PTB 33) x Jyothi] lines with the <i>Bph-3</i> gene	119-121
19	Phenotyping of BC_1F_1 [(Jyothi x IR65482-7-216-1-2) x Jyothi] lines with <i>Bph-18</i> gene	122-124
20	Intercrossing of BC_1F_1 lines	127
21	Details of intercross of BC_1F_1 lines	127
22	Genotyping of Intercrossed BC_1F_1 (ICAB and ICBA) Lines	128
23	The details of backcrossing Intercrossed BC_1F_1 for the development of BC_2F_1 lines	129
24	Genotyping of BC_2F_1 (ICAB and ICBA) Lines	130
25	Phenotyping of Intercrossed BC_1F_1 lines	133-135
26	Phenotyping of BC_2F_1 lines	136-137
27	Proximity dissimilarity matrix analysis using morphological traits of intercrossed BC_1F_1 lines in comparison with Jyothi by Euclidean distance	142-143
28	Proximity dissimilarity matrix analysis using morphological traits of BC_2F_1 lines in comparison with Jyothi by Euclidean distance	144
29	BC_2F_2 lines forwarded to BPH bioassay	147

30	BC ₂ F ₂ segregants showing resistance in BPH bioassay	147
31	Phenotyping of BC ₂ F ₂ segregants derived from ICAB-1/3	150-151
32	Phenotyping of BC ₂ F ₂ segregants derived from ICAB-1/6	152-153
33	Proximity dissimilarity analysis using morphological traits of BC ₂ F ₂ segregants derived from ICAB-1/3 using Euclidean distance	156
34	Proximity dissimilarity analysis using morphological traits of BC ₂ F ₂ segregants derived from ICAB-1/6 using Euclidean distance	157

LIST OF FIGURES

Fig. No.	Title	Pages Between
1.	Backcross breeding program	74-75
2.	Functional Plant Loss Index (FPLI) in the genotypes after BPH infestation	89-90
3.	Dendrogram of intercrossed BC ₁ F ₁ lines based on proximity dissimilarity matrix using morphological traits from the recipient parent Jyothi	145-146
4.	Dendrogram of BC ₂ F ₁ lines based on proximity dissimilarity matrix using morphological traits from the recipient parent Jyothi	145-146
5.	Constellation plot of the intercrossed BC ₁ F ₁ lines based on the proximity dissimilarity matrix using morphological traits	145-146
6.	Constellation plot of the BC ₂ F ₁ lines based on the proximity dissimilarity matrix using morphological traits	145-146
7.	Dendrogram of the BC ₂ F ₂ segregants derived from the ICAB-1/3 based on the proximity dissimilarity matrix using morphological traits	157-158
8.	Dendrogram of the BC ₂ F ₂ segregants derived from the ICAB-1/6 based on the proximity dissimilarity matrix using morphological traits	157-158
9.	Constellation plot of the BC ₂ F ₂ segregants derived from the ICAB-1/3 lines based on the proximity dissimilarity matrix using morphological traits	157-158
10.	Constellation plot of the BC ₂ F ₂ segregants derived from the ICAB-1/6 lines based on the proximity dissimilarity matrix using morphological traits	157-158

11.	The damage scores of rice genotypes in BPH seedling screening	161-162
12.	Biochemical parameters in parent genotypes before and after BPH infestation	165-166
13.	Correlation between biochemical components after infestation and phenotypic reactions to BPH	165-166
14.	PCV and GCV of morphological and biochemical characters in donor and recipient parents	167-168
15.	Heritability and genetic advance of morphological and biochemical characters in donor and recipient parents	167-168
16.	Genotypic correlation matrix of morphological and biochemical characters in donor and recipient parents	169-170
17.	Phenotypic correlation matrix of morphological and biochemical characters in donor and recipient parents	169-170
18.	Path diagram showing the direct and indirect effect of fourteen characters on FPLI	169-170
19.	The frequency of gene combinations in intercrossed BC_1F_1 lines	173-174
20.	The frequency of gene combinations in BC_2F_1 lines	173-174
21.	Distribution, Scatter plot, and Correlation of BC_1F_1 plants derived from PTB-33	175-176
22.	Distribution, Scatter plot, and Correlation of BC_1F_1 plants derived from IR65482-7-216-1-2	175-176
23.	Distribution graph of morphological traits in intercrossed BC_1F_1 lines	175-176
24.	Distribution graph of morphological traits in BC_2F_1 lines	175-176
25.	Distribution graph of BC_2F_2 ICAB-1/3 progenies	175-176

26.	Distribution graph of BC ₂ F ₂ ICAB-1/6 progenies	175-176
27.	Percentage similarity of intercrossed BC ₁ F ₁ and BC ₂ F ₁ lines to recipient parent 'Jyothi' based on Euclidean distance using morphological traits	181-182
28.	Percentage similarity of BC ₂ F ₂ lines (ICAB-1/3 progenies) to recipient parent 'Jyothi' based on Euclidean distance using morphological traits	181-182
29.	Percentage similarity of BC ₂ F ₂ lines (ICAB-1/6 progenies) to recipient parent 'Jyothi' based on Euclidean distance using morphological traits	181-182
30.	Recurrent parent phenotype recovery in BC ₂ F ₂ lines	181-182

LIST OF PLATES

Plate No.	Title	Pages Between
1.	BPH outbreak in (a) Moncompu (b) Wayanad during 2020-2021	11-12
2.	BPH outbreak in Palakkad region during 2020-2021	11-12
3.	Seedlings planted in seedbox for BPH screening	60-61
4.	Experimental setup for Honeydew test	60-61
5.	Plant, seed and kernel morphology of recipient and donor parents	74-75
6.	Crossing block in field and insect-proof net house	74-75
7.	Crossing technique in rice	76-77
8.	Seed set in crossed panicle	76-77
9.	Harvested panicle and dried seeds	76-77
10.	Response of rice genotypes in BPH screening	87-88
11.	Honeydew test in recipient and donor parents	89-90
12.	Culm thickness of parental genotypes	93-94
13.	Kernel colour of parental genotypes	93-94
14.	Amplification profile of SSR markers specific to genes for BPH resistance	107-108
15.	Amplification profile of RM589 specific to <i>Bph-3</i> gene in BC ₁ F ₁ lines [(Jyothi x PTB-33) x Jyothi]	111-112
16.	Amplification profile of RM3331 specific to <i>Bph-3</i> gene in BC ₁ F ₁ lines [(Jyothi x IR65482-7-216-1-2) x Jyothi]	111-112

17.	Amplification profile of RM589 linked to the gene <i>Bph-3</i> and RM3331 linked to the gene <i>Bph-18</i> in intercrossed BC ₁ F ₁ ICAB lines	125-126
18.	Amplification profile of RM589 linked to the gene <i>Bph-3</i> and RM3331 linked to the gene <i>Bph-18</i> in BC ₂ F ₁ ICAB and ICBA lines	127-128
19.	BPH bioassay in BC ₂ F ₂ segregants	145-146
20.	Amplification profile of RM589 specific to <i>Bph-3</i> and RM3331 specific to <i>Bph-18</i> in BC ₂ F ₂ segregants derived from ICAB-1/3 and ICAB-1/6	145-146
21.	BC ₂ F ₂ segregants with the combination of both genes (<i>Bph-3</i> and <i>Bph-18</i>) in homozygous resistant condition	157-158
22.	Panicle of BC ₂ F ₂ segregants with the combination of both genes (<i>Bph-3</i> and <i>Bph-18</i>) in homozygous resistant condition	157-158
23.	Seeds of BC ₂ F ₂ segregants with the combination of both genes (<i>Bph-3</i> and <i>Bph-18</i>) in homozygous resistant condition compared with recipient parent Jyothi	157-158

LIST OF ABBREVIATIONS AND SYMBOLS USED

^o C	Degree Celsius
%	Per cent
@	At the rate of
&	And
BPH	Brown Plant Hopper
CD (0.05)	Critical Difference at 5% level
cm	Centimetre
mg	Milligram
mm	Millimetres
ml	Millilitres
CRD	Complete Randomized Design
DAS	Days After Sowing
<i>et al.</i>	And others
Sec	Seconds
Min.	Minutes
h	Hours
g	Gram
Kg	Kilo gram
SSR	Simple Sequence Repeats
KAU	Kerala Agricultural University
bp	Base pairs
PCR	Polymerase Chain Reaction
ITS	Internal Transcribed Spacer
l	Litres
Temp.	Temperature
<i>Via</i>	Through
No.	Number

RARS	Regional Agricultural Research Station
MSST	Modified Seedbox Screening Test
SES	Standard Evaluation System
t ha ⁻¹	Tonnes per hectare
rpm	Rotation per minute
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
GCV	Genotypic Coefficient of Variation
PCV	Phenotypic Coefficient of Variation
viz.	Namely
d.f	Degrees of freedom
S. E	Standard Error
mg g ⁻¹	Milligram per gram

INTRODUCTION

1. INTRODUCTION

“If you give me rice, I will eat today; if you teach me how to grow rice, I will eat every day.”- Mahatma Gandhi

Rice and its cultivation are integral parts of humankind in and around the world. In efforts to end world hunger and malnutrition, rice is considered the most important crop with significant contributions to the world's primary food production (FAO, 2022). More than half of the global rice production is shared by India and China. The primary challenge confronting rice scientists today is how to continually develop technologies that will eventually result in higher production to commensurate with the increasing population. India has surpassed China to become the world's most populous nation, with 142.86 crore inhabitants (UNFPA, 2023). At the same time, drastic climatic change ensued abiotic and biotic stresses exacerbated the rice production constraints.

Brown plant hopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is the major biotic threat in rice production. In India, the most severe BPH outbreak caused a yield loss in around 50,000 hectares of rice fields in Kerala state between 1971 and 1973-74 (Abraham and Nair, 1975). In recent years, the rice tracts of Kerala have witnessed widespread BPH outbreaks mainly in Palakkad and Kuttanad regions. Unpredictable occurrence, high host specificity, rapid adaptability, migratory capacity, complex insect-plant interactions, quick evolution, and resistance-breaking capacity of the BPH in rice are the major insect factors that challenged the rice breeders and entomologists in developing BPH-resistant rice varieties. Apart from that, indiscriminate insecticide application can have dire consequences on the ecological balance of the rice farming system and lead to the resurgence of BPH.

The major management tactics for the BPH biotype and its development are the periodic release of varieties containing major effect genes, gene pyramiding, horizontal resistance, gene rotation, and varietal blending along with proper agronomic practices, and comprehensive pest monitoring and surveillance. The most reliable, accurate, economical, and environmentally friendly method of managing BPH is to create a persistent host-plant resistance mechanism (Singh *et*

al., 2020). This could be achieved by the development and cultivation of BPH-resistant cultivars. The existing rice varieties with resistance to BPH were developed by conventional breeding strategies mainly by pureline selection from local landraces, hybridization, or mutation. These varieties are not popular among rice farmers due to various reasons such as poor yield, long duration, photosensitivity, and so on. The development of new cultivars that possess BPH resistance mechanisms along with the potential for high yields has become the need of the hour.

There is a possibility that continual planting of crop varieties with monogenic resistance would result in the emergence of virulent BPH biotypes. So the major objective in BPH resistance breeding should be to broaden the genetic base of resistance in terms of both major and minor genes. Gene pyramiding technique can be effectively used in the transfer of R-genes for BPH resistance from variable donor parents to a recipient or recurrent parent which is an elite high-yielding but susceptible variety. Gene pyramiding includes the identification of donor parents with a gene of interest, crossing it with the recipient parent for transferring the desired gene, and repeated backcrossing with the recipient parent for recurrent parent phenotype and genome recovery followed by continuous selfing for fixation of the desired gene in homozygous condition (Dormatey *et al.*, 2020). Conventional breeding strategies include the maintenance of a large population in each crossing, and hectic selection procedures which are time and labour-consuming and reduce the efficiency of gene transfer. The availability of molecular markers for the identification of the desired genes provided easy tracking and selection of resistant plants at an early stage. Marker-assisted backcrossing is an efficient method for transferring and pyramiding genes for BPH resistance (Vignesh *et al.*, 2023).

Around 45 genes imparting resistance to BPH have been identified and mapped, out of which 11 are cloned and characterized (Li *et al.*, 2023). The presence of multiple genes for resistance can provide durable resistance against BPH biotypes and is considered effective as similar to vertical resistance. Either multiple donors or donor parents with more than one gene can be used to introgress

genes for BPH resistance. The gene *Bph-3* reported in KAU rice variety PTB-33 is one of the major genes that confer resistance to all known biotypes of BPH (Akanksha *et al.*, 2019). Introgression lines derived from wild relatives were used to transfer genes to a cultivated variety (Jena *et al.*, 2006). The majority of the genes are tracked using SSR markers for foreground, recombinant, and background selection in the segregating generations to develop or improve a variety with 3 to 4 backcrosses (Hu *et al.*, 2016a).

Recent infestations in rice tracts in Kerala had led to severe crop losses for the farmers growing the most widely accepted popular high-yielding variety Jyothi. To combat BPH attack and improve the variety Jyothi with BPH resistance in the background, the present study was put forth as ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ with the following major objectives,

- i. BPH bioassay, gene validation, morphological and biochemical assessment of rice genotypes for donor parent selection.
- ii. To introgress R genes for resistance to BPH in the background of the elite variety Jyothi using marker-assisted selection.
- iii. Development of BC₂F₂ lines with a combination of genes for resistance to BPH.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

‘Rice is life’

The year 2004 was the *annus mirabilis* in the history of the United Nations General Assembly (UNGA) - an agricultural commodity was globally recognized for the first time and a year was specifically dedicated to it; the ‘International Year of Rice’. The slogan of the year ‘*Rice is life*’ emphasizes that rice is the most important crop among cereals, a primary food source providing livelihoods, and supporting biodiversity thus becoming the way of life. Being the staple food of more than two-thirds of the population, the most exploited crop by human beings, and the first sequenced crop genome, rice is unarguably becoming a model plant of the present era. With utmost importance given to rice crop improvement, the review of literature of the present study entitled ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ is put forth.

2.1. HISTORY OF RICE

The Latin term ‘*Oryza*’ and English word ‘Rice’ was adopted from the Tamil word for rice, ‘*arisi*’ (Sharma, 1991). The Sanskrit words ‘*vrihi*’ and Dravidian ‘*vari*’ and ‘*ari*’ denoted rice (Nair, 2003). In the Malayalam language, rice is called ‘*ari*’. Many of the traditional rice varieties were mentioned in ancient pieces of literature from 2500 BCE, such as ‘*Susruta Samhita*’, and ‘*Ashtanga Hridaya*’ (500 BCE) mentioning njavara rice, Palakkadan matta rice in ‘*Tamil Thirukkural*’ (from 300 BCE), ‘*Krishipattu*’ (17th century), an old recitation during rice planting mentioning Wayanad Gandhakasala and Wayanad Jeerakasala rice, a book named ‘*A journey from Madras through the countries of Mysore, Canara, and Malabar*’ written by Francis Buchanan reported Kaipad rice cultivation during the 18th century, and Pokkali rice cultivation aspects were detailed in Cochin state manual during 1911 (Blakeney *et al.*, 2020). Archaeological evidence of rice in India dating back to around 6400 BCE, found rice grains from the middle Ganges plains, and during 3000-1500 BCE, rice domestication shreds of evidence were found from the Indus Valley civilization (Bates, 2022). During 2000 BCE, remains of charred rice grains were excavated in more than thirty historical sites. The

Sanskrit treatise, *Arthashastra* reported the present-day rice variety ‘sathi rice’ maturing in sixty days. Rice is very well connected with Buddhism culture and Hindu mythology in India, where no rituals or functions are complete without the use of rice grains. Especially in marriage ceremonies, rice is showered over the newlywed couples as a blessing implying that they stay united, flourish like the sturdy grain, and together face all situations in life. This custom is adopted all over the world from India (Sharma, 1991). Rice has become an important ingredient in many rituals and is worshipped in Kerala from the field where it grows to the granary. Southern Travancore celebrates the ‘birthday of rice’ on the day of ‘makam’ asterism in the Malayalam month ‘kanni’ (Nair and Santiaguel, 2014). Thus rice enriched the culture and blended as an inseparable part of life.

2.1.1. Rice cultivation in Kerala

Rice is the most essential and inevitable crop in Kerala state. The state is home to over 500 rice varieties, including traditional varieties with medicinal and aromatic properties, and nutrient-rich varieties. Paddy cultivation over unique and varied agro-climatic conditions i.e., from 3 meters below the sea level to high altitudes is world recognized. Rice occupies 99.9 per cent of food grain production in the state and third place in its contribution to the total cropped area. Palakkad, Alappuzha, Thrissur, and Kottayam districts contribute more than 80 per cent of rice production in Kerala. The agricultural and allied sector accounts for about 10.13 per cent of Kerala’s Gross State Value Added (GOK, 2022).

Kerala rice varieties viz., Njavara, Pokkali rice, Kaipad rice, Wayanad Gandhakasala and Wayanad Jeerakasala, and Palakkadan Matta have obtained geographical indication for its unique characteristics, thus farmers acquiring knowledge and awareness in conserving varieties, gaining reputation and economic benefits (Radhika and Raju, 2021). Farmers partake rice breeder’s role by conserving the specialty rice and traditional varieties. The fourth-highest civilian recognition in the Republic of India, ‘Padma Shri in agriculture’ in the year 2023 was awarded to Mr. Cheruvayal K. Raman, a septuagenarian tribal rice farmer from Wayanad district for his distinguished contribution to conserving more than fifty indigenous rice varieties and was also honored with prestigious National Genome

Saviour Award. Thus traditional rice varieties of Kerala and the farmers conserving them are well-renowned in the agricultural community in the country.

‘What is cooked in earth that’s nice, Cooked in wood will fetch good price, Turns into sweetmeats if cooked thrice? The answer is RICE (Sharma, 1991).

Rice is cooked all over the world and is an inevitable part of the diet of people in Kerala where it is consumed three times a day in one form or another. Rice grains of various colours viz., red, white, black, brown, green, and yellow are under cultivation in different parts of the state but the most preferred for consumption and trading are red and white kernel rice varieties. Red and brown rice are mostly cultivated throughout the state. Rice kernels with a prominent red bran layer are referred to as red rice varieties. The red colour is due to the anthocyanin pigment which possesses antioxidant and free radical scavenging properties. With its highly valuable nutritional content, unique taste, aroma, and medicinal properties, red rice was well recognized from ancient times by Charaka (700 BC), Susruta (400 BC), and Vagbhata (610 AD) in ‘vridhdha trayi’ (a text of indigenous Indian medical system) of Ayurveda, where the red rice variety ‘*Rakthashali*’ of Kerala was considered the best for treating ulcer and fever, best for skin health, diuretic, improves eyesight, fertility, and health (Ahuja *et al.*, 2007). Other widely cultivated and well-known Kerala rice varieties include Palakkadan Matta, Jyothi, Uma, Aiswarya, Prathyasa, Manu Ratna, Shreyas, Bhadra, and Kattamodan. Anthocyanin in coloured rice is responsible for reductase enzyme inhibition causing anti-androgen effects and anti-diabetic activities and has a role in treating urinary tract infections and benign prostatic hyperplasia. The amylose equivalent, glycemic index, glycemic load, triglyceride content, and fatty acid profile of the variety *Jyothi* are comparable with the variety *Njavara* (Deepa *et al.*, 2008). Red rice contains proanthocyanidins that help prevent type 2 diabetes and cancer. The nutritional characteristics of red rice varieties are explored and made use of by including it in the diet as various dishes such as sadhya with rice as a major component, idli, appam, puttu, payasam, and so on (Rathnapriya *et al.*, 2019). Rice variety *Jyothi* has high per cent content of protein, lipid, potassium, iron, zinc and intermediate amylose, manganese, calcium, and copper content (Pillai *et al.*, 2020). Red rice is

rich in total polyphenolic compounds, iron and zinc content, and brown rice is highly nutritious with rich fibre content which fights colon cancer by binding to carcinogenic substances, low calorie, and lysine, a good source of manganese and selenium for fighting cancer by scavenging free radicals, magnesium preventing cardiovascular diseases, sodium balance and blood pressure regulation, vitamin B₆, phosphorous, niacin, aspartic and glutamic acid, thiamine, vitamin E and γ -oryzanol helps in lowering cholesterol levels (Kowsalya *et al.*, 2022).

2.2. THE RICE GENOME

The first scientist to study rice's microsporogenesis, megasporogenesis, and mitosis was Kuwada (1910) who reported that the chromosome number of rice is $2n = 2X = 24$ and has a genome size of 430 Mb with 40,000 to 50,000 functional genes (Kurata *et al.*, 2002). The haploid chromosomes complement was divided into five large, four medium, and three small chromosomes by Rau (1929). Shastry *et al.* (1960) conducted the initial research on rice's pachytene chromosomal complement. Chromosomes were numbered in decreasing order of length, with chromosome 1 being the longest and chromosome 12 being the shortest. The cultivated rice (*Oryza sativa* L.) belongs to the family Gramineae or Poaceae. The species of the *Oryza* genus are classified into four complexes namely, *Sativa*, *Officinalis*, *Ridley*, and *Meyeriana* (Vaughan, 1994). The genus *Oryza* consists of 25 recognized species of which two are cultivated species viz., *Oryza sativa* and *Oryza glaberrima* (Sanchez *et al.*, 2013). These two cultivated species are believed to be evolved independently from *Oryza nivara* and *Oryza barthii* and were domesticated in the South-East Asian region and tropical West Africa respectively. Nine distinct genome groups, viz., A, B, C, D, E, F, G, H, and J are identified in rice (Brar, 2003). The species present in the *Sativa* complex have AA genome and form the primary gene pool. Species belonging to *Officinalis* complex form the secondary gene pool while the *Ridley* and *Meyeriana* complex form the tertiary gene pool. The progenitors of *O. sativa* and *O. glaberrima* belong to the Asian diploid species (AA genome) and African diploid species (AA genome). *Oryza sativa* comprises three subspecies viz., *indica*, *japonica*, and *javanica* (Zhang and Wing, 2013).

2.2.1. Taxonomic classification of Rice

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Poales

Family: Gramineae or Poaceae

Tribe: Oryzeae

Genus: *Oryza*

Species: *sativa*

2.3. CONSTRAINTS IN RICE PRODUCTION

At present, Kerala is witnessing a transformation from an agrarian economy to a market economy. Land use pattern changes from food crops to cash crops, high rainfall intensity, intermittent droughts, less adoption of farm mechanization, the increasing cost of production, inadequate paddy procurement, storage, milling, and marketing facilities, and the unpredictable incidence of pests and diseases are the major impediments to sustainable agriculture progress in Kerala's rice cultivation. The years 1960-80 witnessed the peak production of rice (up to 13.7 lakh tonnes) in Kerala even then the state experienced shortage of 40 per cent of the total demand (GOK, 2016). During 2021-22, the state produced 6 lakh tonnes of rice where the annual requirement is projected around 35 to 40 lakh tonnes (GOK,2022). This jeopardized situation on food security forces the state to depend on other states such as Andhra Pradesh, Tamil Nadu, and Karnataka for meeting the food grain demand.

Apart from this, drastic climatic change is the major crop yield-limiting factor as it brings unexpected abiotic and biotic stresses. Abiotic stresses include salinity, flood, drought, heat, cold, iron toxicity, and so on (Kumar *et al.*, 2021). Biotic stresses include diseases such as bacterial leaf blight, rice blast, sheath blight, grain discoloration, tungro disease, grassy stunt, brown spot, false smut, and many more (Singh *et al.*,2020). Insect pests of rice are the major biotic stress affecting the yield mainly due to the sudden incidence and potential damage-causing ability, and insect population dynamics. Insect pests of rice are classified as major and minor pests. Reports on pest incidence in rice reveal the change in insect pest behavior that the minor pests are sometimes emerging as major pests and vice-versa (Pathak, 2023). Climatic changes are the major cause behind this changing status of insect

pests as they can affect the insect population dynamics by altering the host-plant interaction, and geographical distribution, reducing the efficacy of biocontrol, increasing the biotype development, pest-transmitted diseases incidence, and migratory pest invasion (Hajjar *et al.*,2023).

2.4. INSECT PESTS OF RICE

The rice crop architecture consists of a shoot system with stalks, leaves, panicles, and a fibrous root system. The growth of rice passes through the vegetative, reproductive, and ripening phases from seed germination to the mature grain stage. The crop duration of cultivated varieties in Kerala varies from 75 days to 190 days. The rice crop is attacked by more than 100 species of insects; 20 of them can cause economic damage (Pasalu and Katti, 2006). The average yield loss in rice due to insect pests has accounted for 30% by stem borers, plant hoppers 20%, gall midge 15%, leaf folder 10%, and other pests 25%, respectively (Lisha *et al.*, 2020; Mohapatra *et al.*, 2022). The insect pests in rice crop are classified as leaf-feeding, sap-sucking, stem and ear-damaging, root-damaging, and flower and grain damaging. Mainly two types of feeding modes are seen in insects, chewing type, and piercing and sucking type. The chewing-type insects like leaf folders and stem borers have mouth parts to break and ingest the plant parts. Whereas, insects such as plant hoppers and leaf hoppers, have piercing and sucking type feeding modes with mouth parts that allow sucking up nutrients from vascular tissues through penetration.

Major insect pests in rice are Plant hoppers [mostly brown planthopper (BPH), *Nilaparvata lugens* and white-backed plant hopper (WBPH) *Sogatella furcifera*], Stem borers (yellow stemborer, *Scirphophaga incertulas*, and striped stemborer, *Chilo suppressalis*), Leaf folders (*Marasmia patnalis* and *Cnaphalocrocis medinalis*), Rice gall midge, (*Orseolia oryzae*), Rice bug (*Leptocorisa oratorius* F. and *Leptocorisa acuta* Thunberg.), and Case worm (*Nymphula depunctalis*). These insects can cause direct or indirect damage (Hajjar *et al.*,2023). The direct damage is caused by insect feeding on the plant resources accompanied by insect excretion of honeydew. The pest population size can determine the damage. Indirect damage is caused by the insect transmission of plant

diseases (viruses, mycoplasma, etc.), mainly by sucking pests. Among these, the brown plant hopper acts as a vector for transmitting rice grassy stunt and ragged stunt viruses and has posed a great threat to rice cultivation (Jeevanandham *et al.*, 2023).

2.4.1. The Brown Plant Hopper

The Brown plant hopper was first described by Stål in 1854 as *Delphax lugens* and later renamed as *Nilaparvata lugens* by Distant in 1906. Pioneer studies on the biology and ecology of BPH were done by Caresche in 1933, followed by extensive studies on the effect of nutrient components on BPH feeding (Abraham, 1957; Sakai and Sogawa, 1976), insect polymorphism and its role in BPH population growth (Kisimoto, 1965), fecundity and pre-oviposition period by Suenaga (1963) and Pathak (1968), biochemical basis of resistance to BPH (Kalode, 1971), the feeding behavior of BPH and honeydew secretion by Sogawa (1971) and Mochida (1972), migration and flight activity of BPH (Ohkubo, 1973), and metabolic changes in plants upon BPH feeding (Cagampang *et al.*, 1974).

2.4.1.1. Distribution of brown plant hopper

Brown plant hopper (*Nilaparvata lugens*) is distributed over Asian countries, Pacific islands, and Australia. Historical shreds of evidence recorded the incidence of BPH from AD 701 as a minor pest. Seasonal incidence and migration of BPH were reported in India, China, Fiji, Indonesia, Japan, Korea, Malaysia, Nepal, Philippines, Papua New Guinea, Sri Lanka, Taiwan, Thailand, and Vietnam. The first published report on the widespread occurrence of BPH is from Ceylon (Hutson, 1923). In India, the most severe BPH outbreak caused yield loss of around 50,000 hectares of rice fields in Kerala state during 1971 and 1973-74. (Chakrabarti *et al.*, 1971; Koya, 1974; Nalinakumari and Mammen, 1975). Almost all stages of the crop were affected causing a loss of 70 to 100 per cent in severely affected fields and many farmers were forced to abandon the crop. Around US\$ 12 million was the estimated loss in the 1973-76 period due to the severe BPH outbreak. As many as 10 states in India have reported severe outbursts during the 1970s (Das *et al.*,

1972). In the recent years 2019-2022, Kerala state witnessed the widespread outbreak of BPH infestation mainly in Palakkad district and Kuttanad region in Alappuzha district, the two major rice growing tracts in the state. The infestation was also reported in rice fields of Wayanad district in Kerala (Karthikeyan, K., personal communication, 2022; unreferenced) (Plate 1 and 2). The recent infestations have become a serious threat to the rice-growing farmers as the BPH infests at almost all stages of the crop and the severe damage had even led the farmers to forsake the crop.

2.4.1.2. Taxonomic classification of brown plant hopper(Dupo and Barrion, 2009)

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Family: Delphacidae

Tribe: Delphacini

Genus: *Nilaparvata*

Species: *lugens*

2.4.1.3. Biology and ecology of brown plant hopper

The brown plant hopper, *Nilaparvata lugens* (Stål) is a major pest in rice-growing countries in the world mainly by virtue of the capricious infestation and the severe damage caused by it. Being a delphacid planthopper belonging to the order Hemiptera, the brown plant hopper (BPH) feeds directly by sucking the phloem sap, ovipositing in plant tissues, and acting as a vector of grassy stunt and ragged stunt virus to host plants causing the hopper-burn symptom and drastic yield loss of 20 to 80 per cent for rice farmers. The ontogenetic development is the universal process in the biology of plant hoppers with a total life cycle of about 9 to 26 days and an adult plant hopper may live up to 20 days. BPH is dimorphic in nature with truncate and fully winged forms known as ‘brachypterous’ and ‘macropterous’ respectively. The macropterous BPH population has the potential migratory capacity and produces next-generation with males as macropterous and females as brachypterous. Mating occurs from the day of emergence, and from the

day following mating brachypterous females start to oviposit around 300-350 eggs in the mid-region of the leaf sheath and in leaf blades along a straight line. The egg-laying period ranges from 10 to 28 days. Macropterous females lay less number of eggs. Eggs are elongated and curved with 0.9 mm length and 0.12 mm width. Eggs usually hatch in 6 to 9 days. On hatching, the nymphs will be cottony white, and within an hour, it turns brown colour and pass through five instars till becoming adult BPH. The first instar lasts for 2 to 3 days, the second instar nymphs of brownish black colour have a duration of 2 to 4 days, the third instar remarks the appearance of wings and nymphs will be in light yellow colour, the yellowish-brown colour of nymphs appears in the fourth instar and fifth instar with a length of 2.858 mm. Adult BPH males and females measure 4.5 and 5 mm in length respectively. The brachypterous females have tegmina atrophied into brownish-black scale-like, leathery structures lacking venation and covering only the basal region of the abdomen (Mochida and Okada, 1979).

The maximum survival and reproduction of BPH depend mainly on temperatures ranging from 25-30°C. Egg hatchability and survival of nymphs are optimum at 25°C. BPH nymphs and adults can feed on any stage of the rice plant. The longevity of adult males ranges from 14 to 21 days and that of females from 14 to 30 days. Brachypterous females have very short longevity with an average of 5.6 days (Nalinakumari and Mammen, 1975; Mochida and Okada, 1979).

The insects feed the basal region of the tillers, get rarely noticed by the farmers and the plants get yellowish in the early stages of infestation, leading to round yellow patches with increasing infestation and drying up rapidly causing brown patches in the field. These brown patches are referred to as the 'hopper-burn' symptom of BPH. Endowed with migratory capacity, the brown plant hopper can invade a crop in large numbers under favourable meteorological conditions and is also influenced by the age and sex of BPH (Mochida and Okada, 1979).

The major outbreak of BPH have occurred due to rampant pesticide use causing the insect pest resurgence, improper agronomic practices such as excessive use of nitrogenous fertilizers expediting fecundity of BPH, reduced plant spacing,

prolonged flooding of water in the field, continuous cultivation of susceptible varieties (Dyck *et al.*, 1979).

2.4.1.4. Feeding behavior of brown plant hopper

The BPH is a typical vascular feeder, sucking the phloem sap by 'stylet-sheath feeding' forming a tubular lining by secreting coagulable saliva into the plant (Miles, 1972). BPH has a piercing and sucking type mouth part of about 700 μ long and comprises an outer pair of mandibular and an inner pair of maxillary stylets bundled together for sucking the plant sap. The highly confined feeding activity includes an array of gustatory responses to specific botanical stimuli and many intermediary behavioral reactions that are induced instinctively or in accordance with the internal demands of the insect. Phloem-feeding insects engage in a sequence of feeding behaviours after arriving on a plant to gain access to the underlying phloem-sieve components (Sogawa, 1973).

Three main behavioral phases of feeding are labial exploration of the plant's surface, stylet probing, and sucking (Sogawa and Cheng, 1979). For insects to survive, choosing appropriate host plants and feeding locations is crucial. Hemipterans typically have sensory organs including chemo- or mechanosensory tarsal sensilla, as well as antennal and labial sensilla (Backus, 1988). When BPH comes into contact with a plant, it will walk quickly across its surface while continuously dabbing at it with the tip of its labium to locate a suitable feeding location. BPH has a short time of searching and probing on BPH-susceptible rice plants, followed by an extended period of continuous phloem sap absorption. In contrast, BPH moves more frequently and makes shorter, more frequent probes on BPH-resistant plants. The parenchyma tissues outside the vascular bundles are probed up to 400 μ deep by inserting stylets through a single entry point, secretes coagulable saliva, and deposit stylet sheaths made up of stable lipo proteinaceous substance in the plant tissues in a forking pattern. The damaged cells undergo plasmolysis but do not show any external symptoms but it affects the phloem sap flow to a greater extent. Upon stylet probing and saliva secretion into vascular tissues, BPH starts sucking the phloem sap and nutrients leading to a significant reduction in the translocation of photosynthates to the root system which is

considered the most probable cause of hopper burn symptoms. The physiological disruption in the root system of rice causes leaf senescence. BPH excretes a large amount of honeydew during continuous feeding on plants. The BPH attack leads to the accumulation of amides and free amino acids in the leaves, and a reduction in starch, protein, and moisture content. The decline in moisture content reduces the chlorophyll content in leaves, causing leaf chlorosis in older leaf blades which shows yellowing as the initial symptom and extends to all other plant parts, ultimately causing the brown colour 'hopper burn' and plant death (Sogawa and Cheng, 1979).

The yield loss in rice by BPH is attributed to the reduced number of grains per panicle and the number of panicles per unit area if infested before the maximum tillering stage, and a lower percentage of ripened grain and grain weight if infested after the heading stage. The control measures are taken based on the economic threshold level (ETL) and economic injury level (EIL). The economic threshold level (ETL) is the insect pest density at which control measures need to be taken to restrict the pest population from reaching EIL (Sogawa, 1994). The economic injury level (EIL) is the lowest insect pest population density that will cause economic damage (Stern *et al.*, 1959). ETL of BPH is 5 to 10 insects per hill in non-endemic regions and 2 insects per hill in endemic regions. Farmers prefer chemical pesticides to control BPH if the population crosses the ETL to prevent it from attaining EIL. The pest-defender ratio of 2:1 is followed in rice against BPH to avoid the use of chemical pesticides. Chemical control measures should be withdrawn if the crop is in the maturing or grain-hardening stage (Jena *et al.*, 2018). Farmers prefer insecticides as a control measure of BPH which is not always effective and can have undesirable effects. BPH is more difficult to control using insecticides compared to other plant hoppers in paddy. The insect feeds at the base of the plant making it laborious and time-consuming for farmers. IRRI developed a six-row drop nozzle sprayer for reducing the insecticide application time than the canopy sprays. The granular broadcast was also suggested as an effective method of application. Diazinon was the most widely recommended chemical for BPH control but was banned for manufacture, import, and use in India [GOI, 2022]. The resurgence-

inducing pesticides' ability to enhance BPH's feeding rate at sub-lethal levels, the suppression of natural enemies after intense broad-spectrum insecticide treatment, the pest's ability to stimulate growth and reproduction after insecticide application, and changes in the plant's nutritional composition after insecticidal spraying are significant contributors to the resurgence of BPH (Heinrichs, 1979). Synthetic pyrethroids, a few organophosphates, and carbamates are the major insecticidal groups that cause an increase in the resurgence ratio (Chelliah *et al.*, 1984). Insecticides such as sulfoximines (Ghosh *et al.*, 2013), pymetrozine (Lakshmi *et al.*, 2010; Song *et al.*, 2023), and botanical insecticides such as methyl eugenol (Xu *et al.*, 2015) were reported to be more effective against BPH and less toxic to natural enemies. BPH is known to develop insecticidal resistance within a short period of time. Therefore, evaluating the toxicity and other side effects of newly applied insecticides is crucial for determining the ecological effects of those pesticides in the rice environment.

In India, 93 % of farmers depend more on chemical control of which 51% get their plant protection advice from dealers, while 22% from extension officials, and the majority of the farmers initiate the plant protection based on the first appearance of the pest, irrespective of their population, crop stage, and their damage relationships (Rao and Rao, 2010). Rice plant protection solely depends on insecticides which have tremendous effects on the environment, biodiversity, and human and animal health. Rice alone consumes 18 per cent of chemical pesticides in India (Devi *et al.*, 2017). Considering the after-effects of chemical pesticides, the Government of India banned the use of 55 insect pesticides (GOI, 2022).

2.4.1.5. Abiotic factors affecting biology of BPH

Temperature and carbon dioxide are the primary factors involved in plant-insect interactions. Temperature changes can affect the life cycle, metabolism, metamorphosis, population size, mobility, and geographical distribution of insect pests in rice (De Lucia *et al.*, 2012). The physiology of insects is extremely sensitive to temperature fluctuations, and a rise of 10 °C tends to cause their metabolic rate to roughly double. The rate of the global temperature increase in the upcoming years will determine future changes in insect population dynamics. By the end of the

present century, the average global temperature is expected to rise by 1.5 to 4 °C, according to climate models (Skendžić *et al.*, 2021). Climate change had a sweltering effect in Kerala state as recorded temperatures during 2023 drifted over 32 to 33°C on average throughout the state with a maximum temperature of above 40.1°C in Palakkad district (IMD, 2023). An overall increase of 0.54°C in the daily average temperature is observed in the state (Valappil *et al.*, 2023). Palakkad is the major rice-growing district in the state, with Jyothi as the leading rice variety. Reports from RARS, Pattambi have confirmed the increased incidence of BPH in the rice fields of Palakkad district (Karthikeyan, K., unpublished data, 2023; unreferenced). Peak infestation of BPH was observed within a temperature range of 30-32°C and a relative humidity range of 71-79 per cent. Rice fields of Kerala witnessed widespread BPH attacks during the months of December-January (Abraham and Nair, 1975). A constant temperature of 29°C and 90 to 100 per cent relative humidity favoured the development of brachypterous females (Thomas, 1977). A change in the survival trend of BPH over the years was observed as the upper lethal temperature for BPH nymphs and adults had increased up to 42°C (Piyaphongkul *et al.*, 2012) and infestation had been observed during the months of August to January in rice fields of Kerala (Karthikeyan, K., unpublished data, 2023; unreferenced). Along with temperature fluctuations, rainfall patterns (up to 500 mm) significantly influence the BPH population and its spread. The southern coastal regions of India (Kerala, Karnataka) were predicted as very suitable habitat and high-risk areas for BPH spread which is less than 10 per cent of the total land area at present but it is predicted to be more than 25 per cent by 2050 in the future climatic scenarios. The increasing temperature also creates an asynchrony between natural enemies and insect pests affecting the natural biocontrol system (Pandi *et al.*, 2021).

According to recent studies, the atmospheric CO₂ concentration is now 419 ppm (NOAA, 2021) and is projected to increase to 550 ppm by 2025 and 700 ppm by 2050 (IPCC, 2007). Thus, by 2050, it is anticipated that plants will grow in an environment that contains more CO₂ than it does now, which will have a negative impact on yield. High temperatures with increased CO₂ levels in the atmosphere

can increase the carbohydrate concentration in plants and stimulate insect pests to feed more. The fecundity of brachypterous female BPHs had increased by 29.5 per cent under elevated carbon dioxide and temperature (570 ppm + 3°C in ambient temperature) but the nymphal duration and adult longevity were reduced significantly. This depicted that the population of pests will increase as the life cycle shortens. The higher fecundity rate under elevated conditions increased the BPH population with a shortened life cycle (Pandi *et al.*, 2018).

Water deficits and excesses have an influence on the dynamics between plants and insects. Normally, sucking pests showed inferior performance during water stress conditions whereas it does not have an adverse effect on chewing pests (Huberty and Denno, 2004). BPH outbreaks can also be attributed to rainfall and relative humidity. Abundant rainfall with more than 30 rainy days during the June to September months, followed by higher relative humidity had led to BPH outbreaks. Forecasting the frequency of rain during the monsoon season might give vital information regarding the likelihood that BPH will occur (Chander and Husain, 2018). Water stress in rice plants causes a significant increase in callose deposition and ABA content which in turn hindered BPH feeding, reduced population growth, and had negative physiological changes in BPH (Liang *et al.*, 2022; Liao *et al.*, 2023).

Wind acts as an important agent of insect dispersal and can affect insects both physically and physiologically. Macropterous female adults can migrate and cover a long distance of thousands of kilometres across sea and land (Riley *et al.*, 1991). Wind can act indirectly on plant-insect interactions by reducing predator activity (Carmona *et al.*, 2011). The high migratory ability of BPH enables the pest to invade, settle down, and are sedentary enough to develop a population in its preferred habitat. The microclimate that favours the faster multiplication of immigrant BPH is the closer spacing of plants, continuous flooding, high fertility, and dense canopy with more tillers (Iamba and Dono, 2021).

Soil fertility and fertilizer application can influence insect growth, survival, population density, and fecundity. Reduction in the concentration of secondary metabolites may also occur depending upon the fertilizer application. The dense

BPH population on rice due to the nitrogen application was first reported by Abraham (1957). The nitrogen-rich susceptible plants attracted more female BPH adults and enhanced the survival and multiplication of BPH (Thomas, 1977). The use of nitrogenous fertilizers can increase the growth and reproduction of phytophagous insects (Clemensen *et al.*, 2017). In comparison to other nutrients, nitrogen was revealed to be a more limiting element for BPH in rice plants. While applying phosphorous and potassium fertilizers raised their concentrations in plant tissues alone, not in BPH, applying nitrogen fertilizers to rice plants enhanced the nitrogen concentrations in both the rice plants and BPH. Additionally, nitrogen fertilizer application raised the concentration of soluble proteins and lowered silicon content in rice plants, which boosted BPH feeding and sharply decreased relative water content in rice plants, thus making them more susceptible to BPH. In contrast, potassium fertilizers contribute to tolerance against BPH in rice with reduced nitrogen, soluble protein, and free sugar content, and a minimum reduction of relative water content (Rashid *et al.*, 2016). The strong interaction between silicon and nitrogen accumulation in rice significantly affects rice plants' resistance to insect herbivores when grown in the presence of high nitrogen or silicon-amended soils. High nitrogen fertilization levels result in reduced silicon accumulation, and silicon amendment also enhanced rice resistance to BPH (Wu *et al.*, 2017a). The improvement in BPH ecological fitness characters was observed with increased nitrogen and phosphorous application (Rashid *et al.*, 2017). Silicon application can confer insect resistance to plants by deposition of the silicon layer in epidermal layers acting as a mechanical barrier and inducing elicitors (Roy and Panda, 2020). By triggering plant defence responses against BPH and salicylic acid signalling, silicon actively participates in defence priming in rice (Lin *et al.*, 2022). Therefore, it is important to utilize nitrogen and phosphorous inputs sparingly while growing rice to maintain a low level of BPH ecological fitness potential.

Like other insect pests of rice, BPH depends heavily on environmental factors and seasonal variations for its survival, behaviour, and dispersion. The miraculous capacity of BPH to overcome the resistance of several rice varieties and pesticides, and its monophagous feeding nature on rice make control more challenging. The

abiotic factors can affect BPH insect resistance in plants negatively or positively. The consequences of each factor and its interaction with plants and insects must be understood to develop new methods by using advanced climate and pest monitoring and modelling prediction tools in sustainable pest management strategies

2.5 MECHANISM OF RESISTANCE TO BPH IN RICE

Painter (1951) defined Host Plant Resistance as those characters that enable a plant to avoid, tolerate or recover from attacks of insects under conditions that would cause greater injury to other plants of the same species. The resistance traits are pre-adaptive genetically inherited characters of the plant that helps them survive and resist the selective pressure of the insect population (Sharma and Ortiz, 2002).

The cultural approach of host-plant resistance includes the modification of production practices to make the environment less favourable for pest invasion, reproduction, survival, and dispersal. Its aim is to achieve reductions in pest numbers. In rice, the heavy BPH incidence is avoided by modifying the cropping system, sowing the early maturing varieties, synchronized planting, and adopting agronomic practices such as crop rotation by the simultaneous planting of different resistant varieties, field sanitation by clearing alternate hosts such as *Leersia hexandra*, *Eleusine coracana*, *Zea mays*, *Zizania latifolia* and *Z. longifolia*, and destroying ratoons of harvested crop and removal of rice stubbles to prevent the spread of grassy stunt and ragged stunt virus, proper water management by alternate wetting and drying, optimum plant spacing, and fertilizer management by integrating the cultivation of fertilizer responsive BPH resistant rice varieties along with optimum use of nitrogenous fertilizer (Dyck *et al.*, 1979; Oka, 1979).

The biological control approach to host plant resistance aims at increasing the activity of predators along with the cultivation of BPH-resistant varieties to counterattack the BPH. Biological control relies on the interaction of organisms with the target pests and the environment. It is therefore more complex than certain traditional pest control practices. Though it cannot control all pests, it can be taken as a basic approach to host plant resistance and integrated pest management. The wolf spider (*Pardosa pseudoannulata*), dwarf spider (*Atypena formosana*), orb

spider (*Araneus inustus*), and green mirid bug (*Cyrtorhinus lividipennis*) are the major predators of the nymphs and adults of BPH (Sigsgaard, 2007; Fahad *et al.*, 2015).

The host plant shows diverse kinds of responses upon BPH attack such as avoiding host selection, feeding, and oviposition inhibition, affecting insect survival, and tolerating or recovering from damage caused by the insect population.

Painter (1951) classified the mechanisms of insect resistance as antixenosis, antibiosis, and tolerance.

The resistance mechanism in which the host plant characters lead to non-preference of the insects for colonization, oviposition, or feeding is termed antixenosis, non-acceptance, or non-preference. The term antixenosis coined by Kogan and Ortman (1978) was used for specifying it as a term related to plant character. The degree of antixenosis is variable and can depend on the morphological and biochemical features of the host plant. Allomones or Kairomones can also lead to complete host rejection. In the first attempt of probing, BPH prefer to feed on the long-cell block, a soft, smooth area on the surface of rice leaf sheaths. Because the preservation of this high-density energy channel is essential for plant growth and fitness, plants have developed strong chemical and physical defence mechanisms in the phloem to fend off BPH. One typical plant defence against BPH is sieve-tube sealing either by callose deposition or protein plugging. The sclerenchyma tissue of the rice leaf sheath is the second area where BPH on rice feeds. Above the parenchymal cells, vascular bundle, and below the epidermis of the long-cell block lies the sclerenchyma. Therefore, in order to create a feeding site in the phloem, BPH stylets must penetrate the sclerenchyma. Rice may impede BPH feeding through sclerenchyma strengthening by increased cellulose and hemicellulose deposition (Shi *et al.*, 2021). Antixenosis measured in five wild rice accessions along with varieties TN1 and PTB-33 revealed that the variety PTB-33 possessed minimum settling preference of BPH nymphs and adults, the least ovipositional preference, the maximum feeding or probing marks on the leaf sheath, the maximum trichome density and more number of silica (Sandhu and Sarao, 2021).

The antibiosis resistance mechanism is the ability of the host plant to adversely affect the biology (survival, development, and reproduction) of the insects and their progeny due to the morphological, physiological, or biochemical factors present in it (Smith, 2005). In some cases, allelochemicals (non-nutritional plant chemicals affecting insect behaviour) and primary metabolites causing a nutritional imbalance in insect pests may also be the basis of antibiosis. Varieties with low levels of asparagine cause reduced reproduction of brown plant hopper. Similarly, the host choice test for BPH revealed that BPH was more inclined to feed on plants with low resistance. Antibiosis analysis in rice lines with resistance genes *BPH14* and *BPH15* against BPH showed a significant reduction in population growth rate, survival rate, and emergence rate of BPH nymphs, a significant delay in the developmental duration of nymphs, reduced honeydew excretion of females, and a decreased female ratio, when compared with rice carrying no BPH-resistant genes (Qiu *et al.*, 2020).

Sandhu *et al.* (2020) studied antibiosis parameters in wild rice accessions induced by BPH feeding. The antibiosis parameters such as honeydew excretion, nymph emergence, nymph survival, and development duration, and the potential role of antioxidant enzymes viz., superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and H₂O₂ content were analyzed and found that enhanced enzyme activity and H₂O₂ are involved in BPH insect resistance. The antibiosis investigation by honeydew excretion test in the variety PTB-33 and near-isogenic lines (NILs) developed from the cross between T65 and PTB-33 showed the reduced area of honeydew excretion in the Bph-17-PTB NILs coupled with low plant dry weight loss to dry weight gain in BPH (Nguyen *et al.*, 2021).

The ability of the plant to grow and yield despite pest attacks reveals the tolerance level of a genotype. Tolerant varieties usually show greater recovery from pest damage than susceptible varieties. Factors involved in tolerance are plant vigour, regrowth of damaged tissue, compensatory growth in individual plants or populations, and phytosynthate partitioning. In rice, parameters such as relative growth rates of plant height and fresh weight and the number of days until yellowing were studied to identify the tolerance level in rice varieties against BPH (Qiu *et al.*,

2011). The tolerance mechanism in the BPH screening was studied in nine rice genotypes by computing the functional plant loss index (FPLI) relative dry weight of plants and plant dry weight loss to dry weight gain in BPH (PDWL) based on the relative dry weight of BPH. The genotypes PTB-33 and RP2068-18-3-5 were found to have minimum FLPI and PDWL i.e., high level of tolerance against BPH (Sarao and Bentur, 2016).

The nature of resistance is controlled by morphological factors such as the presence of trichomes, thickness, and roughness of plant tissues, and plant colour, physiological factors such as osmotic concentration of cell sap, and exudates and biochemical factors such as reducing sugars, amino acids, phenolic content, protein content, ascorbic acid, silica content and so on (Ashrith *et al.*, 2017).

Major feeding inhibitors in rice are silicic acid, oxalic acid, and beta-sitosterol. BPH-resistant genotypes have a higher concentration of defensive compounds and a lower concentration of nutritional compounds. The resistant genotypes recorded higher content of crude silica, ascorbic acid, oxalic and phenolic acids (Roy *et al.*, 2022). The endogenous metabolites such as proteinase inhibitors, secondary metabolites (alkaloids, terpenes, flavonoids, etc.), callose, and volatile chemicals (green leaf volatiles, beta-caryophyllene, S-linalool) are released by rice plants in response to BPH attacks (Muduli *et al.*, 2021).

The recent developments in plant breeding to improve grain yield and quality traits have resulted in high-level susceptibility to insect pests. Long and continuing evolutionary processes result in complex insect-plant interactions. The counteracting adaptations by insects towards plant defence mechanisms need to be restricted for which insect factors affecting host plant resistance should be elucidated. Those insect-related factors are insect age and sex, insect preconditioning of host plants, levels of insect infestation, and biotypes of insects.

The response of the BPH to the biophysical and biochemical traits of the variety determines its population establishment. These responses include orientation, feeding, metabolic utilization of ingested food, adult growth and survival, oviposition, and egg hatching (Saxena and Barrion, 1983).

Orientation responses are those that make the BPH get attracted or repelled based on visual, humidity, or odour-based stimuli. The orientation test with BPH biotypes confirmed that the steam distillate extracts of susceptible varieties attracted the specific BPH biotypes for feeding (Saxena and Barrion, 1983). Probing or stylet introduction and duration of feeding determined the feeding response. Seven common amino acids in rice such as valine, asparagine, alanine, serine, glutamic acid, aspartic acid, and leucine had a strong chemosensory specificity in the bioassay of BPH biotypes 1, 2, and 3. The most phagostimulatory were valine and asparagine for biotype 1, alanine for biotype 2, and valine and serine for biotype 3 (Saxena and Barrion, 1983). Food utilization by BPH depends on the weight gain and it depicted the nutritive value of the particular genotype. Growth from nymph to adult through five instars was calculated as growth index, the ratio of the percentage of nymphs growing into an adult to the growth period over an identical period. The oviposition and egg hatching may differ in resistant and susceptible varieties (Saxena and Pathak, 1979).

Host specialization by BPH is also controlled by endosymbionts such as yeast-like endosymbionts (YLS) within the family Clavicipitaceae that occur in fat body cells of BPH. These endosymbionts compensate for the amino acid and protein imbalances while feeding on plants and provide rare nutrients to BPH. YLS plays an important role in both the development of BPH and the adaptation of BPH virulence to resistant plants by uric acid recycling, synthesis of the precursor of cholesterol, and ecdysone (moulting hormone), controls the development of nymph to adult, nymphal survival and feeding behaviour (Chen, 2009; Horgan *et al.*, 2021). The complexity of BPH-rice interactions is aggravated by the presence of mutualistic endosymbionts. All these responses may alter the ability of BPH to infest or attack different varieties of the same host species differing in their resistance to the concerned pest. This leads to the development of insect strains known as biotypes.

2.5.1. Biotype development in brown plant hopper

The term ‘biotype’ refers to insect populations of an infraspecific category with a certain biological trait that has a similar genetic makeup. The interactions between

the genotypes of pest populations and domesticated cultivars, as well as the heterogeneities of their environments, drive the complicated process of biotype evolution. Based on the evolutionary concept, the resistance was classified as sympatric resistance (acquired by coevolution of plant and insect, governed by major genes) and allopatric resistance (not developed by co-evolution of plant and insect, governed by many genes) (Saxena and Barrion, 1987).

Sympatric speciation of the BPH biotype proposed by Bush (1974) presented that the host system causes a sudden shift in the insect's survival locus (loci), giving the genotypes that survive an advantage in terms of fitness. He also suggested that the insect populations are preadapted, and the best genotypes are expressed based on the current environmental conditions. Mutation or dispersal through migration was also considered as the potential cause of insect biotype evolution. When tolerance or non-preference is the plant's innate method of resistance, insects rarely develop biotypes. The antibiosis resistance mechanism causes insect biotype development. The resistant crop variety exerts selection pressure on the BPH population leading to the deaths of many. The biotype populations may be allopatric, parapatric, or partly and transiently sympatric (coincident) with other compatible populations, but they differ in one or more biological characteristics. (Saxena and Barrion, 1987).

Biotypes can be classified as true-resistance-breaking and highly vigorous biotypes. True-resistance-breaking biotypes are adapted to overcome a specific resistance mechanism of the host plant, resulting in a successful attack on host varieties with specific resistance mechanisms. Highly vigorous biotype attacks both resistant and susceptible varieties of the host more successfully than the pre-existing biotypes that lack host-insect pest interaction. The biotypes of BPH are true-resistance-breaking biotypes. Factors influencing the selection of biotypes in BPH are the selection pressure exerted by the resistant crop variety, monogenic resistance, improper management practices, inherent genetic capacity to overcome host resistance, insect species with short life cycles, short generation times, and high fecundities (Muduli *et al.*, 2021).

Biotypes are determined by factors such as host or food preferences, behaviour, genetic makeup and variation, pheromone differences, physiological reactions to environmental stresses (such as changes in light, temperature, relative humidity, insecticides, and host plants), disease vector abilities reproductive analyses (such as fecundity and fertility), diurnal or seasonal behaviour patterns, and migration and distribution tendencies. The concept of insect biotypes specifically refers to the gene-for-gene relation between a pest insect species' genotype for virulence and a host plant's genotype for resistance. There exists a genetic link between resistant rice cultivars and the biotypes of the brown planthopper that infests rice. Biotype 1, Biotype 2, and Biotype 3 populations expressed monogenic virulence towards their respective host varieties. The dominance relationships for the virulence genes B1, B2, and B3 were as follows: B1> B3> B2. Biotype 1 thrived on and infected rice types without any resistance genes. The varieties susceptible to Biotype 1 and those with the Bph-1 resistance gene favoured the development of Biotype 2. Rice varieties susceptible to Biotype 1 and with Bph-2 resistance genes are infested by Biotype 3 (Saxena and Barrion, 1983). The continuous growing of insect-resistant varieties may lead to certain physiological and behavioural changes in insect pests so that they are capable of feeding and developing on the resistant variety (Dhaliwal and Arora, 2009). Insect biotypes are also considered useful tools for studying insect-host interactions, which are crucial for developing crop varieties that are resistant to insects (Kumar *et al.*, 2020).

The reaction of genotypes of rice to the four biotypes of BPH is enlisted in Table 1.

Table 1. The reaction of genotypes of rice to the four biotypes of BPH

SI No.	Variety	Genes	Biotypes				References
			1	2	3	4	
1.	<i>Oryza australiensis</i>	<i>Bph10</i>	+	+	+	#	Pathak and Kush, 1979
2.	<i>Oryza australiensis</i>	<i>Bph4</i>	+	+	+		
3.	<i>Oryza brachyntha</i>		+	+	+		
4.	<i>Oryza brachyntha</i>		+	+	+		
5.	<i>Oryza latifolia</i>	<i>Bph12</i>	+	M+	+		
6.	<i>Oryza latifolia</i>	<i>Bph12</i>	+	+	+		
7.	<i>Oryza punctata</i>		+	+	M+		
8.	Ptb 19	<i>Bph3</i>	+	+	+		
9.	Ptb 20	-	+	+	+		
10.	Ptb 21 (Thekkan)	<i>bph2,Bph3</i>	+	+	+		
11.	Ptb 33	<i>bph2,Bph3, BPH32</i>	+	+	+	+	
12.	Gangala	<i>Bph3</i>	+	+	+		
13.	Heen Rath		+	+	+		
14.	Babawee	<i>bph4</i>	+	+	+		
15.	Kahata Samba	<i>bph4</i>	+	+	+	M+	
16.	Kalu samba		+	+	+		
17.	Muthumanikam	<i>Bph3</i>	+	+	+		
18.	Sinna Sivappu		+	+	+		
19.	<i>Oryza officinalis</i>	<i>Bph6, Bph13</i>	+	+	+	+	
20.	<i>O. latifolia</i>	<i>Bph12</i>	#	+	#	#	Jena and Kim, 2010
21.	<i>O. minuta</i>	<i>Bph20, Bph21</i>	+	#	#	#	
22.	<i>O. australiensis</i>	<i>Bph18</i>	+	+	+	+	
23.	Mudgo	<i>BPH1</i>	+	-	+	-	
24.	Rathu Heenati	<i>BPH3</i>	+	+	+	+	

25.	ARC10550	<i>bph5</i>	-	-	-	+	
26.	ASD 7	<i>bph2</i>	+	+	-	-	
27.	Babawee	<i>bph4</i>	+	+	+	+	
28.	T12	<i>bph7</i>	-	-	-	+	
29.	Swarnalata	<i>Bph6</i>	-	-	-	+	
30.	TN1	<i>none</i>	-	-	-	-	
31.	Balamawee	<i>Bph9</i> , <i>Bph27(t)</i>	+	+	+	#	
32.	China Saba	<i>bph8</i>	+	+	+	#	
33.	<i>O. glaberrima</i>	<i>Bph22</i>	+	+	#	#	
34.	Col 5 Thailand	<i>bph8</i>	-	-	-	+	Cheng <i>et al.</i> , 2013
35.	<i>O. minuta</i>	<i>Bph23</i>	+	+	-	-	
36.	Minghui 63	<i>Bph1</i>	+	+	-	-	
37.	Yagyaw	<i>Bph4</i>	+	+	-	-	Mishra <i>et al.</i> , 2022
38.	IR02W101	<i>Bph3</i>	-	+	-	-	
39.	Salkathi	<i>Bph4</i>	-	-	-	+	
40.	IR26	<i>Bph1</i>	M+	-	+		Cheng and Chang, 1979
41.	IR28	<i>Bph1</i>	M+	#	#		
42.	IR30	<i>Bph1</i>	M+	-	+		
43.	IR32	<i>bph2</i>	M+	+	+		
44.	IR34	<i>Bph1</i>	M+	#	#		
45.	IR38	<i>bph2</i>	+	-	+		
46.	Lekham Samba		+	+	+		
47.	Mudu Kiriya		+	+	+		
48.	Muthukanikam		+	+	+		
49.	Kaharamana	<i>BPH9</i>	+	+	+	-	Muduli <i>et al.</i> , 2021
50.	Pokkali	<i>BPH9</i>	#	#	#	#	
51.	TKM6	<i>Bph1</i>	+	-	+	-	
52.	Kolayal and Poliyal	<i>Bph33</i>	#	#	#	#	

‘+’ Resistant, ‘M+’ Moderately resistant, ‘-’ Susceptible, # Not defined

The virulent variants in the population that persisted interbred to develop a new biotype population, which may make the resistant plant susceptible. Thus biotypes are evolutionary transients in the speciation process that arise from genetic changes in pest populations as a result of natural selection. An insect pest species' specialization and diversification into biotypes enable it to keep up with the evolution and advancement of the host plant's defences through natural selection or by human modification of the host plant's genome through breeding techniques (Saxena and Barrion, 1987).

Radical changes in the pest's gene frequencies were caused by disruptive and directed selects, which were mediated by the resistant rice varieties Mudgo and ASD7, as well as random genetic drift brought on by small sample numbers. Just a few variant forms of the pest's genotypically heterogeneous wild populations were selected by the host resistance. Evidently, the overall Biotype 1 population already had a few genes governing metabolic pathways that imparted virulence against resistant rice varieties due to cumulative effects of selection, recombination, mutation, migration, and genetic drift. Individuals of the brown planthopper carrying virulence genes persisted in the rice cultivars, Mudgo and ASD7. The following generation from the survived BPH displayed a remarkable fixation of favourable virulence alleles versus resistance types. The variant forms of the brown planthopper on the Mudgo and ASD7 varieties evolved with time and continued exposure to certain host kinds, and are considered ancestors of Biotype 2 and Biotype 3, respectively (Barrion, 1985). While biotypes 1 and 2 are found widespread across Southeast and East Asia, biotype 4 is found mainly on the Indian subcontinent and is also referred to as the South Asian biotype.

In 1973, the first BPH-resistant rice variety IR26 was released in the Philippines against BPH biotype-1 but soon it succumbed to the biotype-2 as a result of intensive cultivation and extreme selection pressure on BPH. In the exact same time frame, IR26 was discovered to be susceptible in Kerala, India, and neighbouring country Sri Lanka in field and greenhouse BPH screening tests. This was the first instance of a South Asian Biotype of the BPH having an allopatric virulent population. This initiated IRRI to set up an international brown plant

hopper nursery (IRBPHN) and an international rice testing program (IRTP) to monitor allopatric BPH biotypes and identify novel resistant resources from India and other countries (IRTP, 1976). Further investigations revealed the richness of resistant rice germplasm in India and Sri Lanka, and the development of virulent biotypes occurred as a result of the prolonged coevolution of rice and BPH in this region. The segregation of biotypes was mainly contributed by morphological characters such as the rostrum, legs, and antennae which are common to both morphs and sexes. These morphological characters were mainly used for biotype identification. Apart from this, cytological variations were analyzed for biotype detection. Cytotaxonomy study of sex chromosomes revealed the male diploid chromosome number as $2n=30$ with XY chromosomes and 14 bivalent autosomal pairs. The highest metaphase I cells were observed in biotype 1 followed by biotypes 3 and 2. The autosomes were isolated from sex chromosomes with a higher distance in biotype 2 followed by biotypes 1 and 3. Higher inter and intrachiasmatic connections were observed in biotype 3. Biotype 2 had the longest and widest sex chromosomes, followed by biotypes 3 and 1. Chromosomal aberrations were more frequent in biotype 1 followed by biotypes 3 and 2. Complex polygenic inheritance of virulence was observed in BPH biotypes (Hollander and Pathak, 1981; Saxena and Barrion, 1983).

The DALP (direct amplified length polymorphism) technique was used to investigate genetic polymorphisms in the brown planthopper species complex and to generate high-resolution fingerprints. Eleven populations of *Nilaparvata* spp. were collected from six locations in Malaysia, with two sympatric populations from rice and a weed (*Leersia hexandra*). The (UPGMA) dendrogram based on genetic distances showed that populations belonging to the same species and the same host type grouped together regardless of their geographic regions of capture. The BPH population with high esterase activities, collected from rice, and those with low esterase activities, typically collected from *L. hexandra*, made up the two different clusters. Principal component, molecular variance, and robustness analyses all provided strong support for the cluster analysis results (Latif *et al.*, 2012).

Rice BPH isolated from Kerala was subjected to genomic and phylogenetic research in order to identify genetic variations amongst populations. Mitochondrial COI (Cytochrome Oxidase subunit I) gene partial coding sequence was amplified by PCR, and COI nucleotide sequence comparison revealed similarities in sequences and nucleotide composition with other populations. The genetic divergence investigation revealed no genetic difference between the Kerala population and the Andhra Pradesh population and a few different populations from China and Japan. Phylogenetic analysis also showed comparable outcomes (Mashhoor *et al.*, 2015).

Using SSR markers, the genetic diversity and structure of twenty-two BPH populations collected from twenty-two hotspot locations in India were examined. The thirty chosen SSR markers had a mean genetic diversity of 0.399 and a polymorphic information content (PIC) of 0.337. Additionally, molecular variance (AMOVA) showed a 2 per cent variation among the population and 98 per cent within the population. All twenty-two populations have been grouped into three categories in cluster and population structure analysis. Cluster analysis revealed that the populations of north and west India had a significant degree of genetic resemblance. The BPH population of East and South India was evenly divided into the remaining two groups. Similarly, Principal coordinate analysis (PCoA) revealed that the populations of north and west India were located in the same compartment. This fluctuation may be related to the migration of BPH as a result of the southwest monsoon's wind movement in the Bay of Bengal and the Arabian Sea (Babu *et al.*, 2023).

Comprehensive pest monitoring and surveillance, the periodic release of varieties containing major effect genes, gene pyramiding, horizontal resistance, gene rotation, and varietal blending are the major management tactics for the BPH biotype and its development (Heinrichs and Foster, 2019).

To successfully implement pest resistance into crop improvement initiatives, knowledge about the prevalence of insect biotypes is essential. The host range of insect pests is less predictable due to the occurrence of biotypes. Crop cultivars that were once listed as being resistant to a pest population may become susceptible to

new pest species biotypes. Thus, the stability of resistant crop cultivars is constantly threatened by insect biotypes. Due to the emergence of several biotypes of rice insect pests, the attempt to introduce insect-resistant rice varieties for cultivation is a challenging task. In-depth analyses of the behaviour, sensory and regulatory, physiology, ecology, and genetics of the pure line populations of insect biotypes would necessitate multidisciplinary studies of the inheritance of crop plant defensive compounds as well as the use of sophisticated breeding methodologies for finding novel genetic sources of resistance in the crop germplasm (Jeevanandham *et al.*, 2023).

2.5.2. Molecular basis of BPH resistance in rice

Resistance to insects in plants was classified by Stout (2013) as constitutive or induced and direct or indirect defence responses. Constitutive defence responses include the formation of physical and chemical barriers prior to insect attack. Monitoring, signal transduction, and the generation of defensive chemicals are induced defence responses that are triggered by insect pest attacks. Genes responsible for insect resistance usually encode insect elicitors and effectors, intracellular-localized receptors, plasma membrane-localized receptors, and plant signalling transduction pathways. Inevitably, oviposition fluids and oral secretions such as saliva, and gut regurgitant are released by insects while feeding or ovipositing on plants. These substances are crucial in the interactions between plants and insect pests.

When BPH feeds on rice, the insect secretes salivary proteins into the plant cells. These salivary proteins may act as both effectors and elicitors. The effector proteins that are able to suppress the plant defence mechanism are salivary EF-hand calcium-binding protein, salivary DNaseII, and endo-1,4-glucanase. Few salivary proteins such as mucin-like proteins act as elicitors of plant defence. The first line of defence against BPH is the cell walls of rice plants. Endo-2 -1,4-glucanase, which is released by BPH when it feeds on rice plants, breaks down the celluloses in plant cell walls, allowing the stylet of BPH to access the phloem and destroy the defence of plant cell walls. Some rice cells are destroyed when the BPH stylet passes through the apoplast of the rice, and some DNA fragments are liberated from the

dissolving nuclei of the damaged rice cells. The released DNA fragments (extracellular DNA) function as a molecular pattern that indicates injury (a "wound signal"). The cells near damaged cells detect the wound signal and activate defence. Salivary DNase II, which is released by BPH, breaks down the released DNA fragments in rice, which inhibits the undamaged rice cells from recognizing the damage and suppresses the rice plant's defence. Rice phloem can get plugged by calcium ions (Ca^{2+}) input to stop insects from feeding. The EF-hand calcium-binding domain-containing salivary proteins produced by BPH show Ca^{2+} binding ability, preventing rice phloem clogging and making it suitable for BPH feeding. For BPH to probe the rice and then feed on the rice to suck sap from the rice phloem, it needs the elicitor, a mucin-like protein generated by BPH. When BPH feeds, a mucin-like protein is secreted into the rice tissues, which causes the rice to elicit an immunological response that results in cell death, the production of pathogen-responsive genes, and callose deposition (Ling *et al.*, 2019).

BPH attack or the wound signal is sensed by damage-associated molecular patterns (DAMPs) or Herbivore-associated molecular patterns (HAMPs). Damage-associated molecular patterns (DAMPs) are the plant immune system endogenous elicitors released from the plants as a self-recognition damage response. Herbivore-associated molecular patterns (HAMPs) are the specific plant indirect defence responses to specific herbivore-derived elicitors in oral or ovipositor secretions. Immune receptors localized at the host cell surface known as Pattern Recognition Receptors (PRRs) such as leucine-rich repeats (LRR) detect these DAMPs or HAMPs and activate Mitogen-activated protein kinases (MAPK) cascade followed by pattern-triggered immunity (PTI). PTI is the first line of inducible defence in plants triggered by the detection of DAMPs. Effectors secreted by insect saliva can prevent PTI leading to effector-triggered susceptibility (ETS). Insect resistance proteins such as nucleotide-binding leucine-rich repeats (NB-LRRs) proteins encoded by *R* genes trigger Effector Triggered Immunity (ETI) (Du *et al.*, 2020).

Effector Triggered Immunity (ETI) is the second line of inducible defence response typically activated by the intercellular recognition of insect effector molecules by plant resistance *R* gene proteins. Activation of HAMPs results in plant

susceptibility or resistance by inducing phytohormone-based signalling pathways that activate direct or indirect resistance responses. Direct responses can cause antixenosis, antibiosis, or tolerance against insect pests. The induction of salicylic acid (SA), reactive oxygen species (ROS), methyl jasmonate (MeJA), and 12-oxo-phytodienoic acid (OPDA) can produce antixenosis or antibiosis factors. The induction of abscisic acid (ABA), ethylene (ET), gibberellic acid (GA), or IAA (Indole-3-acetic acid) produces metabolic components contributing to insect pest tolerance (Cheng *et al.*, 2013; Du *et al.*, 2020).

In the case of BPH attack, insect resistance proteins combined with transcription factors activate the salicylic acid (SA) signalling pathway and upregulate SA-responsive genes expression leading to indirect responses such as repelling insect settling and attracting natural enemies provided by increased production of defensive compounds. Proteinase inhibitors such as Trypsin protease inhibitors (TryPIs) and phytoalexin which affect digestive proteases, cause an amino acid deficiency in insect midgut reducing insect growth and development, induced callose deposition prevent BPH feeding, secondary metabolites (terpenes, alkaloids, flavonoids) and green leaf volatiles reduces the insect growth, attraction, survival, and reproduction. (Lu *et al.*, 2018; Du *et al.*, 2020).

Ca²⁺ influx is a frequent plant response to insect feeding that occurs as the initial cellular activity in the early defence response of rice to BPH. A change in Ca²⁺ levels sends a signal to rice plant cells that trigger two defence mechanisms, the formation of reactive oxygen intermediates (ROIs) and the production of callose (Hao *et al.*, 2008; Wu *et al.*, 2017b). Expression of specific defence genes is stimulated by MAPK (Mitogen-Activated Protein Kinase) signalling. Enhanced induction of H₂O₂ and ethylene production by the silencing of *OsWRKY45* increased BPH resistance by lowering insect feeding, survival, nymphal development, and oviposition preferences (Huangfu *et al.*, 2016). MicroRNAs such as *OsmiR156* (Ge *et al.*, 2018) and *OsmiR396* were found to possess negative regulation on BPH resistance (Dai *et al.*, 2019).

Phytohormone signalling pathways play a significant role in defence signalling in rice. In general, BPH infection increases the production of ethylene,

jasmonic acid (JA), and salicylic acid (SA) in rice. Nevertheless, it has been noted that the SA and ethylene signalling pathways have a positive effect on rice resistance to BPH, whilst the JA signalling pathway has a detrimental impact on rice resistance to BPH (Kaya *et al.*, 2023).

2.6. BREEDING FOR BROWN PLANT HOPPER RESISTANCE IN RICE

The most ideal, effective, and economical method of pest control is the development and cultivation of insect-resistant cultivars. The newly developed cultivars should possess insect resistance mechanisms along with the potential for high yields. In the near future, using host plant resistance will continue to be a crucial part of pest management tactics and varietal development. The future focus should be to extend the genetic base of resistance both in terms of major and minor genes due to the possibility that continual planting of crop varieties with monogenic (major gene) resistance would result in the emergence of virulent insect biotypes. Programs for breeding pest-resistant cultivars will benefit from an intensive insect biotype surveillance program.

2.6.1. Screening methods for BPH bioassay

An effective insect-resistance breeding program requires proper and standardized resistance screening techniques. The brown planthopper-rice interactions are studied mainly by screening the varieties (landraces or traditional varieties) within a holistic framework that includes plant and insect phenologies accounting for adaptation and genetic diversity in target organisms.

IRRI initiated BPH resistance evaluation studies in 1966 and introduced the mass screening of varieties at the seedling stage for the identification of resistance sources, studying inheritance, and allelic interaction, and screening of bulk population of germplasm under the greenhouse condition.

An ample population of BPH nymphs and adults is required for the mass screening of varieties. Continuous mass rearing on susceptible varieties is essential for maintaining the BPH population for efficient screening. Two methods of BPH mass rearing include rearing on old potted plants and rearing on seedling mats. Rearing on old potted plants consists of standard wooden or steel cages of size 0.5x 0.5x 1 m with proper aeration ensured using wired mesh or nylon net along the

sides. 40 to 50 days old plants are kept for BPH mass multiplication. This method of rearing can produce more than 2000 late instar nymphs. Plants should be replaced before complete drying and continuous monitoring for maintaining the water level, and removal of natural predators and fungal infections if any are required (Pathak and Khush, 1979).

Rearing on seedling mats is done in a transparent acrylic cage with a top and three sides covered with nylon mesh. Pre-germinated seeds are placed on wet gauze on enamelled trays to make seedling mats. After a few days, when the seedlings have developed and their roots have tangled in the gauze, the mats are simple to cut to the required size. On the seedling mats, the adult insects are caged. One or two days later, the ovipositing insects are removed, and the seedling mats with the eggs are removed from the cages. As nymphs emerge, a brand-new seedling mat is placed on one side of the cage. One seedling mat (about 20x25 cm) that can hold 1,000–2,000 late-instar nymphs can fit within each cage (Pathak and Khush, 1979).

Both rearing methods can provide a continuous batch of BPH nymphs and adults for screening tests. Rearing on potted plants is more efficient compared to seedling mat rearing (Choi and Brady, 1979).

IRRI developed standard procedures and scoring scales for BPH seedling screening (Pathak and Kush, 1977). In 60x45x10 cm seedboxes, the test types or lines are sown in rows 5 cm apart. Each line is positioned along the width of the seedbox in a row that is 20 cm long. Each seedbox contains a susceptible check variety (often Taichung Native 1 or another susceptible variety) and a resistant check variety (usually universal resistant check, PTB-33).

Field screening for BPH resistance was conducted at a hotspot area when a sufficient BPH population was available for screening. A minimum of 25 BPH per hill at the maximum tillering and 100 BPH per hill at the early blooming stage should be ensured for efficient screening at the field level. In the case of reduced population, resurgence-inducing insecticide application was followed for increasing the BPH population (Heinrichs, 1985). The polythene barrier technique of field screening was practiced in India to prevent the migration of BPH nymphs outside the infested field and restrict the entry of natural predators of BPH into the

screening field. Microplot technique in field screening was employed to prevent BPH spread into nearby fields and research plots by using cages over the small plots or in pits taken around the plot (Kalode and Bentur, 1982).

The standard seedbox screening test (SSST) was developed for seedling screening for BPH resistance, where the seeds of each cultivar are sown in a single row along with susceptible and resistant checks in a seedbox of 60x40x10 cm. Twenty seedlings are maintained per row by thinning at seven days after sowing and infested with about eight second instar nymphs per seedling. After one week of infestation (when susceptible checks are killed), plants are rated for damage using a 0-9 scale (Velusamy *et al.*, 1986).

For better detection of field resistance, the modified seedbox screening test (MSST) was developed in which the seedlings are infested with four second instar nymphs per plant twenty days after sowing. BPH affected seedling survival rate in MSST is recorded based on a Standard Evaluation System (SES) score of 0-9 (IRRI, 2013). Low scores indicate the resistant response of genotypes as same as SSST. In MSST, the mortality of susceptible check is usually caused by the F₁ BPH population (first generation of BPH from the released adults).

These insect bioassay methods are remarkably useful for rapid, high-throughput, and cost-effective screening of manifold rice genotypes in bulk to find and isolate resistant genotypes and genes. SSST can be relied upon for nymphal feeding response evaluation whilst MSST allows nymphs to develop into adults, oviposit in response to cultivar suitability. The modified seedbox screening test provides a rapid method for identifying field-resistant cultivars in the greenhouse and can be used to identify field-resistant lines in a rice breeding program for stable resistance to BPH (Velusamy *et al.*, 1986).

Semi-field screening (SMFS) method was used for screening the plants at vegetative and reproductive stages in the field conditions. Twenty-day-old seedlings are transplanted to the field and covered with nylon nets and infested with five insects of 3rd or 4th instar nymphs per hill. The F₂ BPH population causes the mortality of susceptible check and the rest of the plants are scored till the flowering stage. The varieties PTB-33 and Rathu Heenati expressed high resistance to BPH

during the seedling and tillering stage using the SSST, and MSST methods of screening but reported susceptibility in heavy infestation during the grain filling stage (Jairin, 2007).

Standard Seedbox Screening Test (SSST), Modified Standard Seedbox Screening Test (MSST), and Field Screening (FS) methods were employed to confirm the effect of Quantitative trait loci (QTL) associated with BPH resistance. RIL(F₇) mapping population obtained from the cross Swarna x PTB33 was screened with the aid of the SSST, MSST, and FS. The damage scores observed in the mapping population (score 4.0-4.6) were less than that of Swarna (score-9). Significant higher resistance observed in the mapping population was due to the influence of PTB33 alleles and QTLs detected on chromosomes 1, 6, and 12 derived from PTB33 can impart durable resistance against BPH (Akula *et al.*, 2020).

The parents PTB-33, line 9311, and the backcross generations were evaluated for BPH resistance by the SSST method. The rice line 9311 and the susceptible check, TN1 showed high susceptibility with a score of 9.0 whereas PTB-33 showed high resistance with a less than 3.0 score as per the SES scale. The backcross population, BC₂F₂ showed a 3 resistant: 1 susceptible ratio confirming the presence of a single resistance gene (Cheng *et al.*, 2021).

Muduli *et al.* (2023) screened 26 rice varieties using SSST method. The variety PTB-33 scored 1.5 and the damage scores of other varieties varied from 1.8 to 7.5 using the SES scale. Two other varieties found resistant to BPH were Hasanta (damage score 1.8) and Jyotirmayee (damage score 3).

Rapid adaptability to resistant rice varieties and pesticides by the BPH even under laboratory conditions due to continuous rearing of several generations is challenging rice breeders and entomologists intimidate into unveiling the factors underlying planthopper-rice interactions to develop resistant genotypes. The high host specificity is a characteristic feature of brown planthopper.

2.6.2. Genes conferring resistance to BPH

Breeding for pest resistance in crop types involves both short- and long-term techniques. These include the creation of horizontal resistance, extensive

hybridization, tissue culture, the release of major gene-containing varieties in sequence, the pyramiding of major genes, multiline variations, and the identification of novel genes and donors for resistance. It was anticipated that varieties with more durable resistance may inhibit the formation of new biotypes (Saxena and Barrion, 1983). Therefore, the present breeding strategies aim to exploit both major and minor resistance genes for efficient pest control. The abundance of genetic resources available in rice, including wild species, cultivated varieties, and landraces provides a vital resource for creating BPH-resistant cultivars (Du *et al.*, 2020). The wild relatives are exploited in improvement programs as a source of BPH resistance genes. Molecular markers are used with effective breeding techniques to transmit the resistance genes from wild relatives to cultivated varieties. However, the difficulties in cultivar hybridization between distantly related cultivars and the linkage drag from wild species are the two main bottlenecks in the conventional strategy (Ratnasekera *et al.*, 2022).

Tertiary gene pools consist of more distantly related species that have valuable sources of resistance but possess difficulty in hybridization with cultivated species. Advancements in the identification of genes can reduce the phenotype-genotype gaps which facilitate the use of crop wild relatives in insect resistance breeding (Rajpal *et al.*, 2023). *Oryza officinalis* was used as a donor for conferring resistance to brown plant hopper (Jena and Khush, 1990). Species with AA genomes can be crossed with cultivated rice to transfer genes for insect resistance whereas species with other genomes such as BB, CC, and BBCC can be crossed with cultivated rice using the hybrid embryo rescue method (Huang *et al.*, 2000). Wild species of rice such as *O. australiensis*, *Oryza officinalis*, *O. minuta*, *O. eichingeri*, *O. nivara* and *O. latifolia* are the major sources of genes for BPH resistance.

Oryza officinalis possesses 24 per cent of accessions with BPH resistance out of 265 accessions including eighteen wild rice species analyzed. Genes *Bph11*-*Bph15* were identified from the *Oryza officinalis*, *Bph20* and *Bph21* from *Oryza minuta*, and *Bph27* and *bph29* from *Oryza rufipogon*. Wild rice has contributed 13 genes for BPH resistance (Hu *et al.*, 2016a).

The wild species *O. australiensis* was used as a source for the genes *Bph18* and *Bph10*. The introgression line IR65482-7-216-1-12 derived from *O. australiensis* was used for transferring *Bph18* to cultivated varieties using marker-assisted selection (Jena *et al.*, 2006). The protein products of the *Bph18* gene were localized to the endo-membranes in the phloem cells and encodes CC-NBS-NBS-LRR (coiled-coil nucleotide-binding site leucine-rich repeats) protein. The NBS-LRR proteins are one of the largest proteins available in plants for defence mechanisms against pests or pathogens by inducing effector-triggered immunity (ETI). The effectors secreted into the plant by insects are recognized by the NBS-LRR proteins and activate the signal transduction pathway (Ji *et al.*, 2016).

Introgression lines developed by Akanksha *et al.* (2019) from the interspecific cross between the variety Swarna and *O. nivara* were found resistant to BPH biotype 4 at seedling, tillering, and reproductive phases. The genes in the new introgressed lines were tentatively designated as *bph39(t)* and *bph40(t)*.

Athwal *et al* (1971) designated the first gene for BPH resistance in varieties Mudgo, MTU15, and CO22 as *Bph1* but the selection pressure on this gene leads to the involvement of new biotypes in BPH which could tolerate the effect of *bph1* (Myint *et al.*, 2009). Accordingly, around 45 major BPH resistance genes have been reported in several *Oryza sativa* sp. *indica* cultivars and wild relatives (Table 2) (Li *et al.*, 2023). The genetics of BPH resistance revealed the presence of 32 major genes (*Bph1* to *Bph32*) in cultivated rice and seven wild relatives (Sundaram *et al.*, 2018). Whereas, cloning and characterization of nine potential genes (*Bph3/Bph17*, *Bph6*, *BPH9*, *Bph14*, *Bph15*, *Bph18*, *Bph26*, *Bph29*, and *Bph32*) were carried out to date (Muduli *et al.*, 2021).

The presence of *Bph3* and *bph2* in the rice variety ‘PTB-33’ was first reported by Angeles *et al.* (1986). *Bph3* and *bph4* confer resistance to all the biotypes of BPH (Khush and Brar, 1991). The *Bph3* locus consists of a cluster of three genes (OsLecRK1-OsLecRK3) and encodes plasma membrane-localized lectin receptor kinases which are responsible for durable and broad-spectrum BPH resistance (Liu *et al.*, 2015). The *Bph14* gene produces a protein with a leucine-rich repeat (LRR) motif and a coiled-coil nucleotide-binding site (CC-NBS) that regulates a defence

Table 2. List of major BPH resistance genes/QTLs have been reported in several *Oryza sativa* sp. *indica* cultivars and wild relatives

Gene/QTL	Chromosome	Flanking Marker	Donor	References
<i>Bph1</i>	12L	BpE18-3	Mudgo, TKM6	Kim and Sohn, 2005
	12L	XNpb248	IR28	Hirabayashi and Ogawa, 1995
		RG63, RG457	Gayabye	Jeon <i>et al.</i> , 1999
		RZ261, R2708	Norin-PL3	Sharma <i>et al.</i> , 2002
		pBPH4, pBPH14	Cheongcheongbyeo	Cha <i>et al.</i> , 2008
<i>bph2</i>	12L	RM463, RM7102	ASD7	Sun <i>et al.</i> , 2007
		G2140, C449	Norin-PL4	Murata <i>et al.</i> , 1998
		KAM3, KAM5	Norin-PL4	Murai <i>et al.</i> , 2001
		RM1246, RM463	ASD7	Sun <i>et al.</i> , 2006
<i>Bph3</i>	6S	RM469, RM588	Rathu Heenati	Jairin <i>et al.</i> , 2007
		RM589, RM588	PTB33	Jairin <i>et al.</i> , 2007a
<i>QBph3</i>	3L	RM3180, RM2453	Rathu Heenati	Kumari <i>et al.</i> , 2010
<i>bph4</i>	6S	RM190, C76A	Babawee	Kawaguchi <i>et al.</i> , 2001
		RM589, RM586	Babawee	Jairin <i>et al.</i> , 2010
<i>QBph4.1</i>	4S	P17, xc4-27	IR02W101 (<i>O. officinalis</i>)	Hu <i>et al.</i> , 2015b
<i>QBph4.2</i>	4S	RM261, S1	IR65482-17-511 (<i>O. australiensis</i>)	Hu <i>et al.</i> , 2015a

<i>Bph6</i>	4L	Y19, RM119	Swarnalata	Qiu <i>et al.</i> , 2010
<i>bph7</i>	12L	RM3448, RM313	T12	Qiu <i>et al.</i> , 2014
<i>BPH9</i>	12L	RM463, RM5341	Kaharamana	Su <i>et al.</i> , 2006
		OPR04, S2545	Pokkali	Murata <i>et al.</i> , 2001
<i>BPH10</i>	12L	RM260, RM313	<i>O. australiensis</i>	Ishii <i>et al.</i> , 1994
		RG457, CDO459	IR65482-4-136-2-2	
<i>bph11</i>	3L	G1318	IR54742-23-19- 16-12	Hirabayashi <i>et al.</i> , 1998
<i>Bph12</i>	4S	RM16459, RM1305	B14 (<i>O. latifolia</i>)	Qiu <i>et al.</i> , 2012
<i>Bph12(t)</i>	4S	C946, RM261	B14	Yang <i>et al.</i> , 2002
<i>Bph13(t)</i>	3S	AJ09b230, AJ09c	IR54745-2-21 (<i>O. officinalis</i>)	Renganayaki <i>et al.</i> , 2002
<i>Bph14</i>	3L	SM1 G1318	B5	Du <i>et al.</i> , 2009
<i>Bph15</i>	4S	RM261, S16	B5 (<i>O. officinalis</i>)	Lü <i>et al.</i> , 2014
<i>Bph16(t)</i>	12L	RM6732, R10289	M1635-7	Hirabayashi <i>et al.</i> , 2004
<i>Bph17</i>	4S	RM8213, RM5953	Rathu Heenati	Sun <i>et al.</i> , 2005
<i>BPH18</i>	4L	RZ565, RM273	BPH2183	Li <i>et al.</i> , 2006
	12L	RM463, S15552, 7312.T4A	IR65482-7-216 (<i>O. australiensis</i>)	Jena <i>et al.</i> , 2006
<i>bph19</i>	3S	RM6308, RM3134	AS20-1	Chen <i>et al.</i> , 2006
<i>Bph20(t)</i>	4S	MS10, RM5953	IR71033-121-15 (<i>O. minuta</i>)	Rahman <i>et al.</i> , 2009

<i>Bph21(t)</i>	12L	RM3726, RM5479	IR71033-121-15 (<i>O. minuta</i>)	Rahman <i>et al.</i> , 2009
	10S	RM222, RM244	RBPH54	Yang <i>et al.</i> , 2012
<i>Bph22(t)</i>	6S	RM19429	IR71033-62-24	Harini <i>et al.</i> , 2010
<i>BPH25</i>	6S	S00310	ADR52	Myint <i>et al.</i> , 2012
<i>Bph26/bph2</i>	12L	KAM4	IR1154-243	Murai <i>et al.</i> , 2001
<i>Bph27</i>	4L	RM273, RM471	GX2183 (<i>O. rufipogon</i>)	Huang <i>et al.</i> , 2013
<i>Bph27(t)</i>	4L	RM471, RM5742	Balamawee	He <i>et al.</i> , 2013
<i>Bph28(t)</i>	11L	Indel55, Indel66	DV85	Wu <i>et al.</i> , 2014
<i>BPH29</i>	6S	BYL7, BID2	RBPH54 (<i>O. rufipogon</i>)	Wang <i>et al.</i> , 2015
<i>Bph30</i>	4S	RM16294, RM16299	AC-1613 (<i>O. indica</i>)	Wang <i>et al.</i> , 2018
<i>BPH31</i>	3L	PA26, RM2334	CR2711-76 (<i>O. indica</i>)	Prahalada <i>et al.</i> , 2017
<i>Bph33(t)</i>	1	RM488, RM11522	RP2068	Naik <i>et al.</i> , 2018
<i>Bph33</i>	4S	H99, H101	Kolayal and Poliyal	Hu <i>et al.</i> , 2018
<i>Bph34</i>	4L	RM16994, RM17007	IRGC104646 (<i>O. nivara</i>)	Kumar <i>et al.</i> , 2018
<i>Bph35</i>	4L	PSM16, RM413	RBPH660	Zhang <i>et al.</i> , 2020
<i>Bph36</i>	4S	S13, X48	<i>O. rufipogon</i> Griff	Li <i>et al.</i> , 2019
<i>BPH37</i>	1	RM302, YM35	IR64	Yang <i>et al.</i> , 2019
<i>BPH38(t)</i>	1L	SNPs 693, 369, 10, 112, 165	Khazar	Balachiranjeevi <i>et al.</i> , 2019

<i>bph39(t)</i>	-	-	RPBio4918-230S	Akanksha <i>et al.</i> , 2019
<i>bph40(t)</i>				
<i>BPH41</i>	4	SWRm_01617, SWRm_01522	SWD10	Tan <i>et al.</i> , 2022
<i>BPH42</i>		SWRm_01695, SWRm_00328		
<i>Bph43</i>	11	-	IRGC 8678	Kim <i>et al.</i> , 2022
<i>Bph45</i>	4	-	IRGC 102165	Li <i>et al.</i> , 2023

mechanism against insects by activating the salicylic acid (SA)-dependent pathway (Hu *et al.*, 2017). The CC-NBS-LRR protein, which is encoded by the *Bph26* gene, mediates sucking inhibition in the phloem sieve element (Tamura *et al.*, 2014). BPH resistance is provided via the B3 DNA-binding domain of *Bph29*, which activates the SA pathway (Wang *et al.*, 2015). The *Bph32* gene identified in ‘PTB-33’ encodes a protein with an unknown SCR (short consensus repeat) domain and is localized in the plasma membrane cells and highly expressed in leaf sheaths, thus providing antibiosis resistance to BPH (Ren *et al.*, 2016).

Usually, breeders prefer primary and secondary gene pools in a breeding program to confer insect resistance and also improve the crop agronomically. Kerala has a rich diversity of rice genotypes and traditional cultivars/varieties. The KAU-released varieties *PTB19* in 1944, *PTB21*, and *PTB33* in 1952 showed strong resistance to BPH and many other insect pests of rice (Rosamma *et al.*, 2003). Jayalekshmy *et al.* (2017) screened fifty traditional rice varieties from Kerala for locating resistant gene donors for brown plant hopper and identified four varieties *Pallippuram pokkali*, *Karurayima*, *Athikkiraya*, and *Arikkirai* with the presence of both the genes *Bph 17(t)* and *Bph 18(t)*. The promising BPH-resistance sources of rice varieties in Kerala can be well explored for genetic improvement in rice.

The traditional cultivars of Kerala were found to have varied characteristics viz., adaptable to adverse climatic conditions, resistance to major biotic and abiotic stresses, medicinal properties, and high nutritional content (Jayalekshmy *et al.*, 2017; George *et al.*, 2021, Chacko *et al.*, 2023). Rice breeders throughout the world have utilized traditional varieties from Kerala in superior elite variety development. The climatic conditions in Kerala are very much suitable for the sudden proliferation of insect pests in rice. Many of the pests which were considered minor may become major pests at any instant during the crop period as the crop gets intermittent rain, high humidity, and wide variations in temperature.

The sequential varietal release was followed by Kerala Agricultural University as an efficient strategy for rice insect pest management in varying climatic conditions of Kerala. Brown plant hopper-resistant variety, *Bhadra* (MO4) was released in 1978 from the rice research station (RRS), Moncompu as the first

high-yielding rice variety with insect resistance in the state. This was followed by the release of a variety, *Jayathi* from the Regional Agricultural Research Station (RARS), Pattambi in 1992 with improved resistance. Many other varieties namely, *Kanakom*, *Onam*, *Bhagya*, *Makom*, and *Aruna* were also developed. From 1993 onwards, importance was given to the development of multiple resistant rice varieties leading to the release of varieties *Athira*, *Aiswarya*, *Kanchana*, and *Kairali* from RARS, Pattambi. Varieties *Karuna* and *Mangala Mahsuri* were developed with multiple insect resistances in 1998 mainly for cultivation in the central zone.

2.6.3. Inheritance pattern of BPH resistance

The traditional/conventional breeding strategy for insect resistance in rice is done by evaluating the phenotype of F₁ plants or the phenotyping segregation ratio of the F₂ population from a cross between a resistant and susceptible parent, determining whether insect resistance is controlled by dominant or recessive genes or by one or more genes. By assessing the phenotypic segregation ratios of F₂ and F₃ progenies of a cross between resistant cultivars, one could determine if resistant genes in different varieties are allelic, closely linked, or located on different chromosomes.

Among the 45 genes identified, 37 are dominant and 8 are recessive in nature. Almost all the major genes are located on chromosomes 3,4, 6, and 12. The variety IR26 with a dominant *Bph1* gene against biotype 1 was released in 1973. In 1976, biotype 2 evolved, and varieties IR36 and IR38, with recessive *bph2* gene, were released against biotype 2. Biotype 3 evolved by breaking the resistance of *bph2* during 1982 against which varieties IR56 and IR60 with a dominant *Bph3* gene were released. None of the BPH biotypes were capable of damaging varieties with *Bph 3* and *bph 4* genes for resistance, and the variety PTB 33 is resistant to almost all known biotypes. These genes were segregated independently with no recombination occurring between them. In the variety ASD7, recessive gene *bph2* had a close linkage with *Bph3* but no recombination was observed between the genes (Khush, 1979). Genetic analysis by Ikeda and Kaneda (1981) found that the

genes *Bph3*, *bph4*, and *Bph1*, *bph2* segregated independently even though the genes were closely linked.

Khush (1979) reported one recessive and dominant gene based on the reaction to biotype 4 of BPH. The inheritance pattern of BPH resistance in PTB-33 was studied based on damage score and honeydew production. The damage scores from F₁, F₂, and F₃ of the cross between PTB-33 and TN1 revealed the monogenic inheritance of resistance. The F₁ honeydew production was intermediate to parents whereas the F₂ showed skewed distribution towards PTB-33 depicting the polygenic nature of BPH resistance in PTB-33 (Nugaliyadde *et al.*, 2004).

Rice indica variety 'BP60' with BPH resistance was crossed with a japonica BPH susceptible line 02428 for introgression of *Bph3* gene. The F₂ population derived from the cross showed 3:1 resistant: susceptible ratio, indicating the contribution of dominant gene to BPH resistance (Qing *et al.*, 2019).

2.6.4. Breeding methods for developing lines with resistance to BPH

2.6.4.1. Conventional breeding strategy for BPH resistance breeding

Insect resistance breeding program depends on factors such as the availability of stable donor lines with insect resistance, adequate insect population and reliable, easy, and efficient screening techniques for resistance to insects, adequate information on the mechanism of resistance to insects, the biology of the insect, insect-plant and the insect-environment interactions, and information on mode of inheritance of resistance

When existing cultivars or germplasm collections have genes for resistance, hybridization and selection can be adopted by pedigree, bulk, or backcross method of breeding. Recurrent phenotypic selection coupled with screening for insect resistance can be adopted to develop varieties and genetic potential for yield can also be improved with subsequent selection cycles (Hanson *et al.*, 1972).

When resistance is governed by major genes, the pedigree breeding method is preferable. It involves hybridization between selected parents (at least one with strong resistance), selection begins in the F₂ generation and superior plants from superior progeny rows were selected from F₃- F₅ generation. About 92 per cent homozygosity can be achieved by F₇ generation if a trait is governed by five genes.

Segregating lines must be exposed to proper insect pressure so that resistant segregants may be distinguished from susceptible ones. Proper selection of biotypes for screening is necessary. IR 26 rice variety developed at IRRI, Philippines was totally damaged by BPH from Kerala and Hyderabad in 1975. Later it was found that biotypes in South Asia were entirely different from other rice-growing areas (Seshu and Kauffman, 1980).

A simple and convenient method to attain inbreeding in the segregating generations after making an initial cross between desired parents is the bulk method of breeding, proposed by Nilson Ehle in 1908. Since only natural selection operates in early generations, it is desirable to grow the generations in hot spots for insect pests for selection and perpetuation of only resistant plants. A natural insect population may be maintained in the field by using cultural practices that favour the propagation of the insect species. In each generation, the seed is bulked. From F_5 or F_6 generation, single plant selection is practiced. In the case of polygenic resistance, the bulk method is not advisable as selection for resistance to insects in early generations is a difficult task due to the small magnitude of differences in resistance (Khush, 1977). In rice, the polygenic traits were explored using the single-seed descent method. Early generation population from multiple crosses involving three or four parents with minor resistance genes are proposed in bulk breeding. The bulk population is exposed to the target pest pressure at F_5 or F_6 generation and plants with a high level of resistance are selected and evaluated in progeny rows (Khush, 1980).

The importance of breeding for the development of insect resistance in major food crops is not only increasing food productivity but also saving the environment from the hazards of insecticides. Crop wild relatives and primary sources of resistance to insects have been exploited to increase the levels of resistance to insect pests in different crops. Various structural and biochemical traits have been associated with insect resistance and these traits have been successfully incorporated into commercial cultivars with aid of conventional breeding methods. The use of molecular techniques will broaden the insect-resistant gene pool and enable the fast-tracking of their incorporation in to elite germplasm.

2.6.4.2. Molecular breeding strategy for BPH resistance breeding

Identification of genes for BPH resistance from different rice cultivars, the study of resistant mechanisms, and the introgression of resistant genes to elite well-adapted but susceptible rice varieties become needful for sustainable economic yield under the BPH outbreak. Advancement in molecular genetics plays an important role in the search and confirmation of resistant genes from various genotypes and helps in efficient and fast insect-resistant breeding methods. Molecular markers are employed for the selection of plants with desired genomic regions that control the expression of the trait of interest. Marker-assisted selection in plant breeding enables the selection of individual plants based on their genotype. Selection of donor and recipient parents by screening and gene validation, in backcross breeding programs, and other breeding techniques is more efficiently done with aid of molecular markers reducing the cost, time, and resources. Among the molecular markers, SSR markers were the most preferred by plant breeders, geneticists, and molecular biologists due to the codominant nature of inheritance, highly polymorphic, reproducible, multi-allelic, extensive genomic coverage, and high-throughput genotyping capacity (Amiteye, 2021).

The transfer of inherited traits and resistant genes of donor parents to recipient parents is done conventionally by the pedigree method, recurrent selection, or backcross breeding (Hall *et al.*, 2016). The backcross method is mainly used for improving desired trait/s in well-adapted elite varieties, and the method is majorly carried out for resistant gene transfer from the donor parent to the recipient parent. Marker-assisted backcrossing (MABC) is the involvement of molecular markers in locating the linked alleles with desired genes or QTLs instead of phenotypic performance in conventional backcross breeding. MABC reduces the linkage drag, increases the recurrent parent genome recovery, and is ideal for the introgression of large-effect QTLs and oligogenes (Dormatey *et al.*, 2020; Hasan *et al.*, 2021).

2.6.4.3. Gene pyramiding for BPH resistance

The incorporation of two or more major resistant genes in the same variety to provide resistance to two or more biotypes is known as gene pyramiding. Gene pyramiding can be used in many conventional as well as modern breeding techniques to give horizontal persistent resistance against brown plant hopper. Pyramiding multiple resistance genes into a single rice cultivar can provide sustainable control of insect pests. The pyramided lines with two to three pyramided genes providing resistance to BPH have shown additive effects with an order of effectiveness of three-pyramided genes, two pyramided genes followed by a single gene (Hu *et al.*, 2013).

To provide durable resistance to a pest, the genes that contribute a major effect on resistance should be identified, the source of those genes in germplasm should be searched, and transfer those genes to a well-adapted single variety. Gene pyramiding involves three stages, including identification of donor parents with the desired gene, backcrossing with donor parents for accumulation of the desired genes into a single elite genotype, and continuous selfing for the fixation of the desired gene in homozygous condition (Shah *et al.*, 2017).

Some of the varieties have more than one gene providing resistance to BPH, and the gene pyramiding with those varieties introgress multiple resistant genes in a single cross. The presence of multiple resistance genes can impart resistance to different biotypes of BPH. This is considered effective as similar to vertical resistance. Around 45 genes imparting BPH resistance have been mapped, out of which 9 genes have been cloned and characterized. The majority of the genes are tracked using SSR markers for foreground, recombinant, and background selection in the segregating generations in order to develop or improve a variety with 3 to 4 backcrosses (Tanksley *et al.*, 1989., Hu *et al.*, 2016a).

The pioneer attempt reported in gene pyramiding for BPH resistance was done by Sharma *et al.* (2004) and was successful in pyramiding *Bph1* and *bph2* into a susceptible japonica line ‘Tsukushibare’ using resistant donor parents ‘Norin-PL4’ (*Bph1*) and ‘IR1154-243’ (*bph2*).

Marker-assisted gene pyramiding was used to create 25 Near Isogenic Lines (NILs) with 9 single R genes and 16 multiple R gene combinations in the genetic

background of the susceptible indica rice cultivar IR24. Results showed that NILs with two to three pyramided genes had a higher degree of antibiosis against BPH populations. Six significant NIL agronomic data revealed similar performance to IR24. These NILs will provide new genetic resources for BPH resistance breeding and are important gene sources for controlling BPH biotypes in rice-growing regions (Jena *et al.*, 2017).

Two rice varieties, Co51 and PTB-33 were screened with 120 SSR markers for detecting parental polymorphism. Distinct polymorphism was obtained in 51 SSR markers with PIC of 0.5 and 42.5 % polymorphism. These SSR markers can be used in the background selection of recombinants (Gokulakrishnan *et al.*, 2022).

The genes *Bph20* and *Bph21* were introgressed into the background of pre-release high-yielding culture CBMAS14065 by crossing with the resistant donor parent 'IR71033-121-15B'. The BC₁F₃ lines 48-5-3 and 2-3-2 showed high resistance in the BPH seedbox screening test. These backcross inbred lines can be effectively used as a donor for BPH resistance breeding programs (Vignesh *et al.*, 2023). The major gene pyramiding studies are listed in Table 3.

2.6.4.4. Advanced genomic tools in breeding for BPH resistance

With the widespread, inexpensive, and quick sequencing of the hundreds of cultivars and lines that make up the *Oryza* genetic resources, rice biology is currently undergoing a genomics revolution. The rice genome reference sequence is a unique biological resource made available to researchers around the world. It will be used as a basis for research into novel genes, their discovery, and eventually their introduction into cultivars grown under various cultivation conditions. The use of high-throughput phenotyping, transcriptomics, proteomics, metabolomics, miRNA, transcription factors, hormone signalling, and gene editing by CRISPR/Cas9, base editing, and prime editing, have the potential for developing brown plant hopper resistant varieties.

Transgenic rice plants incorporating *Galanthus nivalis* agglutinin (GNA) under phloem-specific promotor and sucrose synthase gene combined with maize ubiquitin promoter significantly improved BPH resistance, reducing nymphal survival and overall fecundity (Nagadhara *et al.*, 2003).

Table 3. List of major gene pyramiding studies in BPH resistance

Sl. No.	Donor parent	Recipient parent	Pyramided genes/QTL	Traits improved	References
1.	Norin-PL4	Tsukushibar e	<i>Bph1</i> + <i>Bph2</i>	BPH-resistance	Sharma <i>et al.</i> , 2004
2.	B14 (<i>Bph12</i>), Swarnalata (<i>Bph6</i>)	93-11, Nipponbare	<i>Bph12</i> + <i>Bph6</i>	BPH-resistance	Qiu <i>et al.</i> , 2012
3.	B5 (<i>Bph14</i> and <i>Bph15</i>)	Minghui 63	<i>Bph14</i> + <i>Bph15</i>	BPH-resistance	Hu <i>et al.</i> , 2012
4.	ADR52 (<i>Bph25</i> and <i>Bph26</i>)	T65	<i>Bph25</i> + <i>Bph26</i>	BPH resistance	Myint <i>et al.</i> , 2012
5.	B5 (<i>Bph14</i> , <i>Bph15</i> , and <i>Stv-bi</i>)	Shengdao 15, Shengdao 16, Xudao 3	<i>Bph14</i> + <i>Bph15</i> + <i>Stv-bi</i>	Rice stripe disease (RSD) + BPH resistance	Xu, 2013
6.	Minghui 63 (<i>Bph14</i> and <i>Bph15</i>); IR65482-7-216-1-2 (<i>Bph18</i>)	93-11	<i>Bph14</i> + <i>Bph15</i> + <i>Bph18</i>	BPH resistance	Hu <i>et al.</i> , 2013
7.	CholSub1 (<i>Sub1A-C</i>) Xa497 (<i>xa5</i> and <i>Xa21</i>)	PinK3	<i>Sub1A-C</i> + <i>xa5</i> + <i>Xa21</i> , <i>TPS</i> + <i>SSIIa</i> + <i>qBph3</i> ,	Submergence tolerance + BB + Blast + BPH resistance	Ruengphaya k <i>et al.</i> , 2015

	RBPiQ (<i>qBL1</i> and <i>qBL11</i>); Bph162 (<i>qBph3</i> and <i>TPS</i>)		<i>qBL1</i> + <i>qBL11</i>		
8.	Balamawee (Ba) (<i>Bph27</i> (<i>t</i>)), R2381 (<i>Bph3</i>)	Ningjing3 (NJ3), 93- 11	<i>Bph3</i> + <i>Bph27</i> (<i>t</i>)	BPH- resistance	Liu <i>et al.</i> , 2016
9.	B5 (<i>Bph14</i> and <i>Bph15</i>) Rathu Heenati (<i>Bph3</i>)	Hemeizhan (HMZ)	<i>Bph3</i> + <i>Bph14</i> + <i>Bph15</i>	BPH- resistance	Hu <i>et al.</i> , 2016
10.	Rathu Heenathi (RH) (<i>Bph3</i>); CBB23 (<i>Xa23</i>); Shuhui 162 (<i>Pita</i>); Zhongzu 14 (<i>Pi1</i> , <i>Pi2</i> and <i>xa5</i>)	HN88	<i>Pita</i> + <i>Pi1</i> + <i>Pi2</i> + <i>xa5</i> + <i>Xa23</i> + <i>Bph3</i>	Blast+ BB + BPH resistance	Ji <i>et al.</i> , 2016
11.	IR49830 (<i>Sub1</i>), ABHAYA (<i>Qbph12</i>)	KDML105	<i>Sub1</i> + <i>Qbph12</i>	Submergence tolerance + BPH resistance	Korinsak <i>et al.</i> , 2016

12.	Pokkali (<i>Bph9</i>), Swarnalata (<i>Bph6</i>)	93–11	<i>Bph9</i> + <i>Bph6</i>	BPH- resistance	Wang <i>et al.</i> , 2017
13.	Luoyang-6 (<i>Bph6</i>); L1880 (<i>Gn8.1</i> , <i>Rf5</i> and <i>Rf6</i>); Shuhui527 (<i>Rf3</i> , <i>Rf4</i> , and <i>Rf5</i>); Luoyang-9 (<i>Bph9</i>)	93-11	<i>Bph6</i> + <i>Bph9</i> + <i>Gn8.1</i> + <i>Rf3</i> + <i>Rf4</i> + <i>Rf5</i> + <i>Rf6</i>	BPH resistance	Fan <i>et al.</i> , 2017
14.	YH (<i>Xa21</i> , <i>Bph14</i> , and <i>Bph15</i>)	Yuehui9113	<i>Xa21</i> + <i>Bph14</i> + <i>Bph15</i>	Bacterial Blight (BB)+ BPH resistance	He <i>et al.</i> , 2019
15.	IR71033- 121-15 (<i>Bph20</i> and <i>Bph21</i>), Rathu Heenati (<i>Bph3</i> and <i>Bph17</i>), PTB33 (<i>Bph2</i> , <i>Bph17-ptb</i> and <i>Bph32</i>)	Taichung 65 (T65)	<i>Bph20</i> + <i>Bph21</i> + <i>Bph3</i> + <i>Bph17</i> + <i>Bph2</i> + <i>Bph17-ptb</i> + <i>Bph32</i>	BPH resistance	Nguyen <i>et al.</i> , 2019

16.	RH and IR72 (<i>Bph3</i>), B5 (<i>Bph14</i>), IR65482-7- 216-1-2 (<i>Bph18</i>) and PTB-33 (<i>Bph32</i>)	Guang 8B (G8)	<i>Bph3</i> + <i>Bph14</i> + <i>Bph18</i> + <i>Bph32</i>	BPH Resistance	He <i>et al.</i> , 2020
17.	PTB-33 (<i>Bph3</i>), BPHR96 (<i>Bph24</i>), HZ, MH63, XYZ, R1238, P59 and MX B, YX B	ZZ B, Qun B, YF B, MMB, 209 B, HF B	<i>Bph3</i> + <i>Bph24</i> + <i>Pi2</i> , <i>Pimh</i> , <i>Pib</i> , <i>Pi9</i> , <i>Pita</i> , <i>Xa23</i> , <i>badh2</i>	BPH resistance, blast, BLB, and aroma	Wang <i>et al.</i> , 2021
18.	Hua1165S (<i>Bph14</i> and <i>Bph15</i>), Hua1015S (<i>Xa23</i>) and Hua1201S (<i>Pi2</i>)	Feng39S	<i>Bph14</i> + <i>Bph15</i> + <i>Xa23</i> + <i>Pi2</i>	BPH resistance, bacterial blight and blast	Yang <i>et al.</i> , 2022
19.	PSL (<i>Bph29</i>), CK30 (<i>Pib</i> , <i>Pikm</i>), MH63 (<i>CRY1C</i> , <i>CRY2A</i>)	CH121, CH891, R205X	<i>Bph29</i> + <i>Pib</i> , <i>Pikm</i> + <i>CRY1C</i> , <i>CR</i> <i>Y2A</i>	BPH, blast, striped stem borer	Li <i>et al.</i> , 2023

The differential expression of genes in BPH-resistant and susceptible rice varieties was compared using gene expression profiling. Four jacalin-related lectin protein genes and one LRR domain-containing gene showed constitutive expression, while antioxidant-related genes, lignin biosynthesis-related genes, and wound-induced response genes were activated upon BPH infestation (Zhou *et al.*, 2019).

The proteomics analysis is a powerful approach in order to comprehending the complicated molecular response against BPH in rice. Jannoey *et al.* (2015) studied the protein expression of PTB33, a BPH-resistant variety, and the susceptible variety TN1, using two-dimensional electrophoresis and mass spectrometry. They found that the protein spots under different categories were upregulated in PTB33, including 2-Cys peroxiredoxin BAS1, IPPase, and EF-1. Vascular sap proteomics is important for BPH-resistant studies due to the sap-sucking nature of the infestation.

MicroRNA (miRNA) plays a critical modulator role in the control of growth, development, and defence response in plants. It was discovered for the first time in rice that miRNA controls BPH resistance (Wu *et al.*, 2017b). The silencing of *miR156* in rice induced the BPH resistance due to reduced production of jasmonic acid than wild types (Ge *et al.*, 2018).

In addition to plant growth and development control mechanisms, transcription factors play a crucial role in numerous abiotic and biotic stress responses. The overexpression of *OsWRKY53* increases resistance to BPH by enhancing H₂O₂ production and reducing ethylene synthesis (Hu *et al.*, 2016c). A variety of secondary metabolites are produced by rice plants exposed to BPH. The quercetin and spermidine contents which are harmful to BPH fitness were significantly elevated in the lines TN1 and IR36 (Kang *et al.*, 2019).

Utilizing genome editing techniques makes it possible to pyramid alleles without linkage drags, add new alleles, remove undesirable alleles/genes, and alter existing alleles. The genome editing approach, CRISPR/Cas9 was used for editing *CYP71A1* (encoding tryptamine 5-hydroxylase) resulting in an increased level of

salicylic acid and a decreased level of serotonin in the rice, which improved the BPH resistance (Lu *et al.*, 2018).

Host-plant resistance is considered an efficient, environment-friendly, and durable resistance mechanism to combat the brown plant hopper attack in rice. The majority of the BPH resistance genes are now marked with closely related molecular markers owing to advancements in molecular marker technology. Utilizing these genes with these markers attached might be a useful strategy for creating potential cultivars with resistance to BPH outbreaks. Despite the adoption of genomic technologies, the understanding of the molecular basis of the host-BPH interaction in rice needs to be well established with precise knowledge regarding the immune responses induced during BPH infestation. The complex interaction of several molecular factors, including genes, proteins, and a number of phytohormones, is what triggers the immunological response in rice that is resistant to infection. Using transcriptomics, proteomics, and metabolomics to restructure the enigmatic system, the precise mechanism of these molecular factors that mediate the resistance response has to be revealed.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ with the objective to introgress R genes for resistance to BPH in the background of the elite variety Jyothi using marker-assisted selection was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Regional Agricultural Research Station (RARS), Pattambi during the years 2020-2023. The materials and methods followed for the study are presented in this chapter.

3.1. BPH BIOASSAY

3.1.1. Plant material

Twelve rice genotypes, viz., Jyothi, PTB 33, PTB 21, IR65482–7–216-1-2, IR7103-121-15-B, RP2068-13-5-8, Kinandong Patong, Apo, Vandana, Way rarem, White Ponni, and Manu Ratna were used in this study. The popular high-yielding but BPH susceptible rice variety Jyothi released from RARS, Pattambi was the recipient parent in the study. Seedlings of rice genotypes were maintained at RARS, Pattambi, Kerala for initial BPH bioassay for identifying the donors. The screening experiment was replicated four times in a completely randomized design. The hybridization and backcross breeding program was done at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani.

3.1.2. BPH collection and rearing

BPH populations were collected from infested rice fields in Palakkad district and mass-reared in the insect rearing and screening net house facility at RARS, Pattambi for BPH bioassay. The nymphs and adults reared from the original population were used for the seedbox screening test. The rice variety Jyothi was used for mass rearing of BPH in the insect net house by following the method of Heinrichs (1985).

3.2.2. Modified seedbox screening test (MSST)

The modified seedbox screening test (MSST), where the seeds of each cultivar were sown in a single row along with susceptible (Jyothi) and resistant (PTB-33) checks in a seedbox of 60x40x10 cm (Plate 3). Maintained 10-20

seedlings per row by thinning at seven days after sowing and infested with about four second-instar nymphs per plant at twenty days after sowing. To distinguish between resistant and susceptible lines, an ideal population of at least 5 insects per seedling was established. Around 5 cm of standing water on the tray is allowed to maintain the high humidity ideal for insect life. The seedlings are gently tapped over by the severely infested plants from the mass-rearing cage. The test varieties were spread with BPH nymphs as evenly as feasible. After one week of infestation (when susceptible checks are killed), plants were rated for damage (Velusamy *et al.*, 1986). The experiment was replicated four times.

BPH-affected seedling survival rate was recorded based on a Standard Evaluation System (SES) score of 0-9 (IRRI, 2013). Low scores indicate the resistance of genotypes. The following rating scale was used for genotype screening.

SCALE	RATING	SYMPTOMS
0	Highly resistant	No injury
1	Resistant	Very Slight Injury
3	Moderately resistant	First and 2nd leaves of most plants partially yellowing
5	Moderately susceptible	Pronounced yellowing and stunting or about 10 to 25% of the plants wilting or dead and the remaining plants severely stunted or dying
7	Susceptible	More than half of the plants dead
9	Highly Susceptible	All plants dead

3.2.3. Nymphal survival rate

The nymphal survival rate determines the antibiosis effect on BPH nymph survival in different rice genotypes. Three leaf stage rice seedlings kept within screening cages were infested with 10-20 first instar nymphs. The survival percentage of BPH was counted after 14 days of infestation.

3.2.4. Honeydew test

In this experiment, the filter paper technique was employed. Bromocresol green indicator (2 mg/ml 70% ethanol) treated Whatman No.1 filter paper which dried for one hour and turned yellow/orange colour and was placed on an inverted



Plate 3. Seedlings planted in seedbox for BPH screening



Plate 4. Experimental setup for Honeydew test

petridish at the base of the plant kept inside a cage (Plate 4). Five hoppers were released for a single plant after five hours of starvation and allowed for feeding for 24 hours. Blue spots on filter paper indicate the phloem-based honeydew excretion upon BPH feeding. The area of honeydew excretion spots was measured and expressed in mm² (Pathak and Heinrichs, 1982).

3.2.5. Functional Plant Loss Index (FPLI)

The tolerance mechanism in the varietal entries was studied by computing the Functional Plant Loss Index (FPLI). The seedlings at the two-three leaf stage maintained in the seedbox were infested with 6-10 BPH nymphs. When the BPH became adults on susceptible genotype, the damage score based on the IRRI SES scale was measured for each genotype. The entire plants, both infested and uninfested were removed without damaging the roots, washed for removing the soil, air dried for 2 hours, and oven dried at 70°C for 48 hours.

The dry weight of uninfested and infested plants from the same replication was measured and FPLI was calculated as follows,

For varietal entries with an SES damage score below 3,

$$FPLI = 1 - \left[\frac{\text{Dry weight of infested plants}}{\text{Dry weight of uninfested plants}} \right] \times 100$$

For varietal entries with an SES damage score above 3,

$$FPLI = 1 - \left[\frac{\text{Dry weight of infested plants}}{\text{Dry weight of uninfested plants}} \right] \left[1 - \frac{\text{Damage rating}}{9} \right] \times 100$$

3.2.6. Tolerance index (TI)

The seeds of each genotype were sown in a separate seedbox of 60x40x10 cm. The seedlings at three leaf stage were infested with 10 first instar nymphs on each plant and BPH fed on the varieties were collected when the susceptible line was fully dried. The collected BPH were oven dried for 48 hours at 55°C and weighed using a precision weighing balance.

$$TI = \frac{\text{BPH dry weight on test entry}}{\text{BPH dry weight on susceptible entry}}$$

3.3. MORPHOLOGICAL CHARACTERS OF PARENTS

3.3.1. Plant height (cm)

The plant height was measured at the time of harvest from the base of the main tiller to the tip of the panicle, apart from the awns, and expressed in centimetres (cm).

3.3.2. Days to 50% flowering

The number of days taken for 50% flowering in the plants was counted from the date of sowing.

3.3.3. Total number of tillers

The number of tillers in the plant was counted at the stage of physiological maturity and expressed in numbers.

3.3.4. Number of productive tillers

The plant tillers with healthy panicles were counted at the stage of physiological maturity and expressed in numbers.

3.3.5. Culm thickness (mm)

The culm thickness was measured at the midpart of the 4th internode from the top at 20 days after heading by taking the cross-section and measured using a Leica microscope and expressed in millimetres (mm).

3.3.6. Panicle length (cm)

The length of the random five panicles was measured at the time of the harvest, and the average value was calculated and expressed in centimetres (cm).

3.3.7. Number of grains per panicle

The filled grains of the selected five panicles were counted, and the mean was computed and expressed in numbers.

3.3.8. 1000 grain weight

The weight of an arbitrarily selected sample of 1000 whole grains was taken using a precision balance and the average was calculated and expressed in grams (g).

3.3.9. Grain length (mm)

The length of the randomly selected grains was measured using a vernier calliper and the average was calculated. It was expressed in millimetres (mm).

3.3.10. Grain breadth (mm)

The breadth of the same grains was calculated using a vernier calliper and the mean value was computed and expressed in millimetres (mm).

3.3.11. Length/breadth (L/B) ratio

The length/breadth ratio was determined by dividing the length by the corresponding breadth of the grains and the average was computed.

3.4. QUALITATIVE PARAMETER

3.4.1. Kernel colour

The rice seeds were manually husked and kernel colour was visually observed based on the following table.

Kernel colour characterization

Sl. No.	colour
1	White
2	Light brown
3	Variegated brown
4	Dark brown
5	Light red
6	Red
7	Variegated purple
8	Purple
9	Dark purple

3.5. BIOCHEMICAL PARAMETERS IN PARENTS

3.5.1. Protein Content (%)

The protein content of plants was estimated by Bradford's method (Bradford, 1976).

Reagents:

- i. Dye-Coomassie brilliant blue G-250 (Bradford reagent): Dissolve 10 mg of Coomassie brilliant blue G-250 in about 5 ml of ethanol, then add 10 ml of orthophosphoric acid (85%). Dilute to 100 ml with water in a volumetric flask and mix thoroughly. Store it in a brown bottle in a cool place.
- ii. Phosphate buffer: pH 7.5
- iii. Dissolve 2.78g of monobasic sodium phosphate in 100 ml of distilled water (solution a).
- iv. Dissolve 5.365 g dibasic sodium phosphate in 100 ml of distilled water (solution b).
 - a. Mix 16 ml solution (a) with 14 ml of solution (b) and dilute to 200 ml with distilled water in the volumetric flask.
- v. Standard protein solution: Dissolve 50mg of Bovine serum albumin (BSA) in distilled water in a 50 ml volumetric flask.

Procedure:

Bradford's protein estimation method involves two major procedures *i.e.*, extraction and estimation. For each genotype, 0.1g of fresh plant sample was homogenized in mortar and pestle with 20 ml of 0.1M phosphate buffer and centrifuged at 5000 rpm for 15 minutes. The 100 μ l of the supernatant was made up to 1.5 ml using 0.1M phosphate buffer and was taken for protein estimation. The aliquots of the standard solution of protein 20, 40, 60, 80, and 100 μ l were pipetted, transferred to test tubes, and diluted to 1.0 ml with phosphate buffer. Add 4 ml of dye solution (Bradford reagent) to all the tubes and mixed thoroughly. The absorbance of the blue colour was read at 595 nm, setting zero with the first tube (blank). Calculated the amount of protein in the sample with the help of a standard curve and expressed it in per cent (%).

3.5.2. Phenol content (mg/100g)

The phenol content in the plant sample was estimated with the Folin-Ciocalteu reagent (Sadasivam and Manickam, 1992).

Reagents:

- i. 80% ethanol
- ii. Folin- Ciocalteu reagent
- iii. 20% Sodium carbonate (Na_2CO_3)
- iv. Standard (100 mg Catechol in 100 ml water), diluted 10 units for working standards.

Procedure:

One gram of fresh plant sample was ground with pestle and mortar at ten times the volume of 80% ethanol and centrifuged the homogenate at 10,000 rpm for 20 minutes for collecting supernatant. Reextracted the residue with five times the volume of 80% ethanol, and centrifuged for supernatant. Evaporated the supernatant to dryness and dissolved the residue in a 5 ml volume of distilled water, pipetted out different aliquots (0.2 to 2 ml) into test tubes and made the volume in each tube with 30 ml of water. 0.5 ml of Folin-Ciocalteu reagent was added and waited for 3 minutes to add 2 ml of 20% Na_2CO_3 solution to each tube. Then, mixed the tube thoroughly and placed it in boiling water for exactly one minute. Cooled and measured the absorbance at 650nm against a reagent blank. A standard curve was prepared using different concentrations of catechol and the concentration of phenols in the test sample was found, which was expressed in mg/100g.

3.5.3. Ascorbic acid (mg/100g)

The ascorbic acid content in the plant sample was determined by the 2, 6-dichlorophenol indophenol (DCPIP) titration method (Rao and Deshpande, 2006).

Reagents:

- i. 4% oxalic acid
- ii. 2, 6-dichlorophenol indophenol (DCPIP)
- iii. Ascorbic acid working standard (500 $\mu\text{g}/5\text{ ml}$)

Procedure:

0.1g of the sample homogenate was mixed with 10 ml of 4% oxalic acid in a standard flask. 5 ml of the ascorbic acid working standard (500 $\mu\text{g}/5\text{ ml}$) and 5 ml

of 4% oxalic acid were pipetted out into a 100 ml conical flask. The contents in the flask were titrated against the dye solution (V_1) until the appearance of a pale pink colour persisted for a few min. 5 ml of the test samples were similarly titrated against the dye solution (V_2). The ascorbic acid content present in the test samples was determined using the formula:

$$\text{Amount of ascorbic acid content (mg/100 g)} = \frac{500 \times V_2 \times 10 \times 100}{V_1 \times 10 \times 0.1 \times 1000}$$

Where,

500 = μg of standard ascorbic acid taken for titration

V_1 = Volume of dye consumed by 500 μg of standard ascorbic acid

V_2 = Volume of dye consumed by 5 ml of the test sample

10 = Corresponds to the total volume of the extract

100 = Ascorbic acid content/100ml of sample

0.1 = weight of sample taken for extraction

10 = Volume of the test liquid sample taken for titration

1000 = conversion factor (μg to mg)

3.5.4. Reducing sugar (g/100g)

The reducing sugar content was estimated using Dinitrosalicylic acid (DNS) reagent (Miller, 1959).

Reagents:

- i. Dinitrosalicylic acid (DNS) reagent: Dissolve simultaneously 1g of dinitrosalicylic acid, 200mg of crystalline phenol and 50mg of sodium sulphite in 100 ml of 1% NaOH solution by stirring. Store the reagent in a stoppered bottle at 4°C.
- ii. 40% Rochelle salt solution (Sodium- potassium tartrate solution). Dissolve 40 g in the final volume of 100 ml in distilled water.
- iii. Standard sugar solution: Weigh accurately 100 mg of dry glucose powder, dissolve it in distilled water and make a final volume of 1000ml in a volumetric flask (100 μg in 1ml or 100 ppm).

Procedure:

100mg of homogenized sample was weighed and transferred in to a clean beaker. 1 ml of warm ethyl alcohol was added and mixed with a glass rod for 15-20 minutes. The supernatant was collected after centrifugation and re-extracted the residue two times with a 3ml portion of ethyl alcohol. Combined the supernatant in the centrifuged tube. Evaporated the ethanol in the water bath (70-80°C) till the residue was dry. 1ml of distilled water was added, which dissolved the sugar, and centrifuged at 1000-1200 rpm for 15-20 minutes. Pipette out the supernatant for estimation. Pipette out the standard solution at a concentration of 50-250 µg/ ml in test tubes and 1.0 ml of the alcohol extract of sample in duplicate and transfer to two numbered test tubes. Made the volume of solution 3.0 ml by adding distilled water. Mixed and added 3ml of alkaline dinitro salicylic acid reagent. Mixed and kept all the test tubes in a boiling water bath for 5 minutes. Added 1.0ml Sodium potassium tartrate solution in all tubes, while they were hot. Then, mixed, cooled and read the absorbance of orange red colour at 510 nm.

3.6. VALIDATION OF GENES FOR BPH RESISTANCE IN THE RECIPIENT AND DONOR PARENTS USING SPECIFIC MOLECULAR MARKERS.

3.6.1. Genomic DNA isolation using the CTAB method

The CTAB technique was used to isolate genomic DNA (Doyle, 1990). Young fresh leaves of rice (200 mg) were ground with an autoclaved pre-chilled pestle and mortar to a fine paste in approximately 500µl of pre-warmed CTAB buffer. Following that, the mixture was transferred to a microcentrifuge tube and continuously shaken every ten minutes for an hour at 60°C in a water bath. After incubation, the mixture was centrifuged for 10 minutes at 12000 rpm to remove cell debris, and the supernatant was then transferred into fresh microcentrifuge tubes. In each tube, an equal amount of 25:24:1 phenol, chloroform, and isoamyl alcohol was added and mixed thoroughly with the solution before being centrifuged at 12000 rpm for 10 minutes. After the supernatant had been collected into sterile microcentrifuge tubes. To precipitate the DNA, an equal volume of ice-cold isopropanol was added, and the tube was gently inverted several times. The tube

was stored at -20°C overnight to allow the DNA to be precipitated by alcohol. The sample was then centrifuged at 12000 rpm for 10 minutes, the supernatant was decanted without disturbing the pellet, and 200 µl of ice-cold, 70% ethanol was added. Decanted the ethanol and dried the pellet just long enough to get rid of the extra alcohol while leaving the DNA wet. The DNA pellet was dissolved in 80 µl of TE buffer (10 mM Tris, pH 8, 1 mM EDTA), and 5 µl of RNase A solution was added. The mixture was then incubated at 37 °C for an hour, dissolved in TE buffer and then kept at -20°C.

Composition of CTAB Buffer (100 ml)

CTAB (cetyl trimethyl-ammonium bromide)	- 2 g
1 M Tris pH 8	- 10 ml
0.5 M EDTA pH 8 (Ethylenediaminetetra Acetic acid Di-sodium salt)	- 4 ml
5 M NaCl	- 28 ml
H ₂ O	- 40 ml
PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer)	- 1 g
β- Mercaptoethanol	- 700 µl
(Adjust all to pH 5.0 with HCL and make up to 100 ml with H ₂ O)	

3.6.2. Agarose Gel Electrophoresis

The genomic DNA was resolved using agarose gel electrophoresis in a submerged horizontal gel electrophoresis unit of Biorad. Agarose (0.8 per cent) was prepared in 0.5X TBE buffer. Ethidium bromide was added to the solution at a rate of 3 µl for every 100 ml after it had cooled to 42–45°C. The solution was then poured onto a preset, gel casting tray with a comb fixed in position, to a height of 3-5 mm. The gel was allowed to solidify for 15-20 min. After the comb was taken out, the casting tray was placed in an electrophoresis tank that contained 0.5X TBE buffer, making sure that the buffer completely covered the gel to a height of 1mm. The micropipette was used to load the slots of the gel with the necessary quantity of DNA sample and loading dye (glycerol 30% + bromophenol blue) in a 5:1 ratio. The power source was connected to the electrophoresis unit's negative (cathode) and positive (anode) terminals, and a voltage of 60V was set for gel running. Once the loading dye reached about 3/4th of the gel, it was taken out of the electrophoresis

unit, and the DNA bands were visualized under UV light using the SYNGENE gel documentation system.

3.6.3. Quantification of genomic DNA

Spectrophotometric UV absorption measurements at wavelengths of 260 and 280 nm were used to quantify the DNA. The TE buffer was used to calibrate the spectrophotometer at 260 and 280 nm wavelengths. Both 260 and 280 nm wavelengths were used to measure the optical density (O.D) of the DNA samples dissolved in TE buffer. By comparing the O. D. values obtained at 260 and 280 nm, the purity of DNA was assessed. The ratio between 1.8 and 2 indicated good quality. The quantity of DNA in the sample was estimated by using the following formula:

$$\text{The concentration of DNA (ng/}\mu\text{l)} = \text{OD}_{260} \times 50 \mu\text{g/ml} \times \text{dilution factor}$$

3.6.4. Validation of genes for BPH resistance using SSR markers

The list of genes, chromosome location, SSR markers, and annealing temperature is enlisted in Table 4.

The genomic DNA of rice genotypes was amplified using four SSR markers. The reaction was executed in a 25 μl reaction mixture consisting of 20ng template DNA, 2 μl of dNTP mix, 2.5 μl of PCR buffer, 2.5 μl of MgCl_2 and 0.3 μl Taq DNA polymerase, and 1 μl of both forward and reverse primers. Amplification compassed in an Eppendorf master cycler nexus PCR. Following PCR conditions were used: An initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94 ° C for 30 seconds, the annealing temperature of 55.3 ° C for 30 seconds, primer extension at 72 ° for 1 minute, and final extension at 72°C for 8 minutes.

3.6.5. Electrophoresis

Metaphor agarose gel electrophoresis was also carried out in a horizontal gel electrophoresis unit of BIORAD for the PCR product analysis. Compared to normal agarose, the metaphor agarose has a low melting temperature (75°C), gelling temperature of less than 35°C, and gel strength of more than 300 g/cm². Metaphor Agarose gel of 4 per cent was used for PCR product separation in 0.5X TBE buffer. 4g of metaphor agarose powder (Lonza, Rockland, USA) was added

to 100 ml of the 0.5X TBE buffer taken in a 500 ml conical flask upon swirling so that the agarose would get mixed up in the solution easily and kept allowed to soak over 5-10 minutes. The conical flask was covered with aluminum foil with a small hole for aeration and the agarose was melted in a microwave oven for 1 min initially followed by cooling for 15 mins and 1-2 mins melting for the complete dissolving of Metaphor agarose. After a few minutes of cooling, 5 µl EtBr was added and mixed thoroughly. The solution was then poured into the casting tray for solidification, cooled at room temperature, and kept at 4°C for 10-15 min for better resolution. The comb was removed after the solidification of the gel and transferred into the BIORAD gel electrophoresis unit. PCR products were loaded into each well (8µl PCR product+2µl loading dye). A voltage of 60V for 2-3 hours was supplied for the electrophoresis of the PCR product. The gel was then visualized under UV light using the SYNGENE gel documentation system in order to analyze the SSR marker amplification. Proper care was taken while preparing the gel and documentation as the gel is very sensitive to breakage.

3.7 HYBRIDIZATION AND BACKCROSS BREEDING PROGRAM

3.7.1. Recipient and donor parents

The popular high-yielding but BPH susceptible variety Jyothi was the recipient parent. Based on BPH screening, resistant reaction study, and gene validation, the KAU released variety 'PTB-33' and IRRI introgression line IR65482-7-216-1-2 were selected as the donors for *Bph-3* and *Bph-18 gene* respectively in the marker-assisted backcross breeding program (Figure 1). The characteristic features of the recipient and donor parents are enlisted in Table 5. These rice genotypes were raised in the nursery by staggered sowing for obtaining synchronization of flowering and transplanted to the field in crossing blocks of 1.5 m² and 30x30 cm spacing (Plate 5 and 6).

3.7.2. Emasculation and pollination

The emasculation was done by clipping the spikelet to half or top 1/3rd of spikelet and removing anthers by hand using needles and also by using a vacuum emasculator. Healthy representative plants of a variety in pots or planted in the field were selected for crossing. Plants in the field were shifted to pot one day prior to

Table 4. List of genes, chromosome location, SSR markers, and annealing temperature

Gene	Chromosome location	Marker	Sequence	Annealing temp. (° C)	Reference
<i>Bph 3</i>	6S	RM589 F	GAGGTTGTTTGGATGGATAGATGG	55.3	Jairin <i>et al.</i> , 2007
		RM589 R	AATCCCGTCCTAGAGTTCTTCTACC		
<i>Bph 18</i>	12L	RM3331 F	CCTCCTCCATGAGCTAATGC	55.3	Jiang <i>et al.</i> , 2018
		RM3331 R	AGGAGGAGCGGATTTCTCTC		
<i>Bph 20</i>	4S	RM8213 F	TGTTGGGTGGGTAAAGTAGATGCTG	55.3	Mohanapriya <i>et al.</i> , 2019
		RM8213 R	CCCAGTGATACAAAGATGAGTTGG		
<i>Bph 21</i>	12L	RM28561 F	CTTCAAGACTGGCCCAATATTACTGC	55.3	Jiang <i>et al.</i> , 2018
		RM28561 R	TGACTGAAGCCTTCTTCACTTGC		

Table 5. Features of recipient and donor parents used in the study

	Variety/breeding line	Characteristic features
Recipient parent	Jyothi	• Most widely accepted, popular high-yielding rice variety. • Released from RARS, Pattambi (1972) • Parentage: PTB 10 x IR 8 • Rich in iron, protein, potassium • Red kernel • Duration: 110-120 days • Yield: 6 t ha⁻¹
Donor parent	PTB-33	• Pureline selection from Arikkirai • Released from RARS, Pattambi (1952) • Photosensitive, tall mundakan variety (>130 cm) • Internationally recognized multiple donor for BPH, GLH, Thrips, WBPH, ZLH • Donor of <i>Bph-3</i> gene • Parent of IR 56 and IR 62 • Red kernel • Duration: 150 days • Yield: 3 t ha⁻¹
	IR65482-7-216-1-2	• <i>Indica</i> introgression line derived from <i>Oryza australiensis</i> • White kernel • Duration: 90-110 days • Donor of <i>Bph18</i> gene • <i>Bph18</i> encodes a CC-NBS-NBS-LRR protein

emasculatation for reducing the transplant shock or wilting. Emasculation was done in the late-evening hours or in the early morning hours well before anthesis. Emasculation in the morning hours had a high risk of self-pollination by bursting the anthers by clipping causing pollen release. Panicles with 50-60 per cent emergence were selected and the young florets from the bottom and that had undergone anthesis if any from the top of the panicle were removed.

The florets were cut obliquely using scissors to expose anthers for vacuum emasculatation. When forceps were engaged for emasculatation, a direct cut across the floret was preferred without damaging stigma. While using a vacuum emasculator, adjusted the suction pressure to 500 mm Hg and extracted the anthers without any stigma damage. Also, ensured that all six anthers are removed. Covered the emasculated panicles with a glassine or butter paper bag with the name of the parents in the cross and the date of emasculatation labeled using a pencil. Folded the bottom edge over and clip folded cover against the stem or used a stick for support in holding the panicle along with the cover (Plate 7).

The emasculated plants were checked for any anthers left prior to pollination and arranged in a manner so that pollination could be done with ease. Anthesis timing of rice spikelets in hot weather conditions was observed at 8 am and lasted for 2 hours whereas in cold weather conditions, cloudy or rainy days, anthesis got delayed and even occurred at 1-2 pm afternoon. The collected panicles with stems were cut back to equal lengths and kept in a beaker of water. The panicles were kept under light (60-100W) for half an hour to fasten the pollen release.

The panicles with anther extrusion were taken for pollination by dusting the pollen by shaking the panicle over an emasculated panicle or collecting the pollen in a petri dish and dusting it over each spikelet using a small pointed paintbrush. After pollination, the panicles were tagged and covered using a butter-paper bag or glassine cover and protected from rain, ants, rodents, and heavy wind. The tag was properly labeled with all details using a pencil. The pollinated panicles were

checked each day for any fungal infection or pest attack. Up to five days, pollen dusted over the emasculated panicles since the stigma remains receptive, or else, the male parent kept them near the recipient parent, and the panicles were tied together, covered with butter paper cover, tapped the panicles at morning hours during pollen release can be effective in pollination. The seed set was observed 2-5 days after pollination and the matured seeds were harvested 30 days after pollination (Plate 8). The harvested half-exposed seeds were dried at 50-55°C for 7 days, glume remnants removed, and stored in the refrigerator (Plate 9).

3.7.3. Development of F₁ and BC₁F₁ lines

Resistant donors (PTB-33 and IR65482-7-216-1-2) for two or three genes identified were crossed independently with the popular but susceptible variety Jyothi to obtain F₁ generation. F₁ obtained were backcrossed with the Jyothi to obtain BC₁F₁. Genotyping of BC₁F₁ for BPH resistance was done using specific molecular markers, RM589 for *Bph-3* and RM3331 for *Bph-18*. PCR and gel electrophoresis was carried out as mentioned in 3.6.4 and 3.6.5 Phenotyping of the BC₁F₁ population was done based on morphological characters mentioned in 3.3.

3.7.4. Intercrossing of BC₁F₁ and development of BC₂F₁ lines

The BC₁F₁ with R genes were intercrossed to develop intercrossed BC₁F₁. Intercrossing of BC₁F₁ lines involved two crosses viz., the PTB-33 derived BC₁F₁ lines with *Bph-3* in heterozygous condition as the female parent and IR65482-7-216-1-2 derived BC₁F₁ lines with *Bph-18* in heterozygous condition as male parent denoted as 'ICAB' and the reciprocal cross denoted as 'ICBA'. Phenotyping of intercrossed BC₁F₁ lines for Jyothi-specific characters was done as mentioned in 3.3. and genotyping for the confirmation of R genes using specific molecular markers was carried out as mentioned in 3.6.4 and 3.6.5. The Euclidean Distance of intercrossed BC₁F₁ lines from the recipient parent Jyothi using proximity dissimilarity matrix analysis was calculated to select the intercrossed BC₁F₁ lines more similar to the recipient parent. The intercrossed BC₁F₁ lines with Jyothi-specific characters and genes for resistance (*Bph-3* and *Bph-18*) in heterozygous condition were backcrossed with Jyothi to develop BC₂F₁ lines. Phenotyping of BC₂F₁ lines for Jyothi-specific characters was done as mentioned in 3.3 and

genotyping for the confirmation of R genes using specific molecular markers was carried out as mentioned in 3.6.4 and 3.6.5.

3.7.5. Development of BC₂F₂ lines, BPH bioassay, phenotyping and genotyping

BC₂F₁ lines with maximum similarity in proximity dissimilarity matrix analysis were selfed to generate BC₂F₂ lines. The BC₂F₂ lines were forwarded to BPH bioassay in the seedling stage at RARS, Pattambi, and COA, Vellayani. The BC₂F₂ lines which showed different levels of resistance in the BPH bioassay were genotyped for the presence of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition as mentioned in 3.6.4 and 3.6.5. Phenotyping of BC₂F₂ lines for Jyothi-specific characters was done as mentioned in 3.3. The similarity per centage of these lines with the recipient parent Jyothi was calculated using Euclidean Distance in proximity dissimilarity matrix analysis.

3.8 STATISTICAL ANALYSIS

The biometric parameters recorded from the donor and recipient parents were statistically analyzed using the following estimations.

3.8.1. Analysis of Variance (ANOVA) for CRD

The observation of the damage score in the parents was transformed using the arc sine transformation method and ANOVA for CRD was calculated.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Treatments	t-1	TrSS	$TrMS = \frac{TrSS}{t-1}$	$\frac{TrMS}{EMS}$
Error	n-t	ESS	$EMS = \frac{ESS}{n-t}$	
Total	n-1	TSS		

Where, n= The total number of observations, t= number of treatments, TrSS= sum of squares for treatments, ESS= sum of squares for error,

$$\text{Critical Difference, } CD = t_{\alpha} \sqrt{EMS \left(\frac{1}{r_i} + \frac{1}{r_j} \right)}$$

Where, t_{α} students' t table value distribution at error d.f with the level of significance α (5% or 1%).

3.8.2. Kruskal Wallis test, one-way ANOVA on ranks

The observation of damage score in donor and recipient parents were subjected to nonparametric equivalent one-way ANOVA, Kruskal-Wallis test. The significant difference between values identified according to Duncan's multiple range test at $\alpha=0.05$.

3.8.3. Estimation of Genetic Parameters

3.8.3.1. Genetic Components of Variance

Phenotypic and genotypic components of variance were estimated for each character by equating the expected value of mean squares (MS) to the respective variance components (Jain, 1982).

- i. Genotypic Variance (V_G), $V_G = \frac{\text{TrMS} - \text{EMS}}{r}$
- ii. Environmental Variance (V_E), $V_E = \text{EMS}$
- iii. Phenotypic Variance (V_P), $V_P = V_G + V_E$

3.8.3.2. Coefficient of Variation

Genotypic, Phenotypic, and Environmental coefficient of variation were estimated from V_P , V_G , and V_E , expressed in per centage for each trait.

- i. Genotypic coefficient of variation, $\text{GCV} = \frac{\sqrt{V_G}}{X} \times 100$
- ii. Phenotypic coefficient of variation, $\text{PCV} = \frac{\sqrt{V_P}}{X} \times 100$
- iii. Environmental coefficient of variation, $\text{ECV} = \frac{\sqrt{V_E}}{X} \times 100$

Where, X = Grand mean

Sivasubrahmanian and Menon (1973) reported the following categories for the range of variation,

- i. High: >20 per cent
- ii. Medium: 10-20 per cent

- iii. Low: <10 per cent

3.8.3.3. Broad Sense Heritability

Ratio of genotypic variance to the total observed variance in the population and calculation expressed in per centage.

$$H^2 = \frac{V_G}{V_P} \times 100$$

Range of Heritability estimation (Johnson *et al.*, 1955) is,

- i. High: >60 per cent
- ii. Medium: 30-60 per cent
- iii. Low: <30 per cent

3.8.3.4. Genetic Advance

The expected genetic gain or improvement in the next generation by selecting a superior genotype under a certain amount of selection pressure. Genetic advance was estimated by using the Burton (1952) formula.

$$GA = KH^2\sqrt{VP}$$

Where K= selection differential, At 5% selection intensity K=2.06,

H²= Heritability, V_p= Phenotypic variance

3.8.3.5. Genetic Advance as Per cent of Mean

$$GAM = GA/X \times 100$$

Where, GA= Genetic Advance, X= Grand Mean

Ranges of genetic advance by Johnson *et al.* (1955) is,

- i. High= >20 per cent
- ii. Medium= 10-20 per cent
- iii. Low= 10 per cent

3.8.3.6. Estimation of Correlation

Degree and direction of association between two variables refers the correlation. Genotypic and phenotypic correlations were calculated by using Falconer (1964) formula.

- i. Genotypic correlation coefficient (r_g) = $r(x_i, x_j)_g = \frac{Cov((x_i, x_j)_g)}{\sqrt{v(x_i)_g v(x_j)_g}}$
- ii. Phenotypic correlation coefficient (r_p) = $r(x_i, x_j)_p = \frac{Cov((x_i, x_j)_p)}{\sqrt{v(x_i)_p v(x_j)_p}}$
- iii. Error correlation coefficient (r_e) = $r(x_i, x_j)_e = \frac{Cov((x_i, x_j)_e)}{\sqrt{v(x_i)_e v(x_j)_e}}$

3.8.3.7. Path Coefficient Analysis

It is a standardized partial regression coefficient which separates the correlation coefficients into direct and indirect effects (Dewey and Lu, 1959).

$$r_{1y} = P_{1y}r_{11} + P_{2y}r_{12} + P_{3y}r_{13} + \dots + P_{ny}r_{1n}$$

$$r_{2y} = P_{2y}r_{21} + P_{2y}r_{22} + P_{3y}r_{23} + \dots + P_{ny}r_{2n}$$

$$r_{ny} = P_{1y}r_{n1} + P_{2y}r_{n2} + P_{3y}r_{n3} + \dots + P_{ny}r_{nn}$$

Where,

1, 2, ..., n = independent variables

y = dependent variable

$r_{1y}, r_{2y}, \dots, r_{ny}$ = coefficient of correlation between independent variables

1 to n on dependent variable y.

$P_{1y}, P_{2y}, \dots, P_{ny}$ = direct effect of character 1 to n on character y.

The above equation can be written in matrix form

$$\begin{bmatrix} r_{1y} \\ r_{2y} \\ \vdots \\ r_{ny} \end{bmatrix} = \begin{bmatrix} 1 & r_{12} & r_{13} & \dots & r_{1n} \\ r_{21} & 1 & r_{23} & \dots & r_{2n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ r_{n1} & r_{n2} & r_{n3} & \dots & 1 \end{bmatrix} \begin{bmatrix} P_{1y} \\ P_{2y} \\ \vdots \\ P_{ny} \end{bmatrix}$$

Then $B = C^{-1}A$ where $C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & \dots & C_{1n} \\ C_{21} & C_{22} & C_{23} & \dots & C_{2n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ C_{n1} & C_{n2} & C_{n3} & \dots & C_{nn} \end{bmatrix}$

Direct effects:

$$P_{1y} = \sum_{i=1}^k c_{1i} r_{iy}$$

$$P_{2y} = \sum_{i=1}^k c_{2i} r_{iy}$$

$$P_{ny} = \sum_{i=1}^k c_{ni} r_{iy}$$

$$\text{Residual effect } PR_y = \sqrt{1 - r^2}$$

$$\text{Where, } r^2 = (P_{1y}r_{1y} + P_{2y}r_{2y} + P_{3y}r_{3y} \dots \dots \dots + P_{ny}r_{ny})$$

P_{iy} = direct effect of X_i on y

r_{iy} = correlation coefficient of X_i on y

$i = 1, 2, 3, \dots, n$

3.8.4. Descriptive Statistics

Observations on the biometric characters were recorded on the recurrent parent, donors, and selected plants in the segregating populations. The descriptive statistical analysis was carried out for each character and it was calculated as follows,

3.8.4.1. Range

It records the highest and lowest value in the observed value for each character in parents and the segregating populations.

3.8.4.2. Arithmetic Mean

It is calculated by the following formula:

$$X = \Sigma X / N$$

Where, X = Mean, ΣX = Sum of all the observations, N = Total number of observations.

3.8.4.3. Variance

$$V = \Sigma (X - \bar{X})^2 / N$$

Where, X = Individual reading, $\bar{X}$ = Mean, N = Sample size

3.8.4.4. Standard Deviation

$$S.D = \sqrt{\Sigma (X - \bar{X})^2 / N}$$

Where, X = Individual reading, $\bar{X}$ = Mean, N = Sample size

3.8.4.5. Standard Error

$$S.E = S.D / \sqrt{N}$$

Where, S.D = Standard Deviation, N = total number of observations.

3.8.4.6. Chi-square test

The goodness of fit for resistant loci to an expected 1:1 segregation ratio in BC₁F₁ population controlled by a single resistant gene was tested by means of chi-square analysis.

Following expression was used to calculate the chi-square values,

$$\chi^2 = \sum (O_i - E_i)^2 / E_i \text{ at d.f.} = n-1$$

Where,

n= number of phenotypic classes

d.f= degrees of freedom

O= Observed number of plants in each phenotypic class

E= Expected number of plants in each phenotypic class

3.8.4.7. Euclidean Distance

The proximity dissimilarity matrix was analyzed using the Euclidean distance method for eleven morphological characters of intercrossed BC₁F₁, BC₂F₁ and BC₂F₂ lines using the formula.

$$\text{Euclidean Distance} = \sum_{k=1}^j \left(\frac{X_{ik} - X_{jk}}{S_k} \right)^2$$

Where,

X_{ik}= performance of the ith parent for kth character

X_{jk}= performance of the jth parent for kth character

S_k= Standard deviation of the kth character

Genetic distance among the intercrossed BC₁F₁, BC₂F₁ and BC₂F₂ lines with the recurrent parent was measured by the Euclidean distance method (Cruz and Regazzi, 1994) using Statistical Package for the Social Sciences (SPSS version 29.0).

RESULTS

4. RESULTS

Brown plant hopper (BPH) is one of the major threats to rice cultivation by causing severe infestation and damage to the host plants showing hopper burn symptoms and also acting as a vector of grassy and ragged stunt viruses. The host plant resistance mechanism in rice genotypes needs to be exploited to curb the BPH attack to improve the genetic background of an elite high-yielding but susceptible variety Jyothi. Using R-genes, the host-plant resistance strategy can be used to combat the BPH attack by introducing it into popular but susceptible cultivars. Marker-assisted backcrossing based gene pyramiding strategy can be effectively used for the transfer of multiple resistant genes from variable donor parents to a single elite variety.

In the present study, the rice genotypes were subjected to BPH bioassay, resistance reaction, and gene validation for donor parent selection. The selected donors and recipient parents were forwarded to the marker-assisted backcross breeding program for gene introgression to develop the BC₂F₂ lines with BPH resistance. The results of the present study are presented in this chapter hereafter under the following headings:

4.1 Bioassay for BPH resistance

4.2 Morphological evaluation of parents

4.3 Biochemical parameters in parents before and after BPH infestation

4.4 Validation of genes for BPH resistance in the donor and recipient parents using specific molecular markers

4.5. Marker-assisted backcross breeding program

4.1 BIOASSAY FOR BPH RESISTANCE

4.1.1. BPH Bioassay

Recipient parent (Jyothi) and probable donors for BPH resistance from the results of a concluded project in the Department of Plant Breeding and Genetics, COA, Vellayani, and other R gene introgressed lines from IRRI were selected for screening. The BPH bioassay was done using the modified seedbox screening test (MSST) developed by Velusamy (1986). BPH-affected seedling survival rate was calculated based on the Standard Evaluation System (SES) scale of 0-9 (IRRI, 2013). Low scales indicate the resistant response of genotypes. SES scale for BPH screening were '0' - No injury, '1'-very slight injury, '3'- first and second leaves of most plants partially yellowing, '5'-pronounced yellowing and stunting or about 10 to 25 per cent of the plants wilting or dead, and remaining plants severely stunted or dying, '7'- more than half of the plants dead, and '9'- all plants dead.

The response of rice genotypes in BPH seedling screening were recorded (Plate 10). Among the twelve rice genotypes screened, PTB-33, PTB-21, and IR7103-121-15-B were classified as highly resistant with a scale of '0' and the genotypes IR65482-7-216-1-2 and RP2068-18-3-5 with a scale of '1' were classified as resistant to BPH infestation. The *japonica* variety Kinandang Patong was moderately resistant to BPH with a scale of '3'. The recipient parent Jyothi and five other genotypes viz., Manu Ratna, White Ponni, Wayrareem, Vandana, and APO with a scale of '9' were classified as highly susceptible to BPH infestation (Table 6).

4.1.2. BPH resistance reaction

Five genotypes, Jyothi (recipient parent), PTB-33, IR7103-121-15-B, IR65482-7-216-1-2, and RP2068-18-3-5 were forwarded for studying the BPH resistance reaction. The antibiosis and tolerance mechanisms of BPH resistance were explored in terms of nymphal survival rate, honey-dew test, functional plant loss index (FPLI), and tolerance index (TI) (Table 7).

Table 6. Response of rice genotypes to BPH infestation

Sl. No.	Variety	Damage Score	Reaction
1.	Jyothi	9	Highly susceptible
2.	Manu Ratna	9	Highly susceptible
3.	White Ponni	9	Highly susceptible
4.	Wayrarem	9	Highly susceptible
5.	Vandana	9	Highly susceptible
6.	APO	9	Highly susceptible
7.	Kinandang Patong	3	Moderately resistant
8.	RP2068-18-3-5	1	Resistant
9.	IR65482-7-216-1-2	1	Resistant
10.	IR7103-121-15-B	0	Highly Resistant
11.	PTB-21	0	Highly Resistant
12.	PTB-33	0	Highly Resistant
	Minimum	0	
	Maximum	9	
	Mean	5.3	
	Std. Dev.	4.33	

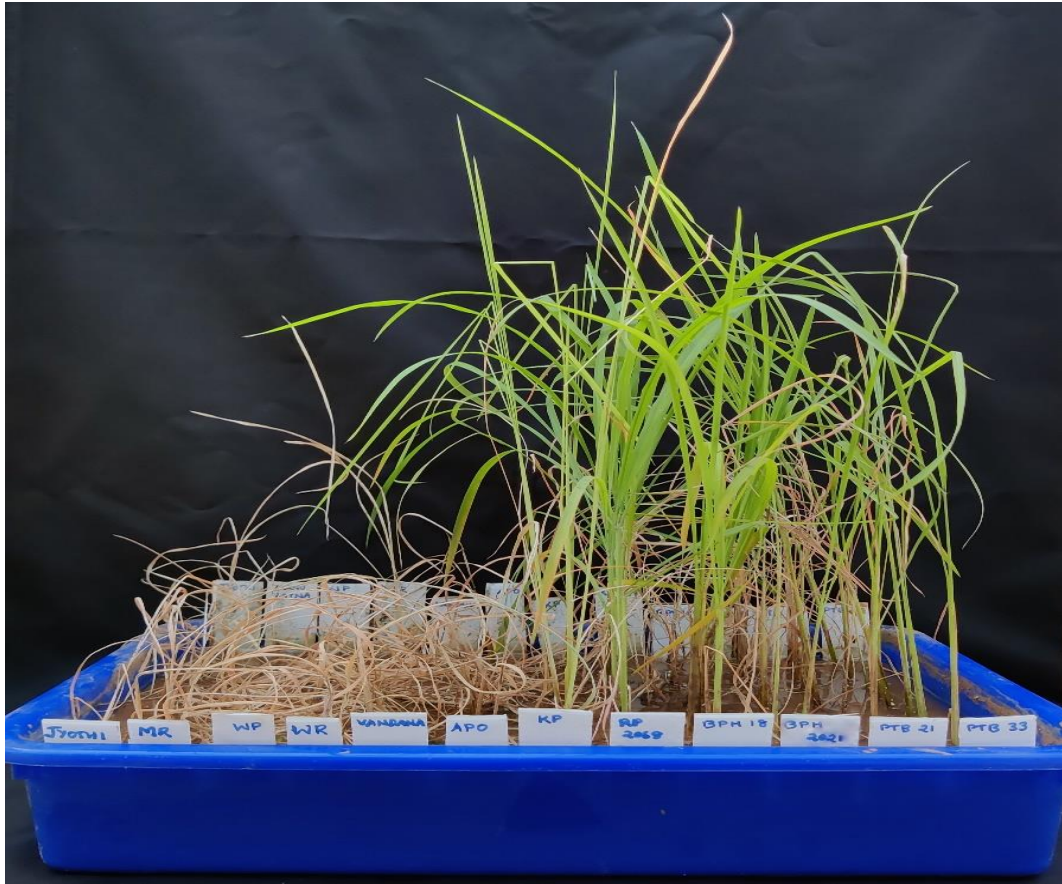


Plate 10. Response of rice genotypes in BPH screening

Table 7. BPH resistance reaction in parent genotypes

Parents	Nymphal survival	Honeydew secretion (mm²)	Functional Plant Loss Index	Tolerance Index
Jyothi	93.33 ^a	340.78 ^a (1.571)*	71.52 ^a	1.00 ^a
PTB33	13.33 ^e	0.000 ^b (0.00)	17.05 ^d	0.24 ^c
IR65482-7-216-1-2	26.67 ^d	0.253 ^b (0.027)	18.12 ^{cd}	0.38 ^{bc}
IR7103-121-15-B	31.67 ^c	0.793 ^b (0.055)	21.87 ^b	0.45 ^b
RP2068-18-3-5	33.33 ^b	0.426 ^b (0.039)	19.71 ^c	0.29 ^c
SE (d)	6.804	0.024	0.969	0.083
SE (m)	4.811	0.017	0.685	0.059
CV (%)	18.863	9.750	3.630	19.227

* Arc Sine transformed data in parenthesis

Alphabetic letters represent significant differences, the same letters represent on-par values

The highest nymphal survival rate was observed in the recipient parent 'Jyothi' followed by RP2068-18-3-5, IR7103-121-15-B, IR65482-7-216-1-2, and lowest in the variety 'PTB-33'.

The feeding activity of BPH on rice seedlings was measured and expressed as mm² of honeydew excretion spot area appearing on filter paper treated with Bromocresol green dye. The variety 'Jyothi' with an average honeydew spot area of 340.78 mm², turned out to be the highly susceptible among the rice varieties screened followed by the genotypes, IR7103-121-15-B (0.793 mm²), RP2068-18-3-5 (0.426 mm²) and IR65482-7-216-1-2 (0.253 mm²). The variety 'PTB-33' showed high resistance to BPH feeding as no honeydew spots were observed in any replicates (Plate 11).

The functional plant loss index (FPLI) was measured as the per cent loss of plant weight on drying as an after effect of BPH feeding on rice varieties. The rice variety Jyothi with the highest FPLI (71.52 per cent) is less tolerant to BPH attack followed by IR7103-121-15-B (21.87 per cent) and RP2068-18-3-5 (19.71 per cent). The FLPI of genotype IR65482-7-216-1-2 (18.12 per cent) was on par with PTB-33 possessing the lowest FPLI (17.05) and was more tolerant to BPH attack. Tolerance and antibiosis mechanisms were studied by constructing an FPLI graph with a regression line generated from the regression of FPLI on the mean value of the dry weight of BPH. The graph was divided into four quadrants by the mean dry weight of the BPH which depicted the level of tolerance and antibiosis. The variety 'Jyothi' with high FPLI and BPH dry weight coordinated in the susceptible quadrant (low tolerance and low antibiosis). The variety PTB-33 with the lowest FPLI and BPH dry weight coordinated in the high tolerance and high antibiosis quadrant along with which the remaining genotypes viz., IR7103-121-15-B, RP2068-18-3-5, and IR65482-7-216-1-2 congregated (Figure 2).

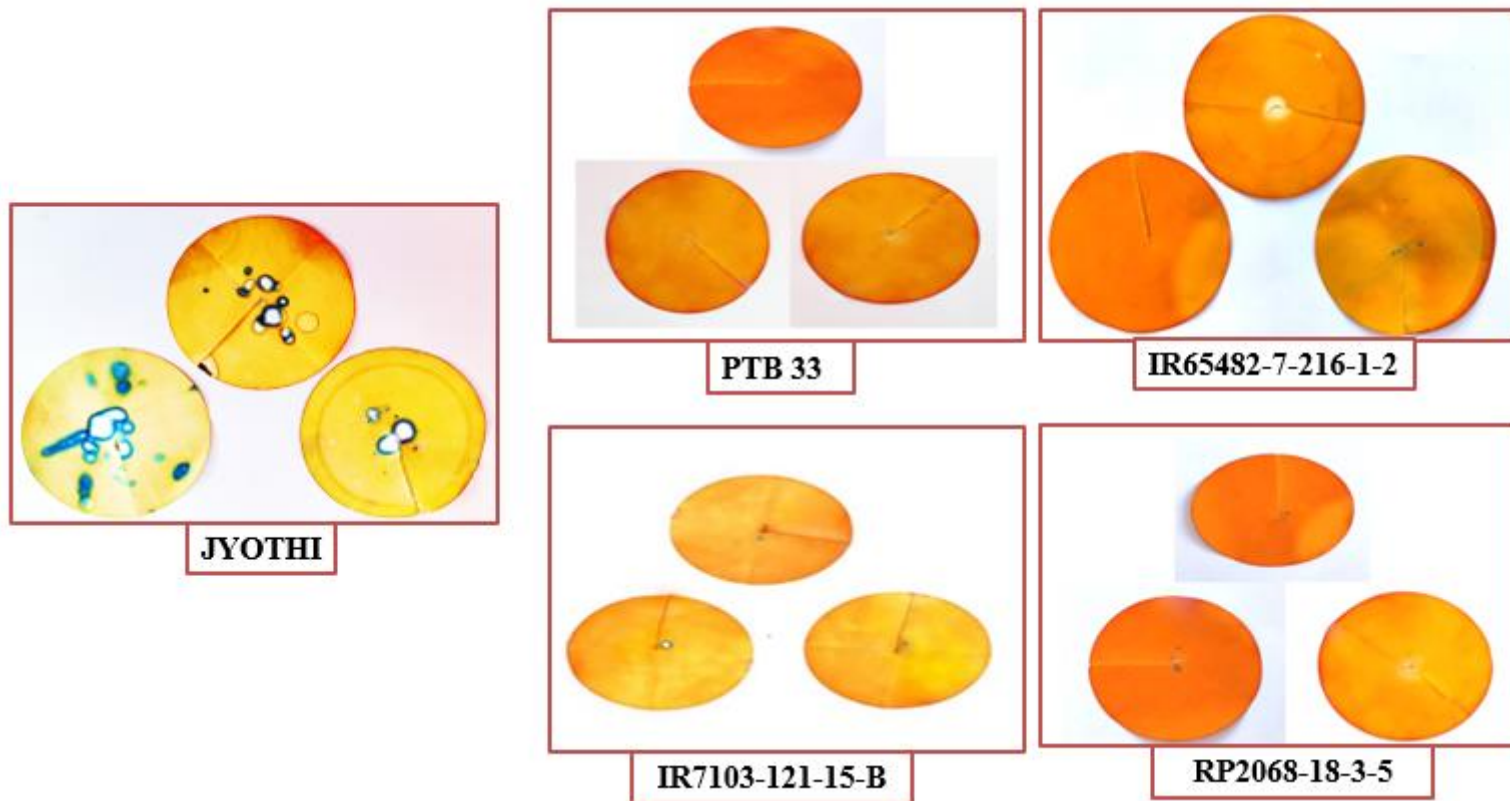


Plate 11. Honeydew test in recipient and donor parents

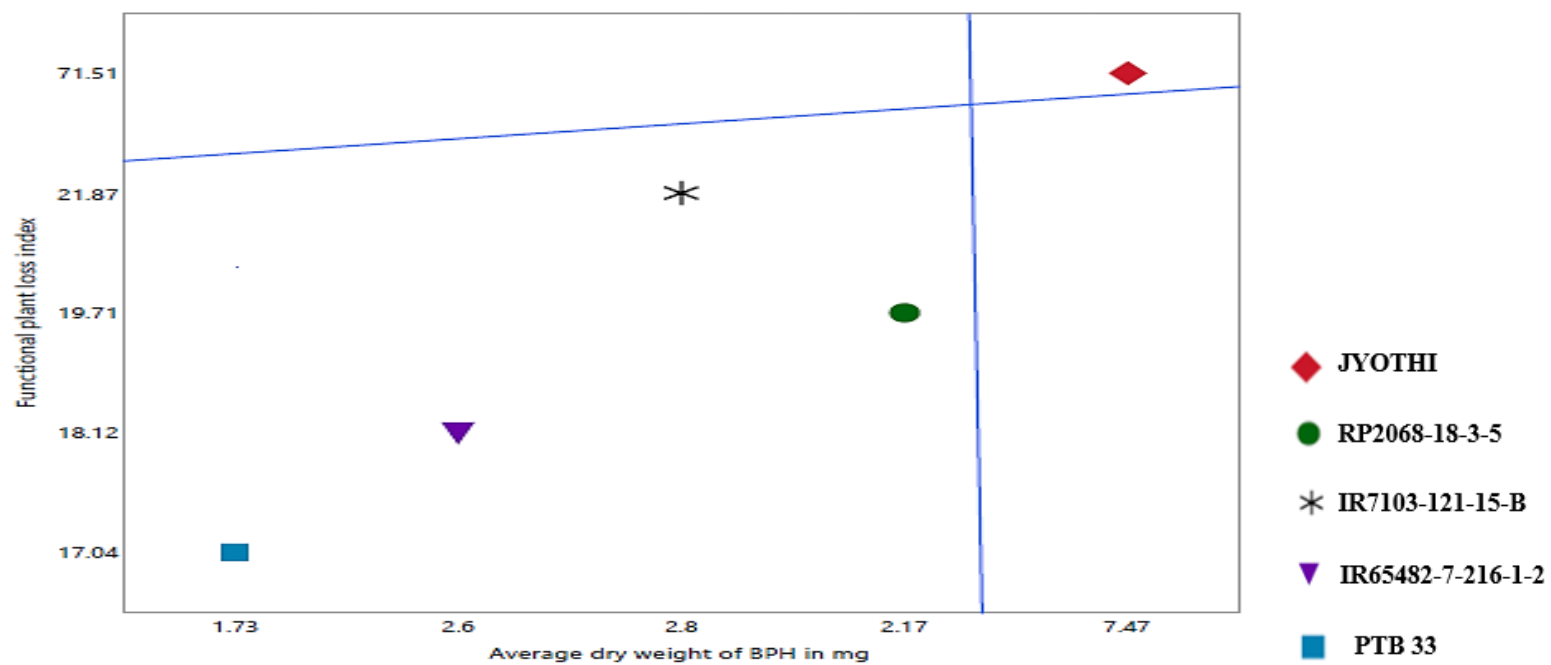


Figure 2. Functional Plant Loss Index (FPLI) in the genotypes after BPH infestation

The tolerance mechanism among rice cultivars was analyzed using the tolerance index based on the dry weight of BPH fed on each rice cultivar. The highest TI value (1) was observed in the rice variety 'Jyothi', followed by IR7103-121-15-B (0.45). The TI values of genotypes IR65482-7-216-1-2 (0.38), and RP2068-18-3-5 (0.29) were on par with that of the variety 'PTB-33' (0.24) possessing the lowest TI value.

4.2 MORPHOLOGICAL EVALUATION OF PARENTS

The morphological observations of the recipient and donor parents are given in Table 8. The results pertaining to phenotypic variance (V_p), genotypic variance (V_g), environmental variance (V_e), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability (h^2), and genetic advance as per cent of the mean (GAM) for all the fourteen characters of recipient and donor parents are furnished in Table 9.

4.2.1. Plant height (cm)

The maximum plant height was observed in PTB-33 (152 cm) and the minimum was observed in RP2068-18-3-5 (81.250 cm) which was on par with Jyothi (92.875 cm). The plant height showed high PCV (29.095%), GCV (27.406%), heritability (88.7%), and genetic advance as per cent of the mean (53.178%).

4.2.2. Number of days to 50% flowering

The genotype PTB-33 recorded a maximum of 113 days to 50% flowering and a minimum of 68 days by RP2068-18-3-5. Moderate PCV (19.491%) and GCV (18.876%) with high heritability (93.8%) and genetic advance as per cent of mean (37.658%) were observed in the character.

4.2.3. Number of productive tillers

The Jyothi produced the highest (7.50) number of productive tillers which was on par with RP2068-18-3-5 (6.250) and IR65482-7-216-1-2 (6.250). The genotypes IR7103-121-15-B and PTB-33 produced the lowest (4.00) number of productive tillers. The trait showed high PCV (38.378%) and GCV (22.952%), moderate heritability (35.8%), and high genetic advance as per cent of mean (28.276%).

Table 8. Morphological evaluation of parents

Parents	Plant height (cm)	Number of days to 50% flowering	Number of productive tillers	Culm thickness (mm)	Panicle length (cm)	Number of grains per panicle	1000 grain weight (g)	Grain length (mm)	Grain breadth (mm)	LB ratio	Kernel colour
Jyothi	92.875 ^{cd}	84.75 ^c	7.500 ^a	0.746 ^a	22.500 ^b	160.25 ^a	28.875 ^a	8.953 ^a	2.680 ^c	3.342 ^b	Red
PTB33	152.000 ^a	113.00 ^a	4.000 ^b	0.744 ^a	27.250 ^a	135.25 ^b	26.200 ^b	7.680 ^b	3.185 ^a	2.415 ^c	Red
IR65482-7-216-1-2	102.750 ^{bc}	88.00 ^c	6.250 ^{ab}	0.665 ^b	18.900 ^c	88.25 ^d	13.575 ^d	7.745 ^b	2.100 ^e	3.695 ^a	White
RP2068-18-3-5	81.250 ^d	68.00 ^d	6.250 ^{ab}	0.609 ^{bc}	21.625 ^b	130.25 ^b	21.375 ^c	7.535 ^b	2.885 ^b	3.308 ^b	White
IR7103-121-15-B	116.500 ^b	103.25 ^b	4.000 ^b	0.586 ^c	21.900 ^b	108.75 ^c	22.000 ^c	7.770 ^b	2.350 ^d	2.618 ^c	White
Maximum	152.000	113.00	7.500	0.746	27.250	160.250	28.875	8.953	3.185	3.695	
Minimum	81.250	68.00	4.000	0.586	18.900	88.250	13.575	7.535	2.100	2.415	
Mean	109.075	91.4	5.600	0.670	22.435	124.550	22.405	7.937	2.64	3.076	
SE (d)	7.604	3.138	1.218	0.031	0.674s	5.278	1.004	0.201	0.085	0.109	
SE (m)	5.377	2.219	0.861	0.022	0.476	3.732	0.71	0.142	0.06	0.077	
CV (%)	9.859	4.856	30.757	6.582	4.247	5.993	6.335	3.578	4.573	5.007	

Table 9. Genetic parameters of fourteen characters in parent genotypes

Characters	Genotypic variance	Phenotypic variance	Env variance	PCV	GCV	Heritability (%)	Genetic Advance as per the mean (%)
Plant height (cm)	852.30	960.61	108.31	29.09	27.40	88.7	53.17
Number of days to 50% flowering	297.65	317.35	19.70	19.49	18.87	93.8	37.65
Number of productive tillers	1.65	4.61	2.96	38.37	22.95	35.8	28.27
Culm thickness (mm)	0.005	0.007	0.002	12.48	10.55	71.4	18.37
Panicle length	8.93	9.83	0.90	13.98	13.32	90.8	26.14
Number of grains per panicle	559.67	581.27	21.60	19.73	19.36	96.3	39.13
1000 grain weight	33.36	35.37	2.01	26.54	25.77	94.3	51.57
Grain length	0.31	0.39	0.08	7.88	7.02	79.3	12.88
Grain breadth	0.18	0.19	0.01	16.71	16.05	92.3	31.78
LB ratio	0.28	0.31	0.02	21.87	20.85	90.9	40.96
Protein content	3.71	4.08	0.37	21.87	20.85	90.9	40.96
Phenol content	0.009	0.01	0.001	31.80	30.12	89.7	58.79
Ascorbic acid	34.61	35.90	1.28	18.22	17.89	96.4	36.19
Reducing sugar	0.19	0.23	0.03	43.98	40.49	84.7	76.78

4.2.4. Culm thickness (mm)

The culm thickness was maximum in Jyothi (0.746 mm) which was on par with PTB-33 (0.744 cm). The minimum culm thickness was observed in IR7103-121-15-B (0.586 mm), which was on par with RP2068-18-3-5 (0.609 mm). The moderate PCV (12.489%) and GCV (10.555%), high heritability (71.4%), and moderate genetic advance as per cent of mean (18.377%) were observed for the culm thickness (Plate 12a to 12e).

4.2.5. Panicle length (cm)

The maximum panicle length was observed in PTB-33 (27.250 cm) and the minimum was recorded in IR65482-7-216-1-2 (18.900 cm). Moderate PCV (13.980%) and GCV (13.320%), high heritability (90.8%), and genetic advance as per cent of mean (26.142%) were observed for the panicle length.

4.2.6. Number of grains per panicle

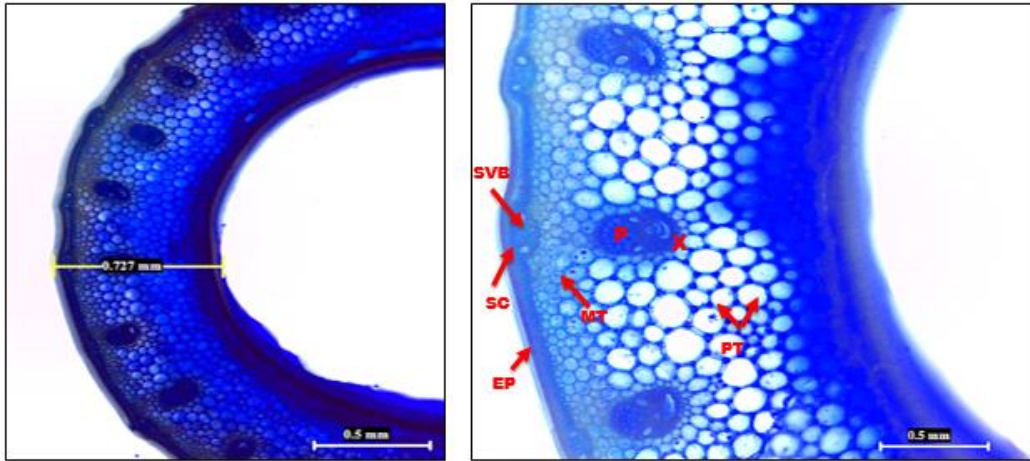
The number of grains per panicle ranged from 88.25 to 160.25. The maximum grains per panicle was observed in Jyothi (160.25) and the minimum was observed in IR65482-7-216-1-2 (88.25). The PCV (19.730%) and GCV (19.360%) were moderate, and heritability (96.3%) and genetic advance as per cent of mean (39.133%) were high for the number of grains per panicle.

4.2.7. 1000 grain weight (g)

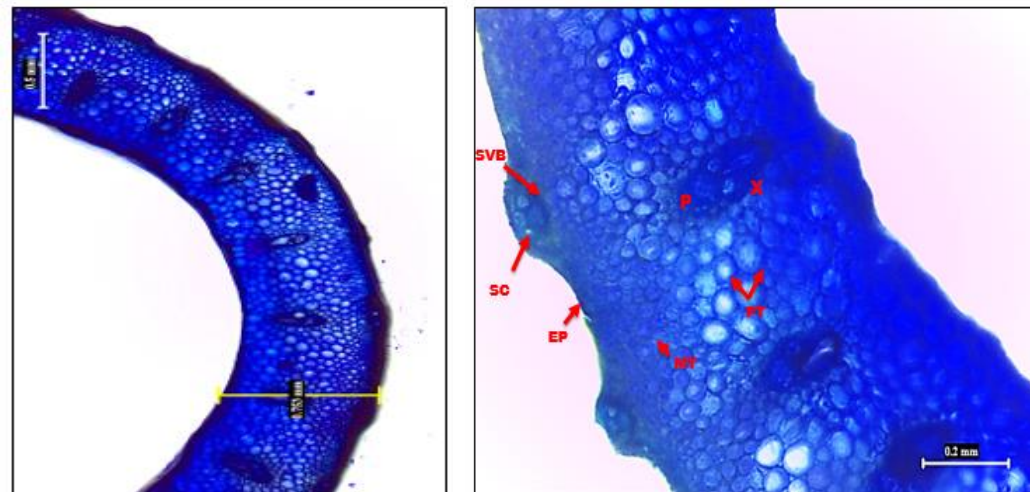
The Jyothi (28.875g) and IR65482-7-216-1-2 (13.575g) recorded the highest and lowest 1000-grain weights respectively. The high PCV (26.546%), GCV (25.779%), heritability (94.3%), and genetic advance as per cent of mean (51.571%) were observed for 1000 grain weight.

4.2.8. Grain length (mm)

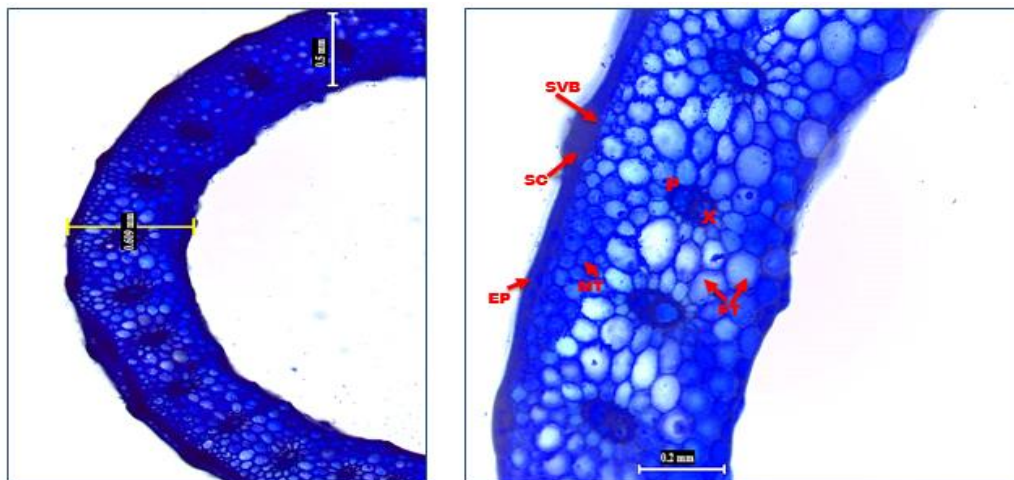
The maximum grain length was observed in Jyothi (8.953 mm) and the minimum was observed in RP2068-18-3-5 (7.535 mm), which was on par with PTB-33 (7.680 mm), IR65482-7-216-1-2 (7.745 mm), and IR7103-121-15-B (7.770 mm). The grain length showed low PCV (7.886%) and GCV (7.024%), high heritability (79.3%), and moderate genetic advance as per cent of mean (12.887%).



(a) Jyothi

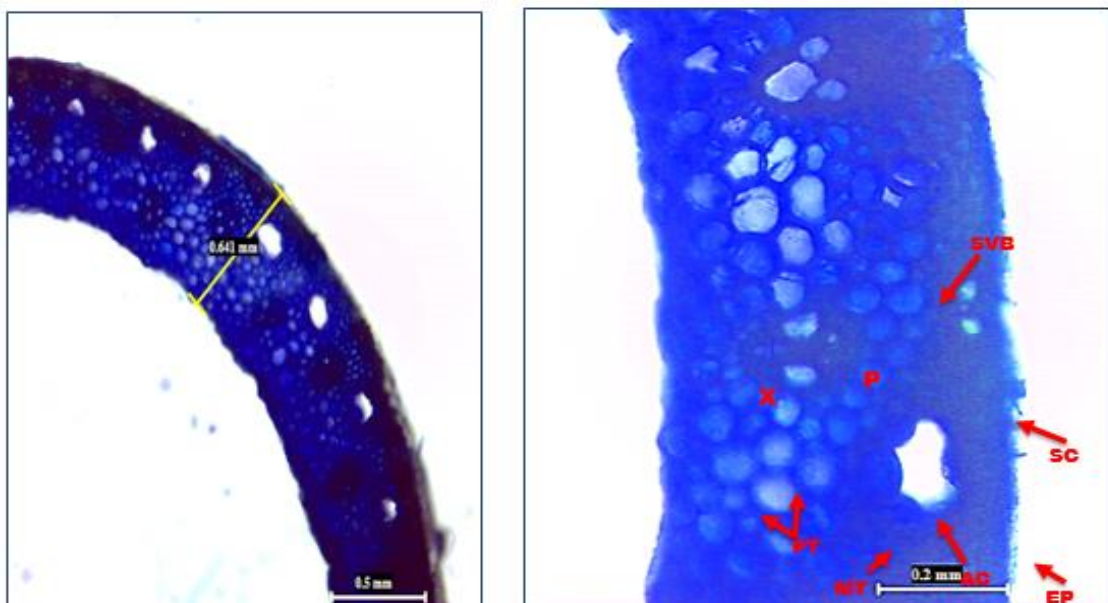


(b) PTB-33

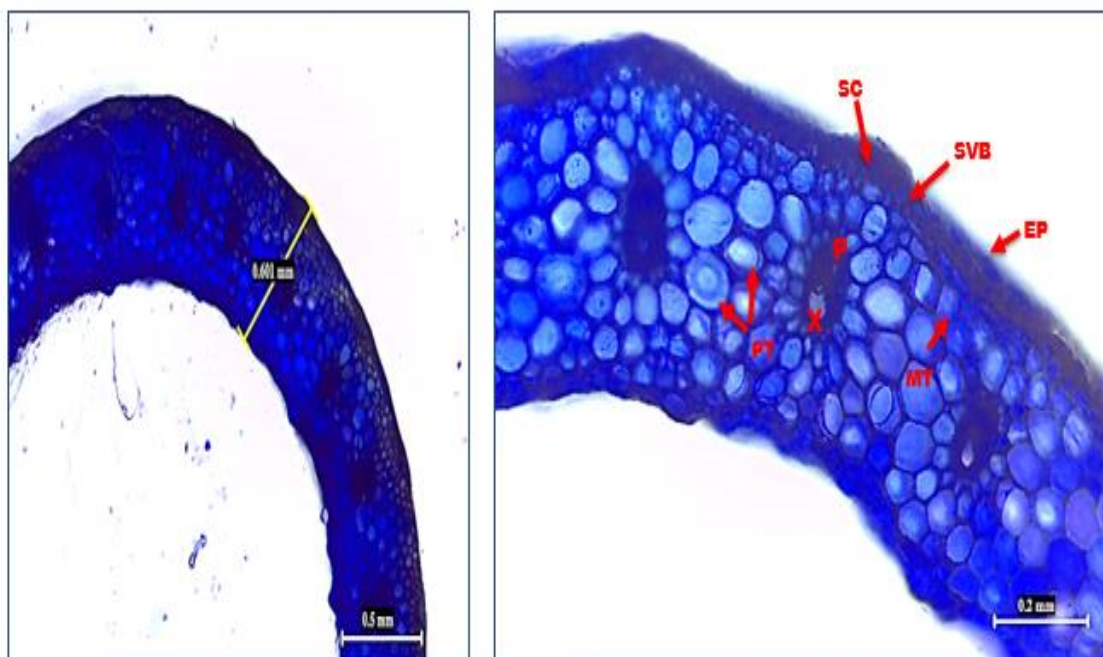


(c) IR65482-7-216-1-2

Plate 12. Culm thickness of parental genotypes



(d) IR7103-121-15-B



(e) RP2068-18-3-5

Plate 12 (contd). Culm thickness of parental genotypes

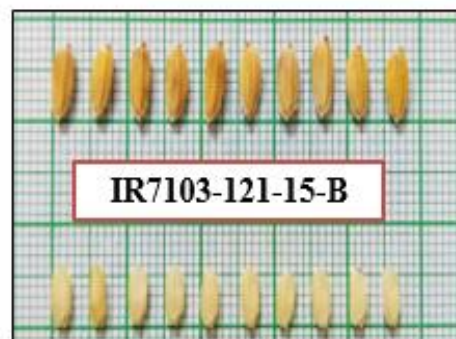
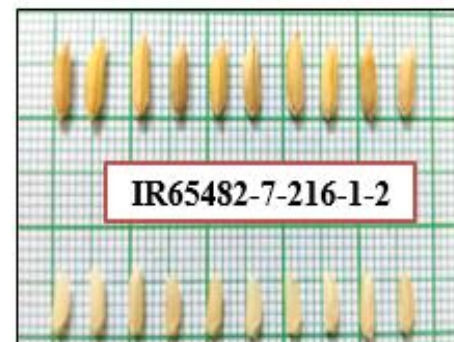
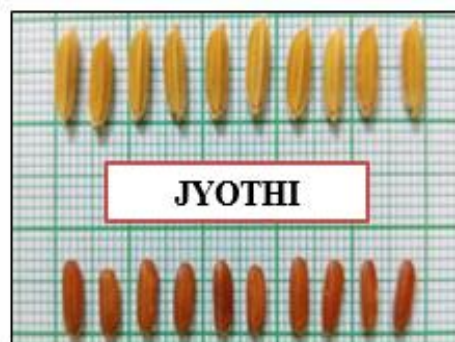
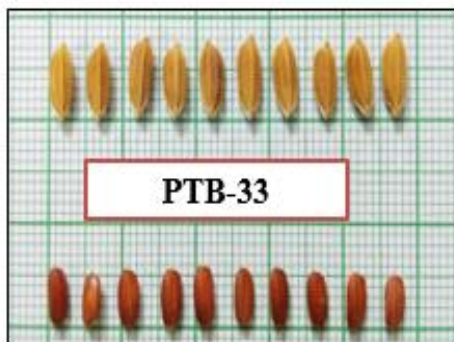


Plate 13. Kernel colour of parental genotypes

4.2.9. Grain breadth (mm)

The grain breadth was maximum in PTB-33 (3.185 mm) and minimum in IR65482-7-216-1-2 (2.100 mm). Moderate PCV (16.716%), GCV (16.059%), high heritability (92.3%), and genetic advance as per cent of mean (31.789%) were recorded for grain breadth.

4.2.10. LB Ratio

The LB ratio was maximum in IR65482-7-216-1-2 (3.695) and minimum in PTB-33 (2.415), which was on par with IR7103-121-15-B (2.618). The LB ratio showed high PCV (21.874%), GCV (20.857%), heritability (90.9%), and genetic advance as per cent of mean (40.969%).

4.2.11. Kernel colour

The genotypes Jyothi and PTB-33 had red-coloured kernels whereas, IR65482-7-216-1-2, RP2068-18-3-5, and IR7103-121-15-B had white-coloured kernels (Plate 13).

4.2.12. Biochemical parameters

4.2.12.1. Protein content (g/100g)

The protein content showed high PCV (21.874%), GCV (20.857%), heritability (90.9%), and genetic advance as per cent of mean (40.969%).

4.2.12.2. Phenol content (mg/100g)

The phenol content showed high PCV (31.803%), GCV (30.128%), heritability (89.7%) and genetic advance as per cent of mean (58.794%).

4.2.12.3. Ascorbic acid (mg/100g)

The ascorbic acid content recorded moderate PCV (18.224%), GCV (17.894%), high heritability (96.4%), and high genetic advance as per cent of mean (36.195%).

4.2.12.4. Reducing sugar (g/100g)

The reducing sugar recorded high PCV (43.989%), GCV (40.492%), heritability (84.7%), and genetic advance as per cent of mean (76.783%).

4.3 BIOCHEMICAL PARAMETERS IN PARENT GENOTYPES BEFORE AND AFTER BPH INFESTATION

The biochemical parameters of recipient and donor parents before and after BPH infestation are enlisted in Table 10.

4.3.1. Protein content (g/100g)

The protein content in healthy plants of Jyothi was 11.66 g/100g and reduced to 9.69 g/100g after BPH infestation with a 16.89 per cent reduction. The protein content in healthy plants of PTB-33 was 8.52 g/100g, which was reduced to 8.14 g/100g after BPH infestation with a 4.46 per cent reduction. The protein content in IR65482-7-216-1-2 was reduced to 7.07 g/100g from 7.27 g/100g after BPH infestation, with a 2.75 per cent reduction. The genotype IR7103-121-15-B had 6.76 g/100g protein and was reduced to 6.59 g/100g after BPH infestation with a 2.51 per cent reduction. A 2.11 per cent reduction of protein content in RP2068-18-3-5 was observed after BPH infestation from 9.50 g/100g to 9.30 g/100g.

4.3.2. Phenol content (mg/100g)

The phenol content in Jyothi was reduced to 0.18 mg/100g from 0.22 mg/100g after BPH infestation (18.18% reduction). In the genotype, PTB-33 the phenol content after BPH infestation was increased from 0.46 mg/100g to 0.65 mg/100g with an increase of 41.30 per cent. The phenol content was increased to 36.11, 31.03, and 33.33 per cent in IR65482-7-216-1-2 (from 0.36 to 0.49 mg/100g), IR7103-121-15-B (from 0.29 to 0.38 mg/100g) and RP2068-18-3-5 (from 0.27 to 0.36 mg/100g) respectively after BPH infestation.

4.3.3. Ascorbic acid (mg/100g)

The ascorbic acid in Jyothi was reduced from 24.09 mg/100 g to 11.59 mg/100 g after BPH infestation (51.88 per cent reduction). A 17.13 per cent of ascorbic acid was reduced in PTB-33 due to BPH infestation, from 39.15 mg/100 g to 32.44 mg/100 g. The genotype IR65482-7-216-1-2 had 36.14 mg/100 g ascorbic acid in healthy plants, which was reduced to 24.85 mg/100 g with a 31.23 per cent reduction after BPH infestation. The ascorbic acid content in IR7103-121-15-B and RP2068-18-3-5 was reduced from 36.14 to 27.23 mg/100 g and from 30.12 to 19.04 mg/100 g with 24.65 per cent and 36.78 per cent respectively.

Table 10. The biochemical parameters of recipient and donor parents before and after BPH infestation

Rice genotypes	Protein content (g/100g)			Phenol content (mg/100g)			Ascorbic acid (mg/100g)			Reducing sugar (g/100g)		
	Healthy plants	Infested plants	% increase	Healthy plants	Infested plants	% increase	Healthy plants	Infested plants	% increase	Healthy plants	Infested plants	% increase
Jyothi	11.66 ^a	9.69 ^a	-16.89	0.22 ^c	0.18 ^c	-18.18	24.09 ^d	11.59 ^c	-51.88	0.048 ^c	0.034 ^c	-29.16
PTB33	8.52 ^c	8.14 ^c	-4.46	0.46 ^a	0.65 ^a	41.30	39.15 ^a	32.44 ^a	-17.13	0.094 ^c	0.058 ^c	-38.29
IR65482-7-216-1-2	7.27 ^d	7.07 ^d	-2.75	0.36 ^b	0.49 ^b	36.11	36.14 ^b	24.85 ^c	-31.23	0.111 ^b	0.050 ^d	-54.95
IR7103-121-15-B	6.76 ^e	6.59 ^e	-2.51	0.29 ^c	0.38 ^c	31.03	36.14 ^b	27.23 ^b	-24.65	0.077 ^d	0.061 ^b	-20.77
RP2068-18-3-5	9.50 ^b	9.30 ^b	-2.11	0.27 ^d	0.36 ^d	33.33	30.12 ^c	19.04 ^d	-36.78	0.186 ^a	0.155 ^a	-16.66
Maximum	11.66	9.69		0.46	0.65		39.15	32.44		0.186	0.155	
Minimum	6.76	6.59		0.22	0.18		24.09	11.59		0.048	0.034	
Mean	8.74	8.15		0.32	0.41		33.12	23.03		0.103	0.070	
SE (d)	0.137	0.106		0.004	0.004		0.424	0.368		0.002	0.001	
SE (m)	0.097	0.075		0.003	0.003		0.299	0.260		0.001	0.001	
CV (%)	2.214	1.843		1.896	1.323		1.808	2.258		2.141	1.642	

4.3.4. Reducing sugar (g/100g)

The reducing sugar in Jyothi was 0.048 g/100 g in healthy plants, which was reduced to 0.034 g/100 g after BPH infestation with a 29.16 per cent reduction. The genotype, PTB-33 had 0.094 g/100 g reducing sugar in healthy plants and reduced to 0.058 g/100 g after BPH infestation (38.29 per cent reduction). The reducing sugar in IR65482-7-216-1-2, IR7103-121-15-B, and RP2068-18-3-5 was reduced from 0.111 to 0.050 g/100 g, 0.077 to 0.061 g/100 g and 0.186 to 0.155 g/100 g with 54.95, 20.77 and 16.66 per cent respectively after BPH infestation.

4.3.5. Correlation between biochemical components after infestation and phenotypic reactions to BPH

The genotypic correlation between biochemical parameters after infestation and phenotypic reactions to BPH is given in Table 11.

The protein content in plants showed a significant and positive correlation with nymphal survival (0.653), excretion of honeydew (0.670), functional plant loss index (0.629), tolerance index (0.475), BPH dry weight (0.559) and damage score (0.643).

The phenol content in plants recorded a significant negative correlation with nymphal survival (-0.925), excretion of honeydew (-0.838), functional plant loss index (-0.843), tolerance index (-0.898), BPH dry weight (-0.882) and damage score (-0.862).

The ascorbic acid content in plants was significantly negatively correlated with nymphal survival (-0.904), excretion of honeydew (-0.833), functional plant loss index (-0.814), tolerance index (-0.793), BPH dry weight (-0.825) and damage score (-0.849).

The reducing sugar showed a significant negative correlation with the tolerance index (-0.524) and a negative correlation with nymphal survival (-0.314), excretion of honeydew (-0.467), functional plant loss index (-0.432), BPH dry weight (-0.481) and damage score (-0.412).

Table 11. The genotypic correlation between biochemical parameters after infestation and phenotypic reactions to BPH

Characters	Nymphal Survival	Excretion of honeydew	Functional Plant loss index	Tolerance index	BPH dry weight	Damage score
Protein content	0.653*	0.67*	0.629*	0.475	0.559*	0.634*
Phenol content	-0.925**	-0.838**	-0.843**	-0.898**	-0.882**	-0.862**
Ascorbic acid	-0.904**	-0.833**	-0.814**	-0.793**	-0.825**	-0.849**
Reducing sugar	-0.314	-0.467	-0.432	-0.524*	-0.481	-0.412

** Significant at 1%, * Significant at 5%

4.3.6. The genotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents

The genotypic correlation among the fourteen characters in donor and recipient parents are presented in Table 12. Only significant correlations are discussed below.

The functional plant loss index showed a positive and significant correlation with culm thickness (0.548), 1000 grain weight (0.620), grain length (0.909) and protein content (0.827) and was significantly negatively correlated with plant height (-0.562), phenol content (-0.513), ascorbic acid (-0.824), and reducing sugar (-0.595).

The plant height was significantly positively correlated with the number of days to 50% flowering (0.936), panicle length (0.715), phenol content (0.870), and ascorbic acid (0.548), and significantly negatively correlated with the number of productive tillers (-0.791), and LB ratio (-0.806).

A significant positive correlation was observed by the number of days to 50% flowering with panicle length (0.628), and phenol content (0.647), and a significant negative correlation with the number of productive tillers (-0.938) and LB ratio (-0.794).

The number of productive tillers showed a significant negative correlation with panicle length (-0.749), 1000 grain weight (-0.538), and reducing sugar (-0.641).

The culm thickness recorded a significant positive correlation with panicle length (0.564), number of grains per panicle (0.572), 1000 grain weight (0.571), grain length (0.637), and phenol content (0.520) and significantly negatively correlated with reducing sugar (-0.703).

A significant positive correlation was observed between panicle length and the number of grains per panicle (0.874), 1000 grain weight (0.738), grain breadth (0.887), and phenol content (0.570). A significant negative correlation was recorded between panicle length and LB ratio (-0.834).

The number of grains per panicle recorded a significant and positive correlation with 1000 grain weight (0.876), grain breadth (0.973), and significant negative correlation with reducing sugar (-0.518).

Table 12. The genotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents

	Plant Height	No. of days to 50% flowering	No. of productive tillers	Culm thickness	Panicle length	No. of grains per panicle	1000 grain weight	Grain length	Grain breadth	LB ratio	Protein content	Phenol content	Ascorbic acid	Reducing sugar	Functional plant loss index
Plant Height	1														
Number of days to 50% flowering	0.936**	1													
Number of productive tillers	-0.791**	-0.938**	1												
Culm thickness	0.263	0.333	0.021	1											
Panicle length	0.715*	0.628*	-0.749*	0.564*	1										
Number of grains per panicle	0.231	0.152	-0.403	0.572*	0.874**	1									
1000 grain weight	0.082	0.226	-0.538*	0.571*	0.738*	0.876**	1								
Grain length	-0.459	-0.124	0.063	0.637*	-0.003	0.227	0.616*	1							
Grain breadth	0.345	0.143	-0.36	0.448	0.887**	0.973**	0.716*	-0.052	1						
LB ratio	-0.806**	-0.794**	0.035	-0.034	-0.834**	-0.2	-0.517*	0.246	-0.538*	1					
Protein content	-0.108	0.284	-0.394	0.498	0.23	0.305	0.728*	0.951**	0.027	0.276	1				
Phenol content	0.87**	0.647*	-0.236	0.52*	0.57*	0.188	-0.117	-0.442	0.343	-0.385	-0.338	1			
Ascorbic acid	0.548*	0.146	0.101	-0.076	0.254	0.05	-0.43	-0.873**	0.322	-0.544*	-0.872**	0.756**	1		
Reducing sugar	0.411	0.485	-0.641*	-0.703*	-0.159	-0.518*	-0.389	-0.504*	-0.475	0.249	-0.139	-0.005	0.051	1	
Functional plant loss index	-0.562*	-0.275	0.193	0.548*	-0.02	0.305	0.62*	0.909	0.051	0.303	0.827**	-0.513*	-0.824**	-0.595*	1

** Significant at 1%, * Significant at 5

A significant and positive correlation was observed between 1000 grain weight and grain length (0.616), grain breadth (0.716), and protein content (0.728) and it was negatively correlated with the LB ratio (-0.517).

The grain length was positively and significantly correlated with protein content (0.951) and negatively correlated with ascorbic acid (-0.873) and reducing sugar (-0.504). The grain breadth was significantly negatively correlated with the LB ratio (-0.538). The LB ratio was significantly negatively correlated with ascorbic acid (-0.544).

A significant positive correlation was observed between phenol content (0.756) and ascorbic acid and a significant negative correlation between ascorbic acid and protein content (-0.872).

4.3.7. The phenotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents

The phenotypic correlation among the fourteen characters in donor and recipient parents are presented in Table 13. Only significant correlations are discussed below. The functional plant loss index was significantly positively correlated with 1000 grain weight (0.606), grain length (0.894), and protein content (0.793), and significantly negatively correlated with plant height (-0.525), ascorbic acid (-0.812) and reducing sugar (-0.595).

A significant positive correlation was recorded between plant height and the number of days to 50% flowering (0.844), panicle length (0.602), and phenol content (0.750) and a significant negative correlation was observed between plant height and the number of productive tillers (-0.513) and LB ratio (-0.719).

The number of days to 50% flowering was significantly positively correlated with panicle length (0.557), and phenol content (0.611), and significantly negatively correlated with the number of productive tillers (-0.574) and LB ratio (-0.727). The number of productive tillers was significantly positively correlated with the LB ratio (0.612) and negatively correlated with (-0.504).

The culm thickness was significantly negatively correlated with reducing sugar (-0.585). A significant positive correlation was observed between panicle length and the number of grains per panicle (0.832), 1000 grain weight (0.683), and

Table 13. The phenotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents

	Plant Height	No. of days to 50%	No. of productive tillers	Culm thickness	Panicle length	No. of grains per panicle	1000 grain weight	Grain length	Grain breadth	LB ratio	Protein content	Phenol content	Ascorbic acid	Reducing sugar	Functional plant loss index
Plant Height	1														
Number of days to 50% flowering	0.844**	1													
Number of productive tillers	-0.513*	-0.574*	1												
Culm thickness	0.168	0.214	0.012	1											
Panicle length	0.602*	0.557*	-0.504*	0.437	1										
Number of grains per panicle	0.22	0.147	-0.279	0.434	0.832**	1									
1000 grain weight	0.084	0.194	-0.413	0.432	0.683*	0.851**	1								
Grain length	-0.353	-0.059	0.112	0.436	-0.033	0.201	0.489	1							
Grain breadth	0.282	0.133	-0.195	0.408	0.811**	0.920**	0.648*	-0.033	1						
LB ratio	-0.719*	-0.727*	0.612*	-0.074	-0.761**	-0.183	-0.467	0.247	-0.532*	1					
Protein content	-0.091	0.216	-0.388	0.399	0.244	0.294	0.703*	0.716*	0.023	0.222	1				
Phenol content	0.750*	0.611*	-0.128	0.365	0.476	0.184	-0.089	-0.342	0.345	-0.357	-0.328	1			
Ascorbic acid	0.479	0.142	0.071	-0.059	0.239	0.041	-0.417	-0.769**	0.324	-0.528*	-0.807**	0.716*	1		
Reducing sugar	0.388	0.466	-0.431	-0.585*	-0.15	-0.511*	-0.379	-0.453	-0.458	0.238	-0.133	-0.009	0.046	1	
Functional plant loss index	-0.525*	-0.27	0.123	0.461	-0.018	0.300	0.606*	0.894**	0.047	0.288	0.793**	-0.488	-0.812**	-0.595*	1

** Significant at 1%, * Significant at 5%

grain length (0.811), and a significant negative correlation with the LB ratio (-0.761).

The number of grains per panicle was significantly positively correlated with 1000 grain weight (0.851), and grain breadth (0.920) and significantly negatively correlated with reducing sugar (-0.511). The 1000-grain weight was significantly positively correlated with grain breadth (0.648) and protein content (0.703). A significant positive and negative correlation was observed between grain length and protein content (0.703) and ascorbic acid (-0.769) respectively.

A significant negative correlation was observed between grain breadth and the LB ratio (-0.532), protein content, and ascorbic acid (-0.807). A significant positive correlation was recorded between phenol content and ascorbic acid (0.716).

4.3.8. The direct and indirect effects of morphological and biochemical characters in donor and recipient parents

Path coefficient analysis splits the genotypic correlation coefficient into direct and indirect effects. The direct and indirect effects of fourteen characters of recipient and donor parents on functional plant loss index are presented in Table 14.

Plant height showed a negative direct effect (-0.101) on the functional plant loss index. A positive indirect effect of plant height through the number of productive tillers (0.113) was observed. A negative direct effect (-0.694) of the number of days to 50% flowering was recorded on the functional plant loss index. A positive indirect effect of the number of days to 50% flowering through the number of productive tillers (0.724), LB ratio (0.553), protein content (0.306), and reducing sugar (0.407), and a negative indirect effect through plant height (-0.649), panicle length (-0.439), phenol content (-0.447) and ascorbic acid (-0.508) was recorded.

A negative direct effect (-0.292) of the culm thickness was recorded on the functional plant loss index. A negative indirect effect of culm thickness through the number of productive tillers (-0.103), panicle length (-0.163), number of grains per panicle (-0.181), 1000 grain weight (-0.166), grain length (-0.190) and protein content (-0.145) was recorded.

Table 14. The direct and indirect effects of morphological and biochemical characters in donor and recipient parents

Characters	Plant Height	No. of days to 50% flowering	Number of productive tillers	Culm thickness	Panicle length	Number of grains per	1000 grain weight	Grain length	Grain breadth	LB ratio	Protein content	Phenol content	Ascorbic acid	Reducing sugar	Genotypic correlation with the main variable
Plant Height	-0.101	-0.649	-0.014	-0.079	0.021	-0.052	-0.031	0.159	0.216	-0.073	0.121	0.363	-0.801	0.357	-0.562*
Number of days to 50% flowering	-0.094	-0.694	-0.013	-0.098	0.018	-0.026	-0.084	0.046	0.089	-0.072	0.099	0.273	-0.66	0.939	-0.275
Number of productive tillers	0.113	0.724	0.012	-0.103	-0.018	0.124	-0.024	-0.245	-0.19	0.099	-0.23	-0.276	0.986	-0.062	0.193
Culm thickness	-0.027	-0.232	0.004	-0.292	0.016	0.161	-0.212	-0.225	0.283	-0.003	-0.139	0.211	0.147	0.856	0.548*
Panicle length	-0.072	-0.439	-0.008	-0.163	0.029	0.147	-0.276	0.001	0.557	-0.076	-0.044	0.245	-0.254	0.331	-0.02
Number of grains per panicle	0.02	0.069	0.006	-0.181	0.016	0.26	-0.349	-0.239	0.452	-0.019	-0.205	-0.084	0.58	0.417	0.305
1000 grain weight	-0.008	-0.156	0.001	-0.166	0.021	0.244	-0.373	-0.217	0.45	-0.047	-0.156	-0.045	0.359	0.713	0.62*
Grain length	0.046	0.092	0.009	-0.19	0.002	0.179	-0.233	-0.347	-0.033	0.022	-0.184	-0.193	0.679	1.163	0.909
Grain breadth	-0.035	-0.099	-0.004	-0.131	0.026	0.187	-0.266	0.018	0.629	-0.049	-0.107	0.149	0.033	-0.299	0.051
LB ratio	0.081	0.553	0.014	0.01	-0.024	-0.053	0.192	-0.086	-0.339	0.091	-0.063	-0.164	0.491	-0.399	0.303
Protein content	0.054	0.306	0.013	-0.179	0.006	0.236	-0.257	-0.282	0.299	0.025	-0.226	-0.143	0.786	0.224	0.827**
Phenol content	-0.086	-0.447	-0.008	-0.145	0.017	-0.052	0.039	0.158	0.221	-0.035	0.076	0.424	-0.683	0.009	-0.513*
Ascorbic acid	-0.089	-0.508	-0.014	0.048	0.008	-0.167	0.149	0.261	-0.023	-0.049	0.197	0.321	-0.902	-0.081	-0.824**
Reducing sugar	0.022	0.407	0.001	0.156	-0.006	-0.068	0.166	0.252	0.117	0.023	0.032	-0.002	-0.046	-1.601	-0.595*

R = 0.048, Y: Functional Plant Loss Index

A positive direct effect of the number of grains per panicle (0.260) and grain breadth (0.626) on the functional plant loss index was observed. An indirect effect of the number of grains per panicle through culm thickness (0.161), 1000 grain weight (0.244), protein content (0.236), and ascorbic acid (-0.167), and an indirect effect of grain breadth through plant height (0.216), culm thickness (0.283), panicle length (0.557), number of grains per panicle (0.452), 1000 grain weight (0.450), protein content (0.299), phenol content (0.221), and LB ratio (-0.339) were recorded. A negative direct effect of 1000 grain weight (-0.373) and grain length (-0.347) on the functional plant loss index was recorded. A negative indirect effect of 1000 grain weight through culm thickness (-0.212), panicle length (-0.276), number of grains per panicle (-0.349), grain length (-0.233), grain breadth (-0.266), and protein content (-0.257) were observed. A positive indirect effect of grain length through ascorbic acid (0.261) and reducing sugar (0.252) and a negative indirect effect through the number of productive tillers (-0.245), culm thickness (-0.225), number of grains per panicle (-0.239), 1000 grain weight (-0.217), and protein content (-0.282) were observed.

A positive direct effect of phenol content (0.424) on the functional plant loss index was recorded. A positive indirect effect of phenol content through plant height (0.363), number of days to 50% flowering (0.273), culm thickness (0.211), panicle length (0.245), and ascorbic acid (0.321), and a negative indirect effect through the number of productive tillers (-0.276), grain length (-0.193), LB ratio (-0.163) and protein content (-0.143) were also observed.

A negative direct effect of protein content (-0.226), ascorbic acid (-0.902), and reducing sugar (-1.601) on the functional loss index was observed. The number of grains per panicle (-0.205), and grain length (-0.184) showed a negative indirect effect and ascorbic acid (0.197) showed a positive indirect effect on the functional plant loss index.

A positive indirect effect of ascorbic acid through number of productive tillers (0.986), number of grains per panicle (0.580), grain length (0.679), 1000-grain weight (0.359), LB ratio (0.491), and protein content (0.786) and a negative

indirect effect through plant height (-0.801), number of days to 50% flowering (-0.660), panicle length (-0.254) and phenol content (-0.683) were recorded.

A positive indirect effect of reducing sugar through plant height (0.357), number of days to 50% flowering (0.939), culm thickness (0.856), panicle length (0.331), number of grains per panicle (0.417), 1000-grain weight (0.713), grain length (1.163) and protein content (0.224), and a negative indirect effect through grain breadth (-0.299) and LB ratio (-0.399) were observed.

The overall residual effect of path coefficient analysis was 0.048.

4.4 VALIDATION OF GENES FOR BPH RESISTANCE IN THE DONOR AND RECIPIENT PARENTS USING SPECIFIC MOLECULAR MARKERS.

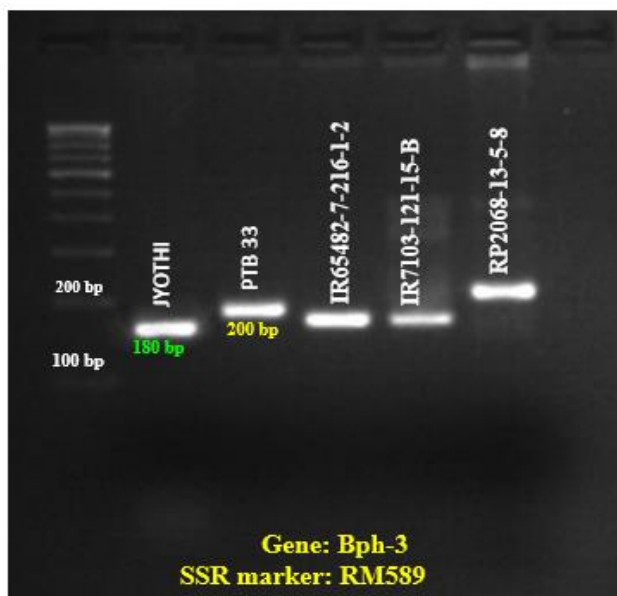
The validation of the presence or absence of genes for BPH resistance in donor and recipient parents was done using SSR markers RM589 for the *Bph-3* gene, RM3331 for *Bph-18*, RM8213 for *Bph-20*, and RM28561 for *Bph-21* (Plate 14).

4.4.1. Polymerase chain reaction

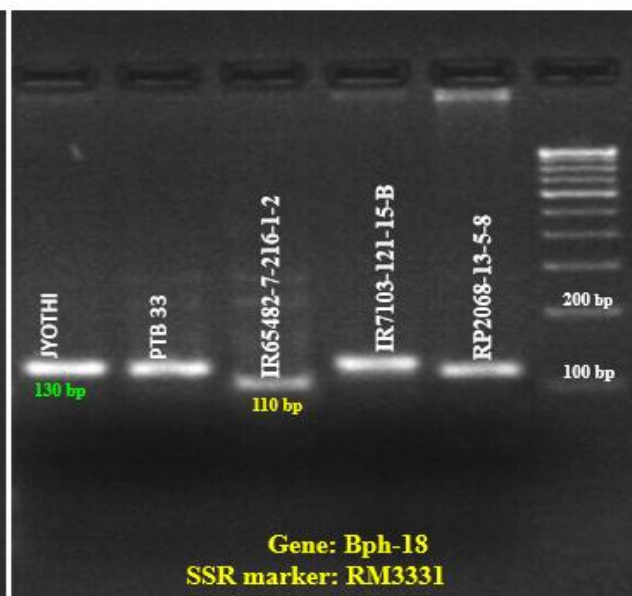
The genomic DNA of five rice varieties was amplified using four SSR markers. All the markers were amplified at an annealing temperature of 55.3°C. The amplification profile of the SSR marker 'RM589' specific to the *Bph-3* gene produced a band at 200 bp in the donor parent PTB-33 which is absent in the recipient parent Jyothi. The susceptible parent Jyothi produced a band at 180 bp. Other genotypes produced bands at 195 bp in IR65482-7-216-1-2, 190 bp in IR7103-121-15-B, and 220 bp in RP2068-18-3-5.

The SSR marker 'RM3331' specific to the *Bph-18* gene produced a band at 110 bp in the donor parent IR65482-7-216-1-2, while the susceptible parent Jyothi produced a band at 130 bp. Other genotypes produced bands at 125 bp in PTB-33, 150 bp in IR7103-121-15-B, and 120 bp in RP2068-18-3-5.

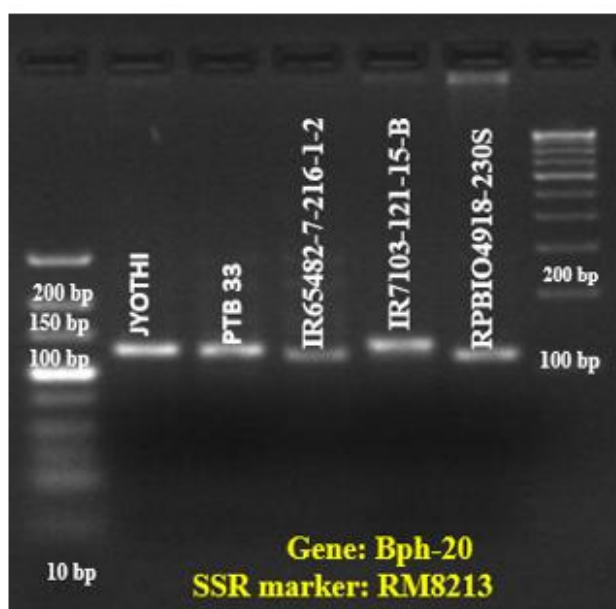
The amplification profile of the SSR marker 'RM8213' specific to the *Bph-20* gene generated the product at 140 bp in the donor parent IR7103-121-15-B and at 130 bp in the recipient parent Jyothi. In other genotypes, the marker was amplified at 120 bp in PTB-33, 110 bp in IR65482-7-216-1-2, and 110 bp in RP2068-18-3-5.



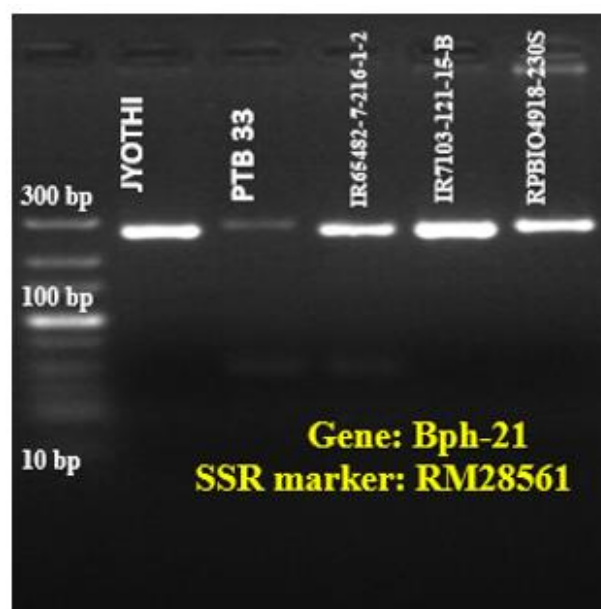
(a) RM589



(b) RM3331



(c) RM8213



(d) RM28561

Plate 14. Amplification profile of SSR markers specific to genes for BPH resistance

The SSR marker 'RM28561' specific to the gene *Bph-21* produced amplicon at 280 bp in the donor parent IR7103-121-15-B, IR65482-7-216-1-2, RP2068-18-3-5, and recipient parent Jyothi. A product of 290 bp size was obtained in the variety PTB-33.

Distinguishable polymorphic bands were obtained in the amplification of SSR markers RM589 specific to the gene *Bph-3* (200 bp for resistance and 180 bp for susceptibility), RM3331 specific to the gene *Bph-18* (110 bp for resistance and 130 bp for susceptibility), and RM8213 specific to *Bph-20* gene (140 bp for resistance and 130 bp for susceptibility).

The gene validation for *Bph-3*, *Bph-18*, and *Bph-20* genes confirmed the presence of these genes for BPH resistance in respective donor parents viz., PTB-33, IR65482-7-216-1-2, and IR7103-121-15-B and the absence of these genes in the recipient parent Jyothi.

4.5. MARKER-ASSISTED BACKCROSS BREEDING PROGRAM

Based on BPH screening, resistant reaction study, and gene validation, the recipient parent, 'Jyothi' turned out to be highly susceptible, and the KAU released variety 'PTB-33' and IRRI introgression line IR65482-7-216-1-2 which showed high resistance to BPH were selected as the donors for *Bph-3* and *Bph-18* gene respectively in the marker-assisted breeding program.

4.5.1. Raising of parents for hybridization program

Staggered sowing of the recipient and donor parents was undertaken to obtain synchronized flowering. The donor parent PTB-33 with long crop duration (150 days) and photosensitivity, was sown one month prior to the other donor parent IR65482-7-216-1-2 and the recipient parent Jyothi. Two to three sowings in 7-15 days intervals were taken up for synchronized flowering. Twenty-one days old seedlings were transplanted to the main field with a plot size of 1.5 m² in 30 x 30 cm spacing and in pots maintained in the insect-proof net house. Plants at the boot leaf initiation stage were transferred to the net house for pollination. Clipping and vacuum suction method of emasculation was followed in the crossing program.

4.5.2. Development of F₁ and BC₁F₁

The donors PTB-33 and IR65482-7-216-1-2 were crossed independently with the recipient parent Jyothi to obtain the F₁ generation followed by backcrossing with Jyothi to develop the BC₁F₁ generation. Details of crossing are enlisted in Table 15.

In the cross, Jyothi x PTB-33 for the development of F₁, 101 crossed seeds were obtained from 28 crossed panicles. The germination per cent of the crossed seeds in F₁ was 32.6 per cent. Out of 33 germinated seeds, 18 seedlings survived and were forwarded for BC₁F₁ development by backcrossing with Jyothi. Fifty-seven panicles were backcrossed with Jyothi from which 218 crossed seeds were obtained. A germination per cent of 35.7 per cent was recorded. Out of 78 germinated seeds, 63 BC₁F₁ seedlings survived.

In the cross, Jyothi x IR65482-7-216-1-2, twenty-nine panicles were crossed, and 153 crossed seeds were obtained. Forty-three seeds out of 153 seeds, germinated with a germination per cent of 28.1 per cent. Out of 43 germinated crossed seeds, 23 survived seedlings were forwarded for BC₁F₁ development. Eighty-nine panicles were backcrossed with Jyothi and 384 crossed seeds were obtained. A germination per cent of 31.2 per cent was recorded in BC₁F₁ seeds. Out of 120 BC₁F₁ seeds germinated, 78 BC₁F₁ seedlings survived.

4.5.2.1. Genotyping of BC₁F₁ Lines

Genotyping of BC₁F₁ lines derived from PTB-33 and IR65482-7-216-1-2 was performed using gene-specific molecular markers, RM589 and RM3331 for the presence of genes *Bph-3* and *Bph-18* respectively.

4.5.2.2. Genotyping of BC₁F₁ Lines [(Jyothi x PTB-33) x Jyothi]

Sixty-three BC₁F₁ lines derived from PTB-33 were screened using SSR markers RM589 for the presence of gene *Bph-3* (Plate 15a -15f). Out of which, 28 plants showed the presence of gene *Bph-3* in heterozygous conditions. The gene was absent in the remaining 35 plants which are susceptible (Table 16).

Table 15. Details of crossing in the development of F₁ and BC₁F₁

Sl. No.	Cross	Generation	No. of panicles crossed	No. of seed set	No. of seeds germinated	Survived Plants	Germination Per cent (%)
1.	Jyothi x PTB 33	F ₁	28	101	33	18	32.6
2.	Jyothi xIR65482-7-216-1-2		29	153	43	23	28.1
3.	[Jyothi x PTB 33] x Jyothi	BC ₁ F ₁	57	218	78	63	35.7
4.	[Jyothi x IR65482-7-216-1-2] x Jyothi		89	384	120	78	31.2

4.5.2.3. Genotyping of BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi]

Seventy-eight BC₁F₁ lines derived from IR65482-7-216-1-2 were subjected to genotyping with the SSR marker RM3331 specific for the gene *Bph-18* (Plate 16). Out of 78 BC₁F₁ lines, 36 plants showed the presence of heterozygous loci for the *Bph-18* gene. The remaining 42 plants showed susceptibility with the absence of the *Bph-18* gene (Table 16).

4.5.2.4. Chi-square test in BC₁F₁ generation

The chi-square test for goodness of fit in BC₁F₁ generation was calculated for identifying the inheritance pattern of the gene for BPH resistance. The expected ratio of resistance: susceptible for both *Bph 3* and *Bph 18* was 1:1 in the BC₁F₁ generation due to dominant gene action. The expected frequency of resistance and susceptibility from 63 BC₁F₁ lines with *Bph 3* resistant gene was 31.5 each and the observed frequency was 28 and 35 respectively. The calculated chi-square value was 0.76 and the table value at 1 degree of freedom (at $\alpha=0.05$) was 3.84. Whereas, the expected frequency of resistant and susceptible from 78 BC₁F₁ lines with *Bph-18* resistant gene was 39 each and the observed frequency was 36 and 42 respectively. The calculated chi-square value was 0.46 and the table value at 1 degree of freedom (at $\alpha=0.05$) was 3.84. Hence, in both cases, the null hypothesis which says that there are no significant differences between observed and expected values was accepted (Table 17).

4.5.2.5. Phenotyping of BC₁F₁ [(Jyothi x PTB 33) x Jyothi] lines

The observations on morphological characters of twenty-eight BC₁F₁ [(Jyothi x PTB 33) x Jyothi] lines identified with the presence of the *Bph-3* gene are given in Table 18.

Plant height (cm)

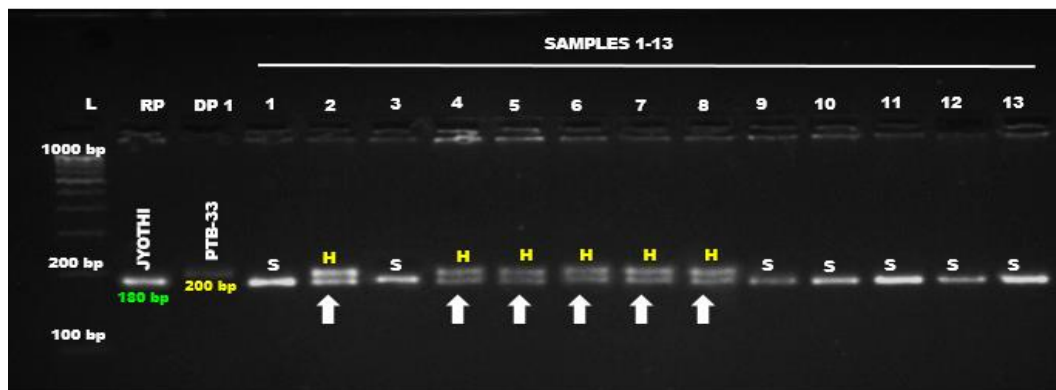
The plant height of parental genotypes Jyothi and PTB-33 was 92 cm and 152 cm respectively. The plant height in BC₁F₁ lines ranged from 80 cm to 153 cm with a mean value of 110.27 cm. The highest plant height (153cm) was observed in the line JP-24 and the lowest was recorded in the line JP-7. The distribution of plant height in BC₁F₁ generation showed positive skewness (0.4333) and negative kurtosis (-1.55).

Table 16. Genotyping of BC₁F₁ lines

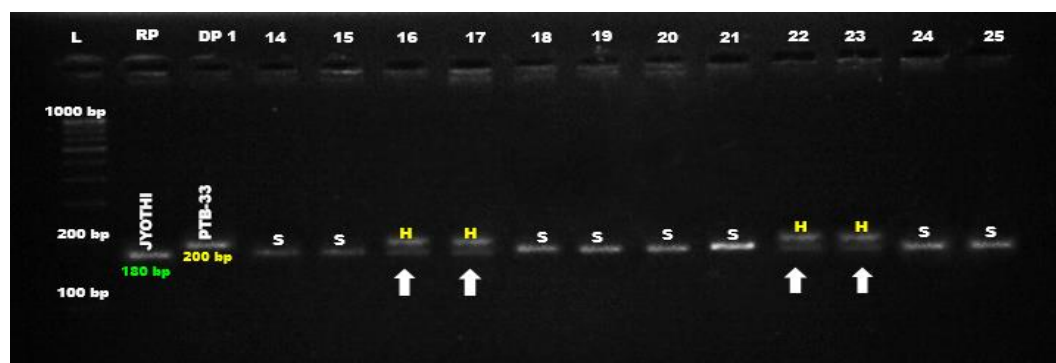
Sl. No.	Cross	Total genotypes screened	Heterozygous lines	Susceptible lines
1.	[Jyothi x PTB 33] x Jyothi	63	28 (<i>Bph3</i>)	35
2.	[Jyothi x IR65482-7-216-1-2] x Jyothi	78	36 (<i>Bph18</i>)	42

Table 17. The chi-square test for goodness of fit in BC₁F₁ generation

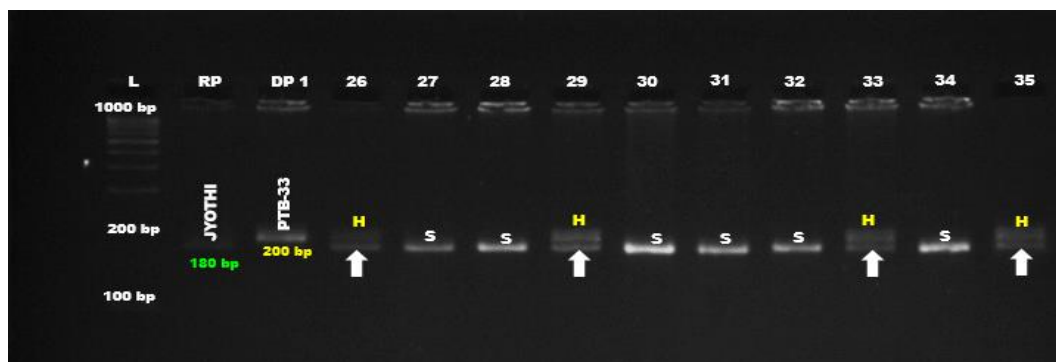
BC ₁ F ₁ generation	Total	Segregation ratio	Observed value		Calculated	Table (5% at $\alpha=1$ df)
			Resistant (Rr)	Susceptible (rr)		
[Jyothi x PTB 33] x Jyothi (<i>Bph-3</i>)	63	1:1	28	35	0.76	3.84
[Jyothi x IR65482-7-216-1-2] x Jyothi (<i>Bph-18</i>)	78	1:1	36	42	0.46	



(a) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 1-13



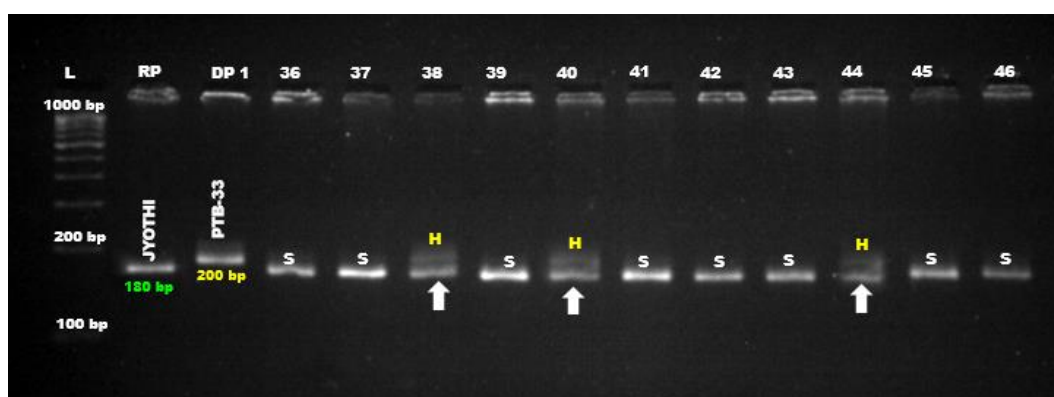
(b) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 14-25



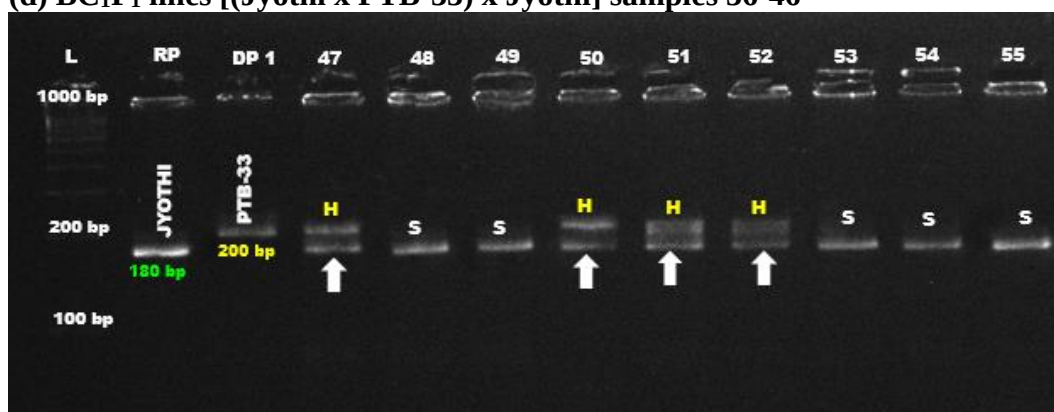
(c) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 26-35

L: Ladder, RP: Recurrent Parent, DP1: Donor parent-1 (PTB-33)
 ↑ H : Heterozygous, S : Susceptible

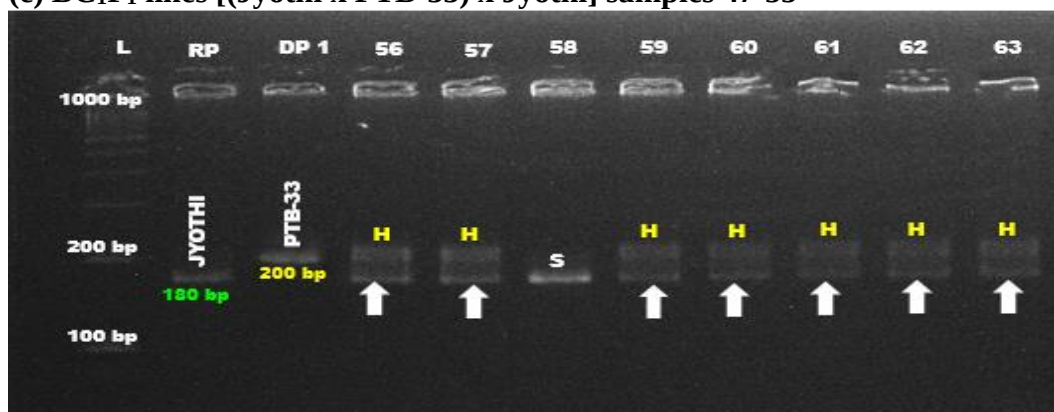
Plate 15. Amplification profile of RM589 specific to *Bph-3* gene in BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi]



(d) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 36-46



(e) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 47-55

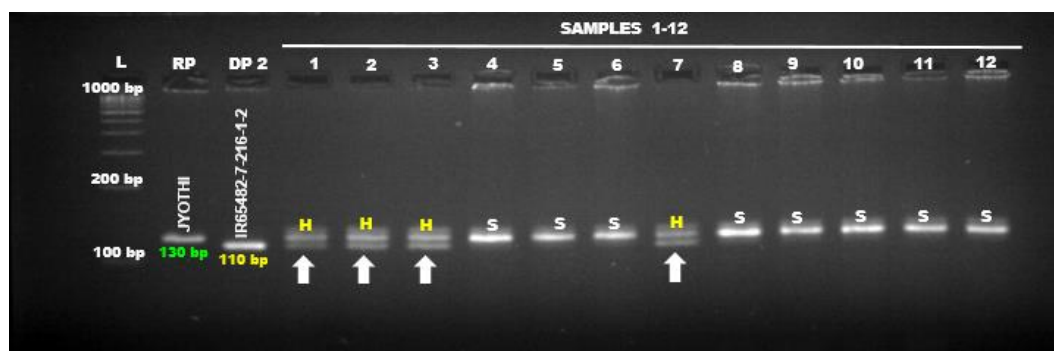


(f) BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi] samples 56-63

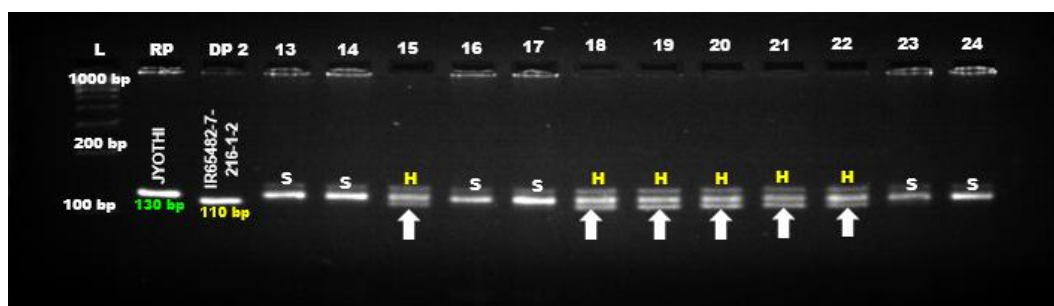
L: Ladder, RP: Recurrent Parent, DP1: Donor parent-1 (PTB-33)

↑ H : Heterozygous, S : Susceptible

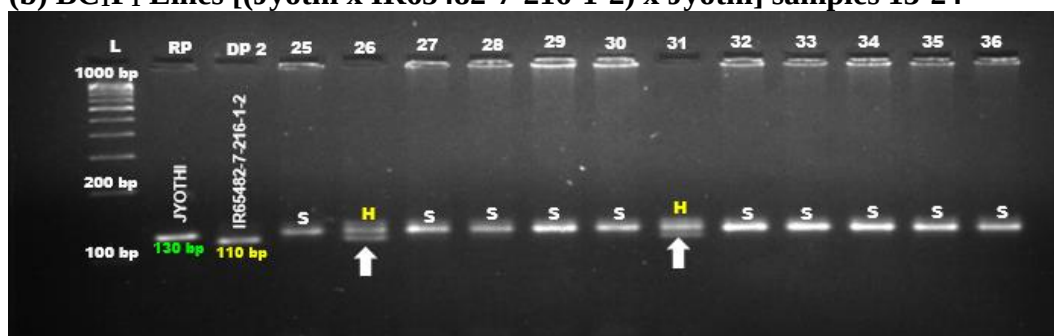
Plate 15 (contd). Amplification profile of RM589 specific to *Bph-3* gene in BC₁F₁ lines [(Jyothi x PTB-33) x Jyothi]



(a) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 1-12



(b) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 13-24

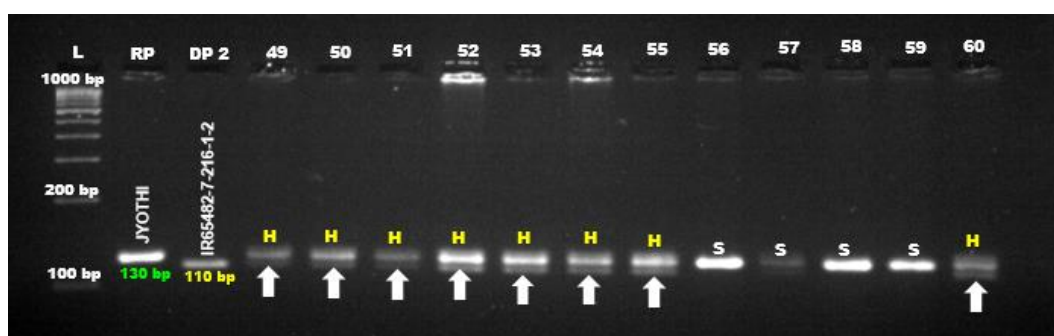


(c) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 25-36

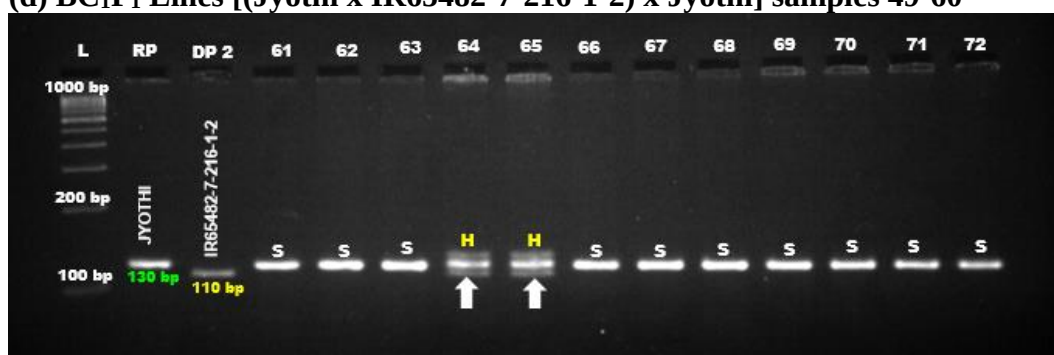
L: Ladder, RP: Recurrent Parent, DP2: Donor parent-2 (IR65482-7-216-1-2)

↑ H: Heterozygous, S: Susceptible

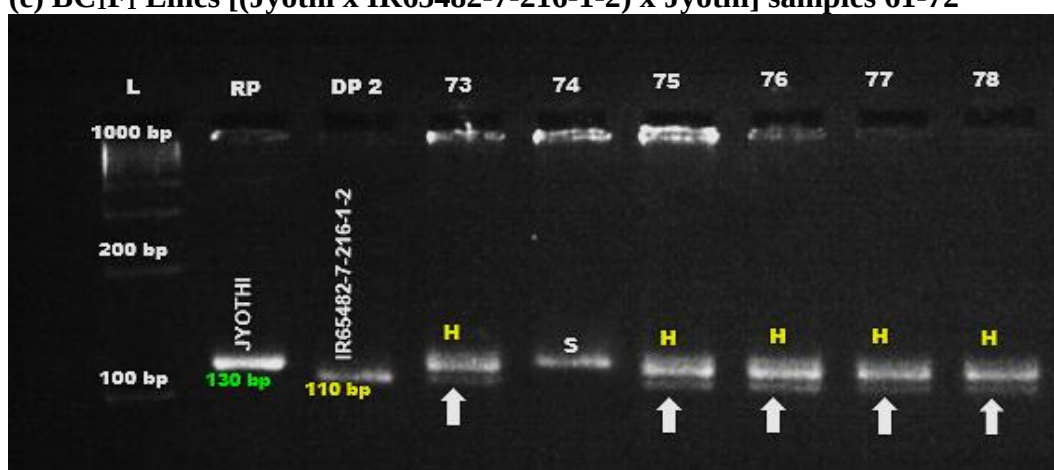
Plate 16. Amplification profile of RM3331 specific to *Bph-3* gene in BC₁F₁ lines [(Jyothi x IR65482-7-216-1-2) x Jyothi]



(d) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 49-60



(e) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 61-72



(f) BC₁F₁ Lines [(Jyothi x IR65482-7-216-1-2) x Jyothi] samples 73-78

L: Ladder, RP: Recurrent Parent, DP2: Donor parent-2 (IR65482-7-216-1-2)
H : Heterozygous, S : Susceptible

Plate 16 (contd). Amplification profile of RM3331 specific to *Bph-3* gene in BC₁F₁ lines [(Jyothi x IR65482-7-216-1-2) x Jyothi]

Table 18. Phenotypic data of BC₁F₁ [(Jyothi x PTB 33) x Jyothi] lines with the *Bph-3* gene

BC₁F₁ line	PH	NDFB	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
JP-1	83.0	80	8	6	20.0	148	25.4	8.42	2.57	3.28	Red
JP-2	86.0	86	6	5	18.0	136	24.9	8.18	2.35	3.48	Red
JP-3	112.5	84	9	8	26.0	133	25.8	7.40	2.60	2.85	Red
JP-4	108.0	103	7	7	19.0	142	26.0	7.80	3.25	2.40	Red
JP-5	87.0	81	11	8	21.0	162	29.2	8.62	3.46	2.49	Red
JP-6	89.0	86	8	6	27.0	158	28.4	9.20	2.34	3.93	Red
JP-7	80.0	99	9	6	22.5	153	28.3	8.63	2.66	3.24	Red
JP-8	85.0	85	9	8	25.0	159	28.7	8.12	3.31	2.45	Red
JP-9	137.0	92	8	7	20.0	142	26.5	7.96	3.42	2.33	Red
JP-10	143.0	99	5	2	26.5	138	24.6	7.30	3.28	2.23	Red
JP-11	152.0	116	7	5	27.0	126	23.4	8.04	3.56	2.26	Red
JP-12	135.5	105	7	4	23.0	126	24.8	8.53	2.98	2.86	Red
JP-13	85.0	86	8	8	23.5	154	27.5	8.94	2.65	3.37	Red

JP-14	95.5	85	8	7	22.0	148	27.0	8.66	2.44	3.55	Red
JP-15	148.0	94	6	6	21.0	133	25.4	7.68	3.12	2.46	Red
JP-16	132.0	88	9	5	26.5	127	25.1	7.74	3.26	2.37	Red
JP-17	86.0	87	5	5	25.0	147	26.8	8.52	2.43	3.51	Red
JP-18	80.5	95	8	7	21.0	153	26.9	8.34	2.67	3.12	Red
JP-19	87.0	87	9	8	22.0	159	27.4	8.96	2.84	3.15	Red
JP-20	86.0	94	6	3	27.0	138	25.3	8.18	2.71	3.02	Red
JP-21	95.0	112	9	5	26.5	141	25.8	7.92	2.66	2.98	Red
JP-22	126.0	90	4	4	23.0	127	24.1	8.36	3.09	2.71	Red
JP-23	148.0	82	6	4	21.0	136	25.2	7.78	3.24	2.40	Red
JP-24	153.0	102	7	4	28.0	124	23.9	7.24	3.49	2.07	Red
JP-25	136.0	86	8	7	25.0	126	24.5	7.76	3.12	2.49	Red
JP-26	124.0	89	9	6	24.0	134	26.0	7.2	2.78	2.59	Red
JP-27	89.0	93	5	5	20.5	162	29.3	8.58	2.56	3.35	Red
JP-28	95.0	87	8	7	22.5	155	28.1	7.74	2.47	3.13	Red

JYOTHI	92.0	85	9	8	23.0	160	28.8	8.95	2.68	3.34	Red
PTB-33	152.0	113	8	4	27.0	135	26.2	7.68	3.18	2.42	Red
Maximum	153.0	116	11	8	28.0	162	29.3	9.2	3.56	3.93	
Minimum	80.0	80	4	2	18.0	124	23.4	7.2	2.34	2.07	
Mean	110.27	92.37	7.53	5.83	23.45	142.73	26.31	8.14	2.91	2.48	
Std. Dev	26.75	9.73	1.59	1.66	2.79	12.47	1.66	0.55	0.38	0.49	
Variance	715.68	94.58	2.53	2.76	7.78	155.71	2.76	0.29	0.14	0.24	
Skewness	0.433	1.05	-0.36	-0.34	-0.06	0.099	0.27	0.03	0.16	0.26	
Kurtosis	-1.55	0.30	-0.14	-0.65	-1.12	-1.35	-0.93	-0.84	-1.36	-1.02	
C V	24.26	10.53	21.13	28.50	11.89	8.74	6.32	6.70	13.01	17.12	

PH: PLANT HEIGHT(cm), **NDFF:** NUMBER OF DAYS TO 50% FLOWERING, **NT:** NUMBER OF TILLERS
NPT: NUMBER OF PRODUCTIVE TILLERS, **PL:** PANICLE LENGTH(cm), **NGPP:** NUMBER OF GRAINS PER PANICLE,
GW: GRAIN WEIGHT(g), **GL:** GRAIN LENGTH(mm), **GB:** GRAIN BREADTH(mm), **LBR:** LENGTH-BREADTH RATIO

Number of days to 50% flowering

The number of days taken to 50% flowering ranged from 80 to 116 in BC₁F₁ lines with 85 and 113 days in parental genotypes Jyothi and PTB-33 respectively. The maximum number of days to 50% flowering was observed in line JP-11 and the minimum in line JP-1 with a mean range of 92.37 days in BC₁F₁ lines. The frequency distribution of the number of days to 50% flowering in BC₁F₁ lines observed a skewness and kurtosis of 1.05 and 0.30 respectively.

Number of tillers

The parental genotypes Jyothi and PTB-33 produced about 9 and 8 tillers respectively. The BC₁F₁ lines showed a range of 4 to 11 tillers with a mean of 7.53. The maximum tillers (11 Nos) were produced by JP-5 and the minimum (4 Nos) by JP-22 in BC₁F₁ lines with negative skewness (-0.36) and kurtosis (-0.14).

Number of productive tillers

The number of productive tillers in Jyothi, PTB-33, and BC₁F₁ lines was 8, 4, and 2 to 8 respectively. The maximum productive tillers (8 Nos) were produced by JP-3, JP-5, JP-8, JP-13, and JP-19, and the minimum (2 Nos) was produced by JP-10 with an average of 5.83 in BC₁F₁ lines. The frequency distribution of the number of productive tillers in BC₁F₁ lines showed negative skewness (-0.34) and kurtosis (-0.65).

Panicle length (cm)

The length of the panicle was 23 cm and 27 cm in Jyothi and PTB-33 genotypes respectively. The panicle length varied from 18 to 28 cm in BC₁F₁ lines with a mean value of 23.45 cm. The lines JP-24 and JP-2 recorded the maximum (28 cm) and the minimum (18 cm) panicle length respectively with negative skewness (-0.06) and positive kurtosis (1.12) in the frequency distribution of BC₁F₁ lines for panicle length.

Number of grains per panicle

The parents Jyothi and PTB-33 produced 160 and 135 grains per panicle respectively. The number of grains per panicle in BC₁F₁ lines ranged from 124 to 162 with an average of 142.73 numbers. The lines JP-27 and JP-5, and JP-24 produced the maximum (162) and the minimum (124) numbers of grains per panicle

in BC₁F₁ lines respectively. The positive skewness (0.099) and the negative kurtosis (-1.12) were shown in the frequency distribution pattern of BC₁F₁ lines for the number of grains per panicle.

1000 grain weight (g)

The 1000-grain weight of Jyothi and PTB-33 was 28.8g and 26.2g respectively. The maximum 1000-grain weight (29.3g) was recorded in JP-27 and the minimum (23.4g) in JP-11 with an average weight of 26.31g in BC₁F₁ lines. The frequency distribution of BC₁F₁ lines for 1000-grain weight showed a skewness of 0.27 with a kurtosis of -0.93.

Grain length (mm)

The grain length of 8.95, 7.68 and 8.14 mm was observed in Jyothi, PTB-33 and BC₁F₁ lines respectively. The highest grain length was observed in the line JP-6 at 9.2 mm and the lowest in JP-26 at 7.2 mm with a mean value of 8.14 mm length in BC₁F₁ lines. The frequency distribution of grain length in BC₁F₁ lines showed 0.03 skewness and -0.84 kurtosis.

Grain breadth (mm)

The grain breadth was maximum in line JP-11 (3.56 mm) and the minimum (2.34 mm) in JP-6 with an average of 2.91 mm in BC₁F₁ lines. The parents Jyothi and PTB-33 recorded 2.68 and 3.18 mm grain breadth respectively. The frequency was distributed with a skewness of 0.16 and a kurtosis of -1.36.

LB ratio

The LB ratio ranged from 2.07 to 3.93 in BC₁F₁ lines with a mean value of 2.48 in which the maximum was observed in the line JP-6 and the minimum in JP-24. The parents Jyothi and PTB-33 showed an LB ratio of 3.34 and 2.42 respectively. The frequency distribution recorded 0.26 skewness and -1.02 kurtosis.

Kernel colour

The parental genotypes Jyothi and PTB-33 had red-coloured kernels, so all BC₁F₁ lines produced red-coloured kernels.

4.5.2.6. Phenotyping of BC₁F₁ [(Jyothi x IR65482-7-216-1-2) x Jyothi] lines with Bph-18 gene

The observations on morphological characters of BC₁F₁ [(Jyothi x IR65482-7-216-1-2) x Jyothi] lines are given in Table 19.

Plant height (cm)

The plant height of Jyothi and IR65482-7-216-1-2 was 92 cm and 102 cm respectively. The maximum plant height (104 cm) was recorded in JB-33 and JB-17, and the minimum (86.5 cm) in JB-1 with an average height of 96.30 cm in BC₁F₁ lines. The frequency distribution of BC₁F₁ lines for plant height showed a skewness of 0.099 with a kurtosis of -0.72.

Number of days to 50% flowering

The number of days taken to 50% flowering was maximum in line JB-33, JB-30, JB-27, -21, JB-18, JB-11, and JB-7 (89 days) and the minimum (81 days) in JB -16 with an average of 86.26 days in BC₁F₁ lines. The parents Jyothi and IR65482-7-216-1-2 recorded 85 and 88 days to 50% flowering respectively. The frequency was distributed with a skewness of -0.49 and a kurtosis of -0.36.

Number of tillers

The number of tillers in Jyothi, IR65482-7-216-1-2 and BC₁F₁ lines was 9, 7 and 5-10 respectively. The maximum tillers (10 Nos) were produced by JB-27 and the minimum (5 Nos) was produced by JB-3, JB-23, and JB-34 with an average of 7.61 in BC₁F₁ lines. The frequency distribution of the number of tillers in BC₁F₁ lines showed negative skewness (-0.56) and positive kurtosis (0.12).

Number of productive tillers

The parental genotypes Jyothi and IR65482-7-216-1-2 recorded about 8 and 6 productive tillers. The BC₁F₁ lines showed a range of 3 to 9 productive tillers with a mean value of 6.11. The maximum productive tillers (9 Nos) were produced by JB-27 and the minimum (3 Nos) by JB-3 in BC₁F₁ lines with negative skewness (-0.07) and kurtosis (-0.55).

Panicle length (cm)

The panicle length ranged from 17 to 25 cm in BC₁F₁ lines with 23 and 19 cm in parental genotypes Jyothi and IR65482-7-216-1-2 respectively. The

maximum panicle length (25 cm) was observed in line JB-8 and the minimum (17 cm) in lines JB-3 and JB-22 with a mean range of 20.55 cm in BC₁F₁ lines. The frequency distribution of the panicle length in BC₁F₁ lines observed a skewness and kurtosis of 0.22 and -1.05 respectively.

Number of grains per panicle

The number of grains per panicle of parental genotypes Jyothi and IR65482-7-216-1-2 was 160 and 88 respectively. The number of grains per panicle in BC₁F₁ lines ranged from 86 to 162 with a mean value of 127.3. The maximum grains per panicle (162 Nos) was observed in the line JB-6 and the minimum (86 Nos) was recorded in the line JB-13. The distribution of the number of grains per panicle in BC₁F₁ generation showed negative skewness (-0.37) and kurtosis (-1.23).

1000 grain weight

The 1000-grain weight of Jyothi and IR65482-7-216-1-2 was 28.8g and 14g respectively. The maximum 1000-grain weight (27.8g) was recorded in JB-30 and the minimum (16.4 g) in JB-13 with an average weight of 22.34 g in BC₁F₁ lines. The frequency distribution of BC₁F₁ lines for 1000-grain weight showed a skewness of -0.19 with a kurtosis of -0.84.

Grain length (mm)

The grain length of 8.95, 7.74 and 8.96 mm was observed in Jyothi, IR65482-7-216-1-2 and BC₁F₁ lines respectively. The highest grain length was observed in line JB-6 at 8.96 mm and the lowest in JB-22 at 7.41 mm with a mean value of 8.28 mm length. The frequency distribution of grain length in BC₁F₁ lines showed -0.24 skewness and -1.28 kurtosis.

Grain breadth (mm)

The grain breadth was maximum in line JB-15 (2.68 mm) and the minimum (2.00mm) in JB-33 with an average of 2.38 mm in BC₁F₁ lines. The parents Jyothi and IR65482-7-216-1-2 recorded 2.68 and 2.10 mm grain breadth respectively. The frequency was distributed with a skewness of -0.06 and a kurtosis of -0.92.

LB ratio

The LB ratio ranged from 3.98 to 3.10 in BC₁F₁ lines with a mean value of 3.48 in which the maximum was observed in the line JB-33 (3.98) and the minimum

Table 19. Phenotyping of BC₁F₁ [(Jyothi x IR65482-7-216-1-2) x Jyothi] lines with *Bph-18* gene

BC₁F₁ line	PH	NDFE	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
JB-1	86.5	87	6	5	20	146	24.5	7.95	2.31	3.44	Red
JB-2	93.5	85	8	6	23	155	26.3	8.03	2.59	3.10	Red
JB-3	95.0	84	5	3	17	125	20.7	8.41	2.64	3.19	Red
JB-4	101.5	86	7	6	22	128	19.4	7.58	2.18	3.48	Red
JB-5	96.0	88	9	8	19	119	21.9	8.82	2.51	3.51	Red
JB-6	92.5	83	8	8	24	162	27.2	8.96	2.64	3.39	Light Red
JB-7	98.0	89	8	7	18	98	18.6	8.30	2.28	3.64	Red
JB-8	94.5	86	8	5	25	156	25.8	8.92	2.35	3.80	Red
JB-9	95.0	87	6	4	20	139	22.1	8.77	2.57	3.41	Red
JB-10	96.0	85	8	7	19	89	17.5	8.65	2.45	3.53	Dark brown
JB-11	103.0	89	9	6	18	91	19.3	7.94	2.28	3.48	Red
JB-12	101.5	86	8	7	21	96	20.1	7.61	2.12	3.59	Red
JB-13	99.0	88	7	4	19	86	16.4	8.66	2.24	3.87	Red
JB-14	91.0	87	6	5	22	137	23.7	8.06	2.33	3.46	Red

JB-15	95.0	84	8	6	21	148	26.6	8.49	2.68	3.17	Red
JB-16	92.0	81	9	7	23	152	24.2	8.83	2.56	3.45	Red
JB-17	104.0	87	7	6	22	119	20.9	7.65	2.34	3.27	White
JB-18	101.0	89	8	5	18	96	18.4	7.98	2.27	3.52	Red
JB-19	91.0	86	9	8	24	148	26.1	8.73	2.59	3.37	Red
JB-20	94.0	88	8	6	23	136	22.5	8.65	2.43	3.56	Red
JB-21	102.0	89	8	7	23	109	16.8	7.79	2.25	3.46	White
JB-22	95.0	84	7	5	17	94	18.3	7.41	2.32	3.19	Red
JB-23	93.0	88	5	4	22	154	27.4	8.79	2.64	3.33	Red
JB-24	101.0	87	7	5	20	103	19.2	7.58	2.13	3.56	Light brown
JB-25	94.0	84	9	8	22	135	25.6	8.44	2.51	3.36	Red
JB-26	91.0	87	8	7	19	149	26.3	8.74	2.38	3.67	Red
JB-27	97.5	89	10	9	18	127	21.7	7.86	2.25	3.49	Red
JB-28	99.0	86	7	5	21	142	24.5	8.43	2.29	3.68	Red
JB-29	101.0	85	8	8	24	97	19.8	7.61	2.11	3.61	Light red
JB-30	94.0	89	8	6	20	157	27.8	8.82	2.41	3.66	Red
JB-31	91.0	84	9	7	18	135	23.4	8.67	2.49	3.48	Red

JB-32	97.0	82	7	6	19	139	23.7	8.34	2.31	3.61	Red
JB-33	104.0	89	8	5	19	113	19.1	7.96	2.00	3.98	White
JB-34	96.0	87	5	4	21	146	25.2	8.45	2.60	3.25	Red
JB-35	92.0	84	8	7	20	141	24.9	8.1	2.30	3.52	Red
JB-36	98.0	86	7	6	18	125	20.4	8.28	2.54	3.26	Red
JYOTHI	92.0	85	9	8	23	160	28.8	8.95	2.68	3.34	Red
IR65482-7-216-1-2	102.0	88	7	6	19	88	16.4	7.74	2.10	3.69	White
Maximum	104.0	89	10	9	25	162	27.8	8.96	2.68	3.98	
Minimum	86.5	81	5	3	17	86	14	7.41	2.00	3.10	
Mean	96.30	86.26	7.61	6.11	20.55	127.3	22.34	8.28	2.38	3.48	
Std. Dev	4.33	2.11	1.19	1.41	2.20	23.94	3.69	0.47	0.18	0.19	
Variance	18.76	4.47	1.43	1.98	4.84	573.5	13.59	0.22	0.033	0.04	
Skewness	0.099	-0.49	-0.56	-0.07	0.22	-0.37	-0.19	-0.24	-0.06	0.27	
Kurtosis	-0.72	-0.36	0.12	-0.55	-1.05	-1.23	-0.84	-1.28	-0.92	0.25	
C V	4.49	2.45	15.75	23.09	10.71	18.80	16.50	5.65	7.71	5.55	

in JB-2 (3.10). The parents Jyothi and IR65482-7-216-1-2 showed an LB ratio of 3.34 and 3.69 respectively. The frequency distribution recorded 0.27 skewness and 0.25 kurtosis.

Kernel colour

The parental genotypes Jyothi and IR65482-7-216-1-2 had red and white coloured kernels respectively. The kernel colour of BC₁F₁ lines varied between white, light red, dark brown, light brown, and red colours. The JB-6 and JB-29 had light red coloured kernels, and JB-10 and JB-24 had dark and light brown coloured kernels respectively. The JB-17, JB-21, and JB-33 produced white-coloured kernels and the remaining 29 BC₁F₁ lines produced red-coloured kernels.

4.5.3. Development of intercrossed BC₁F₁ lines

Based on the genotyping and phenotyping of the BC₁F₁ lines, plants with genes (*Bph-3* or *Bph-18*) for resistance in heterozygous conditions were selected and forwarded for the development of intercrossed BC₁F₁ lines to pyramid the genes into a single line. Intercrossing of BC₁F₁ lines involved two crosses viz., the PTB-33 derived BC₁F₁ lines with *Bph-3* in heterozygous condition as the female parent and IR65482-7-216-1-2 derived BC₁F₁ lines with *Bph-18* in heterozygous condition as male parent denoted as 'ICAB' and the reciprocal cross denoted as 'ICBA' (Table 20). The details of intercrossing of the BC₁F₁ lines are tabulated in Table 21.

In ICAB intercrossed lines, 182 seeds were obtained from 64 panicles crossed. A germination per cent of 29.12 per cent was observed. Among the 53 germinated seeds, 22 seedlings survived as healthy plants.

In ICBA intercrossed lines, 97 panicles were crossed and 223 seeds were obtained. Out of which 62 seeds germinated with a germination percentage of 27.80 per cent. 18 seedlings survived as healthy plants.

4.5.3.1. Genotyping of Intercrossed BC₁F₁ (ICAB and ICBA) Lines

Intercrossed BC₁F₁ (ICAB and ICBA) lines were genotyped using SSR markers RM589 and RM3331 specific for the genes *Bph-3* and *Bph-18* respectively (Plate 17a to 17d).

Table 20. Intercrossing of BC₁F₁ lines

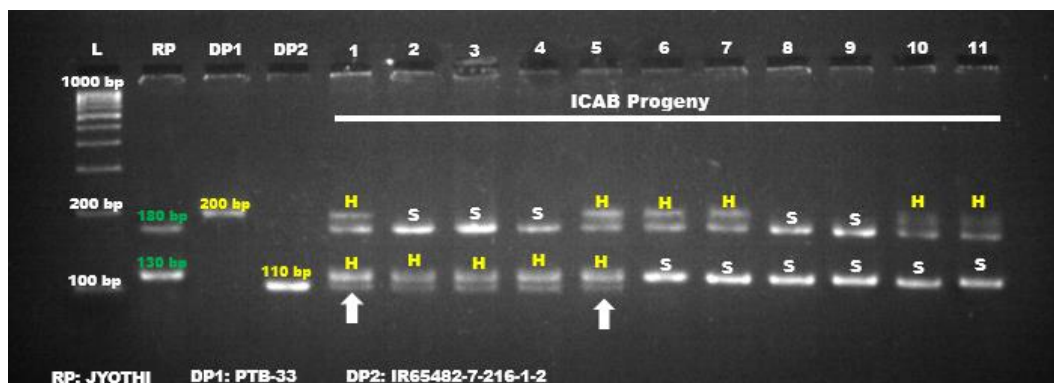
Sl. No.	Cross		Pedigree
	Female parent	Male parent	
1.	[Jyothi x PTB 33] x Jyothi	[Jyothi x IR65482-7-216-1-2] x Jyothi	ICAB
2.	[Jyothi x IR65482-7-216-1-2] x Jyothi	[Jyothi x PTB 33] x Jyothi	ICBA

Population-A: [Jyothi x PTB 33] x Jyothi

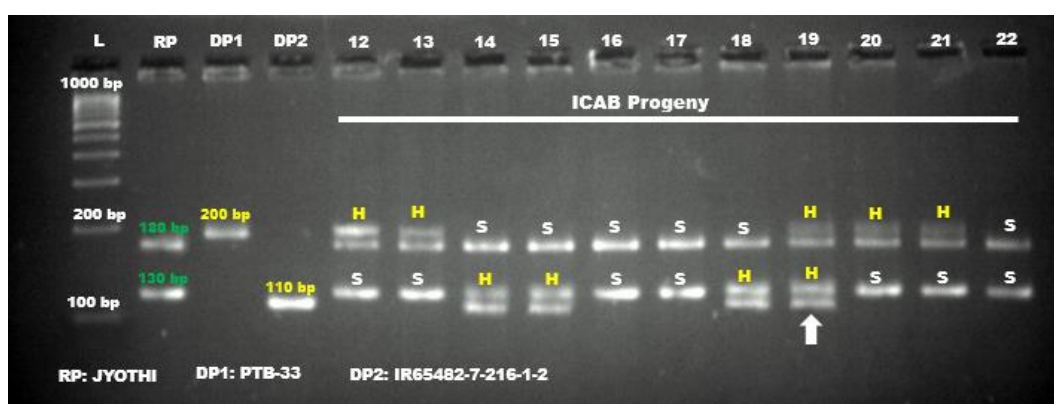
Population-B: [Jyothi x IR65482-7-216-1-2] x Jyothi

Table 21. Details of intercross of BC₁F₁ lines

Sl. No.	Pedigree	No. of panicles crossed	No. of seed set	No. of seeds germinated	Survived	Germination Per cent (%)
1.	ICAB	64	182	53	22	29.12
2.	ICBA	97	223	62	18	27.80



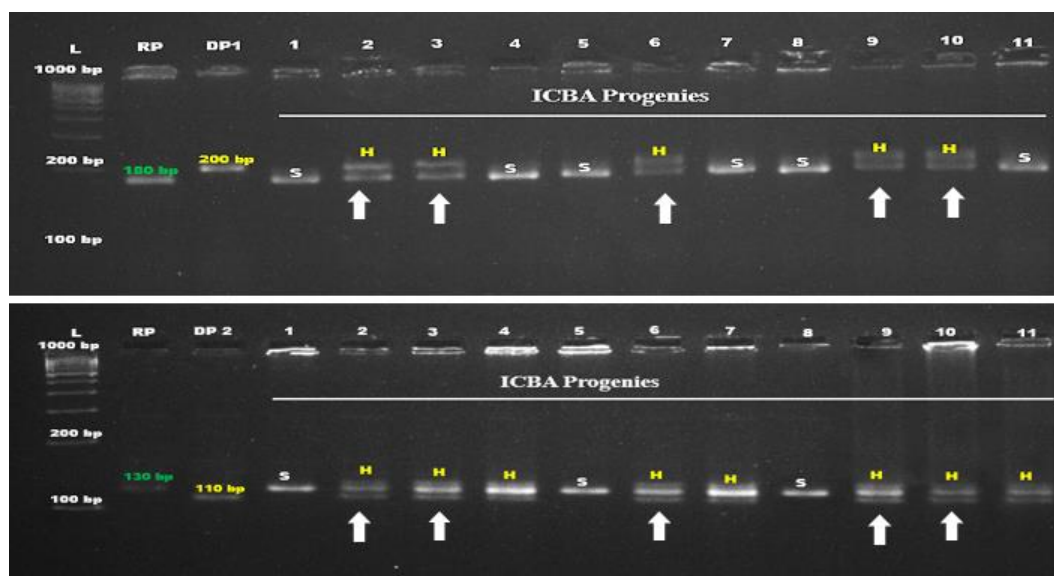
(a) Intercrossed BC₁F₁ samples ICAB-1 to ICAB-11



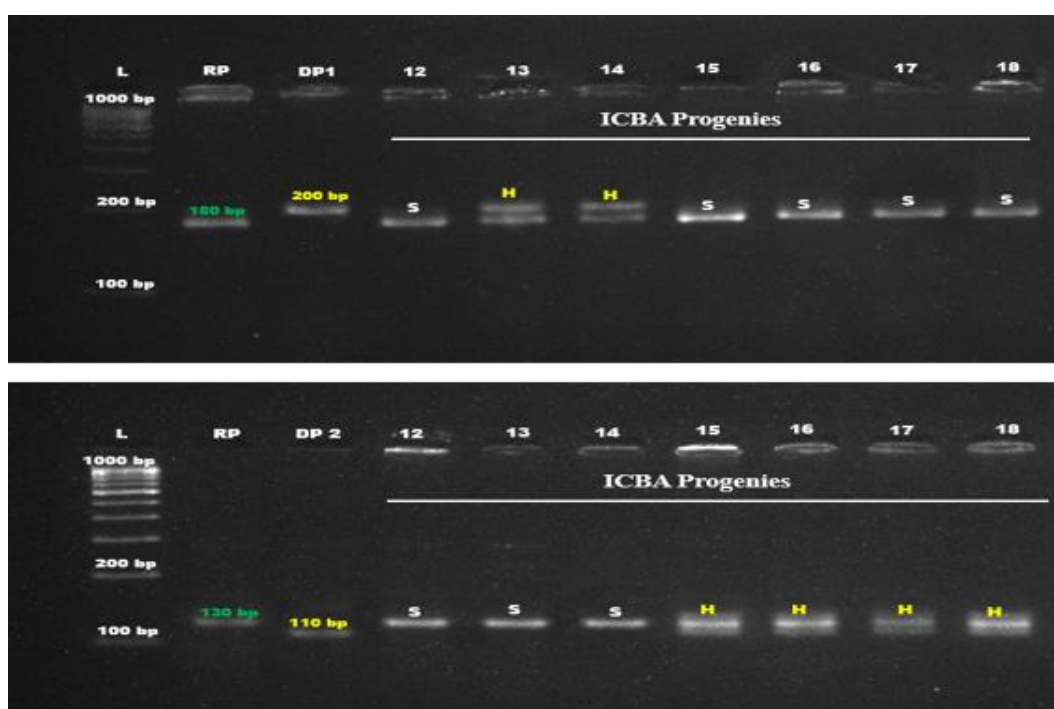
(b) Intercrossed BC₁F₁ samples ICAB-12 to ICAB-22

H: Heterozygous, S: Susceptible
 Arrow mark ↑ : Heterozygous combination of *Bph-3* and *Bph-18*

Plate 17. Amplification profile of RM589 linked to the gene *Bph-3* and RM3331 linked to the gene *Bph-18* in intercrossed BC₁F₁ ICAB lines



(c) Intercrossed BC₁F₁ samples ICBA-1 to ICBA-11



(d) Intercrossed BC₁F₁ samples ICBA-12 to ICBA-18

H: Heterozygous, S: Susceptible
 Arrow mark ↑ : Heterozygous combination of *Bph-3* and *Bph-18*

Plate 17. Amplification profile of RM589 linked to the gene *Bph-3* and RM3331 linked to the gene *Bph-18* in intercrossed BC₁F₁ ICBA lines

Among the twenty-two ICAB lines, three plants viz., ICAB-1, ICAB-5, and ICAB-19 showed the combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition (Table 22). Eight ICAB lines namely, ICAB-6, ICAB-7, ICAB-10, ICAB-11, ICAB-12, ICAB-13, ICAB-20, ICAB-21 exhibited the *Bph-3* gene alone in heterozygous condition. Six ICAB lines viz., ICAB-2, ICAB-3, ICAB-4, ICAB-14, ICAB-15, and ICAB-18 showed the presence of the *Bph-18* gene alone in heterozygous condition. The remaining five ICAB lines viz., ICAB-8, ICAB-9, ICAB-16, ICAB-17, and ICAB-22 were susceptible as it does not show the presence of any genes.

Among the eighteen ICBA progenies, five plants namely, ICBA-2, ICBA-3, ICBA-6, ICBA-9, and ICBA-10 were obtained with the combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition. Two lines viz., ICBA-13 and ICBA-14 were obtained with *Bph-3* alone in heterozygous condition. Heterozygous loci for *Bph-18* alone were observed in seven lines namely, ICBA-4, ICBA-7, ICBA-11, ICBA-15, ICBA-16, ICBA-17, and ICBA-18. The remaining four lines of ICBA did not show the presence of either *Bph-3* or *Bph-18*.

4.5.4. Development of BC₂F₁ Lines

Eight intercrossed BC₁F₁ lines viz., ICAB-1, ICAB-5, ICAB-19, ICBA-2, ICBA-3, ICBA-6, ICBA-9, and ICBA-10 with Jyothi-specific characters and a combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition were backcrossed with Jyothi to develop BC₂F₁ lines. The details of backcrossing for the development of BC₂F₁ lines are tabulated in Table 23.

Four panicles of each intercrossed BC₁F₁ line were backcrossed with Jyothi and the seed set varied from 18-28 seeds. The maximum number of crossed seeds was obtained from the backcross of ICAB-2. The minimum number of crossed seeds was obtained from the backcross of ICBA-3. The germination percentage ranged from 27.27 to 38.88 per cent. Out of 59 seeds that germinated, 26 seedlings survived as healthy plants.

Table 22. Genotyping of Intercrossed BC₁F₁ (ICAB and ICBA) Lines

Sl. No.	Plants with two genes (<i>Bph-3</i> and <i>Bph-18</i>) in heterozygous condition	Plants with gene <i>Bph-3</i> alone in heterozygous condition	Plants with gene <i>Bph-18</i> alone in heterozygous condition	Plants without the presence of any of the genes (<i>Bph-3</i> and <i>Bph-18</i>)
1.	ICAB-1	ICAB-6	ICAB-2	ICAB-8
2.	ICAB-5	ICAB-7	ICAB-3	ICAB-9
3.	ICAB-19	ICAB-10	ICAB-4	ICAB-16
4.	ICBA-2	ICAB-11	ICAB-14	ICAB-17
5.	ICBA-3	ICAB-12	ICAB-15	ICAB-22
6.	ICBA-6	ICAB-13	ICAB-18	ICBA-1
7.	ICBA-9	ICAB-20	ICBA-4	ICBA-5
8.	ICBA-10	ICAB-21	ICBA-7	ICBA-8
9.		ICBA-13	ICBA-11	ICBA-12
10.		ICBA-14	ICBA-15	
11.			ICBA-16	
12.			ICBA-17	
13.			ICBA-18	

Table 23. The details of backcrossing Intercrossed BC₁F₁ for the development of BC₂F₁ lines

Sl. No.	Intercrossed BC₁F₁ parent progeny number	No. of panicles crossed	No. of seed set	No. of seeds germinated	Survived Plants	Germination Per cent (%)
1.	ICAB -1	4	23	8	6	34.78
2.	ICAB -5	4	21	6	2	28.57
3.	ICAB -19	4	22	6	1	27.27
4.	ICBA -2	4	28	10	3	35.71
5.	ICBA -3	4	18	7	4	38.88
6.	ICBA -6	4	20	6	2	30.00
7.	ICBA -9	4	19	7	5	36.84
8.	ICBA-10	4	27	9	3	33.33
TOTAL					26	

4.5.4.1 Genotyping of BC₂F₁ lines

The twenty-six BC₂F₁ lines developed were genotyped using SSR markers RM589 and RM3331 specific for the genes *Bph-3* and *Bph-18* respectively (Plate 18a to 18c). In the genotypic evaluation of BC₂F₁ lines, five lines viz., ICAB-1/3, ICAB-1/6, ICBA-3/2, ICBA-9/3, and ICBA-10/2 showed the combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition. Eight lines namely, ICAB-1/1, ICAB-1/2, ICBA-3/1, ICBA-3/3, ICBA-6/1, ICBA-9/5, ICBA-10/1, and ICBA-10/3 showed heterozygous loci of *Bph-3* gene alone. Six lines viz., ICAB-1/5, ICAB-19/1, ICBA-2/1, ICBA-3/4, ICBA-6/2, and ICBA-9/2 were obtained with *Bph-18* alone in heterozygous condition (Table 24).

The remaining seven BC₂F₁ lines namely, ICAB-1/4, ICAB-5/1, ICAB-5/2, ICAB-2/2, ICAB-2/3, ICAB-9/1, ICAB-9/4 were susceptible lines as these lines did not show the presence of any genes for resistance.

4.5.4.2. Phenotyping of Intercrossed BC₁F₁ lines and BC₂F₁ lines

The observations on morphological characters of intercrossed BC₁F₁ lines and BC₂F₁ lines are given in Table 25 and 26 respectively.

Plant height (cm)

The plant height of parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was 92, 152 and 102 cm respectively. The range of plant height in intercrossed BC₁F₁ varied from 84.5 to 149 cm with an average height of 108.27 cm. The maximum height was observed in ICAB-14 and the minimum was observed in ICAB-6. The frequency distribution in intercrossed BC₁F₁ lines for plant height showed positive skewness (0.99) and negative kurtosis (-0.1752) as in Table 25.

The plant height in BC₂F₁ lines ranged from 88 to 132 cm with a mean value of 99.25 cm. The maximum plant height was observed in ICAB-9/2 and the minimum height in ICAB-1/3. The skewness of frequency distribution was 2.95 with a kurtosis of 8.83 for plant height in BC₂F₁ lines as in Table 26.

Number of days to 50% flowering

The number of days taken to 50% flowering in parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was 85, 113 and 88 days respectively. In intercrossed BC₁F₁ lines, the number of days to 50% flowering ranged from 80 to

Table 24. Genotyping of BC₂F₁ (ICAB and ICBA) Lines

Sl. No.	Plants with two genes (<i>Bph-3</i> and <i>Bph-18</i>) in heterozygous condition	Plants with gene <i>Bph-3</i> alone in heterozygous condition	Plants with gene <i>Bph-18</i> alone in heterozygous condition	Plants without the presence of any of the genes (<i>Bph-3</i> and <i>Bph-18</i>)
1.	ICAB-1/3	ICAB-1/1	ICAB-1/5	ICAB-1/4
2.	ICAB-1/6	ICAB-1/2	ICAB-19/1	ICAB-5/1
3.	ICBA-3/2	ICBA-3/1	ICBA-2/1	ICAB-5/2
4.	ICBA-9/3	ICBA-3/3	ICBA-3/4	ICAB-2/2
5.	ICBA-10/2	ICBA-6/1	ICBA-6/2	ICAB-2/3
6.		ICBA-9/5	ICBA-9/2	ICAB-9/1
7.		ICBA-10/1		ICAB-9/4
8.		ICBA-10/3		

Table 25. Phenotyping of Intercrossed BC₁F₁ lines

Sl. No.	PLANT NO.	PH	NDFE	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
1.	ICAB-1	93.0	81	5	4	25.0	141	27.2	7.32	2.54	2.88	RED
2.	ICAB-2	118.0	80	7	6	19.5	128	23.6	7.60	3.01	2.52	LIGHT RED
3.	ICAB-3	104.0	81	5	3	25.6	121	22.7	6.98	2.97	2.35	RED
4.	ICAB-4	136.0	103	7	4	29.0	142	24.9	7.95	3.10	2.56	DARK BROWN
5.	ICAB-5	91.0	84	9	8	23.0	158	27.5	8.74	2.54	3.44	RED
6.	ICAB-6	84.5	80	6	4	22.5	149	26.4	8.12	2.12	3.83	RED
7.	ICAB-7	109.5	95	6	3	24.0	110	19.2	7.66	2.46	3.11	RED
8.	ICAB-10	89.0	82	7	5	19.0	121	21.7	8.05	2.42	3.33	RED
9.	ICAB-11	132.0	108	9	5	25.5	127	22.5	7.44	3.06	2.43	RED
10.	ICAB-12	96.0	85	7	6	24.0	142	26.8	7.85	2.94	2.67	RED
11.	ICAB-13	91.5	84	6	4	23.0	136	27.3	8.32	2.81	2.96	RED
12.	ICAB-14	149.0	104	8	5	26.0	138	25.1	7.01	2.97	2.36	RED
13.	ICAB-15	104.0	83	6	5	21.0	93	15.3	7.92	2.32	3.41	LIGHT RED
14.	ICAB-18	93.0	82	8	6	22.0	155	26.9	8.36	2.42	3.45	RED
15.	ICAB-19	96.0	87	6	4	24.0	136	27.8	8.37	2.71	3.09	LIGHT RED
16.	ICAB-20	106.0	89	8	7	21.0	96	18.1	7.56	2.34	3.23	WHITE
17.	ICAB-21	93.0	82	8	7	24.0	148	25.6	8.68	2.79	3.11	RED
18.	ICBA-2	108.0	82	7	5	23.0	116	20.1	8.16	2.35	3.47	RED

19.	ICBA-3	98.0	85	6	5	26.0	128	24.3	7.87	2.08	3.78	RED
20.	ICBA-4	125.0	94	7	5	26.0	142	27.4	8.29	2.93	2.83	RED
21.	ICBA-6	91.0	80	8	6	21.0	143	25.7	8.24	2.51	3.28	RED
22.	ICBA-7	119.0	89	7	5	25.0	128	24.2	7.92	2.84	2.79	RED
23.	ICBA-9	137.0	97	8	6	35.0	139	27.8	8.53	2.92	2.92	RED
24.	ICBA-10	96.0	84	7	6	26.0	142	27.1	8.69	2.47	3.52	RED
25.	ICBA-11	105.0	86	8	6	22.0	95	22.5	7.91	2.35	3.37	LIGHT RED
26.	ICBA-13	141.0	91	6	5	25.0	153	28.1	7.74	2.81	2.75	DARK BROWN
27.	ICBA-14	132.0	85	8	7	24.0	147	27.5	7.86	2.93	2.68	RED
28.	ICBA-15	100.5	82	8	6	20.0	128	22.4	8.21	2.38	3.45	LIGHT RED
29.	ICBA-16	96.0	80	7	6	21.0	132	25.2	8.37	2.52	3.32	RED
30.	ICBA-17	102.5	83	6	5	21.0	121	24.6	8.18	2.31	3.54	WHITE
31.	ICBA-18	99.0	81	8	6	23.0	149	27.5	8.36	2.59	3.23	RED
	JYOTHI	92.0	85	9	8	23.0	160	28.8	8.95	2.68	3.34	RED
	PTB-33	152.0	113	8	4	27.0	135	26.2	7.68	3.18	2.42	RED
	IR65482-7-216-1-2	102.0	88	7	6	19.0	88	14.0	7.74	2.10	3.69	WHITE
	Maximum	149.0	108	9	8	35.0	158	27.8	8.74	3.10	3.83	
	Minimum	84.5	80	5	3	19.0	93	15.3	6.98	2.08	2.35	
	Mean	108.27	87.5	7.14	5.38	23.67	131.97	24.47	8.018	2.631	3.091	

	Std. Dev	18.75	8.52	1.076	1.231	3.122	18.692	3.649	0.465	0.307	0.426	
	Variance	351.86	72.62	1.159	1.516	9.751	349.423	13.31	0.216	0.094	0.181	
	Skewness	0.99	1.628	-0.154	0.133	1.344	-0.862	-1.353	-0.270	-0.025	-0.234	
	Kurtosis	-0.1752	2.004	-0.656	-0.089	4.053	0.230	1.493	-0.008	-1.701	-1.009	
	C V	17.323	9.739	15.066	22.87	13.187	14.164	14.912	5.800	11.685	13.799	

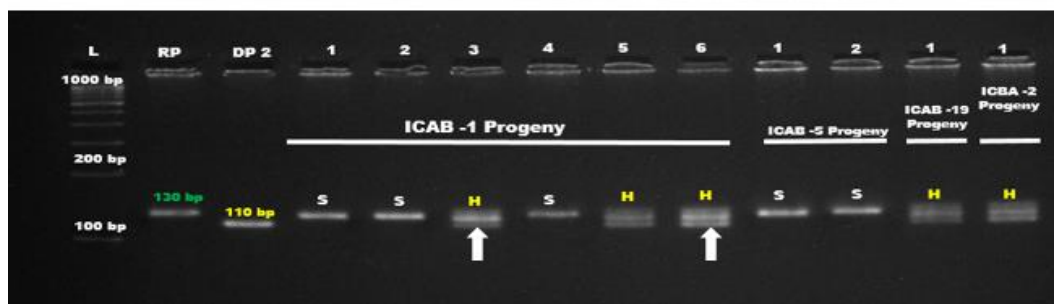
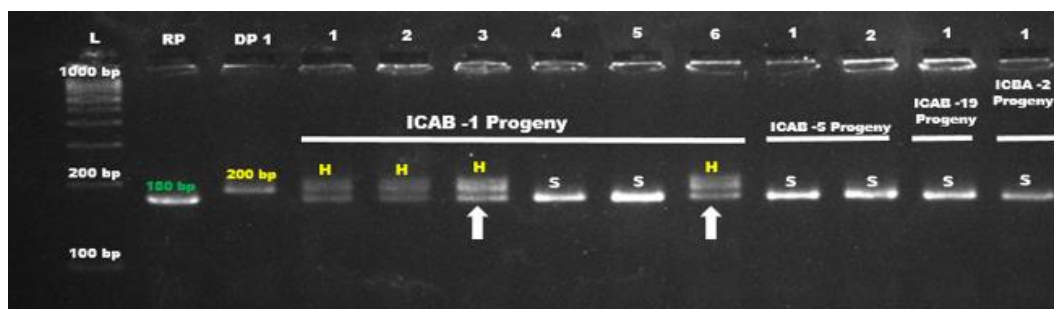
PH: PLANT HEIGHT(cm), **NDF**: NUMBER OF DAYS TO 50% FLOWERING, **NT:** NUMBER OF TILLERS
NPT: NUMBER OF PRODUCTIVE TILLERS, **PL:** PANICLE LENGTH(cm), **NGPP:** NUMBER OF GRAINS PER PANICLE,
GW: GRAIN WEIGHT(g), **GL:** GRAIN LENGTH(mm), **GB:** GRAIN BREADTH(mm), **LBR:** LENGTH-BREADTH RATIO

Table 26. Phenotyping of BC₂F₁ lines

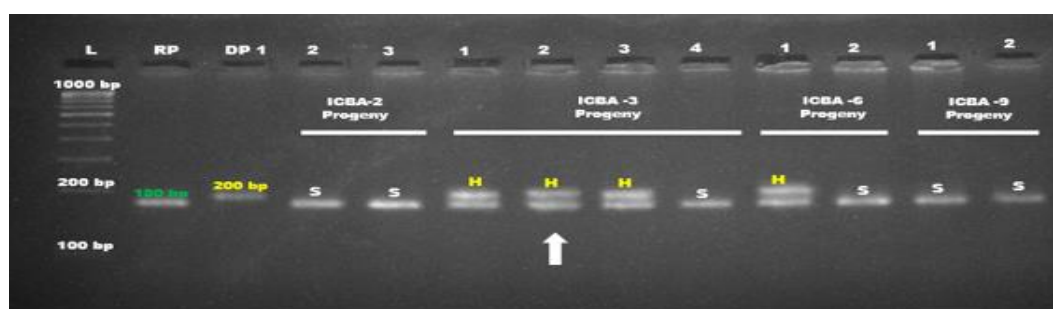
Sl. No.	PLANT NO.	PH	NDF	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
1.	ICAB-1/1	91	83	8	7	21.5	155	28.1	8.02	2.46	3.26	RED
2.	ICAB-1/2	93	84	7	6	24.7	153	27.9	8.32	2.75	3.03	RED
3.	ICAB-1/3	88	84	8	7	26.0	142	27.1	8.31	2.59	3.21	RED
4.	ICAB-1/5	95	83	7	6	26.8	141	26.8	7.96	3.07	2.59	DARK BROWN
5.	ICAB-1/6	94	86	9	8	24.0	152	27.4	8.64	2.78	3.11	RED
6.	ICAB-19/1	97	85	8	7	26.0	149	26.5	8.28	2.53	3.27	RED
7.	ICBA-2/1	101	89	8	6	22.0	117	22.6	8.12	2.42	3.36	RED
8.	ICBA-3/1	97	85	7	5	25.0	144	26.7	8.23	2.56	3.21	DARK BROWN
9.	ICBA-3/2	99	86	9	8	25.0	149	26.2	8.52	2.51	3.39	RED
10.	ICBA-3/3	94	82	8	7	27.0	146	26.1	8.32	2.47	3.37	RED
11.	ICBA-3/4	93	85	6	5	24.0	152	28.3	8.54	2.52	3.39	LIGHT RED
12.	ICBA-6/1	90	83	8	7	24.0	153	28.3	8.61	2.38	3.62	RED
13.	ICBA-6/2	91	81	8	7	25.0	147	27.2	8.42	2.42	3.48	RED
14.	ICBA-9/2	132	103	8	4	26.0	139	25.7	7.99	2.78	2.87	RED
15.	ICBA-9/3	98	84	7	6	25.0	135	24.5	7.92	2.89	2.74	RED
16.	ICBA-9/5	101	86	8	6	26.0	128	25.6	8.05	2.23	3.61	RED

17.	ICBA-10/1	96	82	7	6	24.5	149	27.4	7.76	2.35	3.30	LIGHT RED
18.	ICBA-10/2	93.5	86	8	7	24.0	151	27.8	8.54	2.85	3.00	RED
19.	ICBA-10/3	94	83	8	7	26.0	149	27.4	7.82	2.99	2.62	RED
	JYOTHI	92	85	9	8	23.0	160	28.8	8.95	2.68	3.34	RED
	PTB-33	152	113	8	4	27.0	135	26.2	7.68	3.18	2.42	RED
	IR65482-7-216-1-2	102	88	7	6	19.0	88	14.0	7.74	2.1	3.69	WHITE
	Maximum	132	103	9	8	27.0	155	28.3	8.64	3.07	3.62	
	Minimum	88	81	6	4	21.5	117	22.6	7.76	2.23	2.59	
	Mean	99.25	86.63	7.77	6.36	24.61	142.25	26.21	8.22	2.62	3.18	
	Std. Dev	14.644	7.34	0.75	1.13	1.93	15.57	3.06	0.34	0.27	0.35	
	Variance	214.47	53.86	0.56	1.29	3.74	242.73	9.37	0.12	0.07	0.12	
	Skewness	2.95	2.93	-0.33	-0.59	-1.29	-2.34	-3.31	0.21	0.33	-0.69	
	Kurtosis	8.83	8.71	0.26	0.03	2.17	6.67	12.77	-0.60	-0.29	-0.21	
	C V	14.755	8.47	9.67	17.84	7.86	10.93	11.68	4.12	10.44	10.92	

PH: PLANT HEIGHT(cm), **NDFF:** NUMBER OF DAYS TO 50% FLOWERING, **NT:** NUMBER OF TILLERS
NPT: NUMBER OF PRODUCTIVE TILLERS, **PL:** PANICLE LENGTH(cm), **NGPP:** NUMBER OF GRAINS PER PANICLE,
GW: GRAIN WEIGHT(g), **GL:** GRAIN LENGTH(mm), **GB:** GRAIN BREADTH(mm), **LBR:** LENGTH-BREADTH RATIO



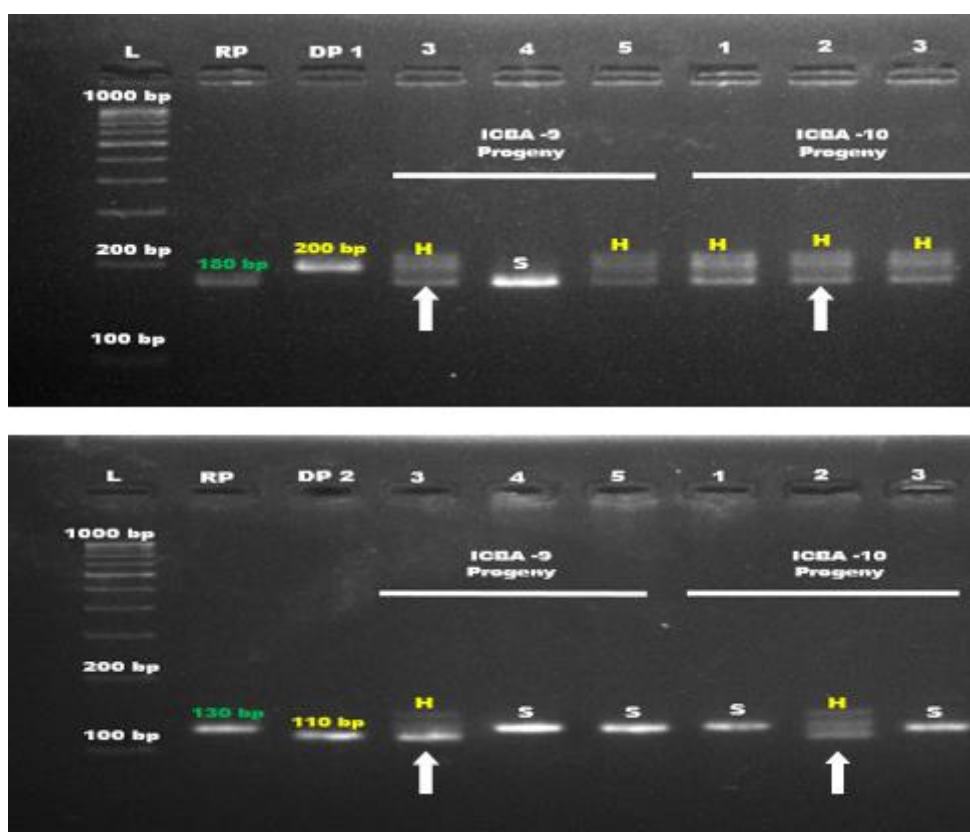
(a) BC₂F₁ lines [ICAB-1/1 to ICAB-1/6, ICAB-5/1, ICAB-5/2, ICAB-19/1, ICBA-2/1]



(b) BC₂F₁ lines [ICBA-2/2, ICBA-2/3, ICBA-3/1 to ICBA-3/4, ICBA-6/1, ICBA-6/2, ICBA-9/1, ICBA-9/2]

H: Heterozygous, S: Susceptible
Arrow mark: Heterozygous combination of *Bph-3* and *Bph-18*

Plate 18. Amplification profile of RM589 linked to the gene *Bph-3* and RM3331 linked to the gene *Bph-18* in BC₂F₁ ICAB and ICBA lines



(c) BC₂F₁ lines [ICBA-2/2, ICBA-2/3, ICBA-3/1 to ICBA-3/4, ICBA-6/1, ICBA-6/2, ICBA-9/1, ICBA-9/2]

↑ **H: Heterozygous, S: Susceptible**
 ↑ Arrow mark: Heterozygous combination of *Bph-3* and *Bph-18*

Plate 18. Amplification profile of RM589 linked to the gene *Bph-3* and RM3331 linked to the gene *Bph-18* in BC₂F₁ ICAB and ICBA lines

108 days with an average of 87.5 days. The line ICAB-11 produced 50% flowering at 108 days and the lines ICAB-2, ICAB-6, ICBA-6, and ICBA-16 produced 50% flowering at 80 days after sowing. The skewness and kurtosis in intercrossed BC_1F_1 lines for the number of days to 50% flowering were 1.628 and 2.004 respectively (Table 25).

The number of days to 50% flowering in BC_2F_1 lines ranged from 81 to 103 with an average of 86.63 days. The highest days to 50% flowering were in line ICBA-9/2 with the lowest being in line ICBA-6/2. The frequency distribution of BC_2F_1 lines for the number of days to 50% flowering showed positive skewness of 2.93 with a positive kurtosis of 8.71 (Table 26).

Number of tillers

The number of tillers in parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was 9, 8 and 7 numbers respectively. The number of tillers in intercrossed BC_1F_1 lines ranged from 5 to 9 with a mean value of 7.14 numbers. The highest number of tillers was produced by ICAB-5 and ICAB-11 and the lowest number of tillers was produced by ICAB-1 and ICAB-3. A negative skewness (-0.154) and kurtosis (-0.656) were observed in intercrossed BC_1F_1 lines for the number of tillers in intercrossed BC_1F_1 lines (Table 25).

The number of tillers in BC_2F_1 lines ranged from 6 to 9 with a mean of 7.77 numbers. The highest number of tillers was produced by ICAB-1/6 and ICBA-3/2 and the lowest was produced by ICBA-3/4. The frequency distribution of BC_2F_1 lines for the number of tillers showed negative skewness of -0.33 with a positive kurtosis of 0.26 (Table 26).

Number of productive tillers

The parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 produced 8, 4 and 6 numbers of productive tillers. The intercrossed BC_1F_1 lines produced a range of 3 to 8 productive tillers, in which ICAB-5 produced the highest and ICAB-3 and ICAB-7 produced the lowest number of productive tillers. The frequency distribution of the number of productive tillers in intercrossed BC_1F_1 lines showed positive skewness (0.133) and negative kurtosis (-0.089) (Table 25).

The number of productive tillers in BC₂F₁ lines ranged from 4 to 8 with a mean of 6.36 numbers. The highest number of productive tillers was produced by ICAB-1/6 and ICBA-3/2 and the lowest was produced by ICBA-9/2. The frequency distribution of BC₂F₁ lines for the number of tillers showed negative skewness of -0.59 with a positive kurtosis of 0.03 (Table 26).

Panicle length

The panicle length in parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was 23, 27 and 19 cm respectively. The panicle length in intercrossed BC₁F₁ lines ranged from 19 to 35 cm with an average of 23.67 cm. The highest panicle length was recorded in line ICBA-9 and the lowest in line ICAB-10. A positive skewness (1.344) and kurtosis (4.053) were observed in intercrossed BC₁F₁ lines for panicle length (Table 25).

The panicle length in BC₂F₁ lines ranged from 21.5 to 27 cm with a mean value of 24.61 cm. The maximum panicle length was observed in ICBA-3/3 and the minimum length in ICAB-1/1. The skewness of frequency distribution was -1.29 with a kurtosis of 2.17 for panicle length in BC₂F₁ lines (Table 26).

Number of grains per panicle

The parental genotypes Jyothi, PTB-33, and IR65482-7-216-1-2 produced 160, 135, and 88 grains per panicle respectively. The intercrossed BC₁F₁ lines produced a range of 93 to 158 grains per panicle, in which ICAB-5 produced the maximum and ICAB-15 produced the minimum number of grains per panicle. The frequency distribution of the number of grains per panicle in intercrossed BC₁F₁ lines showed negative skewness (-0.862) and positive kurtosis (0.230) (Table 25).

The number of grains per panicle in BC₂F₁ lines ranged from 117 to 155 with an average of 142.25 numbers. The highest number of grains per panicle was observed in line ICAB-1/1 and the lowest in line ICBA-2/1. The frequency distribution of BC₂F₁ lines for the number of grains per panicle showed negative skewness of -2.34 with a positive kurtosis of 6.67 (Table 26).

1000 grain weight (g)

The 1000-grain weight of parental genotypes Jyothi, PTB-33, and IR65482-7-216-1-2 was 28.8, 26.2, and 14 g respectively. The range of 1000-grain weight in intercrossed BC₁F₁ varied from 15.3 to 27.8 g with an average weight of 24.47 g. The maximum grain weight was observed in ICAB-19 and the minimum was observed in ICAB-15. The frequency distribution in intercrossed BC₁F₁ lines for 1000-grain weight showed negative skewness (-1.353) and positive kurtosis (1.493) (Table 25).

The 1000-grain weight in BC₂F₁ lines ranged from 22.6 to 28.3 g with a mean of 26.21 g. The maximum weight was observed in ICBA-6/1 and ICBA-3/4 and the minimum was recorded in ICBA-2/1. The frequency distribution of BC₂F₁ lines for the 1000-grain weight showed negative skewness of -3.31 with a positive kurtosis of 12.77 (Table 26).

Grain length (mm)

The grain length in parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was 8.95, 7.68 and 7.74 mm respectively. The grain length in intercrossed BC₁F₁ lines ranged from 6.98 to 8.74 mm with an average of 8.018 mm. The highest grain length was recorded in line ICBA-5 and the lowest in line ICAB-3. A negative skewness (-0.270) and kurtosis (-0.008) were observed in intercrossed BC₁F₁ lines for grain length.

The grain length in BC₂F₁ lines ranged from 7.76 to 8.64 mm with a mean value of 8.22 mm. The maximum grain length was observed in ICAB-1/6 and the minimum length in ICBA-10/1. The skewness of frequency distribution was -0.21 with a kurtosis of -0.60 for grain length in BC₂F₁ lines.

Grain breadth (mm)

The grain breadth in parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 were 2.68, 3.18 and 2.1 mm respectively. The grain breadth in intercrossed BC₁F₁ lines ranged from 2.08 to 3.1 with a mean value of 2.631 mm. The maximum grain breadth was observed in line ICAB-4 and the minimum was recorded in line ICBA-3. A negative skewness (-0.025) and kurtosis (-1.701) were observed in

intercrossed BC_1F_1 lines for the grain breadth in intercrossed BC_1F_1 lines (Table 25).

The grain breadth was maximum (3.07 mm) in BC_2F_1 line ICAB-1/5 and the minimum (2.23 mm) in ICBA-9/5 with a mean value of 2.62 mm. The frequency distribution of BC_2F_1 lines for the grain breadth showed positive skewness of 0.33 with a negative kurtosis of -0.29 (Table 26).

LB ratio

The LB ratio of parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 were 3.34, 2.42 and 3.69 respectively. The range of the LB ratio in intercrossed BC_1F_1 lines varied from 2.35 to 3.83 with an average of 3.091. The maximum LB ratio was observed in ICAB-6 and the minimum was observed in ICAB-3. The frequency distribution in intercrossed BC_1F_1 lines for the LB ratio showed negative skewness (-0.234) and kurtosis (-1.009) (Table 25).

The LB ratio in BC_2F_1 lines ranged from 2.59 to 3.62 with a mean value of 3.18. The maximum LB ratio was observed in ICBA-6/1 and the minimum in ICAB-1/5. The skewness of frequency distribution was -0.69 with a kurtosis of -0.21 for the LB ratio in BC_2F_1 lines (Table 26).

Kernel colour

The kernel colour of parental genotypes Jyothi, PTB-33 and IR65482-7-216-1-2 was red, red, and white respectively. The kernel colour in intercrossed BC_1F_1 lines showed variations with red, light red, dark brown, and white colours (Table 25).

In BC_2F_1 lines ICAB-1/5 and ICBA-3/1 had dark brown, ICBA-10/1 and ICBA-3/4 had light red and the remaining lines had red coloured kernels (Table 26).

4.5.4.3. Proximity dissimilarity analysis in intercrossed BC_1F_1 and BC_2F_1 using Euclidean distance

The proximity dissimilarity matrix was calculated in intercrossed BC_1F_1 and BC_2F_1 lines for the morphological characters except kernel colour using Euclidean distance. The coefficient of dissimilarity value of the intercrossed BC_1F_1 and BC_2F_1

lines with the recipient parent Jyothi was calculated and compared (Tables 27 and 28).

In intercrossed BC₁F₁ lines, the coefficient of dissimilarity values ranged from 6.711 to 69.588. The maximum coefficient of dissimilarity value of 69.588 was observed in ICAB-15 whereas the line ICAB-18 exhibited the minimum coefficient of dissimilarity value of 6.711. The coefficient of dissimilarity values in the intercrossed BC₁F₁ lines with a combination of both genes (*Bph-3* and *Bph-18*) in the heterozygous condition were observed as 12.786 (ICAB-5), 18.28 (ICBA-6), 19.001 (ICBA-10), 20.48 (ICAB-1), 24.968 (ICAB-19), 33.301 (ICBA-3), 47.859 (ICBA-2), and 52.54 (ICBA-9). These lines were forwarded to the development of BC₂F₁ lines.

In BC₂F₁ lines, the coefficient of dissimilarity values ranged from a minimum value of 5.972 in ICAB-1/1 to a maximum value of 49.008 in ICBA-9/2. The five BC₂F₁ lines with a combination of both genes (*Bph-3* and *Bph-18*) in the heterozygous condition had a minimum coefficient of dissimilarity value of 8.492 in ICAB-1/6 and a maximum coefficient of dissimilarity value of 26.343 in ICBA-9/3. Other three lines with gene combinations were observed with a coefficient of dissimilarity value of 9.411 in ICBA-10/2, 13.490 in ICBA-3/2, and 18.850 in ICAB-1/3. These five BC₂F₁ lines with a combination of both genes (*Bph-3* and *Bph-18*) in the heterozygous condition were forwarded to develop BC₂F₂ segregants.

4.5.4.4. The dendrogram based on the Euclidean distance of Intercrossed BC₁F₁ and BC₂F₁ lines

Dendrogram for the intercrossed BC₁F₁ lines and BC₂F₁ lines was constructed based on the Euclidean distance values of lines from the recipient Jyothi (Figures 3 and 4).

The intercrossed BC₁F₁ lines namely, ICBA-9, ICBA-2, ICBA-3, ICBA-10, ICBA-6, ICAB-19, ICAB-1, and ICAB-5 with both genes (*Bph-3* and *Bph-18*) in heterozygous condition clustered along with the recipient parent Jyothi indicating

Table 27. Proximity dissimilarity matrix analysis using morphological traits of intercrossed BC₁F₁ lines in comparison with Jyothi by Euclidean distance

Sl. No.	Intercrossed BC ₁ F ₁ Plants	Euclidean distance
1.	ICAB-1	20.48
2.	ICAB-2	42.129
3.	ICAB-3	42.082
4.	ICAB-4	51.546
5.	ICAB-5	12.786
6.	ICAB-6	15.313
7.	ICAB-7	55.092
8.	ICAB-10	40.24
9.	ICAB-11	57.238
10.	ICAB-12	18.834
11.	ICAB-13	24.598
12.	ICAB-14	64.277
13.	ICAB-15	69.588
14.	ICAB-18	6.711
15.	ICAB-19	24.968
16.	ICAB-20	66.562
17.	ICAB-21	12.937
18.	ICBA-2	47.859
19.	ICBA-3	33.301
20.	ICBA-4	38.971
21.	ICBA-6	18.28
22.	ICBA-7	42.527
23.	ICBA-9	52.54
24.	ICBA-10	19.001

25.	ICBA-11	66.647
26.	ICBA-13	50.103
27.	ICBA-14	42.135
28.	ICBA-15	34.071
29.	ICBA-16	29.16
30.	ICBA-17	40.935
31.	ICBA-18	13.895
	Jyothi	0.000
	PTB-33	71.074
	IR65482-7-216-1-2	74.417

Table 28. Proximity dissimilarity matrix analysis using morphological traits of BC₂F₁ lines in comparison with Jyothi by Euclidean distance

Sl. No.	BC₂F₁ lines	Euclidean distance
1.	ICAB-1/1	5.972
2.	ICAB-1/2	7.950
3.	ICAB-1/3	18.850
4.	ICAB-1/5	20.053
5.	ICAB-1/6	8.492
6.	ICAB-19/1	12.758
7.	ICBA-2/1	44.623
8.	ICBA-3/1	17.406
9.	ICBA-3/2	13.490
10.	ICBA-3/3	15.321
11.	ICBA-3/4	9.189
12.	ICBA-6/1	7.780
13.	ICBA-6/2	13.962
14.	ICBA-9/2	49.008
15.	ICBA-9/3	26.343
16.	ICBA-9/5	33.635
17.	ICBA-10/1	12.639
18.	ICBA-10/2	9.411
19.	ICBA-10/3	11.994
	Jyothi	0.000
	PTB-33	71.074
	IR65482-7-216-1-2	74.417

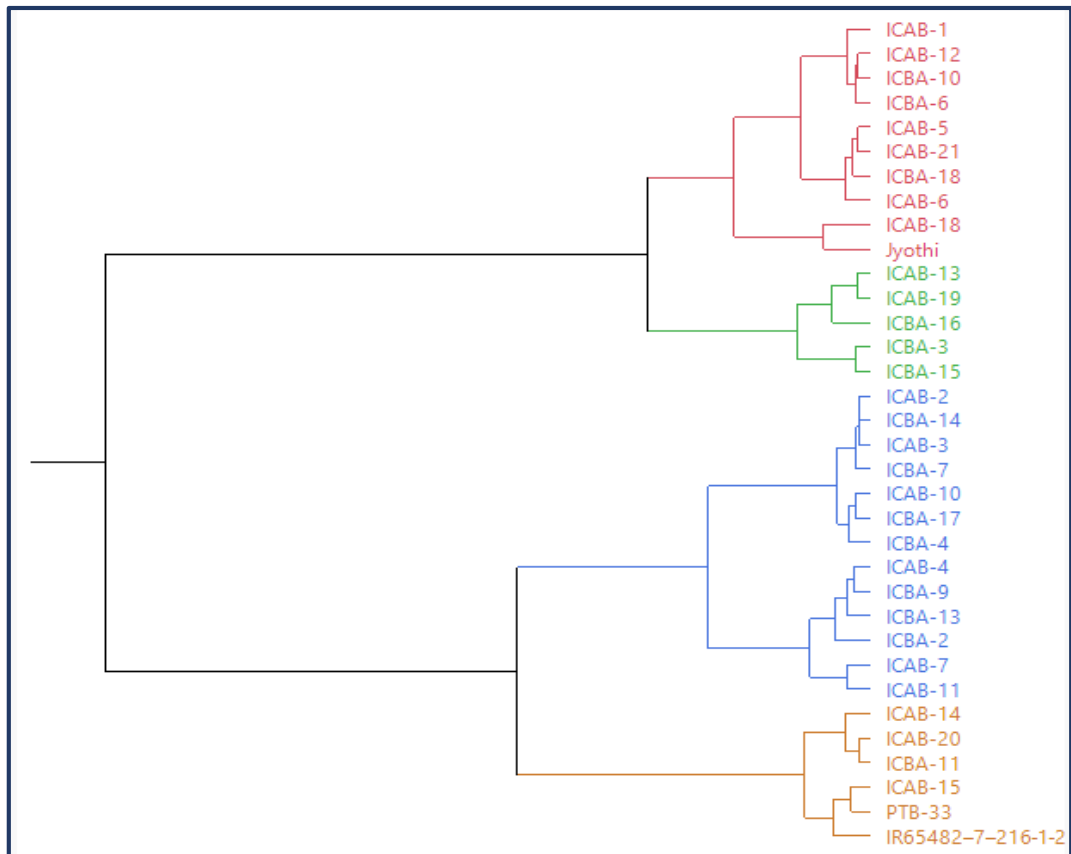


Figure 3. Dendrogram of intercrossed BC₁F₁ lines based on proximity dissimilarity matrix using morphological traits from the recipient parent Jyothi

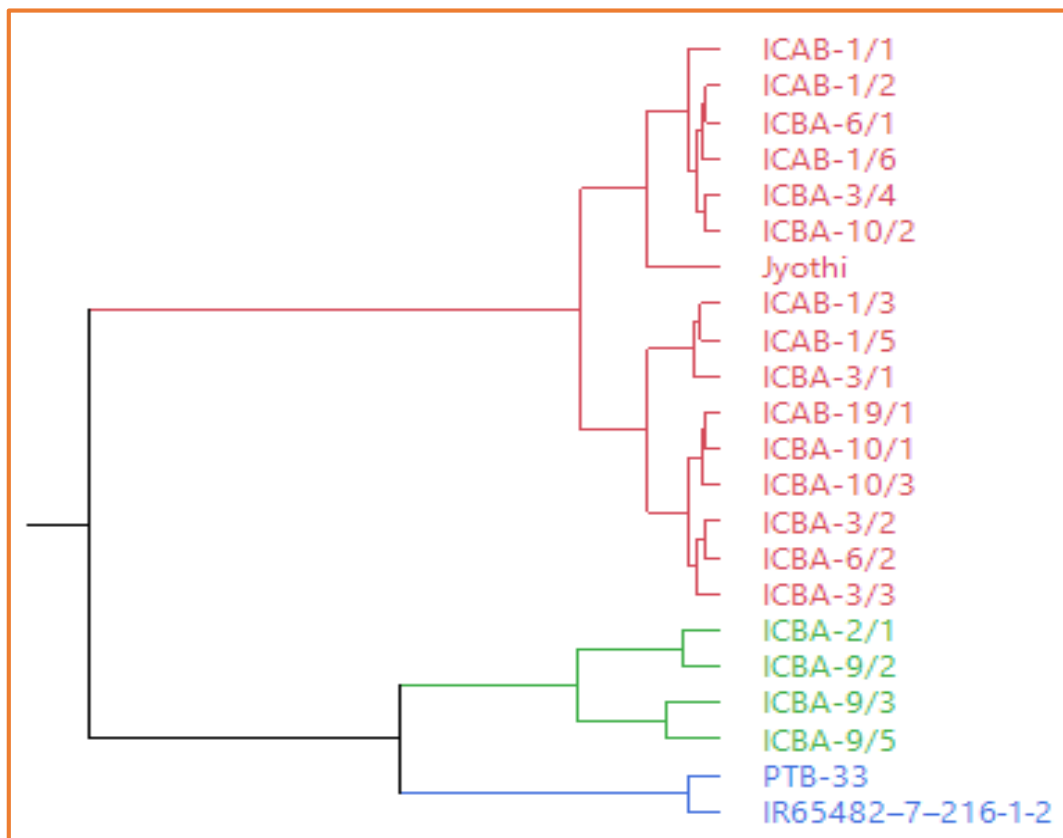


Figure 4. Dendrogram of BC₂F₁ lines based on proximity dissimilarity matrix using morphological traits from the recipient parent Jyothi

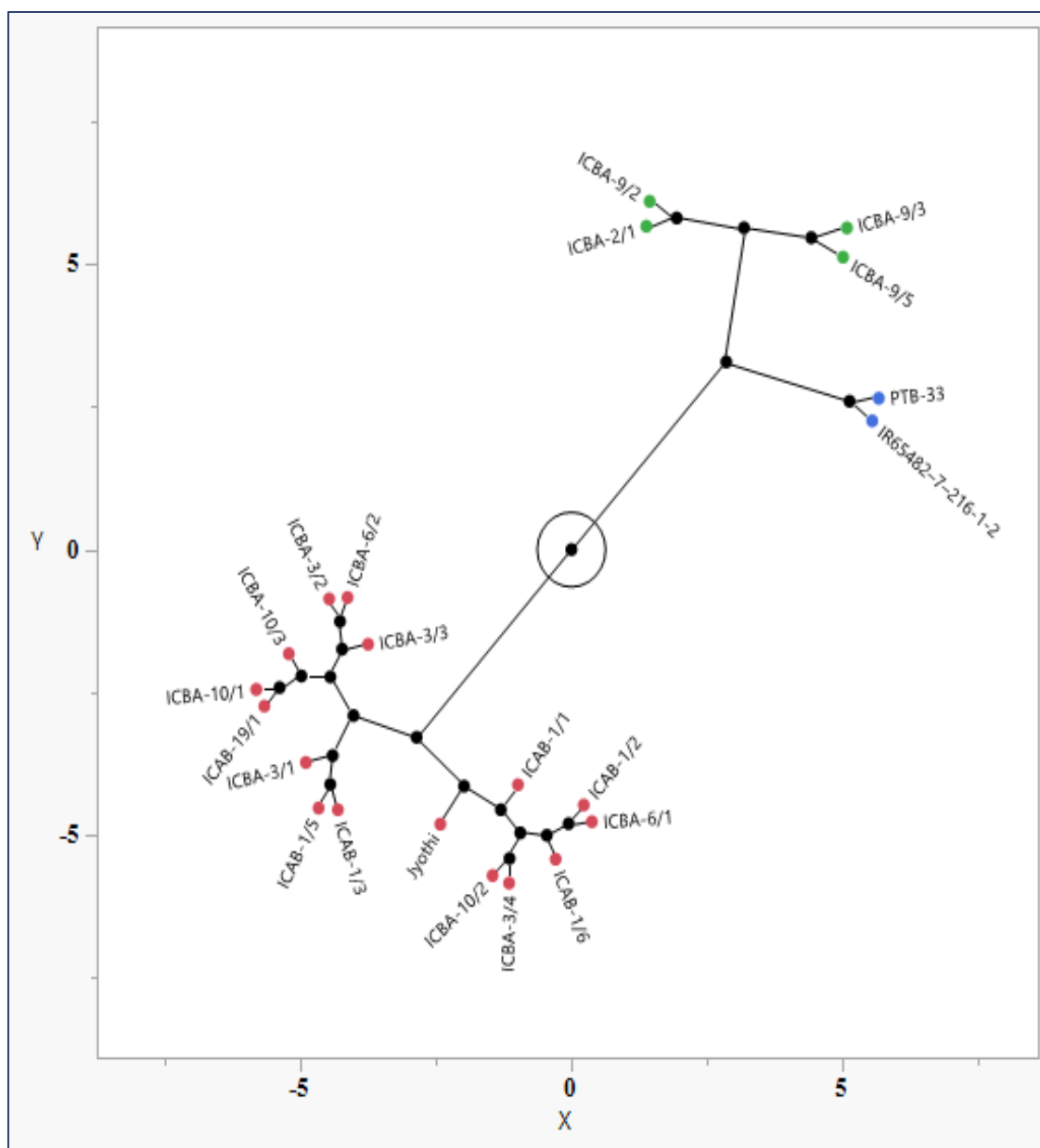


Figure 6. Constellation plot of the BC₂F₁ lines based on the proximity dissimilarity matrix using morphological traits

that these lines were more similar to the recipient parent. The clusters of intercrossed BC₁F₁ lines were also represented in the constellation plot (Figure 5).

The BC₂F₁ lines viz., ICAB-1/3, ICAB-1/6, ICBA-3/2, ICBA-9/3 and ICBA-10/2 with both genes (*Bph-3* and *Bph-18*) in heterozygous condition clustered along with the recipient parent Jyothi indicated that these lines were more similar to the recipient parent. The clusters of BC₂F₁ lines were also represented in the constellation plot (Figure 6).

4.5.5. Development of BC₂F₂ segregants and BPH bioassay

The five BC₂F₁ lines with a combination of both genes (*Bph-3* and *Bph-18*) in the heterozygous condition and maximum similarity in proximity dissimilarity matrix analysis were selfed, and 320 BC₂F₂ segregants were forwarded to BPH bioassay in the seedling stage at RARS, Pattambi, and COA, Vellayani (Table 29). The modified seedbox screening test was followed for BPH seedling screening. Eighty-nine BC₂F₂ segregants showed different levels of resistance in the BPH bioassay (Table 30). The remaining plants were susceptible and completely dried up due to BPH infestation (Plate 19a to 19d).

4.5.5.1. Genotyping of BC₂F₂ segregants

The eighty-nine BC₂F₂ lines that showed resistance reaction in the BPH bioassay were forwarded to genotyping using SSR markers RM589 and RM3331 specific for the genes *Bph-3* and *Bph-18* respectively (Plate 20a to 20c).

Genotyping of these lines obtained the combination of homozygous resistant loci of both genes (*Bph-3* and *Bph-18*) in four lines namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10.

4.5.5.2. Phenotyping of BC₂F₂ segregants

Morphological observations of BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6 lines with both genes (*Bph-3* and *Bph-18*) in homozygous resistant conditions were tabulated in Table 31 and Table 32 respectively.

Plant height

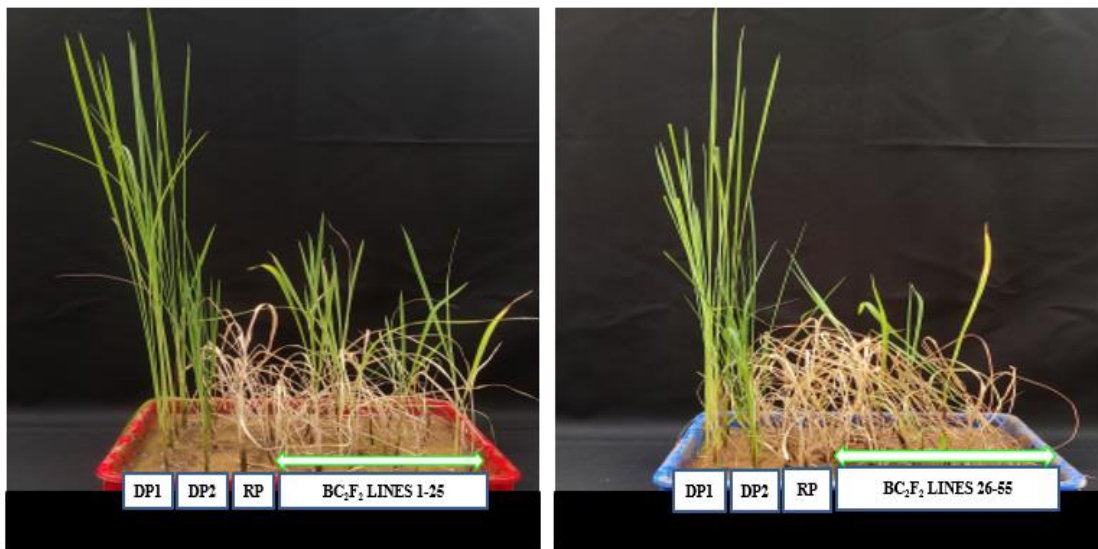
The plant height ranged from 86 to 97 cm in ICAB-1/3 progenies and from 90.5 to 98 cm in ICAB-1/6 progenies of BC₂F₂ segregants. The segregants with both

Table 29. BC₂F₂ lines forwarded to BPH bioassay

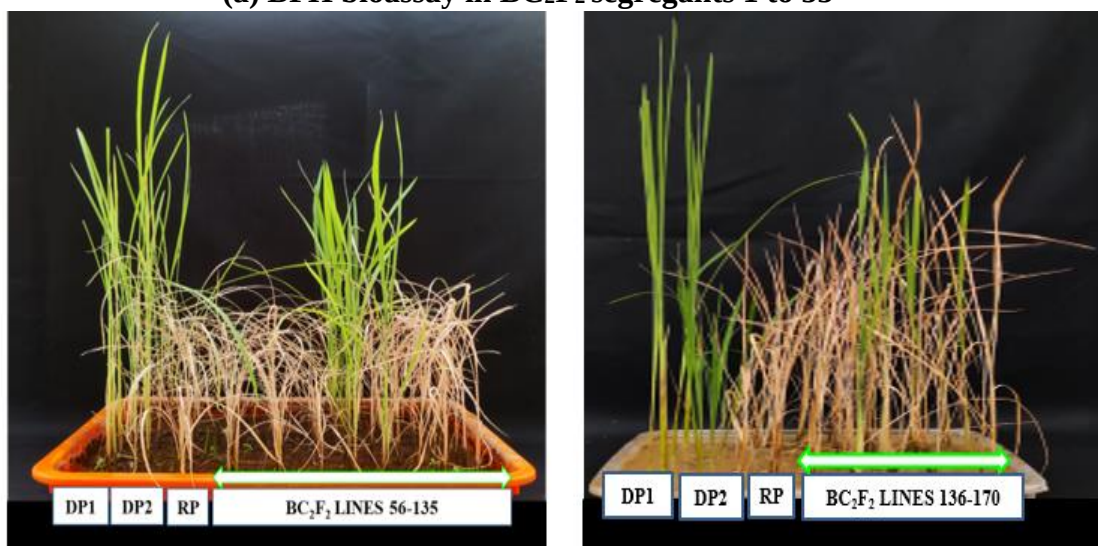
Sl. No.	BC ₂ F ₁ plants with two genes (Bph-3/Bph-18) in heterozygous condition	No. of plant progenies forwarded for BPH seedling screening
	Progeny No.	
1.	ICAB-1/3	60
2.	ICAB-1/6	70
3.	ICBA-3/2	50
4.	ICBA-9/3	60
5.	ICBA-10/2	80
	TOTAL	320

Table 30. BC₂F₂ segregants showing resistance in BPH bioassay

Sl. No.	Progeny No. of parent BC ₂ F ₁	No. of plants forwarded for BPH seedling screening	No. of BC ₂ F ₂ plants showing resistance in BPH seedling screening
1.	ICAB-1/3	60	14
2.	ICAB-1/6	70	20
3.	ICBA-3/2	50	21
4.	ICBA-9/3	60	18
5.	ICBA-10/2	80	16
	TOTAL	320	89



(a) BPH bioassay in BC₂F₂ segregants 1 to 55

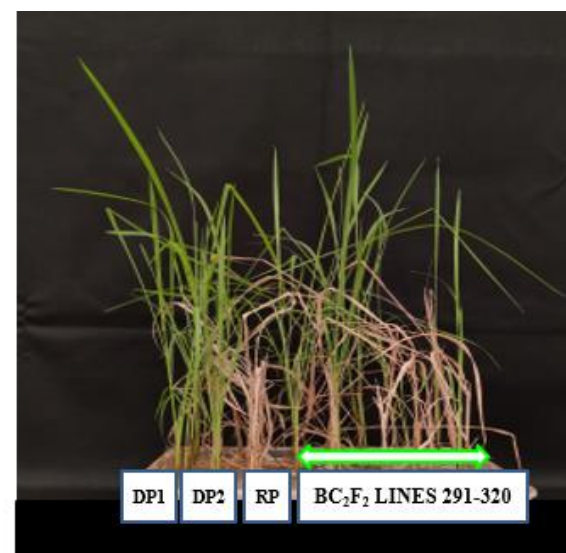
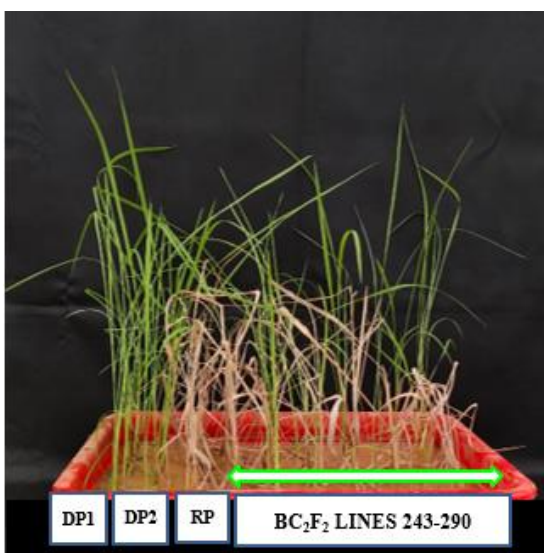
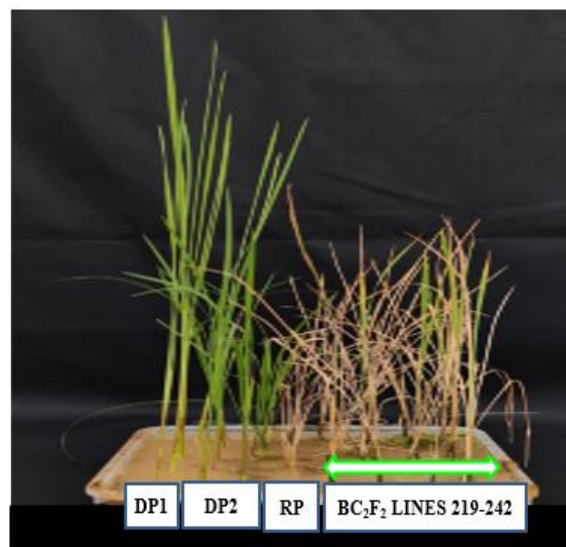
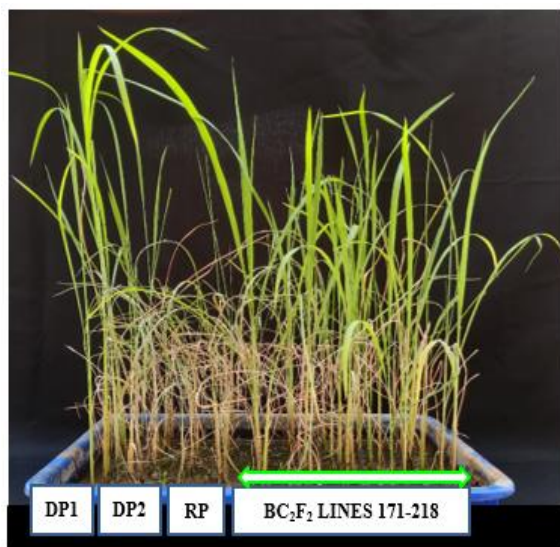


(b) BPH bioassay in BC₂F₂ segregants 55 to 170

DP1: PTB-33,

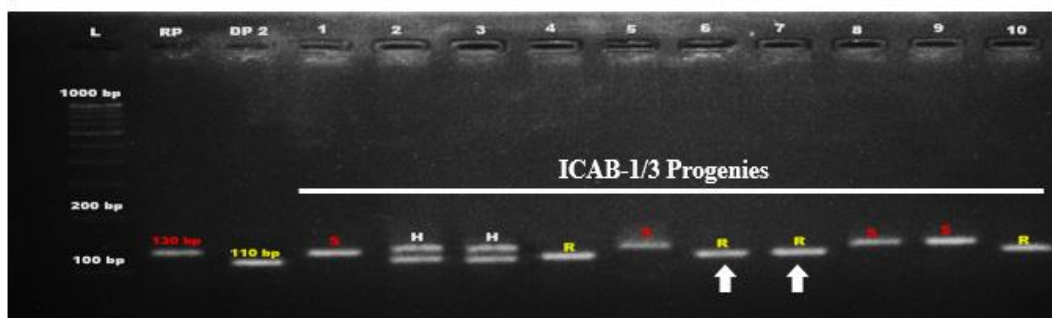
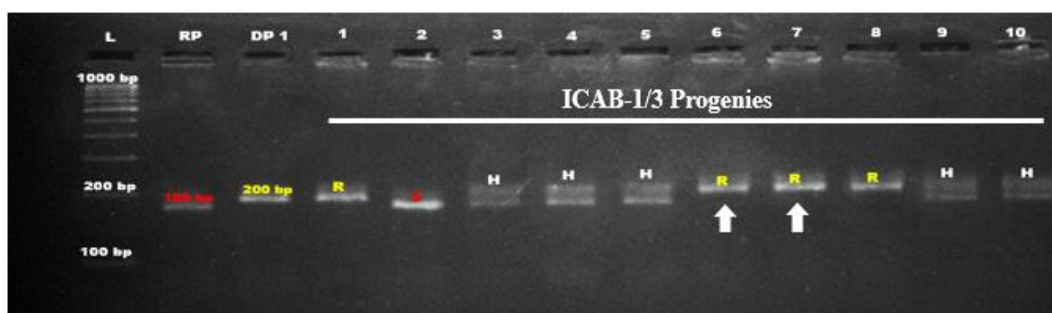
DP2: IR65482-7-216-1-2,

RP: JYOTHI

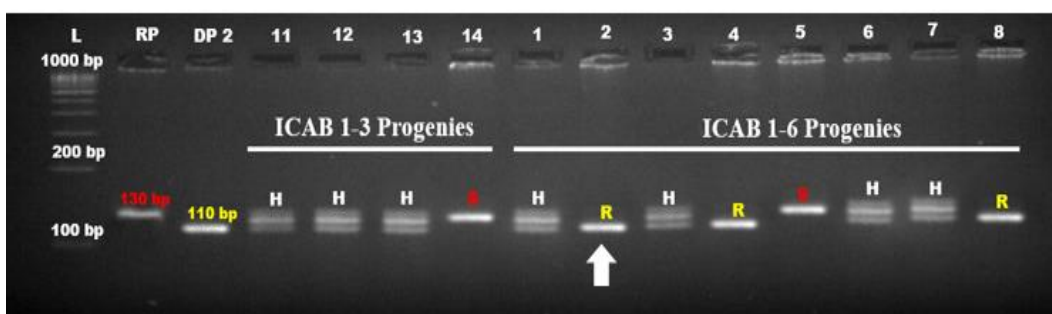
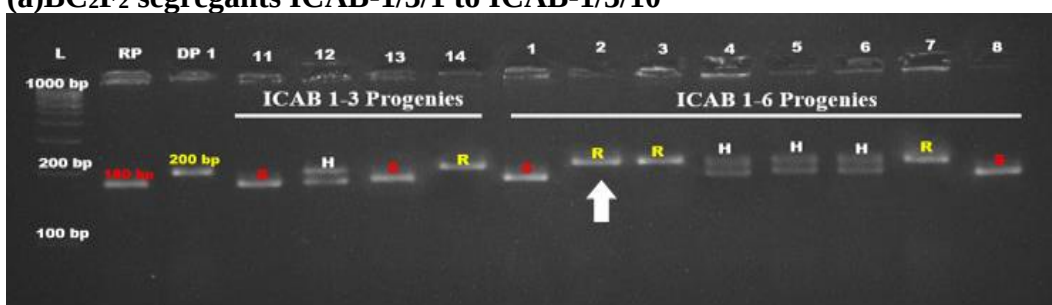


(c) BPH bioassay in BC₂F₂ segregants 171 to 242
 (d) BPH bioassay in BC₂F₂ segregants 243 to 320
 Plate 19 (cont.). BPH bioassay in BC₂F₂ segregants

DP1: PTB-33,	DP2: IR65482-7-216-1-2,	RP: JYOTHI



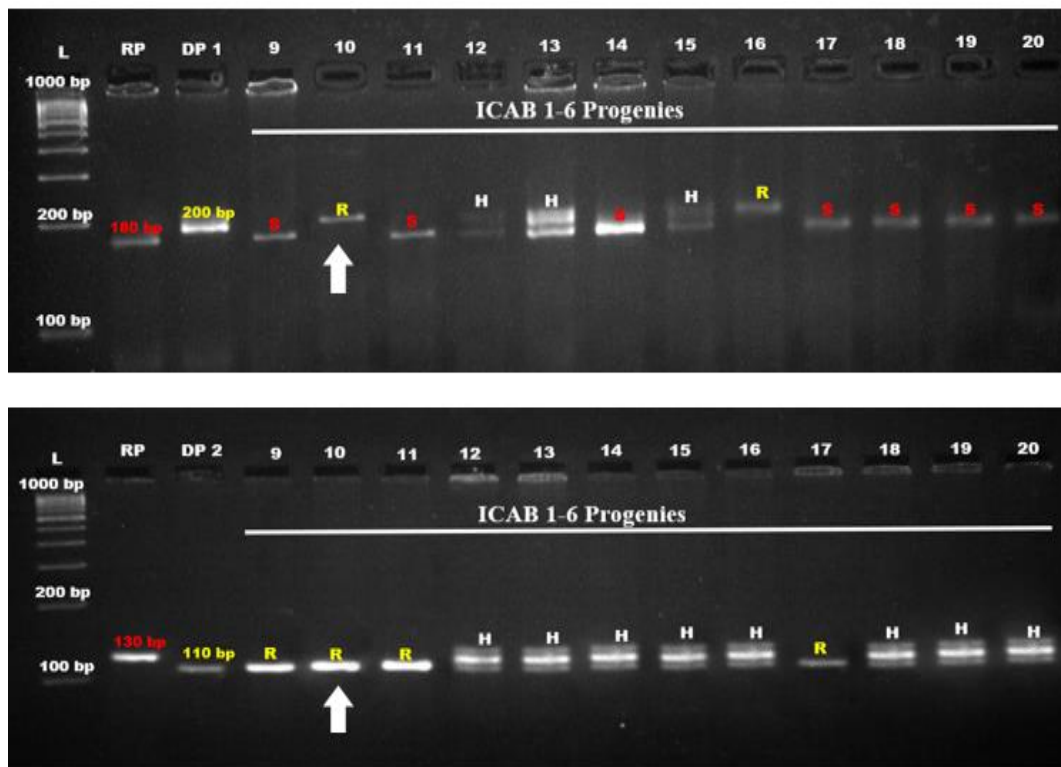
(a) BC₂F₂ segregants ICAB-1/3/1 to ICAB-1/3/10



(b) BC₂F₂ segregants ICAB-1/3/11 to ICAB-1/3/14, ICAB-1/6/1 to ICAB-1/6/8

R: Resistant ↑ **H: Heterozygous** **S: Susceptible**

Plate 20. Amplification profile of RM589 specific to *Bph-3* and RM3331 specific to *Bph-18* in BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6



(c) BC₂F₂ segregants ICAB-1/6/9 to ICAB-1/6/20

Plate 20. Amplification profile of RM589 specific to *Bph-3* and RM3331 specific to *Bph-18* in BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6

genes (*Bph-3* and *Bph-18*) in homozygous conditions viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 recorded the plant height of 90, 93, 94, and 91 cm respectively and that of Jyothi was 92 cm. The skewness of plant height in ICAB-1/3 progenies was -0.53 with a kurtosis of 0.25, whereas in the ICAB-1/6 progenies, the skewness was 0.08 and the kurtosis was -1.00.

Number of days to 50% flowering

The number of days taken to 50% flowering in Jyothi was 85 days and it ranged from 80 to 86 days in ICAB-1/3 progenies and from 80 to 87 days in ICAB-1/6 progenies. The number of days to 50% flowering in BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 was 84, 83, 83 and 82 respectively compared to 85 days in Jyothi. The ICAB-1/3 and ICAB-1/6 progenies showed a skewness of -0.36 and 0.35, and a kurtosis of -0.77 and -1.03 respectively.

Number of tillers

The progenies of ICAB-1/3 and ICAB-1/6 produced 7 to 9 tillers. Jyothi produced 9 tillers whereas the BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 subsequently produced 8, 9, 8 and 8 number of tillers. The ICAB-1/3 and ICAB-1/6 progenies had a skewness and kurtosis of 0.38 and -1.12, and 0.33 and -0.51 respectively.

Number of productive tillers

The number of productive tillers varied from 6 to 8 in the progenies of ICAB-1/3 and ICAB-1/6. The BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/6/2, and ICAB-1/6/10 produced 7 productive tillers whereas the ICAB-1/3/7 produced 8 number of productive tillers as Jyothi. The ICAB-1/3 and ICAB-1/6 progenies showed a skewness of 0.94 and 0.29, and a kurtosis of -0.78 and -0.61 respectively.

Panicle length

The length of the panicle in the recipient parent Jyothi was 23 cm. The panicle length of BC₂F₂ segregants ranged between 21.4 - 27.2 cm and 21.7-26.7 cm in ICAB-1/3 and ICAB-1/6 progenies.

Table 31. Phenotyping of BC₂F₂ segregants derived from ICAB-1/3

Sl. No.	Parent Progeny Number	Plant NO.	PH	NDFB	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
1.	ICAB-1/3	1	93.0	85	8	6	25.5	148	27.3	7.99	2.82	2.83	RED
2.		2	86.0	82	7	6	27.2	151	27.4	7.84	2.76	2.84	RED
3.		3	95.0	81	8	6	25.6	142	26.8	8.01	3.15	2.54	RED
4.		4	91.0	84	8	7	22.0	147	22.1	8.03	2.99	2.69	RED
5.		5	94.0	84	7	6	24.8	152	25.8	8.25	3.12	2.64	RED
6.		6	90.0	84	8	7	25.0	145	26.9	8.63	2.82	3.06	RED
7.		7	93.0	83	9	8	24.0	152	28.2	8.74	2.77	3.16	RED
8.		8	97.0	86	7	6	24.6	140	27.5	7.62	3.11	2.45	RED
9.		9	90.5	82	8	6	25.2	152	26.2	8.33	2.74	3.04	RED
10.		10	94.0	84	7	6	22.7	149	27.4	7.92	2.47	3.21	RED
11.		11	88.0	80	8	7	26.1	148	23.8	7.61	2.31	3.29	RED
12.		12	96.0	80	9	8	25.3	146	25.9	7.82	2.56	3.05	RED
13.		13	91.5	83	7	6	21.4	152	28.1	8.21	2.85	2.88	RED
14.		14	93.5	86	7	6	24.5	148	27.4	8.42	2.54	3.31	RED
JYOTHI			92.0	85	9	8	23.0	160	28.8	8.95	2.68	3.34	RED
Maximum			97.0	86	9	8	27.2	152	28.2	8.74	3.15	3.31	
Minimum			86.0	80	7	6	21.4	140	22.1	7.61	2.31	2.45	

Mean	92.3	83.27	7.8	6.6	24.46	148.8	26.64	8.16	2.78	2.95	
Std. Dev	2.93	1.94	0.77	0.83	1.58	4.79	1.74	0.39	0.25	0.29	
Variance	8.6	3.78	0.6	0.68	2.50	23.03	3.03	0.15	0.06	0.08	
Skewness	-0.53	-0.36	0.38	0.94	-0.49	0.34	-1.53	0.53	-0.12	-0.31	
Kurtosis	0.25	-0.77	-1.12	-0.78	-0.17	1.32	2.53	-0.44	-0.48	-1.06	
C V	3.17	2.34	9.93	12.54	6.47	3.22	6.53	4.88	8.86	9.71	

PH: PLANT HEIGHT(cm), **NDFF:** NUMBER OF DAYS TO 50% FLOWERING, **NT:** NUMBER OF TILLERS
NPT: NUMBER OF PRODUCTIVE TILLERS, **PL:** PANICLE LENGTH(cm), **NGPP:** NUMBER OF GRAINS PER PANICLE,
GW: GRAIN WEIGHT(g), **GL:** GRAIN LENGTH(mm), **GB:** GRAIN BREADTH(mm), **LBR:** LENGTH-BREADTH RATIO

Panicle length of 25 cm, 24 cm, 24.9 cm, and 24.3 cm was recorded in the BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 subsequently. A skewness and kurtosis of -0.49 and -0.17 were observed in ICAB-1/3 progenies while it was -0.35 and -0.90 in ICAB-1/6 progenies.

Number of grains per panicle

The recipient parent Jyothi produced 160 grains per panicle whereas, in BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6, the values ranged between 140-152 and 136-156 grains per panicle respectively. The BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 subsequently produced 145, 152, 152 and 150 grains per panicle. The skewness and kurtosis in ICAB-1/3 were 0.34 and 1.32 respectively, whereas ICAB-1/6 progenies recorded negative skewness (-0.42) and kurtosis (-0.49).

1000 grain weight

The 1000-grain weight of BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6 varied from 22.1-28.2 g and 22.7-28.5 g respectively. The recipient parent Jyothi had a 1000-grain weight of 28.8 g. The 1000-grain weight recorded in the BC₂F₂ segregants with two gene combinations (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 were 26.9 g, 28.2 g, 28.3 g, and 27.8 g respectively.

Grain length

The grain length in the recipient parent Jyothi was 8.95 mm. The grain length of BC₂F₂ segregants ranged between 7.61- 8.74mm and 7.71- 8.45 in ICAB-1/3 and ICAB-1/6 progenies respectively. Grain length of 8.63 mm, 8.74 mm, 8.31 mm, and 8.32 mm was recorded in the BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 subsequently. A skewness and kurtosis of 0.53 and -0.44 were observed in ICAB-1/3 progenies while it was 1.26 and 3.75 in ICAB-1/6 progenies.

Table 32. Phenotyping of BC₂F₂ segregants derived from ICAB-1/6

Sl. No.	Progeny Number	Plant No.	PH	NDFB	NT	NPT	PL	NGPP	1000 GW	GL	GB	LB Ratio	Kernel Colour
1.	ICAB-1/6	1	94.0	82	8	7	21.8	155	27.5	7.94	2.72	2.92	RED
2.		2	94.0	83	8	7	24.9	152	28.3	8.31	2.52	3.30	RED
3.		3	95.5	81	9	7	21.7	146	26.7	8.18	2.44	3.35	RED
4.		4	95.5	82	8	7	26.0	147	27.8	8.01	2.81	2.85	RED
5.		5	96.0	82	7	6	25.3	139	26.5	7.96	2.75	2.89	RED
6.		6	92.1	87	8	6	26.7	136	26.1	7.83	2.52	3.11	RED
7.		7	98.0	86	8	7	25.2	142	27.5	8.21	2.79	2.94	RED
8.		8	94.5	81	7	6	24.1	153	25.2	7.94	2.99	2.66	RED
9.		9	96.0	84	7	6	22.5	149	28.1	8.17	2.45	3.33	RED
10.		10	91.0	82	8	7	24.3	150	27.8	8.32	2.74	3.04	RED
11.		11	92.5	84	7	6	25.0	138	26.9	7.71	3.2	2.41	RED
12.		12	96.0	80	8	7	26.3	152	22.7	7.89	2.78	2.84	RED
13.		13	90.5	82	7	6	22.6	156	28.5	8.22	2.89	2.84	RED
14.		14	93.0	86	8	7	25.1	151	27.8	8.45	2.52	3.35	RED
15.		15	94.0	83	8	7	24.0	149	26.1	8.04	2.81	2.86	RED
16.		16	91.5	86	7	6	25.7	150	27.9	8.21	2.78	2.95	RED
17.		17	95.0	84	7	6	24.7	146	28.2	8.17	2.65	3.08	RED
18.		18	92.0	82	8	7	22.8	153	28.2	8.23	2.57	3.20	RED

19.		19	96.0	86	7	6	26.2	141	27.4	8.26	2.71	3.05	RED
20.		20	91.0	82	8	7	24.1	156	26.8	8.11	2.82	2.88	RED
	JYOTHI		92.0	85	9	8	23.0	160	28.8	8.95	2.68	3.34	RED
	Maximum		98.0	87	9	8	26.7	156	28.5	8.45	3.2	3.35	
	Minimum		90.5	80	7	6	21.7	136	22.7	7.71	2.44	2.41	
	Mean		93.81	83.33	7.71	6.62	24.38	148.62	27.18	8.15	2.72	3.01	
	Std. Dev		2.10	2.01	0.64	0.59	1.49	6.54	1.37	0.26	0.18	0.25	
	Variance		4.42	4.03	0.41	0.35	2.23	41.64	1.88	0.07	0.03	0.06	
	Skewness		0.08	0.35	0.33	0.29	-0.35	-0.42	-1.89	1.26	0.60	-0.36	
	Kurtosis		-1.00	-1.03	-0.51	-0.61	-0.90	-0.49	4.78	3.75	1.04	0.24	
	C V		2.24	2.41	8.34	8.91	6.14	4.34	5.05	3.17	6.74	8.19	

PH: PLANT HEIGHT(cm), **NDFF:** NUMBER OF DAYS TO 50% FLOWERING, **NT:** NUMBER OF TILLERS
NPT: NUMBER OF PRODUCTIVE TILLERS, **PL:** PANICLE LENGTH(cm), **NGPP:** NUMBER OF GRAINS PER PANICLE,
GW: GRAIN WEIGHT(g), **GL:** GRAIN LENGTH(mm), **GB:** GRAIN BREADTH(mm), **LBR:** LENGTH-BREADTH RATIO

Grain breadth

The grain breadth ranged from 2.31 to 3.15 mm in ICAB-1/3 progenies and from 2.44 to 3.2 mm in ICAB-1/6 progenies of BC₂F₂ segregants. The segregants with both genes (*Bph-3* and *Bph-18*) in homozygous conditions viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 recorded the grain breadth of 2.82, 2.77, 2.52, and 2.74 mm respectively and that of Jyothi was 2.68 mm. The skewness of grain breadth in ICAB-1/3 progenies was -0.12 with a kurtosis of -0.48, whereas in the ICAB-1/6 progenies, the skewness was 0.60 and the kurtosis was 1.04.

LB ratio

The LB ratio of BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6 varied from 2.45- 3.31 and 2.41 -3.35. The recipient parent Jyothi had a LB ratio of 3.34. The LB ratio recorded in the BC₂F₂ segregants with two gene combination (*Bph-3* and *Bph-18*) in homozygous resistant condition viz., ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 were 3.06, 3.16, 3.30, and 3.04 respectively.

Kernel colour

The kernel colour of the recipient parent Jyothi was red. All the BC₂F₂ segregants recorded the red coloured kernels.

4.5.5.3. Proximity dissimilarity analysis in BC₂F₂ segregants using Euclidean distance

The proximity dissimilarity matrix was calculated in BC₂F₂ segregants for the morphological characters except kernel colour using Euclidean distance. The coefficient of dissimilarity value and per cent similarity of the BC₂F₂ segregants of the progeny groups ICAB-1/3 and ICAB-1/6 which showed the combination of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition were calculated and compared in Table 33 and Table 34 respectively.

In ICAB-1/3 progenies, the coefficient of dissimilarity value of fourteen ICAB-1/3 progenies ranged from a minimum of 8.393 in ICAB-1/3/7 to a maximum value of 21.00 in ICAB-1/3/8.

Two BC₂F₂ segregants viz., ICAB-1/3/6 and ICAB-1/3/7 with gene combination of homozygous resistant loci of both genes (*Bph-3* and *Bph-18*) showed a coefficient of dissimilarity value of 15.486 and 8.393 respectively.

Table 33. Proximity dissimilarity analysis using morphological traits of BC₂F₂ segregants derived from ICAB-1/3 using Euclidean distance

Sl. No.	BC₂F₂ Plants	Euclidean distance
1.	ICAB-1/3/1	12.637
2.	ICAB-1/3/2	12.453
3.	ICAB-1/3/3	19.144
4.	ICAB-1/3/4	14.841
5.	ICAB-1/3/5	9.509
6.	ICAB-1/3/6	15.486
7.	ICAB-1/3/7	8.393
8.	ICAB-1/3/8	21.00
9.	ICAB-1/3/9	9.609
10.	ICAB-1/3/10	11.712
11.	ICAB-1/3/11	14.951
12.	ICAB-1/3/12	15.877
13.	ICAB-1/3/13	8.949
14.	ICAB-1/3/14	12.64
	Jyothi	0.000
	PTB-33	71.074
	IR65482-7-216-1-2	74.417

Table 34. Proximity dissimilarity analysis using morphological traits of BC₂F₂ segregants derived from ICAB-1/6 using Euclidean distance

Sl. No.	BC₂F₂ Plants	Euclidean distance
1.	ICAB-1/6/1	6.658
2.	ICAB-1/6/2	8.849
3.	ICAB-1/6/3	15.232
4.	ICAB-1/6/4	14.261
5.	ICAB-1/6/5	22.04
6.	ICAB-1/6/6	24.644
7.	ICAB-1/6/7	19.242
8.	ICAB-1/6/8	9.747
9.	ICAB-1/6/9	12.141
10.	ICAB-1/6/10	10.732
11.	ICAB-1/6/11	22.44
12.	ICAB-1/6/12	12.509
13.	ICAB-1/6/13	6.027
14.	ICAB-1/6/14	9.523
15.	ICAB-1/6/15	11.848
16.	ICAB-1/6/16	10.866
17.	ICAB-1/6/17	14.762
18.	ICAB-1/6/18	7.807
19.	ICAB-1/6/19	19.969
20.	ICAB-1/6/20	5.843
	Jyothi	0.000
	PTB-33	71.074
	IR65482-7-216-1-2	74.417

Among the twenty ICAB-1/6 progenies, ICAB-1/6/6 and ICAB-1/6/20 showed the maximum and minimum coefficient of dissimilarity values of 24.644 and 5.843 respectively. Two BC₂F₂ segregants viz., ICAB-1/6/2 and ICAB-1/6/10 with gene combination of homozygous resistant loci of both genes (*Bph-3* and *Bph-18*) showed a coefficient of dissimilarity value of 8.849 and 10.732 respectively.

4.5.8.3. The dendrogram based on the proximity dissimilarity matrix using morphological traits of BC₂F₂ segregants

Dendrogram for the BC₂F₂ segregants derived from the ICAB-1/3 and ICAB-1/6 progenies was constructed based on the proximity dissimilarity matrix using morphological traits of BC₂F₂ segregants from the recipient Jyothi (Figures 7 and 8). The four BC₂F₂ segregants namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10 with both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition clustered along with the recipient parent Jyothi indicated that these lines were more similar to the recipient parent. The clusters of ICAB-1/3 and ICAB-1/6 progenies were also represented in the constellation plot (Figure 9 and 10).

4.5.8.4. The recurrent parent phenotype recovery based on similarity per cent with the recipient parent

The per cent similarity of BC₂F₂ segregants derived from ICAB-1/3 and ICAB-1/6 with the recipient parent Jyothi based on the proximity dissimilarity matrix using morphological traits of BC₂F₂ segregants was calculated.

The BC₂F₂ segregants namely, ICAB-1/3/6 and ICAB-1/3/7 with both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition had a recipient parent phenotypic recovery of 84.51 per cent and 91.6 per cent (Plate 21, 22 and 23).

The BC₂F₂ segregants derived from the ICAB-1/6 progenies viz., ICAB-1/6/2, and ICAB-1/6/10 with both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition had a recipient parent phenotypic recovery of 91.15 per cent and 89.27 per cent (Plate 21, 22 and 23).

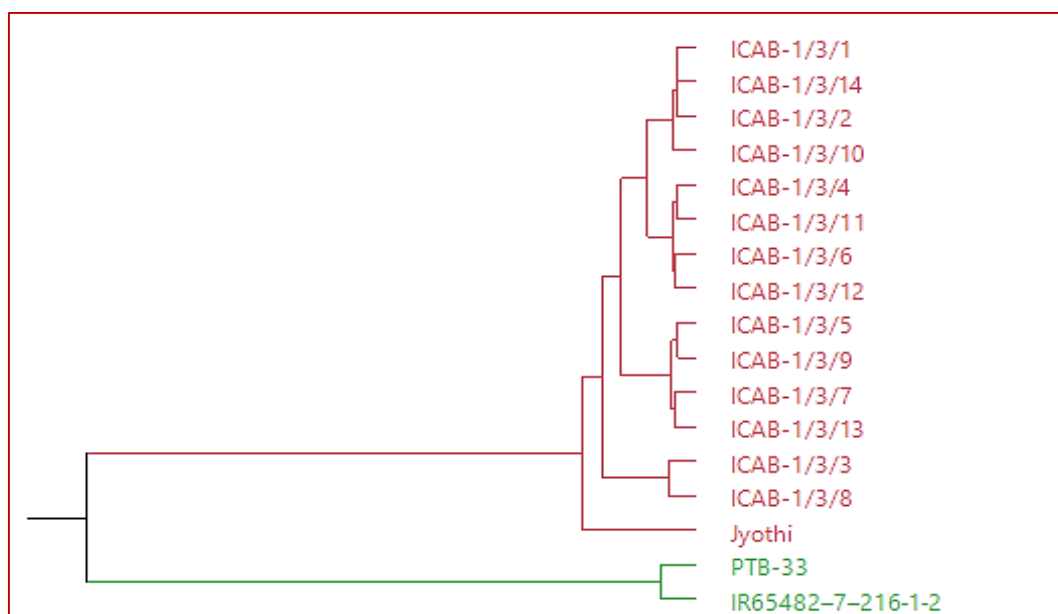


Figure 7. Dendrogram of the BC₂F₂ segregants derived from the ICAB-1/3 based on the proximity dissimilarity matrix using morphological traits

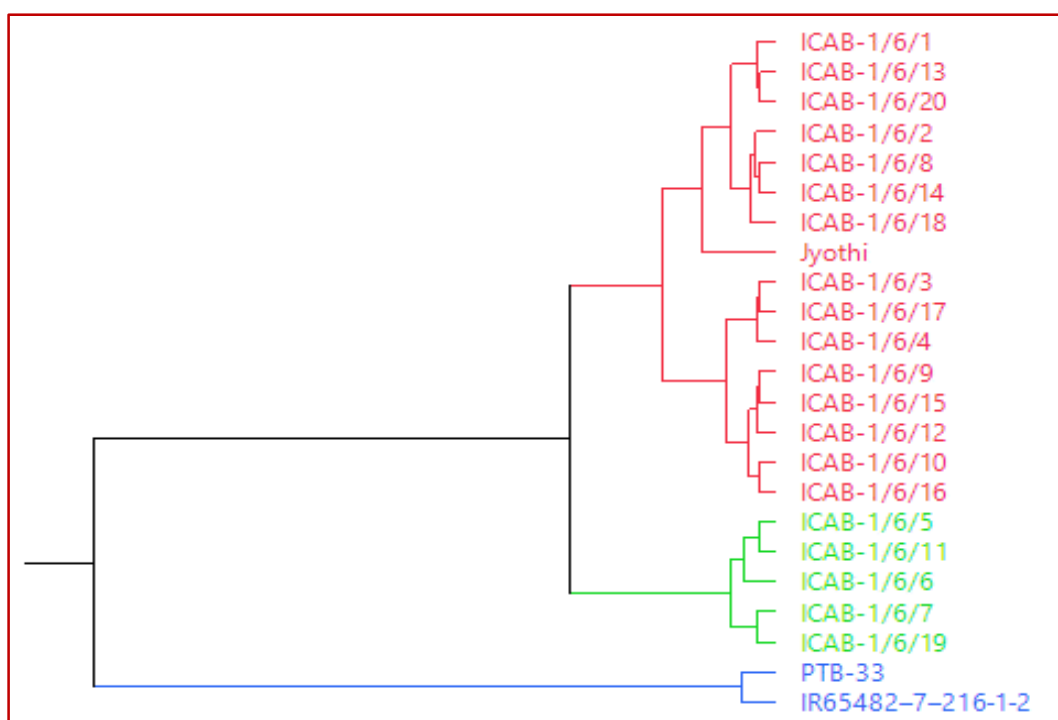


Figure 8. Dendrogram of the BC₂F₂ segregants derived from the ICAB-1/6 based on the proximity dissimilarity matrix using morphological traits

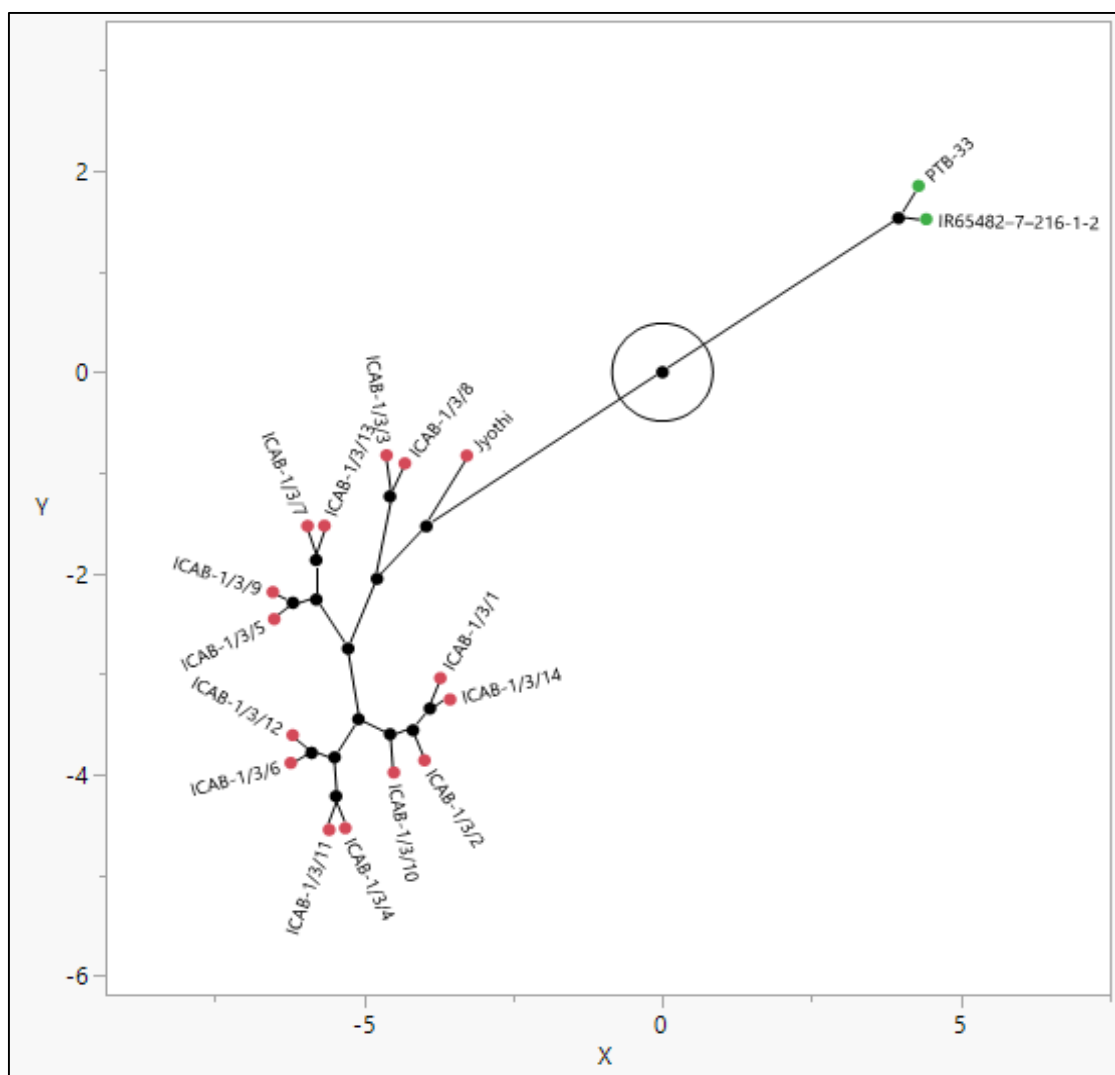


Figure 9. Constellation plot of the BC₂F₂ segregants derived from the ICAB-1/3 lines based on the proximity dissimilarity matrix using morphological traits

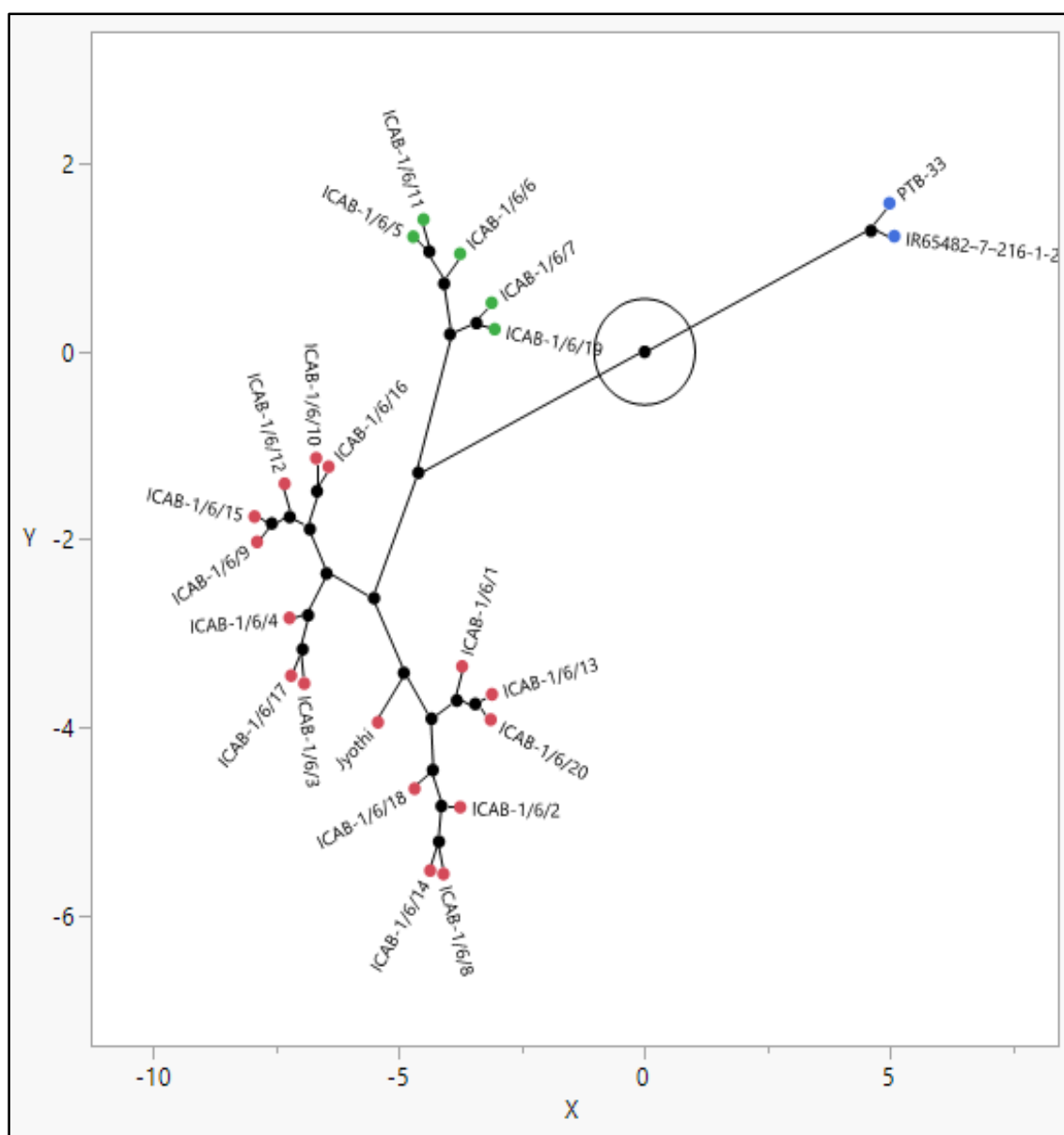


Figure 10. Constellation plot of the BC₂F₂ segregants derived from the ICAB-1/6 lines based on the proximity dissimilarity matrix using morphological traits



Plate 21. BC₂F₂ segregants with the combination of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition



Plate 22. Panicle of BC₂F₂ segregants with the combination of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition

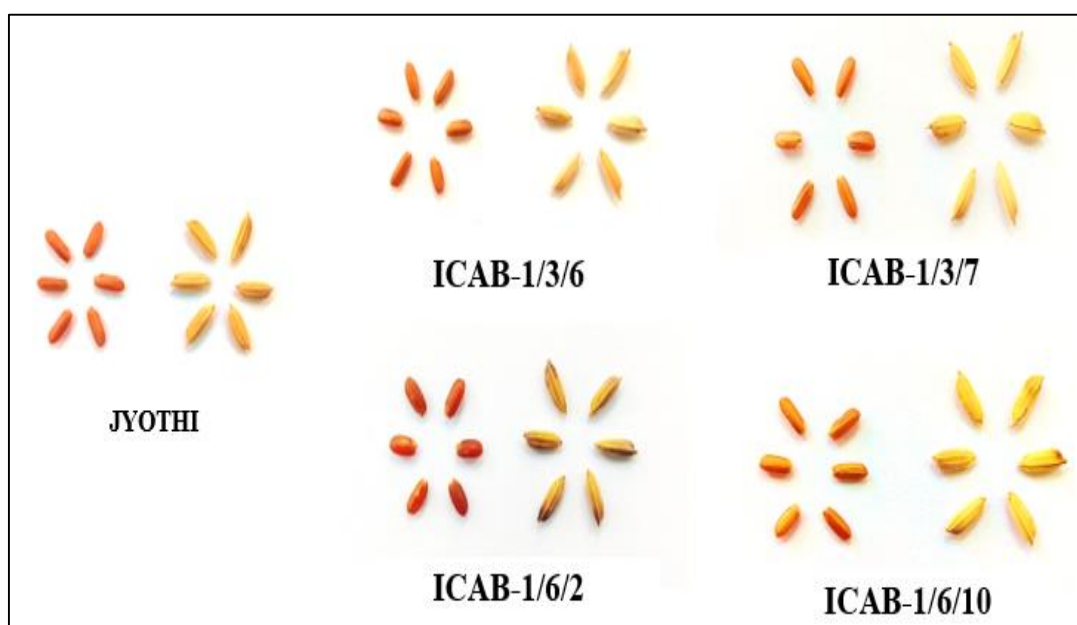


Plate 23. Seeds of BC₂F₂ segregants with the combination of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition compared with recipient parent Jyothi

DISCUSSION

5. DISCUSSION

The Brown plant hopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is increasingly becoming a serious pest in countries that grow rice due to its erratic invasion and severe damage. The brown plant hopper (BPH), a delphacid planthopper of the order Hemiptera, feeds directly by sucking phloem sap, ovipositing in plant tissues, and transmitting grassy stunt and ragged stunt viruses to host plants, which results in hopper-burn symptom and severe yield loss for rice farmers. Employing excessive chemicals to combat BPH is unsafe for human health, not cost-effective, and detrimental to the environment and biodiversity. As an alternative, creating a persistent host-plant resistance mechanism for BPH control is the most reliable, accurate, economical, and environmentally benign course of action. One of the main concerns is the BPH's rapid adaptation to resistant varieties. Therefore, it is necessary to create resistance to BPH using new major and minor genes in an efficient manner in order to achieve broad-spectrum durable resistance.

Among the 45 genes that impart resistance to BPH, 37 are dominant and 8 are recessive and are identified in several wild and cultivated varieties. Almost all the major genes are located on chromosomes 3, 4, 6, and 12 (Carsono *et al.*, 2023). Molecular markers are used to identify and detect the genes for resistance in parent genotypes and in the breeding program. Marker-assisted backcross breeding facilitates the detection and introgression of R genes thus aiding in the development of novel breeding lines and the improvement of existing high-yielding but susceptible varieties (Kamal *et al.*, 2023). Marker-assisted selection can reduce the time required for a variety development by facilitating the hybridization of selected plants within a single season, screening further generations in greenhouses, or off-season nursery breeding (Dormatey *et al.*, 2020).

Pyramiding multiple resistance genes into a single rice cultivar can provide sustainable control of insect pests. The pyramided lines with two to three pyramided R genes providing resistance to BPH have shown additive effects with an order of effectiveness of three-pyramided genes, two pyramided genes followed by a single gene (Muduli *et al.*, 2023). The presence of multiple resistance genes can impart

resistance to different biotypes of BPH. This is considered effective as similar to vertical resistance. The majority of the genes are tracked using SSR markers for foreground, recombinant, and background selection in the segregating generations in order to develop or improve a variety with 3 to 4 backcrosses (Kamaluddin *et al.*, 2022).

In recent years, Kerala had witnessed widespread BPH outbreaks, and the popular high-yielding variety Jyothi was found susceptible to BPH. The present study ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ was put forth in order to improve the genetic background of Jyothi by marker-assisted pyramiding of R-genes. A few probable donors from the results of a concluded project in the Department of Plant Breeding and Genetics, COA, Vellayani, and IRRI lines introgressed with R genes were selected and have undergone BPH bioassay. The morphological and biochemical characterization, and gene validation were carried out in recipient parent Jyothi and the resistant genotypes whose resistance was confirmed in the BPH bioassay study. The selected donors were forwarded to the marker-assisted breeding program for achieving the objective to introgress R genes for resistance to BPH in the background of the elite variety Jyothi using marker-assisted selection. This chapter delves into the discussion of the results along with illustrations, substantiating them with reasons and findings.

5.1 BIOASSAY FOR BPH RESISTANCE

5.1.1 Seedling screening

In this investigation, twelve rice genotypes including a *japonica* cultivar is screened using the modified seedbox screening test (MSST). Being an efficient method for evaluating genotype response to BPH attack, the MSST is extensively used for BPH screening by IRRI and rice scientists (Velusamy *et al.*, 1986; Rath *et al.*, 2022). The damage scores of rice genotypes in BPH seedling screening are illustrated in Figure 11. The results showed that the universal donor and resistant check variety PTB-33, and the varieties PTB-21, and IR7103-121-15-B with a score

of '0-no injury' showed the highest seedling survival rate against BPH. The KAU variety 'PTB-33', traditionally grown in southern India (especially in the Palakkad

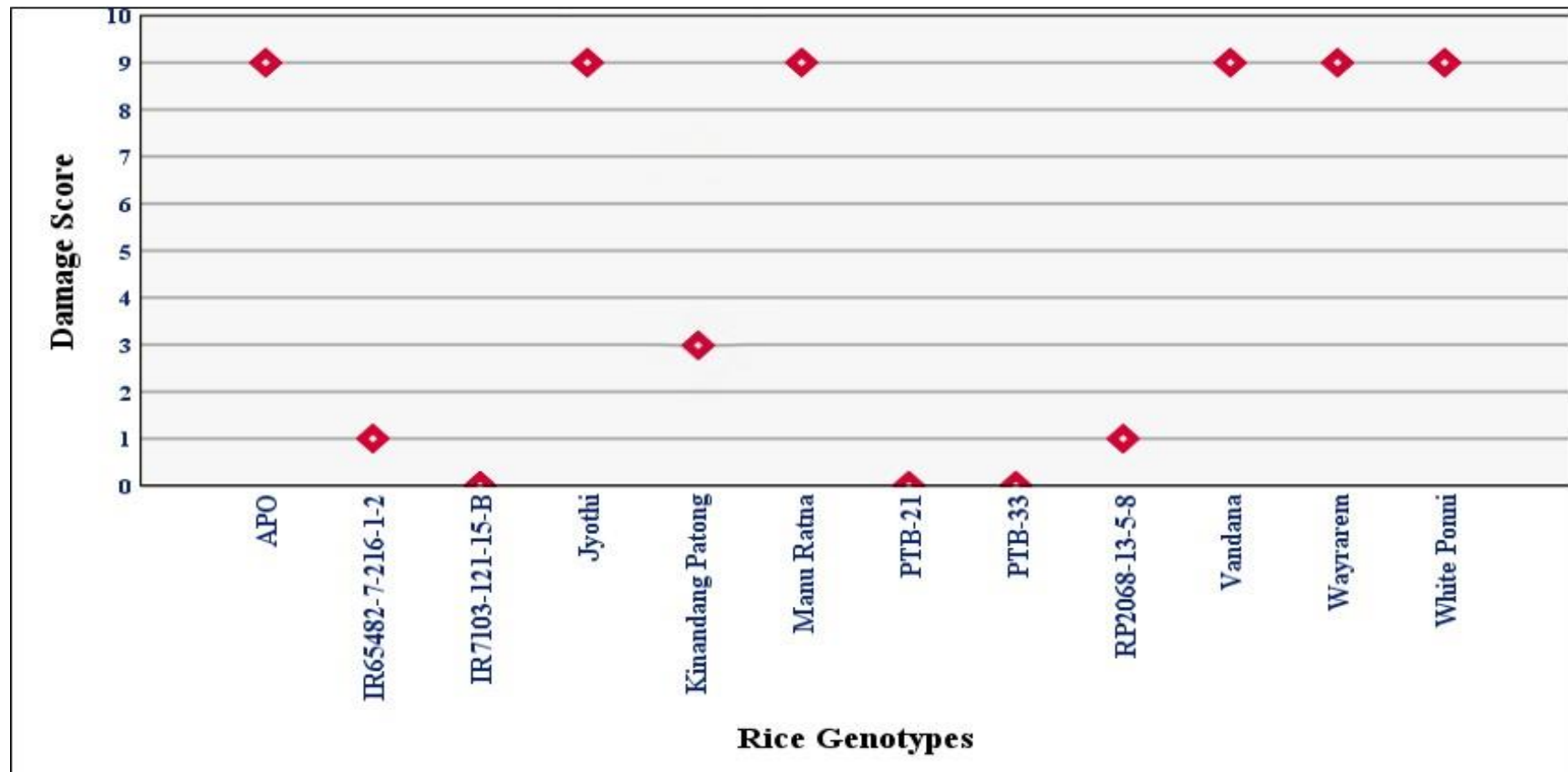


Figure 11. The damage scores of rice genotypes in BPH seedling screening

district of Kerala) can be exploited in breeding programs for transferring its inherent resistance capacity against BPH. *Bph-3*, the major gene in the PTB-33 variety, is the known cause for imparting resistance against BPH biotypes prevailing in India and worldwide (Kumar and Kunhamu, 2021; Carsono *et al.*, 2023). The long duration, photosensitivity, and comparatively less yield are the major reasons for the less preferred cultivation of PTB-33 in Kerala and other parts of India. Such a promising resistant character shown by the PTB-33 needs to be well harnessed for developing breeding lines for imparting resistance against BPH. The KAU variety PTB-21 and the IRRI introgression line IR7103-121-15-B were also reported as promising donors for BPH resistance (Kumar and Kunhamu, 2021; Thulasinathan *et al.*, 2020).

The genotypes IR65482-7-216-1-2 and RP2068-18-3-5 with a scale of ‘1’ were classified as resistant to BPH infestation. The breeding line RP2068-18-3-5, the IRRI introgression lines IR65482-7-216-1-2 (donor of *Bph-18* gene), and IR7103-121-15-B (donor of *Bph-20* and *Bph-21* gene) were reported resistant in seedling screening tests and can be used potential donors (Jena *et al.*, 2015; Naik *et al.*, 2018; Li *et al.*, 2023). The *japonica* variety Kinandang Patong was moderately resistant to BPH with a scale of ‘3’. The recipient parent Jyothi and five other genotypes *viz.*, Manu Ratna, White Ponni, Wayrareem, Vandana, and APO with a scale of ‘9’ were classified as highly susceptible to BPH infestation. The susceptibility of high-yielding varieties to BPH can be due to the plant characters making it more preferred for BPH feeding along with the continuous cultivation of a single variety in a region, agronomic practices such as the use of excess nitrogenous fertilizers, closer planting without proper spacing, and climatic conditions (Bottrell and Schoenly, 2012; Rezvi *et al.*, 2023).

5.1.2. BPH resistance reaction

Plant-based responses based on the mechanism of insect resistance can be classified as antixenosis, antibiosis, and tolerance. The antibiosis and tolerance mechanisms of BPH resistance were explored in terms of nymphal survival rate, honey-dew test, functional plant loss index (FPLI), and tolerance index (TI). Five

genotypes, Jyothi (recipient parent), PTB-33, IR7103-121-15-B, IR65482-7-216-1-2, and RP2068-18-3-5 were forwarded for studying the BPH resistance reaction.

Reduction in the survival rate of BPH nymphs in rice varieties was evident in the varieties PTB-33, IR65482-7-216-1-2, IR7103-121-15-B, and RP2068-18-3-5 which showed the antibiosis activity possessed by these varieties. Similar antibiosis activity was reported for these rice genotypes against BPH infestation (Ji *et al.*, 2016; Thamarai and Soundararajan, 2017; Jiang *et al.*, 2018; Udayasree *et al.*, 2021 and Sukumar *et al.*, 2022). The increased rate of BPH nymph survival on Jyothi indicated that the variety lacked the factors that prevent BPH nymphal growth and feeding and also possess factors that can attract BPH or induce feeding.

Sustained feeding by BPH on rice results in a large amount of honeydew excretion and this honeydew deposition can be used as a tool to measure BPH feeding and the host plant's resistance to BPH attack (Paguia *et al.*, 1980). The difference in honeydew excretion amount in resistant and susceptible varieties might be used as an indirect index of the degree of resistance by the host plant and also the feeding rate of BPH (Zhang *et al.*, 2022). The variety 'Jyothi' turned out to be the highly susceptible among the rice varieties screened. The genotypes IR7103-121-15-B, RP2068-18-3-5, and IR65482-7-216-1-2 showed few honeydew spots. The variety 'PTB-33' showed high resistance to BPH feeding as no honeydew spots were observed in any replicates. These findings were consistent with the previous investigations which concluded that the resistant genotypes produced less amount of honeydew spots compared to the susceptible genotypes (Harini *et al.*, 2013; Prahalada *et al.*, 2017; Tenguri *et al.*, 2023).

The ability of the plant to grow and yield despite BPH attacks reveals the tolerance level of a genotype. A measure of tolerance level is necessary for a rice breeder to develop stable resistance to BPH by successfully utilizing the tolerance mechanism. The functional plant loss index was developed by Panda and Heinrichs (1983) in which the components of resistance were categorized into four groups based on high or low tolerance and antibiosis.

In this experiment, PTB33, IR65482-7-216-1-2, RP2068-18-3-5, and IR7103-121-15-B were found with the lowest FPLI values and high tolerance

against BPH attack. These genotypes were categorized under the group ‘High tolerance, high antibiosis’ as per the FPLI graph. These results were substantiated by the tolerance study done by Sarao and Bentur (2016) who reported similar findings in PTB33 and RP2068-13-5-8. The variety Jyothi with the highest FPLI value of 71.52 per cent showed the least tolerance against BPH attack and was categorized under the group ‘Low tolerance, low antibiosis’ as per the FPLI graph. These results were corroborated by the findings of Panda and Heinrichs (1983), Sarao and Bentur (2016), Prahalada *et al.* (2017), Pati *et al.* (2019), Horgan *et al.* (2021), Sandhu and Sarao (2022) in which the susceptible test entries were observed with high FPLI and high BPH dry weight values whereas resistant test entries with high tolerance and antibiosis showed low FPLI value and low BPH dry weight. The resistant genotypes used in the study, especially the KAU variety PTB-33 with lowest FPLI value and BPH dry weight can be well exploited through various breeding programs for developing new or improving the existing varieties.

The tolerance index as a measure of the tolerance level of the rice genotypes was calculated based on the ratio of BPH dry weight gain on the test genotype to the susceptible genotype (Panda and Heinrichs, 1983). The susceptible recipient parent Jyothi showed the tolerance index value ‘1’ which infers that the dry weight gain was maximum in this variety as a result of sustained feeding. The KAU variety PTB-33 gave the minimum value of tolerance index (0.24) as the dry weight gain of BPH was very less as a result of minimal feeding of the BPH on it. Prahalada *et al.* (2017) also reported that the variety PTB-33 obtained the least tolerance index value and the susceptible genotypes showed a maximum tolerance index value of up to 1.

5.2 MORPHOLOGICAL EVALUATION OF PARENTS

The analysis of variance showed a highly significant difference among the genotypes for all the traits studied implicating the presence of genetic variability among the genotypes. The Jyothi had a maximum number of productive tillers, culm thickness, number of grains per panicle, 1000 grain weight, and grain length whereas PTB-33 recorded maximum plant height, number of days to 50% flowering, panicle length, and grain breadth. The yield and yield-related traits in the

recipient but susceptible parent Jyothi were significantly higher compared to the probable donor parents in this study. The kernel colour of Jyothi and PTB-33 was red while the IRRI introgression lines IR65482-7-216-1-2 and IR7103-121-15-B, and breeding line RP2068-18-3-5 were white-kernelled genotypes.

The cross-section of culm in parental genotypes was analyzed to study the internal anatomical differences if any among the genotypes. The anatomical structure observed under the microscope included the epidermis layer in the outermost region followed by vascular bundles. The presence of sclerenchyma followed by parenchyma and vascular bundles was observed in all the genotypes. The variety PTB-33 possessed a distinguishable thickened appearance of cells at the sclerenchymatous region compared to the culm cross-section of susceptible parent Jyothi. Prominence in the sclerenchymatous region was also observed in other resistant genotypes viz., IR65482-7-216-1-2, IR7103-121-15-B, and RP2068-18-3-5. Harder and thicker sclerenchyma regions may inhibit the stylet penetration on BPH feeding. The previous study done by Shi *et al.* (2021) reported that sclerenchyma acts as a mechanical and protective barrier against BPH stylet probing thus preventing the phloem-feeding. Commensurable findings were documented by Zhang *et al.* (2022) that the genotypes resistant to BPH exhibited thicker sclerenchyma and vascular bundle cells thus preventing the stylet penetration.

5.3 BIOCHEMICAL PARAMETERS IN PARENT GENOTYPES BEFORE AND AFTER BPH INFESTATION

The variations in biochemical parameters in parent genotypes before and after BPH infestation are illustrated in Figure 12a to 12d. The biochemical compounds such as protein content, ascorbic acid, and reducing sugar decreased to a varied extent in all the genotypes after BPH infestation. The reduction in protein content was higher in Jyothi (susceptible parent) when compared to other varieties (resistant parents). The slight reduction of protein and sucrose content in BPH-infested rice plants was reported by Liu *et al.* (2008) and Wang *et al.* (2008). Reducing sugar content in rice is more affected by BPH infestation (Vanitha *et al.*, 2011). BPH feeding was inhibited in rice plants with higher concentrations of

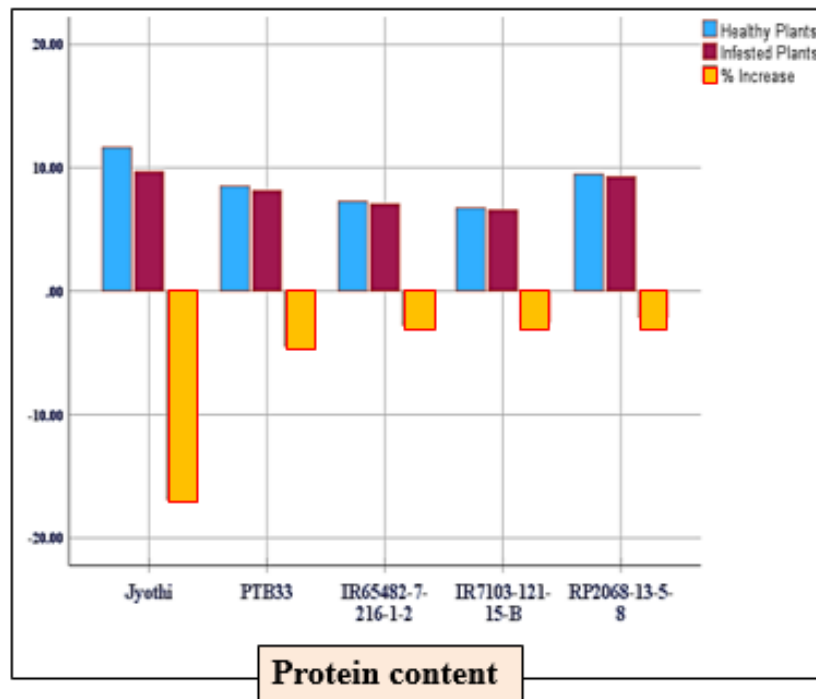
ascorbic acid than in lower concentrations (Sakai and Sogawa, 1976; Ramulamma *et al.*, 2017). The phenol content was decreased in Jyothi (susceptible parent) and it was increased in all other varieties (resistant parents) after BPH infestation. The phenolic compounds were generally found to be feeding deterrents to plant hoppers. The BPH-resistant rice varieties had more phenolic compounds than the susceptible varieties (Pathak and Khush, 1979; Grayer *et al.*, 1993; Kim *et al.*, 2022a).

The biochemical compounds studies in rice genotypes upon BPH infestation resulted in a low level of protein synthesis, production of a higher amount of phenolic compounds, and reduced levels of ascorbic acid and reducing sugar in the resistant genotypes. The probability of these compounds alone or in combination might have a role in antibiosis and non-preference. High protein content, lower availability of phenol content, depletion of ascorbic acid, and reducing sugar might have increased the susceptibility of Jyothi plants to the BPH infestation. Similar results were obtained by Reddy *et al.* (2004), Vanitha *et al.* (2011), Ashrith *et al.* (2017), Ramulamma *et al.* (2017), and Roy *et al.* (2022) and described the important role of biochemical compounds in the BPH-resistant mechanism in rice.

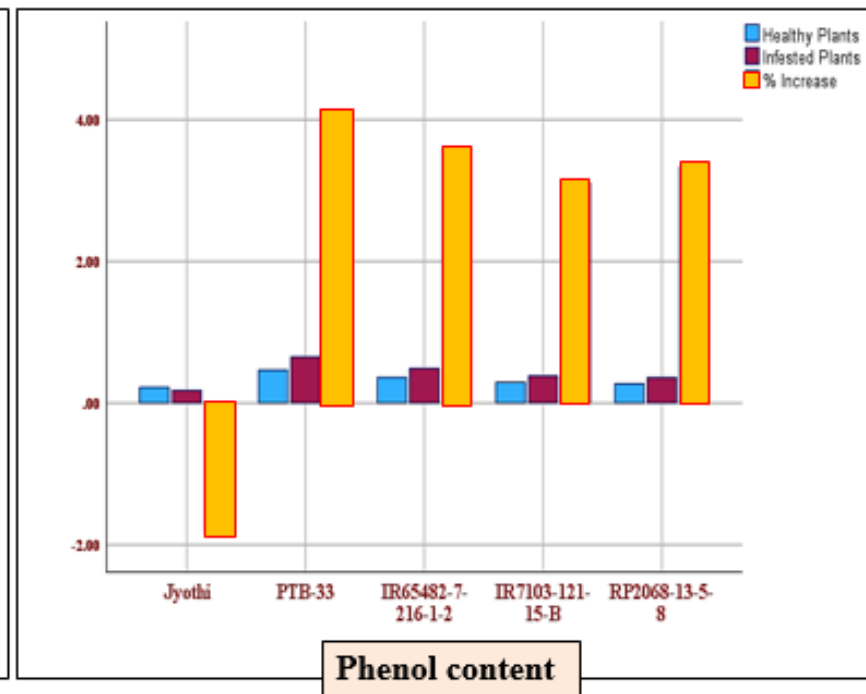
5.3.1. Correlation between biochemical components after infestation and phenotypic reactions to BPH

The correlation between biochemical components after infestation and phenotypic reactions to BPH is illustrated in Figure 13. Pairwise correlation between the tested biochemical components quantified in the rice genotypes after BPH infestation and the phenotypic reactions, resulted in a positive correlation of protein content in plants with nymphal survival, excretion of honeydew, functional plant loss index, tolerance index, BPH dry weight and damage score. The increase in protein content in plants increases the feeding of BPH resulting in the reduction of relative water content leading to the susceptibility to the pest. The application of nitrogen fertilizers to rice plants increased the concentration of nitrogen in plants and the BPH more than other nutrients. Increased nitrogen application improved the level of soluble proteins and decreased the silicon content in rice (Rashid *et al.*, 2017). The nitrogen content was significantly positively correlated with the amount of honeydew secretion by BPH (Lu and Heong, 2009).

The phenol content in plants was negatively correlated with nymphal survival, excretion of honeydew, functional plant loss index, tolerance index, BPH dry weight and damage score, which indicates that the production of phenol content had a positive role in resistance to BPH infestation. The phenolic compounds were discovered to be the feeding inhibitors of BPH in rice and generally have an advantageous association with host plant resistance (Singh, 2004; Roy *et al.*, 2022). Total phenol (TP) levels in the resistant landraces were much lower than those in the susceptible check, however, they increased more quickly in the resistant landraces with BPH infestation (Roy *et al.*, 2022). So, the rice plants' total phenols could be considered one of the selection criteria in selecting BPH-resistant genotypes.

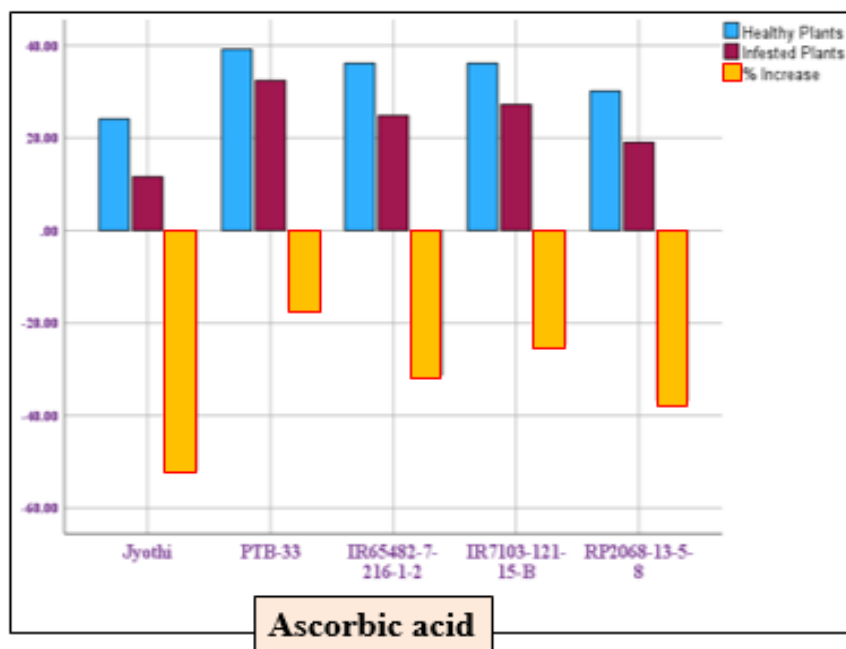


(a)

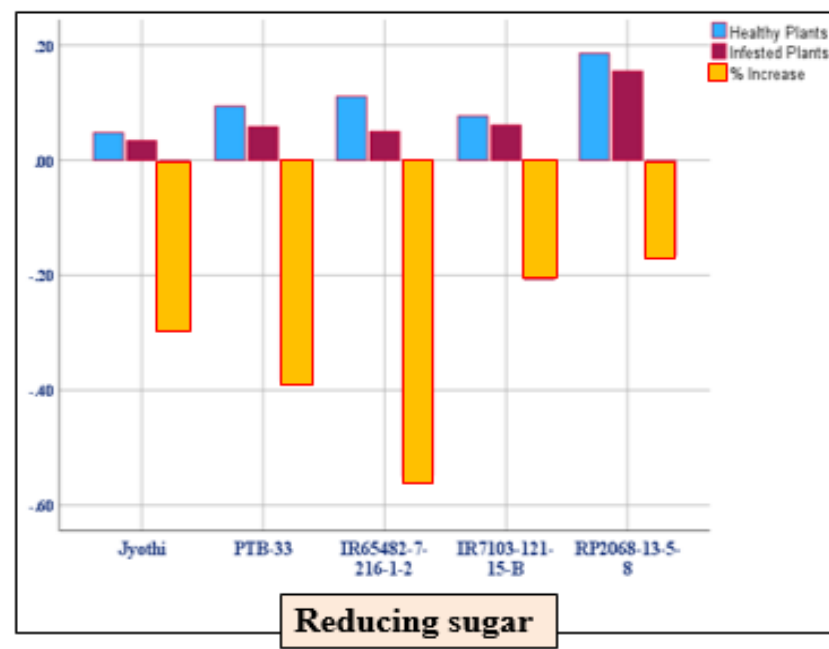


(b)

Figure 12. Biochemical parameters in parent genotypes before and after BPH infestation



(c)



(d)

Figure 12. Biochemical parameters in parent genotypes before and after BPH infestation

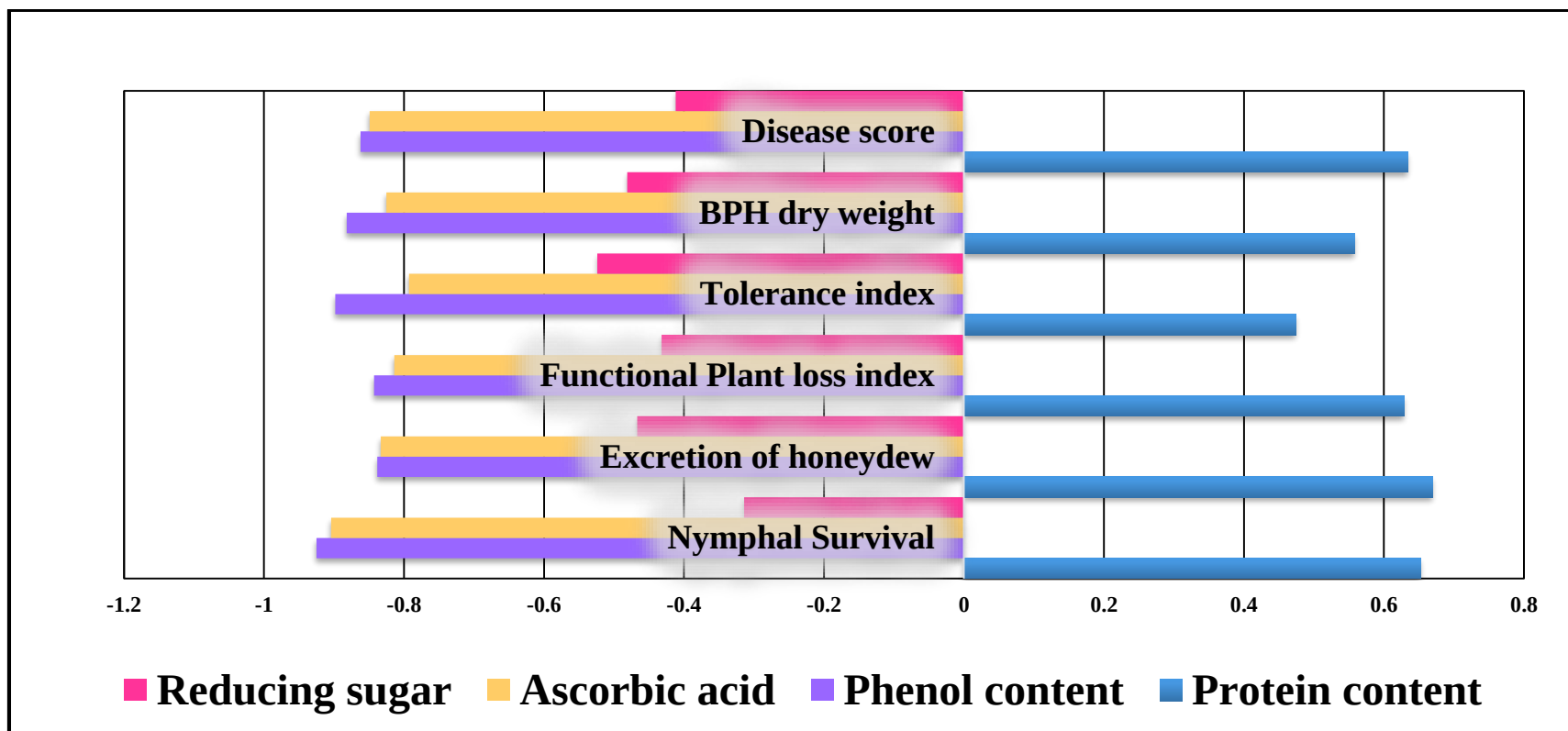


Figure 13. Correlation between biochemical components after infestation and phenotypic reactions to BPH

The ascorbic acid also showed a negative significant correlation with nymphal survival, excretion of honeydew, functional plant loss index, tolerance index, BPH dry weight and damage score. The presence of various metabolites in the resistant rice genotypes hinders the BPH feeding activities due to less preference (He *et al.*, 2013). The reducing sugar content had a negative significant correlation with the tolerance index.

5.3.2. Genetic parameters in donor and recipient parents

The coefficient of variation measures the estimates of genetic variability present in the population. All the traits under study recorded a high phenotypic coefficient of variation (PCV) than the genotypic coefficient of variation (GCV) indicating the presence of environment controlling the traits. But, the less difference between PCV and GCV implies that the involvement had a negligible role in the trait expression (Figure 14). The narrow gap between PCV and GCV in the traits of rice was reported by Sanghamitra *et al.* (2018), Sarif *et al.* (2020), Dhakal *et al.* (2020) and Demeke *et al.* (2023). High PCV and GCV were observed for the character's plant height, the number of productive tillers, 1000 grain weight, L/B ratio, protein content, phenol content and reducing sugar, representing that these characters in genotypes are contributed by additive gene action and the direct selection will improve the trait easily. The same findings were reported by Sanghamitra *et al.* (2018) for plant height, Dhakal *et al.* (2020) and Demeke *et al.* (2023) for the number of tillers, Singh *et al.* (2020) and Mahesh *et al.* (2022) for protein content, Sanghamitra *et al.* (2018) and Sarif *et al.* (2020) for phenolic content, and Parmhansh *et al.* (2011) for reducing sugar in rice genotypes. High PCV and GCV in the L/B ratio and 1000 grain weight were reported by Akshay *et al.* (2022). Moderate PCV and GCV were recorded for the character's number of days to 50% flowering, culm thickness, panicle length, number of grains per panicle, grain breadth and ascorbic acid, indicating the scope for selection and these traits were moderately contributed by the genetic constitution of rice genotypes. Similar results were obtained by Bhati *et al.* (2015) for panicle length, Islam *et al.* (2015) for the number of grains per panicle, Islam *et al.* (2016) for culm thickness

and grain breadth, Keerthiraj and Biju, (2020) and Singh *et al.* (2021) for the number of days to 50% flowering, Keerthiraj and Biju, (2020) and Akshay *et al.* (2022) for panicle length. Low PCV and GCV were reported in the character grain length, in which direct selection of these traits will not be effective for crop improvement. The same results were reported by Divya and Pandey, (2020) in the study on local rice germplasms.

The heritable amount of genetic variation present in a trait is estimated using the measures of heritability. Heritability estimations serve as a predictive tool for expressing the accuracy of phenotypic value. Genetic advancement is a helpful indicator of the advancement that may be anticipated as a result of applying selection to the relevant population. So, both heritability and genetic advance helps in the effective selection of a trait. A more accurate estimate of selection value would result from the combination of heritability and genetic advancement (Johnson *et al.*, 1955). Among the traits studied, high heritability and genetic advance were obtained for plant height, number of days to 50% flowering, panicle length, number of grains per panicle, grain breadth, L/B ratio, protein content, phenol content, and ascorbic acid, representing that these characters are predominant in additive gene action (Figure 15). Similar findings were earlier reported by Chakravorty and Ghosh (2011) for panicle length, grain breadth, and L/B ratio, Islam *et al.* (2015) for the number of grains per panicle, the number of days to 50% flowering, and grain breadth, Sanghamitra *et al.* (2018) for phenolics and plant height, Singh *et al.* (2020) for days to 50% flowering, plant height, panicle length, grains per panicle, grain breadth, L/B ratio, protein content, and total phenol content.

5.3.4. The genotypic and phenotypic correlation coefficient of morphological and biochemical characters in donor and recipient parents

The correlation coefficients estimate the measure of the interrelationship between the traits. That includes both genotypic and phenotypic aspects, in which the genotypic correlation is stronger than phenotypic correlations due to the involvement of the environment in controlling traits.

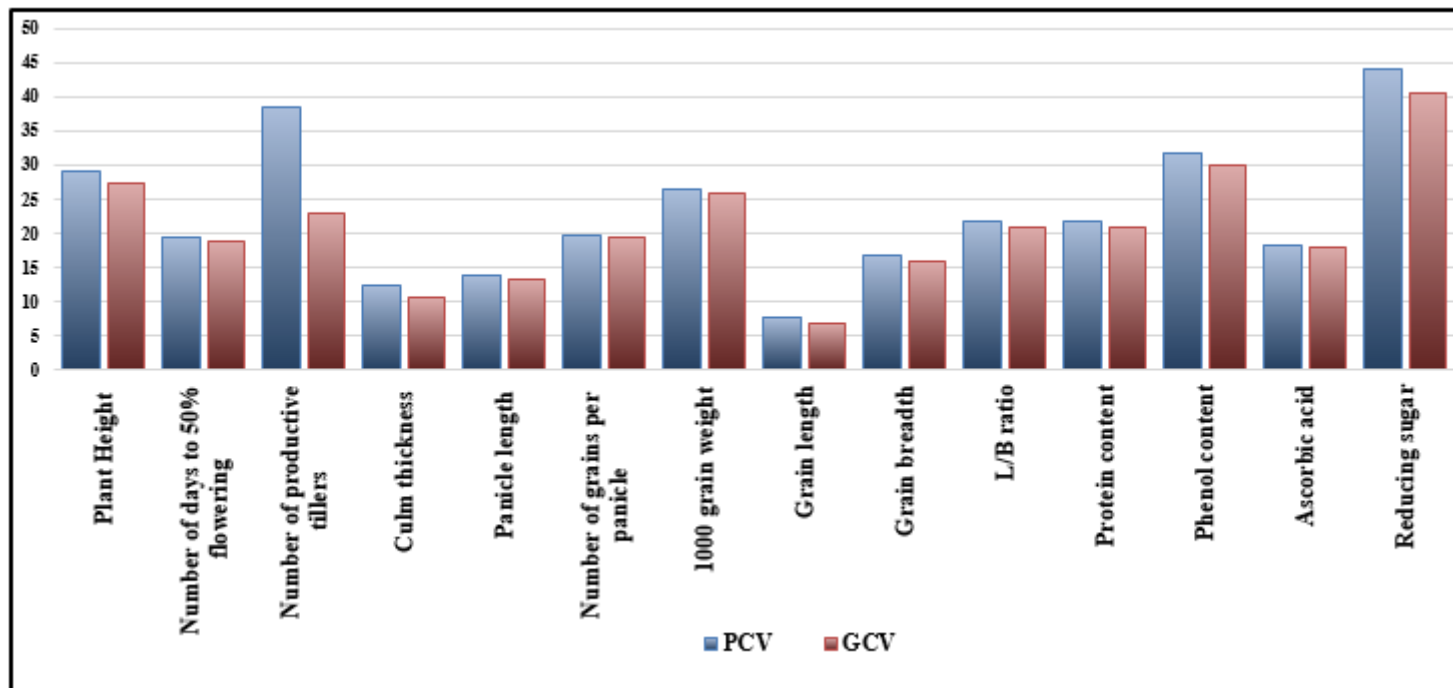


Figure 14. PCV and GCV of morphological and biochemical characters in donor and recipient parents

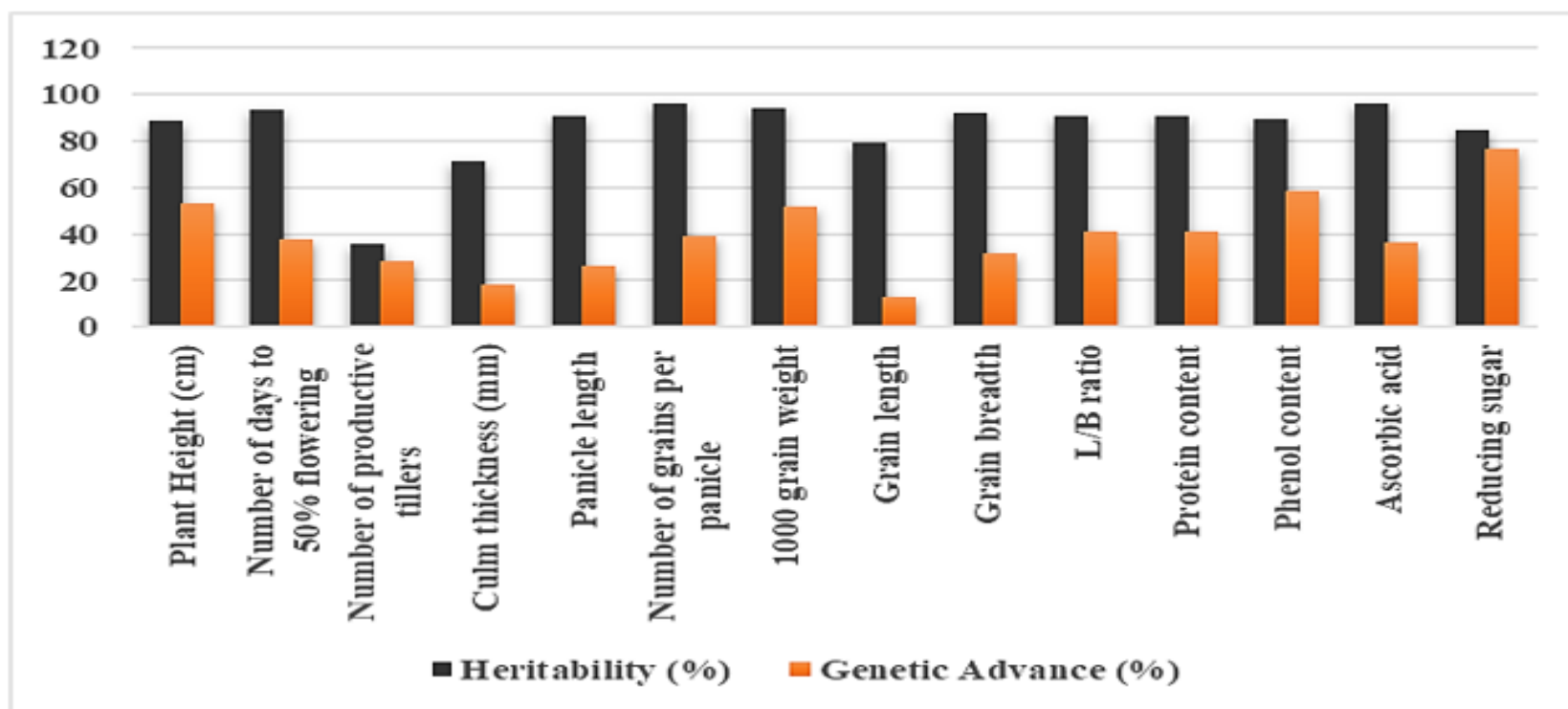


Figure 15. Heritability and genetic advance of morphological and biochemical characters in donor and recipient parents

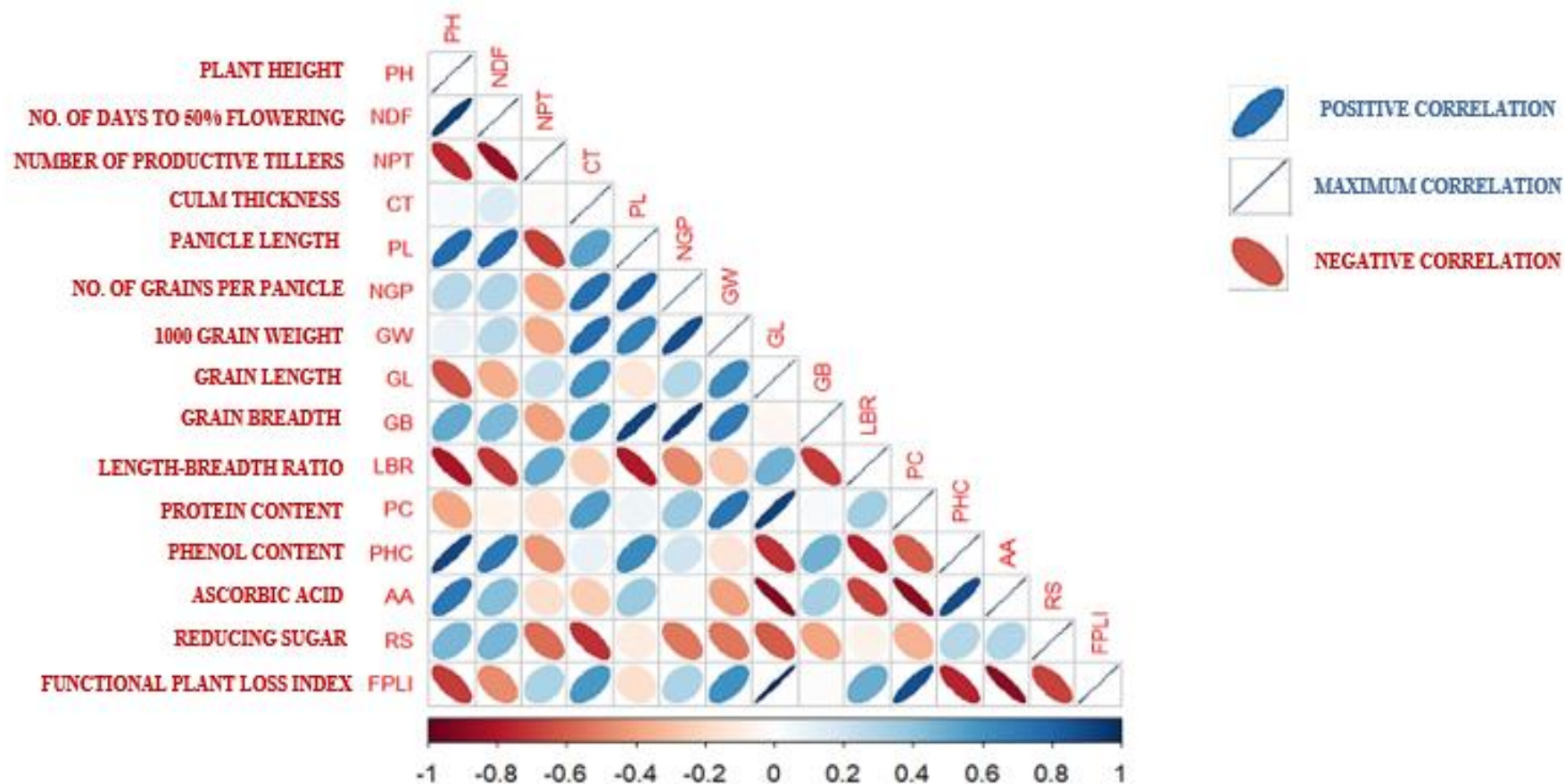


Figure 16. Genotypic correlation matrix of morphological and biochemical characters in donor and recipient parents

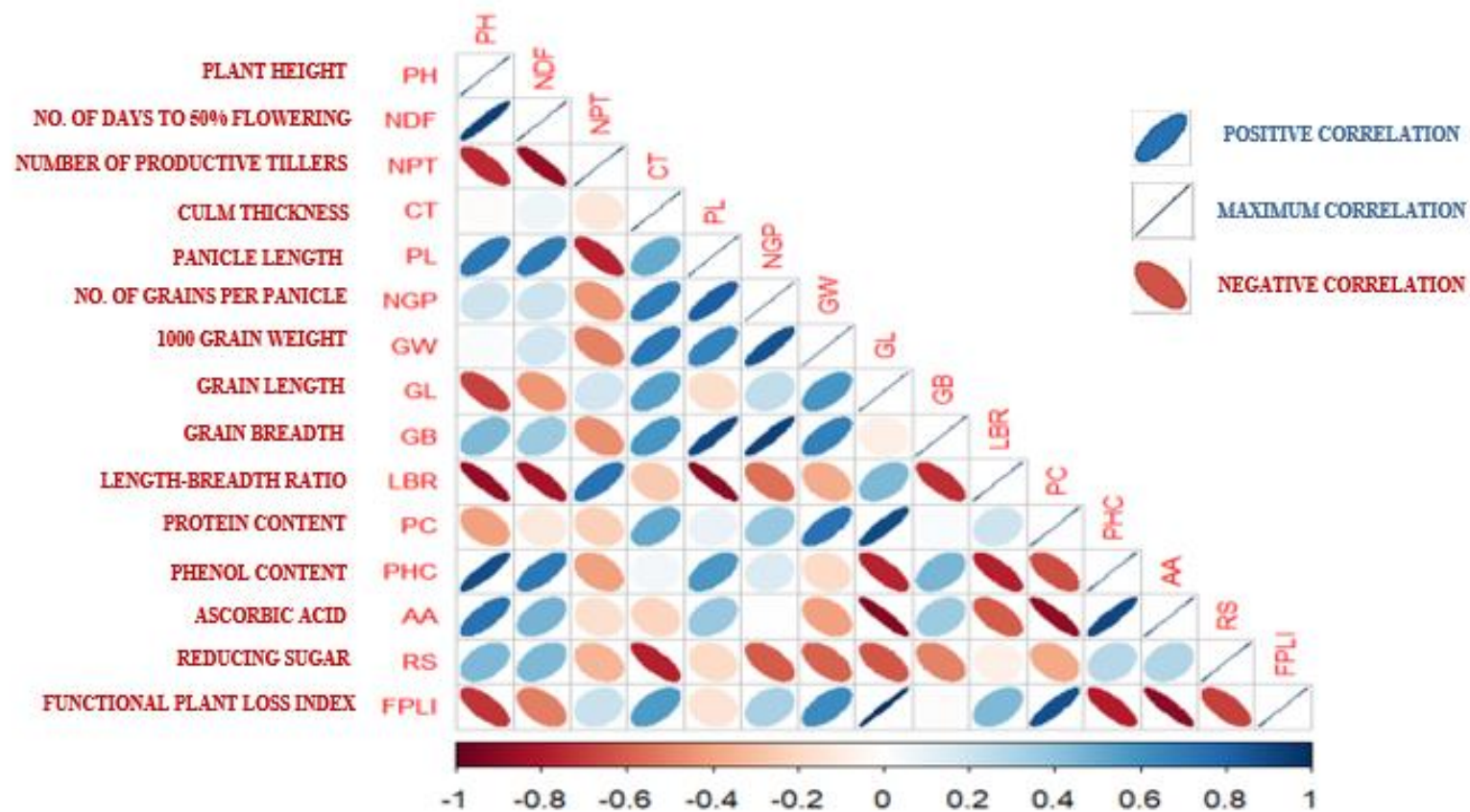


Figure 17. Phenotypic correlation matrix of morphological and biochemical characters in donor and recipient parents

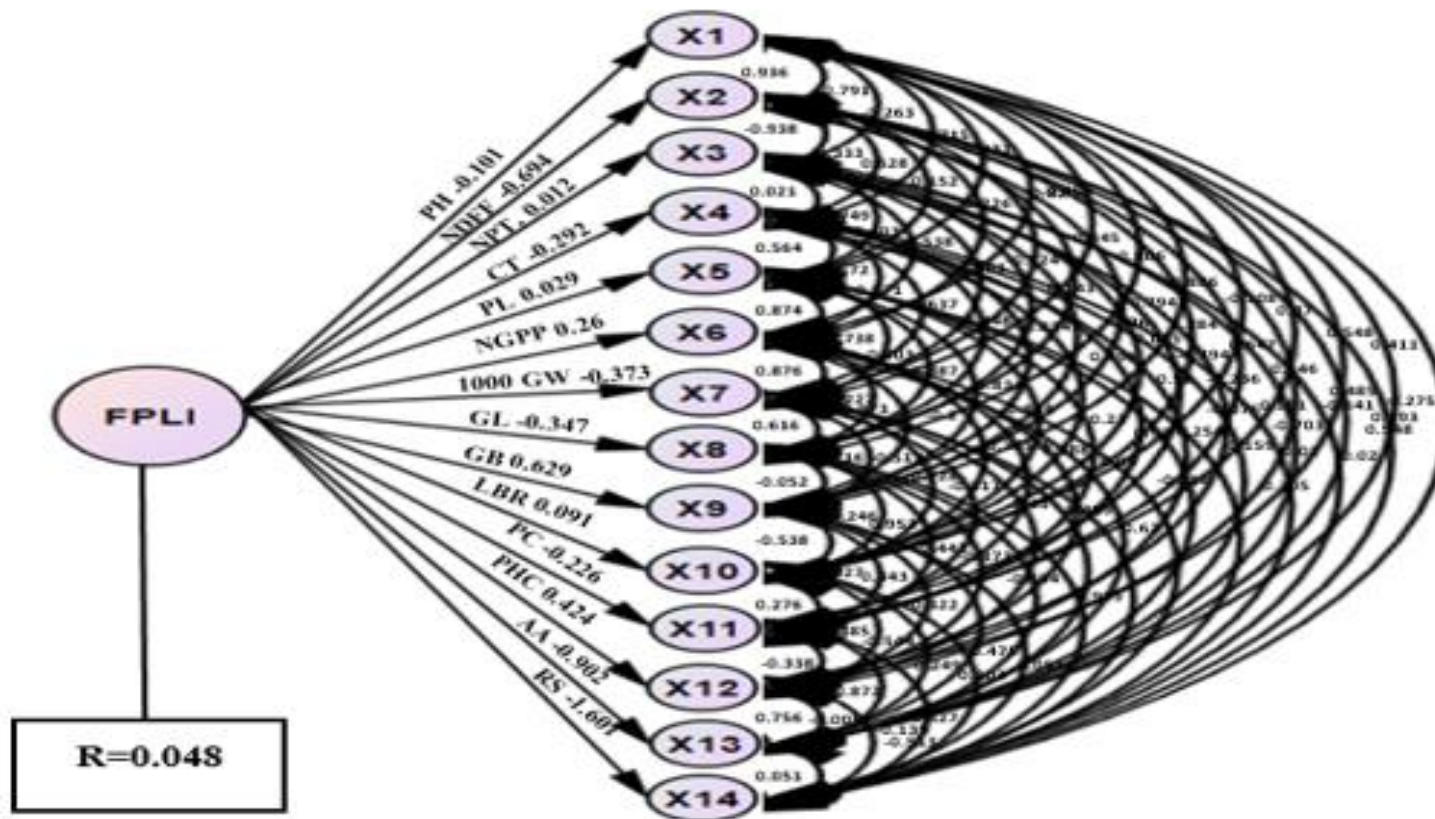


Figure 18. Path diagram showing the direct and indirect effect of fourteen characters on FPLI

From the study, the correlation among the traits was calculated and the tremendous variations were recorded (Figures 16 and 17). Among that, the functional plant loss index was significantly positively correlated with culm thickness, 1000 grain weight, grain length and protein content genotypically and grain length, and protein content phenotypically. The results imply that these traits directly increase the functional plant loss index after BPH infestation. The BPH infested before the maximum tillering stage had fewer panicles per unit area and grains per panicle, while infestation after the heading stage lowered the percentages of ripened grains and grain weight (Sogawa and Cheng, 1979). The positive and significant correlation between protein content and functional plant loss index was reported by Vanitha *et al.* (2011) and Roy *et al.* (2022) in rice.

The functional plant loss index was significantly negatively correlated with plant height, phenol content, ascorbic acid, and reducing sugar at genotypical and phenotypical levels. The phenol content in rice was positively correlated with plant resistance (Sujatha, 1987; Ramulamma *et al.*, 2017). The positive correlation between culm height and BPH resistance was reported by Kaneda and Kisimoto (1979).

5.3.5. The direct and indirect effects of morphological and biochemical characters in donor and recipient parents

The genotypic correlation of the coefficient between the functional plant loss index and other components was partitioned into direct and indirect effects in path coefficient analysis and individually measures the relative importance of the causal factor (Figure 18). In the study, the number of grains per panicle, grain breadth, and phenol content recorded a positive direct effect on the functional plant loss index. The characters such as culm thickness, 1000 grain weight, grain length, protein content, ascorbic acid, and reducing sugars observed a direct negative effect on the functional plant loss index. The overall residual effect of path coefficient analysis was 0.048, which concludes that the characters taken for analysis were appropriate in determining the functional plant loss index.

5.4 VALIDATION OF GENES FOR BPH RESISTANCE IN THE DONOR AND RECIPIENT PARENTS USING SPECIFIC MOLECULAR MARKERS

Screening of rice genotypes and validating the presence or absence of genes for BPH resistance is a prerequisite for the recipient and donor parent selection in breeding programs for the development of resistant varieties (Mishra *et al.*, 2022).

The probable donors selected in this study namely, PTB-33, IR65482-7-216-1-2, IR7103-121-15-B, and RP2068-18-3-5 were identified and reported with the genes *Bph-3*, *Bph-18*, *Bph-20* and *Bph-21*, and *Bph-33t* respectively along with SSR markers linked to it (Jairin *et al.*, 2007; Jiang *et al.*, 2018; Naik *et al.*, 2018; Mohanapriya *et al.*, 2019).

The SSR marker RM589 was utilized in the present investigation for validation of the *Bph-3* gene in the parental genotypes. The amplification profile of the SSR marker ‘RM589’ specific to the *Bph-3* gene produced amplicon at 200 bp in the donor parent PTB-33 which was absent in the recipient parent Jyothi and other genotypes. The susceptible parent Jyothi produced amplicon at 180 bp. The parental polymorphism obtained can be used to screen the backcross generations and aids in the early selection of gene-harboring lines and segregants. The *Bph-3* gene is extensively employed in breeding programs for developing BPH-resistant varieties. The KAU variety PTB-33 was reported resistant against all known biotypes of BPH for the past 70 years which is the most durable resistance shown by any variety (Rosamma *et al.*, 2003; Ren *et al.*, 2016; Akanksha *et al.*, 2019; Akula *et al.*, 2020). The major BPH resistance gene *Bph-3* was localized to the short arm of the 6th chromosome by Jairin *et al.* (2007) who identified the RM589 as a tightly linked SSR marker for the detection of the gene. Further studies by Nguyen *et al.* (2021), Prayoga *et al.* (2022), and Babu *et al.* (2022) effectively employed the SSR marker RM589 for genotyping the backcross populations derived from the parent ‘PTB-33’.

The SSR marker ‘RM3331’ specific to the *Bph-18* gene produced an amplicon at 110 bp in the donor parent IR65482-7-216-1-2 which was absent in other genotypes, while the susceptible parent Jyothi produced a product at 130 bp.

The distinguishable polymorphism obtained between the donor and recipient parent aided the further selection of lines in the backcross breeding program. The result ties well with the previous studies of Jena *et al.* (2006) who developed the introgression line IR65482-7-216-1-2 from the wild species *O. australiensis* and mapped the position of the gene on the long arm of the 12th chromosome. The SSR marker RM3331 was polymorphic among the parental lines and effectively used in the genotyping of the segregating populations. A similar pattern of results was obtained by Jiang *et al.* (2018) and Lakshmi *et al.* (2021) where the SSR marker RM3331 aided in genotyping backcross lines for selection of lines with *Bph-18* gene. The IRRI introgression line IR65482-7-216-1-2 was used as a donor of the *Bph-18* gene for BPH resistance in supporting studies by Hu *et al.* (2013) and Suh *et al.* (2014).

The validation of *Bph20* and *Bph21* genes was done using the SSR markers RM8213 and RM28561 respectively. The absence of distinguishable polymorphism among the parental genotypes limits the use of these markers for further genotyping in the backcross breeding program. A similar pattern of monomorphic bands among the parental genotypes was obtained by Akanksha *et al.* (2019) for the genes *Bph20* and *Bph21*.

5.5. MARKER-ASSISTED BACKCROSS BREEDING PROGRAM

A simple backcrossing method enables the transfer of resistant genes to elite cultivars with BPH resistance. In gene pyramiding, the backcross method is practiced, but more donors are used with respect to the gene of interest. The selection and maintenance of a tremendous plant population in each cross make it difficult in conventional breeding approaches. The selection procedure, time, and labour-consuming with low efficiency of the traditional backcross method introduced the marker-assisted selection (MAS) approach in gene pyramiding (Qiu *et al.*, 2010). MAS is a rapid and efficient method for introducing and pyramiding BPH-resistance genes. Molecular markers are efficient in identifying the resistant gene in a population, which are flanking regions with the gene interest (Miah *et al.*, 2013; Amiteye, 2021). Precise transfer of genes for BPH resistance and gene

pyramiding was accomplished by the marker-assisted backcross breeding method (Wang *et al.*, 2021; Yang *et al.*, 2022, and Li *et al.*, 2023).

Based on BPH screening, resistant reaction study, and gene validation, the KAU released variety ‘PTB-33’ and IRRI introgression line IR65482-7-216-1-2 which showed high resistance to BPH were selected as the donors for *Bph-3* and *Bph-18* gene respectively in the marker-assisted breeding program. The main aim of the present investigation was to introgress the genes *Bph-3* and *Bph-18* into the background of elite high-yielding but susceptible variety Jyothi through marker-assisted backcrossing. The selection of introgressed lines with gene combinations along with maximum recurrent phenotype recovery was followed in the study. Donor identification is a crucial step in gene pyramiding based on the marker-assisted backcross method (Shah *et al.*, 2017; and Kamaluddin *et al.*, 2022).

The stability of resistant genes against BPH is not known before the breeding program and considering of single gene at a time in resistant breeding contains many risk factors and may lead to a susceptible variety in the future. So, gene pyramiding or stacking is the effective approach for stable and durable resistance against BPH in rice. The variety introgressed with a single *Bph14* or *Bph15* resistant gene showed lesser resistance than the variety pyramided with both *Bph14* and *Bph15* resistant genes (Hu *et al.*, 2012). Significantly higher BPH resistance was provided by the combined effect of (*Bph25* + *Bph26*)-NILs in rice rather than either *Bph25*-NILs or *Bph26*-NILs (Myint *et al.*, 2012). Introgression of more than one gene from different donors to an adapted variety shows durable resistance (Hu *et al.*, 2016; and Nguyen *et al.*, 2019).

5.5.1. Raising of parents for hybridization program

In the present study, staggered sowing of the recipient and donor parents was undertaken to obtain synchronized flowering. The donor parent PTB-33 with long crop duration (150 days) and photosensitivity, was sown one month prior to the other donor parent IR65482-7-216-1-2 and the recipient parent Jyothi. Hybridization of parents mainly depends on the synchronization of flowering, compatibility of cross combinations, and climatic conditions. Prior knowledge in floral biology, days to 50 per cent flowering, time of anthesis, and plant morphology

is essential for proper planning of sowing, emasculation, and pollination. Staggered sowing of pollen parents in particular intervals is recommended to ensure sufficient pollen for the crossing program (Gaballah *et al.*, 2021; Chakrabarty *et al.*, 2023).

5.5.2. Genotyping of BC₁F₁, intercrossed BC₁F₁, BC₂F₁ lines, and BC₂F₂ segregants

The donors PTB-33 and IR65482-7-216-1-2 were crossed independently with the recipient parent Jyothi to obtain the F₁ generation followed by backcrossing with Jyothi to develop the BC₁F₁ generation. The germination percentage of the crossed seeds in F₁ and BC₁F₁ generation ranged between 28 to 35 per cent.

Gene-specific molecular markers, RM589 and RM3331 were used for genotyping of BC₁F₁ lines derived from PTB-33 and IR65482-7-216-1-2 for detecting the presence of genes *Bph-3* and *Bph-18* respectively. Out of sixty-three BC₁F₁ lines derived from PTB-33, 28 plants showed the presence of gene *Bph-3* in heterozygous conditions. Among the seventy-eight BC₁F₁ lines derived from IR65482-7-216-1-2, 36 plants showed the presence of heterozygous loci for the *Bph-18* gene. Similar results were reported by Thakur (2022) who screened 120 BC₁F₁ progenies using SSR markers specific to *Bph-33* and *Bph-34* and reported 14 plants heterozygous for both genes, 36 plants with *Bph-33* alone and 34 plants with *Bph-34* alone.

The chi-square analysis test for goodness of fit in the BC₁F₁ generation revealed the simple dominant inheritance pattern of *Bph3* and *Bph18* genes. The contribution of the dominant gene to BPH resistance in rice was reported by Khush (1979), Nugaliyadde *et al.* (2004), and Qing *et al.* (2019). Among the identified genes 45 genes, 37 are dominant and 8 are recessive in nature (Jena and Kim, 2010). A similar segregation pattern of dominant inheritance was observed by Liu *et al.* (2016) for the genes *Bph-3* and *Bph-27(t)*; Hu *et al.* (2016b) for the genes *Bph-3*, *Bph-14*, and *Bph-15*; Wang *et al.* (2017) for the genes *Bph-9* and *Bph-6* in the BC₁F₁ generation. The dominant nature of *Bph3* (Sidhu *et al.*, 1997; Jairin *et al.*, 2007) and *Bph18* (Jena *et al.*, 2006) in different rice genotypes was already reported.

Intercrossing of BC₁F₁ lines with genes (*Bph-3* or *Bph-18*) for resistance in heterozygous conditions was done to pyramid the genes into a single line. A similar

method of pyramiding genes into a single line by intercrossing single gene introgressed lines was followed in the supporting studies of gene pyramiding done by Hu *et al.* (2013) for pyramiding the genes *Bph-14*, *Bph-15*, and *Bph-18*, Liu *et al.* (2016) for pyramiding the genes *Bph-3* and *Bph-27(t)*, Hu *et al.* (2016) for pyramiding the genes *Bph-14*, *Bph-15*, and *Bph-3*, Wang *et al.* (2017) for pyramiding the genes *Bph-9* and *Bph-6*, Nguyen *et al.* (2019) for pyramiding the genes *Bph20*, *Bph21*, *Bph3*, *Bph17*, *Bph2*, *Bph17-ptb*, and *Bph-32*. A similar crossing methodology was also followed in other pyramiding genes for multiple traits of rice such as bacterial leaf blight (Hundekar, 2017), bacterial leaf blight and BPH resistance (He *et al.*, 2019), blast, BLB and BPH resistance (Ji *et al.*, 2016; Wang *et al.*, 2021; Yang *et al.*, 2023), Submergence tolerance + BPH resistance (Korinsak *et al.*, 2016), BPH + blast + striped stem borer resistance (Li *et al.*, 2023).

Eight intercrossed BC₁F₁ lines viz., ICAB-1, ICAB-5, ICAB-19, ICBA-2, ICBA-3, ICBA-6, ICBA-9, and ICBA-10 with Jyothi-specific characters and a combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition were backcrossed with Jyothi to develop BC₂F₁ lines. The gene combinations in intercrossed BC₁F₁ lines are depicted in Figure 19.

The twenty-six BC₂F₁ lines developed were genotyped using SSR markers RM589 and RM3331 specific for the genes *Bph-3* and *Bph-18* respectively. In the genotypic evaluation of BC₂F₁ lines, five lines viz., ICAB-1/3, ICAB-1/6, ICBA-3/2, ICBA-9/3, and ICBA-10/2 showed the combination of both genes (*Bph-3* and *Bph-18*) in heterozygous condition. The gene combinations in BC₂F₁ lines are depicted in Figure 20.

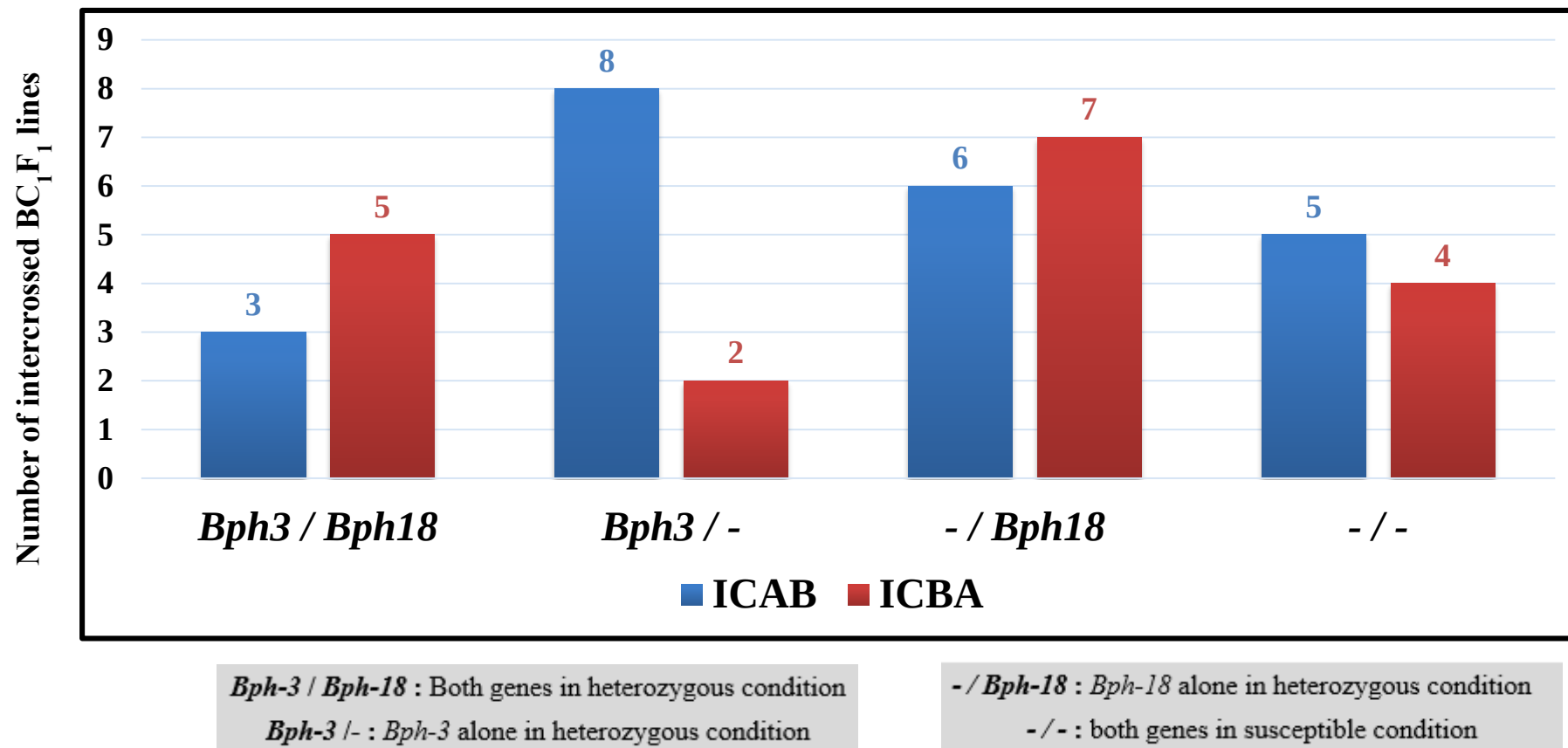
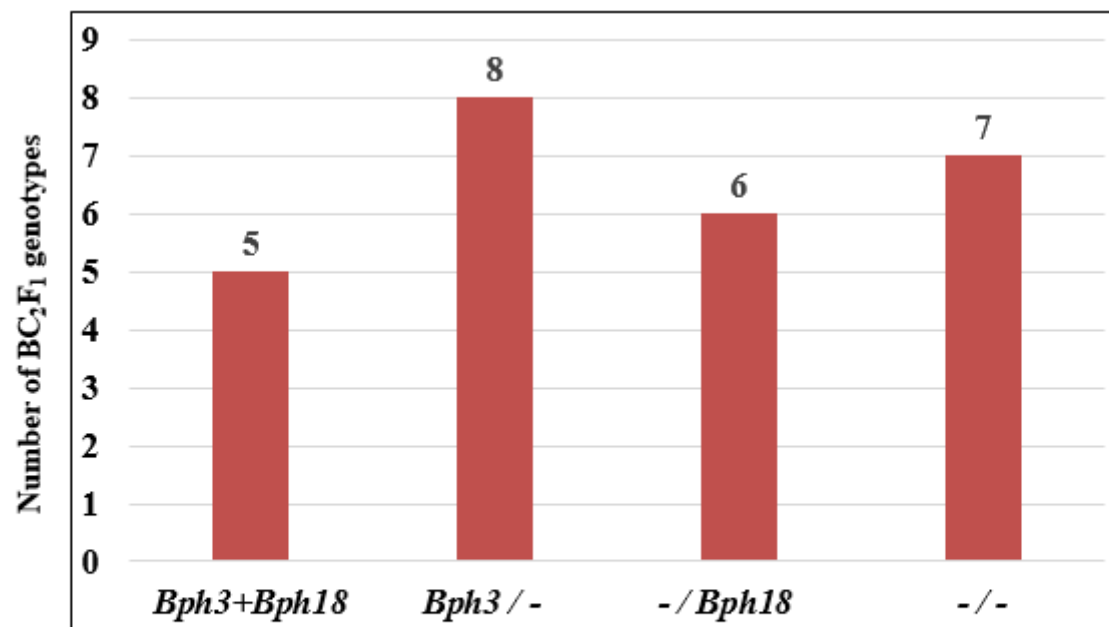


Figure 19.

The frequency of gene combinations in intercrossed BC₁F₁ lines



Bph-3 / Bph-18 : Both genes in heterozygous condition
Bph-3 / - : *Bph-3* alone in heterozygous condition

- / Bph-18 : *Bph-18* alone in heterozygous condition
- / - : both genes in susceptible condition

Figure 20. The frequency of gene combinations in BC₂F₁ lines

The five BC₂F₁ lines with a combination of both genes (*Bph-3* and *Bph-18*) in the heterozygous condition and maximum similarity in proximity dissimilarity matrix analysis were selfed, and the BC₂F₂ segregants were forwarded to BPH bioassay in the seedling stage. The modified seedbox screening test was followed for BPH seedling screening and identified the BC₂F₂ segregants with BPH resistance. The remaining plants were susceptible and completely dried up due to BPH infestation. The results were broadly in line with the BPH bioassay done by Jena *et al.* (2006) in the BC₂F₂ generation derived from the donor IR65482-7-216-1-2 for the *Bph-18* gene in which 433 progenies were screened for BPH resistance in the seedling stage and 97 resistant individuals were genotyped. Similar findings of BPH bioassay in segregating generations for selecting resistant individuals were reported by Jena *et al.* (2017); Balachiranjeevi *et al.* (2019); Kim *et al.* (2022b); Kaur *et al.* (2023); Vignesh *et al.*, 2023.

The eighty-nine BC₂F₂ lines that were found resistant in the BPH bioassay were forwarded to genotyping using SSR markers RM589 and RM3331 specific for the genes *Bph-3* and *Bph-18* respectively. Genotyping of these lines obtained the combination of homozygous resistant loci of both genes (*Bph-3* and *Bph-18*) in four lines namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10. Remaining BC₂F₂ segregants with at least one gene in the homozygous resistant or heterozygous state showed phenotypic resistance which indicates that the BPH resistance is controlled by a simple dominant gene. A similar conclusion was reached by Sidhu *et al.* (1997); Jena *et al.* (2006); Jairin *et al.* (2007); and Qing *et al.* (2019). Analogous to the present study, several studies of genotyping in backcross populations for identifying the homozygous resistant state of pyramided genes were reported. Jena *et al.* (2006) genotyped the BC₂F₂ progenies for the presence of the *Bph-18* gene and reported the presence of marker alleles in homozygous resistant heterozygous resistant and the absence of homozygous susceptible marker allele. Genotyping of BC₂F₂ progenies by Mohanapriya *et al.* (2019) in pyramiding *Bph20* and *Bph21* into the recipient parent CO43Sub1 obtained four segregants with both genes in the homozygous state. Overall the results and methodology of marker-assisted backcrossing, genotyping, and

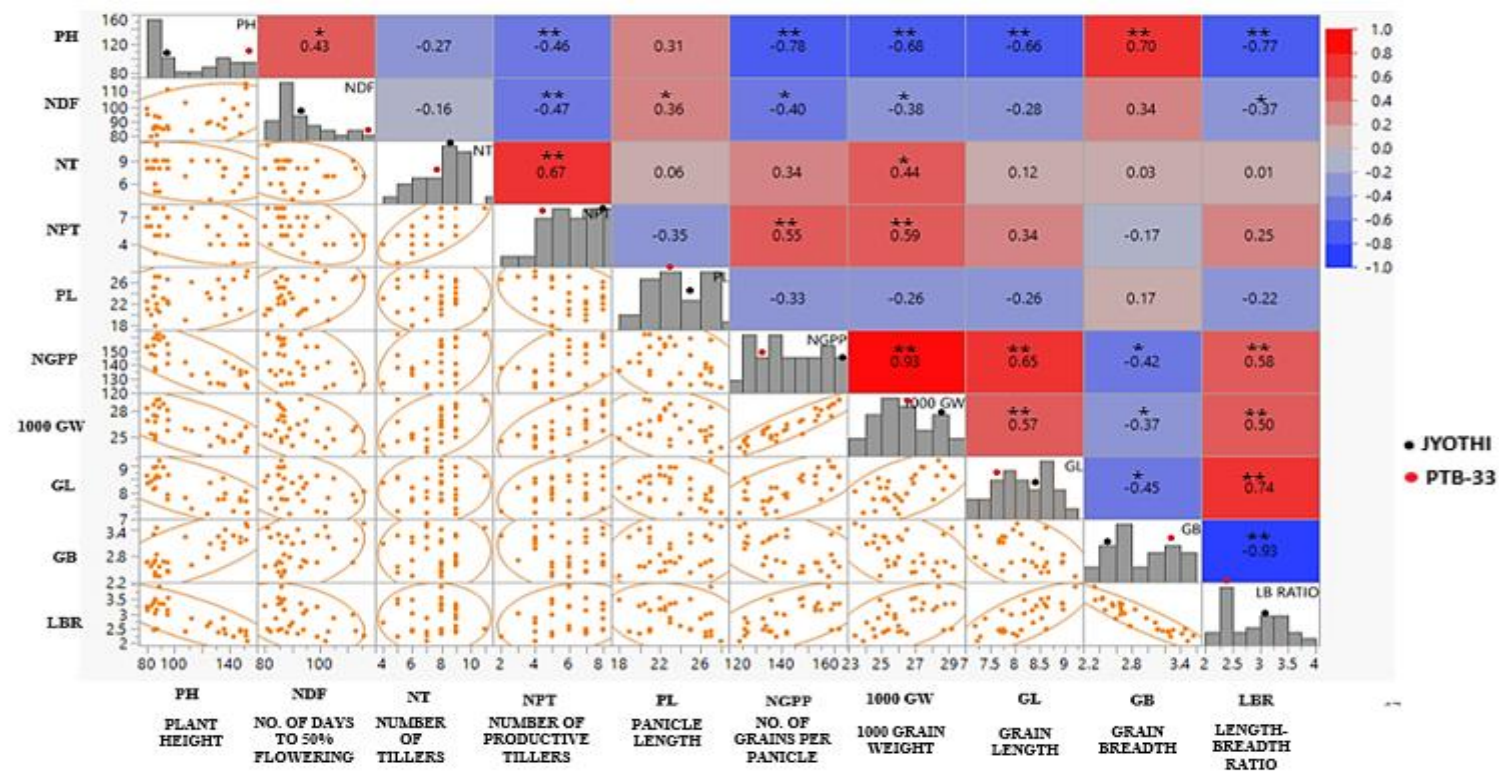


Figure 21. Distribution, Scatter plot, and Correlation of BC₁F₁ plants derived from PTB-33

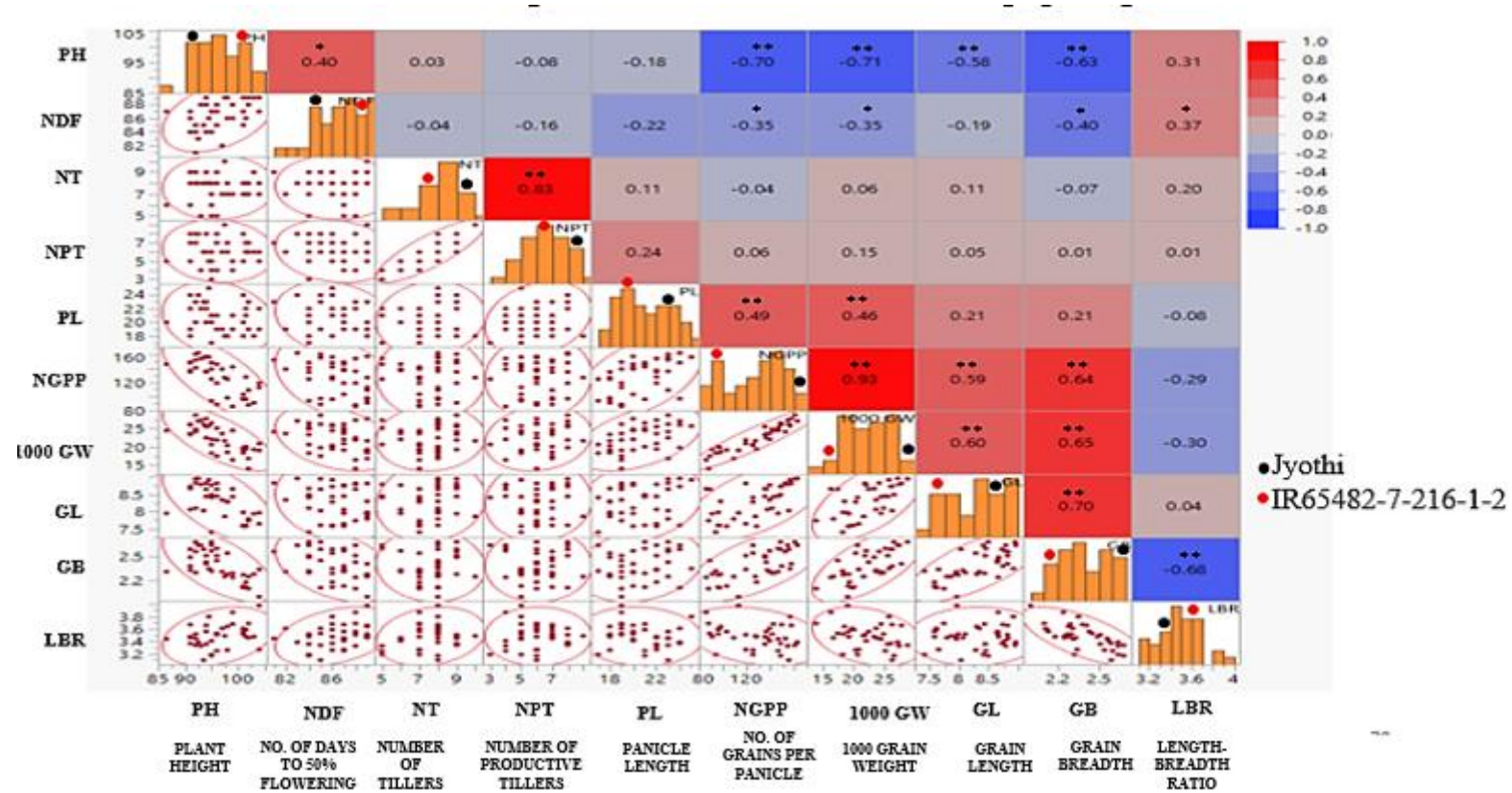


Figure 22. Distribution, Scatter plot, and Correlation of BC₁F₁ plants derived from IR65482-7-216-1-2

phenotyping used in the present study are in accordance with the findings of marker-assisted gene pyramiding studies done by Hu *et al.* (2015); Liu *et al.* (2016); Jena *et al.* (2017); He *et al.* (2020); Wang *et al.* (2021); Yang *et al.* (2022); and Vignesh *et al.* (2023);

Phenotyping of BC₁F₁, intercrossed BC₁F₁, BC₂F₁ lines and BC₂F₂ segregants

Pyramiding several genes and QTL together into a variety aid in the development of lines with tolerance for and resistance to a wide range of abiotic and biotic stressors. But, the recurrent parent phenotype recovery and the trait of interest are crucial in the backcross breeding programme. The marker-aided backcrossing (MAB) technique has been extensively used to introduce genes that increase BPH resistance in rice cultivars (Jena *et al.*, 2006; Korinsak *et al.*, 2016). Reducing the pollen parent's genome content into the recipient parent's genetic makeup is the goal of MAB (Tanweer *et al.*, 2015). So, stringent phenotypic selection based on different morphological and quality characters at each backcross generation is required in the identification of lines with faster recovery of recurrent parent characters (Gopalakrishnan *et al.*, 2008; Pradhan *et al.*, 2015).

A wide range of morphological characters was observed in all the backcrossed generations. The distribution, cluster and correlation of the characters in BC₁F₁ lines of the cross [Jyothi x PTB 33] x Jyothi and [Jyothi x IR65482-7-216-1-2] x Jyothi are depicted in Figure 21 and Figure 22 respectively. The distribution pattern of the morphological characters in intercrossed BC₁F₁ lines, BC₂F₁ lines (Figure 23, 24) and BC₂F₂ segregants are given in the Figure 25 and 26 respectively.

Plant height

The plant height of BC₁F₁ lines of the cross [Jyothi x PTB 33] x Jyothi recorded high variations due to wide differences in the plant height between the parental genotypes Jyothi (92 cm) and PTB-33 (152 cm). Whereas the plant height difference between Jyothi (92 cm) and IR65482-7-216-1-2 (102 cm) was narrow, so the range of variations in BC₁F₁ lines of the cross [Jyothi x IR65482-7-216-1-2] x Jyothi, found to be small. The intermediate plant height of hybrids to that of their parents in BPH-resistant rice was reported by Wang *et al.* (2016). In the intercrossed

BC₁F₁ lines, the plant height varied tremendously due to the involvement of the background of three parents.

In BC₂F₁ lines, most of the lines recorded the height near the recurrent parent Jyothi. The variation in plant height between the parents was also reported in the BC₂F₁ population of the cross between Samba Mahsuri and *Oryza rufipogon* (Basavaraj *et al.*, 2019). All the BC₂F₂ segregants were observed to be similar in plant height to that of Jyothi. The pattern of plant height in each generation of backcross indicates that increasing the number of backcrosses recovered parental plant height in its progenies. The bell-shaped frequency distribution curve for plant height indicated the polygenic nature of the trait. The phenotype recovery of each generation of backcross is due to the no linkage of the characters with undesirable traits (Miah *et al.*, 2015). The BPH resistance from Rathu Heenati was introgressed in rice by backcrossing up to BC₃F₆ generations and recovered the plant height of the recurrent parent, KDML105 (139 cm) in the introgressed lines. The transgressive segregation of plant height in the BC₃F₆ generation of BPH introgressed rice lines was reported by Jairin *et al.* (2009).

Number of days to 50% flowering

The recurrent parent Jyothi flowers at 85 days after sowing while the donor parents PTB-33 and IR65482-7-216-1-2 take 113 and 88 days respectively. The variations in the number of days to 50% flowering in parental genotypes were reflected in their progenies also. The BC₁F₁ lines of the cross [Jyothi x PTB 33] x Jyothi about five lines were flowered after 100 days of sowing. While the BC₁F₁ lines of the cross [Jyothi x IR65482-7-216-1-2] x Jyothi recorded the same range of days to 50% flowering as that of Jyothi. The number of days to 50% flowering in intercrossed BC₁F₁ lines distributed between the three parental genotypes. The frequency distribution curve was bell-shaped implicating that the trait was controlled by polygenes.

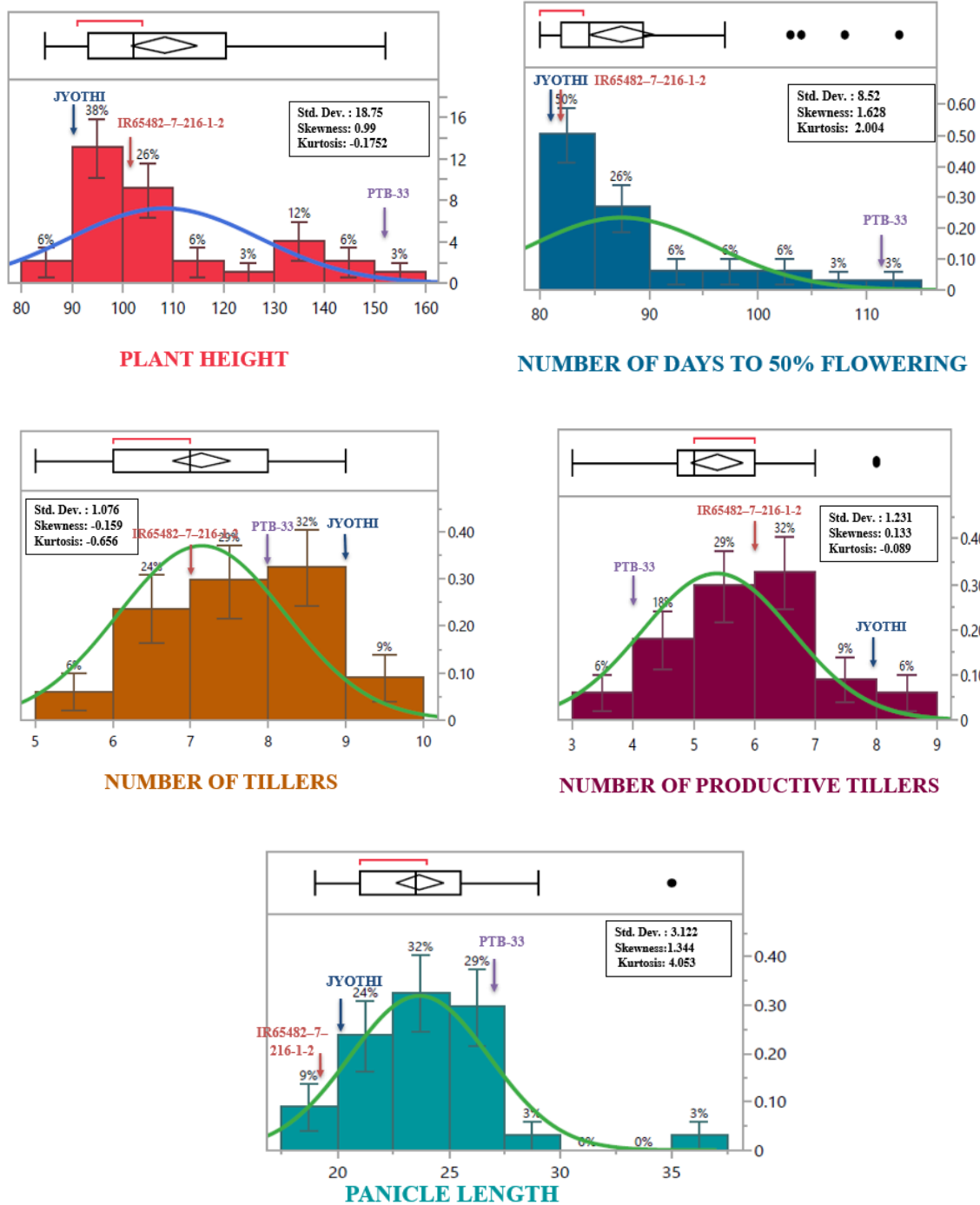


Figure 23. Distribution graph of morphological traits in intercrossed BC₁F₁ lines

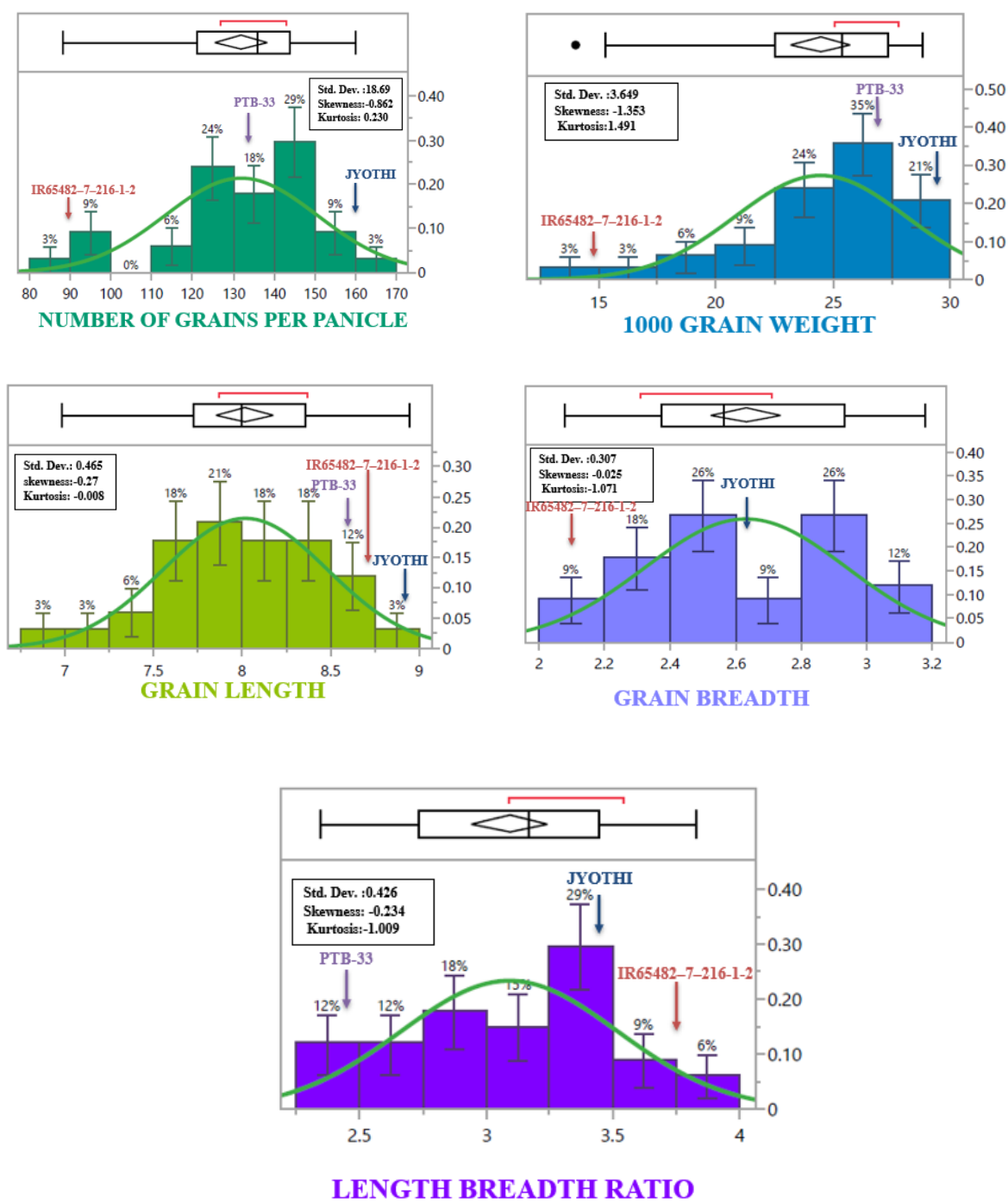


Figure 23. (Contd.) Distribution graph of morphological traits in intercrossed BC₁F₁ lines

A single line (ICAB-9/2) among BC₂F₁ lines crossed 100 days for flowering and the remaining lines were in accordance with the recurrent parent. The number of days to 50% flowering in BC₂F₂ segregants was proximal to recurrent parent characters.

Number of tillers

The number of tillers varied between five to nine in BC₁F₁ lines, intercrossed BC₁F₁ lines, BC₂F₁ lines, and BC₂F₂ segregants. The parental genotypes were also produced in the same range of tiller numbers. The distribution curve of the number of tillers was bell-shaped, indicating that the trait was controlled by polygenes. The distribution of the number of tillers in backcrossed progenies of rice was reported by Hundekar, (2017) and Gopinath (2023).

Number of productive tillers

The recurrent parent Jyothi produced eight productive tillers and the donor parents PTB-33 and IR65482-7-216-1-2 produced four and seven productive tillers. The distribution of productive tillers in BC₁F₁ lines was in between parental genotypes. All the intercrossed BC₁F₁ lines and BC₂F₁ lines produced more than six productive tillers. The BC₂F₂ segregants produced a similar number of productive tillers as that of the recurrent parent. Similar results on the inheritance of the number of productive tillers of R-gene pyramided lines were reported by Joseph *et al.* (2004) in BC₁F₃ lines and Gopalakrishnan *et al.* (2008) in advanced selections.

Panicle length

The difference between the panicle length of the recurrent parent and the donor parent was high, so the distribution of panicle length in BC₁F₁ lines was found to be variable to an extent. The panicle length in Jyothi was 23cm, in which more than 26cm panicle length was recorded in intercrossed BC₁F₁ lines. The panicle length of BC₂F₁ lines varied between parental genotypes and most of the lines were close to the recurrent parent character. The BC₂F₂ segregants showed transgressive segregation for panicle length, in which most of the segregants showed panicle lengths of more than 23 cm. Transgressive segregation can be used to produce genotypes that have desirable characteristics from both parents (Kshirsagar *et al.*,

2013). The high frequency of favourable transgressive segregation in the panicle length in rice was reported by Basavaraj *et al.* (2019). The distribution curve for the panicle length was bell-shaped and the negative skewness and kurtosis indicating the trait was controlled by a greater number of genes.

Number of grains per panicle

The recurrent parent produced the highest number of grains per panicle (160) than the donor parents. The difference between the trait in donors and recurrent parents was huge, and the distribution also showed large variations. More than double the number of grains per panicle was produced in Jyothi compared to the donor IR65482-7-216-1-2. Three lines from the cross [Jyothi x PTB 33] x Jyothi and two lines from the cross [Jyothi x IR65482-7-216-1-2] x Jyothi recorded more than 160 grains per panicle, indicating the transgressive segregation of the trait. The number of grains per panicle was increased in intercrossed BC₁F₁ lines compared to the previous generation. The minimum number of grains per panicle in BC₂F₁ lines was increased to 117, which implicates the recovery of yield characters of Jyothi to the backcrossed progenies. In BC₂F₂ segregants, the number of grains per panicle was almost similar to the recurrent parent. The bell-shaped frequency distribution curve concluded the polygenic control of the trait. The number of grains per panicle was considered an important parameter to improve the recurrent parent genome recovery in backcross progenies (Joseph *et al.*, 2004; Basavaraj *et al.*, 2010; Arunakumari *et al.*, 2016; Baliyan *et al.*, 2018).

1000 grain weight

The 1000-grain weight of the donor IR65482-7-216-1-2 was lower than Jyothi and PTB-33. So, the 1000-grain weight BC₁F₁ lines of the cross [Jyothi x IR65482-7-216-1-2] x Jyothi observed more variation than of BC₁F₁ lines of the cross [Jyothi x PTB 33] x Jyothi. The intercrossed BC₁F₁ lines also showed a tremendous variation in 1000-grain weight, which might be due to more segregation in the trait. The 1000-grain weight in BC₂F₁ lines and BC₂F₂ segregants have been improved through backcrossing towards recurrent parent phenotype. The trait was controlled by polygenes as concluded from the measures of skewness and kurtosis. 1000 grain weight is the unique feature of a variety, which determines the yield potential of the

crop. The recovery of 1000-grain weight in pyramided lines to that of the recurrent parent is a prerequisite in the backcrossing (Jairin *et al.*, 2009).

Grain length

The donor parent PTB-33 had bold grains, whereas Jyothi and IR65482-7-216-1-2 had slender grains. So, the length of grains in PTB-33 was less compared to that of other parents. The variations of grain length in BC₁F₁ lines, intercrossed BC₁F₁ lines, BC₂F₁ lines and BC₂F₂ segregants were between seven and eight millimetres. The distribution curve was bell-shaped for grain length, implicating polygenic control of the trait. The same acceptability as that of the recurrent parent in grain length in the introgressed lines was the foremost priority in gene pyramiding (Basavaraj *et al.*, 2019).

Grain breadth

Due to the bold grains of PTB-33, the grain breadth was high as compared to the other two parents. So, the grain breadth in BC₁F₁ lines in the cross [Jyothi x PTB 33] x Jyothi and cross [Jyothi x IR65482-7-216-1-2] x Jyothi lies in between two to four and two to three millimetres respectively. The grain breadth of other backcrossed generations was improved towards recurrent parent phenotype. The negative skewness and kurtosis in the characters represent that the trait was controlled by a greater number of genes. The same trend in the grain breadth was reported by Gopalakrishnan *et al.* (2008).

LB ratio

The LB ratio in the donors PTB-33 and IR65482-7-216-1-2 and recurrent parent varied to an extent. The LB ratio varied from two to three in BC₁F₁ lines, intercrossed BC₁F₁ lines, BC₂F₁ lines and BC₂F₂ segregants were recorded. The frequency distribution showed a bell-shaped curve which indicates the polygenic inheritance of the trait. The observation of the L/B ratio in backcrossed progenies of rice was studied and the same pattern of distribution was noticed by Hundekar, (2017) and Gopinath (2023).

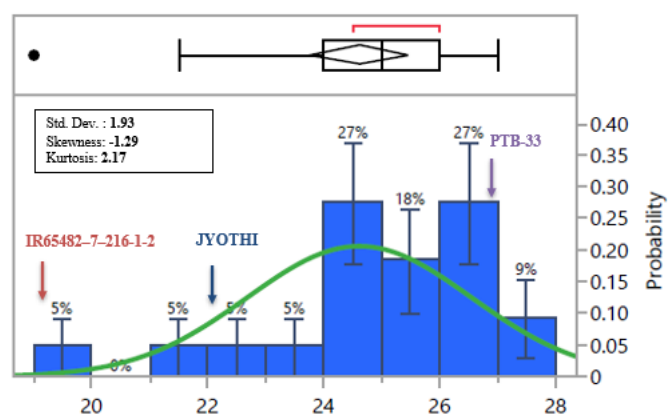
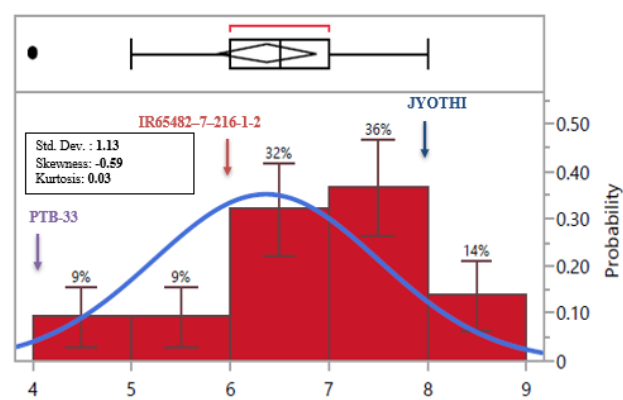
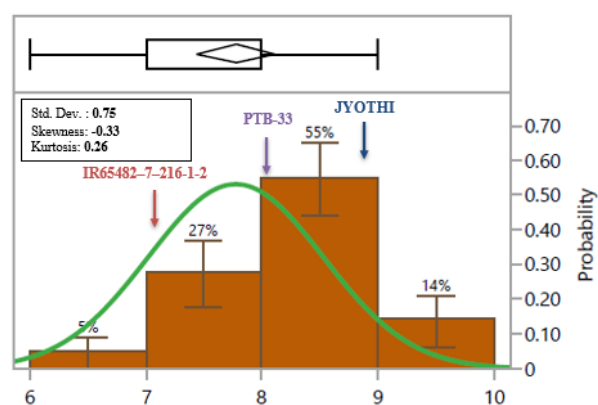
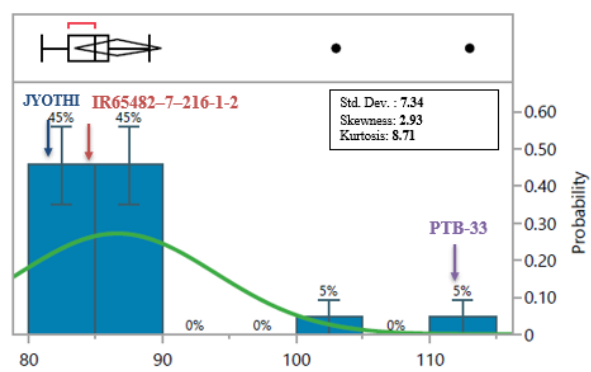
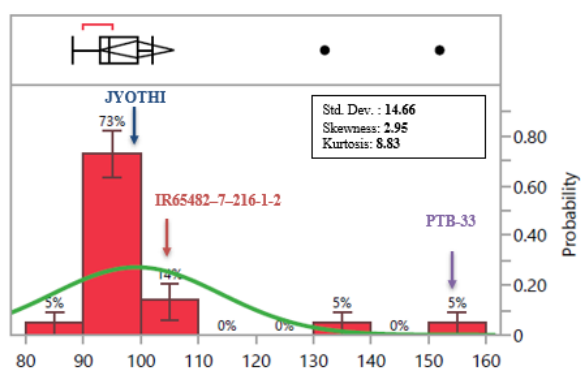


Figure 24. Distribution graph of morphological traits in BC₂F₁ lines

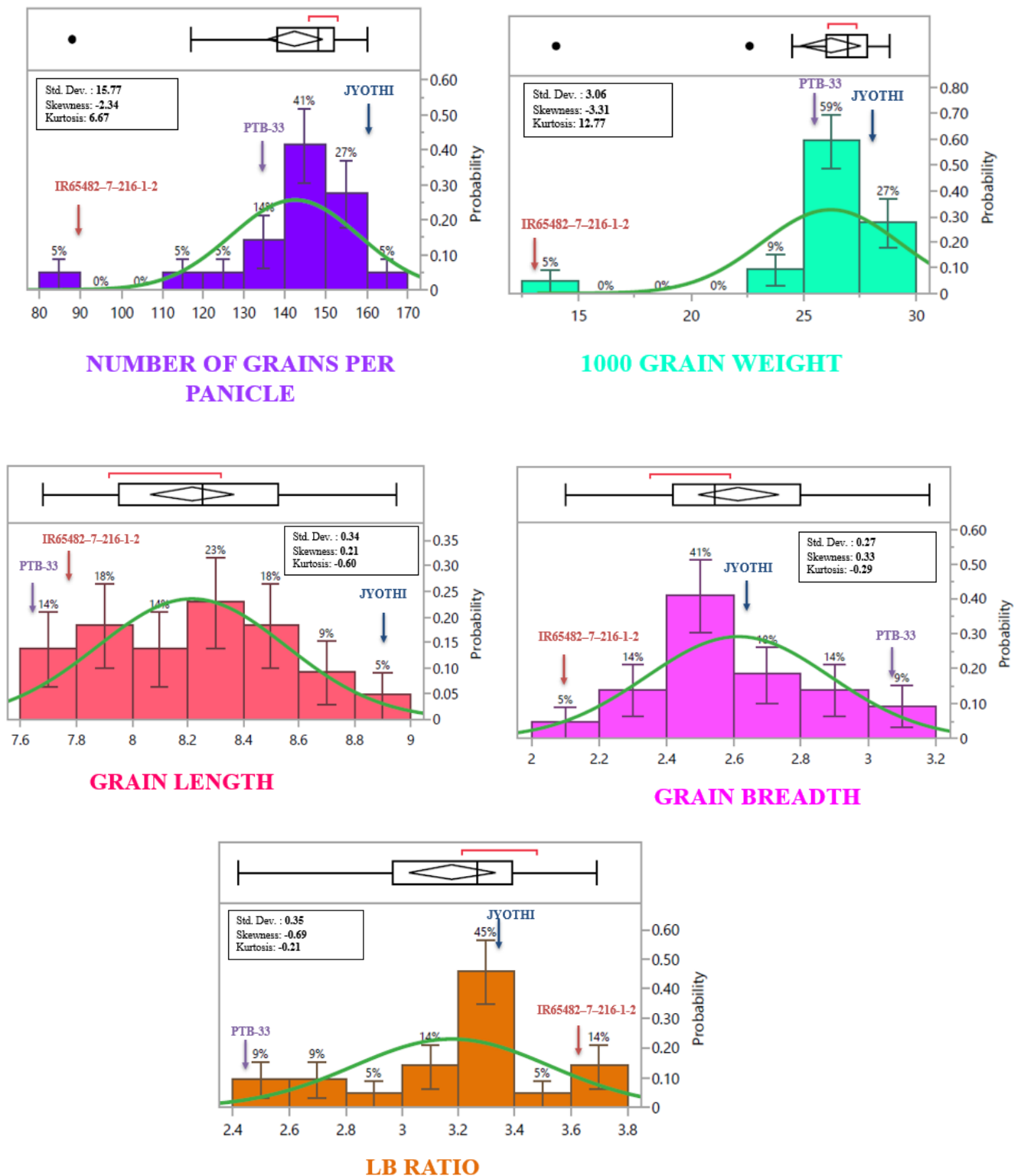


Figure 24. (Contd.) Distribution graph of morphological traits in BC₂F₁ lines

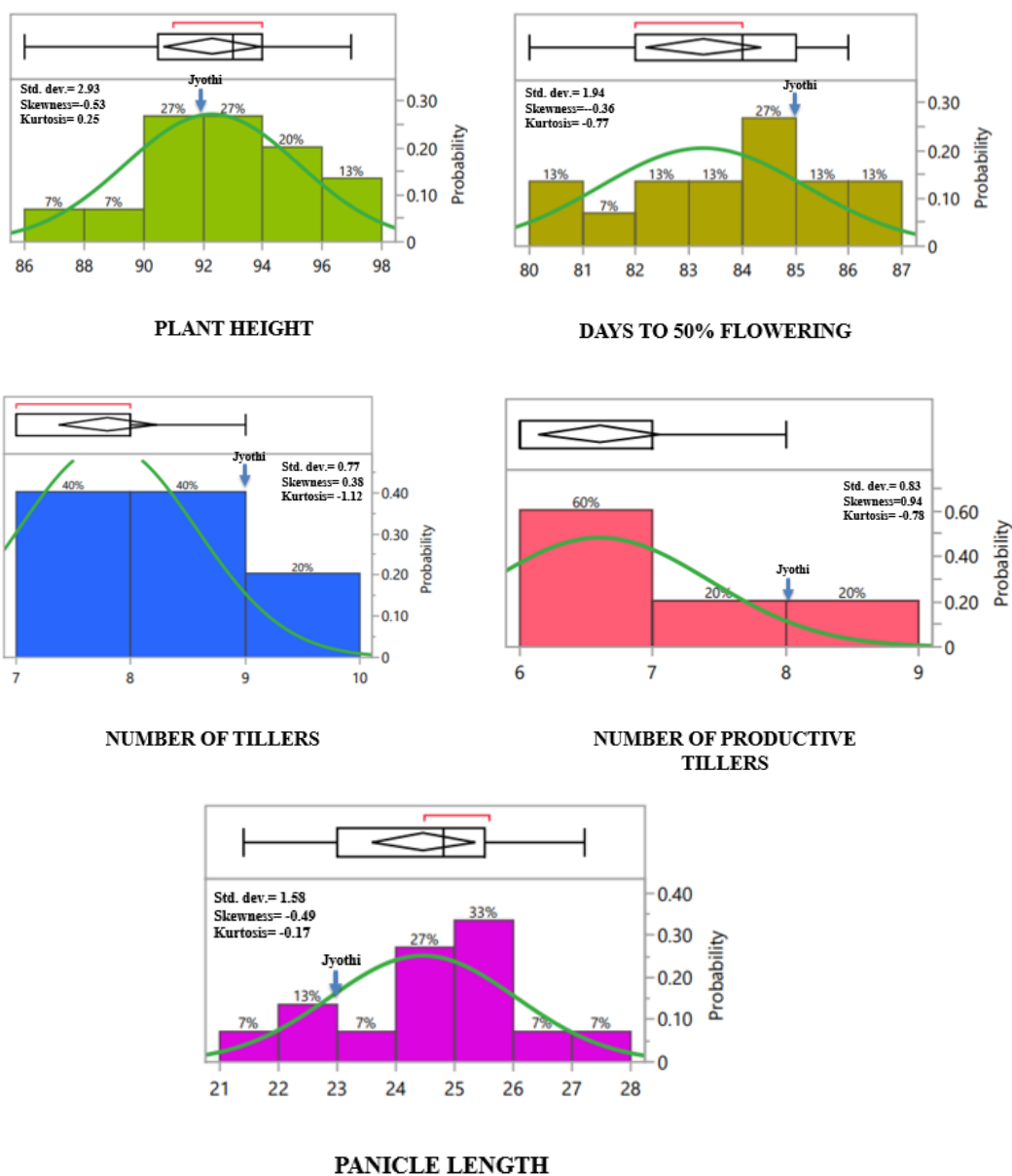


Figure 25. Distribution graph of BC₂F₂ ICAB-1/3 progenies

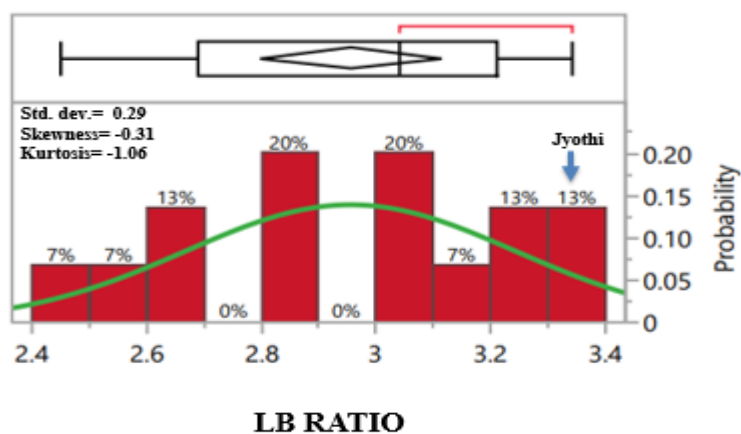
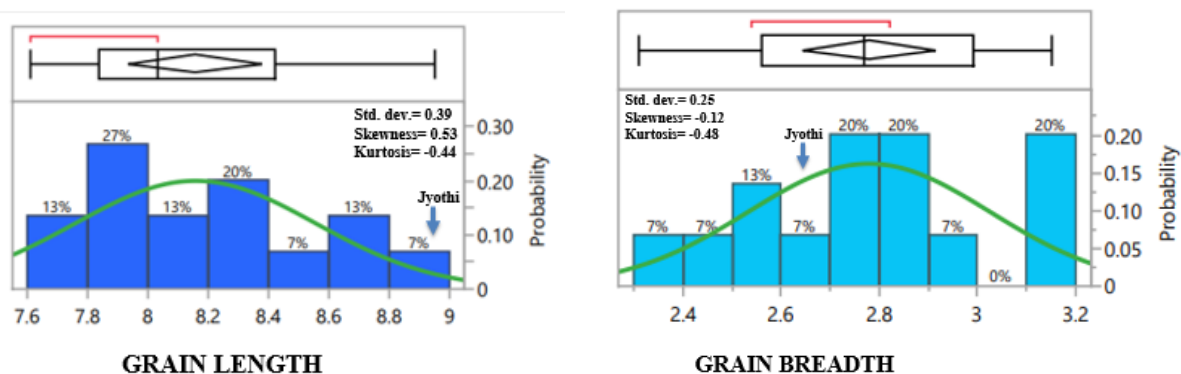
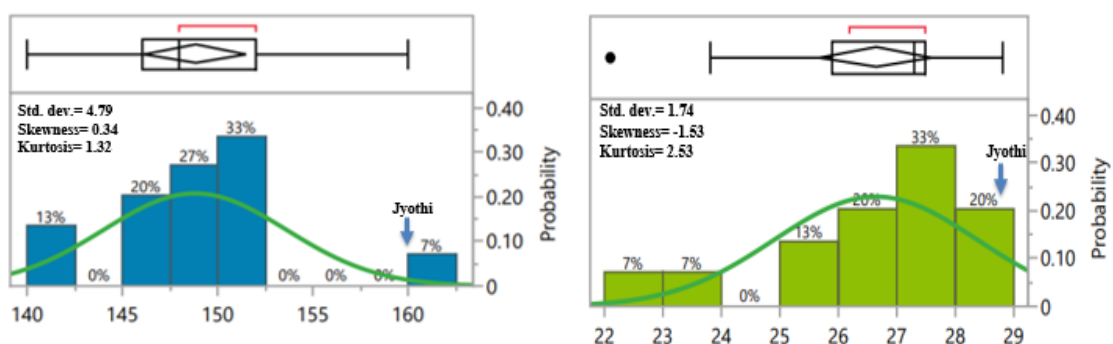


Figure 25. (Contd.) Distribution graph of BC₂F₂ ICAB-1/3 progenies

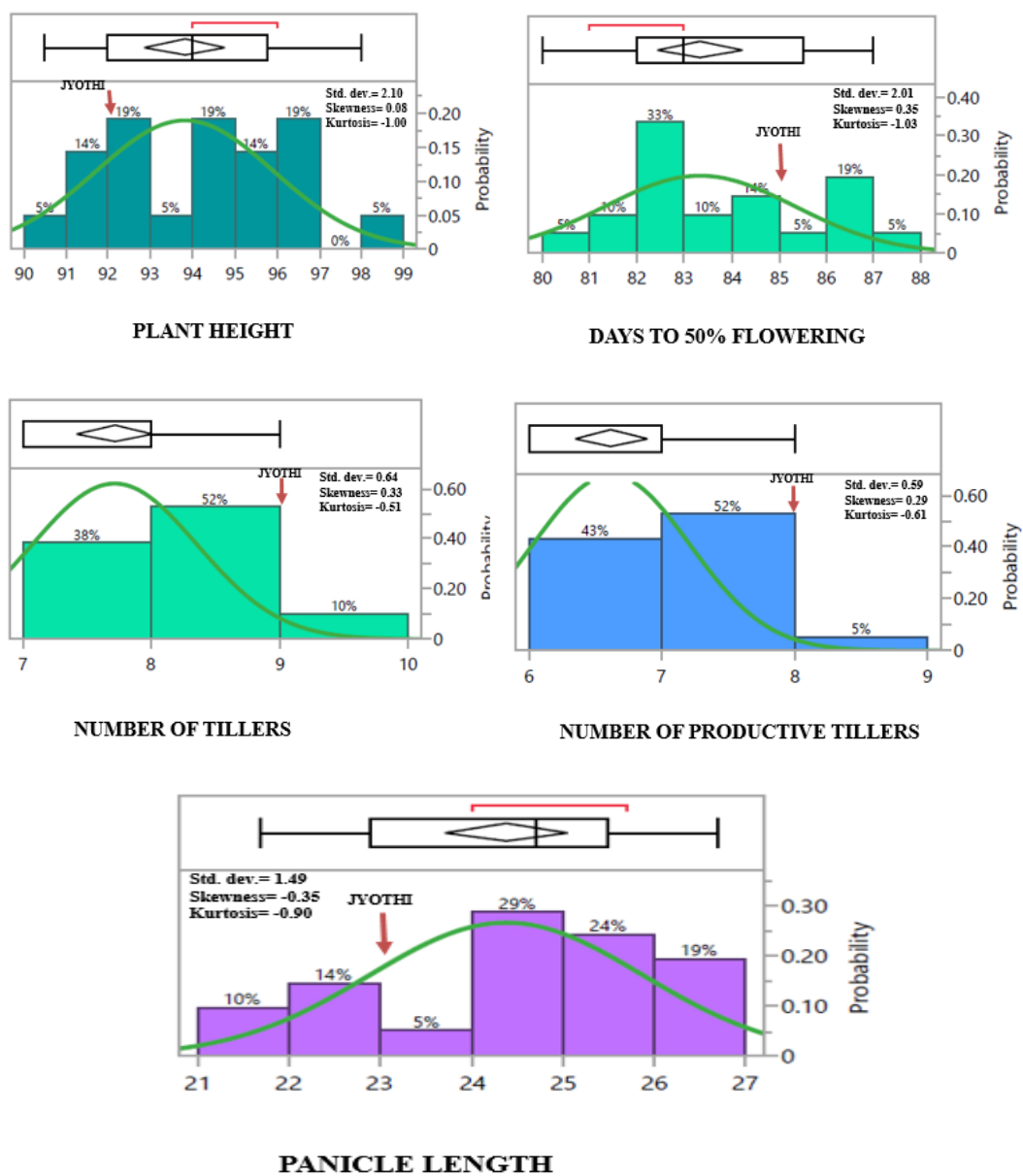
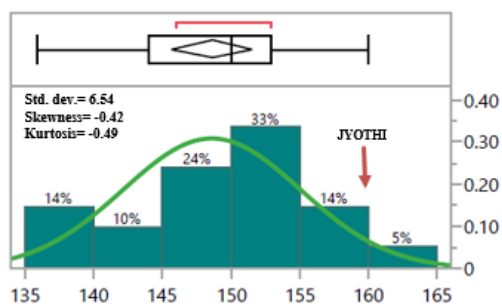
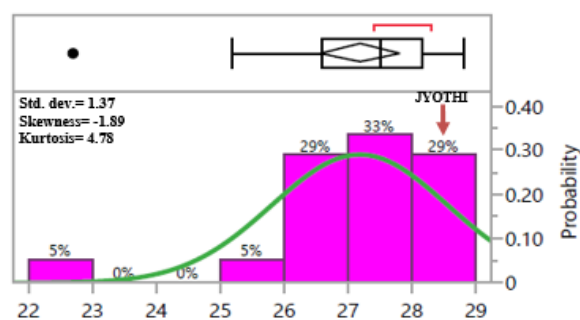


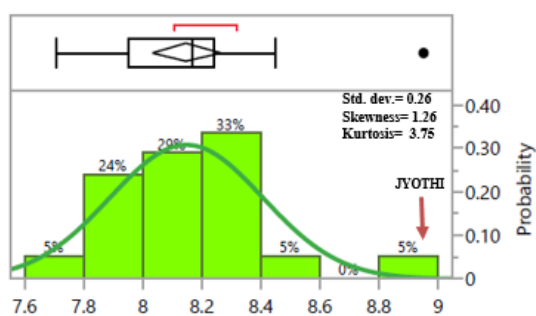
Figure 26. Distribution graph of BC₂F₂ ICAB-1/6 progenies



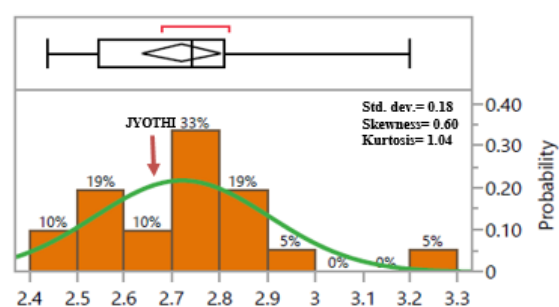
NUMBER OF GRAINS PER PANICLE



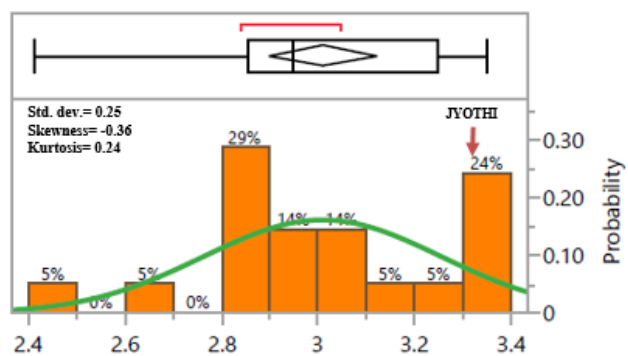
1000 GRAIN WEIGHT



GRAIN LENGTH



GRAIN BREADTH



LB RATIO

Figure 26. (Contd.) Distribution graph of BC₂F₂ ICAB-1/6 progenies

Kernel colour

The kernels of Jyothi, PTB-33 and IR65482-7-216-1-2 were red, red, and white coloured respectively. The light brown to red kernel-coloured lines were recorded in BC₁F₁ lines, intercrossed BC₁F₁ lines, and BC₂F₁ lines, whereas all the BC₂F₂ segregants had red-coloured kernels, in which the recurrent parent phenotype was recovered in the backcrossing programme. The objective of gene pyramiding is to transfer the gene of interest without causing original grain characteristics (Gopalakrishnan *et al.*, 2008). The grain colour greatly depends on its economic value and farmers' acceptance (Basavaraj *et al.*, 2010). Kerala farmers usually prefer red-kernelled rice varieties, which could be achieved in the study through continues backcrossing and directed selection of red-kernelled progenies.

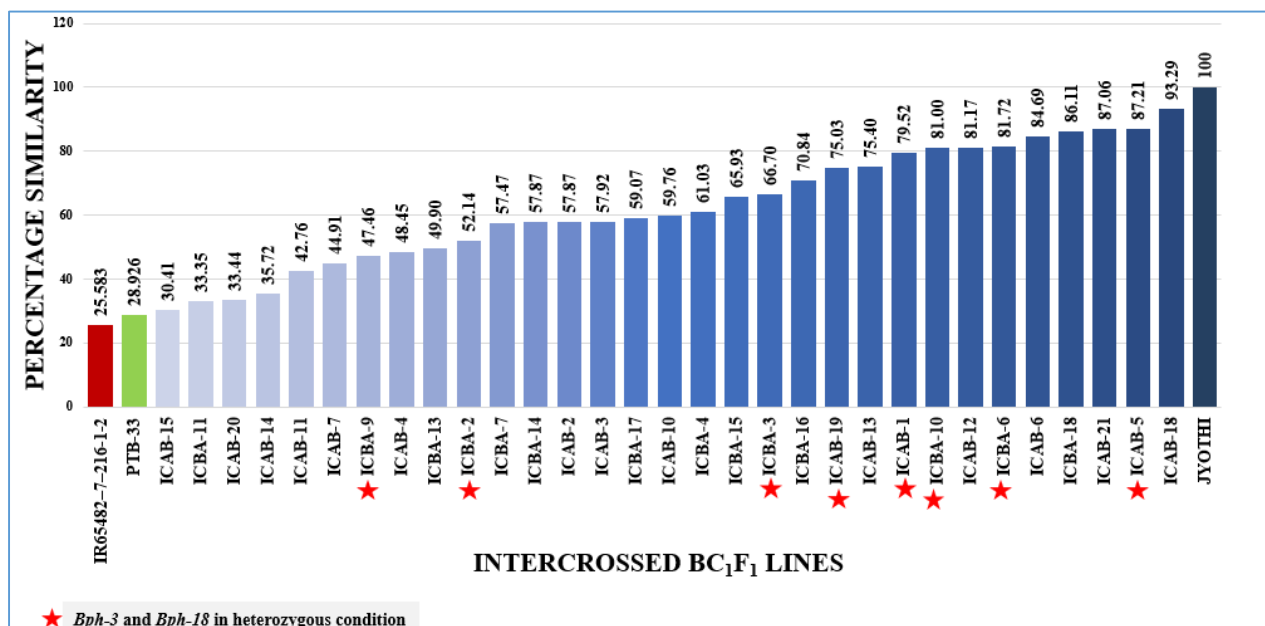
Euclidean Distance of Intercrossed BC₁F₁, BC₂F₁ lines, and BC₂F₂ segregants from Jyothi using Proximity Dissimilarity Matrix Analysis

The Euclidean distance of intercrossed BC₁F₁ lines from Jyothi was higher than that of BC₂F₁ lines measured by the proximity dissimilarity matrix using the morphological traits. The recovery of recurrent parent phenotype increases with the number of back-crosses in breeding programmes. In intercrossed BC₂F₁ lines, similarity to Jyothi ranged from 30 to 93.29 per cent (Figure 27a). About 94.03 per cent similarity to Jyothi was recorded in the BC₂F₁ lines with a minimum of 50 per cent (Figure 27b).

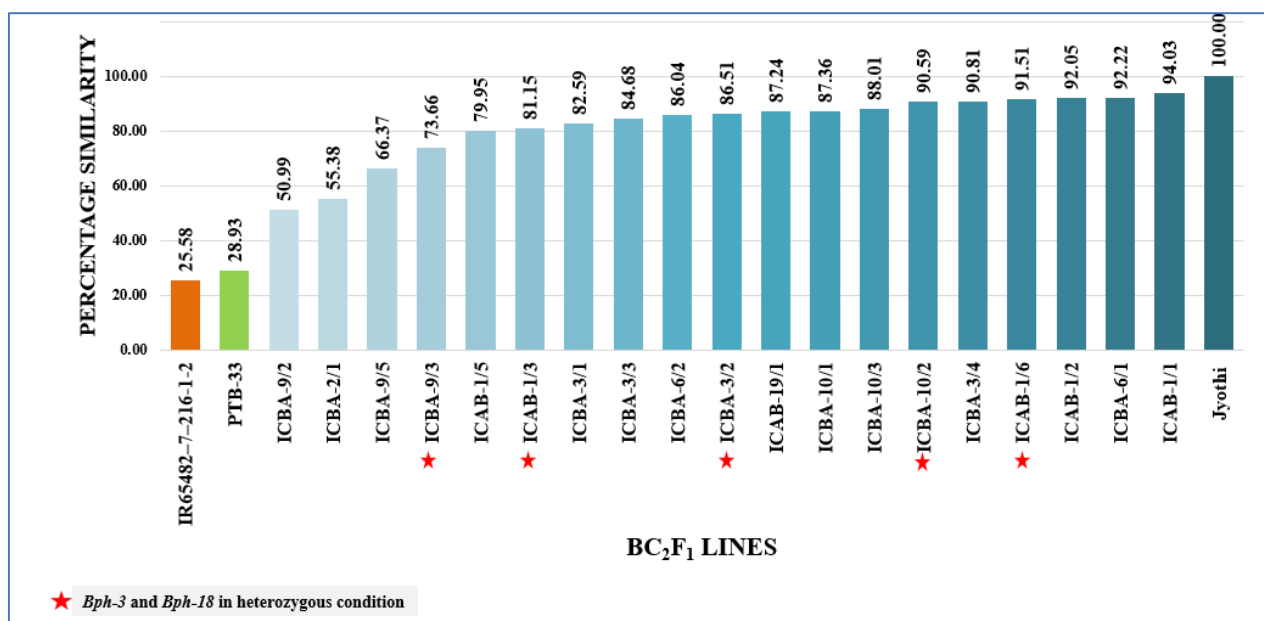
More than 70 per cent similarity to Jyothi was recorded in the BC₂F₁ lines having both the genes (*Bph-3* and *Bph-18*) in heterozygous conditions (Figure 27b). About per cent similarity to Jyothi in the BC₂F₂ lines ranged between 79 to 91.6 per cent in ICAB-1/3 progenies (Figure 28). In ICAB-1/6 progenies, per cent similarity to Jyothi ranged between 75 to 94.16 per cent (Figure 29). BC₂F₂ segregants with both genes in homozygous resistant conditions showed more than 90 per cent phenotype recovery (Figure 30). The results were consistent with the earlier studies of marker-assisted pyramiding that followed the estimation of recurrent parent phenome recovery prior to background screening using molecular markers (Joseph *et al.*, 2004; Suh *et al.*, 2013; Ellur *et al.*, 2016; Krishnamurthy *et al.*, 2020; Zampieri

et al.,2023; Kumar *et al.*, 2023). Recurrent parent phenotype recovery determines the efficacy of the phenotypic selection done in each backcross and can deduct the number of plants in background screening. Superior plants with maximum recurrent parent phenome recovery for important phenotypic characters were advanced from BC₁F₁ onwards to further backcross generations as reported by Ellur *et al.* (2016) in marker-assisted pyramiding of the *Xa38* gene into Pusa Basmati 1121 and Mohapatra *et al.* (2023) in the backcross program for introgression of submergence tolerance into the variety Ranidhan.

The results and discussion of the present study implicated the effectiveness of marker-assisted selection in the development of breeding lines in rice pyramided with R genes for resistance to Brown Plant Hopper (BPH). The R genes *viz.*, *Bph-3* from the donor parent PTB-33 and *Bph-18* from the donor parent IR65482-7-216-1-2 were introgressed successfully into the background of elite high-yielding but susceptible parent Jyothi. The BC₂F₂ generation was subjected to BPH bioassay, phenotyping, and genotyping using gene-specific markers RM589 and RM3331. Four BC₂F₂ segregants with BPH resistance, maximum similarity to recurrent parent Jyothi and both genes in homozygous resistant condition were developed. These breeding lines, possessing pyramided R genes for BPH resistance in the background of Jyothi can be used to develop essentially derived varieties from Jyothi with BPH resistance.



(a) Percentage similarity of intercrossed BC₁F₁ lines to recipient parent 'Jyothi' based on Euclidean distance using morphological traits



(b) Percentage similarity of BC₂F₁ lines to recipient parent 'Jyothi' based on Euclidean distance using morphological traits

Figure 27. Percentage similarity of intercrossed BC₁F₁ and BC₂F₁ lines to recipient parent 'Jyothi' based on Euclidean distance using morphological traits

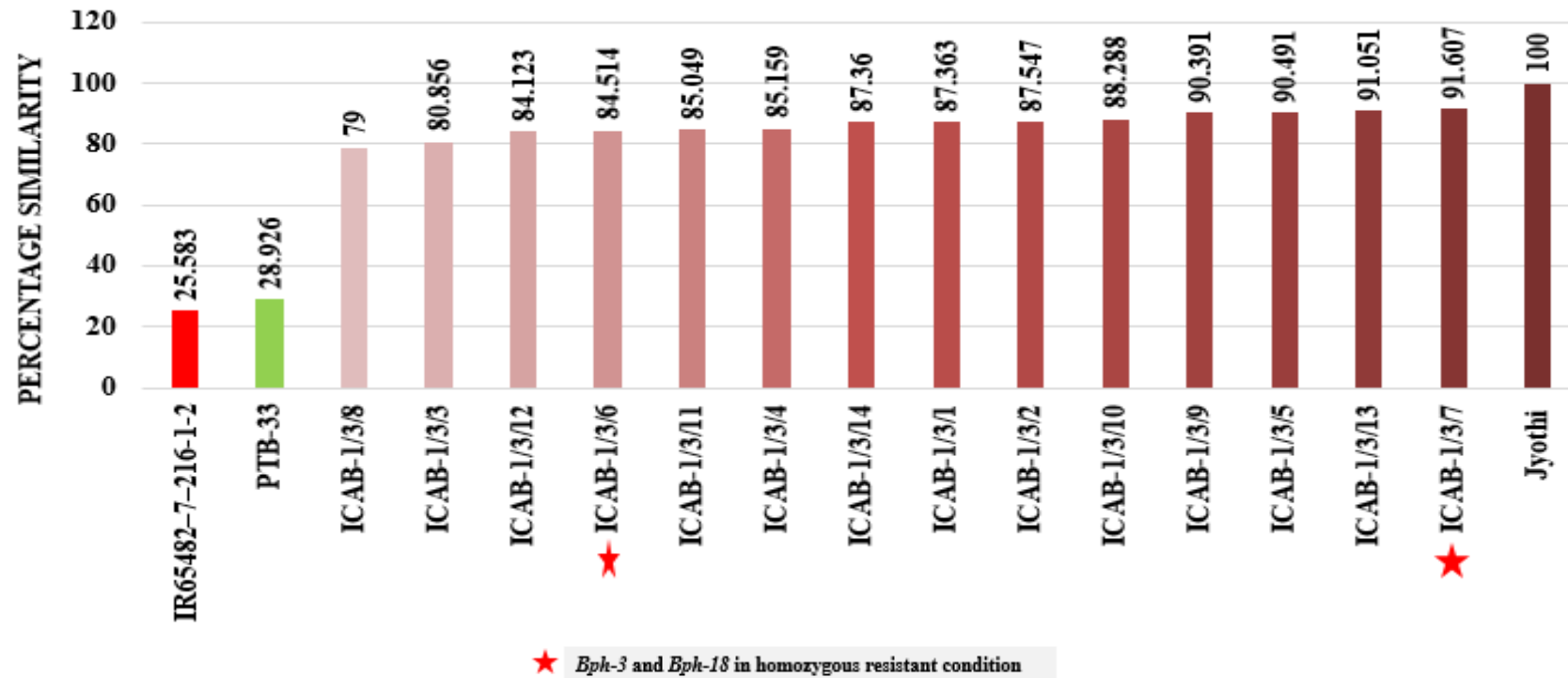


Figure 28. Percentage similarity of BC₂F₂ lines (ICAB-1/3 progenies) to recipient parent 'Jyothi' based on Euclidean distance using morphological traits

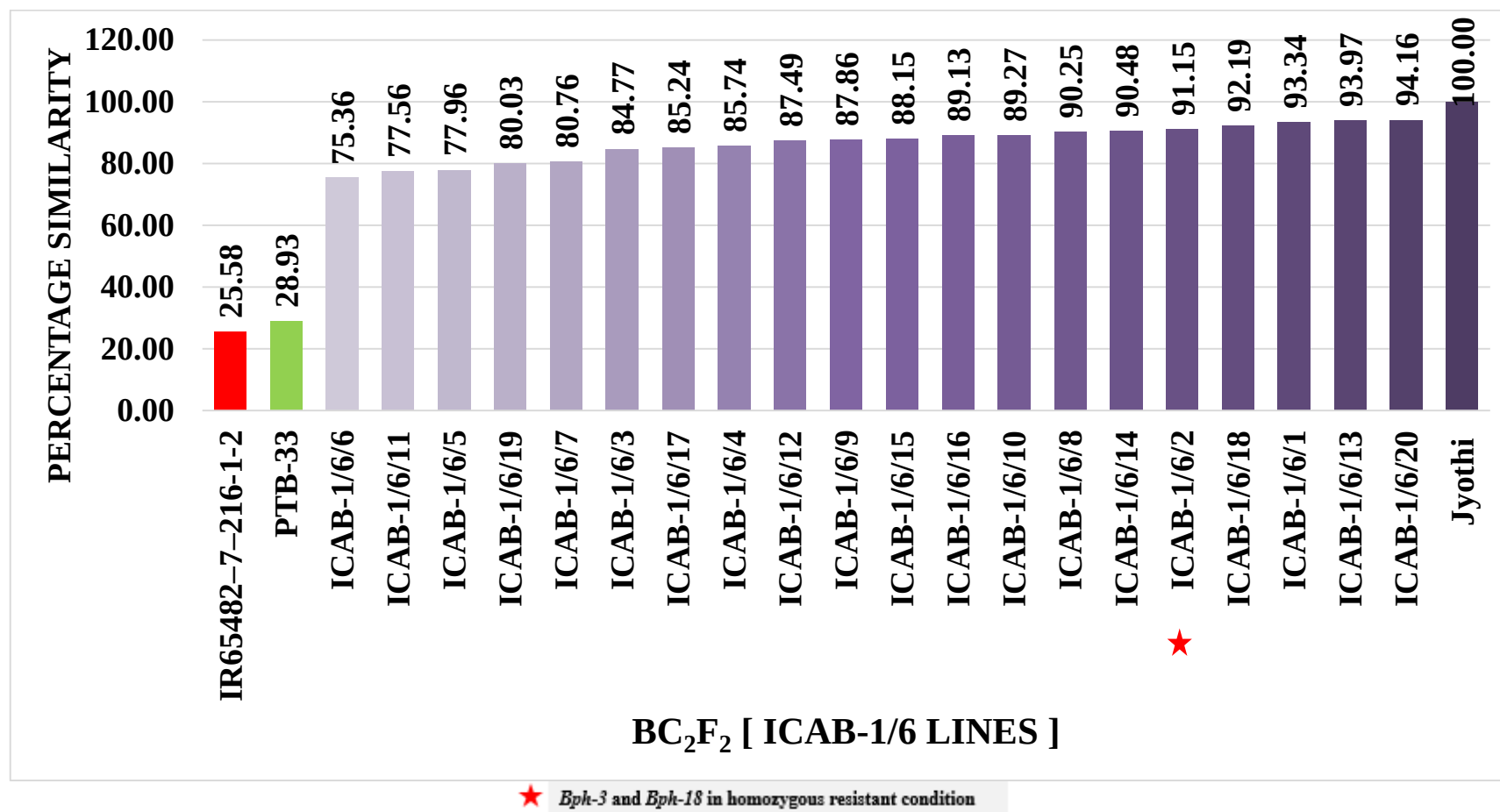
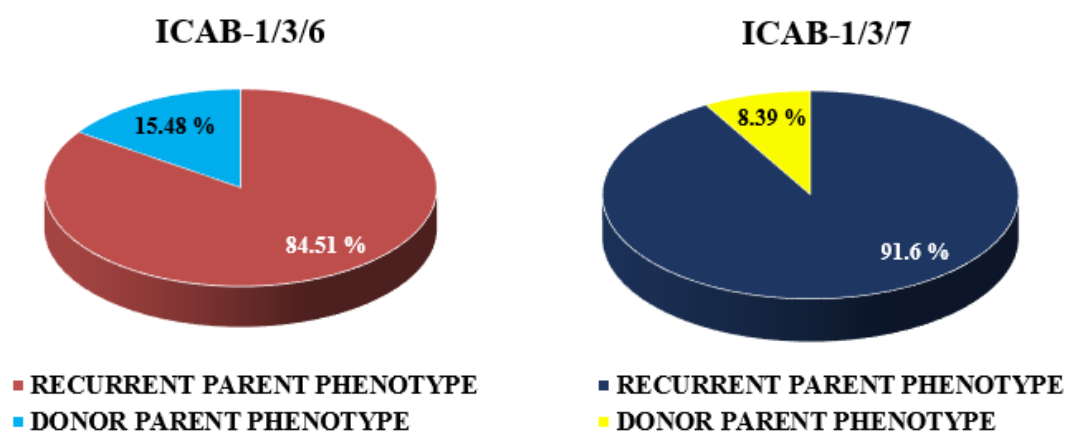
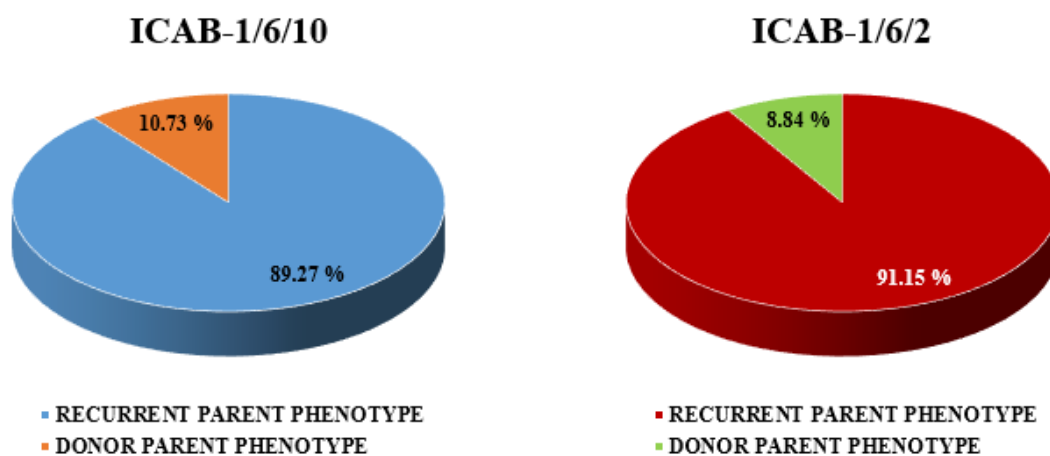


Figure 29. Percentage similarity of BC₂F₂ lines (ICAB-1/6 progenies) to recipient parent 'Jyothi' based on Euclidean distance using morphological traits



(a) ICAB-1/3/6 and ICAB-1/3/7



(b) ICAB-1/6/10 and ICAB-1/6/2

Figure 30. Recurrent parent phenotype recovery in BC₂F₂ lines

SUMMARY

6. SUMMARY

The present study ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ was put forth with an objective to introgress R genes for resistance to BPH in the background of the elite variety Jyothi using marker-assisted selection. The Brown Plant Hopper (BPH) poses a significant threat to rice (*Oryza sativa* L.) production worldwide, leading to substantial yield losses. Recent outbreaks of BPH in Kerala mainly in the Palakkad district had detrimental effects on the rice production affecting the yield potential of the popular high-yielding variety Jyothi.

R gene mediated host plant resistance has proven to be an effective approach to combat BPH infestations. Marker-assisted selection (MAS) offers a promising tool for the efficient and accurate breeding of BPH-resistant rice varieties. To provide durable resistance to BPH, the genes that contribute a major effect on resistance should be identified from the germplasm, and those genes should be transferred to a well-adapted single variety. Pyramiding multiple resistance genes into a single rice cultivar can provide sustainable and durable control against different biotypes of BPH. Around 45 genes imparting BPH resistance have been mapped, out of which 9 genes have been cloned and characterized. The pertinent and considerable information on genes for BPH resistance, gene-specific SSR markers, inheritance patterns, and screening techniques sheds light on the challenges and potential avenues in the development of BPH-resistant varieties.

The study employed BPH bioassay, phenotypic assessment of morphological and biochemical characters linked to BPH resistance in the recipient and donor parents, gene validation using specific molecular markers followed by gene pyramiding based on the marker-assisted backcross breeding program. Recipient parent (Jyothi) and probable donors for BPH resistance from the results of a concluded project in the Department of Plant Breeding and Genetics, COA, Vellayani, and other R gene introgressed lines from IRRI were selected for screening. The BPH bioassay was done using the modified seedbox screening test

(MSST) developed by Velusamy (1986). BPH-affected seedling survival rate was calculated based on the Standard Evaluation System (SES) scale of 0-9 (IRRI, 2013). Among the twelve rice genotypes screened, PTB-33, PTB-21, and IR7103-121-15-B were classified as highly resistant and the genotypes IR65482-7-216-1-2 and RP2068-18-3-5 were classified as resistant to BPH infestation. The *japonica* variety Kinandang Patong was moderately resistant to BPH. The recipient parent Jyothi and five other genotypes viz., Manu Ratna, White Ponni, Wayrareem, Vandana, and APO were classified as highly susceptible to BPH infestation.

Five genotypes, Jyothi (recipient parent), PTB-33, IR7103-121-15-B, IR65482-7-216-1-2, and RP2068-18-3-5 were forwarded for studying the BPH resistance reaction. The antibiosis and tolerance mechanisms of BPH resistance were explored in terms of nymphal survival rate, honey-dew test, functional plant loss index (FPLI), and tolerance index (TI). The recipient parent, ‘Jyothi’ turned out to be highly susceptible, and ‘PTB-33’ showed high resistance to BPH feeding among the rice genotypes screened. Other genotypes, IR65482-7-216-1-2, RP2068-18-3-5, and IR7103-121-15-B showed intermediate values compared to PTB-33 and the highly susceptible variety ‘Jyothi’. The morphological characters and biochemical parameters linked with BPH resistance of five genotypes were assessed in a completely randomized design (CRD) with four replications. The results revealed that BPH feeding, survival, functional plant loss index, and disease score were positively correlated with protein content and negatively correlated with phenol and ascorbic acid content. Reducing sugar content in plants did not show much effect on BPH resistance.

The genetic parameters, phenotypic and genotypic coefficient of variation were higher in seven characters, and moderate in six characters indicating high variability among the parents. High heritability and genetic advance in the characters except for the number of productive tillers, and culm thickness and grain length respectively indicated the presence of additive gene action. The characters, culm thickness, 1000 grain weight, and protein content showed a significant positive correlation with the functional plant loss index (FPLI). Path analysis of morphological and biochemical characters with FPLI as the dependent variable

showed the direct positive effect of the number of productive tillers, panicle length, number of grains per panicle, grain breadth, LB ratio, and phenol content on FPLI.

The validation of the presence or absence of genes for BPH resistance in donor and recipient parents was done using SSR markers RM589 for the *Bph-3* gene, RM3331 for *Bph-18*, RM8213 for *Bph-20*, and RM28561 for *Bph-21*. Distinguishable polymorphic bands were obtained in SSR markers RM589 (200 bp for resistance and 180 bp for susceptibility) and RM3331 (110 bp for resistance and 130 bp for susceptibility). Based on BPH screening, resistant reaction study, and gene validation, the KAU released variety 'PTB-33' and IRRI introgression line IR65482-7-216-1-2 were selected as the donors for *Bph-3* and *Bph-18* gene respectively in the marker-assisted backcross breeding program.

The second experiment was marker-assisted backcrossing to introgress the R genes (*Bph-3* and *Bph-18*) into the background of elite high-yielding but susceptible variety Jyothi. Staggered sowing of the recipient and donor parents was undertaken to obtain synchronized flowering. The donor parent PTB-33 with long crop duration (150 days) and photosensitivity, was sown one month prior to the other donor parent IR65482-7-216-1-2 and the recipient parent Jyothi. The donors PTB-33 and IR65482-7-216-1-2 were crossed independently with Jyothi to obtain the F₁ generation followed by backcrossing with Jyothi to develop the BC₁F₁ generation. The BC₁F₁ lines with genes in the heterozygous state were intercrossed to develop the intercrossed BC₁F₁ generation. The BC₂F₁ lines were developed by backcrossing the intercrossed BC₁F₁ lines with Jyothi. The BC₂F₁ lines with both genes in the heterozygous state were selfed to generate the BC₂F₂ segregants. Phenotyping and genotyping were carried out in all the backcross generations. A wide range of morphological characters was recorded to analyze the distribution, cluster, and correlation of the characters in BC₁F₁ lines of the cross [Jyothi x PTB 33] x Jyothi and [Jyothi x IR65482-7-216-1-2] x Jyothi. The distribution curve of most of the morphological characters in all backcross generations was bell-shaped, indicating that the traits were controlled by polygenes.

Genotyping of 63 BC₁F₁ lines derived from the PTB-33 donor parent with RM589 marker identified 28 plants with the *Bph-3* gene in heterozygous condition.

Genotyping of 78 BC₁F₁ lines derived from the IR65482-7-216-1-2 donor parent with RM3331 marker identified 36 plants with *Bph-18* gene in heterozygous condition. The goodness of fit test (χ^2 test) in BC₁F₁ lines with genotypic data showed that the genes *Bph-3* and *Bph-18* followed the ratio of simple dominance inheritance.

Intercrossing of BC₁F₁ lines involved two crosses viz., the PTB-33 derived BC₁F₁ lines with *Bph-3* in heterozygous condition as the female parent and IR65482-7-216-1-2 derived BC₁F₁ lines with *Bph-18* in heterozygous condition as male parent denoted as 'ICAB' and the reciprocal cross denoted as 'ICBA'. Among the twenty-two ICAB progenies, three plants showed both genes (*Bph-3* and *Bph-18*) in heterozygous condition, and among the eighteen ICBA progenies, five plants were obtained with both genes (*Bph-3* and *Bph-18*) in heterozygous condition. Phenotyping of intercrossed BC₁F₁ lines was carried out to analyze the similarity with the recipient parent. The Euclidean Distance of intercrossed BC₁F₁ lines from the recipient parent Jyothi using proximity dissimilarity matrix analysis was calculated to select the intercrossed BC₁F₁ lines more similar to the recipient parent. Eight intercrossed BC₁F₁ lines with Jyothi-specific characters and both genes in heterozygous condition were backcrossed with Jyothi to develop BC₂F₁ lines.

In the genotypic evaluation of twenty-six BC₂F₁ lines, five lines showed the presence of both genes (*Bph-3* and *Bph-18*) in heterozygous conditions. The five BC₂F₁ lines with maximum similarity in proximity dissimilarity matrix analysis were selfed and three-hundred and twenty BC₂F₂ segregants were forwarded to BPH bioassay in the seedling stage at RARS, Pattambi, and COA, Vellayani. Eighty-nine BC₂F₂ segregants showed different levels of resistance in the BPH bioassay. Genotyping of these lines showed the presence of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition in four lines namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10.

The similarity percentage of these four lines with the recipient parent in proximity dissimilarity matrix analysis was obtained as 84.51 per cent in ICAB-1/3/6, 89.27 per cent in ICAB-1/6/10, 91.15 per cent in ICAB-1/6/2, and 91.6 per

cent in ICAB-1/3/7. The study successfully developed R gene introgressed breeding lines with enhanced BPH resistance and maximum phenotypic similarity to recipient parent Jyothi. These breeding lines can be forwarded to further backcrossing to develop essentially derived varieties from Jyothi. Thus the marker-assisted gene pyramiding proved to be a promising strategy for sustainable BPH management by accelerating the introgression of multiple genes for resistance into a recipient parent.

REFERENCES

7. REFERENCES

- Abraham, C.C. and Nair, M.R.G.K. 1975. The brown planthopper outbreaks in Kerala, India. *Rice Entomol. Newsl.* 2:36.
- Abraham, E.V. 1957. A note on the influence of manuring on the incidence of the fulgorid—*Nilaparvata lugens* S. on paddy. *Madras Agric. J.* 44:530-531.
- Ahuja, U., Ahuja, S.C., Chaudhary, N. and Thakrar, R. 2007. Red rice—past, present and future. *Asian Agri-Hist.* 11(4):291-304.
- Akanksha, S., Lakshmi, V.J., Singh, A.K., Deepthi, Y., Chirutkar, P.M., Balakrishnan, D., Sarla, N., Mangrauthia, S.K. and Ram, T. 2019. Genetics of novel brown planthopper *Nilaparvata lugens* (Stål) resistance genes in derived introgression lines from the interspecific cross *O. sativa* var. Swarna × *O. nivara*. *J. Genet.* 98:1-10.
- Akshay, M., Chandra, B.S., Devi, K.R. and Hari, Y. 2022. Genetic variability studies for yield and its attributes, quality and nutritional traits in rice (*Oryza sativa* L.). *Pharma Innovation J.* 11(5):167-172.
- Akula, S.H., Dass, M.A., Surapaneni, S.K. and Balaravi, P. 2020. Mapping of quantitative trait loci associated with resistance to brown planthopper in background of Swarna from a traditional variety PTB33. *Euphytica*, 216(7):114.
- Amiteye, S. 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon*, 7(10):p.e08093.
- Angeles, E.R., Khush, G.S. and Heinrichs, E.A. 1986. Inheritance of resistance to planthoppers and leafhoppers in rice. In: Banta, S.J. (ed.). *Rice Genetics I: (Parts 2)*. International Rice Research Institute, Philippines, pp. 537-549.
- Arunakumari, K., Durgarani, C.V., Satturu, V., Sarikonda, K.R., Chittoor, P.D.R., Vutukuri, B., Laha, G.S., Nelli, A.P.K., Gattu, S., Jamal, M. and Prasadbabu, A. 2016. Marker-assisted pyramiding of genes conferring

resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. *Rice Sci.* 23(6):306-316.

Ashrith, K.N., Sreenivas, A.G., Guruprasad, G.S., Hanchinal, S.G. and Krishnamurthy, D. 2017. Biochemical basis of resistance in rice against brown and white-backed planthopper. *Int. J. Curr. Microbiol. Appl. Sci.* 6:1699-1706.

Athwal, D.S., Pathak, M.D., Bacalangco, E.H. and Pura, C.D. 1971. Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. *Crop Sci.* 11(5):747-750.

Babu, S. B., Parameswaran, C., Padhi, J., Basana-Gowda, G., Annamalai, M., Patil, N., Meher, C., Sabarinathan, S. and Rath, P.C. 2023. Genetic analysis of brown planthopper, *Nilaparvata lugens* (Stål.)(Hemiptera: Delphacidae) based on DNA markers. *Res. Square*, 1-16.

Babu, S.B., Parameswaran, C., Anant, A.K., Padhi, J., Bansal, R., Priyadarsini, S., Patra, B.C., Basana-Gowda, G., Annamalai, M., Patil, N. and Rath, P.C. 2022. Genomic analysis and finding of candidate genes for *Nilaparvata lugens* (stål) resistance in Indian pigmented and other indigenous rice genotypes. *Crop Protect.* 156:105959.

Backus, E.A. 1988. Sensory systems and behaviours which mediate hemipteran plant-feeding: a taxonomic overview. *J. Insect Physiol.* 34(3):151-165.

Balachiranjeevi, C.H., Prahalada, G.D., Mahender, A., Jamaloddin, M., Sevilla, M.A.L., Marfori-Nazarea, C.M., Vinarao, R., Sushanto, U., Baehaki, S.E., Li, Z.K. and Ali, J. 2019. Identification of a novel locus, *BPH38(t)*, conferring resistance to brown planthopper (*Nilaparvata lugens* Stål.) using early backcross population in rice (*Oryza sativa* L.). *Euphytica*, 215(11): 185.

- Baliyan, N., Mehta, K., Rani, R. and Purushottum, B.K. 2018. Evaluation of pyramided rice genotypes derived from cross between CSR-30 and IRBB-60 basmati variety against bacterial leaf blight. *Int. J. Plant Res.* 29(3):184.
- Barrion, A.A. 1985. Selection on resistant rice varieties and genetics of rice-infesting biotypes of the brown planthopper, *Nilaparvata lugens* (Stal)(Hemiptera: Delphacidae). Ph.D. Thesis, University of Philippines, Laguna, Philippines, 158p.
- Basavaraj, P.S., Gireesh, C., Damodar Raju, C.H., Sheshu Madhav, M. and Jagadeeshwar. R. 2019. Genetic analysis of transgressive segregation in backcross derived introgression population of *Oryza sativa* cv. Samba mahsuri/*O. Rufipogon* for yield enhancing traits. *J. Res. PJTSAU Vol. XLVII No. (3):1-70.*
- Basavaraj, S.H., Singh, V.K., Singh, A., Singh, A., Singh, A., Anand, D., Yadav, S., Ellur, R.K., Singh, D., Gopala Krishnan, S. and Nagarajan, M. 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Mol. Breed.* 26:293-305.
- Bates, J. 2022. The Fits and Starts of Indian Rice Domestication: How the Movement of Rice Across Northwest India Impacted Domestication Pathways and Agricultural Stories. *Frontiers Ecol. Evol.* 535.
- Bhati, M., Babu, G.S. and Rajput, A.S. 2015. Genetic variability, correlation and path coefficient for grain yield and quantitative traits of elite rice (*Oryza sativa* L.) genotypes at Uttar Pradesh. *Electr. J. Plant Breed.* 6(2):586-591.
- Blakeney, M., Krishnankutty, J., Raju, R.K. and Siddique, K.H. 2020. Agricultural innovation and the protection of traditional rice varieties: Kerala a case study. *Frontiers Sustain. Food Syst.* 3:116.

- Bottrell, D.G. and Schoenly, K.G. 2012. Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. *J. Asia-Pacific Entomol.* 15(1):122-140.
- Bradford, M.M. 1976. 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, D.S. 2003. Utilization of wild species of genus *Oryza* in rice improvement. *Monograph on genus Oryza*. Science Publishers, Inc. 134p.
- Burton, G.W. 1952. *Proc. 6th Int. Grassl. Cong.* 1:277-283.
- Bush, G.L. 1974. The mechanism of sympatric host race formation in the true fruit flies (Tephritidae). In: *Genetic Mechanisms of Speciation in Insects: Symposia held at the XIVth International Congress of Entomology, Canberra, Australia August 22–30, 1972, sponsored by the Australian Academy of Science and the Australian Entomological Society*, Springer Netherlands, pp. 3-23.
- Cagampang, G.B., Pathak, M.D. and Juliano, B.O. 1974. Metabolic changes in the rice plant during infestation by the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Appl. Entomol. Zool.* 9(3):174-184.
- Caresche, L., 1933. Note sur les insectes appelés “pucerons du riz” dans le Sud-Indochinois et les moyens de lutte à leur opposer. *Bull. econ. Indochine*, 36: 488-510.
- Carmona, D., Lajeunesse, M.J. and Johnson, M.T. 2011. Plant traits that predict resistance to herbivores. *Functional Ecol.* 25(2):358-367.
- Carsono, N., Tambunan, R.R., Sari, S. and Wicaksana, N. 2023. Molecular and phenotypic markers for pyramiding multiple traits in rice. *Open Agric.* 8(1): p.20220187.

- Cha, Y.S., Ji, H., Yun, D.W., Ahn, B.O., Lee, M.C., Suh, S.C., Lee, C.S., Ahn, E.K., Jeon, Y.H., Jin, I.D., Sohn, J.K., Koh, H.J. and Eun, M. Y. 2008. Fine mapping of the rice *Bph1* gene, which confers resistance to the brown planthopper (*Nilaparvata lugens* Stal) and development of STS markers for ° marker-assisted selection. *Mol. Cells*, 26:146-151.
- Chacko, A., Jayalekshmy, V.G., Shahiba, A.M., and Adarsh, S. 2023. Hidden treasures of rice varieties in Kerala for insect resistance [abstract]. In: International Conference on Biodiversity: Exploration, Exploitation and Conservation for Sustainable Development, 10-11, Feb, 2023, Department of Botany, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Behali, Assam, India. P.22. Abstract No. 38.
- Chakrabarti, N.K., Kulshreshtha, P.J. and Rao, Y.S. 1971. Pests and diseases of new varieties and remedial measures. *Indian Farming*, 21:53-58.
- Chakrabarty, S.K., Basu, S. and Schipprach, W. 2023. *Hybrid Seed Production Technology*. In: Dadlani, M. and Yadava, D.K. (eds). Seed Science and Technology. Springer, Singapore, pp.173-212.
- Chakravorty, A and Ghosh, P.D. 2011. An analysis on genetic parameters of different Land races of rice of West Bengal. *J. Crop Weed*, 7(2), pp.59-63.
- Chander, S. and Husain, M. 2018. Brown plant hopper (BPH) outbreak in rice: analysis of weather parameters during outbreak and non-outbreak years. *Indian J. Entomol.* 80(3):677-682.
- Chelliah, S., Heinrichs, E.A. and Smith, W.H. 1984. *Factors contributing to brown planthopper resurgence*. Judicious and Efficient Use of Insecticides on Rice. IRRI, Philippines, pp.107-115.
- Chen, J.W., Wang, L., Pang, X.F. and Pan, Q.H. 2006. Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph19(t)*. *Mol. Genet. Genomics*, 275(4): 321-329.

- Chen, Y.H. 2009. Variation in planthopper-rice interactions: possible interactions among three species. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Banos (Philippines), pp.315-326.
- Cheng, C.H. and Chang, W.L. 1979. Studies on varietal resistance to the brown planthopper in Taiwan. *Brown planthopper: Threat to rice production in Asia*, International Rice Research Institute, Los Banos (Philippines), pp.251-271.
- Cheng, L., Zhu, Y., Li, J., Jiang, Z., Shu, W. and Qiu, Y. 2021. Mapping and breeding application of the brown planthopper (*Nilaparvata lugens*)-resistance genes derived from a durable resistant PTB33 rice variety (*Oryza sativa*). *Plant Breed.* 140(6):981-989.
- Cheng, X., Zhu, L. and He, G. 2013. Towards understanding of molecular interactions between rice and the brown planthopper. *Mol. Plant*, 6(3):621-634.
- Choi, S.Y. and Brady, N.C. 1979. Screening methods and sources of varietal resistance. *Brown planthopper: Threat to rice production in Asia*, International Rice Research Institute, Los Banos (Philippines), pp.171-185.
- Clemensen, A.K., Provenza, F.D., Lee, S.T., Gardner, D.R., Rottinghaus, G.E., and Villalba, J.J. 2017. Plant secondary metabolites in alfalfa, birdsfoot trefoil, reed canarygrass, and tall fescue unaffected by two different nitrogen sources. *Crop Sci.* 57:964-970.
- Cruz, C.D. and Regazzi, A.J. 1994. Modelos biométricos aplicados ao melhoramento genético. Universidade Federal de Viçosa, Imprensa Universitária, Viçosa, Minas Gerais, Brazil.
- Dai, Z., Tan, J., Zhou, C., Yang, X., Yang, F., Zhang, S., Sun, S., Miao, X. and Shi, Z. 2019. The OsmiR396–Os GRF 8–OsF3H-flavonoid pathway mediates

- resistance to the brown planthopper in rice (*Oryza sativa*). *Plant Biotechnol. J.* 17(8):1657-1669.
- Das, N.M., Mammen, K.V. and Christudas, S.P. 1972. Occurrence of *Nilaparvata lugens* (Stål.) (Delphacidae: Homoptera) as a serious pest of paddy in Kerala. *Agric. Res. J. Kerala* 10(2):191-192.
- De Lucia, E., Nabity, P., Zavala, J. and Berenbaum, M. 2012. Climate Change: Resetting Plant-Insect Interactions. *Plant Physiol.* 160:677-1685.
- Deepa, G., Singh, V. and Naidu, K.A. 2008. Nutrient composition and physicochemical properties of Indian medicinal rice—Njavara. *Food Chem.* 106(1):165-171.
- Demeke, B., Dejene, T. and Abebe, D. 2023. Genetic variability, heritability, and genetic advance of morphological, yield related and quality traits in upland rice (*Oryza Sativa* L.) genotypes at Pawe, northwestern Ethiopia. *Cogent Food Agric.* 9(1):2157099.
- Devi, P.I., Thomas, J. and Raju, R.K. 2017. Pesticide consumption in India: A spatiotemporal analysis. *Agric. Econ. Res. Rev.* 30(1):163-172.
- Dewey, D.R. and Lu, K. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. *Agron. J.* 51(9):515-518.
- Dhakal, A., Sharma, S., Pokhrel, A. and Poudel, A. 2020. Variability and heritability estimate of 30 rice landraces of Lamjung and Tanahun Districts, Nepal. *Indonesian J. Agric. Sci.* 21(1):1-10.
- Dhaliwal, G.S. and Arora, R. 2009. *Integrated pest management: concepts and approaches*. Kalyani publisher, 253p.
- Distant, W.L. 1906. *Rhynchota. The fauna of British India, including Ceylon and Burma*. No. 3. Taylor and Francis, London, England. 266p.

- Divya, T. and Pandey, D.P. 2020. Genetic variability for yield and quality traits in local germplasm of rice of Himachal Pradesh. *J. Cereal Res.* 12(2):157-159.
- Dormatey, R., Sun, C., Ali, K., Coulter, J.A., Bi, Z. and Bai, J. 2020. Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. *Agron.* 10(9):p.1255.
- Doyle, J.J. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
- Du, B., Chen, R., Guo, J. and He, G. 2020. Current understanding of the genomic, genetic, and molecular control of insect resistance in rice. *Mol. Breed.* 40:1-25.
- Du, B., Zhang, W., Liu, B., Hu, J., Wei, Z., Shi, Z., He, R., Zhu, L., Chen, R., Han, B. and He, G. 2009. Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *PNAS*, 106:22163–22168.
- Dupo, A.L.B. and Barrion, A.T. 2009. Taxonomy and general biology of delphacid planthoppers in rice agroecosystems. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Banos (Philippines), pp.3-155.
- Dyck, V.A., Misra, B.C., Alam, S., Chen, C.N., Hsieh, C.Y. and Rejesus, R.S. 1979. Ecology of the brown planthopper in the tropics. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Banos, Philippines, pp.61-98.
- Ellur, R.K., Khanna, A., Bhowmick, P.K., Vinod, K.K., Nagarajan, M., Mondal, K.K., Singh, N.K., Singh, K., Prabhu, K.V. and Singh, A.K. 2016. Marker-aided incorporation of *Xa38*, a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with *xa13+ Xa21*. *Sci. Rep.* 6(1):1-8.

- Fahad, S., Nie, L., Hussain, S., Khan, F., Khan, F.A., Saud, S., Muhammad, H., Li, L., Liu, X., Tabassum, A. and Wu, C. 2015. Rice pest management and biological control. In: Lichtfouse, E. and Goyal, A. (eds), *Sustainable Agriculture Reviews*. Springer, Cham, pp. 85-106.
- Falconer, D.S. 1964. Introduction to Quantitative Genetics. Longmann, London and New York. pp. 294-300.
- Fan, F., Li, N., Chen, Y., Liu, X., Sun, H., Wang, J., He, G., Zhu, Y. and Li, S. 2017. Development of elite BPH-resistant wide-spectrum restorer lines for three and two line hybrid rice. *Frontiers Plant Sci.* 8:986.
- FAO[Food and Agricultural Organisation]. 2022. *World Food and Agriculture – Statistical Yearbook 2022* [online]. Available: <https://doi.org/10.4060/cc2211en>
- Gaballah, M., Hamad, H., Bamagoos, A., Alharby, H., Ahmed, S., Ismail, I.A., Sohikul Islam, M. and EL Sabagh, A. 2021. Flowering synchronization in hybrid rice parental lines at different sowing dates. *Sustain.* 13(6):3229.
- Ge, Y., Han, J., Zhou, G., Xu, Y., Ding, Y., Shi, M., Guo, C. and Wu, G. 2018. Silencing of *miR156* confers enhanced resistance to brown planthopper in rice. *Planta*, 248:813-826.
- George, J., Jayalekshmy, V.G., Shahiba, A.M., Chacko, A. and Lachyan, T.S. 2021. Physical and nutrient characterization of the grains from traditional rice varieties of Kerala and validation of the trait linked molecular markers. *Oryza*, 58 (3):394-408
- Ghosh, A., Das, A., Samanta, A., Chatterjee, M.L. and Roy, A. 2013. Sulfoximine: A novel insecticide for management of rice brown planthopper in India. *Afr. J. Agric. Res.* 8(38):4798-4803.
- GOI [Government of India]. 2022. List of pesticides which are banned, refused registration and restricted in use as on 01.10.2022. [on-line]. Available:

https://ppqs.gov.in/sites/default/files/list_of_pesticides_which_are_banned_refused_registration_and_restricted_in_use_as_on_01.10.2022.pdf.

GOK [Government of Kerala]. 2016. An analytical study on agriculture in Kerala. [on-line]. Available: <https://www.ecostat.kerala.gov.in/publication-detail/an-analytical-study-on-agriculture-in-kerala.pdf> [14 Dec. 2022]

GOK [Government of Kerala]. 2022. Agricultural statistics 2020-21. [on-line]. Available: <https://www.ecostat.kerala.gov.in/storage/publications/622.pdf> [10 Dec. 2022]

Gokulakrishnan, M., Suji, K.K., Priyanka, A.R., Niranjanadevi, J., Balasubramani, V., Saraswathi, R. and Ramalingam, J. 2022. Simple sequence repeats (SSR) polymorphism survey between Co51 and Ptb33 for marker assisted backcross breeding to introgress multiple stress resistance in Rice (*Oryza sativa* L.). *Pharma Innovation J.* 11(8):1247-1252.

Gopalakrishnan, S., Sharma, R. K., Rajkumar, A. K., Joseph, M., Singh, V. P., Singh, A. K., Bhat, K. V., Singh, N. K. and Mohapatra, T. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breed.* 127:131-139.

Gopinath, A.M.P. 2023. Marker-assisted selection in back cross progenies of Prathyasa and Aiswarya pyramided with 2R genes against BLB in rice (*Oryza sativa* L.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 186p.

Grayer, R.J., Harborne, J.B., Kimmins, F.M., Stevenson, P.C. and Wijayagunasekera, H.N.P. 1993. Phenolics in rice phloem sap as sucking deterrents to the brown planthopper, *Nilaparvata lugens*. *Int. Symp. Nat. Phenols Plant Res.* 381:691-694.

Hajjar, M.J., Ahmed, N., Alhudaib, K.A. and Ullah, H. 2023. Integrated Insect Pest Management Techniques for Rice. *Sustain.* 15(5):4499.

- Hall, L.M., Booker, H., Siloto, R.M., Jhala, A.J. and Weselake, R.J. 2016. Flax (*Linum usitatissimum* L.). In: *Industrial oil crops*, AOCS Press, pp.157-194.
- Hanson, C.H., Busbice, T.H. and Hill, R.R. 1972. Directed mass selection for developing multiple pest resistance and conserving germplasm in alfalfa. *J. Environ. Qual.* 1:106-111.
- Hao, P., Liu, C., Wang, Y., Chen, R., Tang, M., Du, B., Zhu, L. and He, G. 2008. Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. *Plant Physiol.* 146(4):1810-1820.
- Harini, A.S., Kumar, S.S., Balaravi, P., Sharma, R., Dass, M.A. and Shenoy, V., 2013. Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. *Afr. J. Biotechnol.* 12(19).
- Harini, A.S., Lakshmi, S.S., Kumar, S.S., Sivaramakrishnan, S. and Kadirvel, P. 2010. Validation and fine-mapping of genetic locus associated with resistance to brown plant hopper [*Nilaparvata lugens* (Stal.)] in rice (*Oryza sativa* L.). *Asian J. Bio. Sci.* 5:32-37.
- Hasan, N., Choudhary, S., Naaz, N., Sharma, N. and Laskar, R.A. 2021. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *J. Genet. Eng. Biotechnol.* 19(1):1-26.
- He, C., Xiao, Y., Yu, J., Li, J., Meng, Q., Qing, X. and Xiao, G. 2019. Pyramiding *Xa21*, *Bph14*, and *Bph15* genes into the elite restorer line Yuehui9113 increases resistance to bacterial blight and the brown planthopper in rice. *Crop Prot.* 115:31-39.
- He, J., Liu, Y.Q., Liu, Y.L., Jiang, L., Wu, H., Kang, H.Y., Liu, S.J., Chen, L.M., Liu, X., Cheng, X.N. and Wan, J.M. 2013. High-resolution mapping of brown planthopper (BPH) resistance gene *Bph27(t)* in rice (*Oryza sativa* L.). *Mol. Breed.* 31(3):549-557.

- He, L., Zou, L., Huang, Q., Sheng, X., Wu, W. and Hu, J. 2020. Development of InDel markers of *Bph3* and pyramiding of four brown planthopper resistance genes into an elite rice variety. *Mol. Breed.* 40:1-11.
- Heinrichs, E.A. 1979. Chemical control of the brown planthopper. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Banos, Philippines, pp.145-167.
- Heinrichs, E.A. and Foster, J.E. 2019. Advances in breeding crops resistant to insect pests: rice as a paradigm. In: Heinrichs, E.A. and Foster, J.E. (eds) *Integrated management of insect pests: Current and future developments*. Burleigh Dodds Science Publishing, pp. 227-282.
- Heinrichs, E.A. 1985. *Genetic evaluation for insect resistance in rice*. International Rice Research Institute, Los Banos, Philippines, 351p.
- Hirabayashi, H. and Ogawa, T. 1995. RFLP mapping of *Bph-1* (Brown planthopper resistance gene) in rice. *Breed. Sci.* 45:369-371.
- Hirabayashi, H., Angeles, E.R., Kaji, R., Ogawa, T., Brar, D.S. and Khush, G.S. 1998. Identification of a brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice. *Breed. Sci.* 48:82.
- Hirabayashi, H., Ideta, O., Sato, H., Takeuchi, Y., Ando, I., Nemoto, H., Imbe, T., Brar, D. S. and Ogawa, T. 2004. Identification of a resistance gene to brown planthopper derived from *Oryza minuta* in rice. *Breed. Res.* 6:285.
- Hollander, D.J. and Pathak, P.K. 1981. The genetics of the “biotypes” of the rice brown planthopper, *Nilaparvata lugens*. *Entomologia experimentalis et applicata*, 29(1):76-86.
- Horgan, F.G., de Freitas, T.F.S., Crisol-Martínez, E., Mundaca, E.A. and Bernal, C.C. 2021. Nitrogenous fertilizer reduces resistance but enhances tolerance

to the brown planthopper in fast-growing, moderately resistant rice. *Insects*, 12(11):989.

Horgan, F.G., Peñalver Cruz, A., Arida, A., Ferrater, J.B. and Bernal, C.C. 2021. Adaptation by the Brown Planthopper to Resistant Rice: A Test of Female-Derived Virulence and the Role of Yeast-like Symbionts. *Insects*, 12:908.

Hu, J., Chang, X.Y., Zou, L., Tang, W.Q. and Wu, W.R. 2018. Identification and fine mapping of *Bph33*, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). *Rice*, 11(1):55.

Hu, J., Cheng, M., Gao, G., Zhang, Q., Xiao, J. and He, Y. 2013. Pyramiding and evaluation of three dominant brown planthopper resistance genes in the elite *indica* rice 9311 and its hybrids. *Pest Manag. Sci.* 69(7):802-808.

Hu, J., Li, X., Wu, C., Yang, C., Hua, H., Gao, G., Xiao, J. and He, Y. 2012. Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. *Mol. Breed.* 29:61-69.

Hu, J., Xiao, C. and He, Y. 2016a. Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. *Rice*, 9(1):1-12.

Hu, J., Xiao, C., Cheng, M., Gao, G., Zhang, Q. and He, Y. 2015. Fine mapping and pyramiding of brown planthopper resistance genes *QBph3* and *QBph4* in an introgression line from wild rice *O. officinalis*. *Mol. Breed.* 35:1-10.

Hu, J., Xiao, C., Cheng, M.X., Gao, G.J., Zhang, Q.L. and He, Y.Q. 2015b. A new finely mapped *Oryza australiensis*-derived QTL in rice confers resistance to brown planthopper. *Gene*, 561(1):132-137.

Hu, J., Zhou, J., Peng, X., Xu, H., Liu, C., Du, B., Yuan, H., Zhu, L. and He, G. 2016. The *Bphi008a* gene interacts with the ethylene pathway and transcriptionally regulates MAPK genes in the response of rice to brown planthopper feeding. *Plant Physiol.* 156(2):856-872.

- Hu, L., Wu, Y., Wu, D., Rao, W., Guo, J., Ma, Y., Wang, Z., Shangguan, X., Wang, H., Xu, C. and Huang, J. 2017. The coiled-coil and nucleotide binding domains of *BROWN PLANTHOPPER RESISTANCE14* function in signalling and resistance against planthopper in rice. *Plant Cell*, 29(12):3157-3185.
- Hu, L., Ye, M., Li, R. and Lou, Y. 2016c. OsWRKY53, a versatile switch in regulating herbivore-induced defense responses in rice. *Plant Signalling Behav.* 11(4):e1169357.
- Hu, W., Xiao, H., Hu, K., Jiang, Y. and Zhang, Y. 2016b. Application of marker-assisted backcross to introgress *Bph3*, *Bph14*, and *Bph15* into an elite *indica* rice variety for improving its resistance to brown planthopper. *Plant Breed.* 135(3):291-300.
- Huang, D., Qiu, Y.F., Zhang, Y., Huang, F., Meng, J., Wei, S., Li, R. and Chen, B.S. 2013. Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). *Theor. Appl. Genet.* 126(1): 219-229.
- Huang, Y.L., Shu, L.H., Zhu, L.L., Liao, L.J., and He, G.C. 2000. Characterization of the intersectional hybrid between *Oryza sativa* and *O. meyeriana* indigenous to China. *J. Wuhan Univ.* 46:739-744.
- Huangfu, J., Li, J., Li, R., Ye, M., Kuai, P., Zhang, T. and Lou, Y. 2016. The transcription factor *OsWRKY45* negatively modulates the resistance of rice to the brown planthopper *Nilaparvata lugens*. *Int. J. Mol. Sci.* 17(6):697.
- Huberty, A.F. and Denno, R.F. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecol.* 85:1383-1398.
- Hundekar, R. 2017. Pyramiding bacterial leaf blight resistance genes into popular rice varieties of Kerala through marker-assisted selection. Ph.D. thesis, Kerala Agricultural University, Thrissur, 176p.

- Hutson, J.C. 1923. Report on the work of entomological division. In: *Administration Report Director Agricultural Ceylon 1922*, 11-20.
- Iamba, K. and Dono, D. 2021. A review on brown planthopper (*Nilaparvata lugens* Stål), a major pest of rice in Asia and Pacific. *Asian J. Res. Crop Sci.* 6:7-19.
- Ikeda, R. and Kaneda, C. 1981. Genetic analysis of resistance to brown planthopper, *Nilaparvata lugens* Stal., in rice. *Jpn. J. Breed.* 31(3):279-285.
- IMD [Indian Meteorological Department]. 2023. Available: <https://mausam.imd.gov.in/thiruvananthapuram> [19 Apr 2023].
- IPCC [Intergovernmental Panel on Climate Change]. 2007. AR4 Climate Change: Impacts, Adaptation, and Vulnerability. [online]. Available: <https://www.ipcc.ch/report/ar4/wg2/>.
- IRRI [International Rice Research Institute]. 2013. Standard Evaluation System for Rice. International Rice Research Institute, Manila, Philippines, 56p.
- IRTP [International Rice Testing Program]. 1976. Standard evaluation system for rice. International Rice Research Institute. 45 p.
- Ishii, T., Brar, D.S., Multani, D.S. and Khush, G.S. 1994. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice *O. sativa*. *Genome*, 37(2):217-221
- Islam, M.A., Raffi, S.A., Hossain, M.A. and Hasan, A.K. 2015. Analysis of genetic variability, heritability and genetic advance for yield and yield associated traits in some promising advanced lines of rice. *Prog. Agric.* 26(1):26-31.
- Islam, M.Z., Khalequzzaman, M., Bashar, M.K., Ivy, N.A., Haque, M.M. and Mian, M.A.K. 2016. Variability assessment of aromatic and fine rice germplasm in Bangladesh based on quantitative traits. *Sci. World J.* 1(2):23-32

- Jain, J.P. 1982. *Statistical techniques in quantitative genetics*. Tata Mc Graw Hill Publishing Company, New Delhi, p.103.
- Jairin, J. 2007. Physical mapping of *Bph3*, a brown plant-hopper resistance locus in rice. *Maejo Int. J. Sci. Technol.* 1:166-177.
- Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A., Toojinda, T. 2007. Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. *Mol Breed.* 19:35-44.
- Jairin, J., Sansen, K., Wongboon, W. and Kothcharerk, J. 2010. Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. *Breed. Sci.* 60:71-75.
- Jairin, J., Teangdeerith, S., Leelagud, P., Kothcharerk, J., Sansen, K., Yi, M., Vanavichit, A. and Toojinda, T. 2009. Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. *Field Crops Res.* 110(3):263-271.
- Jairin, J., Teangdeerith, S.N., Leelagud, P., Phengrat, K., Vanavichit, A. and Toojinda, T. 2007. Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. *Maejo Int. J. Sci. Technol.* 1(2):166-177.
- Jannoey, P., Pongprasert, W., Lumyong, S., Roytrakul, S. and Nomura, M. 2015. Comparative proteomic analysis of two rice cultivars (*Oryza sativa* L.) contrasting in Brown Planthopper (BPH) stress resistance. *Plant Omics*, 8(2).
- Jayalekshmy, V.G., Leenakumary, S. and Hundekar, R. 2017. Locating resistant gene donors for major pests from traditional rice varieties of Kerala. *J. Environ. Biosci.* 31(1):169-175.

- Jeevanandham, N., Raman, R., Ramaiah, D., Senthilvel, V., Mookaiah, S. and Jegadeesan, R. 2023. Rice: *Nilaparvata lugens* Stal interaction—current status and future prospects of brown planthopper management. *J. Plant Dis. Prot.* 130(1):125-141.
- Jena, K.K. and Khush, G.S. 1990. Introgression of genes from *Oryza officinalis* well exWatt to cultivated rice, *O. sativa* L. *Theor. Appl. Genet.* 80:737-745.
- Jena, K.K. and Kim, S.M. 2010. Current status of brown planthopper (BPH) resistance and genetics. *Rice*, 3(2):161-171.
- Jena, K.K., Hechanova, S.L., Verdeprado, H., Prahalada, G.D. and Kim, S.R. 2017. Development of 25 near-isogenic lines (NILs) with ten BPH resistance genes in rice (*Oryza sativa* L.): production, resistance spectrum, and molecular analysis. *Theor. Appl. Genet.* 130:2345-2360.
- Jena, K.K., Jeung, J.U., Lee, J.H., Choi, H.C. and Brar, D.S. 2006. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 112:288-297
- Jena, M., Panda, R.S., Sahu, R.K., Mukherjee, A.K. and Dhua, U. 2015. Evaluation of rice genotypes for rice brown plant hopper resistance through phenotypic reaction and genotypic analysis. *Crop Prot.* 78:119-126.
- Jena, M., Pandi, G.P.G, Adak T., Rath, P.C., Patil, N.K. and Annamalai, M. 2018. *Management of brown plant hopper- an old foe in new form*. NRRI Technology Bulletin-130, National Rice Research Institute, Cuttack.
- Jeon, Y.H., Ahn, S.N., Choi, H.C., Hahn, T.R., and Moon, H.P. 1999. Identification of a RAPD marker linked to a brown planthopper resistance gene in rice. *Euphytica*, 107:23-28.
- Ji, H., Kim, S.R., Kim, Y.H., Suh, J.P., Park, H.M., Sreenivasulu, N., Misra, G., Kim, S.M., Hechanova, S.L., Kim, H. and Lee, G.S. 2016. Map-based

- cloning and characterization of the *BPH18* gene from wild rice conferring resistance to brown planthopper (BPH) insect pest. *Sci. Rep.* 6(1):1-14.
- Jiang, H., Hu, J., Li, Z., Liu, J., Gao, G., Zhang, Q., Xiao, J. and He, Y. 2018. Evaluation and breeding application of six brown planthopper resistance genes in rice maintainer line Jin 23B. *Rice*, 11(1):1-11.
- Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimation of genetic and environmental variability in soybean. *Agric. J.* 47:314-318.
- Joseph, M., Gopalakrishnan, S., Sharma, R.K., Singh, V.P., Singh, A.K., Singh, N.K. and Mohapatra, T. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Mol. Breed.* 13:377-387.
- Kalode, M.B. 1971. Biochemical basis of resistance or susceptibility to brown planthopper and green leafhopper in some rice varieties, IRRI Saturday seminar, Mimeo, p 17.
- Kalode, M.B. and Bentun, J.S., 1982. An improved method for screening cultures resistant to brown planthopper. *Int. Rice Res. Newsl.* 7:6-7.
- Kamal, M.M., Nguyen, C.D., Sanada-Morimura, S., Zheng, S.H. and Fujita, D. 2023. Near-isogenic lines for resistance to brown planthopper with the genetic background of Indica Group elite rice (*Oryza sativa* L.) variety 'IR64'. *Breed. Sci.* p.22093.
- Kamaluddin, P.S., Sharma, V., Kumar, H., Kumar, M., Negi, H.S., Kiran, U., Abdin, M.Z. and Choubey, A.K. 2022. Marker-Assisted Selection for Value Addition in Crop Plants. *Technol. Plant Biotechnol. Breed. Field Crops*, p.23.
- Kaneda, C. and Kisimoto, R. 1979. Status of varietal resistance to the brown planthopper in Japan. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers*:

- new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Banos (Philippines), pp.209-218.
- Kang, K., Yue, L., Xia, X., Liu, K. and Zhang, W. 2019. Comparative metabolomics analysis of different resistant rice varieties in response to the brown planthopper *Nilaparvata lugens* Hemiptera: Delphacidae. *Metabolomics*, 15:1-13.
- Kaur, P., Neelam, K., Sarao, P.S., Dhir, Y.W., Khanna, R. and Singh, K. 2023. Mapping of a novel recessive brown planthopper resistance gene bph46 from wild rice (*Oryza nivara*). *Res. Square* 1-14.
- Kawaguchi, M., Murata, K., Ishii, T., Takumi, S., Mori, N. and Nakamura, C. 2001. Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene bph4 to the rice chromosome 6. *Breed. Sci.* 51(1):13-18.
- Kaya, C., Ugurlar, F., Ashraf, M. and Ahmad, P. 2023. Salicylic acid interacts with other plant growth regulators and signal molecules in response to stressful environments in plants. *Plant Physiol. Biochem.* 196:431-443.
- Keerthiraj, B. and Biju, S. 2020. Genetic variability, heritability and genetic advance of yield and lodging-related traits in rice (*Oryza sativa* L.). *Electr. J. Plant Breed.* 11(04):1093-1098.
- Khush, G. S. 1979. Genetics of and breeding for resistance to the brown planthopper. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos (Philippines), pp.321-332.
- Khush, G.S. 1977. Breeding for resistance in rice. *Ann. New York Acad. Sci.* 287(2):296-308.
- Khush, G.S. 1980. Breeding for multiple diseases and insect resistance in rice. In: Harris, M.K. (ed.), *Biology and breeding for resistance to arthropods and*

pathogens in agricultural plants. Texas Agricultural Experimental Station, Texas, 605p.

Khush, G.S. and Brar, D.S., 1991. Genetics of resistance to insects in crop plants. *Adv. Agron.* 45:223-274.

Kim, E.G., Yun, S., Park, J.R., Jang, Y.H., Farooq, M., Yun, B.J. and Kim, K.M. 2022a. Bio-efficacy of Chrysoeriol, a natural chemical and repellent, against Brown Planthopper in Rice. *Int. J. Mol. Sci.* 23(3):1540.

Kim, J., An, X., Yang, K., Miao, S., Qin, Y., Hu, Y., Du, B., Zhu, L., He, G. and Chen, R. 2022b. Molecular mapping of a new brown planthopper resistance gene *bph43* in rice (*Oryza sativa* L.). *Agron.* 12(4):808.

Kim, S.M. and Sohn, J.K. 2005. Identification of a rice gene (*Bph1*) conferring resistance to brown planthopper (*Nilaparvata lugens* Stål) using STS markers. *Mol. Cells*, 20(1):30-34.

Kisimoto, R. 1965. Studies on the polymorphism and its role-playing in the population growth of the brown planthopper, *Nilaparvata lugens* Stal. *Bull Shikoku Agric. Exp. Stn.* 13:1-106.

Kogan, M. and Ortman, E.F. 1978. Antixenosis—a new term proposed to define Painter's “nonpreference” modality of resistance. *Bulletin of the ESA*, 24(2):175-176.

Korinsak, S., Siangliw, M., Kotcharerk, J., Jairin, J., Siangliw, J.L., Jongdee, B., Pantuwan, G., Sidthiwon, N. and Toojinda, T. 2016. Improvement of submergence tolerance and the brown planthopper resistance of the Thai jasmine rice cultivar KDML105 by pyramiding *Sub1* and *Qbph12*. *Field Crop. Res.* 188:105-112.

Kowsalya, P., Sharanyakanth, P.S. and Mahendran, R. 2022. Traditional rice varieties: A comprehensive review on its nutritional, medicinal, therapeutic and health benefit potential. *J. Food Composition Anal.* P. 104742.

- Koya, V.M., 1974. Brown hopper, the destructive pest. *Kurukshetra*, 22(19):13.
- Krishnamurthy, S.L., Pundir, P., Warraich, A.S., Rathor, S., Lokeshkumar, B.M., Singh, N.K. and Sharma, P.C. 2020. Introgressed saltol QTL lines improves the salinity tolerance in rice at seedling stage. *Frontiers Plant Sci.* 11:833.
- Kshirsagar, D.B., Bhalekar, M.N., Patil, R.S., Kute, N.S and Patil, S.B. 2013. Transgressive segregation in F₃ generation of intervarietal crosses of tomato (*Solanum lycopersicon* L.). *Veg. Sci.* 40(2):240-242.
- Kumar, B.M. and Kunhamu, T.K., 2021. Ecological and historical perspectives of rice cultivation in Kerala: a synthesis. *Oryza*, 58(2):241-261.
- Kumar, K., Kaur, P., Kishore, A., Vikal, Y., Singh, K. and Neelam, K., 2020. Recent advances in genomics-assisted breeding of brown planthopper (*Nilaparvata lugens*) resistance in rice (*Oryza sativa*). *Plant Breed.* 139(6):1052-1066.
- Kumar, K., Sarao, P.S., Bhatia, D., Neelam, K., Kaur, A., Mangat, G.S., Brar, D.S. and Singh, K. 2018. High-resolution genetic mapping of a novel brown planthopper resistance locus, Bph34 in *Oryza sativa* L. × *Oryza nivara* (Sharma & Shastri) derived interspecific F₂ population. *Theor. Appl. Genet.* 131:1163-1171.
- Kumar, M., Kumar, P., Singh, A.G. and Jukanti, A.K. 2021. *Abiotic Stress Tolerance in Rice: Insight in Climate Change Scenario*. Intech Open Publisher, 342p.
- Kumar, M., Singh, R.P., Jena, D., Singh, V., Rout, D., Arsode, P.B., Choudhary, M., Singh, P., Chahar, S., Samantaray, S. and Mukherjee, A.K. 2023. Marker-Assisted improvement for durable bacterial blight resistance in aromatic rice cultivar HUR 917 popular in eastern parts of India. *Plants*, 12(6):1363.

- Kumari, S., Sheba, J.M., Marappan, M., Ponnuswamy, S., Seetharaman, S., Pothi, N., Subbarayalu, M., Muthurajan, R. and Natesan, S. 2010. Screening of IR50 × Rathu Heenati F7 RILs and identification of SSR markers linked to brown planthopper (*Nilaparvata lugens* Stål) resistance in rice (*Oryza sativa* L.). *Mol. Biotechnol.* 46(1): 63-71.
- Kurata, N., Nonomura, K.I. and Harushima, Y. 2002. Rice genome organization: the centromere and genome interactions. *Ann. Bot.* 90(4):427-435.
- Kuwada, Y. 1910. A cytological study of *Oryza sativa* L. *Shokubutsugaku Zasshi*, 24:267-281.
- Lakshmi, V.G.I., Sreedhar, M., Lakshmi, V.J., Gireesh, C., Rathod, S. and Vanisri, S. 2021. Molecular diversity assessment of rice genotypes for brown planthopper resistance using microsatellite markers. *Electr. J. Plant Breed.* 12(2):499-506.
- Lakshmi, V.J., Krishnaiah, N.V., Katti, G.R., Pasalu, I.C. and Chirutkar, P.M. 2010. Screening of insecticides for toxicity to rice hoppers and their predators. *Oryza Int. J. Rice*, 47(4):295-301.
- Latif, M.A., Rafii, M.Y., Mazid, M.S., Ali, M.E., Ahmed, F., Omar, M.Y. and Tan, S.G. 2012. Genetic dissection of sympatric populations of brown planthopper, *Nilaparvata lugens* (Stål), using DALP-PCR molecular markers. *Sci. World J.* 586831:1-11.
- Li, B., Chen, Z., Chen, H., Wang, C., Song, L., Sun, Y., Cai, Y., Zhou, D., Ouyang, L., Zhu, C. and He, H. 2023. Stacking Multiple Genes Improves Resistance to *Chilo suppressalis*, *Magnaporthe oryzae*, and *Nilaparvata lugens* in Transgenic Rice. *Genes*, 14(5):1070.
- Li, C.P., Wu, D.H., Huang, S.H., Meng, M., Shih, H.T., Lai, M.H., Chen, L.J., Jena, K.K., Hechanova, S.L., Ke, T.J. and Chiu, T.Y. 2023. The *Bph45* Gene Confers Resistance against Brown Planthopper in Rice by Reducing the Production of Limonene. *Int. J. Mol. Sci.* 24(2):798.

- Li, R., Li, L., Wei, S., Wei, Y., Chen, Y., Bai, D., Yang, L., Huang, E., Lu, W., Zhang, X., Li, X., Yang, X. and Wei, Y. 2006. The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). *Mol. Plant Breed.* 4:365-371.
- Li, Z.H., Xue, Y.X., Zhou, H.L., Li, Y., Usman, B., Jiao, X.Z., Wang, X.Y., Liu, F., Qin, B.X., Li, R.B. and Qiu, Y.F. 2019. High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza rufipogon* Griff). *Rice*, 12(1):41.
- Liang, X., Chen, L., Lan, X., Liao, G., Feng, L., Li, J., Fan, W., Wang, S. and Liu, J. 2022. Physiological and Population Responses of *Nilaparvata lugens* after Feeding on Drought-Stressed Rice. *Insects*, 13(4):355.
- Liao, G., Li, J., Fan, W., Chen, L. and Liu, J. 2023. Drought stress reduces the feeding preference of *Nilaparvata lugens* due to the accumulation of abscisic acid and callose deposition in rice. *J. Asia-Pacific Entomol.* 26(2):102014.
- Lin, Y., Lin, X., Ding, C., Xia, M., Xue, R., Sun, Z., Chen, D., Zhu-Salzman, K., Zeng, R. and Song, Y. 2022. Priming of rice defense against a sap-sucking insect pest brown planthopper by silicon. *J. Pest Sci.* 95:1371-1385.
- Ling, Y., Ang, L. and Weilin, Z. 2019. Current understanding of the molecular players involved in resistance to rice planthoppers. *Pest Manag. Sci.* 75(10):2566-2574.
- Lisha, J.M., Baskaran, V., Vijay, S. and Vishnu, M. 2020. Status of insect pests in direct seeded and transplanted rice. *J. Entomol. Zool. Stud.* 8(2):1104-1107.
- Liu, J.L., Yu, J.F., Wu, J.C., Yin, J.L. and Gu, H.N. 2008. Physiological responses to *Nilaparvata lugens* in susceptible and resistant rice varieties: Allocation of assimilates between shoots and roots. *J. Econ. Entomol.* 101(2):384-390.

- Liu, Y., Chen, L., Liu, Y., Dai, H., He, J., Kang, H., Pan, G., Huang, J., Qiu, Z., Wang, Q. and Hu, J., 2016. Marker assisted pyramiding of two brown planthopper resistance genes, Bph3 and Bph27 (t), into elite rice cultivars. *Rice*, 9, pp.1-7.
- Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., Sun, Z., Pan, G., Wang, Q., Hu, J. and Zhou, F. 2015. A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nat. Biotechnol.* 33(3):301-305.
- Lu, H.P., Luo, T., Fu, H.W., Wang, L., Tan, Y.Y., Huang, J.Z., Wang, Q., Ye, G.Y., Gatehouse, A.M., Lou, Y.G. and Shu, Q.Y. 2018. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. *Nat. Plants*, 4(6):338-344.
- Lu, W.T., Du, B., Shangguan, X.X., Zhao, Y., Pan, Y.F., Zhu, L.L., He, Y.Q. and He, G.C. 2014. BAC and RNA sequencing reveal the brown planthopper resistance gene *Bph15* in a recombination cold spot that mediates a unique defense mechanism. *BMC Genomics*, 15(1):674.
- Lu, Z.X. and Heong, K.L. 2009. Effects of nitrogen-enriched rice plants on ecological fitness of planthoppers. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos (Philippines), pp. 247-256.
- Mahesh, G., Ramesh, T., Narendar Reddy, S., Meena, A., Rathod, S., Fiyaz, R.A., Badri, J., Rao, L.V.S., Sundaram, R.M. and Jukanti, A.K. 2022. Genetic variability, heritability, genetic advance and path coefficients for grain protein content, quality traits and grain yield in rice (*Oryza sativa* L.) germplasm lines. *Pharma Innovation J.* 11(3):1836-1839.

- Mashhoor, K., Lazar, K.V., Shanas, S. and Ramesh, N. 2015. Genetic structure and phylogenetic status of rice brown plant hopper (bph), *Nilaparvata lugens* isolated from Kerala, India. *J. Appl. Biol. Biotechnol.* 3(5):015-018.
- Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A. and Latif, M.A., 2015. Recurrent parent genome recovery analysis in a marker-assisted backcrossing program of rice (*Oryza sativa* L.). *Comptes rendus biologies*, 338(2):83-94.
- Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., Islam, K.N. and Latif, M.A. 2013. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *Int. J. Mol. Sci.* 14(11):22499-22528.
- Miles, P.W. 1972. The saliva of Hemiptera. In *Advances in insect physiology*, Vol. 9. Academic Press, pp. 183-255.
- Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31(3):426-428.
- Mishra, A., Barik, S.R., Pandit, E., Yadav, S.S., Das, S.R. and Pradhan, S.K. 2022. Genetics, Mechanisms and Deployment of Brown Planthopper Resistance Genes in Rice. *Crit. Rev. Plant Sci.* 41(2):91-127.
- Mochida, O. 1972. Intake of *Nilaparvata lugens* (STÅL)(Hom., Delphacidae) of different sexes and wing-forms from labelled rice seedlings. *Bull. Kyushu Agric. Exp. Stn.* 40(2):69-71.
- Mochida, O. and Okada, T. 1979. Taxonomy and biology of *Nilaparvata lugens* (Hom., Delphacidae). In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos, Philippines, 369p.
- Mohanapriya, B., Jeyaprakash, P., Raveendran, M., Soundararajan, R.P., Ramchander, S., Subashini, G., Prabhakaran, P., Manonmani, K. and Robin,

- S. 2019. Marker assisted introgression for brown planthopper resistance genes *Bph20* and *Bph21* in CO43Sub1 variety of rice. *Electr. J. Plant Breed.* 10(2):645-652.
- Mohapatra, S., Barik, S.R., Dash, P.K., Lenka, D., Pradhan, K.C., Raj K. R.R., Mohanty, S.P., Mohanty, M.R., Sahoo, A., Jena, B.K. and Panda, A.K. 2023. Molecular Breeding for Incorporation of Submergence Tolerance and Durable Bacterial Blight Resistance into the Popular Rice Variety 'Ranidhan'. *Biomolecules*, 13(2):198.
- Mohapatra, S.D., Singh, S. and Giri, G.S. 2022. Field efficacy of triflumezopyrim 5%+ spinetoram 9% against yellow stem borer and brown plant hopper in rice ecosystem and their effects on natural enemies. *Pharma Innovation J.* SP-11(10):1556-1560.
- Muduli, L., Dash, M., Das Mohapatra, S., Mohapatra, K.K., Nayak, H.S., Bastia, D.N., Pradhan, B., Tripathy, S.K., Jena, R.C. and Pradhan, S.K. 2023. Phenotypic and genotypic assessment of elite rice varieties for brown plant hopper (*Nilaparvata lugens* Stål.) resistance. *Cereal Res. Commun.* 1-13.
- Muduli, L., Pradhan, S.K., Mishra, A., Bastia, D.N., Samal, K.C., Agrawal, P.K. and Dash, M. 2021. Understanding brown planthopper resistance in Rice: Genetics, biochemical and molecular breeding approaches. *Rice Sci.* 28(6):532-546.
- Murai, H., Hashimoto, Z., Sharma, P.N., Shimizu, T., Murata, K., Takumi, S., Mori, N., Kawasaki, S. and Nakamura, C. 2001. Construction of a high-resolution linkage map of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph2*. *Theor. Appl. Genet.* 103(4):526-532.
- Murata, K., Fujiwara, M., Kaneda, C., Takumi, S., Mori, N., and Nakamura, C. 1998. RFLP mapping of brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph2* of indica rice introgressed into a *japonica* breeding line 'Norin-PL4'. *Genes Genet. Syst.* 73:359-364.

- Murata, K., Fujiwara, M., Murai, H., Takumi, S., Mori, N. and Nakamura, C. 2001. Mapping of a brown planthopper (*Nilaparvat lugens* Stål) resistance gene *BPH9* on the long arm of rice chromosome 12. *Cereal Res. Commun.* 29:245-250
- Myint, K.K.M., Fujita, D., Matsumura, M., Sonoda, T., Yoshimura, A. and Yasui, H. 2012. Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* [Stål]) in the rice cultivar ADR52. *Theor. Appl. Genet.* 124:495-504.
- Myint, K.K.M., Yasui, H., Takagi, M. and Matsumura, M. 2009. Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (Stål), and whitebacked planthopper, *Sogatella furcifera* (Horváth)(Homoptera: Delphacidae), on rice differential varieties. *Appl. Entomol. Zool.* 44(1):149-153.
- Nagadhara, D., Ramesh, S., Pasalu, I.C., Rao, Y.K., Krishnaiah, N.V., Sarma, N.P., Bown, D.P., Gatehouse, J.A., Reddy, V.D. and Rao, K.V. 2003. Transgenic *indica* rice resistant to sap-sucking insects. *Plant Biotechnol. J.* 1(3):231-240.
- Naik, S.B., Divya, D., Sahu, N., Sundaram, R.M., Sarao, P.S., Singh, K., Lakshmi, V.J. and Bentur, J.S. 2018. A new gene *Bph33* (t) conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. *Euphytica*, 214:1-12.
- Nair, V S. and Santiaguel, A.F. 2014. Rice Today, International Rice Research Institute, Los Banos, Philippines.
- Nair, V.S. 2003. A Study on the Origin of the Word Vrihi. [on-line]. Available: <https://www.boloji.com/articles/688/a-study-on-the-origin-of-the-word-vrihi>.

- Nalinakumari, T. and K. V. Mammen. 1975. Biology of the brown planthopper. *Nilaparvata lugens* (Stål.) (Delphacidae, Hemiptera). *Agric. Res. J. Kerala* 13(1):53-54.
- Nguyen, C.D., Verdeprado, H., Zita, D., Sanada-Morimura, S., Matsumura, M., Virk, P.S., Brar, D.S., Horgan, F.G., Yasui, H. and Fujita, D. 2019. The development and characterization of near-isogenic and pyramided lines carrying resistance genes to brown planthopper with the genetic background of japonica rice (*Oryza sativa* L.). *Plants*, 8(11):498.
- Nguyen, C.D., Zheng, S.H., Sanada-Morimura, S., Matsumura, M., Yasui, H. and Fujita, D. 2021. Substitution mapping and characterization of brown planthopper resistance genes from indica rice variety, ‘PTB33’(*Oryza sativa* L.). *Breed. Sci.* 71(5):497-509.
- NOAA [National Oceanic and Atmospheric Administration]. 2021. Carbon dioxide peaks near 420 parts per million at Mauna Loa observatory. [on-line]. Available:<https://research.noaa.gov/article/ArtMID/587/ArticleID/2764/Coronavirus-response-barely-slows-rising-carbon-dioxide>.
- Nugaliyadde, L., Des Abeysiriwardena, D.S., Samanmalee, L.G.A., Pathirana, R. and Wilkins, R.M. 2004. Inheritance of resistance in rice to brown planthopper: its implications on rice varietal improvement in Sri Lanka. *Melalui* http://www.goviya.lk/agri_learning/Paddy/Paddy_Research/Paddy_pdf P, 3:1-8.
- Ohkubo, N. 1973. Experimental studies on the flight of planthoppers by the tethered flight technique. I. Characteristics of flight of the brown planthopper *Nilaparvata lugens* Stal and effects of some physical factors. *Jpn J. Appl. Entomol. Zool.* 17(1):10-18.
- Oka, I.N. 1979. Cultural control of the brown planthopper. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive*

rice production systems in Asia, International Rice Research Institute, Los Banos, Philippines, pp.357-369.

Paguia, P., Pathak, M.D. and Heinrichs, E.A. 1980. Honeydew excretion measurement techniques for determining differential feeding activity of biotypes of *Nilaparvata lugens* on rice varieties. *J. Econ. Entomol.* 73(1):35-40.

Painter R.H. 1951. Insect resistance in crop plants. *LWW*, 72(6):481.

Panda, N. and Heinrichs, E.A. 1983. Levels of tolerance and antibiosis in rice varieties having moderate resistance to the brown planthopper, *Nilaparvata lugens* (Stål)(Hemiptera: Delphacidae). *Environ. Entomol.* 12(4):1204-1214.

Pandi, G.G.P., Chander, S., Singh, M.P. and Pathak, H. 2018. Impact of elevated CO₂ and temperature on brown planthopper population in rice ecosystem. *Proc. Natl Acad. Sci. India Section B: Biol. Sci.* 88:57-64.

Pandi, G.G.P., Choudhary, L.G., Chemura, J.S., Basana-Gowda, A., Annamalai, G., Patil, M., Adak, N., T. and Rath, P.C. 2021. Predicting the brown planthopper, *Nilaparvata lugens* (Stal)(Hemiptera: Delphacidae) potential distribution under climatic change scenarios in India. *Curr. Sci. India*, 121:1600-1609.

Parmhansh, P., Kumar, S.B., Kumar, A., Mishra, S.B. and Mandal, R.K. 2011. Morphological and biochemical markers for cold tolerance in Boro rice. *Oryza Int. J. Rice*, 48(4):297-303.

Pasalu, I.C. and Katti, G. 2006. Advances in eco-friendly approaches in rice IPM. *J. Rice Res.*1(1):83-90.

Pathak, H. 2023. Impact, adaptation, and mitigation of climate change in Indian agriculture. *Environ. Monit. Assess.*195(1):52.

- Pathak, M.D. 1968. Ecology of common insect pests of rice. *Ann. Rev. Entomol.* 13:257-294.
- Pathak, M.D. and Khush, G.S. 1979. Studies of varietal resistance in rice to the brown planthopper at the International Rice Research Institute. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos, Philippines, pp.285-301.
- Pathak, P.K. and Heinrichs, E.A. 1982. Bromocresol green indicator for measuring feeding activity of *Nilaparvata lugens* on rice varieties. *Philippine Entomol.* 5:197-198.
- Pati, P., Jena, M., Bhattacharya, S., Annamalai, M., Raghu, S., Behera, S.K., Sanghamitra, P., Pandi, G.G. and Meena, S.K. 2019. Evaluation of red rice genotypes against brown planthopper, BPH (*Nilaparvata lugens* Stal.) by phenotypic analysis and study of mechanism of resistance involved. *J. Entomol. Zool. Stud.* 7(5):149-155.
- Pillai, C., Faseela, K.V. and Thampi, H. 2020. Nutritional composition of selected traditional rice varieties of Kerala. *J. Trop. Agric.* 58(1).
- Piyaphongkul, J., Pritchard, J. and Bale, J. 2012. Can tropical insects stand the heat? A case study with the brown planthopper *Nilaparvata lugens* (Stål). *PLoS One*, 7(1): p.e29409.
- Pradhan, S.K., Nayak, D.K., Mohanty, S., Behera, L., Barik, S.R. and Pandit, E. 2015. Pyramiding of three bacterial blight resistance genes for broadspectrum resistance in deep water rice variety, Jalmagna. *Rice*, 8:19.
- Prahalada, G.D., Shivakumar, N., Lohithaswa, H.C., Gowda, D.K.S., Ramkumar, G., Kim, S.R., Ramachandra, C., Hittalmani, S., Mohapatra, T. and Jena, K.K. 2017. Identification and fine mapping of a new gene, *BPH31* conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. *Rice*, 10(1):1-15.

- Prayoga, G.I., Sari, S., Dono, D. and Carsono, N. 2022. Correlation Analysis between Simple Sequence Repeats Molecular Markers and Brown Planthopper Resistance Traits in Five Rice Cultivars. *Agrikultura*, 33(3):331-341.
- Qing, D., Dai, G., Zhou, W., Huang, S., Liang, H., Gao, L., Gao, J., Huang, J., Zhou, M., Chen, R. and Chen, W. 2019. Development of molecular marker and introgression of *Bph3* into elite rice cultivars by marker-assisted selection. *Breed. Sci.* 69(1):40-46.
- Qiu, L., Wang, W., Shi, L., Liu, Q. and Zhan, Z. 2020. Antibiosis effects of rice carrying *Bph14* and *Bph15* on the brown planthopper, *Nilaparvata lugens*. *Agric.* 10(4): 109.
- Qiu, Y., Guo, J., Jing, S., Tang, M., Zhu, L. and He, G. 2011. Identification of antibiosis and tolerance in rice varieties carrying brown planthopper resistance genes. *Entomologia Experimentalis et Applicata*, 141(3):224-231.
- Qiu, Y., Guo, J., Jing, S., Zhu, L. and He, G. 2010. High-resolution mapping of the brown planthopper resistance gene *Bph6* in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. *Theor. Appl. Genet.* 121:1601-1611.
- Qiu, Y.F., Guo, J.P., Jing, S.L., Zhu, L.L. and He, G.C. 2010. High-resolution mapping of the brown planthopper resistance gene *Bph6* in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. *Theor. Appl. Genet.* 121(8):1601-1611.
- Qiu, Y.F., Guo, J.P., Jing, S.L., Zhu, L.L. and He, G.C. 2012. Development and characterization of japonica rice lines carrying the brown planthopper-resistance genes BPH12 and Bph6. *Theor. Appl. Genet.* 124(3):485-494.

- Qiu, Y.F., Guo, J.P., Jing, S.L., Zhu, L.L. and He, G.C. 2014. Fine mapping of the rice brown planthopper resistance gene *bph7* and characterization of its resistance in the 93-11 background. *Euphytica*, 198(3):369-379.
- Radhika, A.M. and Raju, R.K. 2021. Rice GIS in Kerala: Gap in desired and achieved outcomes. *J. Intellectual Property Rights*, 26:83-91.
- Rahman, M.L., Jiang, W.Z., Chu, S.H., Qiao, Y.L., Ham, T.H., Woo, M.O., Lee, J., Khanam, M.S., Chin, J.H., Jeung, J.U., Brar, D.S., Jena, K.K. and Koh, H.J. 2009. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*. *Theor. Appl. Genet.* 119(7):1237-1246.
- Rajpal, V.R., Singh, A., Kathpalia, R., Thakur, R.K., Khan, M.K., Pandey, A., Hamurcu, M. and Raina, S.N. 2023. The Prospects of gene introgression from crop wild relatives into cultivated lentil for climate change mitigation. *Frontiers Plant Sci.* 14.
- Ramulamma, A., Sridevi, D., Jhansilakshmi, V. and Reddy, B.N. 2017. Biochemical analysis of resistance in paddy to BPH, *Nilaparvata lugens*. *Environ. Ecol.* 35(4):2616-2621.
- Rao, B. and Deshpande, V. 2006. *Experimental biochemistry: a student companion*. Tunbridge Wells, Kent, Anshan.
- Rao, R.G.V. and Rao, V.R. 2010. Status of IPM in Indian agriculture: a need for better adoption. *Indian J. Plant Prot.* 38(2):115-121.
- Rashid, M.M., Jahan, M. and Islam, K.S. 2016. Impact of nitrogen, phosphorus, and potassium on brown planthopper and tolerance of its host rice plants. *Rice Sci.* 23(3):119-131.
- Rashid, M.M., Jahan, M., Islam, K.S. and Latif, M.A. 2017. Ecological fitness of brown planthopper, *Nilaparvata lugens* (Stål), to rice nutrient management. *Ecol. Processes*, 6:1-10.

- Rath, P.C., Meher, J., Bose, L.K., Dash, S.K., Sarkar, S. and Subudhi, H.N. 2022. Phenotyping of Rice varieties for Brown Plant Hopper and White backed plant Hopper Resistance in Net house. *Biol. Forum Int. J.* 14(4):1263-1266.
- Rathnapriya, T.S., Eliazar Nelson, A.R.L., Ravichandran, K. and Antony, U. 2019. Nutritional and functional properties of coloured rice varieties of South India: a review. *J. Ethnic Foods*, 6(1):1-11.
- Ratnasekera, D., Tennakoon, A., Sandamal, S. and Wijerathna, P. 2022. Perception of Advanced Techniques in Conservation and Utilization of Wild Genetic Resources of Rice. In *Plant Genetic Resources, Inventory, Collection and Conservation*, Singapore: Springer Nature Singapore, pp. 463-504.
- Rau, N.S. 1929. Further contributions to the cytology of some crop-plants of South India. *J. Indian Bot. Soc.* 8:201-206.
- Reddy, K.L., Pasalu, I.C., Raju, A.S. and Reddy, D.D.R. 2004. Biochemical basis of resistance in rice cultivars to brown plant hopper *Nilaparvata lugens* (Stal.). *J. Entomol. Res.* 28(1):79-85.
- Ren, J., Gao, F., Wu, X., Lu, X., Zeng, L., Lv, J., Su, X., Luo, H. and Ren, G. 2016. *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. *Sci. Rep.* 6:37645.
- Renganayaki, K., Fritz, A.K., Sadasivam, S., Pammi, S., Harrington, S.E., McCouch, S.R., Kumar, S.M. and Reddy, A.S. 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. *Crop Sci.* 42(6):2112-2117.
- Rezvi, H.U.A., Tahjib-Ul-Arif, M., Azim, M.A., Tumpa, T.A., Tipu, M.M.H., Najnine, F., Dawood, M.F., Skalicky, M. and Brestič, M. 2023. Rice and food security: Climate change implications and the future prospects for nutritional security. *Food Energy Security*, 12(1):p.e430.

- Riley, J.R., Xia-Nian, C., Xiao-Xi, Z., Reynolds, D.R., Guo-Min, X.U., Smith, A.D., Ji-Yi, C., Ai-Dong, B. and Bao-Ping, Z. 1991. The long-distance migration of *Nilaparvata lugens* (Stål)(Delphacidae) in China: radar observations of mass return flight in the autumn. *Ecol. Entomol.* 16(4):471-489.
- Rosamma, C.A., Elsy, C.R., Balachandran, P.V. and Francies, R.M. 2003. *Pattambi rice varieties*. Kerala Agricultural University, Thrissur, 165p.
- Roy, D., Biswas, A., Sarkar, S., Chakraborty, G., Gaber, A., Kobeasy, M.I. and Hossain, A. 2022. Evaluation and characterization of indigenous rice (*Oryza sativa* L.) landraces resistant to brown planthopper *Nilaparvata lugens* (Stål.) biotype 4. *PeerJ*, 10:p.e14360.
- Roy, S. and Panda, S.K. 2020. Effect of silicon amendments in enhancing resistance of rice plants against brown plant hopper *Nilaparvata lugens* (Stal.) in Odisha. *Int. J. Curr. Microbiol. Appl. Sci*, 9(9):2171-2177.
- Ruengphayak, S., Chaichumpoo, E., Phromphan, S., Kamolsukyonyong, W., Sukhaket, W., Phuvanartnarubal, E., Korinsak, S., Korinsak, S. and Vanavichit, A. 2015. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. *Rice*, 8(1):1-16.
- Sadasivam, S. and Manickam, A. 1992. Biochemical methods for agricultural sciences, Wiley Eastern Limited, New Delhi, p.246.
- Sakai, T. and Sogawa, K. 1976. Effects of nutrient compounds on sucking response of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Appl. Entomol. Zool.* 11(2):82-88.
- Sanchez, P.L., Wing, R.A. and Brar, D.S. 2013. The wild relative of rice: genomes and genomics. *Genet. Genomics Rice*, 1:9-25.

- Sandhu, R.K. and Sarao, P.S. 2021. Evaluation of antixenosis resistance in wild rice accessions against brown planthopper, *Nilaparvata lugens* (Stål). *Int. J. Trop. Insect Sci.* 41:65-73.
- Sandhu, R.K. and Sarao, P.S., 2022. Tolerance against brown planthopper (*Nilaparvata lugens*) in wild rice (*Oryza sativa*) accessions. *Indian J. Agric. Sci.* 92(2).
- Sandhu, R.K., Sarao, P.S. and Sharma, N. 2020. Antibiosis in wild rice accessions induced by *Nilaparvata lugens* (Stål) feeding. *Phytoparasitica*, 48:801-812.
- Sanghamitra, P., Sah, R.P., Bagchi, T.B., Sharma, S.G., Kumar, A., Munda, S. and Sahu, R.K. 2018. Evaluation of variability and environmental stability of grain quality and agronomic parameters of pigmented rice (*O. sativa* L.). *J. Food Sci. Technol.* 55(3):879-890.
- Sarao, P.S. and Bentur, J.S. 2016. Antixenosis and tolerance of rice genotypes against brown planthopper. *Rice Sci.* 23(2):96-103.
- Sarif, H.M., Rafii, M.Y., Ramli, A., Oladosu, Y., Musa, H.M., Rahim, H.A., Zuki, Z.M. and Chukwu, S.C. 2020. Genetic diversity and variability among pigmented rice germplasm using molecular marker and morphological traits. *Biotechnol. Biotechnol. Equip.* 34(1):747-762.
- Saxena, R.C. and Barrion, A.A. 1983. Biotypes of the Brown Planthopper, *Nilaparvata lugens* (Stal). *Korean J. Appl. Entomol.* 22(2):52-66.
- Saxena, R.C. and Barrion, A.A. 1987. Biotypes of insect pests of agricultural crops. *Int. J. Trop. Insect Sci.* 8(4-5-6):453-458.
- Saxena, R.C. and Pathak, M.D., 1979. Factors governing susceptibility and resistance of certain rice varieties to the brown planthopper. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos, Philippines, pp.303-317.

- Seshu, D.V. and Kauffman, H. E. 1980. *Differential responses of rice varieties to the brown plant hoppers in the international screening tests*. IRRI Research Paper Series, International Rice Research Institute, Los Banos, Philippines, 13p.
- Shah, L., Ali, A., Zhu, Y., Wang, S., Si, H. and Ma, C. 2017. Wheat defense response to Fusarium head blight and possibilities of its improvement. *Physiol. Mol. Plant Pathol.* 98:9-17.
- Sharma, H.C. and Ortiz, R. 2002. Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation. *J. Environ. Biol.* 23(2):111-135.
- Sharma, P.N., Ketipearachchi, Y., Murata, K., Torii, A., Takumi, S., Mori, N. and Nakamura, C. 2002. RFLP/AFLP mapping of a brown planthopper (*Nilaparvata lugens* Stal.) resistance gene *Bph1* in rice. *Euphytica*, 129: 109-117.
- Sharma, P.N., Torii, A., Takumi, S., Mori, N. and Nakamura, C. 2004. Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stal.) resistance genes *Bph1* and *Bph2* on rice chromosome 12. *Hereditas*, 140: 61-69.
- Sharma, R.D. 1991. The story of rice. National Book Trust, India
- Shastri, S.V.S., Kangao Kao, D.R. and Misra, K.N. 1960. Pachytene analysis in *Oryza*. I. Chromosome morphology in *Oryza sativa* L. *Indian J. Genet. Plant. Breed.* 20:15-21.
- Shi, S., Wang, H., Nie, L., Tan, D.I., Zhou, C., Zhang, Q., Li, Y., Du, B., Guo, J., Huang, J. and Wu, D. 2021. *Bph30* confers resistance to brown planthopper by fortifying sclerenchyma in rice leaf sheaths. *Mol. Plant*, 14(10):1714-1732.

- Sidhu, G.S., Khush, G.S. and Medramo, F.G. 1997. A dominant gene in rice for resistance to white-backed planthopper and its relationship to other plant characteristics. *Euphytica*, 28:227-32.
- Sigsgaard, L. 2007. Early season natural control of the brown planthopper, *Nilaparvata lugens*: the contribution and interaction of two spider species and a predatory bug. *Bullet. Entomol. Res.* 97(5):533-544.
- Singh, H.V. 2004. Biochemical transformation in Brassica spp. due to *Pernospora parasitica* infection. *Ann. Plant Protect. Sci.* 12:301-304.
- Singh, K.S., Suneetha, Y., Kumar, G.V., Rao, V.S., Raja, D.S. and Srinivas, T. 2020. Variability, correlation and path studies in coloured rice. *Int. J. Chem. Stud.* 8(4):2138-2144.
- Singh, M., Shweta, Yadav, V.K., Yadav, R.K., Singh, P. and Anjani Singh. K. 2021. Genetic Variability Studies for Yield and Related Attributes in Rice Genotypes (*Oryza sativa* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 10(12):133-138.
- Singh, P., Verma, R.L., Singh, R.S., Singh, R.P., Singh, H.B., Arsode, P., Kumar, M. and Singh, P.K. 2020. Biotic stress management in rice (*Oryza sativa* L.) through conventional and molecular approaches. In: Rakshit, A., Singh, H.B., Singh, A.K., Singh, U.S. and Fraceto, L. (eds). *New frontiers in stress management for durable agriculture*. Berlin, Springer, pp.609-644.
- Sivasubrahmanian, S. and Menon, M. 1973. Genotypic and phenotypic variability in rice. *Madras Agric. J.* 60:1093-1096.
- Skendzic, S., Zovko, M., Zivkovic, I.P., Lesic, V. and Lemic, D. 2021. The impact of climate change on agricultural insect pests. *Insects*, 12(5):440.
- Smith, C.M. 2005. *Plant resistance to arthropods: molecular and conventional approaches*. Springer Press, Dordrecht, The Netherlands, 413p.

- Sogawa, K. 1971. Feeding Behaviors of The Brown Planthopper and Varietal Resistance of Rice to this Insect. In: *Proceedings of a Symposium on Tropical Agriculture Researches, 19-24 July 1971*. Tropical Agricultural Research Center, Ministry of Agriculture and Forestry, No. 5, p. 195.
- Sogawa, K. 1973. Feeding of the rice plant and leafhoppers. *Rev. Plant Prot. Res.* 6:31-43.
- Sogawa, K. 1994. Damage mechanisms of brown planthopper infestation: modelling approaches under a paradigm shift in pest management. In: Elings, A. and Rubia, E.G. (eds), *Analysis of damage mechanisms by pests and diseases and their effects on rice yield*. SARP Research Proceedings, International Rice Research Institute, Los Banos, Philippines, 279p.
- Sogawa, K. and Cheng, C.H. 1979. Economic thresholds, nature of damage, and losses caused by the brown planthopper. In: Heong, K.L. and Hardy, B. (Eds). *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, International Rice Research Institute, Los Banos, Philippines, pp.125-144.
- Song, X.Y., Peng, Y.X., Gao, Y., Zhang, Y.C., Ye, W.N., Lin, P.X., Gao, C.F. and Wu, S.F. 2023. Resistance Monitoring of *Nilaparvata lugens* to Pymetrozine Based on Reproductive Behavior. *Insects*, 14:428.
- Stern, V.M., Smith, R.F., Van Den Bosch, R. and Hagen, K.S. 1959. The integration of chemical and biological control of the spotted alfalfa aphid. Part I. The integrated control concept. *Hilgardia*, 29:81-101.
- Stout, M.J. 2013. Reevaluating the conceptual framework for applied research on host-plant resistance. *Insect Sci.* 20(3):263-272.
- Su, C.C., Zhai, H.Q., Wang, C.M., Sun, L.H. and Wan, J.M. 2006. SSR mapping of brown planthopper resistance gene *Bph9* in Kaharamana, an indica rice (*Oryza sativa* L.). *Acta Genet. Sinica*, 33(3):262-268.

- Suenaga, H. 1963. Analytical studies on the ecology of two species of planthoppers, the white back planthopper (*Sogatia furcifera* Horvath) and the brown planthopper (*Nilaparvata lugens* Stal), with special reference to their outbreaks. *Bull. Kyushu Agric. Exp. Stn.* 8:1-152.
- Suh, J., Jeung, J., Kim, Y., Jena, K.K., Cho, Y., Lee, J., Kim, M., Hong, H., Lee, J., Kim, J. and Choi, I. 2014. A brown planthopper resistant and high grain quality rice variety 'Anmi' developed by molecular breeding method. *Korean J. Breed. Sci.* 46(2):152-159.
- Suh, J.P., Jeung, J.U., Noh, T.H., Cho, Y.C., Park, S.H., Park, H.S., Shin, M.S., Kim, C.K. and Jena, K.K. 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice*, 6:1-11.
- Sujatha, G. 1987. Effect of certain biochemical factors on expression of resistance of rice varieties to brown planthopper (*Nilaparvata lugens* Stal). *J. Res. Andhra Pradesh Agric. Univ.* 15:124-128.
- Sukumar, S., Kennedy, J.S., Raveendran, M., Balasubramani, V. and Pushpam, R. 2022. Antixenosis and antibiosis mechanism of resistance in selected rice entries against brown planthopper, *Nilaparvata lugens* (Stal). *Econ. Enviorn. Conserv.* 28:S367-S372.
- Sun, L.H., Liu, Y., Jiang, L., Su, C.C., Wang, C.M., Zhai, H.R. and Wan, J.M. 2007. Identification of quantitative trait loci associated with resistance to brown planthopper in the indica rice cultivar Col. 5 Thailand. *Hereditas*, 144(2): 48-52.
- Sun, L.H., Su, C.C., Wang, C.M., Zhai, H.Q. and Wan, J.M. 2005. Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. *Breed. Sci.* 55(4):391-396.

- Sun, L.H., Wang, C.M., Su, C.C., Liu, Y.Q., Zhai, H.Q. and Wan, J. 2006. mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). *Acta Genet. Sinica*, 33:717-723.
- Sundaram, R.M., Madhav, M.S., Neeraja, C.N., Balachandran, S.M., Mangrauthia, S.K., Barbadikar, K.B., Divya, P.S., Rao, L.V. and Hariprasad, A.S. 2018. Multi-trait improvement in rice through marker-assisted breeding. *Oryza Int. J. Rice*, 55:24-37.
- Tamura, Y., Hattori, M., Yoshioka, H., Yoshioka, M., Takahashi, A., Wu, J., Sentoku, N. and Yasui, H. 2014. Map-based cloning and characterization of a brown planthopper resistance gene BPH26 from *Oryza sativa* L. ssp. indica cultivar ADR52. *Sci. Rep.* 4(1):1-8.
- Tan, H.Q., Palyam, S., Gouda, J., Kumar, P.P. and Chellian, S.K. 2022. Identification of two QTLs, *BPH41* and *BPH42*, and their respective gene candidates for brown planthopper resistance in rice. *Sci. Rep.* 12(1):18538.
- Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. *Biotechnol.* 7(3):257-264.
- Tanweer F.A., Rafii M.Y., Sijam K., Rahim H.A., Ahmed F., Latif M.A. 2015. Current advance methods for identification of blast resistance genes in rice. *Comptes Rendus Biol.* 338:321-334.
- Tenguri, P., Chander, S., Ellur, R.K., Arya, P.S. and Yele, Y. 2023. Deciphering host plant resistance mechanisms of rice genotypes resistant against Brown Planthopper. *Euphytica*, 219(1):8.
- Thakur, A. 2022. Pyramiding of brown plant hopper resistance genes *Bph34* and *Bph33* in rice. M.Sc. Thesis, Punjab Agricultural University, 87p.

- Thamarai, M. and Soundararajan, R.P. 2017. Evaluation of antibiosis resistance to brown planthopper, *Nilaparvata lugens* (Stål) in rice. *J. Entomol. Zool. Stud.* 5(3):954-957.
- Thomas, M.J. 1977. Biology, ecology and host plant relations of the brown plant hopper *Nilaparvata lugens* Stål. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 162p.
- Thulasinathan, T., Nallathambi, J., Rahman, H., Kambale, R., Ayyenar, B., Venkatasamy, B. and Muthurajan, R. 2020. Marker assisted introgression and validation of resistance genes *Bph20* and *Bph21* for brown plant hopper (*Nilaparvata lugens* Stål) into a popular rice variety of CO51. *J. Pharmacognosy Phytochem.* 9(6):939-944.
- Udayasree, M., Rajanikanth, P., Varma, N.R.G. and Sreedhar, M. 2021. Antibiosis and tolerance of certain elite rice genotypes against brown Planthopper *Nilaparvata lugens* (Stål.). *Pharma Innovation J.* 10(9):1057-1061.
- UNFPA [United Nations Population Fund's]. 2023. State of World Population report.
- Valappil, N.K.M., Hamza, V. and de Oliveira Júnior, J.F. 2023. Evaluation of daily average temperature trends in Kerala, India, using MERRA-2 reanalysis data: a climate change perspective. *Environ. Sci. Pollut. Res.* 1-24.
- Vanitha, K., Suresh, S. and Gunathilagaraj, K. 2011. Influence of brown planthopper *Nilaparvata lugens* (Stål.) feeding on nutritional biochemistry of rice plant. *Oryza Int. J. Rice*, 48(2):142-146.
- Vaughan, D.A. 1994. *The wild relatives of rice: a genetic resources handbook*. International Rice Research Institute, Los Banos, Philippines, 200p
- Velusamy, R., Heinrichs, E.A. and Medrano, F.G. 1986. Greenhouse techniques to identify field resistance to the brown planthopper, *Nilaparvata lugens* (Stål)(Homoptera: Delphacidae), in rice cultivars. *Crop Prot.* 5(5):328-333.

- Vignesh, M., Valarmathi, M., Rakshana, P., Bharathi, A., Dilip, K.R., Sudha, M., Manonmani, S. and Raveendran, M. 2023. Marker assisted pyramiding of major brown plant hopper resistance genes in an elite culture CBMAS14065. *Electr. J. Plant Breed.* 14(1):1-8.
- Wang, H., Ye, S. and Mou, T. 2016. Molecular breeding of rice restorer lines and hybrids for brown planthopper (BPH) resistance using the *Bph14* and *Bph15* genes. *Rice*, 9:1-9.
- Wang, H.Y., Shi, S.J., Guo, Q., Nie, L.Y., Du, B., Chen, R.Z., Zhu, L.L. and He, G.C. 2018. High-resolution mapping of a gene conferring strong antibiosis to brown planthopper and developing resistant near-isogenic lines in 9311 background. *Mol. Breed.* 38(8):107.
- Wang, X., Guo, X., Ma, X., Luo, L., Fang, Y., Zhao, N., Han, Y., Wei, Z., Liu, F., Qin, B. and Li, R. 2021. Development of new rice (*Oryza. sativa* L.) breeding lines through marker-assisted introgression and pyramiding of brown planthopper, blast, bacterial leaf blight resistance, and aroma genes. *Agron.* 11(12):2525.
- Wang, Y., Cao, L., Zhang, Y., Cao, C., Liu, F., Huang, F., Qiu, Y., Li, R. and Luo, X. 2015. Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. *J. Exp. Bot.* 66(19):6035-6045.
- Wang, Y., Jiang, W., Liu, H., Zeng, Y., Du, B., Zhu, L., He, G. and Chen, R. 2017. Marker assisted pyramiding of *Bph6* and *Bph9* into elite restorer line 93–11 and development of functional marker for Bph9. *Rice*, 10(1):1-13.
- Wang, Y., Wang, X., Yuan, H., Chen, R., Zhee, L., He, R. and He, G. 2008. Responses of two contrasting genotypes of rice to brown planthopper. *Mol. Plant-microbe Interactions*, 21(1):122-132.
- Wu, H., Liu, Y.Q., He, J., Liu, Y.L., Jiang, L., Liu, L.H., Wang, C.M., Cheng, X.N. and Wan, J.M. 2014. Fine mapping of brown planthopper (*Nilaparvata*

lugens Stål) resistance gene *Bph28(t)* in rice (*Oryza sativa* L.). *Mol. Breed.* 33:909-918.

Wu, X., Yu, Y., Baerson, S.R., Song, Y., Liang, G., Ding, C., Niu, J., Pan, Z. and Zeng, R. 2017. Interactions between nitrogen and silicon in rice and their effects on resistance toward the brown planthopper *Nilaparvata lugens*. *Frontiers Plant Sci.* 8:28.

Wu, Y., Lv, W., Hu, L., Rao, W., Zeng, Y., Zhu, L., He, Y. and He, G. 2017. Identification and analysis of brown planthopper-responsive microRNAs in resistant and susceptible rice plants. *Sci. Rep.* 7(1):8712.

Xu, H.X., Zheng, X.S., Yang, Y.J., Tian, J.C., Lu, Y.H., Tan, K.H., Heong, K.L. and Lu, Z.X. 2015. Methyl eugenol bioactivities as a new potential botanical insecticide against major insect pests and their natural enemies on rice (*Oryza sativa*). *Crop Protect.* 72:144-149.

Xu, J. 2013. Pyramiding of two BPH resistance genes and Stv-bi gene using marker-assisted selection in japonica rice. *Crop Breed. Appl. Biotechnol.* 13:99-106.

Yang, D., Xiong, L., Mou, T. and Mi, J. 2022. Improving the resistance of the rice PTGMS line Feng39S by pyramiding blast, bacterial blight, and brown planthopper resistance genes. *Crop J.* 10(4):1187-1197.

Yang, H., Ren, X., Weng, Q., Zhu, L., and He, G. 2002. Molecular mapping and genetics of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene. *Hereditas*, 136:39-43.

Yang, L., Li, R.B., Li, Y.R., Huang, F.K., Chen, Y.Z., Huang, S.S., Huang, L.F., Liu, C., Ma, Z.F., Huang, D.H., and Jiang, J.J. 2012. Genetic mapping of *bph20(t)* and *bph21(t)* loci conferring brown planthopper resistance to *Nilaparvata lugens* Stal in rice (*Oryza sativa* L.). *Euphytica*, 183:161-171.

- Yang, M., Cheng, L., Yan, L.H., Shu, W., Wang, X.Y. and Qiu, Y.F. 2019. Mapping and characterization of a quantitative trait locus resistance to the brown planthopper in the rice variety IR64. *Hereditas*, 156(1):22.
- Yang, Z., Xiao, T. and Lu, K. 2023. Contribution of UDP-glycosyltransferases to chlorpyrifos resistance in *Nilaparvata lugens*. *Pesticide Biochem. Physiol.* 190:105321.
- Zampieri, E., Volante, A., Marè, C., Orasen, G., Desiderio, F., Biselli, C., Canella, M., Carmagnola, L., Milazzo, J., Adreit, H. and Tharreau, D. 2023. Marker-assisted pyramiding of blast-resistance genes in a *japonica* elite rice cultivar through forward and background selection. *Plants*, 12(4):757.
- Zhang, Q. and Wing, R.A. 2013. *Genetics and genomics of rice* (Vol. 5). Springer, Science and Business Media, 130p
- Zhang, Q., Li, T., Gao, M., Ye, M., Lin, M., Wu, D., Guo, J., Guan, W., Wang, J., Yang, K. and Zhu, L. 2022. Transcriptome and metabolome profiling reveal the resistance mechanisms of rice against brown planthopper. *Int. J. Mol. Sci.* 23(8):4083.
- Zhang, Y.X., Qin, G., Ma, Q.Q., Wei, M.Y., Yang, X.H., Ma, Z.F., Liang, H.F., Liu, C., Li, Z.J., Liu, F., Huang, D.H. and Li, R.B. 2020. Identification of a major resistance locus *Bph35* to brown planthopper in rice (*Oryza sativa* L.). *Rice Sci.* 27(3):237-245.
- Zhou, S., Chen, M., Zhang, Y., Gao, Q., Noman, A., Wang, Q., Li, H., Chen, L., Zhou, P., Lu, J. and Lou, Y. 2019. OsMKK3, a stress-responsive protein kinase, positively regulates rice resistance to *Nilaparvata lugens* via phytohormone dynamics. *Int. J. Mol. Sci.* 20(12):3023.

**DEVELOPMENT OF BREEDING LINES IN RICE (*Oryza sativa*
L.) PYRAMIDED WITH R GENES FOR RESISTANCE TO
BROWN PLANT HOPPER (BPH) BY MARKER-ASSISTED
SELECTION**

by

ARUN CHACKO

(2019-21-057)

Abstract of the thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University



DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2023

ABSTRACT

The present study ‘Development of breeding lines in rice (*Oryza sativa* L.) pyramided with R genes for resistance to Brown Plant Hopper (BPH) by marker-assisted selection’ was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Regional Agricultural Research Station, Pattambi, during the year 2019-2022, with an objective to introgress R genes for resistance to BPH in the background of the elite variety Jyothi using marker-assisted selection.

The study comprised of two main experiments. Experiment-I aimed at the BPH bioassay, assessment of morphological and biochemical characters linked to BPH resistance in the donor and recipient parents followed by the validation of the presence of genes for resistance using specific molecular markers. Recipient parent (Jyothi) and probable donors for BPH resistance from the results of a concluded project in the Department of Plant Breeding and Genetics, COA, Vellayani, and other R gene introgressed lines from IRRI were selected for screening. The BPH bioassay was done using the modified seedbox screening test (MSST) developed by Velusamy (1986). BPH-affected seedling survival rate was calculated based on the Standard Evaluation System (SES) scale of 0-9 (IRRI, 2013). Among the twelve rice genotypes screened, PTB-33, PTB-21, and IR7103-121-15-B were classified as highly resistant and the genotypes IR65482-7-216-1-2 and RP2068-18-3-5 were classified as resistant to BPH infestation. The *japonica* variety Kinandang Patong was moderately resistant to BPH. The recipient parent Jyothi and five other genotypes viz., Manu Ratna, White Ponni, Wayrareem, Vandana, and APO were classified as highly susceptible to BPH infestation.

Five genotypes, Jyothi (recipient parent), PTB-33, IR7103-121-15-B, IR65482-7-216-1-2, and RP2068-18-3-5 were forwarded for studying the BPH resistance reaction. The antibiosis and tolerance mechanisms of BPH resistance were explored in terms of nymphal survival rate, honey-dew test, functional plant loss index (FPLI), and tolerance index (TI). The recipient parent, ‘Jyothi’ turned out to be highly susceptible, and ‘PTB-33’ showed high resistance to BPH feeding among the rice genotypes screened. Other genotypes, IR65482-7-216-1-2 RP2068-

18-3-5, and IR7103-121-15-B showed intermediate values compared to PTB-33 and the highly susceptible variety 'Jyothi'. The morphological characters and biochemical parameters linked with BPH resistance of five genotypes were assessed in a completely randomized design (CRD) with four replications. The results revealed that BPH feeding, survival, functional plant loss index, and disease score were positively correlated with protein content and negatively correlated with phenol and ascorbic acid content. Reducing sugar content in plants did not show much effect on BPH resistance.

The genetic parameters, phenotypic and genotypic coefficient of variation were higher in seven characters, and moderate in six characters indicating high variability among the parents. High heritability and genetic advance in the characters except for the number of productive tillers, and culm thickness and grain length respectively indicated the presence of additive gene action. The characters, culm thickness, 1000 grain weight, and protein content showed a significant positive correlation with the functional plant loss index (FPLI). Path analysis of morphological and biochemical characters with FPLI as the dependent variable showed the direct positive effect of the number of productive tillers, panicle length, number of grains per panicle, grain breadth, LB ratio, and phenol content on FPLI.

The validation of the presence or absence of genes for BPH resistance in donor and recipient parents was done using SSR markers RM589 for the *Bph-3* gene, RM3331 for *Bph-18*, RM8213 for *Bph-20*, and RM28561 for *Bph-21*. Distinguishable polymorphic bands were obtained in SSR markers RM589 (200 bp for resistance and 180 bp for susceptibility) and RM3331 (110 bp for resistance and 130 bp for susceptibility). Based on BPH screening, resistant reaction study, and gene validation, the KAU released variety 'PTB-33' and IRRI introgression line IR65482-7-216-1-2 were selected as the donors for *Bph-3* and *Bph-18* gene respectively in the marker-assisted breeding program.

The second experiment was marker-assisted backcrossing to introgress the R genes (*Bph-3* and *Bph-18*) into the background of elite high-yielding but susceptible variety Jyothi. The donors PTB-33 and IR65482-7-216-1-2 were crossed independently with Jyothi to obtain the F₁ generation followed by

backcrossing with Jyothi to develop the BC₁F₁ generation. Phenotyping for ten morphological characters and genotyping using gene-specific SSR markers were carried out in all the backcross generations. Genotyping of 63 BC₁F₁ lines derived from the PTB-33 donor parent with RM589 marker identified 28 plants with the *Bph-3* gene in heterozygous condition. Genotyping of 78 BC₁F₁ lines derived from the IR65482-7-216-1-2 donor parent with RM3331 marker identified 36 plants with *Bph-18* gene in heterozygous condition. The goodness of fit test (χ^2 test) in BC₁F₁ lines with genotypic data showed that the genes *Bph-3* and *Bph-18* followed the ratio of simple dominance.

Intercrossing of BC₁F₁ lines involved two crosses viz., the PTB-33 derived BC₁F₁ lines with *Bph-3* in heterozygous condition as the female parent and IR65482-7-216-1-2 derived BC₁F₁ lines with *Bph-18* in heterozygous condition as male parent denoted as 'ICAB' and the reciprocal cross denoted as 'ICBA'. Among the twenty-two ICAB progenies, three plants showed both genes (*Bph-3* and *Bph-18*) in heterozygous condition, and among the eighteen ICBA progenies, five plants were obtained with both genes (*Bph-3* and *Bph-18*) in heterozygous condition. The Euclidean Distance of intercrossed BC₁F₁ lines from the recipient parent Jyothi using proximity dissimilarity matrix analysis was calculated to select the intercrossed BC₁F₁ lines more similar to the recipient parent. Eight intercrossed BC₁F₁ lines with Jyothi-specific characters and both genes in heterozygous condition were backcrossed with Jyothi to develop BC₂F₁ lines.

In the genotypic evaluation of twenty-six BC₂F₁ lines, five lines showed the presence of both genes (*Bph-3* and *Bph-18*) in heterozygous condition. The five BC₂F₁ lines with maximum similarity in proximity dissimilarity matrix analysis were selfed and three-hundred and twenty BC₂F₂ lines were forwarded to BPH bioassay in the seedling stage at RARS, Pattambi, and COA, Vellayani.

Eighty-nine BC₂F₂ lines showed different levels of resistance in the BPH bioassay. Genotyping of these lines showed the presence of both genes (*Bph-3* and *Bph-18*) in homozygous resistant condition in four lines namely, ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, and ICAB-1/6/10. The similarity percentage of these four lines with the recipient parent in proximity dissimilarity matrix analysis was

obtained as 84.51 per cent in ICAB-1/3/6, 89.27 per cent in ICAB-1/6/10, 91.15 per cent in ICAB-1/6/2, and 91.6 per cent in ICAB-1/3/7.

The developed breeding lines, possessing pyramided R genes for BPH resistance in the background of Jyothi, can be used to develop essentially derived varieties from Jyothi with BPH resistance. This will offer an improved and sustainable solution for combating BPH infestations, reducing reliance on chemical insecticides, and enhancing the stability of rice production.

സംഗ്രഹം

“ഡെവലപ്മെന്റ് ഓഫ് ബ്രീഡിങ് ലൈൻസ് ഇൻ റൈസ് (*Oryza sativa* L.) പീരമിഡൽ വിത്ത് R ജീൻസ് ഫോർ റെസിസ്റ്റൻസ് റ്റു ബ്രൗൺ പ്ലാങ്ക് ഹോപ്പർ (ബി പി എച്ച്) ബൈ മാർക്കർ-അസ്സീസ്ഡ് സെലക്ഷൻ” എന്ന പഠനപദ്ധതി 2019-2022 കാലയളവിൽ വെള്ളായണി കാർഷിക കോളേജിലെ സസ്യപ്രജനന ജനിതക ശാസ്ത്ര വിഭാഗത്തിലും പട്ടാമ്പി പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിലുമായി സംഘടിപ്പിക്കുകയുണ്ടായി. മികവുറ്റ ഇനമായ ജ്യോതിയുടെ ജനിതക പശ്ചാത്തലത്തിലേക്ക് മുഞ്ഞയുടെ പ്രതിരോധത്തിനായി R ജീനുകൾ മാർക്കർ അധിഷ്ഠിത തിരഞ്ഞെടുക്കലിലൂടെ ഉൾപ്പെടുത്തുക എന്നതായിരുന്നു ഗവേഷണ പദ്ധതിയുടെ ഉദ്ദേശം.

പ്രധാനമായും രണ്ടു പരീക്ഷണഘട്ടങ്ങൾ ആണ് ഗവേഷണത്തിലുൾപ്പെട്ടിരുന്നത്. ആദ്യ ഘട്ടത്തിൽ മുഞ്ഞയുടെ ജൈവികവിശകലനമാണ് ലക്ഷ്യമാക്കിയിരുന്നത്, അതിനായി ദാതാവായ സസ്യങ്ങളുടെയും സ്വീകർത്താക്കളായ സസ്യങ്ങളുടെയും ബി പി എച്ച്നോടുള്ള പ്രതിരോധശേഷിയുമായി ബന്ധപ്പെട്ടിരിക്കുന്ന രൂപശാസ്ത്രപരമായതും ജീവരസതന്ത്രപരമായതുമായ സവിശേഷതകൾ നിർണ്ണയിക്കുകയുണ്ടായി. തുടർന്ന് നിശ്ചിത മോളികുലാർ മാർക്കറുകൾ ഉപയോഗിച്ച് പ്രതിരോധശേഷി നൽകുന്ന ജീനുകളുടെ സാന്നിധ്യം ഉറപ്പ് വരുത്തുകയും ചെയ്തു. സ്വീകർത്താവായി ജ്യോതി ഇനവും വെള്ളായണി കാർഷിക കോളേജിന്റെ സസ്യപ്രജനന ശാസ്ത്ര വിഭാഗത്തിൽ പൂർത്തിയാക്കിയ ഒരു പ്രോജക്ടിന്റെ കണ്ടെത്തലിൽ നിന്നും അതുപോലെ R ജീനുകൾ ഉൾപ്പെടുത്തിയെടുത്തിട്ടുള്ള ഐ ആർ ആർ ഐ (IRRI) ഇൽ നിന്നുമുള്ള സസ്യ ഇനങ്ങളിൽ നിന്നും ബി പി എച്ച് പ്രതിരോധശേഷിയുള്ള ദാതവുകാർ സാധ്യത ഉള്ള ഇനങ്ങളെയും സ്ക്രീനിംഗ് പ്രക്രിയയ്ക്കായി തിരഞ്ഞെടുത്തു. വേലുസ്വാമി 1986ഇൽ വികസിപ്പിച്ചെടുത്ത മോഡിഫൈഡ് സീഡ്ബോക്സ് സ്ക്രീനിംഗ് ടെസ്റ്റ് (MSST) ഉപയോഗിച്ചാണ് ബി പി എച്ച്ന്റെ ജൈവവിശകലനം നടത്തിയത്. ഐ ആർ ആർ ഐ യുടെ (2013) സ്റ്റാൻഡേർഡ് ഇവാലുയേഷൻ സിസ്റ്റത്തിൽ (SES) 0-9 സ്കെയിൽ ഉപയോഗിച്ചുകൊണ്ട് മുഞ്ഞ ബാധിതമായ തൈ ചെടികളുടെ അതിജീവനത്തിന്റെ നിരക്ക് കണക്കാക്കി. സ്ക്രീനിംഗിന് വിധേയമാക്കിയ 12 നെല്ലിനങ്ങളുടെ ജീനോടൈപ്പ് കളിൽ PTB-33, PTB-21,

IR7103-121-15-B എന്നിവ ബി പി എച്ച്നോട് ഉയർന്ന പ്രതിരോധശേഷി കാണിക്കുന്നതാണെന്നും IR65482-7-216-1-2, RP2068-18-3-5 എന്നീ ജീനോടൈപ്പുകൾ പ്രതിരോധ ശേഷി ഉള്ളവയായും കണ്ടെത്തി. ജപ്പാനിൽ ഇനമായ കിനാൻഡാൻ പടോൻ മിതമായി പ്രതിരോധശേഷി കാണിക്കുന്നവയാണ്. സ്വീകർത്താവായ ജ്യോതിയും മനുരത്ന, വൈറ്റ് പൊന്നി, വേരാറോ, വന്ദന, അപ്പൊ എന്നീ അഞ്ചിനങ്ങൾ ബി പി എച്ച്നോട് എളുപ്പത്തിൽ വിധേയമാകുന്നവയാണ്.

സ്വീകർത്താവായ ജ്യോതി ഉൾപ്പെടെ PTB-33, IR7103-121-15-B, IR65482-7-216-1-2, RP2068-18-3-5 എന്നീ അഞ്ചു ജനിതക ഇനങ്ങളെ ബി പി എച്ച് നോടുള്ള പ്രതിരോധശേഷിയുടെ പ്രതികരണം പഠിക്കുന്നതിനായി പ്രാമുഖ്യപ്പെടുത്തി. നിംഫുകളുടെ അതിജീവനനിരക്ക്, ഹണി-ഡ്യൂ ടെസ്റ്റ്, പ്രവർത്തനക്ഷമമായ സസ്യത്തിനുണ്ടാകുന്ന നഷ്ട സൂചിക (ഫങ്ഷണൽ പ്ലാൻറ് ലോസ് ഇൻഡക്സ് -എഫ്ഐഇൽഐ), സഹിഷ്ണുത സൂചിക (ടോളറൻസ് ഇൻഡക്സ്-ടിഐ)) എന്നിവയുടെ അടിസ്ഥാനത്തിൽ ബിപിഎച്ച് പ്രതിരോധത്തിന്റെ സഹിഷ്ണുത പ്രക്രിയയുടെ പ്രവർത്തന വിധവും ആന്റിബയോസിസ് പ്രക്രിയയും എല്ലാം പര്യവേഷണ വിധേയമാക്കി. സ്ക്രീനിംഗിന് വിധേയമാക്കിയ ജനിതക നെല്ലിങ്ങളിൽ മുത്തയുടെ ആക്രമണത്തിനോട് PTB 33 എന്ന ഇനം ഉയർന്ന പ്രതിരോധശേഷി കാണിച്ചപ്പോൾ സ്വീകർത്താവായ ജ്യോതി അമിതമായി വിധേയത്വം ഉള്ള ഇനമായി കാണപ്പെട്ടു. PTB 33, പ്രതിരോധ ശേഷി ഇല്ലാത്ത ഇനമായ ജ്യോതി എന്നിവയോട് താരതമ്യപ്പെടുത്തുമ്പോൾ IR65482-7-216-1-2 RP2068-18-3-5, IR7103-121-15-B എന്നീ ഇനങ്ങൾ ഇടത്തരം മൂല്യങ്ങളാണ് കാണിച്ചത്. ഈ അഞ്ചു ജനിതക നെല്ലിനങ്ങളുടെ ബി പി എച്ച് പ്രതിരോധവുമായി ബന്ധപ്പെട്ടിരിക്കുന്ന രൂപശാസ്ത്രപരവും ജീവരസതന്ത്രപരവുമായ സൂചകങ്ങൾ കമ്പ്യൂട്ടേറ്റിവി റാണ്ടമയ്സ് ബ്ലോക്ക് ഡിസൈനിൽ നാലു റിപ്ലിക്കേഷനുകളിലായി വിശകലനം ചെയ്തു. തത്ഫലമായി ബി പി എച്ച് ആക്രമണം, അതിജീവനം, പ്രവർത്തനക്ഷമമായ സസ്യത്തിനുണ്ടാകുന്ന നഷ്ട സൂചിക, ഡാമേജ് സ്കോർ എന്നിവ മാംസ്യത്തിന്റെ അളവിനോട് അനുകൂലമായി ബന്ധപ്പെട്ടിരിക്കുന്നതായും ഫീനോളിന്റെ അളവ്, അസ്കോർബിക് ആസിഡിന്റെ അളവ് എന്നിവയുമായി പ്രതികൂലമായി ബന്ധപ്പെട്ടിരിക്കുന്നതായും തെളിഞ്ഞു. ബിപിഎച്ച് പ്രതിരോധശേഷിയെ

സസ്യത്തിലെ റെഡ്യൂസിങ് ഷുഗറിന്റെ അളവ് കാര്യമായി ബാധിക്കുന്നില്ല എന്നും കണ്ടു.

ജനിതകമായ ഗുണനഘടകം, വ്യതിയാനത്തിന്റെ സ്ഥൂലരൂപത്തിലുണ്ടാകുന്ന ഗുണനഘടകം എന്നീ സൂചകങ്ങൾ ഏഴ് സവിശേഷതകളിൽ ഉയർന്ന അളവിലും 6 എണ്ണത്തിൽ മിതമായ അളവിലുമായിരുന്നു. ഇത് ഉൽപ്പാദകരുടെ ഉയർന്ന വൈവിധ്യത്തെ സൂചിപ്പിക്കുന്നതാണ്. തണ്ടിന്റെ നിബിഡത, ഉൽപ്പാദനക്ഷമമായ നെൽക്കതിരുകളുടെ ശിഖരങ്ങളുടെ എണ്ണം, ധാന്യത്തിന്റെ നീളം എന്നിവ ഒഴികെ ഉള്ള പ്രതീകങ്ങളിലെ ഉയർന്ന പൈതൃകക്ഷമതയും ജനിതക മുന്നേറ്റവും ജീനുകളുടെ സങ്കലന പ്രവർത്തനത്തിന്റെ സാന്നിധ്യം സൂചിപ്പിച്ചു. തണ്ടിന്റെ നിബിഡത, 1000 വിത്തുകളുടെ ഭാരം, മാംസ്യത്തിന്റെ അളവ് എന്നീ പ്രതീകങ്ങൾ പ്രർത്തനക്ഷമമായ സസ്യത്തിനുണ്ടാകുന്ന നഷ്ട സൂചികയോട് (എഫ് പി ഐ) അനുകൂലമായുള്ളതും ഗണ്യമായതുമായ ബന്ധം കാണിച്ചു. രൂപശാത്രപരമായതും ജീവരസതന്ത്രപരമായതുമായ സവിശേഷതകളെ എഫ് പി ഐ, ഡിപെൻഡന്റ് വേരിയബിൾ ആയി പരിഗണിച്ചുകൊണ്ട് പാത് അനാലിസിസ് രീതിയിൽ വിശകലനം നടത്തിയതിൽനിന്നും ഉൽപ്പാദനക്ഷമതയുള്ള നെൽക്കതിരുകൾ ഉണ്ടാകുന്ന ശാഖകളുടെ എണ്ണം, നെൽക്കതിരിന്റെ നീളം, ഒരു കതിരിലെ ധാന്യങ്ങളുടെ എണ്ണം, ധാന്യത്തിന്റെ വീതി, നീളം -വീതി അനുപാതം, ഫീനോളിന്റെ അളവ് എന്നീ പ്രതീകങ്ങൾ, പ്രർത്തനക്ഷമമായ സസ്യത്തിനുണ്ടാകുന്ന നഷ്ട സൂചികയിൽ നേരിട്ടും അനുകൂലവുമായ പ്രഭാവം ചെലുത്തുന്നതായി കാണിച്ചു.

ദാതാവിലും സ്വീകർത്താവിലും ബി പി എച്ച് പ്രതിരോധശേഷിക്ക് കാരണമായ ജീനുകളുടെ സാന്നിധ്യം ഉണ്ടോ ഇല്ലയോ എന്ന് നിർണ്ണയിക്കുവാൻ എസ് ആർ മാർക്കറുകൾ ഉപയോഗിച്ചു. RM589 മാർക്കറുകൾ *Bph-3* ജീനിനും RM3331 മാർക്കറുകൾ *Bph-18* ജീനിനായും ഉപയോഗിച്ചപ്പോൾ *Bph-20*, *Bph-21* ജീനുകൾക്കായി യഥാക്രമം RM8213, RM28561 എന്നീ മാർക്കറുകൾ ആണ് ഉപയോഗിച്ചത്. എസ് ആർ മാർക്കറുകളായ RM589 (200bp യിൽ പ്രതിരോധശേഷിയും 180bp വിധേയത്വവും) ലും RM3331 (110bp- പ്രതിരോധശേഷി, 130bp - വിധേയത്വം) ലും വേർതിരിച്ചറിയാൻ കഴിയുന്ന പോളിമോർഫിക് ബാൻഡുകൾ ലഭിച്ചു. ബിപിഎച്ച്

പ്രതിരോധശേഷിയുടെ സ്ക്രീനിംഗ് പ്രക്രിയ, പ്രതിരോധ പ്രതികരണത്തിനെക്കുറിച്ചുള്ള പഠനം, ജീനിന്റെ സാന്നിധ്യം നിർണയിക്കൽ എന്നിവയെ അടിസ്ഥാനമാക്കി മാർക്കർ അധിഷ്ഠിത പ്രജനന പരിപാടിയിലൂടെ കേരള കാർഷിക സർവകലാശാല പുറത്തിറക്കിയ PTB-33 എന്ന ഇനവും IRR-ൽ നിന്നുള്ള IR65482-7-216-1-2 എന്ന ഇനവും യഥാക്രമം *Bph-3*, *Bph-18* എന്നീ ജീനുകളുടെ ദാതാക്കളായി തിരഞ്ഞെടുക്കപ്പെട്ടു.

മികവുറ്റതും ഉയർന്ന ഉൽപ്പാദനക്ഷമതയുള്ളതുമായ എന്നാൽ പ്രതിരോധശേഷി ഇല്ലാത്ത ഇനമായ ജ്യോതിയുടെ ജനിതക പശ്ചാത്തലത്തിലേക്ക് R ജീനുകളെ (*Bph-3*, *Bph-18*) മാർക്കർ അധിഷ്ഠിത ബാക്കിക്രോസ്സിംഗ് രീതിയിലൂടെ പ്രവേശിപ്പിക്കുക എന്നതായിരുന്നു പരീക്ഷണത്തിന്റെ രണ്ടാം ഘട്ടം. ദാതാക്കളായ PTB-33, IR65482-7-216-1-2 എന്നിവയെ ജ്യോതിയുമായി സ്വതന്ത്രമായി സങ്കരണം നടത്തിയുണ്ടായ F_1 തലമുറയെ ജ്യോതിയുമായി ബാക്കിക്രോസ്സിംഗ് നടത്തി BC_1F_1 തലമുറയെ ഉല്പാദിപ്പിച്ചു. എല്ലാ ബാക്കി ക്രോസ്സിംഗ് നടത്തിയുണ്ടാക്കിയ എല്ലാ തലമുറയിലെയും സസ്യങ്ങളിലെ പത്തു രൂപശാസ്ത്രപരമായ സവിശേഷതകളുടെ ഫീനോടൈപ്പിങ്ങും നിശ്ചിത ജീനുകൾക്കുള്ള എസ് ആർ മാർക്കറുകൾ ഉപയോഗിച്ച് ജീനോടൈപ്പിങ്ങും നടത്തുകയുണ്ടായി. PTB-33 ദാതവിൽ നിന്നും രൂപംകൊണ്ട 63 BC_1F_1 സസ്യങ്ങൾ RM589 മാർക്കർ ഉപയോഗിച്ച് ജീനോടൈപ്പിംഗ് നടത്തിയപ്പോൾ അവയിൽ 28 സസ്യങ്ങളിൽ *Bph-3* ജീനിന്റെ സാന്നിധ്യം ഹെറ്ററോസൈഗസ് നിലയിൽ ഉള്ളതായി തിരിച്ചറിഞ്ഞു. ദാതാവായ IR65482-7-216-1-2 ഇനത്തിൽ നിന്നും ഉത്ഭവിച്ച 78 BC_1F_1 സസ്യങ്ങളെ RM3331 മാർക്കർ ഉപയോഗിച്ച് ജീനോടൈപ്പിംഗ് നടത്തിയതിൽ നിന്നും 36 എണ്ണത്തിൽ *Bph-18* ജീൻ ഹെറ്ററോസൈഗസ് നിലയിൽ ഉള്ളതായി കണ്ടെത്തി. BC_1F_1 തലമുറയെ അനുയോജ്യമായവയുടെ മേധാവിത്വം വെളിവാക്കുന്ന കൈ സ്ക്വയർ ടെസ്റ്റിന് വിധേയമാക്കിയപ്പോൾ *Bph-3*, *Bph-18* എന്നീ ജീനുകൾ സിമ്പിൾ ഡോമിനൻസ് അനുപാതമാണ് കാണിച്ചത്.

BC_1F_1 തലമുറയുടെ ഇൻറർക്രോസ്സിംഗിൽ രണ്ട് തരം സങ്കരണമാണ് ഉൾപ്പെടുന്നത്. PTB-33 യിൽ നിന്നുത്ഭവിച്ച *Bph-3* ജീനുള്ള BC_1F_1 സസ്യങ്ങൾ മാതൃസസ്യമായും IR65482-7-216-1-2 നിന്നും ഉത്ഭവിച്ച *Bph-18* ജീനുള്ള BC_1F_1 തലമുറയെ പിതൃ സസ്യമായും എടുക്കുന്ന സങ്കരണം 'ICAB ' എന്നും, അതേ

സമയം ഇവയുടെ വിപരീതരീതിയിൽ ഉള്ള സങ്കരണം 'ICBA' എന്നുമാണ് സൂചിപ്പിച്ചത്. ICAB സങ്കരണത്തിൽ നിന്ന് ലഭിച്ച 22 പ്രോജനിസിൽ 3 എണ്ണത്തിൽ രണ്ടു ജീനുകളും (*Bph-3*, *Bph-18*) ഹെറ്ററോസൈഗസ് നിലയിലാണ് കാണപ്പെട്ടത്. ICBA യുടെ 18 പ്രോജനിസിൽ 5 എണ്ണത്തിലാണ് രണ്ടു ജീനുകളെയും ഹെറ്ററോസൈഗസ് നിലയിൽ കണ്ടെത്തിയത്. സ്വീകർത്താവിനോട് കൂടുതൽ സാമ്യമുള്ള പരസ്പരം സങ്കരണം നടത്തിയ BC_1F_1 സസ്യങ്ങളെ തിരഞ്ഞെടുക്കാൻ ഇവ തമ്മിലുള്ള യുക്ലീഡിയൻ അകലം പ്രോക്സിമിറ്റി ഡിസ്റ്റിമിലാരിറ്റി മാട്രിക്സ് ഉപയോഗിച്ച് കണക്കാക്കപ്പെട്ടു. BC_2F_1 തലമുറ ഉൽപ്പാദിപ്പിക്കുന്നതിനായി ജ്യോതി ഇനത്തിന്റെ പ്രത്യേക സവിശേഷതകളും ഹെറ്ററോസൈഗസ് അവസ്ഥയിൽ നിൽക്കുന്ന രണ്ടു ജീനുകളും ഉൾക്കൊള്ളുന്ന എട്ടു BC_1F_1 സസ്യങ്ങളെ ജ്യോതിയുമായി ബാക്ക്ക്രോസ്സിംഗിന് വിധേയമാക്കി.

ജനിതക ഘടനയുടെ മൂല്യനിർണ്ണയത്തിൽ 26 BC_2F_1 സസ്യങ്ങളിൽ അഞ്ചിനങ്ങൾ രണ്ടു ജീനുകളുടെയും ഹെറ്ററോസൈഗസ് അവസ്ഥയിലുള്ള സാന്നിധ്യം കാണിച്ചു. പ്രോക്സിമിറ്റി ഡിസ്റ്റിമിലാരിറ്റി മാട്രിക്സ് വിശകലനത്തിൽ പരമാവധി സാമ്യം കാണിച്ച അഞ്ച് BC_2F_1 ഇനങ്ങളിൽ സ്വപരാഗണം നടത്തി ലഭിച്ച മൂന്നുറ്റി ഇരുപതു BC_2F_2 സസ്യങ്ങളെ, പട്ടാമ്പി പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിലും കാർഷിക കോളേജ് വെള്ളായണിയിലുമായി തെ പ്രായത്തിലുള്ള ബിപിഎച്ച്ന്റെ ജൈവ പരീക്ഷണങ്ങൾ നടത്തി.

ജൈവ പരീക്ഷണത്തിൽ എൺപത്തി ഒൻപതു BC_2F_2 സസ്യങ്ങൾ പ്രതിരോധത്തിന്റെ വ്യത്യസ്ത തലങ്ങൾ കാണിച്ചു. ഈ തലമുറയുടെ ജനിതക ഘടനയിൽ നടത്തിയ വിശകലനത്തിൽ നിന്നും ICAB-1/3/6, ICAB-1/3/7, ICAB-1/6/2, ICAB-1/6/10 എന്നീ 4 സസ്യഇനങ്ങളിൽ രണ്ടു ജീനുകളും (*Bph-3*, *Bph-18*) ഹോമോസൈഗസ് അവസ്ഥയിലാണെന്നു കണ്ടെത്തി. പ്രോക്സിമിറ്റി ഡിസ്റ്റിമിലാരിറ്റി മാട്രിക്സ് വിശകലനത്തിൽ നിന്നും നാലിനങ്ങളുടെ സ്വീകർത്താവിനോടുള്ള സാമ്യതയുടെ ശതമാനം പരിശോധിച്ചപ്പോൾ ICAB-1/3/6 ന് 84.51 ശതമാനവും, ICAB-1/6/10 89.27 ശതമാനവും, ICAB-1/6/2 91.15 ശതമാനവും ICAB-1/3/7 91.6 ശതമാനവും ആണെന്ന് ലഭിച്ചു.

ജ്യോതിയുടെ ജനിതക പശ്ചാത്തലത്തിൽ ബി പി എച്ച് പ്രതിരോധശേഷി നൽകുന്ന R ജീനുകൾ അടക്കി വികസിപ്പിച്ചെടുത്ത പ്രജനന സസ്യങ്ങൾ ഉപയോഗിച്ചു കൊണ്ട് ജ്യോതിയിൽ നിന്നും

എസ്കൻഷ്യലി ഡിറൈവിഡ് ഇനങ്ങൾ (EDVs) വികസിപ്പിച്ചെടുക്കാൻ കഴിയും. ഇതുവഴി ബിപിഎച്ച് ആക്രമണം ചെറുക്കാനായി മെച്ചപ്പെട്ടതും സുസ്ഥിരവുമായ ഒരു മാർഗമാണ് മുന്നോട്ട് വയ്ക്കുന്നത്, കൂടാതെ രാസകീടനാശിനികളോടുള്ള അമിതമായ ആശ്രയത്വം കുറയ്ക്കുകയും സ്ഥിരതയാർന്ന നെല്ലുൽപ്പാദനത്തിന്റെ വർദ്ധനവിനു കാരണമാകുകയും ചെയ്യുന്നു.