

## Program Based Research Grant (PBRG)

# Sub-project Completion Report

on

## Identification of novel resistant gene(s), gene pyramiding and sustainable management of bacterial blight (BB) disease of rice

### Sub-project Duration

14 February 2018 to 15 November 2021

### Coordinating Organization

Plant Pathology Division  
Bangladesh Rice Research Institute, Gazipur



### Project Implementation Unit

National Agricultural Technology Program-Phase II Project  
Bangladesh Agricultural Research Council  
Farmgate, Dhaka-1215

September 2021

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### Implementing Organization

#### Plant Pathology Division

Bangladesh Rice Research Institute (BRRRI)  
Gazipur-1701

and

#### Department of Plant Pathology

Bangladesh Agricultural University (BAU)  
Mymensingh-2202



#### Project Implementation Unit

National Agricultural Technology Program-Phase II Project  
Bangladesh Agricultural Research Council  
Farmgate, Dhaka-1215

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## Abbreviation and Acronyms

Abbreviation	Elaboration
PSA	Peptone Sucrose Agar
CFU	Colony Forming Unit
HR	Highly Resistant
MR	Moderately Resistant
MS	Moderately Susceptible
HS	Highly Susceptible
IRRI	International Rice Research Institute
CTAB	Cetyl Trimethylammonium Bromide
EDTA	Ethylene Diamine Tetra Acetic acid
HCL	Hydrochloric Acid
PVP	Poly Vinyl Pyrrolidone
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
NBY	Nutrient Broth Yeast
NILs	Near Isogenic Lines
MAS	Marker Assisted Selection
PDA	Potato Dextrose Agar
AEZ	Agro- ecological Zone
BB	Bacterial Blight
LB	Luria Bartani
PDA	Potato Dextrose Agar
<i>X</i>	<i>Xanthomonas</i>
IAA	Indole Acetic Acid
DNA	Deoxyribonucleic acid
DAS	Days after sowing
BAU	Bangladesh Agricultural University
BRRRI	Bangladesh Rice Research Institute
BINA	Bangladesh Institute of Nuclear Agriculture
CRD	Completely Randomized Design
DAT	Days After Transplanting
t/ha	Ton per hectare
SA	Salicylic Acid
JA	Jasmonic Acid
RNA	Ribonucleic acid
PGPB	Plant Growth Promoting Bacteria
cDNA	Complementary DNA
rDNA	Ribosomal DNA
LSD	Least Significant Difference
DMRT	Duncan's Multiple Range Test
PCR	Polymerase Chain Reaction
HAI	Hours After Inoculation
ITS	Internal Transcribed Spacer
SES	Standard Evaluation System

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## Executive Summary

Rice plant infected by 32 diseases and ten are major in Bangladesh at present. Among the diseases three bacterial diseases are of frequent incidence. Among the three diseases, Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) considered as most destructive disease affecting in all Agro Ecological Zones (AEZ) and caused considerable yield loss. It is also an important disease in most of the South and Southeast Asian countries. But till now no effective chemical is available to control the disease. Apart from BB, rice blast become a severe problem in rice cultivation in Bangladesh and it causes upto 100% yield loss. Development of BB resistant variety may not be sustainable without blast resistance. In this circumstance this sub-project was undertaken by Plant Pathology Division, BRRI in collaboration with Department of Plant Pathology, BAU with a general objective, to manage bacterial blight disease through gene pyramiding and biological approaches. The objectives of BRRI component are to: i) Identify the known bacterial blight resistant genes in land races, ii) Identify physiological races across the country and iii) Develop BB resistant varieties coupled with blast resistance in the background of susceptible BRRI released high yielding varieties and the objectives of BAU component are to: i) Isolate and identify the endophytic beneficial fungi and bacteria from rice phylloplane and rhizosphere, ii) Assess the *in vitro* antipathogenic activity of some selected beneficial endophytic fungi and bacteria against BB pathogen, *Xoo*. iii) Formulate selected beneficial endophytic fungi & bacteria and iv) Evaluate the field efficacy of some formulated and non-formulated endophytic fungi and bacteria. To identify resistant genes, 928 rice germplasm (including checks) were screened against the virulent BB isolates by leaf clipping method. Out of 928 rice germplasm, a single entry was evaluated as highly resistant, 71 were resistant and 6 were moderately resistant and the rests were moderately to highly susceptible. The highly resistant to moderately resistant germplasm were evaluated through molecular approach using gene based molecular markers. Based on gene based molecular markers, 10 germplasm contained 4 resistant genes (*Xa4/xa5/Xa7/xa13/Xa21/Xa23*) in 5 different combinations, 15 germplasm contained 3 resistant genes (*Xa4/xa5/Xa7/xa13/Xa23*) in 5 different combinations, 22 germplasm contained 2 resistant genes (*Xa4/xa5/Xa7/xa13/Xa23*) in 7 different combinations and others had single or unknown resistant gene(s). A total of 920 BB infected leaf samples were collected from 40 districts covering almost all AEZs of Bangladesh. From these leaf samples, a total of 300 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates were isolated and purified. All the *Xoo* isolates were tested on 14 NILs (Near Isogenic Lines) of BB. According to the reaction patterns of 300 BB isolates on NILs, 13 races were identified from the reaction patterns of these isolates collected from across the country. Among the resistant genes, *Xa27*, *Xa21*, *xa13* and *Xa7* were effective against *Xanthomonas oryzae* pv. *oryzae* in Bangladesh condition. For the development of BB and blast resistant variety, a crossing program was undertaken using BRRI dhan81, BRRI dhan63, BRRI dhan49 as recipient parent and IRBB58, IRBB60, *Pi9*-[US], *Pb1*-[US] as donor parents. Backcrossed populations were developed upto BC<sub>3</sub>F<sub>2</sub> generation through marker assisted selection. Finally, 10 plants having *Xa21*, *xa13*, *Pb1* and *Pi9* genes in the background of BRRI dhan81, 5 plants containing *Xa21*, *Pb1* and *Pi9* gene in the background of BRRI dhan63 and 4 plants having *Xa21* gene in the background of BRRI dhan49 were selected using pathogenicity test and molecular markers. From these lines, 1-2 resistant varieties having BB and blast resistant genes will be released after the evaluation of agronomic and other performance. To develop environment- friendly sustainable management approach against BB of rice, a total of 63 plant growth promoting bacteria were

identified from rice phylloplane and rhizosphere during boro and aman seasons in 2018 and 2019 that inhibited the growth of *Xanthomonas oryzae* pv. *oryzae* by 20.83% to 76.19%. These bacterial isolates were identified by sequencing PCR products of 16S rDNA belonging to the genera mostly *Pseudomonas*, *Bacillus* and *Serratia*. Out of these bacterial species, 48 bacterial species were found as positive for IAA (Indole Acetic Acid) production, all 63 bacterial species were found positive for siderophore production and 48 were found capable to solubilize insoluble phosphate. Based on the growth inhibition of *X. oryzae* pv. *oryzae* *in vitro* 32 bacterial isolates were selected for plant growth promotion assessment and evaluation under net house and field efficacy. These bacterial species were formulated using talcum powder which remained viable for at least three months of post formulation. Assessment of plant growth promoting determinants revealed that all 32 bacterial isolates enhanced the growth of rice plants as measured by root and shoot length compared to untreated control. The evaluation of net house and field efficacy of the selected 16 bacterial species identified in boro seasons (2018 and 2019) showed 40.83% to 62.20% and 41.46% to 70.16% reduction of lesion length caused by *X. oryzae* pv. *oryzae*, respectively. However, a remarkable (13.33% to 29.94%) yield increase over control was observed by these bacterial species in the same seasons. On the other hand, the evaluation of net house and field efficacy of another 16 bacterial isolates identified in aman seasons (2018 and 2019) showed 31.65% to 69.99% and 47.44% to 60.38% reduction of lesion length caused by *X. oryzae* pv. *oryzae*, respectively. However, significant yield increases (12.25% to 27.03%) over control were observed by these bacterial species in these seasons. Four plant growth promoting antagonistic fungi were identified from rice rhizosphere in boro seasons (2018 and 2019). These fungal species inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* by 23.33% to 67.51%. These four fungal species were identified as BDISOF67R (*Trichoderma paraviridescens*), BDISOF91R (*Trichoderma erinaceuum*), BDISOF08R (*Trichoderma asperellum*) and BDISOF09R (*Trichoderma asperellum*) after sequencing of ITS regions. Growth promotion assessment of rice plants showed that all these *Trichoderma* species promoted the growth significantly compared to control. The evaluation of the performances of these four *Trichoderma* species revealed 45.77% to 61.76% reduction of lesion length caused by *X. oryzae* pv. *oryzae* under net house and 40.14% to 49.12% reduction of lesion length under field condition. However, significant (17.03% to 23.94%) yield increases over control were observed by these *Trichoderma* species in the same seasons. These results of gene expression study by reverse transcriptase (RT)-PCR primarily indicated that these PGPB and fungi induced the expression of some defense related SA and JA responsive genes and thus reduce BB severity. The quantification of Salicylic acid (SA) and Jasmonic acid (JA) in plants treated with these bacterial and fungal bioagents will be required to validate this mechanism. Therefore, patenting and commercialization of some selected formulated potential species of *Pseudomonas*, *Bacillus* and *Trichoderma* can be a step forward strategies for increasing rice yield through sustainable management of BB.

**Keywords:** Bacterial blight, Physiological race, Gene pyramiding, Resistant variety, Antagonistic bacteria, Antagonistic fungi

## **PBRG Sub-Project Completion Report (PCR)**

### **A. Sub-project Description**

- 1. Title of the PBRG sub-project:** Identification of novel resistant gene(s), gene pyramiding and sustainable management of bacterial blight (BB) disease of rice
- 2. Implementing organization (s):**
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#### 4. Sub-project budget (Tk.):

4.1 Total: (in Tk. as approved): 15438152 Taka

4.2 Latest Revised (if any): N/A

#### 5. Duration of the sub-project:

5.1 Start date (based on LoA signed): 14 February 2018

5.2 End date: 15 November 2021

#### 6. Background of the sub-project:

Rice plant infected by 32 diseases of which ten are major in Bangladesh (Latif *et al.*, 2007). Among the diseases three bacterial diseases are frequent in Bangladesh. Among the three diseases, Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) considered as most destructive disease occurring in all Agro Ecological Zones (AEZ) of Bangladesh and mostly in two rice growing seasons namely, Aman (June-July to November-December) and Boro (November-December to April-May) (Latif *et al.*,2011, Miah 1973, Miah *et al.*,1985) and caused considerable yield loss. It is also an important disease in most of the South and Southeast Asian countries (Sharma 1991).

The disease may incur over 50%, 60%, 30% and 57% rice yield reductions in Japan (Soga 1918), India (Srivastava *et al.*,1966), Bangladesh (Shahjahan 1993) and Pakistan (Khan *et al.*, 2015), respectively, in the severely diseased rice fields. *Xoo* is a chief causal agent limiting rice productivity worldwide because of its high epidemic potential (Khan *et al.*,2014, Verdier *et al.*,2012, Xia *et al.*,2012). Increased application of nitrogen fertilizer to high yielding varieties (HYV) favours occurrence and severity in the field (Kim and Cho 1970, Kauffman 1972, Chattopadhyay and Mukharjee 1973, Mohanty *et al.*,1983 and Devadath *et al.*,1987). The disease is also influenced by some climatic factors such as rainfall, humidity, temperature, flood and stormy weather (Soga 1918, Fujikawa *et al.*,1957 and Mizukami and Wakimoto 1969). Heavy rain, high humidity and temperature provide the favourable conditions for high incidence and the severity of the disease (OCTA 1970).

Pathogenic variability of *Xoo* in Bangladesh has been reported (Noda *et al.*,1996, Jalaluddin and Kashem 1999). Twelve races of the *Xoo* have been identified until 1995 in Bangladesh and the study indicated that some virulent strains of *Xoo* occur in Bangladesh (BRRI 2018). The variation of pathogenicity in *Xoo* and resistance genes in rice cultivars has been studied in Japan and at International Rice Research Institute (IRRI) (Noda *et al.*,1990 & 1996, Ogawa 1993, Yamamoto and Ogawa 1990 and Khush *et al.*,1990). Severe outbreak of BB was occurred in Bangladesh in Boro 2007-2008 and both hybrid and inbred varieties were affected. In current T. Aman 2017 Season, BB outbreak was noticed in different regions of Bangladesh and reported by most of the daily newspapers. Control of the disease with copper compounds, antibiotics, and other chemicals has not proven effective (Webster and Gunnell 1992). Regarding host resistance, it is unfortunate that resistance capacity of a particular disease resistant variety deteriorates or totally breaks down because of evolving of new virulent strain in few years. Understanding both pathogen population structure as well as host pathogen resistance is the prerequisite in designing an effective strategy for resistance deployment. Durable resistant varieties can help to minimize the resistance breakdown problem. Gene pyramiding is one of the ways to bred durable resistant variety (Ashkani *et al.*,2015). Breeding for durable resistance to BB requires recent information on the pathogen population and geographical distribution of the races.

Wild plant species are shown to be an important and rich genetic reservoir of resistance sources (Dangl *et al.*, 2013). Several elite BB-resistance genes such as *Xa21* (Song *et al.*, 1995), *Xa23* (Wang *et al.*, 2014) and *Xa27* (Gu *et al.*, 2005) were identified from wild rice species *Oryza longistaminata* A. Chev. & Roehr., *Oryza rufipogon* Griff. and *Oryza minuta* Presl., respectively. To date, at least 41 BB rice resistance genes have been identified, but only a few of them have been successfully deployed for resistance breeding (Zhang *et al.*, 2014, Kim *et al.*, 2015, Hutin *et al.*, 2015), among which *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* appear to be widely used in breeding programs in Bangladesh (Khan *et al.*, 2014). However, this approach is difficult through conventional breeding due to masking effects of genes such as *Xa21*, which provide resistance to many BB races. It is impossible to distinguish between plants having *Xa21* alone and those having *Xa21* and other genes through marker assisted selection (MAS). It allows the identification of plants with multiple resistance genes. Important prerequisites to the deployment of *R* genes are to: 1) have an intensive knowledge of *Xoo* population structure, race distribution and frequency, 2) determine the durability of resistance of *R* genes to be deployed. Most of the *R* genes for BB provide complete race-specific resistance to BB strains. Different combinations of *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* have been incorporated in popular rice commercial varieties in different countries in Asia (Century *et al.*, 1999, Singh *et al.*, 2001, Swamy *et al.*, 2006, Perez *et al.*, 2008, Sundaram *et al.*, 2009, Shanti 2010, Suh *et al.*, 2013, Ruengphayak *et al.*, 2015). Few examples indicated that some *R* genes used for controlling BB disease are overcome by virulent strains in Korea with the resistant gene *Xa21* (Lee *et al.*, 1999, Zhang *et al.*, 2006). Marker assisted selection (MAS) was applied for pyramiding three or four or five BB genes for BB resistance (i.e. *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23*). Pyramid lines IR 129336:11-4 or IR 129336:11-35(*Xa4-xa5-xa13-Xa21-Xa23*) or IRBB60 (*Xa4*, *Xa5*, *xa13* and *Xa21*) having four or five genes were also developed at IRRI. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Huang *et al.*, 1997). MAS is a very useful approach to maximize utilization of the existing gene resources. Genes controlling different agronomic traits can be quickly brought together in an existing variety. Furthermore, genes responsible for resistance to different races or biotypes of a disease or insect pest can be also pyramided together to make a line have multi-race or multi-biotype resistance. Theoretically, such resistances should provide more durability than single-race or single-biotype resistance. Pyramiding means the combining of resistant genes into a single genotype and develops durable resistant variety. Gene pyramiding has been successfully applied in several crop breeding programs and many varieties (rice, wheat, alfalfa) possessing multiple attributes have been developed (Huang *et al.*, 1997, Wang *et al.*, 2001, Samis *et al.*, 2002). Control measures for BB include cultural practices, chemical and biological control, disease forecasting and most importantly, host genetic resistance, typically major gene resistance. But cultural practices are not found effective in all locations and its efficacy mainly depends on disease incidence records. Chemical control of BB in the tropical monsoon climate of Asia is impractical, and no truly effective bactericide is commercially available for disease control (Lee *et al.*, 2003, Ou 1973). Although biological control is an environmentally friendly and cost-effective alternative to chemical control but biological agents have not seen widespread use in controlling BB. Biological control of BB using endophytic fungi and plant growth promoting rhizobacteria (PGPR) has emerged as an effective strategy during last two decades.

Endophytes can bring many effects on their host such as enhancement of stress-, insect- and disease-resistance (Bush *et al.*, 1997, Clay & Holah 1999) and productivity improvement (Quaroni *et al.*, 1997) when in association with their hosts. These facts indicate that endophytes can be potential biological control agents and will play an important role in ecological agriculture. Moreover, endophytic fungi from rice plants were reported to be effective *in vitro* against rice pathogens such as *Magnaporthe oryzae*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Xanthomonas oryzae* (Tian *et al.*, 2004)

Commercial applications of PGPR are being tested and are found promising (Gupta *et al.*, 2015). However, a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications. Some species of *Bacillus* suppress the pathogen inoculum at the infection site due to (1) antibiosis, (2) competition for space and nutrients, (3) inhibition of pathogen-related enzymes or toxins and (4) parasitism (Wang *et al.*, 2009).

Selected strains of beneficial PGPR trigger a plant-mediated ISR response that is effective against a broad spectrum of plant pathogens. Several studies exemplified the use of antagonistic bacteria as PGPR (Almoneafy *et al.*, 2014, Kakar *et al.*, 2014a, 2014b). Many reports have described either the use of PGPR for growth promotion or the antagonistic bacteria to control pathogens but rice PGPR that can be used both as biofertilizer as well as biopesticide is not available in Bangladesh. In this study, we designed a single “dual-purpose inoculum” based upon native endophytic fungi and antagonistic-PGPR that can promote rice growth on one hand and control *Xoo* attack on the other hand. This dual-purpose inoculum may serve as rice supplement for sustainable rice growth of the country.

BRRI released premium quality varieties BRRI dhan63 and BRRI dhan81 are become popular among the farmers but the varieties are highly susceptible to BB. Another, popular modern variety BRRI dhan49 in T. Aman season is highly susceptible to BB. The incidence and severity of BB is increasing day by day due to unfavorable environment (BRRI 2016). So, combination of BB resistant genes of *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* in the background of BRRI dhan63 or BRRI dhan81 or BRRI dhan49 would help much to reduce rice yield loss as well as improve livelihood of the resource poor.

In this study, BB resistant gene(s) will be identified from native germplasm. Physiological races of BB pathogens and its distribution throughout the country will be identified using the near isogenic lines. As well as bacterial blight and blast resistance genes will introgressed into the popular rice varieties BRRI dhan63, BRRI dhan81 and/or BRRI dhan49 having high yield potential through marker-assisted backcrossing. Simultaneously, environment friendly and sustainable management package will be developed against BB of rice.

#### **7. Sub-project general objective (s):**

Manage bacterial blight disease through gene pyramiding and biological approaches

#### **8. Sub-project specific objectives (component wise):**

##### **BRRI component:**

- i. Identify the novel/known bacterial blight resistant genes in land race
- ii. Identify physiological races across the country and
- iii. Develop BB resistant varieties along with blast resistant gene in the background of susceptible BRRI released high yielding varieties.

**BAU component:**

- i. Isolate and identify the endophytic beneficial fungi and bacteria from rice phylloplane and rhizosphere.
- ii. Assess the *in vitro* antipathogenic activity of beneficial endophytic fungi and bacteria against bacterial blight pathogen.
- iii. Formulate selected beneficial endophytic fungi and bacteria against BB pathogen.
- iv. Evaluate the field efficacy of some formulated and some non-formulated endophytic fungi and bacteria against BB disease of rice.

**9. Implementing location (s):**

Plant Pathology Division, BRRI, Gazipur and Department of Plant Pathology, BAU, Mymensingh-2202.

**10. Methodology:****A) BRRI component****Identification of known/novel BB resistant genes**

**Screening of land races and cultivars:** In total 928 rice germplasm including land races and cultivars were collected from the Genetic Resources and Seed Division, BRRI. These materials along with two susceptible checks (Purbachi and IR24) and resistant check (IRBB60) were screened against bacterial blight was conducted during T. Aman and Boro seasons. Artificial inoculation was carried out using most virulent and widely distributed three representative bacterial blight isolates (*Bxo67*, *Bxo87*, *Bxo91*) of major races of Bangladesh. Bacterial blight isolates were transferred to PSA plates from preserved cultures and incubated at 28°C for 48 h. A two days old culture of each isolate was used for to inoculum preparation. Inoculum was prepared by suspending the bacterial cells with distilled water and adjusting to a concentration of 10<sup>8</sup> CFU/ml before inoculation. Artificial inoculation was done at maximum tillering stage of the plants by leaf clipping method and at least five to ten leaves were beibg inoculated. Data of mean lesion length were collected at 14 days after inoculation. Disease reactions of the genotypes were evaluated based on lesion length following Standard Evaluation System, where <0.5 cm was considered as highly resistant (HR), 0.5-3.0 cm was considered as resistant(R), 3.0-5.0 cm was considered as moderately resistant (MR), 5.0-10.0 cm was considered as moderately susceptible (MS), 10.0-15.0 cm was considered as susceptible (S), and >15.0 cm was rated as highly susceptible (HS) (IRRI 2013; Kim *et al.*, 2015).

**Molecular screening of land races and cultivars:** Resistant germplasm found after screening were selected for molecular screening. Gene-based markers were used to explore the known bacterial blight resistant genes in the resistant germplasms. Based on phenotyping and molecular screening presence of known resistant genes were confirmed in the land races.

**Genomic DNA purification**

The leaves of selected germplasm were collected from 21 days old seedlings. The modified CTAB method was used in the DNA extraction. Firstly, about 150 mg of young leaves along with 300 µl 2X CTAB buffer [2% (w/v) CTAB, 20 mM EDTA pH 8.0, 100mM Tris-HCL pH 8.0, 2% (w/v) PVP, 1.4 mM NaCl] were grinded into a micro centrifuge tube (2.0 ml) using Geno grinder (Retsch MM400). 300µl mixture of chloroform: isoamyl alcohol: phenol

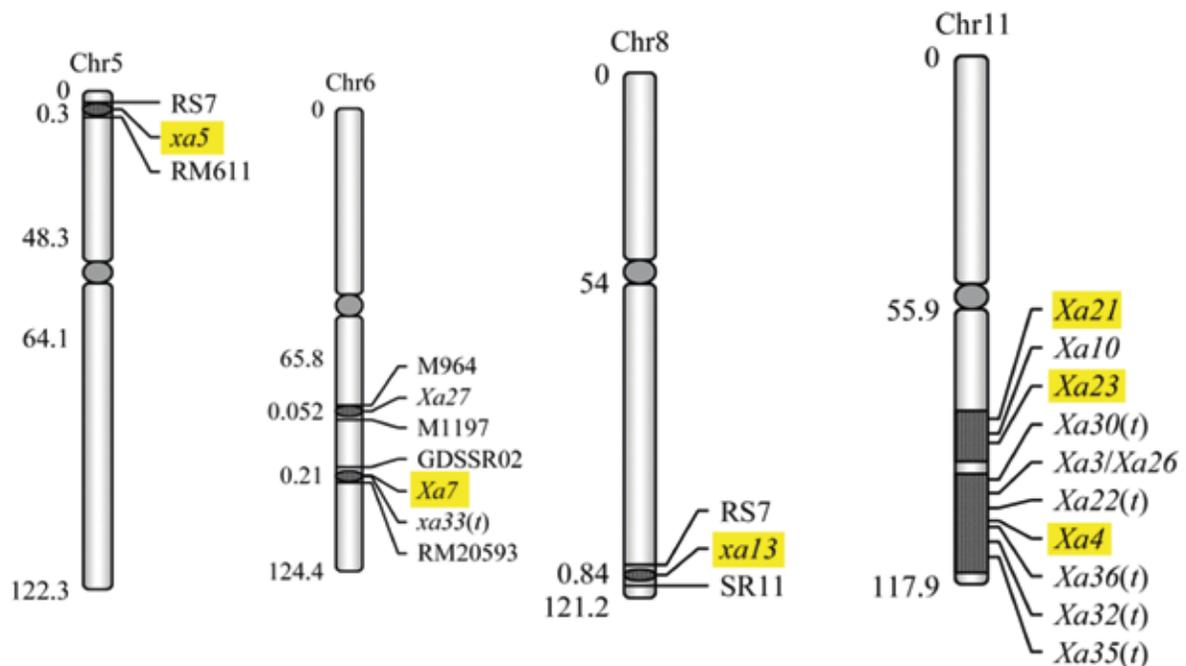
(25:24:1) was added and was centrifuged the mixtures at 13000rpm for 10 minutes. After that 200µl supernatant was transferred into a micro centrifuge tube (1.5ml) and was added 200µl ice cooled isopropanol. The solution was centrifuged at 13,000 rpm for 10 minutes which was preceded by invert shaking and incubated at room temperature (25°C) for 10 minutes. DNAs were precipitated at the lower portion of the tube, which was cleaned with 700µl of 70% ethanol. Alcohol was removed carefully and dried the DNA pellet. Finally, 50µl TE buffer (1 mM EDTA pH 8.0, 10 mM Tris-HCL pH 8.0) with 1µl RNase was added and incubated at 37°C for 1 hour to remove the RNA and to dissolve the DNA.

### PCR amplification and gel documentation

A total of six gene base markers were used in this study (Table 1) to explore the presence of known resistant genes in the resistant germplasm. The PCR mixture included 1µl of 60ng DNA template, 7.4µl PCR master mix (Promega, USA), 1µl primer and 5.6µl nuclease-free water for making 15µl PCR reactions. PCR reaction was done following program- primary denaturation at 95°C for 3 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 45 sec and the elongation at 72°C for 60 seconds. Gel electrophoresis was completed through agarose (1.5%) gel and polyacrylamide gel electrophoresis technique (8%). Gel image was taken after staining the gel with ethidium bromide for 20 minutes followed by distilled water for several times and visualized by gel documentation unit. Size and positions of the six bacterial blight resistant genes in different rice chromosome is demonstrated (Figure 1).

**Table 1:** Gene based markers for the detection of BB resistant genes

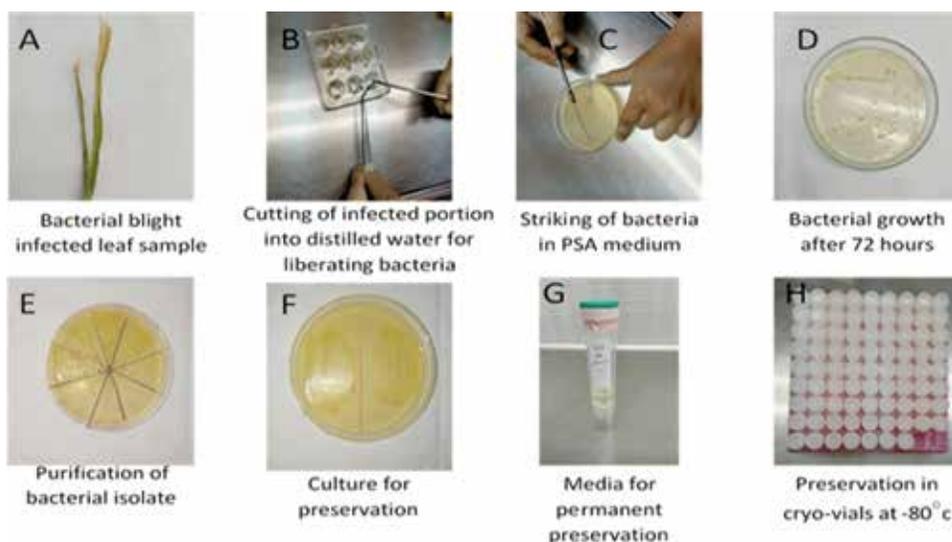
Gene	Marker	Sequence
<i>Xa4</i>	MP1	ATCGATCGATCTTCACGAGG
	MP2	TCGTATAAAAAGGCATTCGGG
<i>xa5</i>	xa5-pro S-F	GTCTGGAATTTGCTCGCGTTCG
	xa5-pro S-R	TGGTAAAGTAGATACCTTATCAAACCTGGA
	xa5-pro R-F	AGCTCGCCATTCAAGTTCTTGAG
	xa5-pro R-R	TGACTTGGTTCTCCAAGGCTT
<i>Xa7</i>	Xa7-STS F	CTGGATACGGAACCTTCTAAC
	Xa7-STS R	AGAGAACCTTCTCCTTCAGTG
<i>xa13</i>	xa13-prom F	GGCCATGGCTCAGTGTTTAT
	xa13-prom R	GAGCTCCAGCTCTCCAAATG
<i>Xa21</i>	pTA248 F	AGCCGCGGAAGGGTGGTTCCCGGA
	pTA248 R	AGACGCGGTAATCGAAAGATGAAA
<i>Xa23</i>	Xa23-STS F	CTCGGTTTCCGTCTTCTCAG
	Xa23-STS R	GACTTTGCGTGCTTTCAGC



**Figure 1.** Approximate size and positions of the six bacterial blight resistant genes (yellow highlighted) in different rice chromosome

**Identification of physiological races of bacterial blight and its distribution patterns**

**Collection, isolation and purification of bacterial blight isolates:** In total, 920 bacterial blight infected leaf samples were collected from 40 districts covering almost all AEZs of Bangladesh. The collected leaf samples were preserved in refrigerator. Then *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were isolated from the infected leaf samples following the procedure described in the Figure 2. A total of 300 *Xoo* isolates were isolated from the diseased samples on peptone sucrose agar (PSA) medium. The bacterial blight isolates were permanently preserved in 40 % Glycerin NBY (Nutrient Broth Yeast) medium for further use (Figure 2).



**Figure 2:** Activities of isolation, purification and preservation of bacterial blight isolates (A-H)

**Inoculum preparation and inoculation:** Preserved bacterial blight isolates were transferred to PSA plates and incubated at 28°C for 48 h. A two days old culture of each isolate was used to prepare inoculum preparation. Inoculum was prepared by suspending the bacterial cells with water at a concentration of 10<sup>8</sup> CFU/ml prior to inoculation. Differential varieties (Near Isogenic Lines of IR24 harboring 14 bacterial blight resistant genes individually) were transplanted in field with three replications. Artificial inoculation using 300 bacterial blight isolates were conducted at maximum tillering stage of NILs along with susceptible and resistant checks following leaf clipping method (Kaufman *et al.*, 1973 and Khan *et al.*, 2010).

**Disease assessment and identification of races/pathotypes:** Identification of the races were recognized based on disease reaction of different *Xoo* isolates to near isogenic lines (harboring single bacterial blight resistant gene) in artificial inoculation condition. A total of 14 near isogenic lines is used in this study (Table 2) developed by IRRI. Identification of the race(s) was recognized based on disease reaction to differential varieties following gene for gene theory. The percentage of diseased leaf areas from the cut leaf tip was measured at 14 days after inoculation. Disease reactions were categorized according to percent (%) diseased leaf areas. The percentages of diseased leaf areas were classified into 1 to 9 scales (IRRI, 2015).

**Table 2:** List of near isogenic lines (NILs) used in disease assessment and identification of races

Sl. No.	Near isogenic Lines (NILs)	Harboring BB resistant gene	Source
1	IRBB1	<i>Xa1</i>	IRRI
2	IRBB2	<i>Xa2</i>	IRRI
3	IRBB3	<i>Xa3</i>	IRRI
4	IRBB4	<i>Xa4</i>	IRRI
5	IRBB5	<i>xa5</i>	IRRI
6	IRBB7	<i>Xa7</i>	IRRI
7	IRBB8	<i>Xa8</i>	IRRI
8	IRBB10	<i>Xa10</i>	IRRI
9	IRBB11	<i>Xa11</i>	IRRI
10	IRBB13	<i>xa13</i>	IRRI
11	IRBB14	<i>Xa14</i>	IRRI
12	IRBB21	<i>Xa21</i>	IRRI
13	IRBB23	<i>Xa23</i>	IRRI
14	IRBB27	<i>Xa27</i>	IRRI

### Gene pyramiding for the development of BB and blast resistant varieties

Backcross breeding followed by marker assisted selection were followed for introgressing resistant gene(s) into highly susceptible Boro and Aman varieties, BRRI dhan63, BRRI dhan81 and BRRI dhan49 by crossing with highly resistant IRRI developed pyramid lines IRBB58 (*Xa4*, *xa5* and *Xa21*) and/or IRBB60 (*Xa4*, *xa5*, *xa13* and *Xa21*) through marker assisted selection (MAS). As the test boro varieties were susceptible to blast, so in accordance with the suggestion of the inception workshop blast resistant parents i.e., *Pi9*-[US], *Pb1*-[US] were included in the gene pyramiding programme. These blast resistant parents were crossed and inter-crossed with the previously mentioned crossing lines for the bridging of bacterial blight and blast resistant genes in the background of popular BRRI varieties.

**Polymorphism studies of foreground primer between parents:** The DNA of two parents (donor and recipient) were extracted following modified CTAB method. 3 (Three) polymorphic primers linked with bacterial blight and 2 (Two) polymorphic primers linked with blast resistant genes were selected for foreground selection (Table 3). The PCR mixture included 1µl of 60ng DNA template, 7.4µl PCR master mix (Promega, USA), 1µl primer and 5.6µl nuclease-free water for making 15µl PCR reactions. PCR reaction was done following program- primary denaturation at 95°C for 3 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 45 sec and the elongation at 72°C for 60 seconds. Gel electrophoresis was completed through agarose (1.5%) gel and polyacrylamide gel electrophoresis technique (8%). Gel image was taken after staining the gel with ethidium bromide for 20 minutes followed by distilled water for several times and visualized by gel documentation unit.

**Table 3:** List of polymorphic primers along with respective genes

Gene	Marker	Sequence
<i>xa5</i>	xa5-pro S-F	GTCTGGAATTTGCTCGCGTTCG
	xa5-pro S-R	TGGTAAAGTAGATACCTTATCAAAGTGG
	xa5-pro R-F	AGCTCGCCATTCAAGTTCTTGAG
	xa5-pro R-R	TGACTTGGTTCTCCAAGGCTT
<i>xa13</i>	xa13-prom F	GGCCATGGCTCAGTGTTTAT
	xa13-prom R	GAGCTCCAGCTCTCCAAATG
<i>Xa21</i>	pTA248 F	AGCCGCGGAAGGGTGGTTCCCGGA
	pTA248 R	AGACGCGGTAATCGAAAGATGAAA
<i>Pi9</i>	NmSM-Pi9 F	ATGGTCCTTTATCTTTATTG
	NmSM-Pi9 R	TTGCTCCATCTCCTCTGTT
<i>Pb1</i>	RM206 F	CCCATGCGTTTAACTATTCT
	RM 206 R	CGTTCCATCGATCCGTATGG

**Hybridization for bacterial blight and blast resistance:**

Three sets of parental line (BRRRI dhan81, BRRRI dhan49, BRRRI dhan63 and IRBB58, IRBB60, *Pi9*-[US], *Pbl*-[US]) were planted at 7 days interval for synchronization of flowering. Crosses were made between the recipient parent and donor parent to produce  $F_1$  population.

**Growing and confirmation of  $F_1$  population and production of  $BC_1F_1$ :** Three sets of parental line *viz.* BRRRI dhan81, BRRRI dhan49, BRRRI dhan63 and IRBB58, IRBB60, *Pi9*-[US], *Pbl*-[US] were planted along with  $F_1$  seeds. The leaf samples from the  $F_1$  plants were collected and after confirmed the heterozygous  $F_1$  plants using gene based molecular markers (Table 3). The selected  $F_1$  plants were backcrossed with the parental lines to generate  $BC_1F_1$  population.

**Growing and confirmation of  $BC_1F_1$  and production of  $BC_2F_1$  seeds:** One set of  $BC_1F_1$  population and three sets of parents *viz.* BRRRI dhan81, BRRRI dhan49, BRRRI dhan63 and IRBB58, IRBB60, *Pi9*-[US], *Pbl*-[US] were planted at 7 days interval for synchronization of flowering. After confirmation of the heterozygous plants of  $BC_1F_1$  using molecular markers (Table 3), the selected  $BC_1F_1$  plants were then backcrossed with the parents to generate  $BC_2F_1$  population.

**Intercrossing between  $BC_1F_1$  population harbouring bacterial blight and blast resistant genes:** Intercrosses were made between the  $BC_1F_1$  plants having bacterial blight and blast resistant genes in the background of BRRRI dhan63 and BRRRI dhan81 to produce  $BC_2F_1$  population.

**Growing and confirmation of  $BC_2F_1$  and production of  $BC_3F_1$  seeds by back crossing:** One set of  $BC_2F_1$  population and three sets of parental line *viz.* BRRRI dhan81, BRRRI dhan49, BRRRI dhan63 and IRBB58, IRBB60, *Pi9*-[US], *Pbl*-[US] were planted at different time of planting for synchronization of flowering in hybridization programme. After confirmation of the heterozygous plants of  $BC_2F_1$  generation using molecular markers (Table 3). The selected  $BC_2F_1$  plants were backcrossed with the parental lines to produce  $BC_3F_1$  population having bacterial blight and blast resistant genes.

**Growing of  $BC_2F_1$  &  $BC_3F_1$  and production of  $BC_2F_2$  &  $BC_3F_2$  seeds by selfing:** Seeds of  $BC_3F_1$  and  $BC_2F_1$  population produced in previous season were planted and heterozygous plants were selected through marker assisted selection. The selected plants were undergone for selfing to produce  $BC_3F_2$  and  $BC_2F_2$  generations.

**Pathological and molecular screening of the progenies:** Backcross populations of  $BC_3F_2$  and  $BC_2F_2$  generations from different cross combinations of BRRRI dhan63, BRRRI dhan81, BRRRI dhan49 with IRBB58, IRBB60, *Pi9*-[US], *Pbl*-[US] were screened against virulent bacterial isolates (*Bxo67*, *Bxo87*, *Bxo91*). The plants showing resistant reaction were selected for molecular screening to ensure the presence of resistant genes in the progenies using gene based tightly linked molecular markers (Table 3).

**Statistical analyses:** Data were analyzed using Alphaease and NTSYS software for the identification of genes and races. Different agronomic and yield attributing characters were analyzed using CropStat software.

## B) BAU component

### Isolation and identification of fungi and bacteria from rice phylloplane and rhizosphere antagonistic to BB pathogen

**Plant sample collection:** The healthy rice plants with root system with soils of different rice cultivars were collected from 40 districts representing 30 Agro ecological Zones (AEZs) of Bangladesh from the vicinity of BB infected rice plants during boro and aman season, 2018 and 2019 at maximum tillering stage to pre-ripening stage and brought into the laboratory in labeled polybags.

**Isolation and purification of beneficial fungi:** For isolation of beneficial fungi both from phylloplane and rhizosphere, 100µl of serially diluted phylloplane (leaf and shoot) and 100µl of rhizosphere soil solution were spread on PDA plates containing 100µg ampicillin separately. Ampicillin was added to PDA medium to suppress bacterial growth. These plates were then incubated at 25°C for 5-10 days. Hyphal tips of the developing fungal colonies were then transferred onto PDA for purification. After purifying the isolates were spread on PDA medium at 4°C refrigerator for subsequent studies.

**Determination of antagonistic activity of fungi against *X. oryzae* pv. *oryzae* by dual culture method:** The antagonistic activity of the purified fungal isolates against BB pathogen *X. oryzae* pv. *oryzae* was performed following dual culture method (Tian *et al.* 2004). In this method, *X. oryzae* pv. *oryzae* cells were suspended in sterile water up to a cell density at 10<sup>8</sup> CFU/ml in this method (~ optical density: 0.3). Then 8 mm mycelial blocks of the fungi were placed on NBY medium inoculated with 100 µl *X. oryzae* pv. *oryzae* cell suspension by spreading with a cotton swab. Then the plates were incubated until 7 days post inoculation at 28°C. Then the growth inhibition of *X. oryzae* pv. *oryzae* by the fungi were measured in mm as indicated by the presence of clear inhibition zones around fungal mycelial block placed into medium. The percent growth inhibition of BB pathogen *X. oryzae* pv. *oryzae* by fungal isolates was calculated as follows:

**Growth inhibition (%)** = [Total diameter (Colony diameter + clear halo zones) - Colony diameter] x 100/Total diameter

**Isolation and purification of beneficial bacterial spp.:** The phylloplane bacteria were isolated using washing method. Freshly harvested 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> leaves were vortexed in sterile saline solution for 12 minutes with two or three brief intervals. Then 100µl solution was placed at the center of both Luria Bertani (LB) and King's B agar plate and the solution was spread with glass spreader. The inoculated plates were incubated for 3-5 days at room temperature. After incubation of the inoculated plates, bacterial colonies appeared with various types of colors. Then the bacterial colonies were selected and isolated depending on their color and were streaked on LB media separately. Again the streaked LB plates were incubated at room temperature for 2 days. For isolation of antagonistic bacteria from rhizosphere, 1 g roots with rhizospheric soils were taken and then it was shaken with 100 ml sterile water for about 10-15 min to obtain soil suspension. Isolation of bacteria and fungi were carried out from rhizospheric soil by serial dilution technique up to 10<sup>-5</sup> to 10<sup>-6</sup> using LB (Luria Bertani) medium. Then 20-30 solution was placed at the center of Luria Bertani (LB) or King's B agar plate and the solution was spread with glass spreader. The inoculated plates

were incubated for 3-5 days at room temperature. After incubation of the inoculated plates, bacterial colonies appeared with various types of colors. Then the bacterial colonies were selected and isolated depending on their color and were streaked on LB media separately. Again the streaked LB plates were incubated at room temperature for two days.

**Assay of antagonism of bacterial spp. to *X. oryzae* pv. *oryzae* by dual culture method:**

Antimicrobial activity of antagonistic strains of fluorescent pseudomonads/*Pseudomonas* spp./*Bacillus* spp. were determined by agar diffusion technique method (Monteiro *et al.* 2005) with some modifications. Antagonistic bacterial suspension was spot inoculated (5 µl of 10<sup>8</sup> CFU/ml) at three places on the NBY plates that were prior inoculated with a *X. oryzae* pv. *oryzae* cell suspension (10<sup>8</sup> CFU/ml ~ optical density: 0.3). The plates were incubated for 7 days post inoculation at 28°C. Then *X. oryzae* pv. *oryzae* growth inhibition by the antagonistic bacterial isolates indicated by clear halo zones measured with a ruler in mm. The percent growth inhibition of *X. oryzae* pv. *oryzae* by bacterial isolates was calculated as follows:

**Growth inhibition (%)** = [Total diameter (Colony diameter + clear halo zones) - Colony diameter] x100/Total diameter

**Assessment of plant growth promoting determinants of bacteria antagonistic to *X. oryzae* pv. *oryzae***

Active isolates with antagonistic potentials against *X. oryzae* pv. *oryzae* were further evaluated for their ability to produce plant growth promoting determinants *viz.* siderophore production, Indole acetic acid (IAA) production and phosphate solubilization as follows:

**Assay for siderophore production:** Siderophore productions by antagonistic bacterial isolates were tested qualitatively as described by Alexander and Zuberer (1991). 5 µl of antagonistic bacterial cell suspension (5 × 10<sup>8</sup> CFU mL<sup>-1</sup>) was spot inoculated on Chrome azurol S (CAS) agar plate. The plates were then incubated at 30°C for 5 days. Development of yellow-orange halo zone around the bacterial growth was considered as positive for siderophore production. Experiment was performed with a completely randomized design with 3 replications. CAS agar was prepared from 4 solutions. Solution 1 (Fe-CAS indicator solution) was prepared by mixing 10 mL of 1 mmol L<sup>-1</sup> FeCl<sub>3</sub> .6H<sub>2</sub>O (in 10 mmol L<sup>-1</sup> HCl) with 50 mL of an aqueous solution of CAS (1.21 g L<sup>-1</sup>). The resulting dark purple mixture was added slowly with constant stirring to 40 mL of aqueous solution of hexadecyl trimethyl ammonium bromide (1.821 g L<sup>-1</sup>). The yielded of dark blue solution which was autoclaved, then cooled to 50°C. The entire reagent was freshly prepared for each batch CAS agar. Solution 2 (buffer solution) was prepared by dissolving 30.24 g of piperazine-N, N-bis (2-ethane sulfonic acid) (PIPES) in 750 mL of salt solution containing 0.3 g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g NaCl and 1.0 g NH<sub>4</sub>Cl. The pH was adjusted to 6.8 with 50% (w/v) KOH, and water was added to bring the volume 800 mL. The solution was autoclaved after adding 15 g of agar then cooled to 50°C. Solution 3 contained 2 g glucose, 2 g mannitol, 493 mg MgSO<sub>4</sub>.7H<sub>2</sub>O, 11 mg CaCl<sub>2</sub>, 1.17 mg MnSO<sub>4</sub>.2H<sub>2</sub>O, 1.4 mg H<sub>3</sub>BO<sub>3</sub>, 0.04 mg CuSO<sub>4</sub>.5H<sub>2</sub>O, 1.2 mg ZnSO<sub>4</sub>.7H<sub>2</sub>O, 1.0 mg NaMoO<sub>4</sub>.2H<sub>2</sub>O in 70 mL water, autoclaved, cooled to 50°C. Solution 4 was 30 mL filter sterilized 10% (w/v) casamino acid. Finally, solution 3 added to solution 2 along with solution 4, solution 1 was added last, with sufficient water.

**Assay for Indole acetic acid (IAA) production:** IAA production of antagonistic bacterial isolates was carried out following Patten and Glick (1996). Every isolate was grown in LB media supplemented with (0.005%) L-tryptophan and incubated in shaker at 30°C with 160 rpm for 48 h. Then bacterial culture was centrifuged at 8000 rpm for 15 min and 1 mL culture filtrate was mixed with 4 mL salkowski's reagent (1.5 mL FeCl<sub>3</sub>.6H<sub>2</sub>O 0.5M solution in 80 mL 60% H<sub>2</sub>SO<sub>4</sub>) and the mixture was incubated at room temperature for 30 min, presence of pink color indicate qualitatively that isolate produced IAA. Formation of pink color indicated the presence of indoles (Gordon and Weber, 1951).

**Phosphate solubilization assay by antagonistic bacterial isolates:** Phosphate solubilization was determined according to the method of *Azman et al.* (2017). Sterile filter papers (5.0 mm) were soaked in antagonistic bacterial cell suspension ( $5 \times 10^8$  CFU mL<sup>-1</sup>) was dispensed using pipette onto sterile filter paper (6.0 mm) that was placed on National Botanical Research Institute's phosphate (NBRIP) agar plate (Glucose (10 g L<sup>-1</sup>), Ca<sub>3</sub> (PO<sub>4</sub>)<sub>2</sub> (5 g L<sup>-1</sup>), MgCl<sub>2</sub>.6H<sub>2</sub>O (5 g L<sup>-1</sup>), MgSO<sub>4</sub>.H<sub>2</sub>O (0.25 g L<sup>-1</sup>), KCl (0.2 g L<sup>-1</sup>), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.1 g L<sup>-1</sup>), Bacteriological Agar (15 g L<sup>-1</sup>) (Nautiyal, 1999). The plates were then incubated at 28°C for 7 days. Phosphate solubilization was assessed by observing the clear halo zone. The experiment was performed with a completely randomized design (CRD) with three replications.

#### **Identification of selected plant growth promoting antagonistic bacterial isolates by sequence analyses of 16S rDNA**

**Extraction of genomic DNA:** Bacterial culture from NA media was transferred in LB broth and shaken for 18 h at 28°C. Then genomic DNA of antagonistic bacteria was extracted according to Wizard<sup>®</sup> Genomic DNA purification Kit (Promega, Madison, USA). Obtaining the DNA pellet was rehydrated by adding 25µL DNA rehydration solution and kept it overnight at 4°C. Finally, the genomic DNA samples of the isolates were preserved at -20°C for further use.

**Primers and PCR conditions:** To identify the antagonistic bacterial isolates, the primer sets 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1518R (5'-AAG GAG GTG ATC CAN CCR CA-3') were used for 16S rDNA amplification from the prepared genomic DNA template (Gio-vannoni, 1991). The PCR condition was as follows: initial denaturation at 95°C for 5 min, 35 cycles denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and finally a 7 min extension at 72°C. PCR products were visualized by electrophoresis on 1.0% agarose gel containing 0.5% of ethidium bromide using a Gel Documentation System after separating the PCR products in the agarose gel for 50 min at 80 volts.

**Sequencing of PCR products:** A partial nucleotide sequencing of 16Sr DNA was carried out from amplified PCR products using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1518R (5'-AAG GAG GTG ATC CAN CCR CA-3') in the Macrogen Lab, South Korea via Biotech Concern Bangladesh. The sequencing was done directly from PCR products in both orientations according to the standard protocols for the ABI 3730xl DNA genetic analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye<sup>®</sup> Terminator v1.1 and 3.1 Cycle Sequencing Kits.

### **Identification of antagonistic fungal isolates by sequencing of ITS region**

**Extraction of genomic DNA from antagonistic fungal isolates:** Fungal isolates were grown on Potato Dextrose Agar (PDA) for 10 days. A 5mm fungal culture block was taken from a 10 days old culture for each fungal isolate in 100 ml potato dextrose (PD) broth in a conical flask. The flasks were kept at 25°C for 7 days. Then the mycelium was harvested from each flask separately for each isolate. Then 100 mg fungal tissues were taken for each isolate and grind with liquid nitrogen. Genomic DNA of the fungal isolates were extracted by using wizard® genomic DNA purification kit (Promega, Madison, WI, USA). Finally, the all isolated genomic DNA samples were stored at -20°C in deep freeze for further use.

**Primers and PCR:** After isolation, fungal species were identified as described by White *et al.*, 1990 using universal primers ITS1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') specific to internal transcribed spacer (ITS) regions of rDNA from all fungal isolates. PCR reactions were carried out Hotstart Master Mix (Promega, USA) with genomic DNA as template. PCR conditions were: initial denaturation at 95°C for 5 min, 35 cycles at denaturation at 95°C for 30 secs, annealing at 55°C for 1 min and extension 72°C for 1 min. The final extension was 72°C for 6 min. PCR products were visualized in 1.5% agarose gel containing ethidium bromide using a Gel Documentation System after separating the PCR products in the agarose gel for 50 min at 80 volts.

**Sequencing of PCR products:** Sequencing of the PCR products amplified with forward ITS1(5'-TCCGTAGGTGAACCTGCGG-3') primer. For sequencing, PCR products were purified using SV Total Clean up System (Promega, Madison, USA). Sequencing reactions was performed using ABI 3730xl DNA genetic analyzer at Macrogen, Korea, Malaysia via. Biotech Concern, Dhaka as mentioned above.

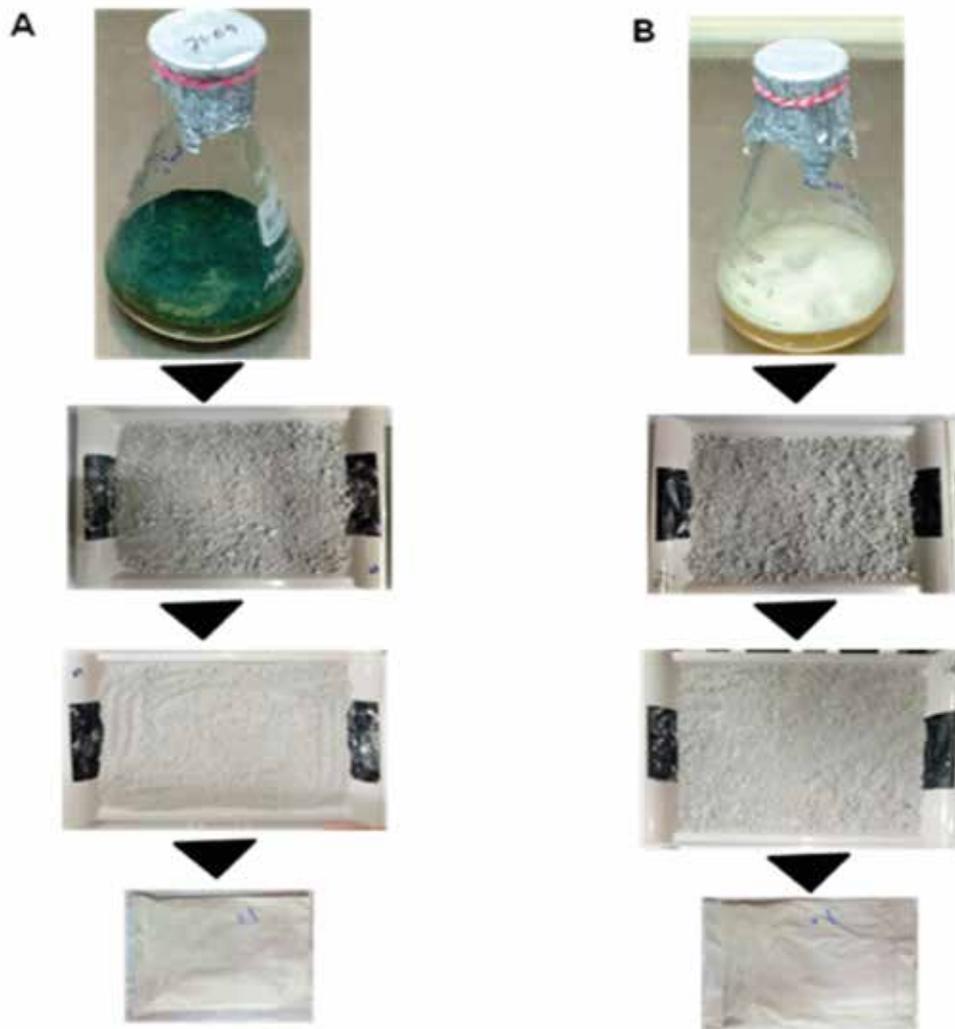
**Processing of sequence data:** The sequencing data were processed and nucleotide sequence data was exported using Chromas software version 2.6.4. The quality of nucleic acid sequences was evaluated using Chromas (Version 2.6) software to avoid the use of low-quality bases.

**Analyses of nucleotide sequences:** The nucleotide sequences were analyzed using online bioinformatics tools. The DNA sequences of 16S rDNA of the bacterial isolates were compared with 16S rDNA of the bacterial spp. and the sequences of ITS region of the fungal isolates were compared with ITS region of the fungal spp. that were available in the NCBI database using Basic Local Alignment Search Tool (BLAST) algorithm to identify closely related sequences (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### **Formulation of some selected plant growth promoting antagonistic fungi and bacteria against, *X. oryzae* pv. *oryzae***

**Multiplication and formulation of fungal bioagents:** Based on the performance in enhancing plant growth and inhibiting the growth of *X. oryzae* pv. *oryzae* under *in vitro* condition, four fungal isolates viz. BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) were selected for formulation development and subsequent

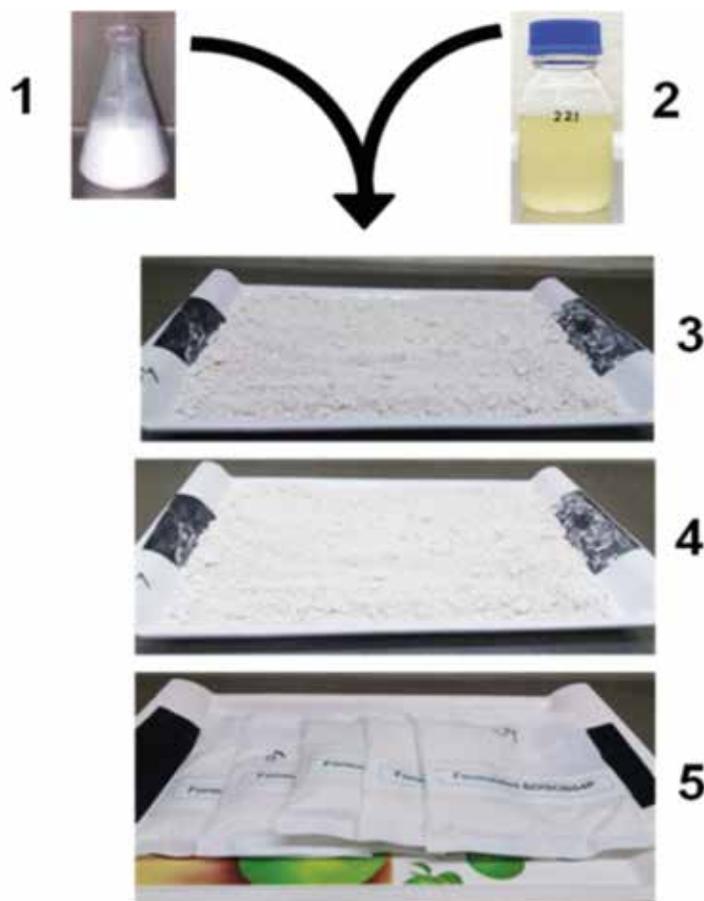
evaluation. A mycelia disc (6 mm diameter) of each fungal isolate was inoculated in 100 ml Potato Dextrose Broth (PDB) broth. Conidia production was counted after 7-10 days and the mycelial mat along with conidia from PDB were mixed thoroughly with autoclaved talcum powder pre-treated with 0.5 % carboxy methyl cellulose (CMC) and additional 100 ml pure PDB. The mixtures were then air-dried over night. The formulations were then powdered with hand, the formulated fungal antagonists were packed in plastic bags. The formulated fungal antagonists were then kept at both room and 4-8°C temperature.



**Figure 3.** Formulation of BDISOF67R (*Trichoderma paraviridescens*) (A) and BDISOF91R (*Trichoderma erinaceum*) (B)

**Formulation of plant growth promoting antagonistic bacterial spp.:** The pure cultures of thirty-two selected potential bacterial antagonists were grown on LB agar medium for 24 hrs. Then the bacterial isolates were transferred in LB broth for about six hours by taking a loopful of bacteria from the LB agar plate. After that the liquid culture were then centrifuged and resuspended the pellet in previously prepared 200 ml peptone broth aimed to fortify the carrier materials. This culture broth was then grown for another two hours with shaking. After that 5

ml of sterile 100% glycerol was added to this 200 ml culture. Then the cultures of the bacterial antagonists (200ml fortified with 1% peptone and 1% glycerol) was added to the mixture of 500g talcum powder amended with 5g carboxy methyl cellulose (CMC) and 7.5g Calcium carbonate which were autoclaved for two consecutive days at 121°C under 15PSI pressure for 30 min each. The formulations were then being dried overnight in the tray. After that the formulations were powdered with hand, the formulated bacterial antagonists were packed in plastic bags. The formulated bacterial antagonists were then kept at both room and 4-8°C temperature in the refrigerator.



**Figure 4** Formulation of some selected potential antagonistic bacterial isolates. **1:** Autoclaved talc powder with CMC and  $\text{CaCO}_3$ , **2:** Bacterial culture in Peptone broth, **3:** Mixture Autoclaved talc powder with CMC and  $\text{CaCO}_3$ , **4:** Air dried formulation of the bacterial antagonists and **5:** Formulated Packets of the bacterial antagonists.

**Assessment of viability of the formulated fungal and bacterial antagonists:** The viability of the bacterial and fungal antagonists was checked by drawing 1g of the formulated products in sterile water in every 30 days after formulation and diluted serially up to  $10^{-4}$  or  $10^{-5}$ . The numbers of viable cells (colony forming unit) were counted per gram formulations stored in both room temperature ( $25^\circ\text{C}$ ) and  $4-8^\circ\text{C}$  temperature in the refrigerator.

## Assessment of plant growth promotion induced by antagonistic bacterial and fungal isolates

Rice seeds (cv. IR24) were surface sterilized, dried the sterilized rice seeds were treated with formulated bacterial and fungal antagonists (10g/kg seeds) and the treated seeds were left for 1h under shade. The rice seeds were then sown in the plastic pots previously filled with sterile soils. Fifty seeds were sown in each pot and three replications were maintained. Then the germination of seeds was recorded at 7 DAS. The seedlings were uprooted at 7 DAS, 14 DAS and 28 DAS to measure the root length, shoot length and to calculate the vigor index = [(root length + shoot length) × germination percentage] were measured.

## Evaluation of the efficacy of some formulated plant growth promoting antagonistic bacteria and fungi against BB disease of rice under net house and field condition

**Experimental period:** Both field and net house experiments were conducted in two boro (2018-2019 and 2019-2020) and two aman (2019 and 2020) seasons in Farm Management Section, Bangladesh Agricultural University (BAU), Mymensingh.

### Treatments

**Efficacies of plant growth promoting antagonistic 32 bacterial isolates were tested with the following treatment combinations under net house and field conditions:**

**Boro season 2018-2019:** T<sub>0</sub>= Control; T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide); T<sub>2</sub>= [BDISOB04P (*Pseudomonas putida*)]; T<sub>3</sub>= [BDISOB05P (*Pseudomonas putida*)]; T<sub>4</sub>= [BDISOB219R (*Pseudomonas taiwanensis*)]; T<sub>5</sub>= [BDISOB221R (*Pseudomonas* sp.)]; T<sub>6</sub>= [BDISOB222R (*Pseudomonas plecoglossicida*)]; T<sub>7</sub>= [BDISOB258R (*Pseudomonas putida*)]; T<sub>8</sub>= [BDISOB186R (*Pseudomonas* sp.)]; T<sub>9</sub>= [BDISOB283R (*Pseudomonas fluorescens*)]

**Aman season 2019:** T<sub>0</sub>= Control; T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)]; T<sub>2</sub>= BDISO04P (*Pseudomonas putida*); T<sub>3</sub>= BDISO45R (*Bacillus paramycooides*); T<sub>4</sub>= BDISO198P (*Serratia plymuthica*); T<sub>5</sub>= BDISO135R (*Bacillus* sp.); T<sub>6</sub>= BDISO148P (*Serratia marcescens*); T<sub>7</sub>= BDISOB01R (*Bacillus amyloliquefaciens*); T<sub>8</sub>= BDISO145P (*Serratia marcescens*); T<sub>9</sub>= BDISO158R (*Serratia marcescens*)

**Boro season 2018-2020:** T<sub>0</sub>= Control; T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)]; T<sub>2</sub>= [BDISOB37R (*Pseudochrobactrum asaccharolyticum*)]; T<sub>3</sub>= [BDISOB16R (*Pseudochrobactrum asaccharolyticum*)]; T<sub>4</sub>= [BDISOB92R (*Pseudomonas fluorescens*)]; T<sub>5</sub>= [BDISOB21R (*Stenotrophomonas maltophilia*)]; T<sub>6</sub>= [BDISOB17R (*Limnolyngbya circumcreta*)]; T<sub>7</sub>= [BDISOB15R (*Pseudochrobactrum asaccharolyticum*)]; T<sub>8</sub>= [BDISOB86R (*Enterobacter aerogenes*)] and T<sub>9</sub>= [BDISOB30R (*Pseudochrobactrum asaccharolyticum*)]

**Aman season 2020:** T<sub>0</sub>= Control; T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)]; T<sub>2</sub>= [BDISOB07R (*Serratia nematodiphila*)]; T<sub>3</sub>= [BDISOB12R (*Serratia marcescens*)]; T<sub>4</sub>= [BDISOB31R (*Serratia marcescens*)]; T<sub>5</sub>= [BDISOB36R (*Serratia marcescens*)]; T<sub>6</sub>= [BDISOB46R (*Serratia marcescens*)]; T<sub>7</sub>= [BDISOB54R (*Burkholderia gladioli*)]; T<sub>8</sub>= [BDISOB70R (*Serratia marcescens*)] and T<sub>9</sub>= [(BDISOB172R (*Bacillus aerophilus*)]

**Efficacies of plant growth promoting antagonistic fungal isolates were tested with the following treatment combinations in both net house and field conditions:**

T<sub>0</sub>= Control; T<sub>1</sub>= T<sub>1</sub> for Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution)+Hemoxy (Copper fungicides) @ 4g/L, 2ml/L and 4g/L of water respectively]; T<sub>2</sub>= BDISOF67R (*Trichoderma paraviridescens*); T<sub>3</sub>= BDISOF91R (*T. erinaceum*); T<sub>4</sub>= BDISOF08R (*T. asperellum*); T<sub>5</sub>= BDISOF09R (*T. asperellum*)

**Rice varieties used:** For boro season, rice varieties viz. BRRI dhan28, BRRI dhan29, Hybrid HERA-2 & ArizeTej Gold and for aman season, rice varieties viz. Dhanigold, BRRI dhan49, BINA dhan7 and BINA dhan11 were used in this study. Rice varieties were selected based on the utilization by each variety and popularity. Hybrid varieties were selected for this study because these varieties are susceptible to bacterial blight of rice.

**Seed treatment:** Rice seeds (BRRI dhan28, BRRI dhan29, Hybrid Hera 2 & ArizeTej Gold) were treated with fungal and bacterial formulations @10g/kg seeds for 1h in case of boro season (2018-2019 and 2019-2020). In aman season (2019 and 2020) the rice seeds (Dhanigold, BRRI dhan49, BINA dhan7 and BINA dhan11) were treated with bacterial formulations @10g per kg/seeds for 1h.

**Preparation of seedling nursery bed and raising of seedlings:** A small piece of medium low land (2m x 3m) was selected for raising seedlings. The land was puddled well with country plough followed by labeling with a ladder. The treated seed was sown in the nursery bed. Proper care was taken to raise the healthy seedlings in the nursery bed. Then 40 days old seedlings were transplanted in boro seasons and 35 days old seedlings were transplanted in aman seasons in the experimental plots.

**Preparation of the experimental land and pots:** The field was ploughed with tractor followed by laddering. The layout of the field was made after final land preparation. Weeds and stubbles were removed from individual plots. All the experimental pots were filled with well-prepared sterilized soils.

**Fertilizer application:** The experimental plots were fertilized with urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and zinc sulphate at the rate of 90, 52, 60, 45 & 4 kg ha<sup>-1</sup>, respectively. The entire amounts of triple super phosphate, muriate of potash, gypsum and zinc sulphate were applied at the time of final land preparation. Urea was applied in three equal installments at 15, 30 and 45 days after transplanting (DAT) @ 166 kg ha<sup>-1</sup>.

**Uprooting of seedlings:** The nursery bed was made wet by application of water one day before uprooting seedlings. The seedlings were uprooted without causing much mechanical injury to the roots and they were immediately transferred to the main field and pots.

### **Transplanting of the seedlings in the experimental plots**

**Pot experiment:** The seedlings raised from the above treated seeds were transplanted in plastic pots following CRD design with three replications. The experiment was carried out in Net house, Seed Pathology Centre, Bangladesh Agricultural University.

**Field experiment:** Forty days old seedlings were transplanted in well prepared puddled field at the rate of 3 seedlings hill maintaining 25 cm x 15 cm spacing in boro seasons. Field experiment was conducted following RCBD design with three replications.

**Irrigation and drainage:** The experimental plots were irrigated and drained out water as and when it was necessary.

**Weeding:** During the whole growth two hand weeding were done, first weeding was done at 25 days after transplanting followed by second weeding at 50 days after transplanting.

**Preparation and application of the formulated bacterial antagonists in the field:** In boro seasons (2017-2018 and 2018-2019), the fungal and bacterial formulations were sprayed at 40, 55, 75, 90 and 105 DAT. Formulated fungal and bacterial powders were suspended in water to prepare the formulated bacterial and fungal solution (0.5% w/v). Fungal and Bacterial formulation was sprayed on the plant surface with the help of a sprayer. Every time the sprayer was washed and spray each isolate separately. In aman seasons (2019 and 2020), the bacterial formulations were sprayed at 40, 50, 60, 70 and 80 DAT. Formulated bacterial powders were sprayed on the plant surface with the help of a sprayer @ 0.5% (w/v). Every time the sprayer was washed and spray separately of each isolate. Similar spray schedules and concentrations were maintained for the formulated fungal and bacterial antagonists in case of pot experiments. Bactroban (4g/L), Hemoxy (7g/L) and SICOGREEN® (2ml/L) were applied as positive control.

**Artificial inoculation of *X. oryzae* pv. *oryzae*:** *X. oryzae* pv. *oryzae* strains were cultured in NBY agar medium at 28°C for 48 hours and then resuspended in sterile distilled water at cell density 10<sup>8</sup> cells/ml. The leaves of each plants were inoculated by clip-inoculation method of Kauffman *et al.*, (1973). Artificial inoculation of *X. oryzae* pv. *oryzae* was performed at 73 DAT in boro season and 60 DAT in aman season. In case of pot experiment the leaf of each hill in each replication were inoculated and in case of field experiment the leaves of plants of a single row in each plot were inoculated by clip-inoculation method. For leaf clipping, IRRI leaf clipper was used.

**Harvesting:** The crops were harvested at full maturity stage. Maturity of crops was determined when 90% of the grains became golden yellow in color. Then the harvested crops of each plot were bundled separately, properly tagged and brought to threshing floor and subsequent data were collected.

### **Data recorded at different growth stage**

Data were collected on the following parameters.

- Lesion length (cm)
- Yield/ treatment (t/ha)

In boro season lesion length data were collected at 14, 21 and 28 days after inoculation and in aman season lesion length data were collected at 14 and 21 days after inoculation for both net house and field experiment. In case of yield, data was collected at the time of harvest in each variety.

### **Yield data conversion**

Fresh yield data were converted using the following formula: Yield (t/ha) at 14% moisture content =  $100 - \text{Moisture content at harvest maturity (35\%)} \times \text{Weight at harvest} / 100 - \text{Moisture content at consumption (14\%)}$ . That is the conversion factor was 0.75.

### **Mechanisms of plant growth promoting fungi and bacteria mediated induced resistance in rice against *X. oryzae* pv. *oryzae***

**Expression of defense related gene in rice induced by plant growth promoting fungi and bacteria against *X. oryzae* pv. *oryzae*:** To study the Salicylic acid (SA) and Jasmonic acid (JA) pathway mediated induced resistance in rice by PGP bacteria and fungi, a susceptible check variety (IR24) was used.

**Seed priming, raising of seedlings and transplanting:** Seeds of IR24 were treated with 32 selected formulated PGP antagonistic bacterial isolates and four formulated PGP antagonistic fungal isolates. The treated seeds were left for 1 hr for adherence of the bacterial and fungal isolates with the treated seed surface. The treated seeds were then sown in the plastic pots filled with sterilized soils. One-month old seedlings were then transplanted in the plastic pots filled with puddle soils.

**Foliar spray of formulated PGP bacterial and fungal isolates:** Formulated PGP antagonistic bacterial and fungal isolates were sprayed two times (at 50 and 55 DAS) before inoculation and two times after inoculation i.e 65 and 70 DAS.

**Inoculation of the rice plant with *X. oryzae* pv. *oryzae*:** Rice plants were inoculated with a strain of *X. oryzae* pv. *oryzae* by Scissor clip method as described above at 60 DAS.

**Sampling of rice leaf samples for RNA extraction:** Ten rice leaf samples for each treatment were collected at 24, 48, 72 and 144 hrs after inoculation in zipper bags. The leaf samples were then immediately brought in the laboratory. The collected leaf samples were frozen with liquid nitrogen and ground in powder for either RNA extraction immediately or the ground samples were stored at -80°C for future use.

**Data Collection:** Data on lesion length was recorded at 7 and 14 days after inoculation using plastic ruler.

**Extraction of RNA:** Total RNA was extracted from 20mg ground rice leaves powder using SV total RNA kit (Promega, USA) according to manufacturers' instruction. The extracted RNA was then stored at -80°C for future use.

**Synthesis of complementary DNA (cDNA):** The following procedure was designed to convert up to 5µg of total RNA into the first-strand cDNA. The following components were mixed before used and combined as follows. Each tube of the reaction mix was closed tightly. The tubes were placed into a Thermocycler at 70°C for 5 minutes. Immediately the tubes were chilled in ice-water for at least 5 minutes. Each tube was centrifuged for 10 seconds in a microcentrifuge to collect the condensate, and the original volume was maintained. The tubes were closed and were placed on ice until the reverse transcription reaction mix is added.

Component	Volume
Experimental RNA (up to 5µg/reaction)	1.0 µl
Primer [Oligo(dT) 15 (0.5µg/reaction) and/or Random Primer (0.5µg/reaction) or gene-specific primer (10–20pmol/reaction)]	1.0 µl
Nuclease-Free Water	3.0 µl
Final volume	5.0 µl

**Reverse Transcription:** The reverse transcription reaction mix was prepared by combining the following components of the GoScript™ Reverse Transcription System in a sterile microcentrifuge tube on ice. Sufficient mix was prepared to allow 15 µl for each cDNA synthesis reaction to be performed. The volumes were prepared needed for each component and combined in the order listed below. The reaction mix was vortexed gently to mix and was kept on ice prior to dispensing into the reaction tubes. 15 µl aliquots of the reverse transcription reaction mix were added to each ice reaction tube. 5µl of RNA and primer mix was then added to each reaction for a final reaction volume of 20µl per tube. The tubes were placed in a controlled-temperature water bath equilibrated at 25°C and incubate at 5 minutes. The tubes were incubated in a controlled-temperature heat block at 42°C for up to one hour for extension. The reaction tubes in a controlled-temperature were placed in the Thermocycler at 70°C for 15 minutes. Finally, the cDNA was stored at -20°C.

Component	Amount
Nuclease-Free Water (to a total volume of 15µl)	6.0 µl
GoScript™ 5X Reaction Buffer	4.0 µl
MgCl <sub>2</sub> (final concentration 1.5–5.0mM)1	2.5 µl
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)	1.0 µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5 µl
GoScript™ Reverse Transcriptase	1.0 µl
Total volume	15.0 µl
RNA + Primer mix	5.0 µl
Final volume	20.0 µl

**Primers and Reverse Transcription (RT)-PCR:** RT-PCR was performed using Hotstart Go Taq master mix (Promega, USA) following the instruction of kit's manual. The following primers (Table 4) were used for the analyses of the expression of some selected marker genes of SA and JA-pathway. The PCR amplification reaction was performed under the following thermal cycling conditions: 95°C for 10 min; 45 cycles of 95°C for 10s; 60°C for 10s and 72°C for 10s. The expression level of different defense related genes was compared based on the intensity of the band as compared with the untreated control. 18S rRNA gene was used as the internal control. The following primers were used.

**Table 4.** List of the primers for expression study of some selected defense related genes of rice against *X. oryzae* pv. *oryzae*.

Genes	Primer name	Sequence (5'-3')	Pathway
OsPR1	OsPR1 forward	TTATCCTGCTGCTTGCTGGT	SA pathway
	OsPR1 reverse	GATGTTCTCGCCGTA CTCC	
OsPR10	OsPR10 forward	GGCACCATCTACACCATGAA	SA pathway
	OsPR10 reverse	TTGTCCG CTGTGATGA ATGT	
OsWRKY45	OsWRKY45 forward	CCGGCATGGAGTTCTTCAAG	SA pathway
	OsWRKY45 reverse	TATTT CTGTACACACGCGTGGAA	
OsWRKY62	OsWRKY62 forward	AGGATGGGTACCAATGGA	SA pathway
	OsWR KY62 reverse	ACGAGTTGATGGAGATGGA	
OsWRKY71	OsWRKY71 forward	AGCCCAA GA TCTCC AAGCTC	SA pathway
	OsWRKY71 reverse	ACGAGGATCGTGTTGTCCTC	
OsACS2	OsACS2 forward	GGAATAAAGCTGC TGCCGAT	SA pathway
	OsACS2 reverse	TGAGCCTGAAG TCGTTGAAGC	
OsHI-LOX	OsHI-LOX forward	GCATCCCCAACAGCACATC	JA Pathway
	OsHI-LOX reverse	AATAAAGATTTGGGA	
18S rRNA	18S rRNA Forward	CTACGTCCCTGCCCTTTGTACA	Internal control
	18S rRNA Reverse	ACACTTCACCGGACCATTCAA	

**Statistical analysis:** The data on various parameters obtained from both net house and field experiments were analyzed statistically using MStatC program. Means of the treatments were compared with either DMRT and/or LSD.

## 11. Results and discussion:

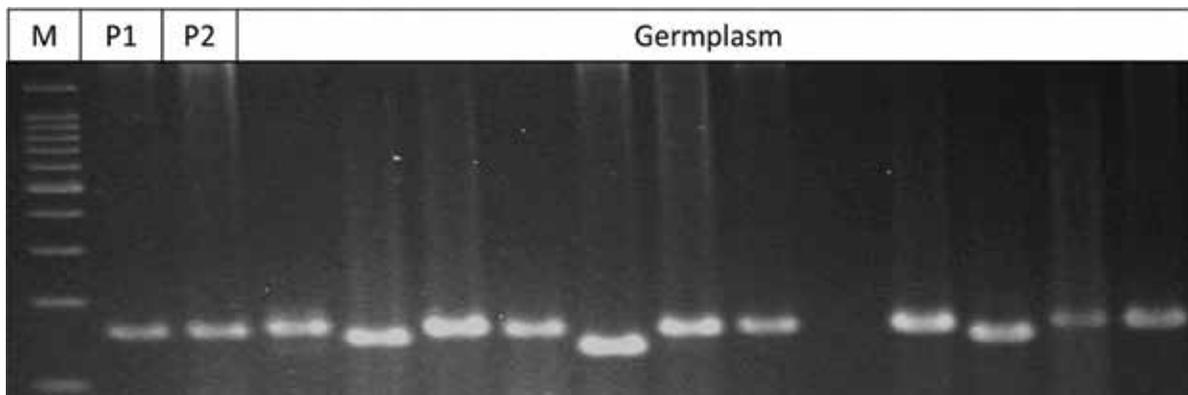
### BRR1 component

#### Identification of known bacterial blight resistant genes

**Phenotyping screening of land races and cultivars:** For the identification of known bacterial blight resistant gene(s), a total of 928 landraces (including checks) were screened with virulent BB isolates (*Bxo67*, *Bxo87*, *Bxo91*). Mean values of lesion length along with disease reaction of 928 land races were shown in Appendix 1. The lesion length data of the genotypes ranged from 0.5 to 51.25 cm. The lowest lesion length was found on Acc. No. 4786 and the highest lesion length was found on the susceptible check 'BRR1 dhan28. On the basis of lesion length data, out of the 928-rice germplasm (with checks), a single entry showed highly resistant (HR), 77 showed resistant (R) to moderately resistant (MR) reaction against the virulent BB isolates. The check variety 'IRBB60' showed highly resistant to resistant reaction against all the 3 virulent isolates of *Xanthomonas oryzae* pv. *oryzae* tested in the present experiment. In addition, other materials showed moderately susceptible to highly susceptible reaction against the pathogen. The susceptible check variety BRR1 dhan28 showed highly susceptible reaction to all 3 isolates of *Xanthomonas oryzae* pv. *oryzae*. Researchers from Nepal (Karki 1991), China (Zhang *et al.*,1994, Zhao *et al.*,1994), India (Mohanty *et al.*,1996), India (Mohanty *et al.*,1996), Bangladesh (Mondal and Hossain 1997), and Pakistan (Ali *et al.*,2009, Shah *et al.*,2009) screened large number of genotypes and found rice genotypes with resistant genes against the BB pathogens.

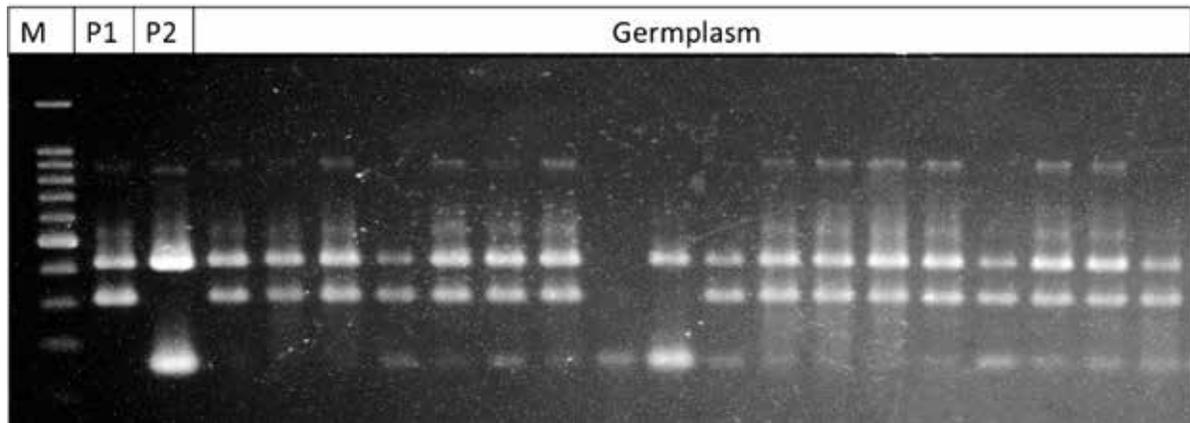
**Molecular detection of BB resistant gene(s) in selected germplasm:** A total of 78 highly resistant to moderately resistant germplasm those were found by pathogenicity test were used for molecular detection of genes. These germplasms were confirmed for resistance genes using molecular markers. The known resistant genes those are responsible for bacterial blight resistance, *Xa4*, *xa5*, *Xa7*, *xa13*, *Xa21*, *Xa23* were confirmed using gene-based markers *i.e.* MP1/MP2, *xa5*-pro(multiplex), *Xa7*-STS, *xa13*-prom, pTa248 and *Xa23*-STS, respectively.

**Detection of *Xa4* gene in germplasm:** MP1/MP2 primer (*Xa4* linked) amplified two different DNA fragments of 150bp (resistant band) and 120 bp (susceptible band) length. compared to the 100bp DNA ladder. IRBB4 along with 41 germplasm showed amplified resistant allele; whereas, 33 germplasm along with BRR1 dhan28 showed susceptible band (Figure 5).



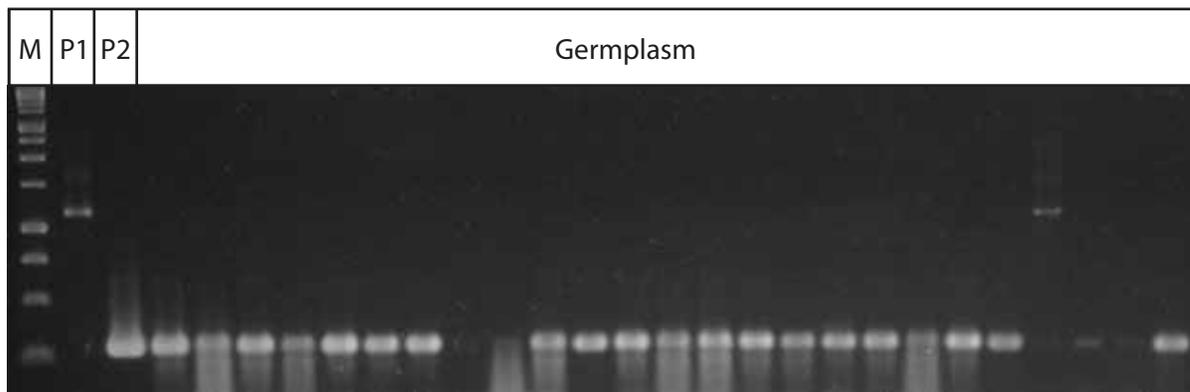
**Figure 5.** Partial gel picture to detect *Xa4* gene in test germplasm (M= 100 bp ladder, P1= resistant and P2 = susceptible parent)

**Detection of *xa5* gene in germplasm:** *xa5* multiplex primer (*xa5* linked) amplified three different DNA fragments of 160bp (resistant allele), 300bp (susceptible allele) and 420bp (common allele) length compared to the 100bp DNA ladder. IRBB5 along with 15 germplasm (20.27%) showed resistant allele. however, 59 germplasm (79.73%) along with BRR1 dhan28 showed susceptible reaction (Figure 6).



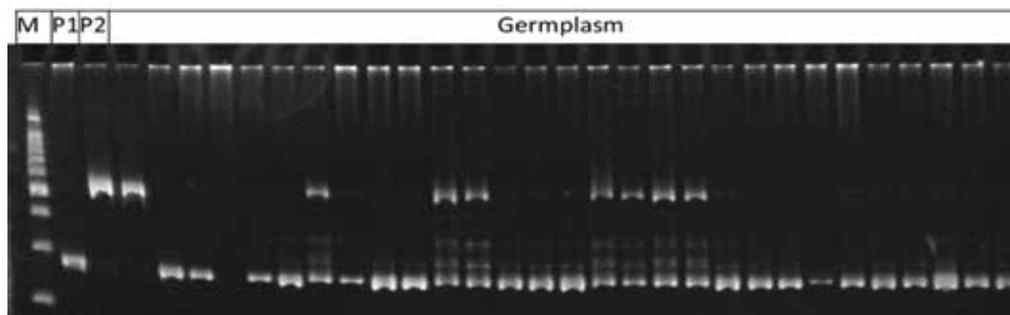
**Figure 6.** Partial gel picture of detection of *xa5* gene in germplasm (M= 100 bp ladder, P1= resistant and P2 = susceptible parent)

**Detection of *Xa7* gene in germplasm:** *Xa7* STS primer (*Xa7* linked) amplified three different fragments of 294bp (resistant allele) and 1170bp (susceptible allele) length compared to the 1kb DNA ladder. IRBB7 along with 62 germplasm (80.78%) showed resistant allele. while, 16 germplasm (19.22%) along with BRR1 dhan28 showed susceptible allele (Figure 7).



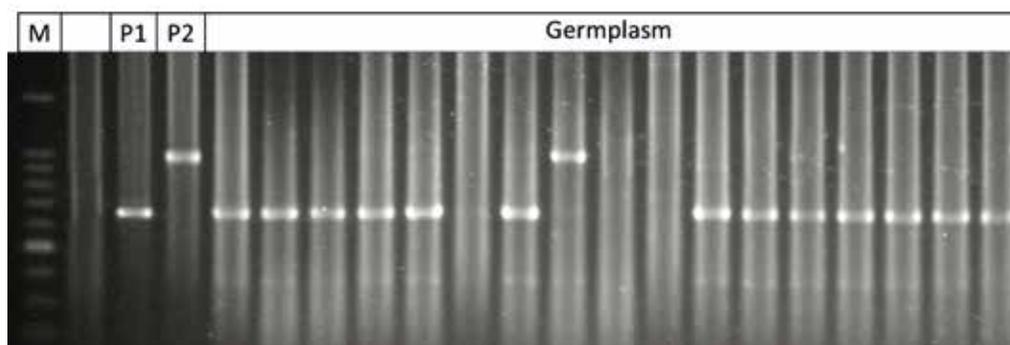
**Figure 7.** Partial gel picture of detection of *Xa7* gene in germplasm (M= 100 bp ladder, P1= resistant and P2 = susceptible parent)

**Detection of *xa13* gene in germplasm:** *xa13*-pro STS primer (*xa13* linked) amplified two different DNA fragments of 250 bp (Susceptible allele) and 500 bp (resistant allele) length compared to the 1kb DNA ladder. IRBB13 along with 33 germplasm (44.59%) showed resistant allele. where, 41 germplasm (55.41%) along with BRR1 dhan28 showed susceptible allele (Figure 8).



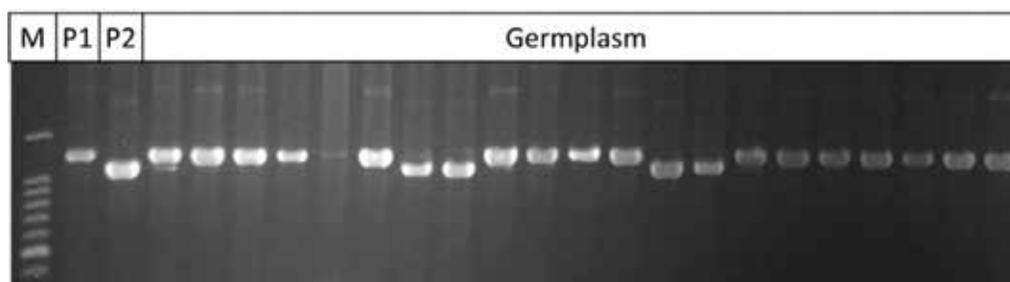
**Figure 8.** Partial gel picture of detection of *xa13* gene in germplasm (M= 100 bp ladder, P1= resistant and P2= susceptible parent)

**Detection of *Xa21* gene in germplasm:** pTA248 primer (*Xa21* linked) amplified two different DNA fragments of 750bp (Susceptible allele) and 1000 bp (resistant allele) length compared to the 1kb DNA ladder. Although, 73 germplasm (98.65%) along with BRRI dhan28 showed susceptible allele, only one germplasm (1.35%) along with IRBB21 showed resistant allele (Figure 9).



**Figure 9.** Partial gel picture of detection of *Xa21* gene in germplasm (M= 100 bp ladder, P1= resistant and P2= susceptible parent)

**Detection of *Xa23* gene in germplasm:** *Xa23* STS primer (*Xa23* linked) amplified two different DNA fragments of 1100 bp (resistant allele) and 1200 bp (susceptible allele) length compared to the 1kb DNA ladder. IRBB23 along with 19 germplasm (25.68%) showed resistant reaction. while, 55 germplasm (74.32%) along with BRRI dhan28 showed susceptible allele (Figure 10).



**Figure 10.** Partial gel picture of detection of *Xa23* gene in germplasm (M= 100 bp ladder, P1= resistant and P2 = susceptible parent)

Based on all molecular detection for specific gene using specific primers, ten (10) germplasm contained 4 resistant genes, 15 germplasm contained 3 resistant genes, 22 germplasm contained 2 resistant genes and others had a single or unknown resistant gene (Table 5 & Figure 11).

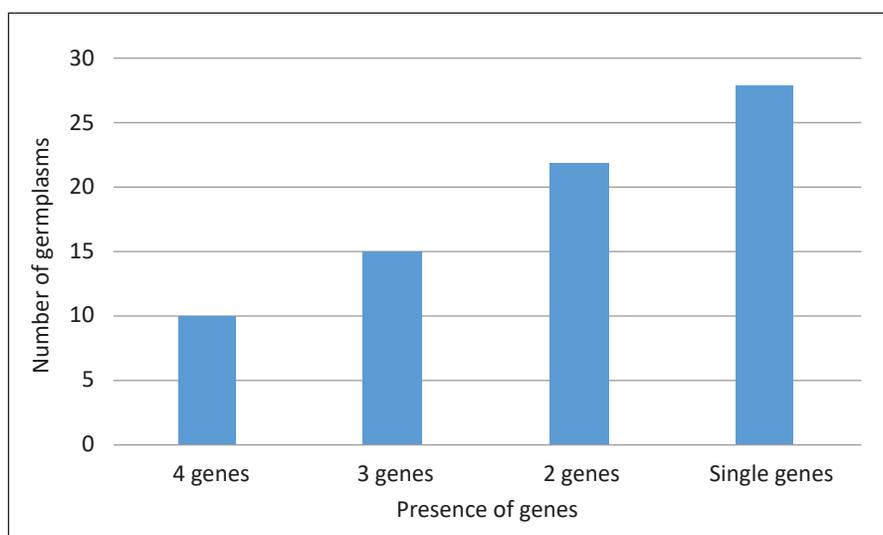
**Table 5:** List of germplasm having resistant genes for BB along with pathogenicity test

Serial No.	Acc. No	Reaction pattern	Resistant genes for BB					
			<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>	<i>xa13</i>	<i>Xa21</i>	<i>Xa23</i>
1	4786	HR	+	-	+	-	-	-
2	4214	MR	-	-	+	-	*	-
3	4216	MR	+	-	+	+	+	-
4	4380	MR	+	-	+	-	-	-
5	5043	R	+	-	+	+	*	+
6	991	R	-	-	+	+	-	+
7	1050	R	+	-	+	+	-	+
8	1051	R	*	*	*	*	*	*
9	1753	R	+	+	+	+	*	-
10	1795	R	*	+	+	*	*	-
11	1797	R	+	-	+	+	*	-
12	1800	R	-	-	+	-	-	+
13	1862	R	+	-	+	-	*	-
14	1864	R	+	-	+	-	-	-
15	2266	R	*	-	+	+	*	-
16	2268	R	+	-	+	+	*	-
17	3962	R	*	-	+	+	*	-
18	3981	R	*	-	+	+	*	-
19	3982	R	*	-	+	-	*	-
20	3987	R	*	+	+	-	*	+
21	4004	R	+	-	+	+	-	-
22	4009	R	+	-	+	+	-	-
23	4057	MR	+	-	-	-	-	-
24	3293	R	+	-	+	-	-	-
25	3106	R	*	-	+	+	*	-
26	4217	R	-	-	+	+	-	-
27	4370	R	+	-	+	-	-	-
28	4374	R	-	-	+	-	-	-
29	4378	R	+	-	+	-	-	-

Serial No.	Acc. No	Reaction pattern	Resistant genes for BB					
			<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>	<i>xa13</i>	<i>Xa21</i>	<i>Xa23</i>
30	3215	R	+	-	+	-	-	-
31	4967	MR	+	-	+	-	*	-
32	4980	R	-	-	+	+	-	-
33	4982	R	+	-	-	-	-	-
34	4985	MR	+	-	-	+	-	-
35	4995	R	*	+	+	+	*	-
36	5101	R	*	-	+	+	*	-
37	6851	R	*	-	-	+	*	+
38	7367	R	+	-	+	-	-	-
39	7370	R	+	+	+	-	-	-
40	570	R	*	-	+	-	*	-
41	571	R	+	+	+	+	-	-
42	572	R	+	-	+	+	-	-
43	1523	R	+	+	-	+	-	+
44	1525	R	+	+	+	-	-	+
45	3530	R	+	+	+	-	-	+
46	7244	R	+	+	+	-	*	+
47	7444	R	+	+	+	-	-	+
48	7446	R	*	+	+	-	-	+
49	7353	R	*	-	+	+	-	+
50	7352	R	+	-	+	-	-	-
51	1925	R	-	-	+	+	-	-
52	1904	R	*	-	+	-	*	-
53	3846	R	-	+	-	-	-	-
54	1007	R	-	-	+	+	-	-
55	3828	R	+	-	-	-	*	+
56	1897	R	+	-	-	-	-	+
57	5296	R	+	*	*	+	-	*
58	4246	R	-	-	*	-	-	*
59	1896	R	-	-	+	+	-	+
60	7356	R	-	*	*	*	-	*
61	7351	R	-	-	+	-	-	-
62	3134	R	-	-	+	-	+	-
63	3101	R	+	-	+	+	-	-

Serial No.	Acc. No	Reaction pattern	Resistant genes for BB					
			<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>	<i>xa13</i>	<i>Xa21</i>	<i>Xa23</i>
64	3103	R	+	-	-	-	+	-
65	3105	R	+	-	+	-	-	-
66	3124	R	+	-	-	-	-	+
67	3155	R	*	-	+	-	-	+
68	3160	R	-	-	+	+	-	+
69	3161	R	+	+	+	-	-	-
70	3163	R	-	+	+	+	-	-
71	3165	R	+	-	+	+	+	-
72	3169	R	-	-	+	-	-	-
73	3179	R	+	-	-	+	-	-
74	3277	R	+	-	+	+	+	-
75	3409	R	*	-	-	+	+	-
76	3463	R	*	-	+	*	+	-
77	3487	R	*	-	+	*	+	-
78	3493	R	+	-	+	-	-	-

+ = Presence of resistant genes, - = Absence of resistant genes, \* = allele not amplified



**Figure 11:** Distribution of resistant genes among test germplasm

The present result was supported by several researchers all over the world. According to Dangl *et al.*, 2013, wild plant species or land races are shown to be an important and rich genetic reservoir of resistance sources. Several elite bacterial blight-resistance genes such as *Xa21* (Song *et al.*, 1995), *Xa23* (Wang *et al.*, 2014) and *Xa27* (Gu *et al.*, 2005), were identified from wild rice species *Oryza longistaminata* A. Chev. & Roehr., *Oryza rufipogon* Griff. and

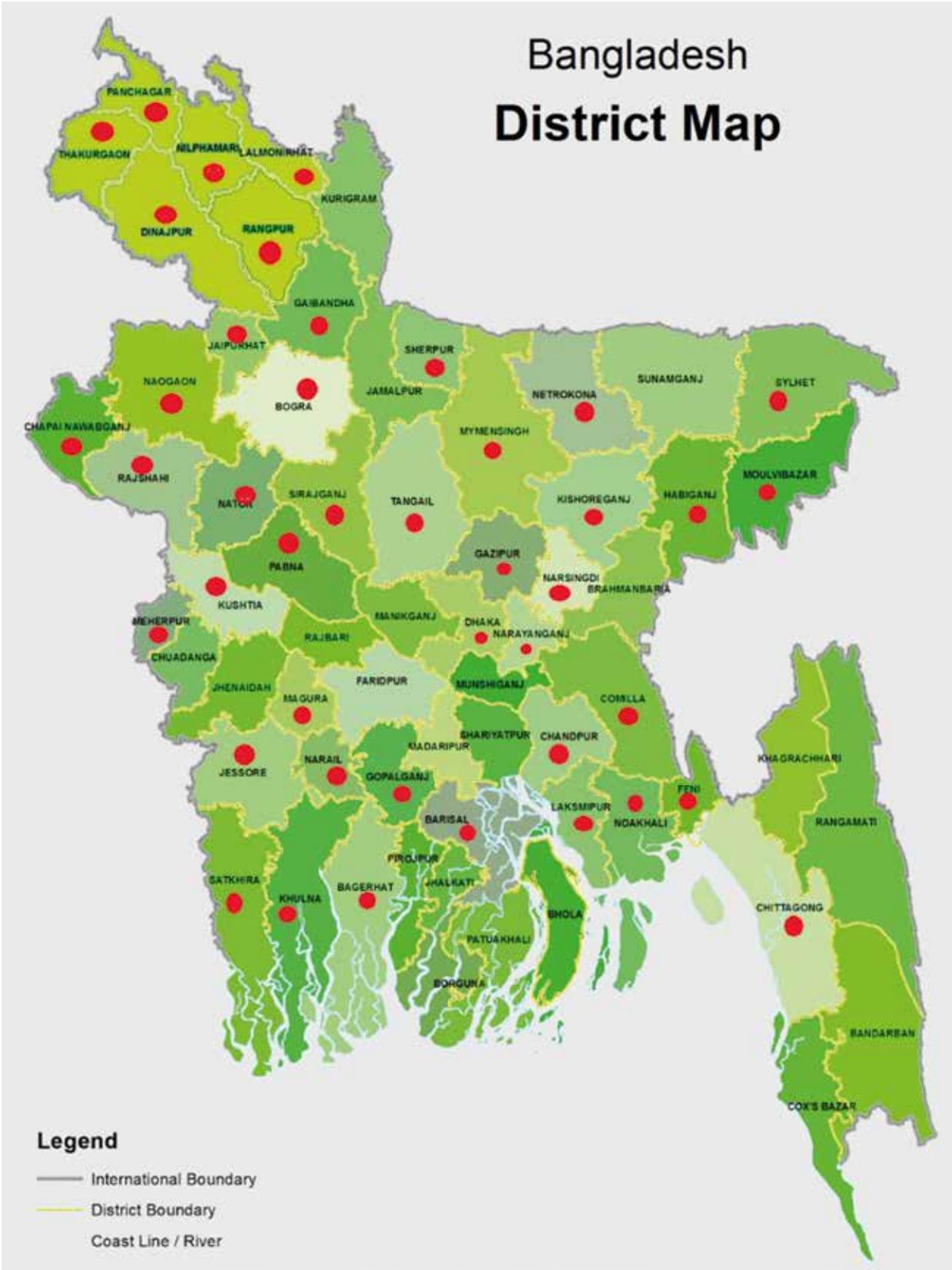
*Oryza minuta* Presl., respectively. To date, at least 41 bacterial blight of rice resistant genes have been identified, but only a few of them have been successfully deployed for resistance breeding (Kinoshita 1995, Lin *et al.*,1996, Kihupi *et al.*,1999, Wang *et al.*,2000, Lee *et al.*,2002, Khan *et al.*,2014, Zhang *et al.*,2014, Kim *et al.*,2015, Hutin *et al.*,2015), among which *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* are widely used in breeding programs across Asia (Khan *et al.*,2014).

### Collection, isolation and purification of bacterial blight isolates

In total, 920 bacterial blight infected samples were collected from the 40 different districts covering mostly all AEZs of Bangladesh (Figure 12) during the project period. The collected samples were used for the isolation of bacterial blight isolates. A total of 300 bacterial blight isolates were isolated and purified from the collected samples (Table 6). The bacterial blight isolates were tested against bacterial blight susceptible varieties (BRRI dhan28, Purbachi, BR11 etc.). Afterwards the isolates were re-isolated from the inoculated plant and preserved in 40% NYB glycerol medium in -80°C for long term preservation for further use.

**Table 6:** District wise list of sample collection and isolation of BB isolates

Sl No.	District	Collected samples	Isolated samples	Sl No.	District	Collected samples	Isolated samples
1	Panchagar	25	6	21	Meherpur	19	5
2	Thakurgaon	31	8	22	Kustia	21	6
3	Nilphamari	23	5	23	Gazipur	54	20
4	Lalmonirhat	24	7	24	Kishoreganj	24	9
5	Dinazpur	18	4	25	Habiganj	15	6
6	Rangpur	15	6	26	Moulavibazar	10	5
7	Gaialleleha	11	3	27	Jessore	20	7
8	Jaipurhat	21	8	28	Dhaka	25	8
9	Naogoan	37	11	29	Narayanganj	21	5
10	Chapai Nawabganj	18	5	30	Narail	18	3
11	Bogura	39	12	31	Gopalganj	18	9
12	Jamalpur	25	6	32	Chandpur	27	6
13	Sherpur	23	6	33	Comilla	41	16
14	Rajshahi	20	5	34	Shatkhira	30	12
15	Natore	22	8	35	Khulna	27	8
16	Sirajganj	24	6	36	Bagerhat	25	5
17	Tangail	23	7	37	Barisal	19	8
18	Mymensingh	20	11	38	Noakhali	15	6
19	Netrokona	19	6	39	Feni	14	6
20	Shylet	21	7	40	Chattagram	18	13



**Figure 12:** District demarcation for sample collections and isolation BB isolates

### Identification of physiological races of *Xanthomonas oryzae* pv. *oryzae* and distribution patterns

To identify the physiological races, pathogenicity tests of 300 bacterial blight isolates were done on NILs and pyramid lines for Bacterial blight resistance in T. Aman 2020 season. A total of 13 races were identified according to the reaction pattern of the BB isolates against NILs (Table 7). From this study *Xa21*, *Xa27*, *xa13* & *Xa7* identified as effective gene for the deployment of bacterial blight resistance in Bangladesh.

**Table 7:** Identification of bacterial blight races using NILs

Races	No. of isolates	% of isolates	IRBB1 ( <i>Xa1</i> )	IRBB2 ( <i>Xa2</i> )	IRBB3 ( <i>Xa3</i> )	IRBB4 ( <i>Xa4</i> )	IRBB5 ( <i>Xa5</i> )	IRBB7 ( <i>Xa7</i> )	IRBB8 ( <i>Xa8</i> )	IRBB10 ( <i>Xa10</i> )	IRBB11 ( <i>Xa11</i> )	IRBB13 ( <i>xa13</i> )	IRBB14 ( <i>Xa14</i> )	IRBB21 ( <i>Xa21</i> )	IRBB23 ( <i>Xa23</i> )	IRBB27 ( <i>Xa27</i> )
1	71	23.67	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	78	26.00	S	S	S	S	S	S	S	S	S	S	S	R	S	S
3	13	4.33	S	S	S	S	S	S	S	S	S	S	S	R	S	R
4	21	7.00	S	S	S	S	S	S	S	S	S	R	S	R	S	R
5	29	9.67	S	S	S	S	S	S	S	S	S	R	S	S	S	R
6	18	6.00	S	S	S	S	S	S	S	S	S	R	S	S	S	S
7	26	8.67	S	S	S	S	S	R	S	S	S	S	S	R	S	R
8	5	1.67	S	S	S	R	R	R	R	R	S	R	R	R	R	R
9	10	3.33	S	S	S	S	R	R	S	S	S	R	S	R	S	R
10	7	2.33	S	S	S	S	S	S	R	S	S	S	S	R	S	R
11	13	4.33	S	S	S	S	S	S	S	S	S	S	S	S	R	R
12	5	1.67	S	S	S	S	S	S	S	R	S	S	S	S	S	R
13	4	1.33	S	S	S	S	S	R	R	S	S	R	S	R	S	R
<b>Resistance frequency</b>			0.00	0.00	0.00	1.67	5.00	15.0	5.33	3.33	0.00	29.0	1.67	54.7	6.00	44.3

Pathogenic variability of *Xoo* in Bangladesh has been reported (Noda *et al.*, 1996, Jalaluddin and Kashem 1999). Twelve races of the *Xoo* have been identified in Bangladesh and the study indicated that some aggressive strains of *Xoo* prevails in Bangladesh (BRRI, 2018). The variation of pathogenicity in *Xoo* and resistance genes in rice cultivars has been studied in Japan and at International Rice Research Institute (IRRI) (Noda *et al.*, 1990 & 1996, Ogawa 1993, Yamamoto and Ogawa 1990 and Khush *et al.*, 1990). Severe outbreak of BB was occurred in Bangladesh in Boro 2007-2008 and both hybrid and inbred varieties were affected. In T. Aman 2017 Season, bacterial blight outbreak was occurred in different regions of Bangladesh which are documented in most of the daily newspapers. Control of the disease with copper compounds, antibiotics, and other chemicals has not proven effective (Webster and Gunnell 1992). Regarding host resistance, it is unfortunate that resistance capacity of a particular disease resistant variety deteriorates or totally breaks down because of evolving race in few years. Understanding both pathogen population structure as well as host pathogen resistance is the prerequisite in designing of effective strategy for gene deployment. Durable resistant varieties can help to minimize the resistance breakdown problem. Therefore, the pyramiding of *Xa27*, *Xa21*, *xa13* and *Xa7* genes could be introgressed for the development of durable bacterial blight resistant variety, as it is one of the ways to develop durable resistant variety (Ashkani *et al.*, 2015).

#### **Gene pyramiding for the development of bacterial blight and blast resistant varieties**

Backcross breeding followed by marker assisted selection were conducted for introgressing resistant gene(s) into BRRI dhan63, BRRI dhan81 and BRRI dhan49 by crossing with IRBB58 (*Xa4*, *xa5* and *Xa21*) and/or IRBB60 (*Xa4*, *xa5*, *xa13* and *Xa21*) and *Pi9*-[US], *Pb1*-[US]. Crossing and backcrossing were performed for development of bacterial blight and blast resistant variety/pre-breeding lines throughout the project period. Every year crossing was carried out in two seasons *i.e.*, T. aman and boro season. In every season the progenies were evaluated with gene based molecular markers (Table 3) for the BB and blast resistant genes. Progenies with resistant genes were selected for the further advancement in the next season.

#### **Hybridization for bacterial blight and blast resistance:**

Three sets of parents *viz.* BRRI dhan63, BRRI dhan81, BRRI dhan49 and IRBB58, IRBB60, *Pi9*-[US], *Pb1*-[US] were planted for hybridization.

**Production of F<sub>1</sub> population:** Six crosses were performed in T. Aman 2018 season, in different combinations. BRRI dhan81, BRRI dhan63, BRRI dhan49 were used as recipient parent and IRBB58, IRBB60, *Pi9*-[US], *Pb1*-[US] were used as donor parent. The cross combination and number of produced seeds represented in Table 8.

**Table 8:** List of crosses and the number of seeds for respective cross combinations (T. Aman, 2018)

Generation	Cross combination	No. of seeds
F <sub>1</sub>	BRRRI dhan49*IRBB60	22
F <sub>1</sub>	BRRRI dhan63-Pb1*IRBB60	33
F <sub>1</sub>	BRRRI dhan81*IRBB60	42
F <sub>1</sub>	BRRRI dhan81*Pb1-[US]	23
F <sub>1</sub>	BRRRI dhan63-Pb1* Pi9-[US]	90
F <sub>1</sub>	BRRRI dhan81* Pi9-[US]	189

**Confirmation of F<sub>1</sub> population and production of BC<sub>1</sub>F<sub>1</sub> population:** F<sub>1</sub> seeds produced in T. Aman 2018 were grown in boro 2018-19. Each plant was confirmed with gene specific molecular markers (Figure 13). The selected plants were backcrossed with the recipient parent to produce BC<sub>1</sub>F<sub>1</sub> population. The cross combination and number of produced seeds are presented in Table 9.

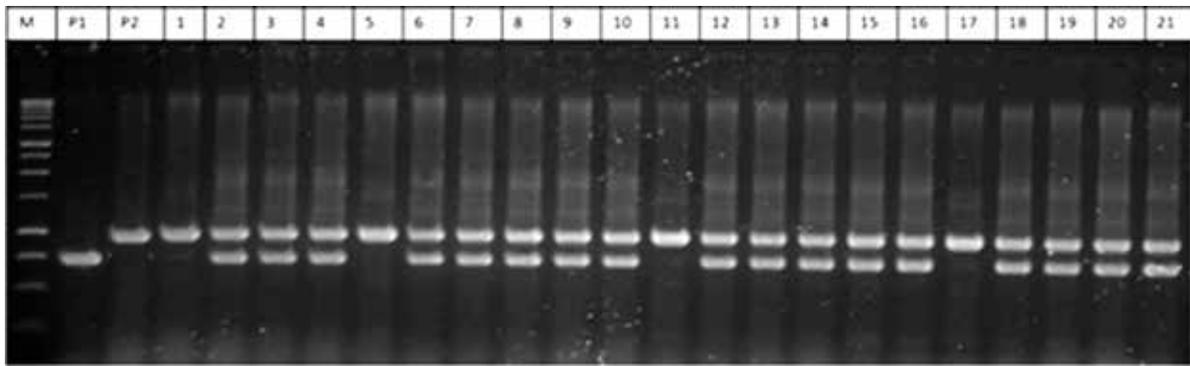


**Figure 13:** Confirmation of F<sub>1</sub> population (M= 100bp ladder, P1= BRRRI dhan81, P2= IRBB60 & P3=IRBB58)

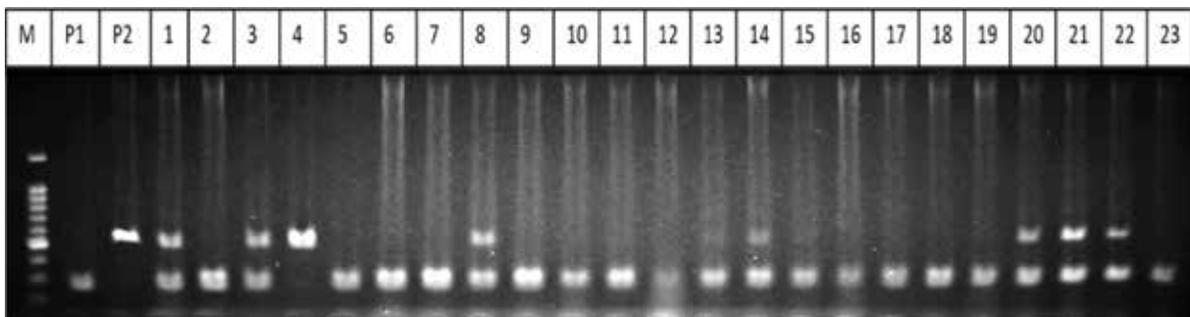
**Table 9:** List of backcrosses and number of seeds for respective cross (Boro 2018-19)

Generation	Cross combination	No. of seeds
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan49*IRBB60	40
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan63-Pb1*IRBB60	75
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan81*IRBB60	42
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan81*Pb1-[US]	50
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan63-Pb1* Pi9-[US]	21
BC <sub>1</sub> F <sub>1</sub>	BRRRI dhan81* Pi9-[US]	37

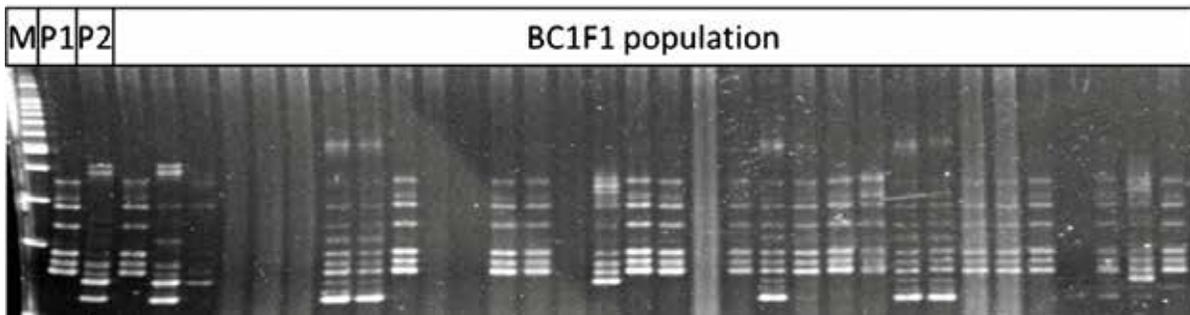
**Confirmation of BC<sub>1</sub>F<sub>1</sub> and production of BC<sub>2</sub>F<sub>1</sub> population:** BC<sub>1</sub>F<sub>1</sub> seeds from boro 2018-19 were grown in T. aman 2019. Each plant was confirmed with gene specific molecular markers (Figure 14, 15 & 16). The selected plants were backcrossed with the recipient parent to produce BC<sub>2</sub>F<sub>1</sub> population. The cross combination and number of produced seeds are presented in Table 12.



**Figure 14:** Confirmation of *Xa21* gene in BC<sub>1</sub>F<sub>1</sub> population (M= 1kb ladder, P1= BRRIdhan63-Pb1, P2= IRBB60)



**Figure 15:** Confirmation of *xa13* gene in BC<sub>1</sub>F<sub>1</sub> population (M= 100bp ladder, P1= BRRIdhan81, P2= IRBB60)



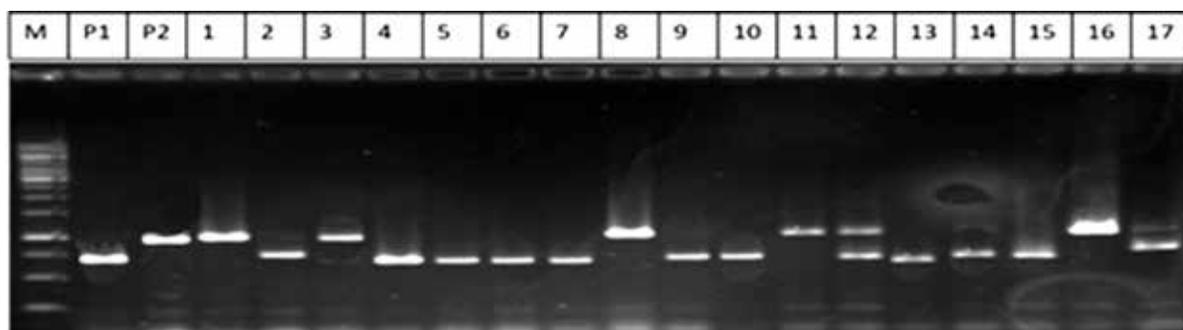
**Figure 16:** Confirmation of *Pbl* gene in BC<sub>1</sub>F<sub>1</sub> population (M= 100bp ladder, P1= BRRIdhan81, P2= Pb1-US)

**Intercrossing (Bridging) of BC<sub>1</sub>F<sub>1</sub> population for the combination of bacterial blight and blast resistant genes:** Intercrosses were made between BC<sub>1</sub>F<sub>1</sub> populations of BRRIdhan81 and BRRIdhan63 (Table 10).

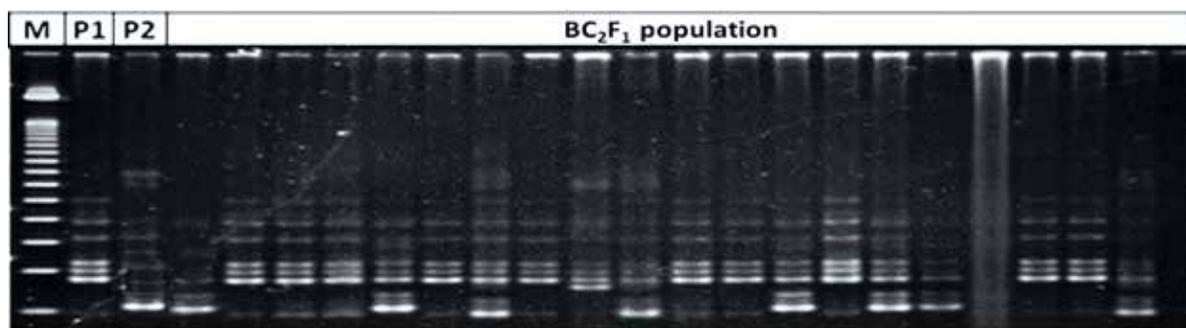
**Table 10:** List of crosses and the number of seeds for respective cross combinations (T. Aman, 2019)

Generation	Cross combination	No. of seeds
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan49*IRBB60	32
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan81*IRBB60/BRRI dhan81*Pi9-[US]/BRRI dhan81*Pb1	45
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan81*IRBB58/BRRI dhan81*Pb1	50
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan63-Pb1*IRBB60/BRRI dhan63*Pi9-[US]	19
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan63-Pb1*IRBB60	22
BC <sub>2</sub> F <sub>1</sub>	BRRI dhan81*IRBB60	16

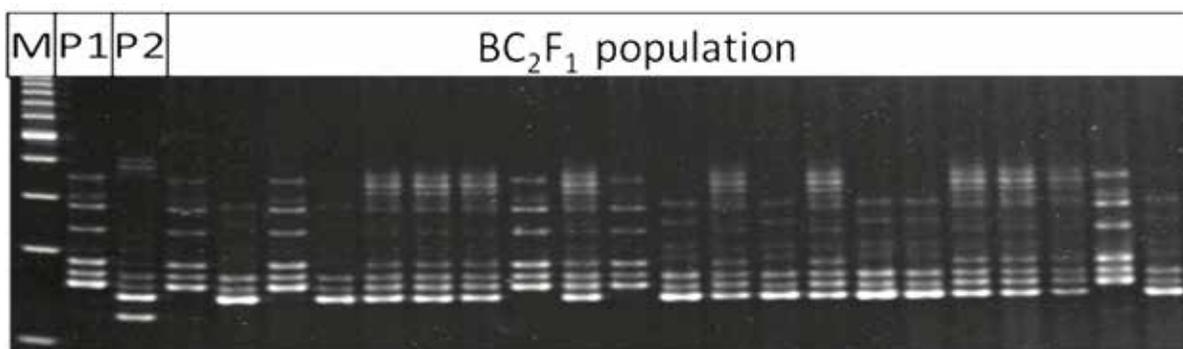
**Confirmation of BC<sub>2</sub>F<sub>1</sub> and production of BC<sub>3</sub>F<sub>1</sub> population:** BC<sub>2</sub>F<sub>1</sub> seeds from T. aman 2019 were grown in boro 2019-20. Each plant was confirmed with gene specific molecular markers (Figure 17, 18 & 19). The selected plants were backcrossed with the recipient parent to produce BC<sub>3</sub>F<sub>1</sub> and was selfed to produce BC<sub>2</sub>F<sub>2</sub> population. The cross combination and number of produced seeds are presented in Table 11.



**Figure 17:** Confirmation of *Xa21* gene in BC<sub>2</sub>F<sub>1</sub> population (M= 1kb ladder, P1= BRRI dhan49, P2= IRBB60)



**Figure 18:** Confirmation of *Pi9* gene in BC<sub>2</sub>F<sub>1</sub> generation (M= 100 bp ladder, P1= BRRI dhan63, P2= *Pi9*-[US])

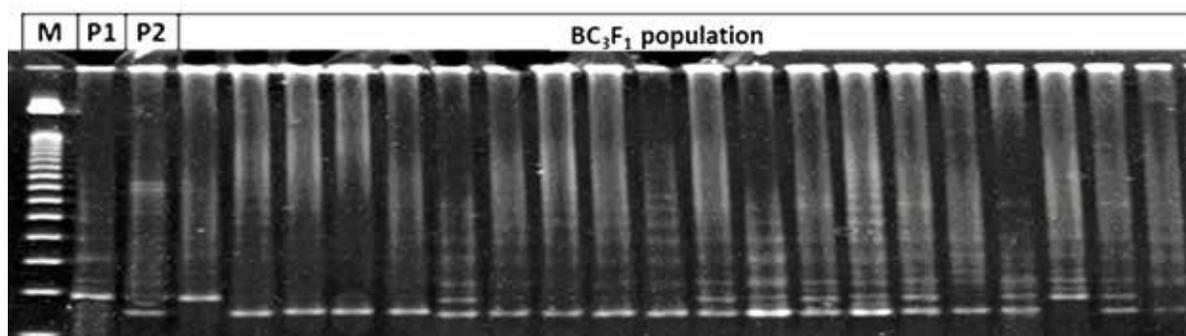


**Figure 19:** Confirmation of *Pbi* gene in  $BC_2F_1$  generation (M= 100 bp ladder, P1= BRRIdhan63, P2= *Pbi*-[US])

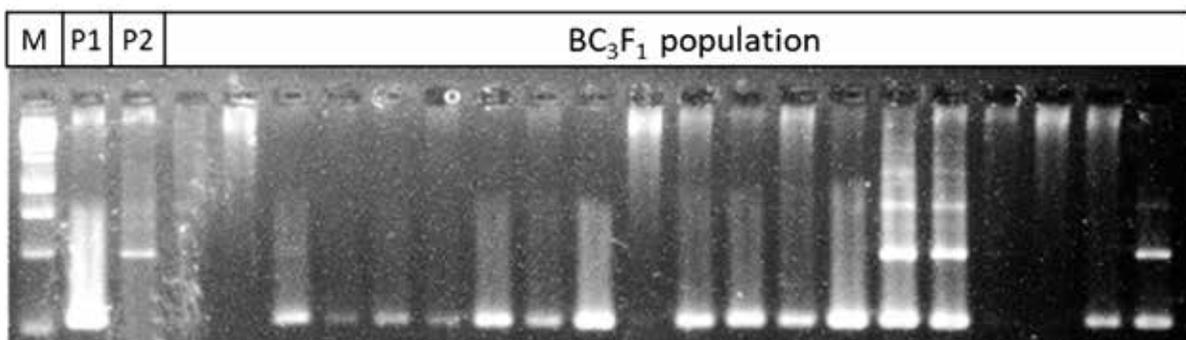
**Table 11:** List of backcrosses and selfed population and number of seeds for respective cross (Boro 2019-20)

Generation	Cross combination	No. of seeds
$BC_3F_1$	BRRIdhan49*IRBB60	35
$BC_3F_1$	BRRIdhan81*IRBB60/BRRIdhan81*Pi9-[US]/BRRIdhan81*Pb1	23
$BC_3F_1$	BRRIdhan81*IRBB58/BRRIdhan81*Pb1	29
$BC_3F_1$	BRRIdhan63-Pb1*IRBB60/BRRIdhan63*Pi9-[US]	70
$BC_2F_2$	BRRIdhan81*IRBB60/BRRIdhan81*Pi9-[US]/BRRIdhan81*Pb1	
$BC_2F_2$	BRRIdhan81*IRBB58/BRRIdhan81*Pb1	
$BC_2F_2$	BRRIdhan63-Pb1*IRBB60/BRRIdhan63*Pi9-[US]	
$BC_2F_2$	BRRIdhan63-Pb1*IRBB60	52
$BC_2F_2$	BRRIdhan81*IRBB60	46

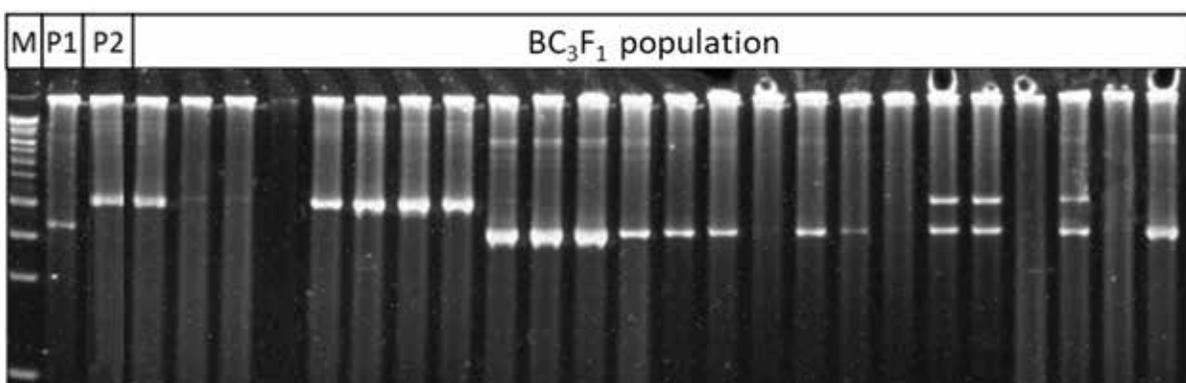
**Confirmation of  $BC_3F_1$  and Production of  $BC_3F_2$  population:**  $BC_3F_1$  seeds produced in boro 2019-20 were grown in T. aman 2020. Each plant was confirmed with gene specific molecular markers (Figure 20, 21 & 22). The selected plants were undergone for selfing to produce  $BC_3F_2$  population. The cross combination and number of produced seeds are presented in Table 12.



**Figure 20:** Confirmation of *Pi9* gene in  $BC_3F_1$  generation (M= 100bp ladder, P1= BRRIdhan81, P2= *Pi9*-[US])



**Figure 21:** Confirmation of *xal3* gene in BC<sub>3</sub>F<sub>1</sub> generation (M= 100bp ladder, P1= BRRI dhan63, P2= IRBB60)



**Figure 22:** Confirmation of *Xa21* gene in BC<sub>3</sub>F<sub>1</sub> generation (M= 100bp ladder, P1= BRRI dhan49, P2= IRBB60)

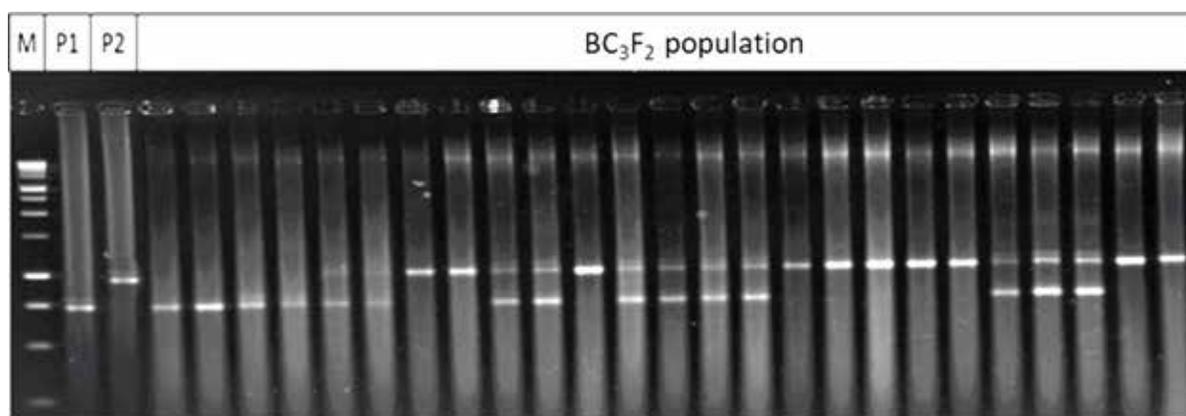
**Table 12:** List of BC<sub>3</sub>F<sub>2</sub> population along with resistant plants

Generation	Cross combination	No. of seeds
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan49*IRBB60	20
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan81*IRBB60/BRRI dhan81*Pi9-US/BRRI dhan81*Pb1	25
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan81*IRBB58/BRRI dhan81*Pb1	15
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan63-Pb1*IRBB60/BRRI dhan63*Pi9-US	9

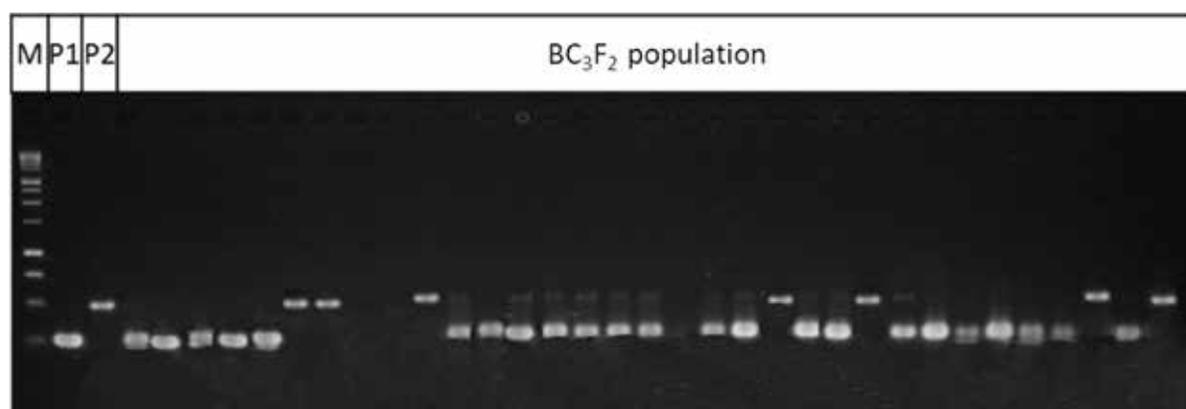
**Pathological and molecular screening of the progenies:** Backcross progenies of BC<sub>3</sub>F<sub>2</sub> and BC<sub>3</sub>F<sub>2</sub> generations from the different cross combinations (Table 13) were screened against virulent isolates (*Bxo67*, *Bxo87*, *Bxo91*) of bacterial blight. The selfing plants were screened following leaf clipping method. A total of 55 plants of different cross combinations (Table 13) were showed resistant reaction against bacterial blight. The plants showing resistant reaction were subjected to molecular screening to ensure the presence of resistant genes in the progenies using gene based tightly linked molecular markers (Table 3). Finally, a total of 10 plants having *Xa21*, *xal3*, *Pb1* and *Pi9* genes in the background of BRRI dhan81; 5 plants of containing *xal3*, *Pb1* and *Pi9* gene in the background of BRRI dhan63 and 4 plants of having *Xa21* gene in the background of BRRI dhan49 were selected using molecular markers (Figure 23-30).

**Table 13:** List of selfing population along with resistant plants

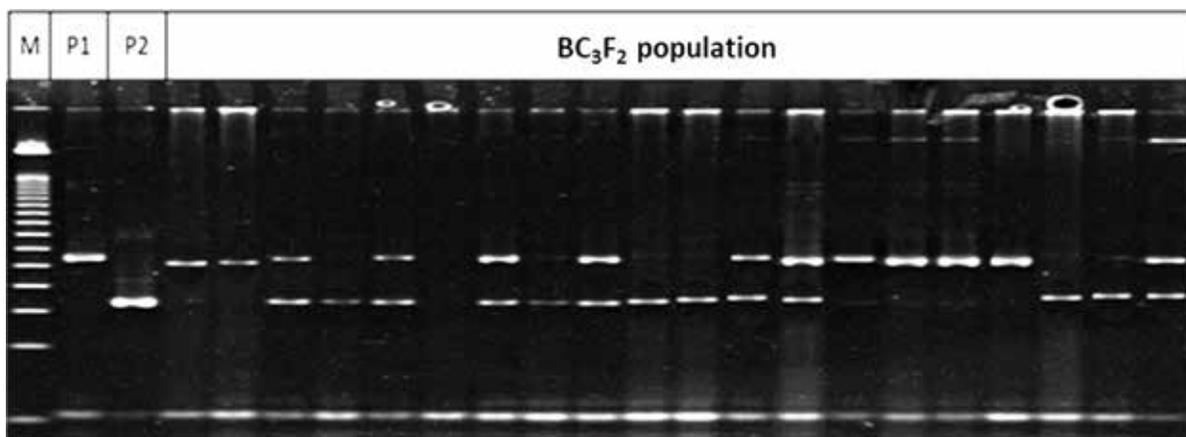
Generation	Cross combination	No. of Plants showing resistant reaction	No. of plant selected	Containing genes
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan49*IRBB60	6	4	<i>Xa21</i>
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan81*IRBB60/BRRI dhan81*Pi9-US/BRRI dhan81*Pb1	10	7	<i>xa13, Xa21, Pi9, Pb1</i>
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan81*IRBB58/BRRI dhan81*Pb1	12	3	<i>xa13, Xa21, Pb1</i>
BC <sub>3</sub> F <sub>2</sub>	BRRI dhan63-Pb1*IRBB60/BRRI dhan63*Pi9-US	13	4	<i>xa13, Pi9, Pb1</i>
BC <sub>2</sub> F <sub>2</sub>	BRRI dhan81*IRBB58/BRRI dhan81*Pb1	9	0	<i>Xa21, Pb1</i>
BC <sub>2</sub> F <sub>2</sub>	BRRI dhan63-Pb1*IRBB60/BRRI dhan63*Pi9-US	2	1	<i>xa13, Pi9, Pb1</i>



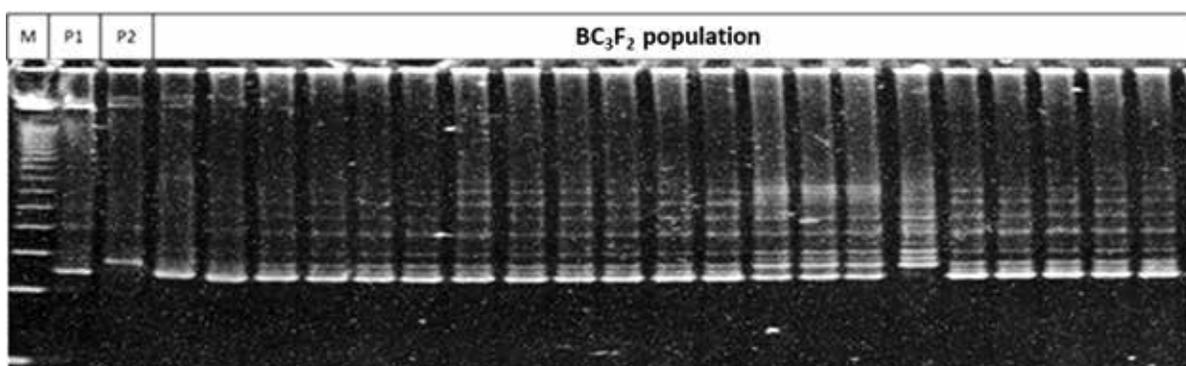
**Figure 23.** Confirmation of *Xa21* gene in BC<sub>3</sub>F<sub>2</sub> population (M= 1000 bp DNA marker, P1= BRRI Dhan81, P2= IRBB60)



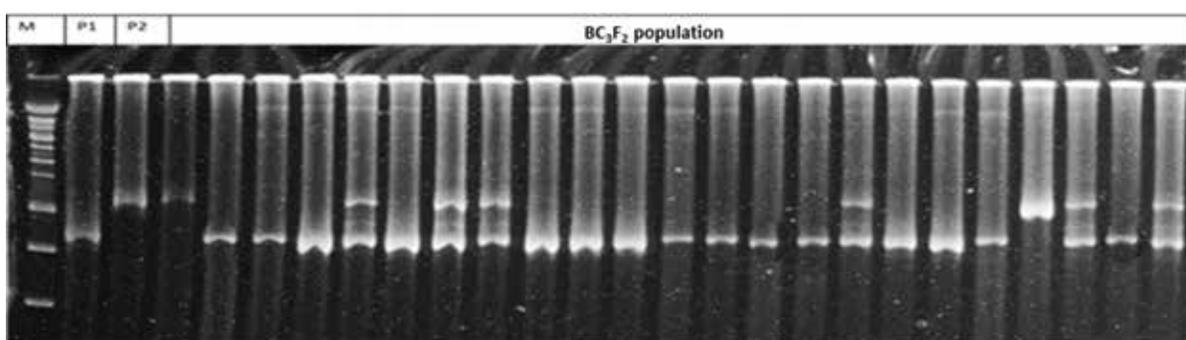
**Figure 24.** Confirmation of *xa13* gene in BC<sub>3</sub>F<sub>2</sub> population (M= 1000 bp DNA marker, P1= BRRI Dhan81, P2= IRBB60)



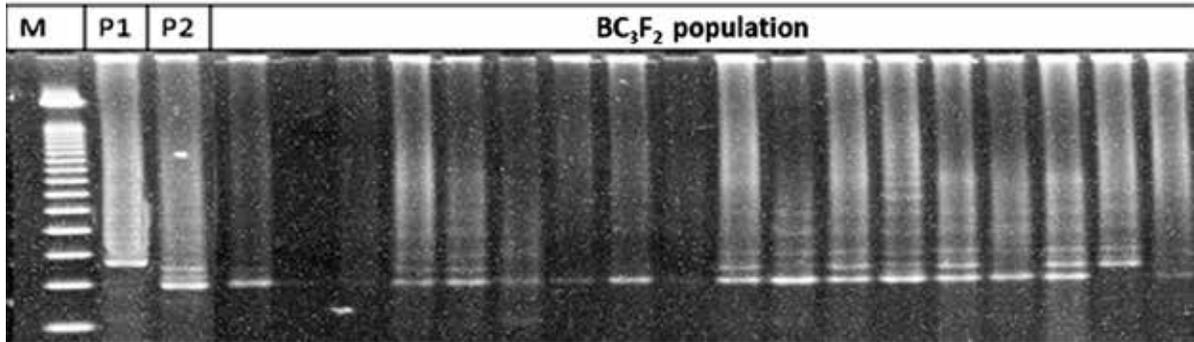
**Figure 25.** Confirmation of *Pi9* gene in  $BC_3F_2$  population (M= 100 bp DNA marker, P1= BRRi Dhan81, P2= *Pi9*-[US])



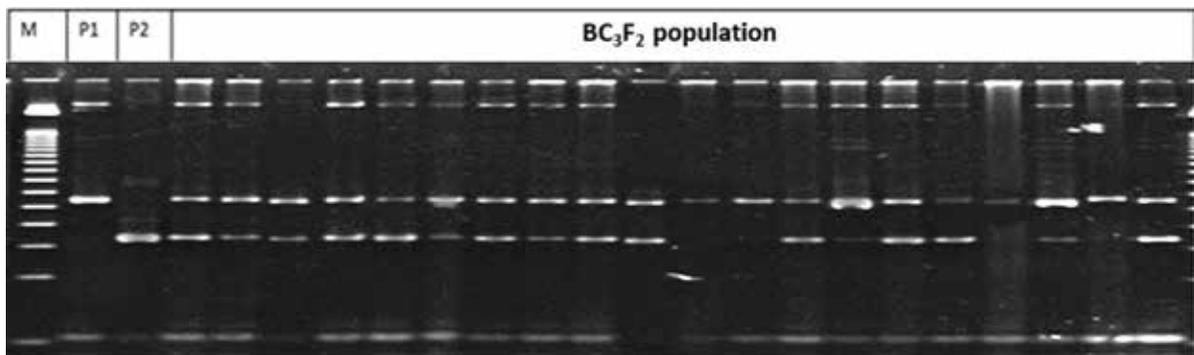
**Figure 26.** Confirmation of *Pbi* gene in  $BC_3F_2$  population (M= 100 bp DNA marker, P1= *Pbi*-[US], P2= BRRi Dhan81)



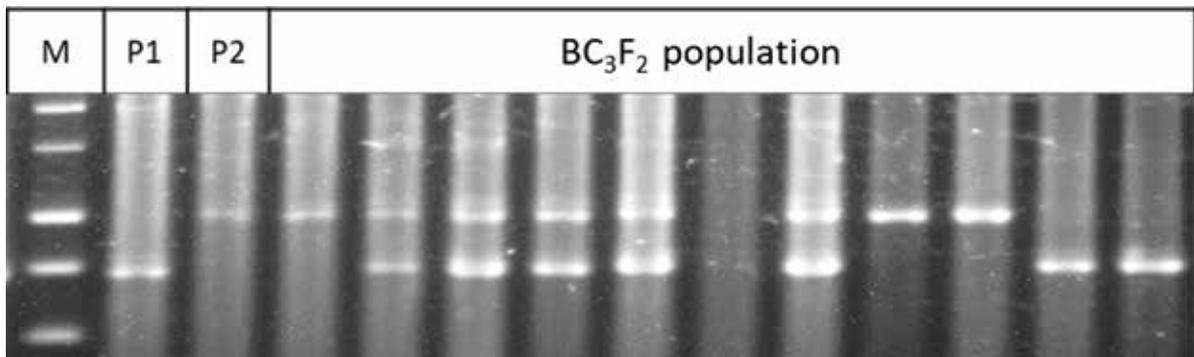
**Figure 27.** Confirmation of *Xa21* gene in  $BC_3F_2$  population (M= 1000 bp DNA marker, P1= BRRi Dhan63, P2= IRBB60)



**Figure 28.** Confirmation of *Pbl* gene in  $BC_3F_2$  population (M= 100 bp DNA marker, P1= BRR I Dhan63, P2= *Pbl*-[US])



**Figure 29.** Confirmation of *Pi9* gene in  $BC_3F_2$  population (M= 100 bp DNA marker, P1= BRR I Dhan63, P2= *Pi9*-[US])



**Figure 30.** Confirmation of *Xa21* gene in  $BC_3F_2$  population (M= 100 bp DNA marker, P1= BRR I Dhan49, P2= IRBB60)

Pyramiding of multiple resistance genes into rice varieties is one way to develop durable resistance to BB and blast. However, this approach is difficult through conventional breeding due to masking effects of genes such as *Xa21*, which provide resistance to many BB races. It is impossible to distinguish between plants having *Xa21* alone and those having *Xa21* and other genes. Marker-assisted selection allows the identification of plants with multiple resistance genes. Resistance (R) genes are largely used in rice breeding programs in Asia to control BB disease. Important prerequisites to the deployment of R genes are as follows: 1) to have an intensive knowledge of *Xoo* population structure, race distribution and frequency 2) to determine the durability of resistance of R genes to be deployed. Most of the Bacterial blight R genes provide complete race-specific resistance to *Xoo* strains. Different combinations of *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* have been incorporated in popular rice commercial varieties in different countries in Asia (Century *et al.*, 1999; Singh *et al.*, 2001; Swamy *et al.*, 2006; Perez *et al.*, 2008; Sundaram *et al.*, 2009; Shanti 2010; Suh *et al.*, 2013; Ruengphayak *et al.*, 2015). Few examples indicate that some R genes used for controlling BB disease are overcome by virulent strains in Korea with the resistant gene *Xa21* (Lee *et al.*, 1999; Zhang *et al.*, 2006).

Marker assisted selection (MAS) was applied for pyramiding three or four or five genes for BB resistance (*i.e.*, *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23*) and blast resistance (*Pi9* and *Pb1*). Pyramid lines IR 129336:11-4 or IR 129336:11-35 (*Xa4-xa5-xa13-Xa21-Xa23*) or IRBB60 (*Xa4*, *xa5*, *xa13* and *Xa21*) four or five genes were also developed at IRRI. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Huang *et al.*, 1997).

## **BAU component**

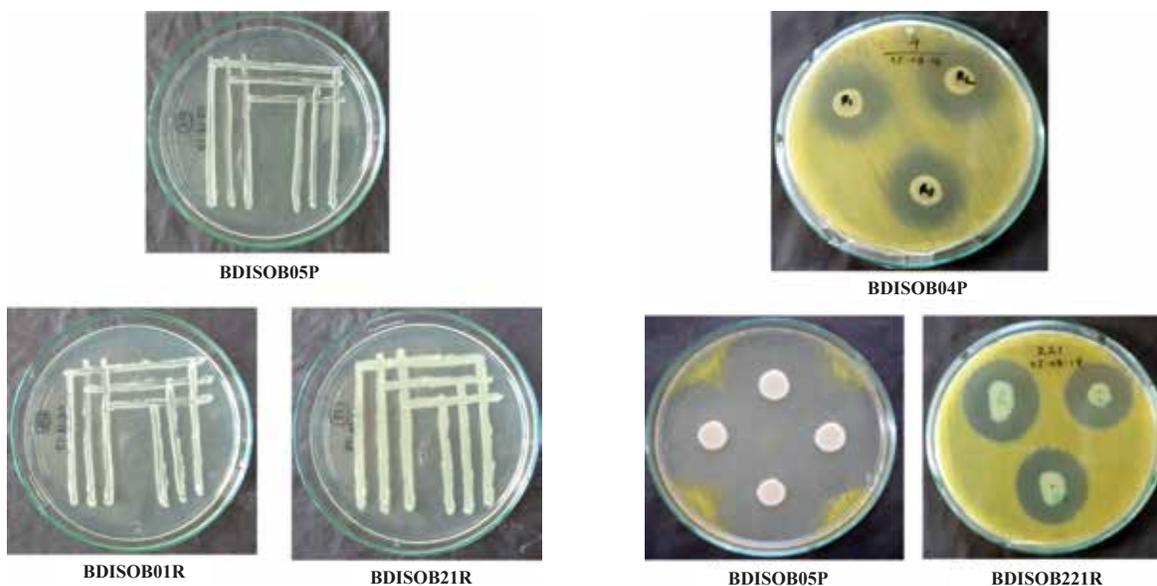
### **Isolation and identification of antagonistic bacteria against *Xanthomonas oryzae* pv. *oryzae***

Rice plant samples were collected from 40 districts of Bangladesh representing 30 AEZs during boro season (2017-2018 & 2018-2019) and aman season (2018 & 2019). To develop environment- friendly sustainable management approach against BB of rice, a total of 63 plant growth promoting bacteria were identified from rice phylloplane and rhizosphere during boro and aman seasons in 2018 and 2019 that inhibited the growth of *Xanthomonas oryzae* pv. *oryzae* by 20.83% to 76.19% (Figure. 31, 32 & 33).

Eighteen bacterial isolates were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* which was ranged by 28.39%-76.19% in boro season 2017-2018 (Table 14 & Figure. 34). The maximum (76.14%) growth inhibition of *X. oryzae* pv. *oryzae* *in vitro* was recorded by BDISOB05P while the minimum (28.59%) growth inhibition was exhibited by BDISOB272R. These antagonistic bacterial isolates were identified by sequencing of PCR products of 16S rDNA gene (Figure 34). In aman season, 2018, seventeen bacterial isolates were identified that inhibited 38.33-60.66% growth of *X. oryzae* pv. *oryzae* (Table 15 & Figure 35). The maximum (60.66%) growth inhibition of *X. oryzae* pv. *oryzae* was exhibited by BDISO147P and the minimum (38.33%) growth inhibition was shown by BDISO135P. However, in boro season 2018-2019, fourteen were identified as antagonist against *X. oryzae* pv. *oryzae*. These bacterial isolates inhibited 20.83%-60.87% growth of *X. oryzae* pv. *oryzae* *in vitro* (Table 16 & Figure. 36). The maximum (60.87%)

growth inhibition of *X. oryzae* pv. *oryzae* in vitro was recorded by BDISOB37R while the minimum (20.83%) growth inhibition was exhibited by BDISOB14R. On the other hand in aman season 2019, fourteen bacterial isolates were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* in vitro which was ranged by 50.83%-61.545% (Table 17 and Figure 37). The maximum (61.54%) growth inhibition of *X. oryzae* pv. *oryzae* in vitro was recorded by BDISOB54R while the minimum (50.93%) growth inhibition was exhibited by BDISOB12R. The results of the assessment of plant growth promoting determinants conceded that 48 bacterial species were found as positive for IAA (Indole Acetic Acid) production, all 63 bacterial species were found positive for siderophore production and 48 were found capable to solubilize insoluble phosphate out of these bacterial species (Table 14, 15, 16 & 17 and Figure 33).

The present findings were supported by Yasmin *et al.*, (2017). They showed consistent suppression of BLB pathogen in rice by different bacterial isolates. The findings of the present study also underpinning by the findings of Rahman *et al.*, (2007), who showed three bacterial isolates exhibited comparatively higher growth inhibition of *X. oryzae* pv. *oryzae*. Antagonistic bacteria can suppress plant pathogens either by directly or indirectly. Antibiotics, enzymes like chitinases, glucanases, proteases, and siderophores produce directly or indirect mechanisms in which the antagonistic bacteria compete with the pathogen for a niche or nutrient site (Bardin *et al.*, 2015). Isolates of *Pseudomonas* spp. have widely studied and exploited bacterial species as biocontrol agents (Kloepper *et al.*, 1989; Okon and Labandera-Gonzalez, 1994). It has been reported that *P. fluorescens* PDY7 can control BLB and enhance the growth of rice variety IR24 (Velusamy *et al.*, 2013). Forty-eight bacterial species were found positive for IAA production out of 69 antagonistic bacterial species identified in this study. IAA also has been speculated to improve the fitness of plant-microbe interactions (Patten and Glick, 2002). It was proved that many plant-associated bacteria have the ability to produce IAA take part in the most important role in plant growth promotion by stimulating plant roots development and improving absorption of water and nutrients from soil (Aslanta's *et al.*, 2007; Wu *et al.*, 2005). The IAA producing bacteria encouraged adventitious root formation, produced the greatest roots and shoots weight (Cakmakci *et al.*, 2007). All bacterial species were found to produce siderophore in the present study. It was known that microorganism that can produce siderophore provided Fe nutrition to enhance plant growth when iron element bioavailability was low (Crowley, 2006). It was also known for more than three decades that different bacterial species were capable to improve plant growth, contributed into plant Fe nutrition and promoted roots and shoots growth by producing siderophores (Verma *et al.*, 2011). Siderophore is particularly important when evaluating the potential of a strain for biocontrol (Manninen and Mattila-Sandholm, 1994). Siderophores have been suggested to be an environmentally friendly alternative to hazardous pesticides (Schenk *et al.*, 2012). The biological control mechanism depended on the role of siderophore as competitors for Fe in order to reduce Fe availability for the phytopathogen (Beneduzi *et al.*, 2012). Siderophores produced by numerous bacteria had a significant role in the biocontrol and negatively affected the growth of several pathogens (Yu *et al.*, 2011; Beneduzi *et al.*, 2012).

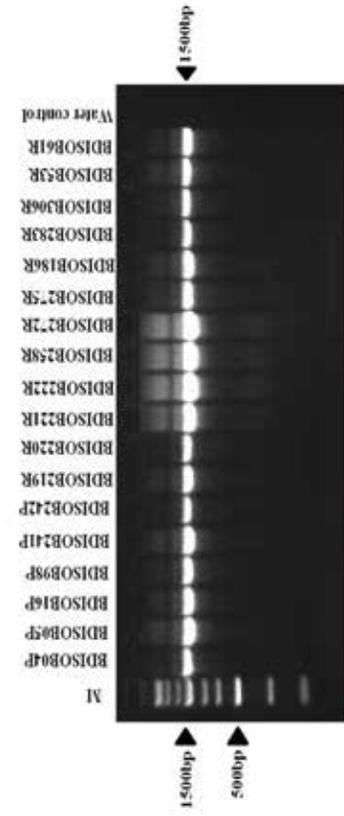


**Figure 31.** Representative photographs of purified bacterial isolates obtained from rice phylloplane and rhizosphere. BDISOB05P: an isolate from Mymensingh, BDISOB01R: an isolate from Mymensingh and BDISOB21R: an isolate from Chattagram.

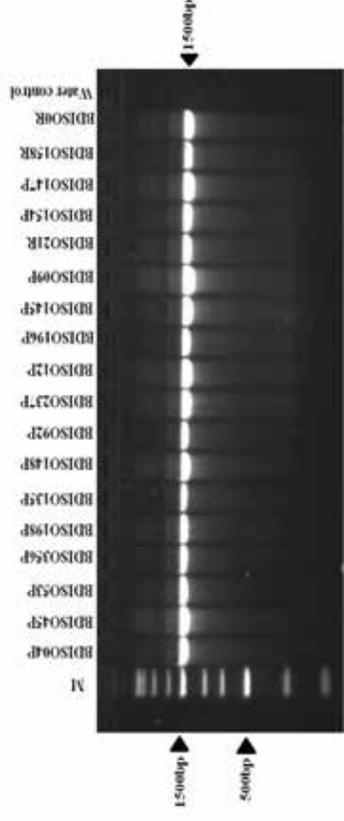
**Figure 32.** Representative photographs of in vitro growth inhibition of *X. oryzae* pv. *oryzae* by different potential bacterial isolates. BDISOB04P: an isolate from Cox's Bazar, BDISOB05P: an isolate from Mymensingh and BDISOB221R: an isolate from Chattagram.



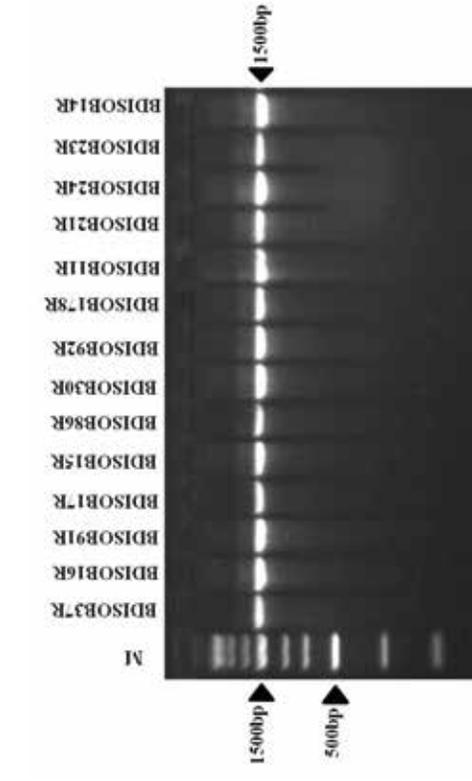
**Figure 33.** Representative photographs showing the assessment of different plant growth promoting determinants. **Siderophore production:** Antagonistic bacterial isolates showed positive siderophore production activity as indicated by orange halo zone around bacterial colony on CAS agar plates, **Phosphate solubilization:** Antagonistic bacterial isolates showed positive phosphate solubilizing activity by producing clear halo zone around the bacterial colony on National Botanical Research Institute's Phosphate (NBRIP) agar plates and **Indole acetic acid (IAA) production:** IAA activity by different antagonistic bacterial isolates indicated by the presence of pink color when bacterial culture supernatant mixed with Salkowskis reagent. BDISOB05P: isolate from Mymensingh.



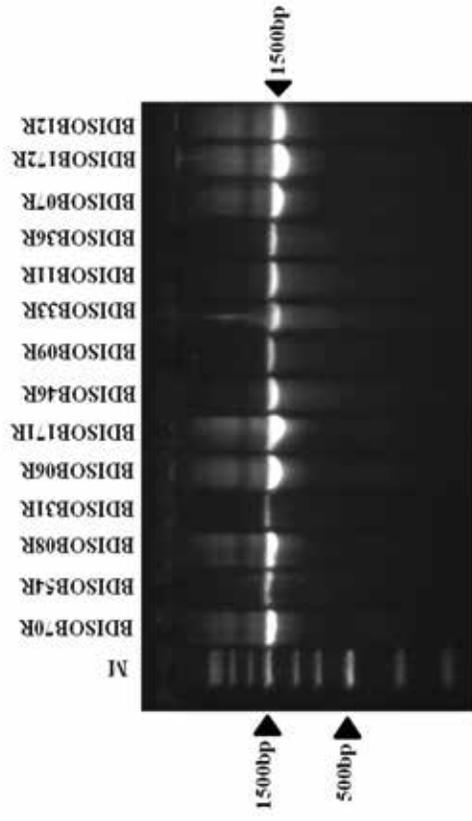
**Figure 34.** PCR confirmation of the antagonistic bacterial isolates by amplification of 16S rDNA using primers 27F and 1518R obtained from plant samples collected in boro season 2017-2018. These PCR products were then used for sequencing.



**Figure 35.** PCR confirmation of the antagonistic bacterial isolates by amplification of 16S rDNA using primers 27F and 1518R obtained from plant samples collected in aman season 2018. These PCR products were then used for sequencing.



**Figure 36.** PCR confirmation of the antagonistic bacterial isolates by amplification of 16S rDNA using primers 27F and 1518R obtained from plant samples collected in boro season 2018-2019. These PCR products were then used for sequencing.



**Figure 37.** PCR confirmation of the antagonistic bacterial isolates by amplification of 16S rDNA using primers 27F and 1518R obtained from plant samples collected in aman season 2019. These PCR products were then used for sequencing.

**Table 14.** List of antagonistic bacterial isolates (obtained in boro season 2017-2018) identified by homology search of sequences of 16S rDNA by BLAST program

Isolates	Closest relatives	Accession no.	Alignment	Homology (%)	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)	Plant Growth Promoting Determinants		
						IAA	Siderophore	Phosphate Solubilization
BDISOB04P	<i>Pseudomonas putida</i> strain PF41	MF838698.1	968/1086	89	61.67	+	+	+
BDISOB05P	<i>Pseudomonas putida</i> strain TB3	MH085459.1	931/1140	82	76.14	+	+	+
BDISOB16P	<i>Bacillus</i> sp. (in: Bacteria) strain VPS44	MH819972.1	702/738	95	59.94	+	+	+
BDISOB98P	<i>Stenotrophomonas maltophilia</i> strain AUX077_Japan	AY486381.1	1224/1271	96	33.04	+	+	-
BDISOB241P	<i>Burkholderia</i> sp. RBA1	GU979224.1	1154/1222	94	63.64	+	+	+
BDISOB242P	<i>Burkholderia gladioli</i> strain LMG 2121	MH748602.1	1186/1239	96	51.18	+	+	+
BDISOB219R	<i>Pseudomonas taiwanensis</i> strain GGRJ11	KC293831.1	913/969	94	63.12	-	+	-
BDISOB220R	<i>Serratia</i> sp. B2-254	FM875872.1	150/186	81	61.77	+	+	+
BDISOB221R	<i>Pseudomonas</i> sp. strain M2.2.1	MG021242.1	303/341	89	68.33	-	+	+
BDISOB222R	<i>Pseudomonas plecoglossicida</i> strain HFgGr	KC864769.1	614/751	82	64.79	-	+	-
BDISOB258R	<i>Pseudomonas putida</i> strain B-18	MF417798.1	917/1050	87	64.40	+	+	+
BDISOB272R	<i>Stenotrophomonas maltophilia</i> strain JC178	KJ534495.1	794/923	86	28.59	-	+	-
BDISOB275R	<i>Pseudomonas putida</i> strain P6	KT984874.1	1201/1229	98	71.86	+	+	+
BDISOB186R	<i>Pseudomonas</i> sp. E10 strain	JQ977022.1	29/29	100	64.43	+	+	+
BDISOB283R	<i>Pseudomonas fluorescens</i> strain B8	KF010368.1	969/1006	96	66.04	+	+	+
BDISOB306R	<i>Pseudomonas putida</i> strain DNCA01	KF030905.1	1298/1374	94	44.97	+	+	+
BDISOB53R	<i>Pseudomonas putida</i> strain p49_G07	JQ833720.1	53/60	88	48.19	+	+	+
BDISOB61R	<i>Delftia tsuruhatensis</i> strain As-23	MF353931.1	976/1168	84	38.54	+	+	+

‘+’ indicates the capability of antagonistic bacteria to produce siderophore, Indole Acetic Acid (IAA) and phosphate solubilization

‘-’ indicates the bacteria having no capability of producing siderophore, Indole Acetic Acid (IAA) and phosphate solubilization

**Table 15.** List of antagonistic bacterial isolates (obtained in aman season 2018) identified by homology search of sequences of 16SrDNA by BLAST program

Isolates	Closest relatives	Accession no.	Alignment	Homology (%)	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)	Plant Growth Promoting Determinants		
						IAA	Siderophore	Phosphate Solubilization
BDISO04P	<i>Pseudomonas putida</i>	FR749878.1	827/1080	96	46.37	+	+	+
BDISO45P	<i>Bacillus paramycoides</i>	MK467557.1	1027/1133	91	50.00	+	+	+
BDISO356P	<i>Pseudomonas hibiscicola</i>	KJ396817.1	1125/1148	98	46.83	+	+	-
BDISO198P	<i>Serratia plymuthica</i>	KU821695.1	472/530	89	50.00	+	+	+
BDISO135P	<i>Bacillus</i> sp.	KU146461.1	189/237	80	38.33	+	+	+
BDISO148P	<i>Serratia marcescens</i>	MN691926.1	929/990	94	54.26	-	+	-
BDISO92P	<i>Serratia marcescens</i>	MG996733.1	568/616	92	44.18	+	+	+
BDISO237P	<i>Alcaligenes faecalis</i>	KR827435.1	1048/1102	95	57.19	-	+	+
BDISO12P	<i>Alcaligenes faecalis</i>	MN513225.1	927/1094	85	57.44	-	+	-
BDISO196P	<i>Alcaligenes faecalis</i>	MN513225.1	901/1111	81	46.18	+	+	+
BDISO145P	<i>Serratia marcescens</i>	MF360051.1	545/630	87	40.00	-	+	-
BDISO09P	<i>Serratia marcescens</i>	MN252007.1	171/185	92	44.47	+	+	+
BDISO21R	<i>Serratia marcescens</i>	MG557818.1	194/200	97	54.60	+	+	+
BDISO154P	<i>Pseudomonas taiwanensis</i>	MN416314.1	161/178	90	47.22	+	+	+
BDISO147P	<i>Serratia marcescens</i>	MF716688.1	1086/1130	96	60.66	+	+	+
BDISO158R	<i>Serratia marcescens</i>	MK346258.1	866/953	91	47.27	+	+	+
BDISO00R	<i>Bacillus amyloliquefaciens</i>	KC888017.1	1151/1153	99	50.00	+	+	+

‘+’ indicates the capability of antagonistic bacteria to produce siderophore, Indole Acetic Acid (IAA) and phosphate solubilization  
‘-’ indicates the bacteria having no capability of producing siderophore, Indole Acetic Acid (IAA) and phosphate solubilization

**Table 16.** List of antagonistic bacterial isolates (obtained in boro season 2018-2019) identified by homology search of sequences of 16SrDNA by BLAST program

Isolates	Closest relatives	Accession no.	Alignment	Homology (%)	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)	Plant Growth Promoting Determinants		
						IAA	Siderophore	Phosphate Solubilization
BDISOB37R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456599.1	275/298	92	60.87	+	+	+
BDISOB16R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456599.1	275/298	92	57.09	+	+	+
BDISOB91R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456543.1	748/841	89	56.55	+	+	+
BDISOB17R	<i>Limnolyngbya circumcreta</i>	KR697754.1	86/105	82	43.42	+	+	+
BDISOB15R	<i>Pseudochrobactrum asaccharolyticum</i>	KM921740.1	399/535	75	49.94	-	+	-
BDISOB86R	<i>Enterobacter aerogenes</i>	KM503142.1	444/483	92	45.75	+	+	+
BDISOB30R	<i>Pseudochrobactrum asaccharolyticum</i>	MK100767.1	166/177	94	47.73	-	+	+
BDISOB92R	<i>Pseudomonas fluorescens</i>	KJ027533.1	29/29	100	45.44	-	+	-
BDISOB178R	<i>Serratia marcescens</i>	MN691653.1	635/679	94	45.91	+	+	+
BDISOB11R	<i>Pseudochrobactrum saccharolyticum</i>	MK377096.1	770/827	93	40.00	+	+	+
BDISOB21R	<i>Stenotrophomonas maltophilia</i>	MN173472.1	994/1084	92	38.42	+	+	+
BDISOB24R	<i>Pseudochrobactrum accharolyticum</i>	FJ950551.1	994/1084	92	36.55	+	+	+
BDISOB23R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456600.1	1082/1122	96	32.46	+	+	+
BDISOB14R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456600.1	535/541	99	20.83			

‘+’ indicates the capability of antagonistic bacteria to produce siderophore, Indole Acetic Acid (IAA) and phosphate solubilization  
‘-’ indicates the bacteria having no capability of producing siderophore, Indole Acetic Acid (IAA) and phosphate solubilization

**Table 17.** List of antagonistic bacterial isolates (obtained in aman season 2019) identified by homology search of sequences of 16S rDNA by BLAST program

Isolates	Closest relatives	Accession no.	Alignment	Homology (%)	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)	Plant Growth Promoting Determinants		
						IAA	Siderophore	Phosphate Solubilization
BDJSOB70R	<i>Serratia marcescens</i>	MG571677.1	239/300	80	52.38	+	+	+
BDJSOB54R	<i>Burkholderia gladioli</i>	MH748601.1	1050/1108	95	61.54	+	+	-
BDJSOB08R	<i>Serratia marcescens</i>	KU963569.1	100/114	88	59.31	+	+	+
BDJSOB31R	<i>Serratia marcescens</i>	MN691926.1	929/990	94	59.17	-	+	-
BDJSOB06R	<i>Serratia marcescens</i>	MG571677.1	111/127	87	59.26	+	+	+
BDJSOB171R	<i>Alcaligenes faecalis</i>	MN513225.1	927/1094	85	57.37	-	+	-
BDJSOB46R	<i>Serratia marcescens</i>	MF360051.1	545/630	87	55.53	-	+	-
BDJSOB09R	<i>Serratia marcescens</i>	MN252007.1	171/185	92	55.92	+	+	+
BDJSOB33R	<i>Serratia marcescens</i>	KJ535346.1	127/143	89	52.27	+	+	+
BDJSOB11R	<i>Serratia marcescens</i>	MK806681.1	88/98	90	53.57	+	+	+
BDJSOB36R	<i>Serratia marcescens</i>	MK961214.1	787/910	86	58.33	+	+	+
BDJSOB07R	<i>Serratianem atodiphila</i>	MN691930.1	572/639	90	52.00	+	+	+
BDJSOB172R	<i>Bacillus aerophilus</i>	KY307912.1	874/1043	84	51.19	+	+	+
BDJSOB12R	<i>Serratia marcescens</i>	MH074778.1	780/841	93	50.93	+	+	+

‘+’ indicates the capability of antagonistic bacteria to produce siderophore, Indole Acetic Acid (IAA) and phosphate solubilization  
‘-’ indicates the bacteria having no capability of producing siderophore, Indole Acetic Acid (IAA) and phosphate solubilization

### **Plant growth promotion by bacterial isolates antagonistic to *X. oryzae* pv. *oryzae***

Based on the growth inhibition of *X. oryzae* pv. *oryzae* by these antagonistic bacterial species, 32 bacterial isolates were selected for the assessment of their performances in plant growth promotion, in reducing BB severity and in increasing yield of rice.

Different plant growth promoting bacterial antagonists enhanced the root length, shoot length and vigour index at 14, 21 and 28 DAS (Table 18). Among 32 bacterial isolates, the maximum vigour index (4198.29) was recorded in seedlings raised from seeds treated with BDISOB45PanR (*Bacillus paramycooides*) followed by BDISOB283R (*Pseudomonas fluorescens*) (4087.60), BDISOB222R (*Pseudomonas plecoglossicida*) (4040.97) while the minimum (2418.03) vigour index was obtained in BDISOB135SheR (*Bacillus* sp.) followed by BDISOBP (*Serratia marcescens*) (2449.53) and BDISOB54R (*Burkholderia gladioli*) (2449.53) at 30 DAS. However, all the antagonistic bacterial isolates exhibited the increase of vigour index ranged by 0.01 to 71.41. This result implies that some of the selected antagonistic bacterial isolates have the potentiality in enhancing plant growth.

In sustainable agriculture, certain plant pathogens can be controlled by biological agents like plant growth promoting bacteria (PGPB) and PGPB can also be used as bio-fertilizer (Shanthi and Vittal, 2013). There are a lot of PGPB strains that reported to suppress numerous of plant pathogen, reduce the disease incidence, stimulate the plant growth factor and supplies the nutrition for the growth of the plant (Hariprasad *et al.*, 2009; Yasmin *et al.*, 2017). Therefore, it has been shown considerable research interest in the potential use of antagonistic bacteria as PGPB (Babalola, 2010; Kumar *et al.*, 2012). Different plant growth promoting bacterial antagonists having significant impact in increasing root length, shoot length and vigour index. were selected based on their antagonistic capability and also in increasing plant growth compared to control. Similarly, Sakthivel *et al.* (1986) and Mishra and Sinha (1998) also reported enhanced growth of rice seedling with bioagent application. van Peer and Schippers (1989) stated that shoot, root and fresh weight was increased for tomato, cucumber, lettuce, and potato as a result of bacterization with *Pseudomonas* strains. The results of the present study depict that the effect of plant growth promoting bacterial isolates on growth and vigour of rice plants was significantly higher over control.

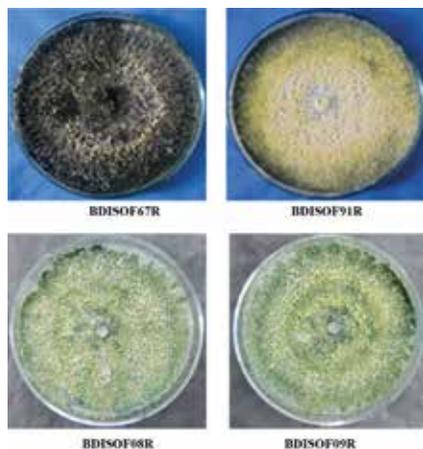
**Table 18.** Effect of different antagonistic bacteria on plant growth promotion of rice (cv. IR24)

Treatments	Root length (cm)						% increase of root length over control						Shoot length (cm)						% increase of shoot length over control						Vigour Index						% increase of vigour Index over control																	
	Days after sowing (DAS)												Days after sowing (DAS)												Days after sowing (DAS)												Days after sowing (DAS)											
	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28												
Control	6.76	9.20	11.28	0.00	0.0	0.0	10.72	11.97	17.23	0.00	0.00	0.00	13.16	32.20	46.56	2046.56	2449.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1316.32	2046.56	2449.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00												
BDISOB04P	9.12	12.31	13.20	34.91	33.80	17.02	12.37	16.77	23.07	15.39	40.10	33.89	1697.18	2306.48	2877.95	28.93	12.70	17.50																														
BDISOB05P	8.23	12.22	12.84	21.75	32.83	13.83	12.37	16.53	18.32	15.39	38.10	6.33	1634.27	2549.46	2658.42	24.15	24.57	8.54																														
BDISOB219R	8.69	12.22	12.58	28.55	32.83	11.52	12.40	16.53	18.88	15.67	38.10	9.58	1869.68	2549.46	2790.04	42.04	24.57	13.91																														
BDISOB221R	8.43	11.13	11.30	24.70	20.98	0.18	11.90	15.65	19.53	11.01	30.74	13.35	1647.00	2169.45	2497.50	25.12	6.00	1.97																														
BDISOB222R	10.63	14.95	16.23	57.25	62.50	43.88	15.12	21.15	27.85	41.04	76.69	61.64	2360.42	3309.17	4040.97	79.32	61.69	64.98																														
BDISOB258R	9.12	13.04	13.37	34.91	41.74	18.53	12.37	17.60	23.42	15.39	47.03	35.93	1697.18	2420.82	2906.41	28.93	18.29	18.66																														
BDISOB186R	8.12	11.75	13.50	20.12	27.72	19.68	12.00	17.38	22.32	11.94	45.20	29.54	1595.92	2311.51	2841.72	21.24	12.95	16.02																														
BDISOB283R	10.90	14.87	16.11	61.24	61.63	42.82	14.68	21.22	29.65	36.94	77.28	72.08	2285.44	3223.44	4087.60	73.62	57.51	66.89																														
BDISOB04P	7.72	12.42	12.84	14.20	35.00	13.83	11.88	17.37	18.32	10.82	45.11	6.33	1672.53	2541.40	2658.42	27.06	24.18	8.54																														
BDISOB45P	10.32	14.25	15.00	52.66	54.89	32.98	14.18	21.73	30.33	32.28	81.54	76.03	2237.67	3286.48	4198.29	69.99	60.59	71.40																														
BDISOB198P	8.65	11.38	63.00	27.96	23.70	458.51	11.43	13.35	20.07	6.62	11.53	16.48	1687.00	2127.07	2689.20	28.16	3.93	9.79																														
BDISOB135P	7.82	11.45	12.33	15.68	24.46	9.31	12.90	15.53	20.05	20.34	29.74	16.37	1788.00	2329.56	2418.03	35.83	13.83	-1.28																														
BDISOB148P	8.65	11.38	12.05	27.96	23.70	6.83	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB01R	8.33	11.38	13.36	23.22	23.70	18.44	12.72	13.35	23.65	18.66	11.53	37.26	1810.30	2127.07	3187.73	37.53	3.93	30.15																														
BDISOB145P	8.65	11.38	13.42	27.96	23.70	18.97	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB158R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB37R	8.13	12.66	12.33	20.27	37.61	9.31	12.18	16.52	20.07	13.62	38.01	16.48	1632.11	2324.41	2689.20	23.99	13.58	9.79																														
BDISOB16R	8.34	11.95	12.12	23.37	29.89	7.45	11.57	18.52	24.45	7.93	54.72	41.90	1585.63	2528.73	3071.60	20.46	23.56	25.41																														
BDISOB92R	7.10	13.06	12.38	5.03	41.96	9.75	12.02	15.87	20.28	12.13	32.58	17.70	1587.24	2429.56	2613.33	20.58	18.71	6.70																														
BDISOB21R	8.65	11.62	13.52	27.96	26.30	19.86	11.43	12.50	19.43	6.62	4.43	12.77	1687.00	1792.92	2449.53	28.16	-12.39	0.01																														
BDISOB17R	7.10	11.45	13.36	5.03	24.46	18.44	12.02	15.53	20.57	12.13	29.74	19.38	1587.24	2329.56	2567.37	20.58	13.83	4.82																														
BDISOB15R	8.13	12.66	12.33	20.27	37.61	9.31	12.18	16.52	20.07	13.62	38.01	16.48	1632.11	2324.41	2689.20	23.99	13.58	9.79																														
BDISOB86R	8.13	12.66	12.33	20.27	37.61	9.31	12.18	16.52	20.07	13.62	38.01	16.48	1632.11	2324.41	2689.20	23.99	13.58	9.79																														
BDISOB30R	8.13	12.66	12.33	20.27	37.61	9.31	12.18	16.52	20.07	13.62	38.01	16.48	1632.11	2324.41	2689.20	23.99	13.58	9.79																														
BDISOB7R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB12R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB31R	8.49	11.38	13.36	25.59	23.70	18.44	12.72	13.35	20.57	18.66	11.53	19.38	1604.39	2127.07	2567.37	21.88	3.93	4.82																														
BDISOB36R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB46R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB54R	7.87	11.62	13.52	16.42	26.30	19.86	11.77	12.50	19.43	9.79	4.43	12.77	1459.41	1792.92	2449.53	10.87	-12.39	0.01																														
BDISOB70R	8.65	11.38	13.36	27.96	23.70	18.44	11.43	13.35	20.57	6.62	11.53	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82																														
BDISOB172R	8.40	12.35	12.84	24.26	34.24	13.83	13.00	16.92	22.92	21.27	41.35	33.02	1719.13	2351.09	2872.18	30.60	14.88	17.26																														

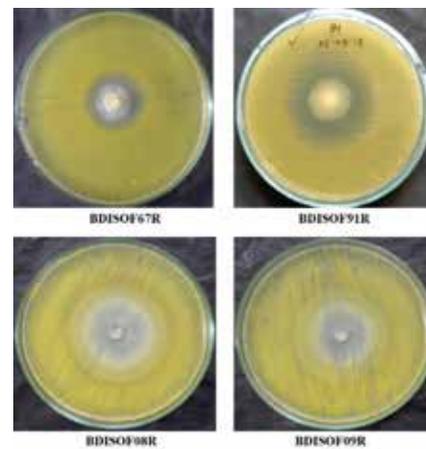
### Isolation and identification of plant growth promoting fungi antagonistic to *X. oryzae* pv. *oryzae*

A total of 100 fungal isolates were isolated and purified from rice plant samples during boro season, 2017-2018 and around 100 fungal isolates were isolated and purified in boro season, 2018-2019. Out of these fungal isolates, two fungal isolates were identified as antagonists against *X. oryzae* pv. *oryzae* in boro season 2017-2018 that inhibited the growth of *X. oryzae* pv. *oryzae* by 67.51% and 52.19% and two were identified as antagonists against *X. oryzae* pv. *oryzae* in boro season 2018-2019 that inhibited the growth of *X. oryzae* pv. *oryzae* by 24.06% and 23.33% (Table 19, Figure 38 & 39). These four fungal isolates were identified by sequencing of ITS region. Sequence analyses by BLAST program revealed that the fungal isolates were BDFISO67R (*Trichoderma paraviridescens*), BDFISO91R (*Trichoderma erinaceum*), BDISOF08R (*Trichoderma asperellum*) and BDISOF09R (*Trichoderma asperellum*) (Table 19 & Figure 40). Previously identified fungal species were also used as effective biocontrolling agents in various types of crops, the use of *Trichoderma* was reported to control *X. oryzae* pv. *oryzae* causing bacterial leaf blight of rice by Gangwar *et al.*, (2010) they screened fifty-two isolates of *Trichoderma* spp. obtained from different sources *viz.* soil, rice rhizosphere and rice leaves for their bio-control ability against *X. oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of rice.

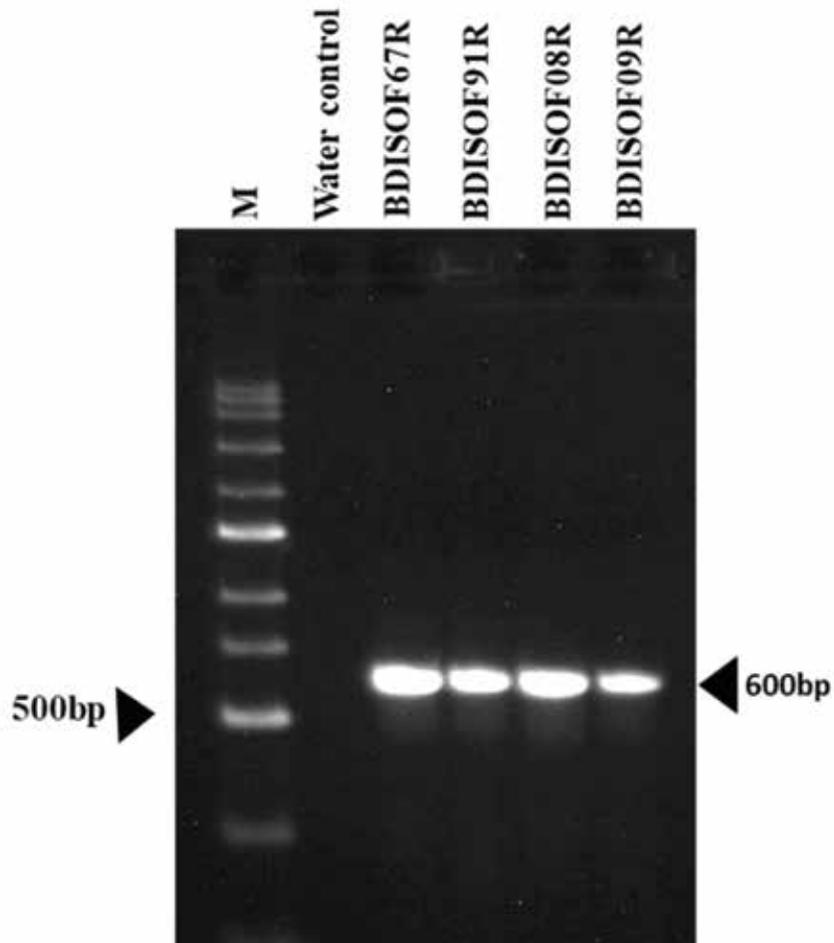
The growth promoting analyses showed that BDISOF08R (*Trichoderma asperellum*) exerted maximum root and shoot length with vigour index (2633.40) which is 10.49% increase of vigour index over control followed by BDFISO67R (*Trichoderma paraviridescens*) (2623.50). The minimum root and shoot length with vigour index were recorded in seedlings raised from seeds treated with BDISOF09R (*Trichoderma asperellum*) (Table 20).



**Figure 38.** Pure culture of beneficial fungi identified from rhizosphere soil samples collected in boro seasons (2017-2018 & 2018-2019) antagonistic to *X. oryzae* pv. *oryzae*. BDISOF67R: isolate from Bogura, BDISOF91R: isolate from Habiganj, BDISOF08R, a rhizosphere isolate, Jashore and BDISOF09R, a rhizosphere isolate, Jashore. Photographs were taken after 72 hrs of inoculation.



**Figure 39.** Pure culture of beneficial fungi identified from rhizosphere soil samples collected in boro seasons (2017-2018 & 2018-2019) antagonistic to *X. oryzae* pv. *oryzae*. BDISOF67R: isolate from Bogura, BDISOF91R: isolate from Habiganj, BDISOF08R, a rhizosphere isolate, Jashore and BDISOF09R, a rhizosphere isolate, Jashore. Photographs were taken after 72 hrs of inoculation.



**Figure 40.** PCR confirmation by amplification of ITS region of the beneficial fungi antagonistic to *X. oryzae* pv. *oryzae* identified from rhizosphere samples collected in boro seasons (2017-2018 & 2018-2019). M: DNA ladder, W: Water control, BDISOF67R: isolate from Bogura, BDISOF91R: isolate from Habiganj, BDISOF08R and BDISOF09R: rhizosphere isolates from Jessore.

**Table 19.** List of beneficial fungi (obtained in boro seasons 2017-2018 & 2018-2019) identified by homology search of sequences of ITS region by BLAST program

Isolates	Closest relatives	Accession no.	Alignment	Homology (%)	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)
BDFISO67R	<i>Trichoderma paraviridescens</i>	MF782827.1	602/605	99	67.51
BDFISO91R	<i>Trichoderma erinaceum</i>	KY2225644.1	605/610	99	52.19
BDISOF08R	<i>Trichoderma asperellum</i>	KU878976.1	298/318	94	24.06
BDISOF09R	<i>Trichoderma asperellum</i>	KY2225660.1	185/205	90	23.33

**Table 20.** Effect of different antagonistic fungi on plant growth promotion of rice (cv. IR24)

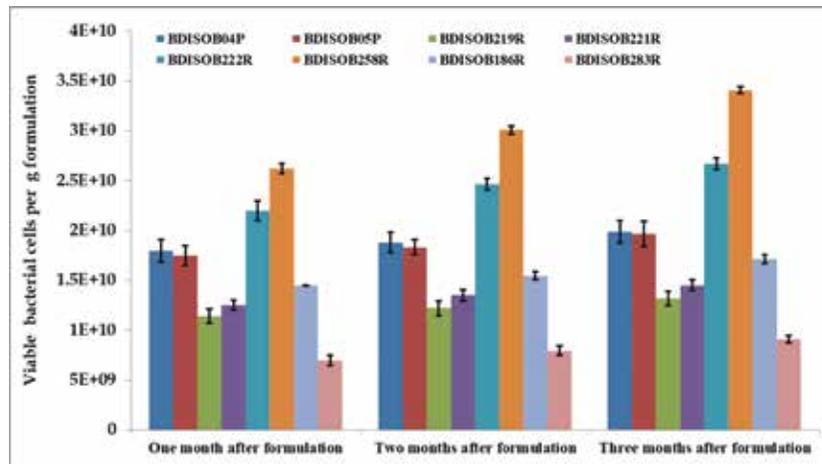
Treatments	Root length (cm)		% increase of root length over control		Shoot length (cm)			% increase of shoot length over control			Figure Index			% increase of figure Index over control				
	Days after sowing (DAS)																	
	7	14	30	7	14	30	7	14	30	7	14	30	7	14	30	7	14	30
Control	4.00	7.05	7.20	0.00	0.00	0.00	9.03	18.49	19.30	0.00	0.00	1172.20	2297.02	2383.38	0.00	0.00	0.00	
BDISOF67R	4.94	7.25	8.12	23.50	2.84	12.78	10.65	19.51	21.03	17.94	5.52	1403.40	2408.70	2623.50	19.72	4.86	10.07	
BDISOF91R	4.26	7.20	7.56	6.50	2.13	5.00	9.77	18.87	20.64	8.19	2.06	1263.00	2346.30	2537.70	7.75	2.15	6.47	
BDISOF08R	4.94	7.07	8.04	23.50	0.28	11.67	10.65	19.51	21.22	17.94	5.52	1403.40	2392.50	2633.40	19.72	4.16	10.49	
BDISOF09R	4.12	7.20	7.40	3.00	2.13	2.78	9.37	19.30	20.36	3.77	4.38	1214.40	2384.70	2498.10	3.60	3.82	4.81	

### **Field efficacy of formulated antagonistic bacterial isolates (identified in boro seasons 2017-2018 and 2018-2019) in reducing BB severity and in increasing rice yield**

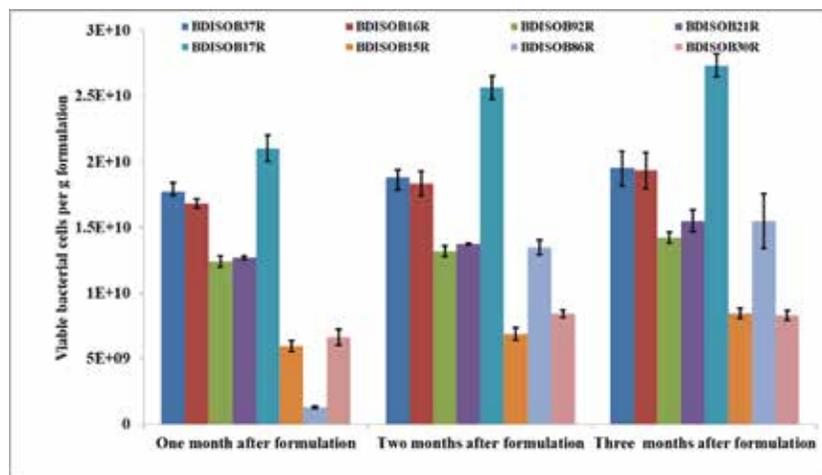
Depending on the weather conditions and AEZs mega inbred rice varieties such as BRRIdhan28 and BRRIdhan29 as well as hybrid rice varieties are generally susceptible to BB disease in boro season. Therefore, the efficacy of some selected identified antagonistic plant growth promoting bacteria were evaluated against BB of rice in two selected hybrids viz. Hybrid Hera 2 and ArizeTej Gold and two mega inbred viz. BRRIdhan28 and BRRIdhan29 rice varieties under both net house and field condition during boro seasons 2018-2019 and 2019-2020. Bacterial strains identified in boro season 2017-2018 and 2018-2019 were formulated and these formulated bacterial strains could survive for at least three months after talc-based formulation (Figure 41 & Figure 42). The formulated bacterial bioagents identified in boro season 2017-2018 reduced lesion length by 48.38% to 62.20% compared to control under net house condition (Table 21) and by 46.93% to 50.03% under field condition considering all four varieties (Table 22). These same formulated bacterial bioagents increased yield by 13.58% and 29.94% compared to control (Table 23). On the other hand, formulated bacterial bioagents identified in boro season 2018-2019 reduced lesion length by 40.83% to 54.64% under net house condition (Table 24) and by 41.46% to 70.16% compared to control under field condition (Table 25). However, these formulated bacterial bioagents increased yield by 13.33% and 20.37% compared to control (Table 26). The plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide) [T<sub>1</sub>] reduced lesion length significantly and was considered as positive control in the study (Table 21, 22, 27 & 28).

The results of the present study clearly indicate the antagonistic potential of some species of *Pseudomonas*, *Bacillus* and *Serratia* against *X. oryzae* pv. *oryzae* and suppressive activity against BB of rice under both net house and field condition. *Pseudomonas putida* was identified as a potential antagonistic bacterial isolate to *X. oryzae* pv. *oryzae* and observed significant suppression of BB pathogen (Yasmin *et al.*, 2016). They speculated that these suppressions of BB pathogen by *Pseudomonas putida* through production of secondary metabolites. Strong activities of *P. fluorescens* against *X. oryzae* pv. *oryzae* were also observed (Ramanamma *et al.*, 2017). Seven *P. fluorescens* isolates were found significantly superior to other isolates in inhibiting *X. oryzae* pv. *oryzae* out of thirty-five isolates (Meera and Balabaskar, 2012). However, Velusamy *et al.* (2006) reported 59%–64% suppression of bacterial blight in rice under net-house and field experiments. In another study, the antagonistic potential of *Pseudomonas fluorescens* isolate RRb-11 against BB pathogen of rice was also observed (Jambhulkar and Sharma (2014). In field study, the best results they obtained when talc based bioformulation of *P. fluorescens* RRb-11 was applied as seed treatment, seedling root dip and soil application in combination which reduced the disease by 92.3% and 88.5% over control. This treatment also produced maximum yield of 3.88 t/ha *i.e.*, 61% greater than control. However, in our study we applied the formulated bacterial isolates as seed treatment and foliar application. Our study suggests that rice associated *Bacillus* spp. are predominant in rice plants as antagonists against BB pathogen, *X. oryzae* pv. *oryzae*. *Bacillus* spp. has been reported to control many crop diseases (Jacobsen *et al.*, 2004; Perez-Garcia *et al.*, 2011). Recent examples include citrus canker caused by *X. axonopodis* pv. *citri* (Huang *et al.*, 2012), bacterial wilt and late blight in tomato caused by *Ralstonia solanacearum* and *Phytophthora infestans*, respectively (Chen *et al.*, 2013; Kabir *et al.*, 2013;

Tan *et al.*, 2013), bacterial blight in rice caused by *X. oryzae* pv. *oryzae* (Chithrashree 2011), and wheat root rot caused by *Fusarium graminearum* (Moussa *et al.*, 2013). Shrestha *et al.*, (2016) reported *B. amyloliquefaciens* as potential bioagents against sheath blight and panicle blight of rice. In addition, *B. amyloliquefaciens* were found as a good biological control agent for other crop diseases, including bacterial wilt and powdery mildew of tomato (Tan *et al.*, 2013; Yamamoto *et al.*, 2015), stem rot of canola (Wu *et al.*, 2014), Wang and Liang 2014), bacterial wilt of peanut (Wang and Liang, 2014), ring rot of apple (Li *et al.*, 2013), and bacterial soft rots of vegetables (Zhao *et al.*, 2013).



**Figure 41.** Viability of formulated plant growth promoting antagonistic bacteria. T<sub>2</sub>= BDISOB04 (*Pseudomonas putida*), T<sub>3</sub>= BDISOB05P (*Pseudomonas putida*), T<sub>4</sub>= BDISOB219R (*Pseudomonas taiwanensis*), T<sub>5</sub>= BDISOB221R (*Pseudomonas* sp.), T<sub>6</sub>= BDISOB222R (*Pseudomonas plecoglossicida*), T<sub>7</sub>= BDISOB258R (*Pseudomonas putida*), T<sub>8</sub>= BDISOB186R (*Pseudomonas* sp.) and T<sub>9</sub>= BDISOB283R (*Pseudomonas fluorescens*)



**Figure 42.** Viability of formulated plant growth promoting antagonistic bacteria. BDISOB37R (*Pseudochrobactrum asaccharolyticum*), BDISOB16R (*Pseudochrobactrum asaccharolyticum*), BDISOB92R (*Pseudomonas fluorescens*), BDISOB21R (*Stenotrophomonas maltophilia*), BDISOB17R (*Limnolyngbya circumcreta*), BDISOB15R (*Pseudochrobactrum asaccharolyticum*), BDISOB86R (*Enterobacter aerogenes*) and BDISOB30R (*Pseudochrobactrum asaccharolyticum*).

**Table 21.** Effect of different formulated bacterial antagonists (identified in boro season 2017-2018) in reducing lesion length of rice caused by *X. oryzae* pv. *oryzae* under net house condition in boro season 2018-2019

Treatments	Lesion length (cm)												**Mean lesion length reduction (%)				
	Hybrid Hera-2				Arize Tej Gold				BRRI dhan 28					BRRI dhan 29			
	Days after inoculation (DAI)		Days after inoculation (DAI)		Days after inoculation (DAI)		Days after inoculation (DAI)		Days after inoculation (DAI)		Days after inoculation (DAI)			Days after inoculation (DAI)		Days after inoculation (DAI)	
	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28		
T <sub>0</sub>	0.85a	1.4a	4.48a	0.88a	1.47a	7.65a	0.87a	1.47a	2.85a	0.88a	1.48a	4.23a	0.88a	1.48a	4.23a	0.00	
T <sub>1</sub>	0.68bc	1.08b	1.02c	0.67b	1.07b	4.45bc	0.72b	1.1bc	1.4bcd	0.7bc	1.15b	2.3bc	0.7bc	1.15b	2.3bc	55.29	
T <sub>2</sub>	19.65	22.68	84.04	24.40	27.31	42.13	17.21	24.73	49.80	20.70	22.39	45.18	20.70	22.39	45.18		
T <sub>3</sub>	0.68bc	1.12b	2.82abc	0.62b	1.1b	5.77abc	0.65b	1.05bc	1.1d	0.73b	1.12bc	1.4bc	16.88	24.62	66.88	51.62	
T <sub>4</sub>	19.65	20.25	54.55	30.17	24.94	23.69	24.94	28.44	61.37	16.88	24.62	66.88	16.88	24.62	66.88		
T <sub>5</sub>	0.70bc	1.12b	3.02ab	0.63b	1.17b	3.32c	0.72b	1.13bc	1.13d	0.68bc	1.01c	1.5bc	0.68bc	1.01c	1.5bc	58.30	
T <sub>6</sub>	17.58	20.25	52.00	28.32	23.86	56.52	17.10	22.55	59.47	22.54	31.29	65.21	22.54	31.29	65.21		
T <sub>7</sub>	0.65c	1.13b	1.87bc	0.65b	1.08b	5.15abc	0.63b	1.13bc	1.42bcd	0.7bc	1.01c	1.1c	0.7bc	1.01c	1.1c	56.54	
T <sub>8</sub>	-23.3	-18.97	-69.88	-26.36	-26.16	-31.75	-26.8	-22.58	-50.81	-20.58	-31.29	-73.7	-20.58	-31.29	-73.7		
T <sub>9</sub>	0.67bc	1.13b	1.82bc	0.65b	1.03b	4.02bc	0.68b	1.03c	1.36bcd	0.7bc	1.06bc	1.65bc	0.7bc	1.06bc	1.65bc	57.49	
T <sub>10</sub>	-21.39	-18.93	-71.57	-26.47	-29.65	-46.32	-21.02	-29.48	-50.91	-20.69	-27.77	-61.14	-20.69	-27.77	-61.14		
T <sub>11</sub>	0.65c	1.13b	3.47ab	0.67b	1.12b	4.75bc	0.70b	1.08bc	1.3cd	0.7bc	1.13bc	1.57bc	0.7bc	1.13bc	1.57bc	49.3	
T <sub>12</sub>	-23.35	-18.97	-43.38	-24.5	-23.79	-37.54	-19.06	-25.72	-54.31	-20.91	-23.54	-61.96	-20.91	-23.54	-61.96		
T <sub>13</sub>	0.72bc	1.08b	2.53bc	0.65b	1.07b	4.33bc	0.70b	1.07bc	1.3cd	0.73b	1.12bc	1.55bc	0.73b	1.12bc	1.55bc	54.54	
T <sub>14</sub>	-15.5	-22.42	-58.62	-26.36	-27.12	-44.05	-19.06	-26.96	-52.94	-16.88	-24.62	-62.56	-16.88	-24.62	-62.56		
T <sub>15</sub>	0.73b	1.1b	3.05ab	0.68b	0.98b	4.17bc	0.68b	1.15b	1.72bc	0.68bc	1.03bc	1.7c	0.68bc	1.03bc	1.7c	48.38	
T <sub>16</sub>	-13.64	-21.5	-50.64	-22.65	-32.95	-45.81	-21.02	-21.31	-38.56	-22.54	-30.29	-58.51	-22.54	-30.29	-58.51		
T <sub>17</sub>	0.68bc	1.07b	1.73bc	0.65b	1.05b	3.78bc	0.68b	1.05bc	1.38bcd	0.67c	1.17bc	1.15c	0.67c	1.17bc	1.15c	62.20	
T <sub>18</sub>	-19.43	-23.91	-72.37	-26.47	-28.35	-51.9	-21.13	-27.87	-51.83	-24.5	-24.62	-72.7	-24.5	-24.62	-72.7		
Level of significance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
CV (%)	6.37	5.32	4.73	6.62	8.15	6.77	7.56	5.76	22.2	5.39	6.38	7.19	5.39	6.38	7.19		
LSD	0.076	0.1	0.14	0.08	0.15	0.19	0.09	0.1	0.57	0.07	0.11	0.21	0.07	0.11	0.21		

In each column values with same letters indicate statistically similar. Data in the parentheses are the reduction of lesion length by each treatment over control. \* indicates the difference among the treatment means are significant at 5% level of significance. \*\* Mean lesion length reduction by each treatment over control in four varieties at 28 DAI. T<sub>0</sub> = Control, T<sub>1</sub> = Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub> = BDISOB04 (*Pseudomonas putida*), T<sub>3</sub> = BDISOB05P (*Pseudomonas putida*), T<sub>4</sub> = BDISOB219R (*Pseudomonas taiwanensis*), T<sub>5</sub> = BDISOB221R (*Pseudomonas* sp.), T<sub>6</sub> = BDISOB222R (*Pseudomonas plecoglossicida*), T<sub>7</sub> = BDISOB258R (*Pseudomonas putida*), T<sub>8</sub> = BDISOB186 R (*Pseudomonas* sp.) and T<sub>9</sub> = BDISOB283R (*Pseudomonas fluorescens*)

**Table 22.** Effect of different formulated bacterial antagonists (identified in boro season 2017-2018) in reducing lesion length of rice caused by *X. oryzae* pv. *oryzae* under field condition in boro season 2018-2019

Treatments	Lesion length (cm)												**Mean lesion length reduction (%)				
	Hybrid Hera-2				Arize Tej Gold				BRRI dhan 28					BRRI dhan 29			
	Days after inoculation (DAI)				Days after inoculation (DAI)				Days after inoculation (DAI)					Days after inoculation (DAI)			
	14	21	28	14	21	28	14	21	28	14	21	28	14	21	28		
T <sub>0</sub>	1.05a 0.00	1.597a 0.00	3.03a 0.00	1.03a 0.00	1.59a 0.00	3.07a 0.00	1.03a 0.00	1.57a 0.00	3.07a 0.00	1.03a 0.00	1.55a 0.00	3.36a 0.00	1.03a 0.00	1.55a 0.00	3.36a 0.00	0	
T <sub>1</sub>	0.64cde -38.46	0.75b -52.86	1.62c -46.5	0.61e -41.33	0.82b -48.55	1.49bc -51.35	0.61c -40.22	0.95b -39.62	1.59b -48.26	0.65c -37.47	0.89b -42.71	1.61b -51.97	0.65c -37.47	0.89b -42.71	1.61b -51.97	49.52	
T <sub>2</sub>	0.64de -39.09	0.72b -54.51	1.74bc -42.51	0.63cd -39.41	0.83b -48.16	1.63b -47.16	0.63c -38.96	0.9bc -32.79	1.53bc -50.22	0.63c -39.38	0.86bc -44.65	1.58b -52.79	0.63c -39.38	0.86bc -44.65	1.58b -52.79	48.17	
T <sub>3</sub>	0.69b -34.02	0.74b -53.62	1.69bc -44.09	0.62cde -39.72	0.83b -48.18	1.52bc -50.57	0.63c -38.94	0.87cd -44.48	1.55bc -49.46	0.64c -38.42	0.82cde -46.79	1.56b -53.26	0.64c -38.42	0.82cde -46.79	1.56b -53.26	49.35	
T <sub>4</sub>	0.68bc -34.97	0.75b -52.84	1.78b -40.93	0.65b -37.17	0.83b -47.94	1.66b -46.07	0.65c -37	0.87cd -44.7	1.63b -47	0.65c -37.45	0.83cde -46.13	1.48b -53.73	0.65c -37.45	0.83cde -46.13	1.48b -53.73	46.93	
T <sub>5</sub>	0.65bcde -37.51	0.76b -52.2	1.71bc -43.47	0.65b -36.84	0.83b -47.96	1.42c -53.83	0.68b -33.77	0.83de -47.03	1.53bc -50	0.70b -32.03	0.80e -48.5	1.63b -51.2	0.70b -32.03	0.80e -48.5	1.63b -51.2	49.63	
T <sub>6</sub>	0.64de -39.09	0.78b -51.16	1.71bc -43.3	0.62de -40.05	0.85b -46.71	1.55bc -49.76	0.63c -38.62	0.83cde -46.83	1.44c -53.19	0.63c -39.06	0.81de -47.44	1.6b -52.49	0.63c -39.06	0.81de -47.44	1.6b -52.49	49.69	
T <sub>7</sub>	0.67bcd -35.92	0.746b -53.27	1.71bc -43.32	0.64de -38.13	0.83b -48.18	1.49bc -51.5	0.63c -38.63	0.85cde -45.75	1.56b -49	0.63c -38.72	0.84cd -45.7	1.47b -56.3	0.63c -38.72	0.84cd -45.7	1.47b -56.3	50.03	
T <sub>8</sub>	0.65cde -38.14	0.78b -51	1.65bc -45.19	0.65bc -36.84	0.80b -49.62	1.60bc -47.88	0.64c -37.97	0.83cde -46.81	1.52bc -50.32	0.64c -38.42	0.83cde -46.55	1.63b -51.28	0.64c -38.42	0.83cde -46.55	1.63b -51.28	48.67	
T <sub>9</sub>	0.62e -41	0.73b -54.29	1.74bc -42.45	0.63cd -39.09	0.81b -49.52	1.58bc -48.46	0.64c -37.34	0.8e -49.15	1.57b -48.78	0.64c -38.09	0.81de -47.85	1.61b -52.09	0.64c -38.09	0.81de -47.85	1.61b -52.09	47.95	
Level of significance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
CV (%)	3.43	6.07	4.8	1.68	6.4	6.13	2.25	4.05	4.27	2.25	2.67	5.78	2.25	2.67	5.78	-	
LSD	0.04	0.08	0.15	0.02	0.1	0.17	0.02	0.06	0.12	0.03	0.04	0.17	0.03	0.04	0.17	-	

In each column values with same letters indicates statistically similar. Data in the parentheses are the reduction of lesion length by each treatment over control. \* indicates the difference among the treatment means are significant at 5% level of significance. \*\* Mean lesion length reduction by each treatment over control in four varieties at 28 DAI. T<sub>0</sub> = Control, T<sub>1</sub> = Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub> = BDISOB04 (*Pseudomonas putida*), T<sub>3</sub> = BDISOB05P (*Pseudomonas putida*), T<sub>4</sub> = BDISOB219R (*Pseudomonas taiwanensis*), T<sub>5</sub> = BDISOB221R (*Pseudomonas* sp.), T<sub>6</sub> = BDISOB222R (*Pseudomonas plecoglossicida*), T<sub>7</sub> = BDISOB258R (*Pseudomonas putida*), T<sub>8</sub> = BDISOB186 R (*Pseudomonas* sp.) and T<sub>9</sub> = BDISOB283R (*Pseudomonas fluorescens*)

**Table 23.** Effect of different formulated bacterial antagonists (identified in boro season 2017-2018) on yield of rice under field condition in boro season 2018-2019

Treatments	Hybrid Hear-2		Hybrid Hear-2		Hybrid Hear-2		Hybrid Hear-2		**Mean yield increase (%)
	Yield (t/ha)	Yield increase (%)							
T0 (Control)	5.20d	0.00	5.53b	0.00	5.08c	0.00	5.60d	0.00	0.00
T <sub>1</sub>	6.29b	20.99	6.13ab	10.86	6.22ab	22.30	6.38c	13.99	17.03
T <sub>2</sub>	6.23b	19.71	6.55a	18.55	6.73ab	32.30	7.33ab	30.80	25.34
T <sub>3</sub>	6.25b	20.19	6.30a	14.03	5.19c	2.05	7.35ab	31.25	16.88
T <sub>4</sub>	5.63cd	8.17	6.25ab	13.12	6.42ab	26.23	6.83bc	21.88	17.35
T <sub>5</sub>	5.31cd	2.08	6.18ab	11.76	5.83bc	14.59	7.05b	25.89	13.58
T <sub>6</sub>	5.68c	9.13	6.71a	21.42	6.90a	35.74	6.93bc	23.66	22.49
T <sub>7</sub>	6.25b	20.19	6.30a	14.03	5.18c	1.80	7.10b	26.79	15.70
T <sub>8</sub>	6.95a	29.26	6.65a	20.36	6.38ab	25.41	7.38ab	31.70	26.68
T <sub>9</sub>	6.83a	31.25	6.41a	15.99	6.75ab	32.79	7.83a	39.73	29.94
Level of significance	*	-	*	-	*	-	*	-	-
CV (%)	3.94	-	6.23	-	8.08	-	4.50	-	-

In each column values with same letters indicates statistically similar. \* \* Mean yield increase by each treatment over control in four varieties \*indicates the different treatment means are significant at 5% level of significance. Here, T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>= BDISOB04 (*Pseudomonas putida*), T<sub>3</sub>= BDISOB05 (*Pseudomonas putida*), T<sub>4</sub>= BDISOB219 (*Pseudomonas taiwanensis*), T<sub>5</sub>= BDISOB221 (*Pseudomonas* sp.), T<sub>6</sub>= BDISOB222 (*Pseudomonas plecoglossicida*), T<sub>7</sub>= BDISOB258 (*Pseudomonas putida*), T<sub>8</sub>= BDISOB186 (*Pseudomonas* sp.) and T<sub>9</sub>= BDISOB283 (*Pseudomonas fluorescens*)

**Table 24.** Effect of different formulated bacterial antagonists (identified in boro season 2018-2019) in reducing lesion length of rice caused by *Xanthomonas oryzae* pv. *oryzae* under net house condition in boro season 2019-2020

Treatments	Lesion length (cm)												**Mean lesion length reduction (%)
	Hybrid Hera-2			ArizeTej Gold			BRR1 dhan 28			BRR1 dhan 29			
	Days after inoculation			Days after inoculation			Days after inoculation			Days after inoculation			
	14	21	28	14	21	28	14	21	28	14	21	28	
T <sub>0</sub>	1.17a (0.00)	1.68a (0.00)	3.51a (0.00)	1.33a (0.00)	2.86a (0.00)	3.93a (0.00)	2.30a (0.00)	2.94a (0.00)	4.52a (0.00)	2.01a (0.00)	3.80a (0.00)	4.71a (0.00)	0.00
T <sub>1</sub>	0.51b (56.57)	0.54b (67.86)	1.21b (65.55)	0.52b (61.00)	0.57b (80.20)	1.29b (67.25)	0.58b (74.88)	0.65b (77.78)	2.38bc (47.38)	0.52b (74.25)	0.54 b (85.79)	1.50c (68.10)	62.07
T <sub>2</sub>	0.52b (55.71)	0.53b (68.25)	2.17b (38.14)	0.51b (61.50)	0.55b (80.67)	1.85b (52.97)	0.55b (75.90)	0.56b (80.84)	2.38bc (47.31)	0.59b (70.43)	0.76b (80.09)	2.37bc (49.79)	47.05
T <sub>3</sub>	0.51b (56.00)	0.59b (64.68)	2.07b (41.14)	0.52b (61.00)	0.58b (79.73)	1.84b (53.14)	0.57b (75.32)	0.69b (76.54)	2.39bc (46.99)	0.60b (70.09)	0.54b (85.88)	1.88bc (60.08)	50.34
T <sub>4</sub>	0.52b (55.43)	0.56b (66.87)	1.49b (57.5)	0.55b (58.75)	0.60b (78.92)	2.29b (41.86)	0.59b (74.16)	0.70b (76.08)	2.23bc (50.57)	0.70b (65.12)	0.55b (85.53)	2.08bc (55.84)	54.64
T <sub>5</sub>	0.53b (54.86)	0.60b (64.29)	2.18b (38.01)	0.54b (59.75)	0.56b (80.32)	2.30b (41.59)	0.62b (72.85)	0.67b (77.10)	3.39abc (25.02)	0.57b (71.43)	0.56b (85.18)	1.51c (68.03)	43.16
T <sub>6</sub>	0.51b (56.00)	0.56b (66.47)	1.65b (53.15)	0.53b (60.50)	0.57b (79.97)	1.75b (55.61)	0.63b (72.56)	0.69b (76.54)	3.57ab (21.03)	0.58b (71.10)	0.73b (80.79)	2.46bc (47.74)	44.38
T <sub>7</sub>	0.51b (56.00)	0.55b (67.06)	1.77b (49.72)	0.53b (60.50)	0.59b (79.27)	2.19b (44.36)	0.57b (75.32)	0.67b (77.33)	2.46bc (45.56)	0.61b (69.60)	0.79b (79.29)	2.73b (42.08)	45.43
T <sub>8</sub>	0.53b (54.29)	0.58b (65.48)	2.04b (41.86)	0.52b (60.75)	0.57b (80.08)	1.21b (69.15)	0.62b (73.14)	0.63b (78.58)	2.02c (55.20)	0.53b (73.59)	0.66b (82.63)	2.37bc (49.70)	53.98
T <sub>9</sub>	0.51b (56.00)	0.57b (65.87)	2.39ab (31.94)	0.53b (60.50)	0.57b (79.97)	1.69b (57.12)	0.59b (74.16)	0.72b (75.67)	2.81bc (37.89)	0.56b (71.93)	0.60b (84.30)	3.00b (36.35)	40.83
Level of significance	*	*	*	*	*	*	*	*	*	*	*	*	*
CV (%)	15.49	28.01	33.90	15.24	35.82	33.93	9.01	11.58	25.64	40.64	29.31	25.28	

Values in the parentheses indicate the percent reduction of lesion length over control by each treatment. Values with same letters in each column indicate statistically similar. \* indicates the treatment means are significant at 5% level of significance, \*\* average lesion length reduction by each treatment over control in four varieties at 28 DAL, T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>= BDISOB37R (*Pseudochrobactrum asaccharolyticum*), T<sub>3</sub>= BDISOB16R (*Pseudochrobactrum asaccharolyticum*), T<sub>4</sub>= BDISOB92R (*Pseudomonas fluorescens*), T<sub>5</sub>= BDISOB21R (*Stenotrophomonas maltophilia*), T<sub>6</sub>= BDISOB17R (*Limnolyngbya circumcreta*), T<sub>7</sub>= BDISOB15R (*Pseudochrobactrum asaccharolyticum*), T<sub>8</sub>= BDISOB86R (*Enterobacter aerogenes*) and T<sub>9</sub>= BDISOB30R (*Pseudochrobactrum asaccharolyticum*).

**Table 25.** Effect of different formulated bacterial antagonists (identified in boro season 2018-2019) in reducing lesion length of rice caused by *Xanthomonas oryzae* pv. *oryzae* under field condition in boro season 2019-2020

Treatments	Lesion length (cm)												**Mean lesion length reduction (%)
	Hybrid Hera-2			ArizeTej Gold			BRRi dhan 28			BRRi dhan 29			
	Days after inoculation			Days after inoculation			Days after inoculation			Days after inoculation			
	14	21	28	14	21	28	14	21	28	14	21	28	
T <sub>0</sub>	1.42a (0.00)	3.00a (0.00)	5.35a (0.00)	2.07a (0.00)	2.67a (0.00)	3.32a (0.00)	1.50a (0.00)	2.53a (0.00)	3.34a (0.00)	1.57a (0.00)	2.61a (0.00)	3.93a (0.00)	0.00
T <sub>1</sub>	0.52b (63.06)	0.54b (82.00)	1.16b (78.39)	0.51b (75.42)	0.69b (74.16)	1.62b (51.25)	0.50b (66.44)	0.54b (78.81)	0.58c (82.71)	0.68b (56.36)	0.73b (72.00)	1.61c (58.96)	67.83
T <sub>2</sub>	0.50b (64.47)	0.54b (81.89)	1.81b (66.19)	0.50b (75.90)	0.50b (81.27)	0.50c (84.95)	0.54b (63.77)	0.58b (77.17)	0.68c (79.62)	0.70b (55.51)	0.57b (78.01)	1.97bc (49.88)	70.16
T <sub>3</sub>	0.51b (64.24)	0.53b (82.44)	1.81b (66.10)	0.52b (74.77)	0.57b (78.78)	1.61b (51.41)	0.55b (63.55)	0.65b (74.33)	0.70c (79.12)	0.53b (66.16)	0.55b (79.03)	1.73c (55.90)	63.13
T <sub>4</sub>	0.52b (63.06)	0.60b (79.89)	2.22b (58.55)	0.55b (73.49)	0.58b (78.40)	1.35b (59.40)	0.56b (62.65)	0.63b (74.99)	0.78bc (76.62)	0.53b (66.37)	0.58b (77.88)	2.25bc (42.67)	59.31
T <sub>5</sub>	0.51b (64.00)	0.58b (80.77)	1.52b (71.23)	0.54b (74.13)	0.56b (79.15)	1.28b (61.60)	0.52b (65.10)	0.58b (77.09)	0.62c (81.41)	0.52b (66.58)	0.55b (79.03)	1.54c (60.88)	68.78
T <sub>6</sub>	0.51b (64.00)	0.56b (81.33)	1.54b (74.22)b	0.53b (74.45)	0.53b (80.15)	1.32b (60.38)	0.53b (64.44)	0.83b (67.36)	1.04b (68.92)	0.69b (56.15)	0.63b (75.71)	2.13bc (45.71)	62.31
T <sub>7</sub>	0.51b (64.00)	0.56b (81.22)	1.38b (74.22)	0.51b (75.26)	0.57b (78.78)	1.48b (55.33)	0.51b (65.88)	0.58b (77.04)	0.73bc (78.21)	0.71b (54.45)	0.84b (67.78)	2.51bc (36.18)	60.70
T <sub>8</sub>	0.51b (64.00)	0.60b (80.00)	2.26b (57.85)	0.52b (74.77)	0.57b (78.53)	1.39b (58.17)	0.53b (64.88)	0.64b (74.73)	0.68c (79.72)	0.51b (67.22)	0.79b (69.70)	2.21bc (43.86)	59.90
T <sub>9</sub>	0.51b (64.00)	0.59b (80.22)	2.01b (62.42)	0.51b (75.26)	0.55b (79.40)	1.95b (41.32)	0.52b (65.11)	0.68b (72.96)	0.85bc (74.52)	0.67b (57.00)	0.63b (75.71)	2.85c (27.58)	41.46
Level of significance	*	*	*	*	*	*	*	*	*	*	*	*	*
CV (%)	27.78	18.83	32.56	16.82	26.22	36.40	21.92	26.97	16.82	26.92	43.73	22.29	

Values in the parentheses indicate the percent reduction of lesion length over control by each treatment. Values with same letters in each column indicate statistically similar. \* indicates the treatment means are significant at 5% level of significance, \*\* average lesion length reduction by each treatment over control in four varieties at 28 DAL, T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>= BDISOB37R (*Pseudochrobactrum asaccharolyticum*), T<sub>3</sub>= BDISOB16R (*Pseudochrobactrum asaccharolyticum*), T<sub>4</sub>= BDISOB92R (*Pseudomonas fluorescens*), T<sub>5</sub>= BDISOB21R (*Stenotrophomonas maltophilia*), T<sub>6</sub>= BDISOB17R (*Limnolyngbya circumcreta*), T<sub>7</sub>= BDISOB15R (*Pseudochrobactrum asaccharolyticum*), T<sub>8</sub>= BDISOB86R (*Enterobacter aerogenes*) and T<sub>9</sub>= BDISOB30R (*Pseudochrobactrum asaccharolyticum*).

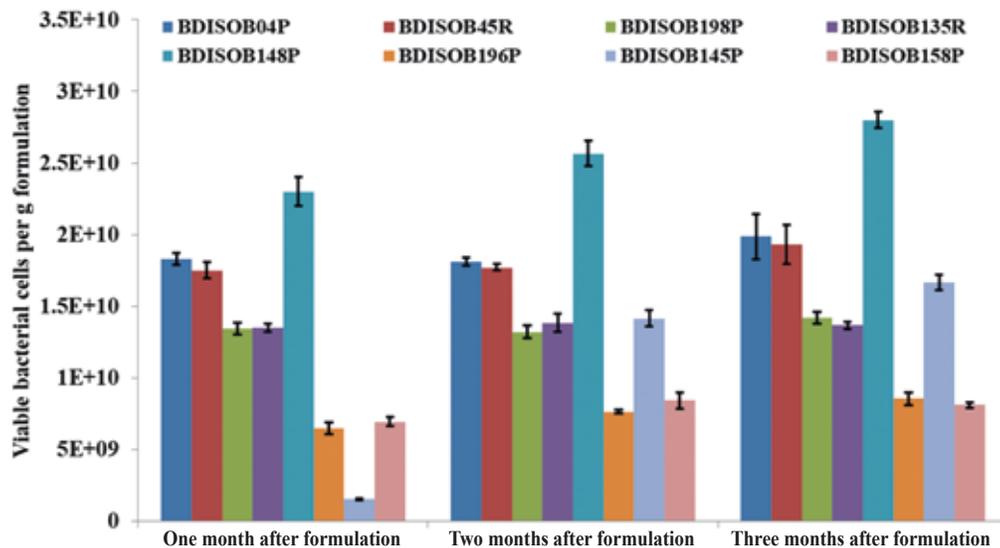
**Table 26.** Effect of different formulated bacterial antagonists (identified in boro season 2017-2018) on yield of rice under field condition in boro season 2018-2019

Treatments	Hybrid Hear-2		Arize Tejgold		BRRIdhan28		BRRIdhan29		**Mean yield increase (%)
	Yield (t/ha)	Yield increase (%)	Yield (t/ha)	Yield increase (%)	Yield (t/ha)	Yield increase (%)	Yield (t/ha)	Yield increase (%)	
T0 (Control)	8.63c	0.00	7.38d	0.00	5.83	0.00	5.54	0.00	0.00
T <sub>1</sub>	9.67abc	12.08	8.29bc	12.43	6.54	12.14	5.67	2.26	9.73
T <sub>2</sub>	10.25a	18.84	7.67cd	3.95	7.08	21.43	6.42	15.79	15.00
T <sub>3</sub>	9.83ab	14.01	9.29a	25.99	6.54	12.14	7.17	29.32	20.37
T <sub>4</sub>	9.25abc	7.25	8.63ab	16.95	7.83	34.29	6.17	11.28	17.44
T <sub>5</sub>	9.46abc	9.66	8.13bcd	10.17	7.04	20.71	6.25	12.78	13.33
T <sub>6</sub>	9.96ab	15.46	8.33bc	12.99	7.67	31.43	6.63	19.55	19.86
T <sub>7</sub>	10.08a	16.91	8.58ab	16.38	7.17	22.86	5.63	1.50	14.41
T <sub>8</sub>	10.13a	17.39	8.04bcd	9.04	7.29	25.00	5.67	2.26	13.42
T <sub>9</sub>	8.92bc	3.38	8.54abc	15.82	6.88	17.86	6.63	19.55	14.15
Level of significance	*		*		NS		NS		
CV (%)	5.93		5.50		16.84		17.21		

Values with same letters indicates statistically similar. \*indicates the treatment means are significant at 5% level of significance. NS-Not significant. \*\* average yield increase by each treatment over control. \*\*\* average yield increase by each treatment over control considering four varieties. T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>= BDISOB37R (*Pseudochrobactrum asaccharolyticum*), T<sub>3</sub>= BDISOB16R (*Pseudochrobactrum asaccharolyticum*), T<sub>4</sub>=BDISOB92R (*Pseudomonas fluorescens*), T<sub>5</sub>= BDISOB21R (*Stenotrophomonas maltophilia*), T<sub>6</sub>= BDISOB17R (*Limnolyngbya circumcreta*). T<sub>7</sub>= BDISOB15R (*Pseudochrobactrum asaccharolyticum*), T<sub>8</sub>= BDISOB86R (*Enterobacter aerogenes*) and T<sub>9</sub>= [BDISOB30R (*Pseudochrobactrum asaccharolyticum*).

### Field efficacy of formulated antagonistic bacterial isolates (identified aman seasons 2018 and 2019) in reducing BB severity and in increasing rice yield

Bacterial blight appears as potential threat for high yielding aman rice varieties (both hybrids and inbreds) depending on poor management practices and late monsoon in Bangladesh and in other Asian countries. One hybrid viz. Dhanigold and three inbreds viz. BRRI dhan49, BINA dhan7 and BINA dhan11 rice varieties were selected based on their nature of susceptibility and area coverage in the season. The efficacy of some selected plant growth promoting bacterial antagonists were evaluated against BB of rice under both net house and field condition during aman seasons 2019 and 2020. Eight bacterial strains identified in aman season 2018 and eight identified in aman season 2019 were formulated and these formulated bacterial strains could survive for at least three months after talc-based formulation (Figure 43-46)). The eight formulated bacterial bioagents significantly reduced lesion length by 31.65% to 33.93% compared to control under net house condition (Table 27) and by 47.44% to 48.59% compared to control considering four varieties under field condition (Table 28). However, these eight formulated bacterial bioagents increased yield by 17.26% to 23.15% compared to control under field condition (Table 29). On the other hand, eight formulated bacterial bioagents (identified in aman season 2019) reduced lesion length significantly by 68.82% to 69.99% as compared to control under net house condition (Table 30) and these eight formulated bacterial bioagents reduced lesion length by 51.26% to 60.38% as compared to control under field condition (Table 31). However, these formulated bacterial bioagents increased yield by 12.25% to 27.03% as compared to control under field condition considering four varieties (Table 32). Plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide) [T<sub>1</sub>] was considered as positive control for comparison (Table 27, 28, 30 & 31).



**Figure 43.** Viability of formulated plant growth promoting antagonistic bacteria. BDISO04P (*Pseudomonas putida*), BDISO45R (*Bacillus paramycoides*), BDISO198P (*Serratia plymuthica*), BDISO135R (*Bacillus* sp.), BDISO148P (*Serratia marcescens*), BDISOB01R (*Bacillus amyloliquefaciens*), BDISO145P (*Serratia marcescens*) and BDISO158R (*Serratia marcescens*)

**Table 27.** Effect of different formulated bacterial bioagents (identified in aman season 2018) in reducing lesion length caused by *X. oryzae* pv. *oryzae* under net house condition during aman, 2019

Treatments	Lesion length (cm)										**Mean lesion length reduction (%)		
	BRRIdhan49			DhaniGold			BINAdhan7			BINAdhan11			
	Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			
	14	21	14	21	14	21	14	21	14	21			
T <sub>0</sub>	1.25 a (0.00)	1.83 a (0.00)	1.10a (0.00)	1.77 a (0.00)	1.10 a (0.00)	1.80 a (0.00)	1.25 a (0.00)	1.75 a (0.00)	0.00				
T <sub>1</sub>	0.82 b (33.76)	1.20 b (34.38)	0.77b (30.30)	1.17 b (33.94)	0.75 b (31.72)	1.22 b (32.37)	0.78 b (37.24)	1.25 b (28.57)	33.32				
T <sub>2</sub>	0.63 c (48.80)	1.20 b (34.26)	0.62 d (43.94)	1.17 b (33.88)	0.65 c (40.83)	1.20 b (33.27)	0.67 c (46.21)	1.23 b (29.52)	32.73				
T <sub>3</sub>	0.65 c (47.22)	1.23 b (32.50)	0.62 d (43.94)	1.18 b (32.99)	0.65 c (40.76)	1.23 b (31.42)	0.63 c (49.28)	1.17 cd (33.33)	32.56				
T <sub>4</sub>	0.63 c (48.90)	1.23 b (32.58)	0.65 cd (40.91)	1.18 b (32.99)	0.60 cd (45.38)	1.23 b (31.39)	0.60 c (51.84)	1.15 d (34.29)	32.81				
T <sub>5</sub>	0.63 c (48.80)	1.23 b (32.45)	0.63 cd (42.42)	1.17 b (33.97)	0.63 cd (42.34)	1.18 b (34.22)	0.67 c (46.54)	1.22 bc (30.48)	32.78				
T <sub>6</sub>	0.63 c (48.50)	1.22 b (33.44)	0.65 cd (40.91)	1.18 b (32.94)	0.65 c (41.03)	1.20 b (33.32)	0.63 c (49.28)	1.25 b (28.57)	31.65				
T <sub>7</sub>	0.63 c (48.41)	1.20 b (34.29)	0.60 d (45.45)	1.13 b (35.71)	0.60 cd (45.38)	1.20 b (33.32)	0.65 c (47.83)	1.22 bc (30.48)	33.45				
T <sub>8</sub>	0.63 c (48.90)	1.20 b (34.21)	0.68 cd (37.88)	1.10 b (37.67)	0.65 c (40.83)	1.25 b (30.52)	0.65 c (47.83)	1.17 cd (33.33)	33.93				
T <sub>9</sub>	0.60 c (51.37)	1.20 b (34.26)	0.62 d (43.94)	1.13 b (35.77)	0.58 d (46.83)	1.20 b (33.32)	0.58 c (53.12)	1.22 bc (30.48)	33.46				
Level of significance	*	*	*	*	*	*	*	*	-				
LSD	0.108	0.093	0.054	0.132	0.054	0.076	0.076	0.054	-				
CV (%)	8.79	4.63	5.08	6.38	5.55	3.78	5.91	2.31	-				

In each column values with same letters indicate statistically similar. Data in the parentheses are the reduction of lesion length by each treatment over control. \* indicates the difference of the treatment means are significant at 5% level of significance and \*\* Mean lesion length reduction at 21 DAI in four varieties T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) +Hemoxy (Copper fungicide)], T<sub>2</sub>=BDISO04P (*Pseudomonas putida*), T<sub>3</sub>= BDISO45R (*Bacillus paramycooides*), T<sub>4</sub>= BDISO198P (*Serratia plymuthica*), T<sub>5</sub>= BDISO135R (*Bacillus* sp.), T<sub>6</sub>= BDISO148P (*Serratia marcescens*), T<sub>7</sub>= BDISOB01R (*Bacillus amyloliquefaciens*), T<sub>8</sub>= BDISO145P (*Serratia marcescens*) and T<sub>9</sub>= BDISO158R (*Serratia marcescens*)

**Table 28.** Effect of different formulated bacterial bioagents (identified in aman, 2018) in reducing lesion length caused by *X. oryzae* pv. *oryzae* under field condition during aman, 2019

Treatments	Lesion length (cm)						**Mean lesion length reduction (%)		
	BRRIdhan49		DhaniGold		BINAdhan7			BINAdhan11	
	Days after inoculation (DAI)		Days after inoculation (DAI)		Days after inoculation (DAI)			Days after inoculation (DAI)	
14	21	14	21	14	21	14	21		
T <sub>0</sub>	1.06 a (0.00)	1.90 a (0.00)	1.11 a (0.00)	1.86 a (0.00)	1.32 a (0.00)	2.51 a (0.00)	1.93 a (0.00)	2.42 a (0.00)	0.00
T <sub>1</sub>	0.52 b (38.46)	1.18 b (38.17)	0.52 b (41.33)	1.08 bc (42.16)	0.52 d (40.22)	1.11 b (55.73)	0.51 d (37.47)	1.12 b (53.71)	47.44
T <sub>2</sub>	0.52 b (39.09)	1.15 c (39.58)	0.53 b (39.41)	1.06 c (43.07)	0.51 e (38.96)	1.11 b (55.87)	0.52 c (39.38)	1.11 b (54.11)	48.16
T <sub>3</sub>	0.52 b (34.02)	1.15 c (39.58)	0.53 b (39.72)	1.09 bc (41.64)	0.52 d (38.94)	1.09 b (56.54)	0.52 b (38.42)	1.11 b (54.13)	47.97
T <sub>4</sub>	0.52 b (34.97)	1.12 e (41.33)	0.51 b (37.17)	1.07 bc (42.70)	0.52 d (37.00)	1.08 b (56.77)	0.52 b (37.45)	1.12 b (53.57)	48.59
T <sub>5</sub>	0.52 b (37.51)	1.11 f (41.51)	0.53 b (36.84)	1.08 bc (41.80)	0.53 b (33.77)	1.10 b (55.98)	0.52 c (32.03)	1.10 b (54.41)	48.43
T <sub>6</sub>	0.52 b (39.09)	1.15 d (39.76)	0.53 b (40.05)	1.12 bc (40.04)	0.51 f (38.62)	1.08 b (57.05)	0.52 b (39.06)	1.11 b (54.02)	47.72
T <sub>7</sub>	0.52 b (35.92)	1.11 g (41.86)	0.51 b (38.13)	1.12 b (39.68)	0.52 c (38.63)	1.08 b (57.04)	0.51 d (38.72)	1.10 b (54.55)	48.28
T <sub>8</sub>	0.52 b (38.14)	1.12 e (41.32)	0.52 b (36.84)	1.07 bc (42.34)	0.51 f (37.97)	1.10 b (56.6)	0.51 d (38.42)	1.11 b (54.01)	48.57
T <sub>9</sub>	0.52 b (40.99)	1.12 f (41.33)	0.53 b (39.09)	1.06 bc (42.87)	0.52 c (37.34)	1.10 b (55.98)	0.52 c (38.09)	1.10 b (54.40)	48.65
Level of significance	*	*	*	*	*	*	*	*	-
CV (%)	4.11	1.21	3.97	2.08	2.95	2.82	2.19	3.23	-

In each column values with same letters indicate statistically similar. Data in the parentheses are the reduction of lesion length by each treatment over control. \* indicates the difference of the treatment means are significant at 5% level of significance and \*\* Mean lesion length reduction at 21 DAI in four varieties T<sub>0</sub> = Control, T<sub>1</sub> = Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub> = BDISO04P (*Pseudomonas putida*), T<sub>3</sub> = BDISO45R (*Bacillus paramycoides*), T<sub>4</sub> = BDISO198P (*Serratia plymuthica*), T<sub>5</sub> = BDISO135R (*Bacillus sp.*), T<sub>6</sub> = BDISO148P (*Serratia marcescens*), T<sub>7</sub> = BDISOB01R (*Bacillus amyloliquefaciens*), T<sub>8</sub> = BDISO145P (*Serratia marcescens*) and T<sub>9</sub> = BDISO158R (*Serratia marcescens*)

**Table 29.** Effect of different formulated bacterial antagonists (identified in aman season 2018) on yield of rice under field condition in aman, 2019

Treatments	BRRIdhan49		Dhanigold		BINAdhan7		BINAdhan11		**Mean yield increase (%)
	Yield (t/ha)	Yield increase (%)							
T <sub>0</sub> (Control)	5.05b	0.00	5.00b	0.00	5.54	0.00	5.00b	0.00	0.00
T <sub>1</sub>	6.06a	19.97	6.67a	33.33	5.83	5.26	5.83ab	16.67	18.81
T <sub>2</sub>	6.02a	19.22	6.25a	25.00	6.25	12.78	5.83ab	16.67	18.42
T <sub>3</sub>	6.15a	21.70	5.83ab	16.67	6.46	16.54	5.83ab	16.67	17.89
T <sub>4</sub>	6.08a	20.46	6.46a	29.17	6.46	16.54	6.25a	25.00	22.79
T <sub>5</sub>	6.17a	22.11	6.25a	25.00	5.83	5.26	5.83ab	16.67	17.26
T <sub>6</sub>	5.98a	18.48	5.83ab	16.67	6.33	14.29	6.04a	20.83	17.57
T <sub>7</sub>	6.07a	20.13	6.04a	20.83	6.25	12.78	5.83ab	16.67	17.60
T <sub>8</sub>	6.28a	24.42	6.46a	29.17	5.83	5.26	6.25a	25.00	20.96
T <sub>9</sub>	6.37a	26.07	6.25a	25.00	6.46	16.54	6.25a	25.00	23.15
Level of significance	*	-	*	-	NS	-	*	-	-
CV (%)	4.47	-	7.63	-	8.98	-	7.53	-	-

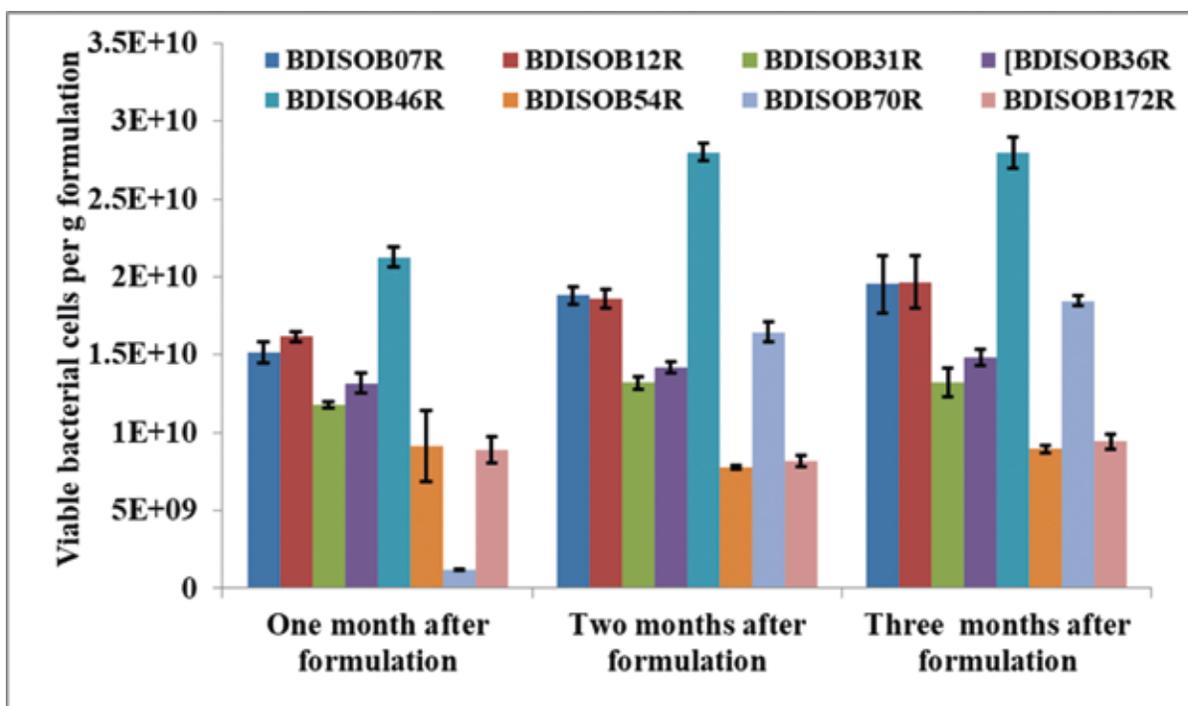
In each column values with same letters indicate statistically similar, \* indicates the difference of the treatment means are significant at 5% level of significance and \*\* Mean yield increase by each treatment over control in four varieties, T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>=BDISO04P (*Pseudomonas putida*), T<sub>3</sub>=BDISO45R (*Bacillus paramycoides*), T<sub>4</sub>=BDISO198P (*Serratia phymuthica*), T<sub>5</sub>=BDISO135R (*Bacillus sp.*), T<sub>6</sub>=BDISO148P (*Serratia marcescens*), T<sub>7</sub>=BDISOB01R (*Bacillus amyloliquefaciens*), T<sub>8</sub>=BDISO145P (*Serratia marcescens*) and T<sub>9</sub>=BDISO158R (*Serratia marcescens*).



**Figure 44.** Photographs showing A) the treatment of seeds with antagonistic bacterial isolates, B) Sowing treated seeds, C) Seedlings for field trial treated with antagonistic bacterial isolates, D) Field experiment and E-F) Net house experiment.



**Figure 45.** Photographs showing field experiment activities.



**Figure 46.** Viability of formulated plant growth promoting antagonistic bacteria. BDISOB07R (*Serratia nematodiphila*), BDISOB12R (*Serratia marcescens*), BDISOB31R (*Serratia marcescens*), BDISOB36R (*Serratia marcescens*), BDISOB46R (*Serratia marcescens*), BDISOB54R (*Burkholderia gladioli*), BDISOB70R (*Serratia marcescens*) and BDISOB172R (*Bacillus aerophilus*).

**Table 30.** Effect of different formulated bacterial antagonists (identified in aman season 2019) in reducing lesion length under net house condition in aman, 2020

Treatments	Lesion length (cm)										**Mean lesion length reduction (%)		
	BRRI dhan49			DhaniGold			BINA dhan7			BINA dhan11			
	Days after inoculation			Days after inoculation			Days after inoculation			Days after inoculation			
	14	21		14	21		14	21		14		21	
T <sub>0</sub>	1.98a (0.00)	3.80a (0.00)		2.39a (0.00)	2.94a (0.00)		1.37a (0.00)	2.86a (0.00)		1.21a (0.00)	1.68a (0.00)		0.00
T <sub>1</sub>	0.50b (74.70)	0.54b (85.79)		0.58b (75.55)	0.65b (77.78)		0.65b (52.44)	0.57b (80.20)		0.58b (52.21)	0.54b (67.86)		70.81693
T <sub>2</sub>	0.51b (74.37)	0.76b (80.09)		0.71b (70.38)	0.56b (80.84)		0.70b (48.54)	0.55b (80.67)		0.62b (48.34)	0.53b (68.25)		68.9351
T <sub>3</sub>	0.51b (74.37)	0.54b (85.88)		0.57b (76.11)	0.69b (76.54)		0.79b (42.44)	0.58b (79.73)		0.59b (50.83)	0.59b (64.68)		68.82202
T <sub>4</sub>	0.52b (73.86)	0.55b (85.53)		0.73b (69.27)	0.70b (76.08)		0.71b (47.80)	0.60b (78.92)		0.51b (58.01)	0.56b (66.86)		69.54199
T <sub>5</sub>	0.51b (74.20)	0.56b (85.17)		0.58b (75.69)	0.67b (77.10)		0.80b (41.70)	0.56b (80.32)		0.57b (52.76)	0.60b (64.28)		68.90511
T <sub>6</sub>	0.51b (74.37)	0.73b (80.79)		0.60b (74.85)	0.69b (76.54)		0.63b (54.15)	0.57b (79.97)		0.60b (50.00)	0.56b (66.46)		69.64104
T <sub>7</sub>	0.51b (74.03)	0.79b (79.30)		0.61b (74.29)	0.67b (77.32)		0.58b (57.80)	0.59b (79.27)		0.60b (50.00)	0.55b (67.06)		69.88618
T <sub>8</sub>	0.50b (74.54)	0.66b (82.63)		0.81b (65.91)	0.63b (78.57)		0.66b (51.71)	0.57b (80.08)		0.58b (52.21)	0.58b (65.48)		68.89171
T <sub>9</sub>	0.51b (74.03)	0.60b (84.30)		0.61b (74.56)	0.72b (75.67)		0.66b (51.71)	0.57b (79.97)		0.56b (53.87)	0.57b (65.87)		69.99884
Level of significance	*	*		*	*		*	*		*	*		*
CV (%)	39.24	29.31		15.71	11.58		22.96	35.74		16.45	28.01		

Values in the parentheses are the reduction of lesion length by each treatment over control. Values in each column with same letters indicates statistically similar. \* indicates the treatment means are significant at 5% level of significance and \*\* Mean reduction of lesion length (%) over control considering four rice varieties at 28 DAI. T<sub>0</sub> = Control, T<sub>1</sub> = Positive control [where the plants sprayed with Bacitroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub> = BDISOB07R (*Serratia nematodiphila*), T<sub>3</sub> = BDISOB12R (*Serratia marcescens*), T<sub>4</sub> = BDISOB3 IR (*Serratia marcescens*), T<sub>5</sub> = BDISOB36R (*Serratia marcescens*), T<sub>6</sub> = BDISOB46R (*Serratia marcescens*), T<sub>7</sub> = BDISOB54R (*Burkholderia gladioli*), T<sub>8</sub> = BDISOB70R (*Serratia marcescens*) and T<sub>9</sub> = (BDISOB172R (*Bacillus aerophilus*))

**Table 31.** Effect of different formulated bacterial antagonists (identified in aman season 2019) in reducing lesion length under field condition in aman, 2020

Treatments	Lesion length (cm)										**Mean lesion length reduction (%)
	BRRRI dhan49		DhaniGold		BINA dhan7		BINA dhan11				
	Days after inoculation		Days after inoculation		Days after inoculation		Days after inoculation		Days after inoculation		
	14	21	14	21	14	21	14	21	14	21	
T <sub>0</sub>	1.49a (0.00)	3.04a (0.00)	2.13a (0.00)	3.86a (0.00)	2.64a (0.00)	5.95a (0.00)	1.74a (0.00)	6.51a (0.00)	0.00		
T <sub>1</sub>	0.51b (65.79)	0.94c (69.12)	0.52c (75.44)	1.39b (63.90)	1.19b (55.03)	2.45c (58.81)	0.74cd (55.02)	2.29c (58.81)	63.79		
T <sub>2</sub>	0.52b (65.12)	0.69c (77.19)	0.84b (60.74)	1.14b (70.55)	1.50b (43.27)	3.02bc (49.21)	1.50ab (43.27)	4.52b (49.21)	51.26		
T <sub>3</sub>	0.53b (64.45)	1.47bc (51.75)	0.59c (72.47)	1.20b (68.82)	1.17b (55.78)	3.01bc (49.43)	1.45abc (55.78)	4.04bc (49.43)	52.15		
T <sub>4</sub>	0.53b (64.34)	0.85c (71.90)	0.57c (73.41)	0.86b (77.80)	1.00b (61.97)	3.69bc (37.94)	1.22abcd (61.97)	2.24c (37.94)	60.38		
T <sub>5</sub>	0.51b (65.91)	1.45bc (52.30)	0.64bc (69.81)	0.91b (76.51)	1.34b (49.21)	3.42bc (42.54)	0.77bcd (49.21)	2.90bc (42.53)	58.42		
T <sub>6</sub>	0.59b (60.24)	1.65bc (54.73)	0.68bc (68.09)	1.00b (74.00)	1.26b (52.11)	3.83bc (35.60)	1.09abcd (52.11)	3.18bc (35.60)	53.05		
T <sub>7</sub>	0.68b (54.01)	2.54ab (16.55)	0.62c (70.75)	0.87b (77.46)	1.23b (53.25)	3.25bc (45.39)	0.93bcd (53.25)	3.42bc (45.39)	51.39		
T <sub>8</sub>	0.52b (65.24)	1.84abc (39.63)	0.61c (71.37)	0.83b (78.49)	1.82ab (30.89)	4.30b (27.69)	0.66d (30.89)	2.43c (27.69)	54.73		
T <sub>9</sub>	0.56b (62.65)	1.77bc (41.86)	0.68bc (68.09)	0.78b (79.87)	1.99ab (24.45)	3.68bc (38.11)	0.68d (24.45)	2.40c (38.11)	54.91		
Level of significance	*	*	*	*	*	*	*	*	*	*	
CV (%)	23.27	41.27	13.48	35.90	38.63	21.36	36.23	29.57			

Values in the parentheses are the reduction of lesion length by each treatment over control. Values in each column with same letters indicates statistically similar. \* indicates the treatment means are significant at 5% level of significance. \*\*Mean reduction of lesion length (%) over control considering four rice varieties at 28 DAI. T<sub>0</sub> = Control, T<sub>1</sub> = Positive control [where the plants sprayed with Bacitroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub> = BDISOB07R (*Serratia nematodiphila*), T<sub>3</sub> = BDISOB12R (*Serratia marcescens*), T<sub>4</sub> = BDISOB31R (*Serratia marcescens*), T<sub>5</sub> = BDISOB36R (*Serratia marcescens*), T<sub>6</sub> = BDISOB46R (*Serratia marcescens*), T<sub>7</sub> = BDISOB54R (*Burkholderia gladioli*), T<sub>8</sub> = BDISOB70R (*Serratia marcescens*) and T<sub>9</sub> = BDISOB172R (*Bacillus aerophilus*)

**Table 32.** Effect of different formulated bacterial antagonists (identified in aman season 2019) on rice yield in aman, season 2020

Treatments	BRR I dhan49		Dhanigold		BINA dhan7		BINA dhan11		**Mean yield increase (%)
	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	
T <sub>0</sub>	3.38b	0.00	3.83b	0.00	2.83b	0.00	3.33b	0.00	0.00
T <sub>1</sub>	4.13ab	22.22	4.33ab	13.04	3.25ab	14.71	4.25a	27.50	19.37
T <sub>2</sub>	4.38ab	29.63	4.67ab	21.74	3.88a	36.76	4.00ab	20.00	27.03
T <sub>3</sub>	4.17ab	23.46	4.21ab	9.78	3.63ab	27.94	4.13a	23.75	21.23
T <sub>4</sub>	4.58a	35.80	4.63ab	20.65	3.21ab	13.24	4.08a	22.50	23.05
T <sub>5</sub>	4.54ab	34.57	4.46ab	16.30	3.42ab	20.59	4.96a	30.00	25.37
T <sub>6</sub>	3.29b	0.00	4.50ab	17.39	3.38ab	19.12	3.75ab	12.50	12.25
T <sub>7</sub>	3.83ab	13.58	3.88b	1.09	3.42ab	20.59	3.79ab	13.75	12.25
T <sub>8</sub>	4.33ab	28.40	4.00ab	4.35	3.25ab	14.71	3.75ab	12.50	14.99
T <sub>9</sub>	4.25ab	25.93	4.88a	27.17	3.25ab	14.71	4.00ab	20.00	21.95
Level of significance	*		*		*		*		
CV (%)	14.95		13.54		11.43		9.39		

Values with same letters indicate statistically similar. \* indicates the treatment means are significant at 5% level of significance. NS-Not significant. \*\* average yield increase by each treatment over control considering in four varieties. T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)], T<sub>2</sub>=BDISOB07R (*Serratia nematodiphila*), T<sub>3</sub>= BDISOB12R (*Serratia marcescens*), T<sub>4</sub>= BDISOB31R (*Serratia marcescens*), T<sub>5</sub>= BDISOB36R (*Serratia marcescens*), T<sub>6</sub>= BDISOB46R (*Serratia marcescens*), T<sub>7</sub>= BDISOB54R (*Burkholderia gladioli*), T<sub>8</sub>= BDISOB70R (*Serratia marcescens*) and T<sub>9</sub>= BDISOB172R (*Bacillus aerophilus*)

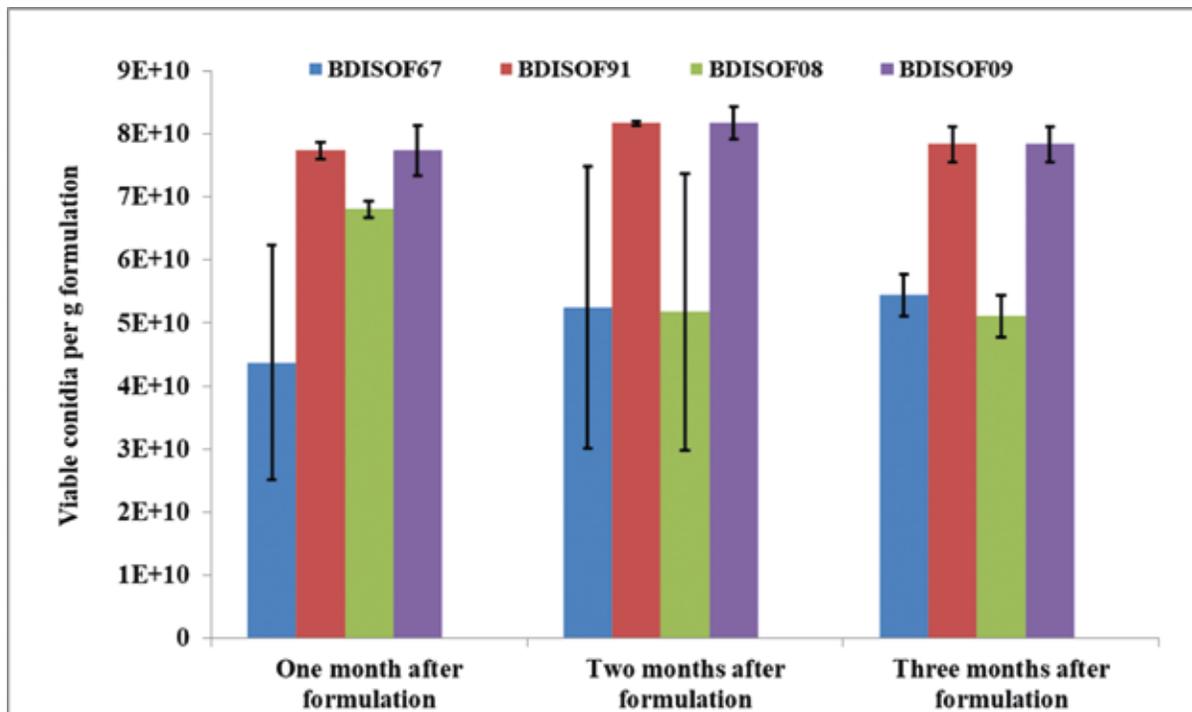
### Field efficacy of formulated antagonistic fungal isolates identified from rhizosphere soil samples collected in two boro seasons

The efficacy of four formulated antagonistic and plant growth promoting fungi identified from rhizosphere soil samples collected in boro season 2018 and 2019 were evaluated against BB of rice in two hybrids viz. Hybrid Hera-2 and ArizeTejGold and in two inbreed viz. BRRIdhan28 and BRRIdhan29 rice varieties under both net house and field condition during boro seasons 2019 and 2020.

Four fungal strains identified in boro season 2018 and 2019 viz. BDISOF67R (*Trichoderma paraviridescens*), BDISOF91R (*T. erinaceum*), BDISOF08R (*T. asperellum*) and BDISOF09R (*T. asperellum*) were formulated and these formulated fungal strains can survive for at least three months after formulation (Figure 47).

The effect of these four formulated fungal bioagents such as T<sub>2</sub>= BDISOF67R (*Trichoderma paraviridescens*), T<sub>3</sub>= BDISOF91R (*T. erinaceum*), T<sub>4</sub>= BDISOF08R (*T. asperellum*) and T<sub>5</sub>= BDISOF09R (*T. asperellum*) significantly reduced lesion length by 45.77% to 61.76% caused by *X. oryzae* pv. *oryzae* in four varieties under net house condition at 28 days after inoculation (Table 33). The effect of these formulated fungal bioagents reduced lesion length by 40.14 to 49.12% caused by *X. oryzae* pv. *oryzae* in four varieties under field condition at 28 DAI (Table 34).

However, these four formulated fungal bioagents increased yield by 17.03% to 23.94% as compared to control under field condition considering four varieties (Table 35).



**Figure 47.** Viability of formulated plant growth promoting fungal isolates antagonistic to *X. oryzae* pv. *oryzae*. BDISOF67R (*Trichoderma paraviridescens*), BDISOF91R (*T. erinaceum*), BDISOF08R (*T. asperellum*) and BDISOF09R (*T. asperellum*)

**Table 33.** Effect of formulated plant growth promoting fungi on the reduction of lesion length caused by *X. oryzae* pv. *oryzae* under net house condition

Treatments	Hybrid Hera-2			ArizeTej Gold			BRR1 dhan28			BRR1 dhan29			**Mean lesion length reduction (%)
	Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			
	14	21	28	14	21	28	14	21	28	14	21	28	
T <sub>0</sub>	0.85a (0.00)	1.40a (0.00)	6.37a (0.00)	0.88a (0.00)	1.47 (0.00)	7.65a (0.00)	0.87a (0.00)	1.47 (0.00)	2.85a (0.00)	0.88a (0.00)	1.48 (0.00)	4.23a (0.00)	0.00
T <sub>1</sub>	0.68b (19.60)	1.08b (22.62)	1.02b (84.03)	0.67b (24.53)	1.07 (27.27)	4.45b (41.83)	0.72b (17.31)	1.10 (25.00)	1.40c (50.88)	0.70b (20.75)	1.15 (22.47)	2.30b (45.66)	55.60
T <sub>2</sub>	0.67b (21.56)	1.08b (22.62)	2.17b (65.97)	0.65bc (26.42)	1.08 (26.14)	2.11c (72.46)	0.67bc (23.08)	1.13 (22.73)	1.62bc (43.27)	0.65b (26.42)	1.08 (26.97)	1.47b (65.35)	61.76
T <sub>3</sub>	0.7b (17.65)	1.08b (22.62)	1.65b (74.08)	0.62bc (30.19)	1.10 (25.00)	1.99c (73.99)	0.65c (25.00)	1.15 (21.59)	1.93bc (32.16)	0.70b (20.75)	1.05 (29.21)	1.53b (63.78)	61.00
T <sub>4</sub>	0.52c (39.22)	1.10b (21.43)	2.29b (63.98)	0.60c (32.45)	1.44 (1.59)	3.00bc (60.78)	0.55d (36.54)	1.31 (10.68)	2.38ab (16.65)	0.57c (35.47)	1.34 (9.44)	2.47b (41.70)	45.77
T <sub>5</sub>	0.51c (39.61)	1.23ab (11.90)	2.33b (63.40)	0.53d (40.00)	1.33 (8.86)	2.12c (72.29)	0.56d (35.77)	1.07 (26.82)	2.39ab (16.00)	0.56c (36.60)	1.21 (18.70)	2.28b (46.14)	49.45
Level of significance	*	*	*	*	NS	*	*	*	*	*	NS	*	*
CV (%)	5.03	8.86	26.44	5.40	26.20	25.18	5.28	25.23	20.70	4.59	20.58	26.14	

Values with same letters indicates statistically similar. \* indicates the treatment means are significant at 5% level of significance. NS=Not significant. \*\*Mean reduction in lesion length over control considering four varieties. T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicides), T<sub>2</sub>= BDISOF67R (*Trichoderma paraviridescens*), T<sub>3</sub>= BDISOF9IR (*T. erinaceum*), T<sub>4</sub>= BDISOF08R (*T. asperellum*) and T<sub>5</sub>= BDISOF09R (*T. asperellum*)

**Table 34.** Effect of formulated plant growth promoting fungi on the reduction of lesion length caused by *X. oryzae* pv. *oryzae* under field condition

Treatments	Hybrid Hera-2			ArizeTej Gold			BRRi dhan28			BRRi dhan29			**Mean lesion length reduction (%)
	Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			Days after inoculation (DAI)			
	14	21	28	14	21	28	14	21	28	14	21	28	
T <sub>0</sub>	1.05 a (0.00)	1.59 a (0.00)	3.03 a (0.00)	1.03 a (0.00)	1.59 a (0.00)	3.07 a (0.00)	1.03 a (0.00)	1.58 a (0.00)	3.07 a (0.00)	1.03 a (0.00)	1.56 a (0.00)	3.36 a (0.00)	0.00
T <sub>1</sub>	0.64 b (38.46)	0.73 b (54.24)	1.62 c (46.53)	0.61 d (41.34)	0.74 b (53.57)	1.49 b (51.35)	0.61 b (40.24)	0.96 b (39.24)	1.59 c (46.53)	0.65 bc (37.46)	0.92 b (41.22)	1.61 b (52.08)	49.12
T <sub>2</sub>	0.66 b (36.87)	0.86 b (46.10)	1.60 c (47.08)	0.64 c (38.14)	0.76 b (52.31)	1.66 b (45.93)	0.65 b (36.37)	0.81 bc (48.73)	1.60 c (47.08)	0.66 bc (36.50)	0.79 b (49.53)	1.70 b (49.31)	47.35
T <sub>3</sub>	0.67 b (35.60)	0.88 b (44.84)	1.56 c (48.51)	0.67 b (35.25)	0.80 b (49.80)	1.62 b (47.24)	0.66 b (36.04)	0.79 bc (50.00)	1.61 c (48.51)	0.65 bc (37.14)	0.81 b (48.25)	1.64 c (51.09)	48.83
T <sub>4</sub>	0.51 c (51.78)	1.01 b (36.69)	1.98 b (34.65)	0.51 e (51.28)	0.50 c (68.63)	1.14 b (62.84)	0.54 c (47.35)	0.50 c (68.35)	1.96 b (34.65)	0.72 b (30.73)	1.52 ab (2.89)	1.97 b (41.47)	43.40
T <sub>5</sub>	0.51 c (51.15)	1.01 b (36.69)	1.93 b (36.19)	0.51 e (51.28)	0.60 c (62.35)	1.58 b (48.71)	0.55 c (47.02)	1.50 a (5.06)	1.90 b (36.19)	0.52 c (50.29)	1.49 b (4.81)	2.03 b (39.48)	40.14
Level of significance	*	*	*	*	*	*	*	*	*	*	*	*	*
CV (%)	3.39	19.48	5.76	1.90	7.81	19.03	4.72	31.67	4.36	14.36	20.24	6.95	

Values with same letters indicates statistically similar. \*indicates the treatment means are significant at 5% level of significance. \*\*Mean reduction in lesion length over control considering four varieties. T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicides) @ 4g/L, 2ml/L and 4g/L of water respectively], T<sub>2</sub>= BDISOF67R (*Trichoderma paraviridescens*), T<sub>3</sub>= BDISOF91R (*T. erinaceum*), T<sub>4</sub>= BDISOF08R (*T. asperellum*) and T<sub>5</sub>= BDISOF09R (*T. asperellum*)

**Table 35.** Effect of formulated plant growth promoting fungi on rice yield in boro, 2018-2019

Treatments	Hybrid Hear-2		ArizeTej Gold		BRR1 dhan28		BRR1 dhan29		**Mean yield increase (%)
	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	Yield (t/ha)	Yield increase over control (%)	
T <sub>0</sub> (Control)	5.20b	0.00	5.53b	0.00	5.08b	0.00	5.60b	0.00	0.00
T <sub>1</sub>	6.29a	20.99	6.13ab	10.86	6.22a	22.30	6.38a	13.99	17.03
T <sub>2</sub>	6.71a	29.01	6.99a	26.55	6.25a	22.95	6.50a	16.07	23.64
T <sub>3</sub>	6.68a	28.53	6.80ab	23.08	6.49a	27.64	6.53a	16.52	23.94
T <sub>4</sub>	6.53a	25.64	6.5ab	17.65	6.16a	21.11	5.99ab	7.08	17.87
T <sub>5</sub>	6.5a	25	6.9a	24.89	6.3a	23.93	6.18a	10.48	21.07
Level of significance	*		*		*		*		
CV (%)	5.26		10.20		7.47		4.71		

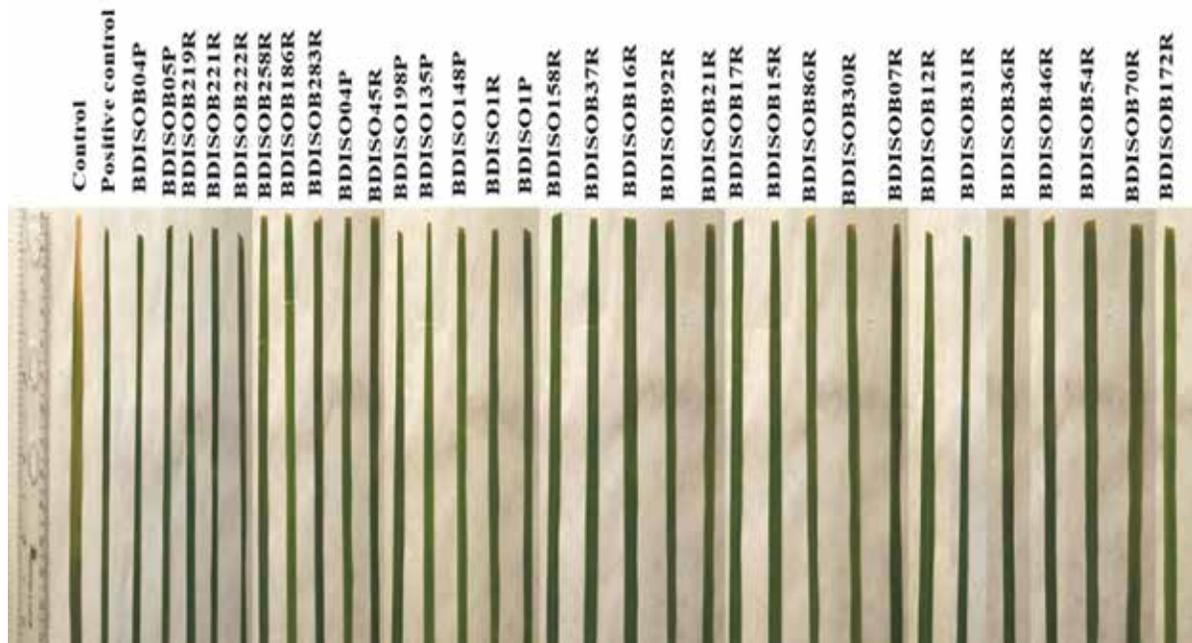
Values with same letters indicate statistically similar. \* indicates the treatment means are significant at 5% level of significance. \*\*Mean yield increase by each treatment over control considering four varieties. T<sub>0</sub>= Control, T<sub>1</sub>= Positive control [where the plants sprayed with Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicides), T<sub>2</sub>= BDISOF67R (*Trichoderma paraviridescens*), T<sub>3</sub>= BDISOF91R (*T. erinaceum*), T<sub>4</sub>= BDISOF08R (*T. asperellum*) and T<sub>5</sub>= BDISOF09R (*T. asperellum*)

### Effect of some selected antagonistic bacterial isolates on the reduction of lesion length in susceptible check cultivar (IR24) caused by *X. oryzae* pv. *oryzae*

To study the mechanisms of BB severity reduction by plant growth promoting antagonistic bacteria, susceptible check variety IR24 was used. The results of plant inoculation showed a significant reduction of lesion length in plants sprayed with formulated bacterial bioagents as compared with untreated control (Table 36).

The maximum (96.56%) reduction of lesion length were observed in plants sprayed with BDISOB222R (*Pseudomonas plecoglossicida*) followed by BDISOB05P (*Pseudomonas putida*) (95.71), BDISOB283R (*Pseudomonas fluorescens*) (94.38), BDISOB21R (*Serratiamarcescens*) (93.80), BDISOB258R (*Pseudomonas putida*) (93.61), BDISOB04P (*Pseudomonas putida*) (92.61), BDISO45P (*Bacillus paramycoides*) (91.55) and BDISO1R (*Bacillus amyloliquefaciens*) (90.16). The minimum (50.145%) reduction of lesion length were observed in plants sprayed with BDISO158R (*Serratia marcescens*) followed by BDISO198P (*Serratia plymuthica*) (52.36) and BDISOB15R (*Pseudochrobactrum asaccharolyticum*) (54.03). However, all other bacterial isolates reduced lesion length significantly compared to the untreated plants (Table 36 & Figure 48)

Four plant growth promoting bacteria viz. BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISO45P (*Bacillus paramycoides*) and BDISO1R (*Bacillus amyloliquefaciens*) were selected to study the mechanisms of plant growth promoting bacteria mediated induced resistance in rice. Seven SA and JA-pathway related genes involved in resistance in rice were considered in this study.



**Figure 48.** Reduction of lesion length by some selected antagonistic bacteria in susceptible check cultivar (IR24). Photographs were taken at 14 days after inoculation.

**Table 36.** Effect of some selected antagonistic bacterial isolates on the reduction of lesion length in susceptible check cultivar (IR24) caused by *X.oryzae* pv. *oryzae*

\*Lesion lengths were measured at 14 DAI.

Isolate ID	Name of Bacteria	*Lesion length (mm)	Reduction of lesion length (%)
Control	-	23.67a	0
Positive control	-	6.33b-d	73.31
BDISOB04P	<i>Pseudomonas putida</i>	1.50ij	92.61
BDISOB05P	<i>Pseudomonas putida</i>	1.00j	95.71
BDISOB219R	<i>Pseudomonas taiwanensis</i>	5.67c-f	76.04
BDISOB221R	<i>Pseudomonas</i> sp.	5.00d-g	78.85
BDISOB222R	<i>Pseudomonas plecoglossicida</i>	0.83j	96.56
BDISOB258R	<i>Pseudomonas putida</i>	1.50ij	93.61
BDISOB186R	<i>Pseudomonas</i> sp.	5.33c-g	77.38
BDISOB283R	<i>Pseudomonas fluorescens</i>	1.33ij	94.38
BDISOB04P	<i>Pseudomonas putida</i>	5.83c-e	75.25
BDISOB45R	<i>Bacillus paramycoides</i>	2.00ij	91.55
BDISOB198P	<i>Serratia plymuthica</i>	5.83c-e	52.36
BDISOB135R	<i>Bacillus</i> sp.	2.83hi	88.08
BDISOB148P	<i>Serratia marcescens</i>	5.83c-e	75.69
BDISOB1R	<i>Bacillus amyloliquefaciens</i>	2.33ij	90.16
BDISOB145P	<i>Serratia marcescens</i>	6.83bc	71.12
BDISOB158R	<i>Serratia marcescens</i>	6.83bc	50.14
BDISOB37R	<i>Pseudochrobactrum asaccharolyticum</i>	5.33c-g	77.44
BDISOB16R	<i>Pseudochrobactrum asaccharolyticum</i>	5.17d-g	78.01
BDISOB92R	<i>Pseudomonas fluorescens</i>	4.50e-g	80.85
BDISOB21R	<i>Serratia marcescens</i>	2.17ij	93.80
BDISOB17R	<i>Limnolyngbya circumcreta</i>	4.00gh	83.33
BDISOB15R	<i>Pseudochrobactrum asaccharolyticum</i>	5.33c-g	54.03
BDISOB86R	<i>Enterobacter aerogenes</i>	4.00gh	83.33
BDISOB30R	<i>Pseudochrobactrum asaccharolyticum</i>	4.33e-h	81.64
BDISOB07R	<i>Serratia nematodiphila</i>	4.00gh	83.33
BDISOB12R	<i>Serratia marcescens</i>	4.00gh	83.06
BDISOB31R	<i>Serratia marcescens</i>	5.00d-g	78.97
BDISOB36R	<i>Serratia marcescens</i>	5.83c-e	75.49
BDISOB46R	<i>Serratia marcescens</i>	4.17f-h	82.28
BDISOB54R	<i>Burkholderia gladioli</i>	4.17f-h	82.41
BDISOB70R	<i>Serratia marcescens</i>	2.83hi	87.96
BDISOB172R	<i>Bacillus aerophilus</i>	7.50b	68.21
Level of significance		*	
CV (%)		16.80	

BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB219R (*Pseudomonas taiwanensis*), BDISOB221R (*Pseudomonas* sp.), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*), BDISOB186R (*Pseudomonas* sp.), BDISOB283R (*Pseudomonas fluorescens*), BDISO04P (*Pseudomonas putida*), BDISO45R (*Bacillus paramycoides*), BDISO198P (*Serratia plymuthica*), BDISO135R (*Bacillus* sp.), BDISO148P (*Serratia marcescens*), BDISOB01R (*Bacillus amyloliquefaciens*), BDISO145P (*Serratia marcescens*), BDISO158R (*Serratia marcescens*), BDISOB37R (*Pseudochrobactrum asaccharolyticum*), BDISOB16R (*Pseudochrobactrum asaccharolyticum*), BDISOB92R (*Pseudomonas fluorescens*), BDISOB21R (*Stenotrophomonas maltophilia*), BDISOB17R (*Limnolyngbya circumcreta*), BDISOB15R (*Pseudochrobactrum asaccharolyticum*), BDISOB86R (*Enterobacter aerogenes*), BDISOB30R (*Pseudochrobactrum asaccharolyticum*), BDISOB07R (*Serratia nematodiphila*), BDISOB12R (*Serratia marcescens*), BDISOB31R (*Serratia marcescens*), BDISOB36R (*Serratia marcescens*), BDISOB46R (*Serratia marcescens*), BDISOB54R (*Burkholderia gladioli*), BDISOB70R (*Serratia marcescens*) and BDISOB172R (*Bacillus aerophilus*)

## Differential expression of some SA and JA pathway related genes in plants treated with plant growth promoting bacteria

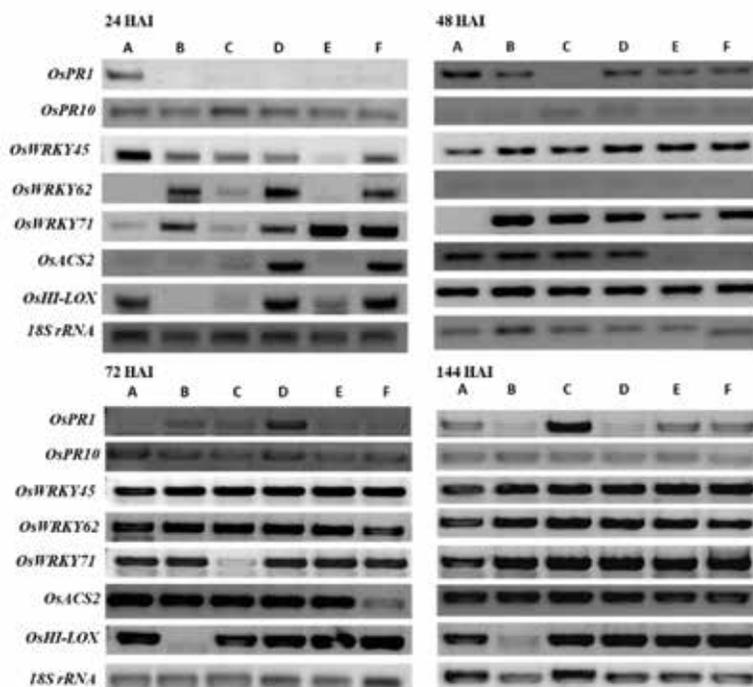
At 24 hours after inoculation (HAI) the higher levels of expression of *OsPRI0*, *OsWRKY45*, *OsWRKY62*, *OsWRKY71*, *OsACS2* and *OsHI-LOX* were observed in plants sprayed with, BDISOB222R (*Pseudomonas plecoglossicida*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared to untreated control. However, the expression levels of *OsPRI0*, *OsWRKY45* and *OsWRKY62* were found higher in plants sprayed with BDISOB05P (*Pseudomonas putida*), and BDISOB45P (*Bacillus paramycoides*). The expression of *OsPRI0*, *OsWRKY71* and *OsHI-LOX* was observed higher in plants treated with BDISOB45R (*Bacillus paramycoides*) (Figure 49).

At 48 HAI, the higher level of expression of *OsWRKY45* was found in plants treated with BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45P (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated control. The expression of *OsWRKY71* was observed in plants treated with BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated control. The expression of *OsACS2* was observed in plants treated with BDISOB45R (*Bacillus paramycoides*) and BDISOB222R (*Pseudomonas plecoglossicida*) compared to untreated control.

At 72 HAI, the higher expression level of *OsWRKY62* was found in plants sprayed BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45P (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated and positive control. The expression of *OsWRKY71* was noticed higher in plants treated with BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated control. The higher level of expression of *OsHI-LOX* was also observed in plants sprayed with BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) compared to untreated and positive control.

At 144 HAI, the higher expression of *OsPRI* was observed in plants sprayed with BDISOB05P (*Pseudomonas putida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated and positive control. The gene *OsWRKY45*, *OsWRKY62*, *OsWRKY71*, *OsACS2* and *OsHI-LOX* expressed at higher levels in plants treated with BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) compared to untreated control (Figure 49).

These results primarily indicated that plant growth promoting bacteria reduced bacterial blight severity in rice through inducing the expression of some selected SA and JA pathway related genes. The quantification of SA and JA in plants treated with these bacterial bioagents will clarify our observation. However, the possibility of induction of defense related enzymes is under investigation in our Laboratory.



**Figure 49.** Expression levels of some selected marker genes involved in Salicylic acid (SA) and Jasmonic acid (JA) pathway by RT-PCR. Total RNA was extracted from rice leaves and cDNA was synthesized. PCR was performed using cDNA as template. A. Untreated control, B. Positive control (Plants sprayed with Bactroban, Hemoxy and SICOGREEN®), C. BDISOB05P (*Pseudomonas putida*), D. BDISOB222R (*Pseudomonas plecoglossicida*), E. BDISBO45P (*Bacillus paramycooides*) and F. BDISBO1R (*Bacillus amyloliquefaciens*).

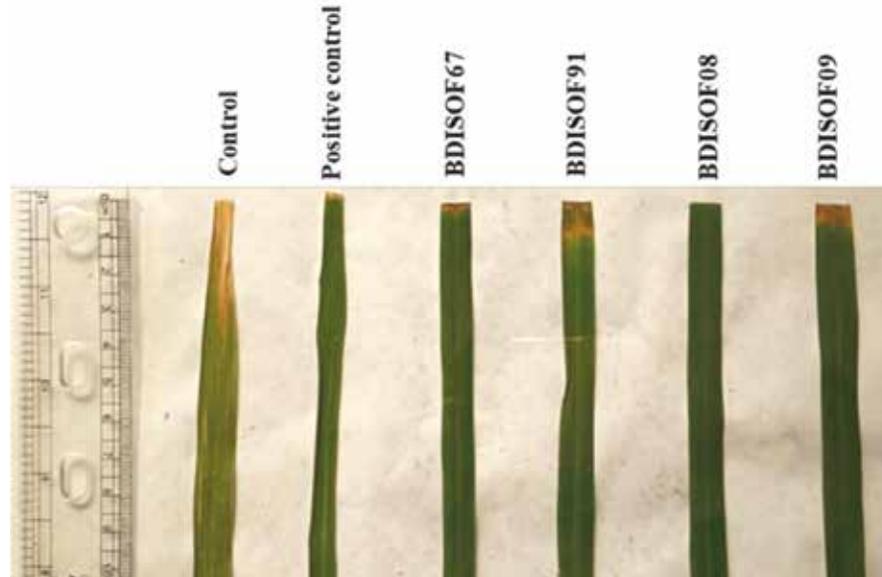
**Effect of some selected antagonistic fungal isolates on the reduction of lesion length in susceptible check cultivar (IR24) caused by *X. oryzae* pv. *oryzae***

The maximum (87.50%) reduction of lesion length were observed in plants sprayed with BDISOF08 (*Trichoderma asperellum*) followed by BDISOF67 (*Trichoderma paraviridescens*) (75.00%) and BDISOF91 (*Trichoderma erinaceum*) (60.42%) and the minimum (31.25%) reduction of lesion length were observed in plants sprayed with BDISOF09 (*Trichoderma asperellum*) as compared with the untreated plants (Table 37 & Figure 50).

**Table 37.** Reduction of lesion length by plant growth promoting antagonistic fungal isolates in susceptible check cultivar (IR24).

Treatments	Name of the fungi	Lesion length (mm)	Reduction of lesion length (%)
Control		23.67a	0.00
Positive control		6.33d	75.00
BDISOF67	<i>Trichoderma paraviridescens</i>	5.50de	75.00
BDISOF91	<i>Trichoderma erinaceum</i>	10.83c	60.42
BDISOF08	<i>Trichoderma asperellum</i>	3.83e	87.50
BDISOF09	<i>Trichoderma asperellum</i>	15.50b	31.25
Level of significance		*	-
CV (%)		10.31	-

\*Lesion lengths were measured at 14 DAI.



**Figure 50.** Reduction of lesion length by plant growth promoting antagonistic fungal isolates in susceptible check cultivar (IR24). Photographs were taken at 14 days after inoculation.

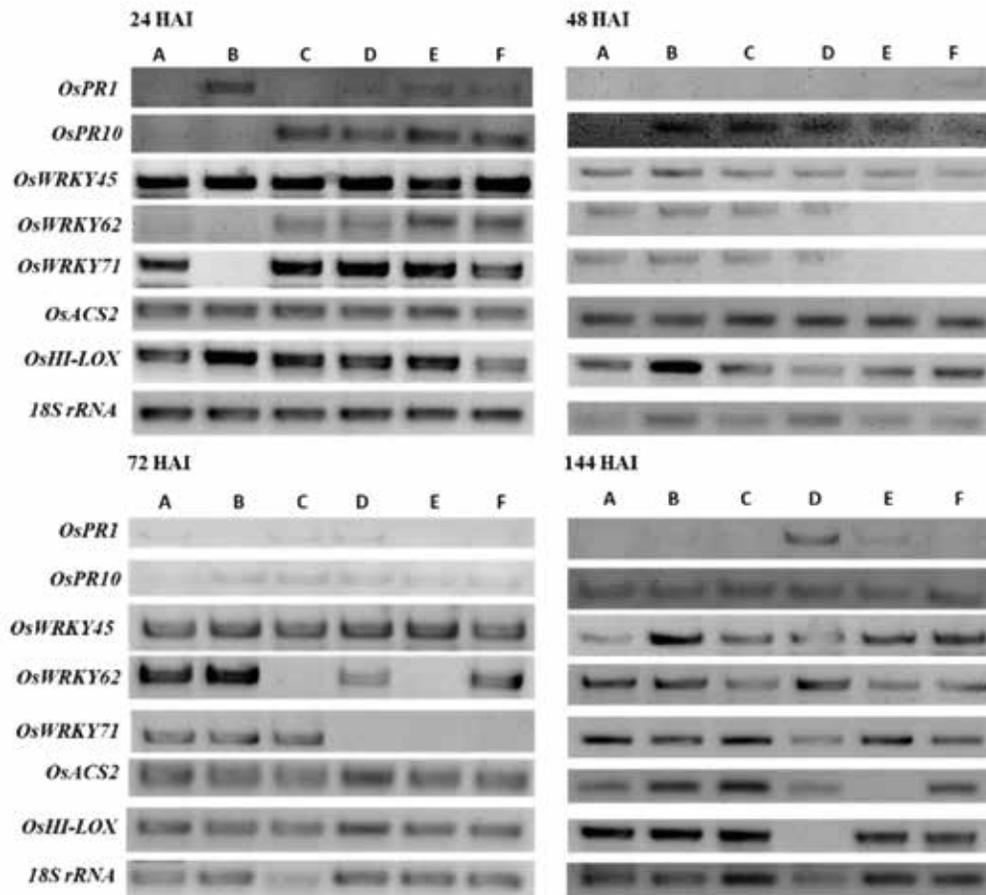
### **Differential expression of some SA and JA pathway related genes in plants treated with plant growth promoting fungi**

At 24 HAI, the higher levels of expression of *OsPR10*, *OsWRKY45*, *OsWRKY62*, *OsWRKY71*, *OsACS2* and *OsHI-LOX* were recorded in plants sprayed with BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) as compared with untreated control. However, at 48 HAI no significant differences were observed in expression of these genes in plants treated with BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) (Figure 51).

At 72 HAI, the higher levels of expression of *OsWRKY45*, *OsACS2* and *OsHI-LOX* were found in plants sprayed with BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) as compared with untreated control. However, the gene *OsWRKY71* was expressed slightly higher in plants treated with BDISOF67 (*Trichoderma paraviridescens*).

At 144 HAI, the gene *OsPR1* was expressed slightly higher in plants treated with BDISOF91 (*Trichoderma erinaceum*) and BDISOF08 (*Trichoderma asperellum*). However, the higher levels of expression of *OsWRKY45* and *OsACS2* was noticed in plants sprayed with BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) as compared with untreated and positive control.

These results indicated that plant growth promoting *Trichoderma* reduced bacterial blight severity in rice possibly through induced of the expression of some selected genes related to SA and JA pathway. The quantification of SA and JA in plants treated with these fungal bioagents will validate our gene expression data. However, the possibility of induction of defense related enzymes is under investigation in our Laboratory (Figure 51).



**Figure 51.** Expression levels of some selected marker genes involved in Salicylic acid (SA) and Jasmonic acid (JA) pathway by RT-PCR. Total RNA was extracted from rice leaves and cDNA was synthesized. PCR was performed using cDNA as template. A. Untreated control, B. Positive control (Plants sprayed with Bactroban, Hemoxy and SICOGREEN®), C. BDISOF67 (*Trichoderma paraviridescens*), D. BDISOF91 (*Trichoderma erinaceum*), E. BDISOF08 (*Trichoderma asperellum*) and F. BDISOF09 (*Trichoderma asperellum*).



**Figure 52.** Photographs showing the activities of net house experiment for mechanisms study.

## 12. Research highlights:

**Title of the sub-project:** Identification of novel resistant gene(s), gene pyramiding and sustainable management of bacterial blight (BB) disease of rice

**Background:** Among 32 diseases of rice in Bangladesh at present, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) considered as a most destructive disease occurs in all Agro Ecological Zones (AEZs) of Bangladesh and cause considerable yield loss (30%) (Shahjahan 1993). It is also an important disease in most of the South and Southeast Asian countries (Sharma *et al.*, 1991), as it can cause over 50%, 60% and 57% rice yield reductions in Japan (Soga 1918), India (Srivastava *et al.*, 1966) and Pakistan (Khan *et al.*, 2015) respectively in the severely diseased rice fields.

Pathogenic variability of *Xoo* in Bangladesh has been reported (Noda *et al.*, 1996, Jalaluddin and Kashem 1999). Twelve races of the *Xoo* have been identified until 1995 in Bangladesh and the study indicated that some aggressive strains of *Xoo* occur in Bangladesh (BRRRI 2018). Few examples indicate that some *R* genes used for controlling BB disease are overcome by virulent strains in Korea with the resistant gene *Xa21* (Lee *et al.*, 1999, Zhang *et al.*, 2006).

Understanding both pathogen population structure as well as host pathogen resistance is the prerequisite in designing of effective strategy for deployment of resistance. Durable resistant varieties can help to minimize the resistance breakdown problem. Breeding for durable resistance to BB in rice requires recent information on the pathogen population and the geographical distribution of the races.

Wild plant species and land races are shown to be an important and rich genetic reservoir of resistance sources (Dangl *et al.*, 2013). To date, at least 46 BB rice resistance genes have been identified, but only a few of them have been successfully deployed for resistance breeding (Zhang *et al.*, 2014, Kim *et al.*, 2015, Hutin *et al.*, 2015), among which *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* appear to be widely used in breeding programs in Bangladesh (Khan *et al.*, 2014). Pyramiding of multiple resistance genes into rice varieties is one way to develop durable resistance to BB. Marker assisted selection (MAS) was applied for pyramiding three or four or five genes for BB resistance (i.e., *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23*). Pyramid lines IR 129336:11-4 or IR 129336:11-35 (*Xa4-xa5-xa13-Xa21-Xa23*) or IRBB60 (*Xa4*, *Xa5*, *xa13* and *Xa21*) four or five genes were also developed at IRRI. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Huang *et al.*, 1997).

BRRRI recently released Boro varieties BRRRI dhan63 and BRRRI dhan81 (background is BRRRI dhan28) both are premium quality rice and farmer's acceptance was observed as high but these varieties are highly susceptible to BB. Another, popular modern variety BRRRI dhan49 in T. Aman season is highly susceptible to BB. So, combination of BB resistant genes *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* in the background of BRRRI dhan63 or BRRRI dhan81 or BRRRI dhan49 will help much to reduce yield loss of rice as well as livelihood improvement of the poor people in Bangladesh.

On the contrary, biological control of BB using endophytic fungi and plant growth promoting rhizobacteria (PGPR) has emerged as an effective strategy during last two decades. In the recent days, biological control is considered as a best alternative way of reducing the use of chemicals in agriculture (Misk and Franco 2011). Endophytes can have many effects on their host such as enhancement of stress-, insect- and disease-resistance (Bush *et al.*, 1997, Clay & Holah 1999) and productivity improvement (Quaroni *et al.*, 1997) when in association with their hosts. These facts indicate that endophytes can be potential biological control agents and

will play an important role in ecological agriculture. Moreover, endophytic fungi from rice plants were reported to be effective *in vitro* against rice pathogens such as *Magnaporthe oryzae*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Xoo* (Tian *et al.*,2004).

In this study, known bacterial blight resistant gene(s) will be identified from native germplasm. Races and its' distribution of *Xanthomonas oryzae* pv. *oryzae* will be identified as well as bacterial blight resistance genes will be introgressed into the popular rice varieties BRR1 dhan63. BRR1 dhan81 or BRR1 dhan49 having high yield potential through marker-assisted backcrossing. Simultaneously, the use of a single “dual-purpose inoculum” based upon native endophytic fungi and antagonistic-PGPR that can promote rice growth and control *Xoo* attack for environment friendly and sustainable management of bacterial blight.

### **Objectives:**

**Sub-project general objective (s):** Manage bacterial blight disease through gene pyramiding and biological approaches

### **Sub-project specific objectives (component wise):**

#### **BRR1 component:**

- i. Identify the novel/known bacterial blight resistant genes in land race
- ii. Identify physiological races across the country and
- iii. Develop BB resistant varieties along with blast resistant gene in the background of susceptible BRR1 released high yielding varieties.

#### **BAU component:**

- i. Isolate and identify the endophytic beneficial fungi and bacteria from rice phylloplane and rhizosphere.
- ii. Assess the *in vitro* antipathogenic activity of beneficial endophytic fungi and bacteria against bacterial blight pathogen.
- iii. Formulate selected beneficial endophytic fungi and bacteria against BB pathogen.
- iv. Evaluate the field efficacy of some formulated and some non-formulated endophytic fungi and bacteria against BB disease of rice.

### **Methodology followed:**

#### **BRR1 component**

#### **Identification of known/novel BB resistant genes**

**Phenotyping screening of land races and cultivars:** In total, 928 rice germplasm (including checks) were received from the Genetic Resources and Seed Division, BRR1. Screening of these land races along with two susceptible check varieties (Purbachi and IR24) and resistant check (IRBB60) against BB was conducted during T. Aman and Boro seasons, 2018 and 2019. Artificial inoculation was carried out using most virulent and widely spread 3 major BB isolates (*Bxo67*, *Bxo87*, *Bxo91*).

**Molecular screening of land races and cultivars:** Resistant germplasm found after phenotypic screening were selected for molecular screening. Gene-based markers were used to explore the known bacterial blight resistant genes in the resistant germplasms. Based on phenotyping and molecular screening presence of known resistant genes were confirmed in the land races.

### **Identification of physiological races of BB and its distribution patterns**

**Collection, isolation and purification of BB isolates:** A total of 920 BB disease samples of BB were collected from 40 districts of different AEZs of Bangladesh. Then *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were isolated from the bacterial blight infected leaf samples. In total 300 *Xoo* were isolated from the diseased samples using peptone sucrose agar (PSA) media. The BB isolates were permanently preserved in 40% Glycerin NBY (Nutrient Broth Yeast) medium at -80°C.

**Inoculum preparation and inoculation:** Preserved BB isolates were transferred to PSA plates and incubated at 28°C for 48 h. A two days old culture of each isolate were used to prepare inoculum. Inoculum was prepared by suspending the bacterial cells with water and adjusting to a concentration of 10<sup>8</sup> CFU/ml prior to inoculation.

**Disease Assessment and identification of races/pathotypes of BB:** Identification of the races were recognized based on disease reaction of different *Xoo* isolates on 14 Near Isogenic lines (harboring single BB resistant gene) based on gene for gene theory. The percentages of diseased leaf areas were classified into 1 to 9 scales (SES, 2015).

### **Gene pyramiding for the development of durable resistant varieties**

Crossing and backcrossing were conducted for introgressing resistant gene(s) into highly susceptible Boro varieties BRR1 dhan63, BRR1 dhan81 and T. Aman variety BRR1 dhan49 by crossing with highly resistant IRRI developed pyramided lines IRBB58 and/or IRBB60 (*Xa4*, *xa5*, *xa13* and *Xa21*) through marker assisted selection (MAS). Since test varieties were also susceptible to blast, so in accordance with the suggestion of the inception workshop blast resistant parents i.e., *Pi9*-[US], *Pbl*-[US] were included in the gene pyramiding programme.

**Hybridization of the genotypes for producing backcross population:** The crossing population population were hybridized up to BC<sub>2</sub>F<sub>2</sub> or BC<sub>3</sub>F<sub>2</sub> population.

**Parental survey for the identification of polymorphic foreground primers:** A total of five gene based and tightly linked polymorphic foreground primers were used for the selection of resistant plants. advanced lines with BB and blast resistant genes were selected for the development of resistant varieties/pre-breeding lines.

**Pathological and molecular screening of the progenies:** Backcross populations of BC<sub>2</sub>F<sub>2</sub> or BC<sub>3</sub>F<sub>2</sub> generation from the different cross combinations were screened against virulent isolates (*Bxo67*, *Bxo87*, *Bxo91*) of BB. The plants were screened following leaf clipping method. The plants showing resistant reaction were also screened by molecular markers.

## **(BAU component)**

### **Identification of fungi and bacteria from rice phylloplane and rhizosphere antagonistic to bacterial blight pathogen, *X. oryzae* pv. *oryzae***

**Plant sample collection:** The healthy rice plants with root system of different rice cultivars were collected from 40 districts representing 30 AEZs of Bangladesh.

**Isolation and purification of fungi and bacteria:** Fungal isolates were isolated from both phylloplane and rhizosphere following dilution plate technique on PDA medium. The phylloplane bacteria were isolated by spreading 100µl solution obtained by washing 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> leaves of rice plant and bacterial isolates were isolated from both rhizospheres following dilution plate technique on PDA medium on Luria Bartani (LB) or King's B agar plate.

**Assay of antagonism of bacterial spp. and fungi against *X. oryzae* pv. *oryzae* by dual culture method:** The antagonistic activity of the purified fungal isolates against *X. oryzae* pv. *oryzae* was performed following dual culture method as described by Tian *et al.*, 2004. Antimicrobial activity of antagonistic strains of fluorescent pseudomonas/ *Pseudomonas* spp./ *Bacillus* spp. were determined by agar diffusion technique method (Monteiro *et al.*, 2005) with some modifications.

**Assessment of plant growth promoting determinants of bacteria antagonistic to *X. oryzae* pv. *oryzae*:** Active isolates with antagonistic potential against *X. oryzae* pv. *oryzae* were further evaluated for their ability to produce plant growth promoting determinants *viz.* siderophore production, Indole acetic acid (IAA) production and phosphate solubilization.

**Identification of selected plant growth promoting antagonistic bacterial isolates by sequence analyses of 16S rDNA:** The genomic DNA of antagonistic bacteria was extracted according to Wizard<sup>®</sup> Genomic DNA purification Kit (Promega, Madison, USA). To identify the antagonistic bacterial isolates, PCR products of 16S rDNA were sequenced which were amplified with primer sets 27F (5'-AGA GTT TGATCM TGG CTC AG-3') and 1518R (5'-AAG GAG GTG ATC CAN CCR CA-3') (Gio-vannoni, 1991).

**Sequencing of PCR products:** A partial nucleotide sequencing of 16Sr DNA was performed from amplified PCR products using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1518R (5'-AAG GAG GTG ATC CAN CCR CA-3') in the Macrogen Lab, South Korea via Biotech Concern Bangladesh.

**Identification of antagonistic fungal isolates by sequencing of ITS region:** Genomic DNA of the fungal isolates were extracted by using wizard<sup>®</sup> genomic DNA purification kit (Promega, Madison, WI, USA) from 100mg ground mycelium. Fungal isolates were identified by sequencing using PCR products of ITS regions which were amplified with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990)

### **Formulation of some selected plant growth promoting antagonistic fungi and bacteria against, *X. oryzae* pv. *oryzae***

Talc based formulation of 32 selected plant growth promoting antagonistic bacteria and four fungi were developed.

**Assessment of viability of the formulated fungal and bacterial antagonists:** The viability of the formulated bacterial and fungal antagonists was checked following dilution plate technique by drawing 1g of the formulated products in sterile water in every 30 days after formulation.

Assessment of plant growth promotion induced by antagonistic bacterial and fungal isolates: Rice seeds (cv. IR24) were surface sterilized and dried. Then the sterilized rice seeds were treated with formulated bacterial and fungal antagonists (10g/kg seeds). Then the germination of seeds was recorded at 7 DAS. The seedlings were uprooted at 7 DAS, 14 DAS and 28 DAS to measure the root length, shoot length and to calculate the vigor index [= (root length + shoot length) × germination percentage].

### **Evaluation of the efficacy of some formulated plant growth promoting antagonistic bacteria and fungi against BB disease of rice under net house and field condition**

Both field and net house experiments were conducted in two boro (2018-2019 and 2019-2020) and two aman (2019 and 2020) seasons on Farm Management Section, BAU, Mymensingh. For boro season, rice varieties viz. BRR1 dhan28, BRR1 dhan29, Hybrid HERA-2 & ArizeTejGold and for aman season, Dhanigold, BRR1 dhan49, BINA dhan7 and BINA dhan11 were used. Rice seeds were treated with fungal and bacterial formulations @10g/kg seeds. The seedlings were raised in the seedbed. Field experiments were conducted following RCBD design with three replications. Net house experiments were conducted at Professor Golam Ali Fakir Seed Pathology Centre, BAU following CRD design with three replications.

**Application of formulated bacterial and fungal antagonists:** In boro seasons (2017-2018 and 2018-2019) and aman seasons (2019 and 2020) the fungal and bacterial formulations were sprayed at 40, 55, 75, 90 and 105 DAT and in aman seasons (2019 and 2020) at 40 DAT, 50 DAT, 60 DAT, 70 DAT and 80 DAT. The leaves of each plants were inoculated by clip-inoculation method of Kauffman *et al.*, 1973.

**Harvesting and data collection:** Data were collected on the following parameters: lesion length(cm) and yield/ treatment (t/ha). Fresh yield data were converted using the formula: Yield (t/ha) at 14% moisture content = 100-Moisture content at harvest maturity (35%) x Weight at harvest /100- Moisture content at consumption (14%). That is the conversion factor was 0.75.

### **Mechanisms of plant growth promoting fungi and bacteria mediated induced resistance in rice against *X. oryzae* pv. *oryzae***

In studying the Salicylic acid (SA) and Jasmonic acid (JA) pathway mediated induced resistance in rice by PGP bacteria and fungi, a susceptible check variety (IR24) was used. Formulated PGP antagonistic bacterial and fungal isolates were sprayed twice (at 50 and 55 DAS) before inoculation and twice after inoculation *i.e* at 65 and 70 DAS. Rice plants were inoculated with a strain of *X. oryzae* pv. *oryzae* by Scissor following clipping method as described at 60 DAS. Ten rice leaf samples for each treatment were collected at 24, 48, 72 and 144 hrs after inoculation in zipper bags. Total RNA was extracted from 20 mg ground rice leaves powder using SV total RNA kit (Promega, USA) according to manufacturers' instruction. cDNA was synthesized from 5µg of total RNA. The reverse transcription reaction was performed using GoScript™ Reverse Transcription System (Promega, Madison, USA) using primers for the genes *OsPRI*, *OsPRI0*, *OsWRKY45*, *OsWRKY62*, *OsWRKY71*, *OsACS2*, *OsHI-LOX* related to SA and JA-pathway. *18S rRNA* was used as internal control for the analyses of the expression studies of test marker genes.

### **Statistical analysis**

The data on various parameters obtained from both net house and field experiments were analyzed statistically using MStatC program. Means of the treatments were compared with either DMRT and/or LSD.

## Key findings

### BRRRI component

#### Identification of known BB resistant genes

A highly resistant, 71 resistant and six moderately BB resistant germplasm was identified out of 928 test germplasms. Based on evaluation of gene based molecular marker, ten (10) germplasm contained 4 resistant genes, 15 germplasm contained 3 resistant genes, 22 germplasm contained 2 resistant genes and others had a single or unknown resistant gene.

#### Identification of physiological races of BB and its distribution patterns

Thirteen (13) races were identified across the country and the resistant genes, *Xa27*, *Xa21*, *xa13* and *Xa7* were effective against *xanthomonas oryzae* pv. *oryzae* under Bangladesh condition.

#### Gene pyramiding for the development of durable bacterial blight and blast resistant varieties

- A total of 10 plants of BC<sub>3</sub>F<sub>2</sub> generation having *Xa21*, *pb1* and *pi9* genes were selected in the background of BRRRI dhan81 using molecular marker.
- 5 plants of BC<sub>3</sub>F<sub>2</sub> generation containing *Xa21*, *pb1* and *pi9* gene were selected in the background of BRRRI dhan63 using molecular marker.
- In total, 4 plants of BC<sub>3</sub>F<sub>2</sub> generation having *Xa21* gene were selected in the background of BRRRI dhan49 using molecular marker.

### BAU Component

#### Identification of plant growth promoting antagonistic bacteria against *X. oryzae* pv. *oryzae*

The potential plant growth promoting bacteria viz. BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB283R (*Pseudomonas fluorescens*), BDISOB21R (*Serratia marcescens*), BDISOB258R (*Pseudomonas putida*), BDISOB04P (*Pseudomonas putida*), BDISO45P (*Bacillus paramycoides*) and BDISO1R (*Bacillus amyloliquefaciens*) were identified based on their performances in enhancing plant growth, reducing BB severity as well as in increasing rice yield.

#### Identification of plant growth promoting fungi antagonistic to *X. oryzae* pv. *oryzae*

Four potential fungal isolates viz. BDFISO67R (*Trichoderma paraviridescens*), BDFISO91R (*Trichoderma erinaceum*), BDISOF08R (*Trichoderma asperellum*) and BDISOF09R (*Trichoderma asperellum*) were identified based on their performances in increasing plant growth, reducing BB severity and increasing rice yield.

#### Mechanism of plant growth promoting bacteria and fungi mediated induced resistance in rice

Four plant growth promoting bacteria (PGPB) viz. BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISO45P (*Bacillus paramycoides*) and BDISO1R (*Bacillus amyloliquefaciens*) were selected to study the PGPB mediated induced resistance in rice against BB. Selected SA and JA-pathway related genes involved in resistance in rice were considered for expression study. The higher expression levels of some selected genes such as, *OsPRI*, *OsWRKY45*, *OsWRKY62*, *OsWRKY71*, *OsACS2* and

*OsHI-LOX* in plants sprayed with BDISOB05P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB45R (*Bacillus paramycoides*) and BDISOB01R (*Bacillus amyloliquefaciens*) as compared with untreated control indicated the PGPB mediated induced resistance in rice against BB pathogen.

On the other hand, the higher expression levels of *OsWRKY45* and *OsACS2* was noticed in plants sprayed with BDISOF67 (*Trichoderma paraviridescens*), BDISOF91 (*Trichoderma erinaceum*), BDISOF08 (*Trichoderma asperellum*) and BDISOF09 (*Trichoderma asperellum*) as compared with untreated and positive control clearly indicated the SA and JA mediated resistance in rice against BB pathogen. The quantification of SA and JA in plants treated with these bacterial and fungal bioagents will validate our gene expression data. However, the possibility of induction of defense related enzymes is under investigation in our Laboratory.

**Key words:** Bacterial blight, Physiological race, Gene pyramiding, Resistant variety, Antagonistic bacteria, Antagonistic fungi

## B. Implementation Status

### 1. Procurement (Component wise):

#### BRR component

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
<b>(a) Office equipment</b>	<b>12</b>	<b>261500</b>	<b>12</b>	<b>260750</b>	
1. Computer Table	1	5000	1	7900	
2. Computer Chair	1	3500	1	7350	
3. Executive Table	1	20000	1	16000	
4. Executive Chair	1	10000	1	10900	
5. File Cabinet	1	20000	1	17300	
6. Visitor Chair	1	4000	1	2600	
7. Steel Almira	1	24000	1	24200	
8. Desktop computer	1	60000	1	54400	
9. Laptop	1	60000	1	75900	
10. Laser Printer	1	20000	1	17900	
11. Digital camera	1	25000	1	19400	
12. Scanner	1	10000	1	6900	
<b>(b) Lab &amp;field equipment</b>	<b>7</b>	<b>2500000</b>	<b>7</b>	<b>2488870</b>	
1. Door type cold Incubator	1	250000	1	350000	
2. Four (4) degree freezer	1	190000	1	120000	
3. Digital micropipette	1	60000	1	29870	
4. UV-VIS Spectrophotometer	1	550000	1	548000	
5. Digital Gel doc. system	1	800000	1	795000	
6. Door type -200 freezer	1	400000	1	398000	
7. Drying Oven +20 to 3000 C	1	250000	1	248000	
<b>(c) Other capital items</b>					

### BAU component

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
<b>(a) Office equipment</b>					
Executive Table	01	20,000	01	20,000	
Executive Chair	01	10,000	01	10,000	
File Cabinet	01	20,000	01	20,000	
Steel Almira	01	24,000	01	24,000	
Visitor/Front Chair	01	4,000	01	4,000	
Computer Table	01	5,000	01	5,000	
Computer Chair	01	3,500	01	3,500	
<b>(b) Lab &amp;field equipment</b>					
Spectrophotometer	01	7,20,000	01	7,20,000	
Centrifuge Machine	01	2,00,000	01	2,00,000	
Magnetic stirrer with hotplate	01	60,000	01	60,000	
Laptop Computer	01	60,000	01	60,000	
Laser Printer	01	20,000	01	20,000	
Scanner	01	10,000	01	10,000	
Digital Camera	01	25,000	01	25,000	
<b>(c) Other capital items</b>					

### 2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

### 3. Training/study tour/ seminar/workshop/conference organized: BRR component

Description	Number of participants			Duration (Days/weeks/months)	Remarks
	Male	Female	Total		
(a) Training	65	14	79	1 day (3 batch)	
(b) Workshop	85	25	110	1 day	

### BAU component

Description	Number of participants			Duration (Days/weeks/months)	Remarks
	Male	Female	Total		
(a) Training	-	-	-	-	
(b) Workshop	40	10	50	1 day	

**C. Financial and physical progress (Combined & Component wise)  
Combined**

Fig in Tk.

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	3382258	3262603	3160153	102450	96.86	Recruitment of contractual staff was delayed
b. Field research/lab expenses and supplies	5533342	5451988	5570633	-118645	102.18	Chemical and reagents prices were higher than the estimated budget
c. Operating expenses	912361	932755	892380	40375	95.67	Due to COVID-19 pandemic
d. Vehicle hire and fuel, oil & maintenance	518925	515075	567075	-52000	110.10	Vehicle hire was increased during lockdown period
e. Training/workshop/ seminar etc.	552470	534700	534700	0	100.00	-
f. Publications and printing	240000	199126	199126	0	100.00	-
g. Miscellaneous	367676	367626	342656	24970	93.21	Due to COVID-19 pandemic, some renovation work was not done
h. Capital expenses	3931120	3931120	3928270	2850	99.93	Competitive tender pricing.
<b>Total</b>	<b>15438152</b>	<b>15194993</b>	<b>15194993</b>	<b>0</b>	<b>100</b>	

**BRR component**

Fig in Tk.

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2209146	2209146	2209146	0	100.00	-
b. Field research/lab expenses and supplies	3888392	3807038	3806173	865	99.98	Extra bank charge was paid from this line item
c. Operating expenses	552361	572755	573620	-865	100.15	Bank Charge was more than the allocated budget
d. Vehicle hire and fuel, oil & maintenance	218925	215075	215075	0	100.00	-
e. Training/workshop/ seminar etc.	455000	439700	439700	0	100.00	-
f. Publications and printing	200000	199126	199126	0	100.00	-
g. Miscellaneous	207676	207626	207626	0	100.00	-
h. Capital expenses	2749620	2749620	2749620	0	100.00	-
<b>Total</b>	<b>10481120</b>	<b>10400086</b>	<b>10400086</b>	<b>0</b>	<b>100.00</b>	

## BAU component

Fig in Tk.

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1173112	1053457	951007	102,450	90.27	Recruitment of contractual staff was delayed
b. Field research/lab expenses and supplies	1644950	1644950	1764460	-119,510	107.27	Chemical and reagents prices were higher than the estimated budget
c. Operating expenses	360000	360000	318760	41,240	88.54	Due to COVID-19 pandemic
d. Vehicle hire and fuel, oil & maintenance	300000	300000	352000	-52,000	117.33	Vehicle hire was increased during lockdown period
e. Training/workshop/ seminar etc.	97470	95000	95000	0	100.00	-
f. Publications and printing	40000	0	0	0	0.00	-
g. Miscellaneous	160000	160,000	135030	24970	84.39	Due to COVID-19 pandemic, some renovation work was not done
h. Capital expenses	1181500	1181500	1178650	2850	99.76	Competitive tender pricing.
<b>Total</b>	<b>4957032</b>	<b>4794907</b>	<b>4794907</b>	<b>0</b>	<b>100</b>	

## D. Achievement of Sub-project by objectives (Tangible form): Technology generated/ developed

### BRRRI component

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e., product obtained, visible, measurable)	Outcome (short term effect of the research)
Develop bacterial blight and blast resistant varieties in the background of susceptible BRRRI released high yielding varieties.	Hybridization was performed between BRRRI dhan63, BRRRI dhan81, BRRRI dhan49 and IRBB58, IRBB60, <i>Pi9</i> -[US], <i>Pb1</i> -[US] for bacterial blight and blast resistant pre-breeding lines development. Pathogenicity test and MAS were used for the selection of plants in BC <sub>3</sub> F <sub>2</sub> generation.	<ul style="list-style-type: none"> <li>➤ A total of 10 plants of BC<sub>3</sub>F<sub>2</sub> generation having <i>Xa21</i>, <i>Pb1</i> and <i>Pi9</i> genes were selected in the background of BRRRI dhan81 using molecular marker.</li> <li>➤ 5 plants of BC<sub>3</sub>F<sub>2</sub> generation containing <i>Xa21</i>, <i>Pb1</i> and <i>Pi9</i> gene were selected in the background of BRRRI dhan63 using molecular marker.</li> <li>➤ In total, 4 plants of BC<sub>3</sub>F<sub>2</sub> generation having <i>Xa21</i> gene were selected in the background of BRRRI dhan49 using molecular marker.</li> </ul>	Nineteen advanced lines (BC <sub>3</sub> F <sub>2</sub> ) having BB and blast resistant genes were developed, which have the potential as candidate variety and donor.

## BAU component

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e., product obtained, visible, measurable)	Outcome (short term effect of the research)
1. Isolate and identify the fungi and bacteria from rice phylloplane and rhizosphere antagonistic to bacterial blight pathogen, <i>X. oryzae</i> pv. <i>oryzae</i>	Collection of plant samples from 30 AEZs of Bangladesh. Isolation, purification and identification of plant growth promoting and beneficial fungi and bacteria from rice phylloplane and rhizosphere <i>in vitro</i> antagonistic activity assay of fungi and bacteria against <i>X. oryzae</i> pv. <i>oryzae</i>	63 antagonistic bacterial and 4 antagonistic fungal isolates were identified against <i>X. oryzae</i> pv. <i>oryzae</i>	63 antagonistic bacteria and 4 antagonistic fungi were identified
2. Identify the plant growth promoting fungi and bacteria antagonistic to <i>X. oryzae</i> pv. <i>oryzae</i> .	Characterization of plant growth promoting bacteria and beneficial fungi obtained from rice phylloplane and rhizosphere Molecular based identification of plant growth promoting and beneficial fungi by sequencing of ITS region and bacteria by sequencing of 16S rDNA	63 antagonistic bacterial and 4 antagonistic fungal isolates were identified against <i>X. oryzae</i> pv. <i>oryzae</i> by sequencing of 16S rDNA and ITS region, respectively. 32 bacterial out of 63 and 4 fungal isolates out of 4 were identified as plant growth promoter.	63 antagonistic bacteria and 4 antagonistic fungi isolates were identified
3. Develop bioformulation of some selected antagonistic and plant growth promoting fungi and bacteria against, <i>X. oryzae</i> pv. <i>oryzae</i> .	Formulation of selected bioagents for field application against BB of rice	Talc based formulations of 32 bacterial and 4 fungal isolates were developed	Talc based formulations of 32 bacteria and 4 fungi were identified

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e., product obtained, visible, measurable)	Outcome (short term effect of the research)
4. Evaluate the efficacy of some formulated antagonistic and plant growth promoting fungi and bacteria against BB disease of rice under net house and field condition.	Disease protection by plant growth promoting and beneficial fungi and bacteria against BB of rice under net house condition Expression studies of defense related rice genes by RT-PCR Field evaluation of the formulated bioagents against BLB of rice	Based on antagonistic nature, plant growth promoting capability, disease protection capacity, seven potential bacterial isolates were identified: BDISOB04KhaP ( <i>Pseudomonas putida</i> ), BDISOB05MymP ( <i>Pseudomonas putida</i> ), BDISOB222GaiR ( <i>Pseudomonas plecoglossicida</i> ), BDISOB258GaiR ( <i>Pseudomonas putida</i> ), BDISO45PanP ( <i>Bacillus paramycooides</i> ), BDISO1MymR ( <i>Bacillus amyloliquefaciens</i> ) and BDISOB21ChaR ( <i>Serratia marcescens</i> ) and three potential fungal isolates viz. BDISOF67 ( <i>Trichoderma paraviridescens</i> ), BDISOF91 ( <i>Trichoderma erinaceum</i> ) and BDISOF08 ( <i>Trichoderma asperellum</i> ) were identified.	Formulated Bacterial and Fungal Biopesticides and Biofertilizers reduced BB severity

**E: Information/knowledge generated/policy generated  
BRRRI component**

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Identify the bacterial blight resistant genes in land race.	A total of 928 germplasm was screened against virulent isolates ( <i>Bxo67</i> , <i>Bxo87</i> , <i>Bxo091</i> ) of bacterial blight to identify the bacterial blight resistant genes in land race.	➤ Out of 928 germplasm, a single entry was highly resistant, 71 were resistant & 6 were moderately resistant.	Forty-nine germplasm were found having two or more BB resistant genes, those can be used as donor.

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
		<ul style="list-style-type: none"> <li>➤ Based on evaluation of gene based molecular marker, ten (10) germplasm contained 4 resistant genes, fifteen (15) germplasm contained 3 resistant genes, twenty-two (22) germplasm contained 2 resistant genes and others had a single or unknown resistant gene(s).</li> </ul>	
Identify physiological races across the country	A total of 920 bacterial blight infected leaf samples were collected from 40 different districts of Bangladesh. From them, 300 isolates were isolated and preserved. Pathogenicity test of these isolates were performed against 14 NILs of bacterial blight.	<ul style="list-style-type: none"> <li>➤ 13 races were identified based on the reaction patterns of these isolates collected across the country.</li> <li>➤ Among the resistant genes, <i>Xa27</i>, <i>Xa21</i>, <i>xa13</i> and <i>Xa7</i> were effective against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> in Bangladesh condition.</li> </ul>	A variation among races prevails across the country while <i>Xa27</i> , <i>Xa21</i> , <i>xa13</i> and <i>Xa7</i> genes were found effective against BB.

### BAU component

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
1. Isolate and identify the fungi and bacteria from rice phylloplane and rhizosphere antagonistic to bacterial blight pathogen, <i>X. oryzae</i> pv. <i>oryzae</i> .	Collection of plant samples from 30 AEZs of Bangladesh. Isolation, purification and identification of plant growth promoting and beneficial fungi and bacteria from rice phylloplane and rhizosphere. In vitro antagonistic activity assay of fungi and bacteria against <i>X. oryzae</i> pv. <i>oryzae</i>	63 antagonistic bacterial and 4 antagonistic fungal isolates were identified against <i>X. oryzae</i> pv. <i>oryzae</i>	63 antagonistic bacteria and 4 antagonistic fungi were isolated & identified

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
2. Identify the plant growth promoting fungi and bacteria antagonistic to <i>X. oryzae</i> pv. <i>oryzae</i> .	Characterization of plant growth promoting bacteria and beneficial fungi obtained from rice phylloplane and rhizosphere Molecular based identification of plant growth promoting and beneficial fungi by sequencing of ITS region and bacteria by sequencing of 16S rDNA	63 antagonistic bacterial and 4 antagonistic fungal isolates were identified against <i>X. oryzae</i> pv. <i>oryzae</i> by sequencing of 16S rDNA and ITS region, respectively. 32 bacterial out of 63 and 4 fungal isolates out of 4 were identified as plant growth promoter.	63 antagonistic bacteria and 4 antagonistic fungi isolates were identified
3. Develop bioformulation of some selected antagonistic and plant growth promoting fungi and bacteria against, <i>X. oryzae</i> pv. <i>oryzae</i> .	Formulation of selected bioagents for field application against BB of rice	Talc based formulations of 32 bacterial and 4 fungal isolates were developed.	Talc based formulations of 32 bacteria and 4 fungi were developed
4. Evaluate the efficacy of some formulated antagonistic and plant growth promoting fungi and bacteria against BB disease of rice under net house and field condition.	Disease protection by plant growth promoting and beneficial fungi and bacteria against BB of rice under net house condition Expression studies of defense related rice genes by RT-PCR Field evaluation of the formulated and non-formulated bioagents against BLB of rice	Based on antagonistic nature, plant growth promoting capability, disease protection capacity, seven potential bacterial isolates were identified: BDISOB04KhaP ( <i>Pseudomonas putida</i> ), BDISOB05MymP ( <i>Pseudomonas putida</i> ), BDISOB222GaiR ( <i>Pseudomonas plecoglossicida</i> ), BDISOB258GaiR ( <i>Pseudomonas putida</i> ), BDISO45PanP ( <i>Bacillus paramycoides</i> ), BDISO1MymR ( <i>Bacillus amyloliquefaciens</i> ) and BDISOB21ChaR ( <i>Serratiamarcescens</i> ) and three potential fungal isolates viz. BDISOF67 ( <i>Trichoderma paraviridescens</i> ), BDISOF91 ( <i>Trichoderma erinaceum</i> ) and BDISOF08 ( <i>Trichoderma asperellum</i> ) were identified.	Formulated Bacterial and Fungal Biopesticides and Biofertilizers reduced BB severity

**F. Materials Development/Publication made under the Sub-project:  
BRRRI component**

Publication	Number of publications		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/ leaflet/flyer etc.	0	0	
Journal publication	0	1 (Under review)	Exploring bacterial blight resistance in landraces, mining of resistant gene(s) and genetic divergence of resistant germplasm through molecular markers and quantitative traits.
Video clip/TV program	0	0	
News Paper/Popular Article	1	0	
Other publications, if any	0	3	BRRRI Annual report, 2017-18, BRRRI Annual report, 2018-19, BRRRI Annual report, 2019-20

**BAU component**

Publication	Number of publications		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/ leaflet/flyer etc.	02	0	
Journal publication	06	1 (accepted)	Potential role of rice plant growth promoting phylloplane and rhizospheric bacteria in controlling <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> .
Video clip/TV program	0	0	
News Paper/Popular Article	02	0	
Other publications, if any	Master's thesis: 06 PhD dissertation: 01	2	Appendix-2

## **G. Description of generated Technology/knowledge/policy:**

### **i. Technology Fact Sheet**

#### **BRRRI component**

**Title of the technology:** Multiple disease resistant advance lines for combating bacterial blight and blast disease in rice.

**Introduction:** Among 32 rice major diseases, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and blast caused by *Pyricularia oryzae* frequently occurs in all Agro Ecological Zones (AEZ) of Bangladesh and cause huge yield loss. But till now no resistant variety is so far developed in Bangladesh. Development of resistant variety is the most economic and eco-friendly way to combat these two destructive diseases for sustainable food security of Bangladesh.

**Description:** A crossing program was undertaken for the development of bacterial blight and blast resistant advance lines. Where, BRRRI dhan81 and BRRRI dhan63 were used as recipient parents and IRBB58, IRBB60, *Pi9*-[US], *Pb1*-[US] as donor parents. Crosses were made between the donor and recipient parents. F<sub>1</sub> progenies were selected using gene base molecular markers for each gene followed by subsequent backcrossing. Progenies of BC<sub>1</sub>F<sub>1</sub> generation were intercrossed (bridged) for the introgression of bacterial blight and blast resistant genes in the background of BRRRI dhan63 and BRRRI dhan81. The progenies were backcrossed and forwarded up to BC<sub>3</sub>F<sub>2</sub> or BC<sub>2</sub>F<sub>2</sub> generations. Finally, a total of 10 plants having *Xa21*, *Pb1* and *Pi9* genes in the background of BRRRI dhan81, 5 plants containing *Xa21*, *Pb1* and *Pi9* genes in the background of BRRRI dhan63 of BC<sub>3</sub>F<sub>2</sub> or BC<sub>2</sub>F<sub>2</sub> generation were selected based on pathogenicity test and Marker Assisted Selection.

**Suitable location:** Almost all areas of Bangladesh.

**Benefits:** Nineteen advanced lines (BC<sub>3</sub>F<sub>2</sub> and BC<sub>2</sub>F<sub>2</sub>) having BB and blast resistant genes were developed, which have the potentiality as candidate variety as well as donor source.

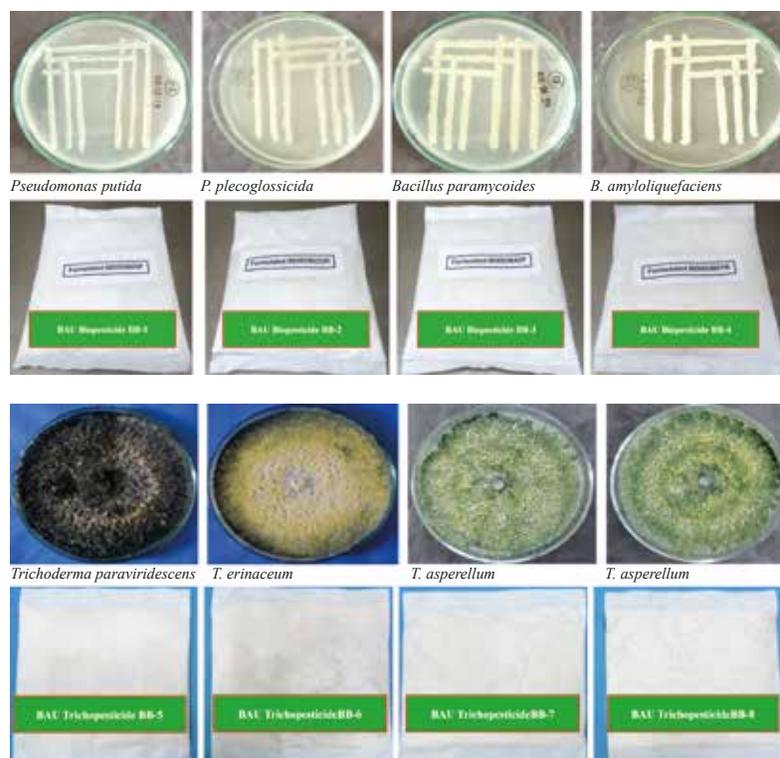
**Name and contact address of author:** Dr. Md. Abdul Latif; Chief Scientific Officer and Head; Plant Pathology Division; Bangladesh Rice Research Institute (BRRRI), Gazipur-1701; Mobile.: +8801715034094; Email: alatif1965@yahoo.com

## BAU component

**Title of the technology:** Novel plant growth promoting (PGP) bacterial and fungal biopesticides for sustainable management of bacterial blight disease of rice

**Introduction:** Rice (*Oryza sativa L.*) is one of the top ranked cereals in the country in terms of its acreage and production. Bangladesh ranked 4<sup>th</sup> with regard to rice production in the world. Although the country has achieved the food sufficiency but natural disaster and outbreak of emerging pest and diseases creates some obstacles to this achievement of food self-sufficiency of the country. Among the diseases, Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is emerged as one of important threats for rice production in the country due to the intensive use of high yielding nitrogen responsive rice varieties. There is no bactericide available in the country against BB of rice. Therefore, it is mandatory to develop new sustainable alternatives. The purpose of the development of this technology is to minimize the yield losses through sustainable management of BB of rice especially in modern varieties and to maximize the rice yield through the use of plant growth promoting rhizobacteria (PGPR) and fungi based biopesticides with a view to sustain the food sufficiency of the country.

**Description:** Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) considered as a most destructive disease of rice (Latif *et al.*, 2011) and it causes considerable yield losses around 30-50% depending on the outbreak (Shahjahan 1993). None of the control measures *viz.* host resistance, cultural and chemical was found effective due to dynamic change of *Xoo* population and variation in regional outbreak of BB. Plant growth promoting rhizobacteria (PGPR) and fungi are now considered as the best option in developing new biological pesticides for sustainable management of BB of rice (Yasmin *et al.*, 2016). Four potential bacterial *viz.* BAU Biopesticide BB-1, BAU Biopesticide BB-2, BAU Biopesticide BB-3 and BAU Biopesticide BB-4 and four potential fungal *viz.* BAU Trichopesticide BB-5, BAU Trichopesticide BB-6, BAU Trichopesticide BB-7 and BAU Trichopesticide BB-8 have been developed to reduce BB severity and thus increased rice yield by 10 to 30% through seed treatment and foliar application. Patenting, formulation, registration and field application of these biopesticides in different zones will be the target-oriented dissemination of the technology.



**Figure.1** Four bacterial viz. BAU Biopesticide BB-1 (*Pseudomonas putida*), BAU Biopesticide BB-2 (*Pseudomonas plecoglossicida*), BAU Biopesticide BB-3 (*Bacillus paramycoides*), BAU Biopesticide BB-4 (*Bacillus amyloliquefaciens*) and four fungal viz. BAU Trichopesticide BB-5 (*Trichoderma paraviridescens*), BAU Trichopesticide BB-6 (*T. erinaceum*), BAU Trichopesticide BB-7 (*T. asperellum*) and BAU Trichopesticide BB-8 (*T. asperellum*). Seed treatment: 10g/kg seed and foliar spray: 0.5% (w/v) at 40, 55, 75, 90 and 105 days after transplanting (DAT) in boro season and at 40, 50, 60, 70 and 80 DAT in aman season.

**Suitable location/ecosystem:**

The technology is suitable for application in all 30 Agro-Ecological Zones (AEZs) of Bangladesh.

**Benefits:**

These bacterial and fungal biopesticides are potentially effective in reducing BB severity and in increasing rice yield. The technology will certainly contribute to ensuring food security in the country. There is no potential risk or negative impacts of the technology to human health, animals and environment. Scale up and validation of the application of these bacterial and fungal biopesticides is almost completed. Therefore, it is recommended to apply this technology at field level after patenting, registration and large-scale production for increasing rice production through sustainable management of BB of rice in modern varieties.

**Name and contact address of author**

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**ii. Effectiveness in Policy Support (if applicable)**

## H. Technology/Knowledge generation/Policy Support (as applied):

### i. Immediate impact on generated technology (commodity & non-commodity)

The advance lines carrying BB and blast resistant genes in BRR1 dhan81 and BRR1 dhan63 are potential to be candidate variety and donor.

### ii. Generation of new knowledge that help in developing more technology in future BRR1 component

- The germplasm consist of two or more resistant genes can be used as donor parent in crossing scheme for the development of BB resistant variety.
- Location specific bacterial blight resistant variety can be developed by exploiting the knowledge of physiological races of BB and its distribution across the country.
- The newly developed bacterial blight and blast resistant lines can be used as donor in future in order to develop multiple disease resistant varieties.

### BAU component

New and potential plant growth promoting antagonistic bacteria and fungi identified in this sub-project will help in generating new technology for increasing rice yield through sustainable management of bacterial blight disease in Bangladesh.

### iii. Technology transferred that help increased agricultural productivity and farmers' income: N/A

### iv. Policy Support: N/A

## I. Information regarding Desk and Field Monitoring

### i. Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.): N/A

### ii. Field Monitoring (date& no. of visit, name and addresses of team visit and output):

Date	No. of Visit	Name and Addresses of Team Visit	Output
20/03/2019	1 (BAU component)	1. Dr. ASM Anowarul Hoque Member Director (Administration and Finance), BARC 2. Dr. Md. Mosarrof Uddin Mollah CSO (AERS), BARC 3. Ajit Kumar Chakrabarty Director (Finance), BARC 4. Dipok Kumar Monitoring Associate, PIU-BARC	The ongoing sub-project work is satisfactory.
31/03/2019	1 (BAU component)	1. Dr. Md. Aziz Zilani Choudhury Member Director (Crop), BARC 2. Dr. Harunur Rashid Director (Human Resource and Training Unit), BARC 3. DR. Zakiah Rahman Moni, SSO, TTMU 4. Md. Abdur Rahman Monitoring Associate, PIU-BARC-NATP-2	The ongoing sub-project work is satisfactory.

Date	No. of Visit	Name and Addresses of Team Visit	Output
11/11/2020	1 (BAU component)	1. Dr. Harunur Rashid, Director, PIU-BARC 2. Dr. Md. Abdul Jalil Bhuyan RMS, PIU-BARC 3. Dr. Nowsher Ali Sarder M & E Specialist, PIU-BARC	The ongoing sub-project work is satisfactory.

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities: N/A

#### J. Sub-project auditing (covers all types of audit performed)

##### BRRRI component

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
GoB Audit (FAPAD)	No queries or observations	26,14,202	1 <sup>st</sup> Year	
GoB Audit (FAPAD)	No queries or observations	42,86,460	2 <sup>nd</sup> Year	
<b>Total Taka</b>		<b>69,00,662</b>		

##### BRRRI component

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
GoB Audit (FAPAD)	No queries or observations	28,75,031	1 <sup>st</sup> Year	
Do	No queries or observations	10,00,546	2 <sup>nd</sup> Year	
<b>Total Taka</b>		<b>38,75,577</b>		

#### K. Lessons Learned:

- i) Learned how to develop bacterial blight and blast resistant materials using Marker Assisted Selection.
- ii) Gathered knowledge how to identify physiological races using monogenic lines based on gene for gene interaction.
- iii) How to identify resistant genes in landraces using both pathogenicity test and molecular approach.
- iv) Learn how to develop inter-institutional collaboration.
- v) Learned how to design a new research proposal for achieving the ultimate goal based on the present findings.

#### **L. Challenges:**

- i) Conducting the sub-project activities under COVID-19 pandemic situation was troublesome.
- ii) Delay release of funds was a challenge to maintain proper flow of work.

#### **M. Suggestions for future planning:**

- i) The bacterial blight and blast resistant materials developed from this sub-project will be carried out in varietal development pipeline for releasing.
- ii) The potential plant growth promoting antagonistic bacteria and fungi need to be formulated in large scale to introduce in the farmers field.

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**Appendix-1:** List of germplasm screened with virulent bacterial blight isolates

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
1	98	32.5	35	33.75	33.75	9	HS
2	137	21.5	23	22.25	22.25	9	HS
3	499	30	23	26.5	26.50	9	HS
4	594	31.5	23.5	27.5	27.50	9	HS
5	647	24	29	26.5	26.50	9	HS
6	781	26	18.5	22.25	22.25	9	HS
7	989	4.5	5.5	7	5.67	5	MS
8	997	55	47.5	51.25	51.25	9	HS
9	999	25	34.5	29.75	29.75	9	HS
10	1008	9	15	9	11.00	7	S
11	1017	19.5	21.5	20.5	20.50	9	HS
12	1021	26.5	36.5	31.5	31.50	9	HS
13	1027	32	44.5	38.25	38.25	9	HS
14	1043	47.5	17.5	32.5	32.50	9	HS
15	1138	21.5	28.5	25	25.00	9	HS
16	1581	43	28.5	35.75	35.75	9	HS
17	1867	24.5	28	26.25	26.25	9	HS
18	1867	24.5	23.5	24	24.00	9	HS
19	1870	17.5	16	16.75	16.75	9	HS
20	1871	22	23.5	22.75	22.75	9	HS
21	1872	23.5	28	25.75	25.75	9	HS
22	1781	20	27	23.5	23.50	9	HS
23	1884	37.5	31.5	34.5	34.50	9	HS
24	1885	42.5	49.5	46	46.00	9	HS
25	1891	42.5	23	32.75	32.75	9	HS
26	1890	30	35	32.5	32.50	9	HS
27	1895	28	32	30	30.00	9	HS
28	1796	28	30.5	29.25	29.25	9	HS
29	1899	32.5	35	33.75	33.75	9	HS
30	1875	41.5	35.5	38.5	38.50	9	HS
31	1902	32.5	24	28.25	28.25	9	HS
32	1910	26.5	29	27.75	27.75	9	HS
33	1909	23.5	31.5	27.5	27.50	9	HS
34	1908	21.5	19	20.25	20.25	9	HS
35	1907	27.5	22.5	25	25.00	9	HS
36	1906	27.5	21.5	24.5	24.50	9	HS
37	1916	22	21.5	21.75	21.75	9	HS
38	1905	18.5	12	15.25	15.25	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
39	1903	26	19	22.5	22.50	9	HS
40	1920	27	29	28	28.00	9	HS
41	2121	26	20	23	23.00	9	HS
42	2122	23.5	27	25.25	25.25	9	HS
43	1923	28	24	26	26.00	9	HS
44	1924	33	37.5	35.25	35.25	9	HS
45	2126	34.5	38	36.25	36.25	9	HS
46	2127	36.5	23	29.75	29.75	9	HS
47	1928	41.5	33.5	37.5	37.50	9	HS
48	1930	30	20.5	25.25	25.25	9	HS
49	1932	26.5	37.5	32	32.00	9	HS
50	1936	24	37	30.5	30.50	9	HS
51	1937	21.5	46.5	34	34.00	9	HS
52	1988	33	27	30	30.00	9	HS
53	2041	32	30.5	31.25	31.25	9	HS
54	3281	26	28.5	27.25	27.25	9	HS
55	3632	31	37.5	34.25	34.25	9	HS
56	3843	19.5	15	17.25	17.25	9	HS
57	3844	17.5	22.5	20	20.00	9	HS
58	3845	40	30	35	35.00	9	HS
59	3849	26.5	31	28.75	28.75	9	HS
60	3850	17	18.5	17.75	17.75	9	HS
61	3854	25.5	25.5	25.5	25.50	9	HS
62	3841	22	16	19	19.00	9	HS
63	3842	26	19	22.5	22.50	9	HS
64	3840	21	29.5	25.25	25.25	9	HS
65	3839	29.5	22.5	26	26.00	9	HS
66	3838	34	53	43.5	43.50	9	HS
67	3837	32.5	29	30.75	30.75	9	HS
68	3836	39.5	47.5	43.5	43.50	9	HS
69	3835	28.5	43	35.75	35.75	9	HS
70	3834	28.5	23.5	26	26.00	9	HS
71	3833	28.5	26	27.25	27.25	9	HS
72	3832	24.5	34.5	29.5	29.50	9	HS
73	3831	44.5	26	35.25	35.25	9	HS
74	3830	22.5	22.5	22.5	22.50	9	HS
75	3872	29	17.5	23.25	23.25	9	HS
76	3829	19	20.5	19.75	19.75	9	HS
77	3874	25.5	29	27.25	27.25	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
78	3828	2	2	3	2.33	1	R
79	3827	19.5	22	20.75	20.75	9	HS
80	3877	29.5	26	27.75	27.75	9	HS
81	3778	25.5	25	25.25	25.25	9	HS
82	3779	27.5	30.5	29	29.00	9	HS
83	3780	36	42.5	39.25	39.25	9	HS
84	3781	-	-	-	-	-	-
85	3782	30	37	33.5	33.50	9	HS
86	3783	19.5	18	18.75	18.75	9	HS
87	4051	20.5	23	21.75	21.75	9	HS
88	4055	22.5	22	22.25	22.25	9	HS
89	4159	41.5	25	33.25	33.25	9	HS
90	4169	26	23	24.5	24.50	9	HS
91	4241	29	40.5	34.75	34.75	9	HS
92	4146	29	41.5	35.25	35.25	9	HS
93	4256	14.5	16	15.25	15.25	9	HS
94	4272	16	15.5	15.75	15.75	9	HS
95	4341	14.5	17.5	16	16.00	9	HS
96	4782	26	15.5	20.75	20.75	9	HS
97	4786	0.5	0.5	0.5	0.50	0	HR
98	4787	28	32.5	30.25	30.25	9	HS
99	4788	17.5	14.5	16	16.00	9	HS
100	4806	12.5	18.5	15.5	15.50	9	HS
101	5208	15.5	12	13.75	13.75	7	S
102	5295	22	14.5	18.25	18.25	9	HS
103	5297	14.5	23	18.75	18.75	9	HS
104	5344	14.5	12.5	13.5	13.50	7	S
105	6007	19	20	19.5	19.50	9	HS
106	7354	5.5	3.5	4.5	4.50	3	MR
107	7357	6.5	6	6.25	6.25	5	MS
108	7358	13.5	12	12.75	12.75	7	S
109	7359	18	28.5	23.25	23.25	9	HS
110	7350	29	19.5	24.25	24.25	9	HS
111	BRR1 dhan49	32.5	30	31.25	31.25	9	HS
112	IRBB60	31.5	27	29.25	29.25	9	HS
113	IRBB65	36.5	33	34.75	34.75	9	HS
114	IRBB58	35	26.5	30.75	30.75	9	HS
115	TN1	31	25.5	28.25	28.25	9	HS
116	BR11	26	26.5	26.25	26.25	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
117	Purbachi	17.5	14.5	16	16.00	9	HS
118	261	12	14.5	13.25	13.25	7	S
119	271	13	12.5	12.75	12.75	7	S
120	274	14	14.5	14.25	14.25	7	S
121	377	22.5	20.5	21.5	21.50	9	HS
122	377	24.5	38.5	31.5	31.50	9	HS
123	379	23	33.5	28.25	28.25	9	HS
124	505	23.5	23	23.25	23.25	9	HS
125	561	26	32	29	29.00	9	HS
126	562	23	19.5	21.25	21.25	9	HS
127	569	17.5	26.5	22	22.00	9	HS
128	572	0.5	3	3	2.17	1	R
129	574	27.5	30	28.75	28.75	9	HS
130	575	25	27	26	26.00	9	HS
131	576	24.5	14.5	19.5	19.50	9	HS
132	577	35.5	29.5	32.5	32.50	9	HS
133	578	42	32.5	37.25	37.25	9	HS
134	579	33	39.5	36.25	36.25	9	HS
135	1522	28.5	27	27.75	27.75	9	HS
136	1123	14	14	14	14.00	7	S
137	1515	19	19	19	19.00	9	HS
138	1528	15	15	15	15.00	7	S
139	1531	19	19	19	19.00	9	HS
140	1532	21	21	21	21.00	9	HS
141	1533	40	43	41.5	41.50	9	HS
142	1563	23	32.5	27.75	27.75	9	HS
143	1565	37	30	33.5	33.50	9	HS
144	1688	21	19.5	20.25	20.25	9	HS
145	2149	14	13.5	13.75	13.75	7	S
146	2150	25.5	23.5	24.5	24.50	9	HS
147	2153	13.5	24.5	19	19.00	9	HS
148	2157	31	28.5	29.75	29.75	9	HS
149	2154	26	20.5	23.25	23.25	9	HS
150	2158	19.5	25	22.25	22.25	9	HS
151	2159	38.5	37.5	38	38.00	9	HS
152	2162	21.5	32.5	27	27.00	9	HS
153	2163	26	36.5	31.25	31.25	9	HS
154	2164	35	39	37	37.00	9	HS
155	3484	32	35	33.5	33.50	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
156	3485	34.5	37	35.75	35.75	9	HS
157	3509	32	31	31.5	31.50	9	HS
158	3512	43.5	34.5	39	39.00	9	HS
159	3513	30	30	30	30.00	9	HS
160	3523	21	27	24	24.00	9	HS
161	3524	33	23.5	28.25	28.25	9	HS
162	3525	-	-	-	-	-	-
163	3526	30.5	26	28.25	28.25	9	HS
164	3527	12.5	13.5	13	13.00	7	S
165	3529	10.5	16	13.25	13.25	7	S
166	3532	34.5	18.5	26.5	26.50	9	HS
167	3531	36.5	0	18.25	18.25	9	HS
168	3539	16.5	14.5	15.5	15.50	9	HS
169	4220	19	19	19	19.00	9	HS
170	4221	21.5	15.5	18.5	18.50	9	HS
171	4222	22.5	22	22.25	22.25	9	HS
172	4224	-	-	-	-	-	-
173	4225	35	33.5	34.25	34.25	9	HS
174	4588	32	33.5	32.75	32.75	9	HS
175	7243	37	27	32	32.00	9	HS
176	7245	31	30.5	30.75	30.75	9	HS
177	7247	19.5	21.5	20.5	20.50	9	HS
178	7248	20.5	23.5	22	22.00	9	HS
179	7246	0	32.5	16.25	16.25	9	HS
180	7250	30.5	0	15.25	15.25	9	HS
181	7252	30	30	30	30.00	9	HS
182	7257	37.5	39.5	38.5	38.50	9	HS
183	7258	35.5	26	30.75	30.75	9	HS
184	7255	27	21.5	24.25	24.25	9	HS
185	7256	-	-	-	-	-	-
186	7431	27.5	20.5	24	24.00	9	HS
187	7437	22	24	23	23.00	9	HS
188	7438	23	26.5	24.75	24.75	9	HS
189	7434	27	23.5	25.25	25.25	9	HS
190	7435	25.5	25	25.25	25.25	9	HS
191	7436	40	25.5	32.75	32.75	9	HS
192	7439	30	23	26.5	26.50	9	HS
193	7420	25.5	46.5	36	36.00	9	HS
194	7421	47.5	35.5	41.5	41.50	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
195	7422	31.5	19	25.25	25.25	9	HS
196	7424	17	25	21	21.00	9	HS
197	7448	15	35.5	25.25	25.25	9	HS
198	7449	21	36.5	28.75	28.75	9	HS
199	7447	27.5	35.5	31.5	31.50	9	HS
200	7450	23.5	34.5	29	29.00	9	HS
201	7451	19.5	20	19.75	19.75	9	HS
202	7452	33.5	32	32.75	32.75	9	HS
203	7473	32	26.5	29.25	29.25	9	HS
204	7474	37	24	30.5	30.50	9	HS
205	7475	30	22.5	26.25	26.25	9	HS
206	7476	17	18	17.5	17.50	9	HS
207	7457	19.5	20.5	20	20.00	9	HS
208	7459	15	12.5	13.75	13.75	7	S
209	7461	21	24.5	22.75	22.75	9	HS
210	7462	32.5	44.5	38.5	38.50	9	HS
211	7463	0	2.5	1.25	1.25	1	R
212	7465	9	8	8.5	8.50	5	MS
213	7466	-	-	-	-	-	-
214	7467	-	-	-	-	-	-
215	7468	-	-	-	-	-	-
216	7470	-	-	-	-	-	-
217	1052	28.5	19	23.75	23.75	9	HS
218	1053	35.5	32	33.75	33.75	9	HS
219	1752	-	-	-	-	-	-
220	1755	28.5	47	37.75	37.75	9	HS
221	1991	17	20	18.5	18.50	9	HS
222	1792	20	35.5	27.75	27.75	9	HS
223	1793	28	19.5	23.75	23.75	9	HS
224	1798	-	-	-	-	-	-
225	1796	30.5	23.5	27	27.00	9	HS
226	1801	22.5	30	26.25	26.25	9	HS
227	1802	16.5	15.5	16	16.00	9	HS
228	1803	-	-	-	-	-	-
229	1850	39	0	19.5	19.50	9	HS
230	1851	-	-	-	-	-	-
231	1852	25	33	29	29.00	9	HS
232	1863	30	20	25	25.00	9	HS
233	1865	40	23	31.5	31.50	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
234	1867	25.5	32	28.75	28.75	9	HS
235	1972	37.5	22	29.75	29.75	9	HS
236	1973	33	39.5	36.25	36.25	9	HS
237	2253	47	28	37.5	37.50	9	HS
238	2254	35	37.5	36.25	36.25	9	HS
239	2259	32	21	26.5	26.50	9	HS
240	2261	19.5	22.5	21	21.00	9	HS
241	2262	24.5	15.5	20	20.00	9	HS
242	2263	-	-	-	-	-	-
243	2265	20.5	17	18.75	18.75	9	HS
244	2269	23.5	19.5	21.5	21.50	9	HS
245	3961	32	23	27.5	27.50	9	HS
246	3964	30.5	20.5	25.5	25.50	9	HS
247	3965	18	26.5	22.25	22.25	9	HS
248	3966	-	-	-	-	-	-
249	3967	32.5	25.5	29	29.00	9	HS
250	3968	-	-	-	-	-	-
251	3969	18	22	20	20.00	9	HS
252	3970	-	-	-	-	-	-
253	3976	-	-	-	-	-	-
254	3977	32.5	22	27.25	27.25	9	HS
255	3978	24.5	22.5	23.5	23.50	9	HS
256	3990	35	33	34	34.00	9	HS
257	3992	23	26.5	24.75	24.75	9	HS
258	3993	-	-	-	-	-	-
259	3994	-	-	-	-	-	-
260	4002	32.5	34.5	33.5	33.50	9	HS
261	4001	43	44	43.5	43.50	9	HS
262	4005	37.5	26	31.75	31.75	9	HS
263	4010	33	28	30.5	30.50	9	HS
264	4011	37.5	41.5	39.5	39.50	9	HS
265	4008	-	-	-	-	-	-
266	4012	22	18	20	20.00	9	HS
267	4055	22.5	24	23.25	23.25	9	HS
268	4056	39.5	32	35.75	35.75	9	HS
269	4202	23	38	30.5	30.50	9	HS
270	4203	17.5	29	23.25	23.25	9	HS
271	4211	-	-	-	-	-	-
272	4210	29	32	30.5	30.50	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
273	4215	39.5	22	30.75	30.75	9	HS
274	4218	-	-	-	-	-	-
275	4219	-	-	-	-	-	-
276	4369	24	42	33	33.00	9	HS
277	4371	35	17	26	26.00	9	HS
278	4372	-	-	-	-	-	-
279	4373	22	27.5	24.75	24.75	9	HS
280	4375	0	0	0	0.00	0	
281	4376	27.5	30	28.75	28.75	9	HS
282	4532	33	47	40	40.00	9	HS
283	4538	14.5	29.5	22	22.00	9	HS
284	4951	23	38.5	30.75	30.75	9	HS
285	4952	32.5	23.5	28	28.00	9	HS
286	4953	-	-	-	-	-	-
287	4954	-	-	-	-	-	-
288	4955	30.5	31.5	31	31.00	9	HS
289	4981	24	16	20	20.00	9	HS
290	4983	34	31.5	32.75	32.75	9	HS
291	4994	21	24	22.5	22.50	9	HS
292	5042	-	-	-	-	-	-
293	5041	41	19	30	30.00	9	HS
294	5091	41	26.5	33.75	33.75	9	HS
295	5102	29	30.5	29.75	29.75	9	HS
296	5181	27.5	30.5	29	29.00	9	HS
297	6852	18	24.5	21.25	21.25	9	HS
298	6864	26.5	28.5	27.5	27.50	9	HS
299	7364	-	-	-	-	-	-
300	7366	30.5	39	34.75	34.75	9	HS
301	7365	-	-	-	-	-	-
302	7368	11	19	15	15.00	7	S
303	7369	18	26	22	22.00	9	HS
304	7373	24.5	30.5	27.5	27.50	9	HS
305	7374	13.5	28.5	21	21.00	9	HS
306	7371	21	26	23.5	23.50	9	HS
307	7668	17	32.5	24.75	24.75	9	HS
308	8104	23.5	17	20.25	20.25	9	HS
309	BR8189-10-2-3-1-5-RAN7	27	39	33	33.00	9	HS
310	BR9392-6-2-1B-RAN5	28	32	30	30.00	9	HS
311	BR10238-5-1-RAN6	18.5	39.5	29	29.00	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
312	BR11 (CK)	19.5	29	24.25	24.25	9	HS
313	BRRRI dhan52 (CK)	28.5	18	23.25	23.25	9	HS
314	BR8521-30-3-1	18.5	24	21.25	21.25	9	HS
315	BR8841-38-1-2-2	13.5	34	23.75	23.75	9	HS
316	IR11L433	40	32.5	36.25	36.25	9	HS
317	IR 13F352	33.5	12	22.75	22.75	9	HS
318	IR13F402	29	29.5	29.25	29.25	9	HS
319	BRRRI dhan39 (CK)	34.5	32.5	33.5	33.50	9	HS
320	BRRRI dhan49 (CK)	45	41	43	43.00	9	HS
321	BR8526-25-4-2-2-1-HR1	30.5	30.5	30.5	30.50	9	HS
322	BR8526-38-3-2-1-HR2	37.5	21.5	29.5	29.50	9	HS
323	Habudhan	36	23.5	29.75	29.75	9	HS
324	Latabalam	41.5	35	38.25	38.25	9	HS
325	BR8526-L8	37	23	30	30.00	9	HS
326	HPB (PQR-TLA3) Red Rice	17	21.5	19.25	19.25	9	HS
327	BRRRI dhan49 (CK)	27	38.5	32.75	32.75	9	HS
328	IR04A428	24.5	18	21.25	21.25	9	HS
329	IR11N202	16	28.5	22.25	22.25	9	HS
330	IR14L362	31	23.5	27.25	27.25	9	HS
331	IR94391-131-358-19-B-1-1-1	24.5	37.5	31	31.00	9	HS
332	IR96321-1099-402-B-4-1-2	23.5	30.5	27	27.00	9	HS
333	BRRRI dhan56 (CK)	34	35	34.5	34.50	9	HS
334	BRRRI dhan66 (CK)	31.5	18.5	25	25.00	9	HS
335	BRRRI dhan71 (CK)	33	32	32.5	32.50	9	HS
336	BR8850-10-12-2-3	42.5	19	30.75	30.75	9	HS
337	BR8850-10-12-8-3-3	29.5	24	26.75	26.75	9	HS
338	BR8493-12-7-4 (Com)	20	19	19.5	19.50	9	HS
339	BR8493-3-5-1 (Com)	12.5	31.5	22	22.00	9	HS
340	Kalizira (Local CK)	26	22	24	24.00	9	HS
341	BINA dhan13 (CK)	14	25.5	19.75	19.75	9	HS
342	BR8846-32-2-4-2	36.5	27.5	32	32.00	9	HS
343	BR8846-38-2-4-2	25.5	24.5	25	25.00	9	HS
344	BRRRI dhan37 (CK)	20	14.5	17.25	17.25	9	HS
345	Kataribhog (Local CK)	6	16	11	11.00	7	S
346	BR8535-2-1-2	19	29	24	24.00	9	HS
347	BRRRI dhan34 (Std. CK)	19	40.5	29.75	29.75	9	HS
348	263	7	9	9	8.33	5	MS
349	273	7	9	9	8.33	5	MS
350	378	-	9	9	9.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
351	563	9	1	7	5.67	5	MS
352	564	-	-	-	-		
353	565	3	6	7	5.33	5	MS
354	566	9	5	9	7.67	5	MS
355	567	3	7	6	5.33	5	MS
356	568	3	8	7	6.00	5	MS
357	570	1	1	3	1.67	1	R
358	571	2	1	3	2.00	1	R
359	1523	3	1	5	3.00	1	R
360	1522	3	9	5	5.67	5	MS
361	1124	5	4	9	6.00	5	MS
362	1525	3	1	3	2.33	1	R
363	1528	-	-	-	-		
364	1534	9	3	9	7.00	5	MS
365	1564	9	7	5	7.00	5	MS
366	1689	9	7	7	7.67	5	MS
367	2151	9	7	9	8.33	5	MS
368	2152	7	9	5	7.00	5	MS
369	2155	5	9		7.00	5	MS
370	2156	3	7	6	5.33	5	MS
371	2160	9	9	5	7.67	5	MS
372	2161	9	-	-	9.00	5	MS
373	3485	9	-	9	9.00	5	MS
374	3510	5	9	9	7.67	5	MS
375	3511	7	9	5	7.00	5	MS
376	3514	5	9	9	7.67	5	MS
377	3515	7	7	3	5.67	5	MS
378	3517	5	9	9	7.67	5	MS
379	3518	9	7	7	7.67	5	MS
380	3528	-	9	9	9.00	5	MS
381	3530	1	1	5	2.33	1	R
382	7244	1	1	7	3.00	1	R
383	7249	5	8	9	7.33	5	MS
384	7251	9	-	9	9.00	5	MS
385	7253	9	9	7	8.33	5	MS
386	7254	5	5	7	5.67	5	MS
387	7432	-	-	-	-		
388	7433	-	-	7	7.00	5	MS
389	7440	7	7	7	7.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
390	7442	-	7	7	7.00	5	MS
391	7443	-	-	7	7.00	5	MS
392	7444	3	3	3	3.00	1	R
393	7446	3	1	1	1.67	1	R
394	7447	7	3	9	6.33	5	MS
395	7453	7	-	9	8.00	5	MS
396	7454	9	-	9	9.00	5	MS
397	7455	9	-	3	6.00	5	MS
398	7456	7	6	7	6.67	5	MS
399	7458	1	6	9	5.33	5	MS
400	7464	9	5	9	7.67	5	MS
401	7465	3	5	9	5.67	5	MS
402	7469	1	9	9	6.33	5	MS
403	138	1	9	9	6.33	5	MS
404	593	3	9	7	6.33	5	MS
405	646	1	8	9	6.00	5	MS
406	990	5	9	5	6.33	5	MS
407	991	3	2	2	2.33	1	R
408	1000	9		7	8.00	5	MS
409	1007	1	1	3	1.67	1	R
410	1016	9	5	5	6.33	5	MS
411	1028	9		7	8.00	5	MS
412	1044	5	9	9	7.67	5	MS
413	1137	9	9	9	9.00	5	MS
414	1580	9	7	9	8.33	5	MS
415	1867	9	5	9	7.67	5	MS
416	1876	9	5	7	7.00	5	MS
417	1877	5	9	5	6.33	5	MS
418	1878	3	7	9	6.33	5	MS
419	1882	9	7	9	8.33	5	MS
420	1883	9		7	8.00	5	MS
421	1885	9	9	9	9.00	5	MS
422	1892	3	6	9	6.00	5	MS
423	1893	5	7	9	7.00	5	MS
424	1894	5	7	7	6.33	5	MS
425	1896	5	1	1	2.33	1	R
426	1897	1		5	3.00	1	R
427	1898	9	7	7	7.67	5	MS
428	1904	1	1	7	3.00	1	R

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
429	1911	9	5	9	7.67	5	MS
430	1912	7	9	9	8.33	5	MS
431	1913	9	9	7	8.33	5	MS
432	1914	9		9	9.00	5	MS
433	1915	5	3	9	5.67	5	MS
434	1917	7	3	9	6.33	5	MS
435	1918	9	9	9	9.00	5	MS
436	1919	9	3	7	6.33	5	MS
437	1921	9	5	7	7.00	5	MS
438	1922	7	9	9	8.33	5	MS
439	1925	3	1	3	2.33	1	R
440	1926	9	7	9	8.33	5	MS
441	1927	7	9	7	7.67	5	MS
442	1929	9	-		9.00	5	MS
443	1931	9	-	5	7.00	5	MS
444	1933	7	9	9	8.33	5	MS
445	1934	9	7	9	8.33	5	MS
446	1935	9	-	5	7.00	5	MS
447	1998	7	-	9	8.00	5	MS
448	2043	9	9	9	9.00	5	MS
449	3283	9	1	9	6.33	5	MS
450	3846	3	1	2	2.00	1	R
451	3847	9	-	9	9.00	5	MS
452	3848	9	-	7	8.00	5	MS
453	3851	9	9	9	9.00	5	MS
454	3853	9	-	7	8.00	5	MS
455	3855	9	-	7	8.00	5	MS
456	3856	9	-	9	9.00	5	MS
457	3857	9	9	7	8.33	5	MS
458	3858	9	9	7	8.33	5	MS
459	3859	9	9	7	8.33	5	MS
460	3860	9	9	7	8.33	5	MS
461	3861	5	7	5	5.67	5	MS
462	3862	9	7	5	7.00	5	MS
463	3863	9	9	7	8.33	5	MS
464	3864	7	-	9	8.00	5	MS
465	3866	9	-	5	7.00	5	MS
466	3868	5	7	5	5.67	5	MS
467	3870	7	7	5	6.33	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
468	3871	-	-	7	7.00	5	MS
469	3873	-	9	9	9.00	5	MS
470	3875	-	9	9	9.00	5	MS
471	3876	7	9	7	7.67	5	MS
472	3878	9	5	5	6.33	5	MS
473	3879	5	7	7	6.33	5	MS
474	3880	9	5	5	6.33	5	MS
475	3892	9	7	5	7.00	5	MS
476	3894	9	-	5	7.00	5	MS
477	3895	9	-	5	7.00	5	MS
478	4160	3	9	7	6.33	5	MS
479	4170	9	9	9	9.00	5	MS
480	4246	1	1	5	2.33	1	R
481	4271	9	-	7	8.00	5	MS
482	4340	9	-	9	9.00	5	MS
483	4781	9	3	7	6.33	5	MS
484	4783	7	9	3	6.33	5	MS
485	4784	7	9	9	8.33	5	MS
486	4785	7	5	7	6.33	5	MS
487	4805	3	7	7	5.67	5	MS
488	5209	9	-	9	9.00	5	MS
489	5296	3	1	3	2.33	1	R
490	5298	9		9	9.00	5	MS
491	6006	9	9	5	7.67	5	MS
492	7351	5	-	1	3.00	1	R
493	7352	3	3	1	2.33	1	R
494	7353	3	3	1	2.33	1	R
495	7355	9	-	7	8.00	5	MS
496	7356	3	5	1	3.00	1	R
497	1050	3	3	1	2.33	3	R
498	1051	4	3	1	2.67	1	R
499	1753	3	3	1	2.33	1	R
500	1754	9	9	3	7.00	5	MS
501	1794	7	7	7	7.00	5	MS
502	1795	4	3	1	2.67	1	R
503	1797	5	3	1	3.00	1	R
504	1799	9	-	9	9.00	5	MS
505	1800	3	5	1	3.00	1	R
506	1804	7	7	7	7.00	5	MS
507	1812	7	-	9	8.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
508	1860	9	7	9	8.33	5	MS
509	1861	9	3	7	6.33	5	MS
510	1862	3	1	3	2.33	1	R
511	1864	3	3	1	2.33	1	R
512	1866	7	9	7	7.67	5	MS
513	1970	7	5	9	7.00	5	MS
514	1971	9	7	5	7.00	5	MS
515	2252	5	7	7	6.33	5	MS
516	2258	9	9	5	7.67	5	MS
517	2260	7	5	7	6.33	5	MS
518	2264	9	9	3	7.00	5	MS
519	2266	3	1	3	2.33	1	R
520	2267	7	7	7	7.00	5	MS
521	2268	5	3	1	3.00	1	R
522	3960	9	7	5	7.00	5	MS
523	3962	3	1	4	2.67	1	R
524	3963	9	-	9	9.00	5	MS
525	3980	5	-	7	6.00	5	MS
526	3981	3	1	3	2.33	1	R
527	3982	3	1	3	2.33	1	R
528	3983	9	7	9	8.33	5	MS
529	3984	9	3	7	6.33	5	MS
530	3985	9	3	9	7.00	5	MS
531	3986	9	9	9	9.00	5	MS
532	3987	4	3	2	3.00	1	R
533	3988	7	7	7	7.00	5	MS
534	3989	9	3	5	5.67	5	MS
535	3991	9	3	9	7.00	5	MS
536	3995	9	-	9	9.00	5	MS
537	4003	9	5	7	7.00	5	MS
538	4004	3	2	3	2.67	1	R
539	4006	9	3	5	5.67	5	MS
540	4007	9	7	9	8.33	5	MS
541	4009	3	1	5	3.00	1	R
542	4055	9	9	7	8.33	5	MS
543	4057	5	6	1	4.00	3	MR
544	4096	9	7	9	8.33	5	MS
545	4201	9	7	5	7.00	5	MS
546	4212	9	9	5	7.67	5	MS
547	4213	7	-	5	6.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
548	4214	6	1	5	4.00	3	MR
549	4216	6	1	7	4.67	3	MR
550	4217	3	1	3	2.33	1	R
551	4370	3	1	3	2.33	1	R
552	4374	5	1	2	2.67	1	R
553	4378	2	1	5	2.67	1	R
554	4379	7	3	7	5.67	5	MS
555	4380	5	1	6	4.00	3	MR
556	4533	9	9	3	7.00	5	MS
557	4539	9	5	3	5.67	5	MS
558	4956	9	3	9	7.00	5	MS
559	4957	7	5	9	7.00	5	MS
560	4962	7	5	9	7.00	5	MS
561	4966	5	7	9	7.00	5	MS
562	4967	9	1	3	4.33	3	MR
563	4968	9	9	9	9.00	5	MS
564	4969	7	-	9	8.00	5	MS
565	4980	3	5	1	3.00	1	R
566	4982	3	3	1	2.33	1	R
567	4985	7	7	1	5.00	3	MR
568	4995	3	5	1	3.00	1	R
569	5043	3	3	3	3.00	1	R
570	5092	7	7	5	6.33	5	MS
571	5101	7	1	1	3.00	1	R
572	5182	7	9	7	7.67	5	MS
573	6851	3	3	1	2.33	1	R
574	7367	5	3	1	3.00	1	R
575	7370	1	1	5	2.33	1	R
576	7372	7	9	9	8.33	5	MS
577	7667	7	7	7	7.00	5	MS
578	8105	9	5	7	7.00	5	MS
579	3101	3	3	1	2.33	1	R
580	3102	5	7	9	7.00	5	MS
581	3103	2.5	3	2	2.50	1	R
582	3104	4	5	7	5.33	5	MS
583	3105	1	1.5	2	1.50	1	R
584	3106	2	1	1	1.33	1	R
585	3107	5	6	7	6.00	5	MS
586	3108	40	28	30	32.67	5	HS
587	3109	5	7	6	6.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
588	3110	5	7	4	5.33	5	MS
589	3111	6	17	18	13.67	7	S
590	3112	4	5	7	5.33	5	MS
591	3113	30	28	28	28.67	9	HS
592	3114	10	8	12	10.00	5	MS
593	3115	15	10	12	12.33	7	S
594	3116	12	15	17	14.67	7	S
595	3117	20	22	18	20.00	9	HS
596	3118	8	10	12	10.00	5	MS
597	3119	15	20	18	17.67	9	HS
598	3120	20	15	17	17.33	9	HS
599	3121	10	12	8	10.00	5	MS
600	3122	5	4	7	5.33	5	MS
601	3123	7	10	8	8.33	5	MS
602	3124	2.5	3	3	2.83	1	R
603	3125	5	5.5	7	5.83	5	MS
604	3126	5	6	8	6.33	5	MS
605	3127	5	6	7	6.00	5	MS
606	3128	4	7	7	6.00	5	MS
607	3129	10	12	13	11.67	5	MS
608	3130	8	10	7	8.33	5	MS
609	3131	20	22	23	21.67	9	HS
610	3132	10	12	14	12.00	7	S
611	3133	28	30	26	28.00	9	HS
612	3134	1	1.5	2	1.50	1	R
613	3135	5	5	8	6.00	5	MS
614	3136	18	20	17	18.33	9	HS
615	3137	20	22	23	21.67	9	HS
616	3138	28	30	26	28.00	9	HS
617	3139	30	40	28	32.67	9	HS
618	3140	10	12	14	12.00	7	S
619	3141	20	22	23	21.67	9	HS
620	3142	10	12	13	11.67	7	S
621	3143	8	10	12	10.00	7	S
622	3144	10	11	15	12.00	7	S
623	3145	13	12	10	11.67	7	S
624	3146	6	7	9	7.33	5	MS
625	3147	8	10	12	10.00	5	MS
626	3148	6	7	9	7.33	5	MS
627	3149	20	22	21	21.00	9	HS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
628	3150	18	20	25	21.00	9	HS
629	3151	28	30	36	31.33	9	HS
630	3152	30	28	32	30.00	9	HS
631	3153	28	26	22	25.33	9	HS
632	3154	18	20	21	19.67	9	HS
633	3155	3	3	3	3.00	1	R
634	3156	2	7	7	5.33	5	MS
635	3157	4	5	9	6.00	5	MS
636	3158	9	7	2	6.00	5	MS
637	3159	6	2	9	5.67	5	MS
638	3160	2.5	3	2	2.50	1	R
639	3161	2	3	4	3.00	1	R
640	3162	8	10	12	10.00	5	MS
641	3163	2	2.5	3	2.50	1	R
642	3164	5	5	6	5.33	5	MS
643	3165	1	1.5	2	1.50	1	R
644	3166	7	8	10	8.33	5	MS
645	3167	13	14	15	14.00	7	S
646	3168	6	5	6	5.67	5	MS
647	3169	2	3	4	3.00	1	R
648	3170	4	5	7	5.33	5	MS
649	3171	4	7	6	5.67	5	MS
650	3172	5	5	8	6.00	5	MS
651	3173	8	10	12	10.00	5	MS
652	3174	14	15	16	15.00	7	S
653	3175	10	12	13	11.67	7	S
654	3176	8	7	6	7.00	5	MS
655	3177	10	12	13	11.67	7	S
656	3178	12	13	10	11.67	7	S
657	3179	2	3	4	3.00	1	R
658	3180	12	11	10	11.00	7	S
659	3181	6	5	6	5.67	5	MS
660	3182	8	7	6	7.00	5	MS
661	3183	10	12	4	8.67	5	MS
662	3184	7	8	9	8.00	5	MS
663	3185	12	10	9	10.33	5	MS
664	3186	8	7	10	8.33	5	MS
665	3187	8	10	9	9.00	5	MS
666	3188	12	11	10	11.00	7	S
667	3189	8	12	10	10.00	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
668	3190	10	12	8	10.00	5	MS
669	3191	10	12	13	11.67	7	S
670	3192	12	9	8	9.67	5	MS
671	3193	0	0	0	0.00	-	-
672	3194	0	0	0	0.00	-	-
673	3195	8	7	6	7.00	5	MS
674	3196	10	12	11	11.00	7	S
675	3197	5	6	7	6.00	5	MS
676	3198	14	15	13	14.00	7	S
677	3199	8	7	6	7.00	5	MS
678	3200	0	0	0	0.00	-	-
679	3201	6	5	6	5.67	5	MS
680	3202	6	8	8	7.33	5	MS
681	3203	4	5	8	5.67	5	MS
682	3204	12	15	15	14.00	7	S
683	3205	0	0	0	0.00	-	-
684	3206	7	8	9	8.00	5	MS
685	3207	0	0	0	0.00	-	-
686	3208	0	0	0	0.00	-	-
687	3209	15	14	16	15.00	7	S
688	3210	0	0	0	0.00	-	-
689	3211	8	9	7	8.00	5	MS
690	3212	0	0	0	0.00	-	-
691	3213	8	7	10	8.33	5	MS
692	3214	16	17	15	16.00	9	HS
693	3215	3	2	2	2.33	1	R
694	3216	12	13	11	12.00	7	S
695	3217	0	0	0	0.00	-	-
696	3218	10	12	11	11.00	7	S
697	3219	12	13	16	13.67	7	S
698	3220	8	7	6	7.00	5	MS
699	3221	0	0	0	0.00	-	-
700	3222	0	0	0	0.00	-	-
701	3223	0	0	0	0.00	-	-
702	3224	7	8	9	8.00	5	MS
703	3225	0	0	0	0.00	-	-
704	3226	10	12	8	10.00	5	MS
705	3227	0	0	0	0.00	-	-
706	3228	0	0	0	0.00	-	-
707	3229	0	0	0	0.00	-	-

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
708	3230	5	7	6	6.00	5	MS
709	3231	0	0	0	0.00	-	-
710	3232	4	5	8	5.67	5	MS
711	3233	0	0	0	0.00	-	-
712	3234	0	0	0	0.00	-	-
713	3235	0	0	0	0.00	-	-
714	3236	0	0	0	0.00	-	-
715	3237	4	7	8	6.33	5	MS
716	3238	0	0	0	0.00	-	-
717	3239	0	0	0	0.00	-	-
718	3240	0	0	0	0.00	-	-
719	3241	4	5	6	5.00	5	MS
720	3242	4	6	7	5.67	5	MS
721	3243	5	6	7	6.00	5	MS
722	3244	5	4	10	6.33	5	MS
723	3245	7	5	6	6.00	5	MS
724	3246	10	12	14	12.00	7	S
725	3247	7	8	9	8.00	5	MS
726	3248	8	10	12	10.00	7	S
727	3249	12	13	10	11.67	7	S
728	3250	10	12	15	12.33	7	S
729	3251	7	8	9	8.00	5	MS
730	3252	0	0	0	0.00	-	-
731	3253	5	6	7	6.00	7	S
732	3254	0	0	0	0.00	-	-
733	3255	6	7	8	7.00	5	MS
734	3256	8	7	10	8.33	5	MS
735	3257	10	12	13	11.67	7	S
736	3258	0	0	0	0.00	-	-
737	3259	7	8	9	8.00	5	MS
738	3260	5	6	7	6.00	5	MS
739	3261	12	13	11	12.00	7	S
740	3262	0	0	0	0.00	-	-
741	3263	0	0	0	0.00	-	-
742	3264	7	8	9	8.00	5	MS
743	3265	10	12	11	11.00	7	S
744	3266	0	0	0	0.00	-	-
745	3267	12	13	15	13.33	7	S
746	3268	10	12	13	11.67	7	S
747	3269	0	0	0	0.00	-	-

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
748	3270	0	0	0	0.00	-	-
749	3271	0	0	0	0.00	-	-
750	3272	12	13	10	11.67	7	S
751	3273	0	0	0	0.00	-	-
752	3274	15	20	22	19.00	9	HS
753	3275	10	8	12	10.00	5	MS
754	3276	0	0	0	0.00	-	-
755	3277	3	3	3	3.00	1	R
756	3278	0	0	0	0.00	-	-
757	3279	0	0	0	0.00	-	-
758	3280	12	13	14	13.00	7	S
759	3281	10	12	14	12.00	7	S
760	3282	16	17	20	17.67	9	HS
761	3283	12	13	10	11.67	7	S
762	3284	0	0	0	0.00	-	-
763	3285	7	8	9	8.00	5	MS
764	3286	10	12	9	10.33	7	S
765	3287	9	10	12	10.33	7	S
766	3288	8	7	6	7.00	5	MS
767	3289	12	10	13	11.67	7	S
768	3290	8	7	6	7.00	5	MS
769	3291	12	13	11	12.00	7	S
770	3292	12	13	20	15.00	7	S
771	3293	1	2	1	1.33	1	R
772	3294	15	14	16	15.00	7	S
773	3295	12	10	9	10.33	7	S
774	3296	0	0	0	0.00	-	-
775	3297	0	0	0	0.00	-	-
776	3298	0	0	0	0.00	-	-
777	3299	11	10	12	11.00	7	S
778	3300	0	0	0	0.00	-	-
779	3301	12	13	11	12.00	7	S
780	3302	8	7	9	8.00	5	MS
781	3303	12	13	15	13.33	7	S
782	3304	0	0	0	0.00	-	-
783	3305	0	0	0	0.00	-	-
784	3306	8	7	10	8.33	5	MS
785	3307	10	12	7	9.67	5	MS
786	3308	12	13	11	12.00	7	S
787	3309	10	8	7	8.33	5	MS

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
788	3310	12	13	11	12.00	7	S
789	3311	17	18	16	17.00	9	HS
790	3312	15	20	22	19.00	9	HS
791	3313	10	12	9	10.33	7	S
792	3314	6	7	8	7.00	5	MS
793	3315	0	0	0	0.00	-	-
794	3316	0	0	0	0.00	-	-
795	3317	12	13	14	13.00	7	S
796	3318	11	12	13	12.00	7	S
797	3319	8	7	10	8.33	5	MS
798	3320	8	7	9	8.00	5	MS
799	3321	10	12	9	10.33	7	S
800	3322	0	0	0	0.00	-	-
801	3323	12	13	15	13.33	7	S
802	3324	20	22	18	20.00	9	HS
803	3325	7	8	10	8.33	5	MS
804	3326	10	12	13	11.67	7	S
805	3327	16	17	18	17.00	9	HS
806	3328	14	16	17	15.67	9	HS
807	3329	17	18	20	18.33	9	HS
808	3330	14	15	17	15.33	9	HS
809	3331	7	8	10	8.33	5	MS
810	3332	0	0	0	0.00	-	-
811	3333	0	0	0	0.00	-	-
812	3334	10	12	9	10.33	7	S
813	3335	15	17	18	16.67	9	HS
814	3336	16	17	20	17.67	9	HS
815	3337	12	13	11	12.00	7	S
816	3338	16	17	20	17.67	9	HS
817	3339	0	0	0	0.00	-	-
818	3340	7	8	9	8.00	5	MS
819	3341	12	13	11	12.00	7	S
820	3342	7	8	9	8.00	5	MS
821	3343	12	13	20	15.00	7	S
822	3344	7	8	9	8.00	5	MS
823	3345	0	0	0	0.00	-	-
824	3346	0	0	0	0.00	-	-
825	3347	0	0	0	0.00	-	-
826	3348	0	0	0	0.00	-	-
827	3231	0	0	0	0.00	-	-

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
828	3350	10	8	12	10.00	5	MS
829	3401	28	27	26	27.00	9	S
830	3402	14	15	13	14.00	7	S
831	3403	16	17	28	20.33	9	S
832	3404	9	5	6	6.67	5	MS
833	3405	28	27	30	28.33	9	S
834	3406	30	32	35	32.33	9	S
835	3407	28	30	32	30.00	9	S
836	3408	34	35	36	35.00	9	S
837	3409	2	2	3	2.33	1	R
838	3410	22	20	25	22.33	9	S
839	3411	20	22	23	21.67	9	S
840	3412	20	18	23	20.33	9	S
841	3413	25	26	30	27.00	9	S
842	3414	28	26	22	25.33	9	S
843	3415	26	28	30	28.00	9	S
844	3416	5	6	7	6.00	5	MS
845	3417	34	35	38	35.67	9	S
846	3418	25	26	30	27.00	9	S
847	3419	28	26	30	28.00	9	S
848	3420	45	40	48	44.33	9	S
849	3421	20	22	25	22.33	9	S
850	3422	22	25	26	24.33	9	S
851	3423	40	42	45	42.33	9	S
852	3424	22	25	23	23.33	9	S
853	3425	45	40	38	41.00	9	S
854	3426	25	26	22	24.33	9	S
855	3427	10	12	13	11.67	7	S
856	3428	12	13	16	13.67	7	S
857	3429	40	45	38	41.00	9	S
858	3430	22	23	25	23.33	9	S
859	3431	14	15	16	15.00	7	S
860	3432	19	20	17	18.67	9	S
861	3433	20	22	30	24.00	9	S
862	3434	45	48	40	44.33	9	S
863	3435	28	26	30	28.00	9	S
864	3436	26	28	30	28.00	9	S
865	3437	22	25	21	22.67	9	S
866	3438	26	27	25	26.00	9	S
867	3439	20	22	24	22.00	9	S

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		<i>Bxo67</i>	<i>BXo87</i>	<i>BXo91</i>			
868	3440	12	13	14	13.00	7	S
869	3441	14	15	13	14.00	7	S
870	3442	16	17	18	17.00	9	S
871	3443	20	22	23	21.67	9	S
872	3444	11	12	10	11.00	7	S
873	3445	10	12	9	10.33	7	S
874	3446	16	17	18	17.00	9	S
875	3447	25	26	30	27.00	9	S
876	3448	12	13	14	13.00	7	S
877	3449	14	12	16	14.00	7	S
878	3450	20	22	23	21.67	9	S
879	3451	28	26	30	28.00	9	S
880	3452	12	10	11	11.00	7	S
881	3453	16	17	20	17.67	9	S
882	3454	20	22	25	22.33	9	S
883	3455	28	30	26	28.00	9	S
884	3456	16	17	20	17.67	9	S
885	3457	10	12	9	10.33	7	S
886	3458	19	20	17	18.67	9	S
887	3459	12	13	10	11.67	7	S
888	3460	7	9	10	8.67	5	MS
889	3461	20	22	21	21.00	9	S
890	3462	14	15	16	15.00	7	S
891	3463	3	1	3	2.33	1	R
892	3464	12	13	14	13.00	7	S
893	3465	20	21	22	21.00	9	S
894	3466	8	10	9	9.00	5	MS
895	3467	13	12	11	12.00	7	S
896	3468	20	22	28	23.33	9	S
897	3469	8	10	9	9.00	5	MS
898	3470	14	15	17	15.33	9	S
899	3471	12	13	10	11.67	7	S
900	3472	24	25	23	24.00	9	S
901	3473	26	28	30	28.00	9	S
902	3474	28	26	25	26.33	9	S
903	3475	14	15	17	15.33	9	S
904	3476	14	12	11	12.33	7	S
905	3477	20	17	18	18.33	9	S
906	3478	34	32	35	33.67	9	S
907	3479	12	13	14	13.00	7	S

Entry Sl.	Designation/Accisison number	Lesion length (cm)			Avg.	Score	Reaction
		Bxo67	BXo87	BXo91			
908	3480	19	20	21	20.00	9	S
909	3481	13	14	15	14.00	7	S
910	3482	20	22	19	20.33	9	S
911	3483	14	15	18	15.67	9	S
912	3484	35	34	38	35.67	9	S
913	3485	20	22	18	20.00	9	S
914	3486	26	28	30	28.00	9	S
915	3487	3	3	1	2.33	1	R
916	3488	14	15	13	14.00	7	S
917	3489	20	22	21	21.00	9	S
918	3490	26	27	28	27.00	9	S
919	3491	9	10	11	10.00	5	MS
920	3492	30	35	38	34.33	9	S
921	3493	3	3	3	3.00	1	R
922	3494	16	17	19	17.33	9	S
923	3495	13	14	15	14.00	7	S
924	3496	19	17	18	18.00	9	S
925	3497	16	17	18	17.00	9	S
926	3498	20	21	22	21.00	9	S
927	3499	15	16	17	16.00	9	S
928	3500	16	17	20	17.67	9	S

**Appendix-2:** List of PhD dissertation and Master's thesis

**PhD dissertation (Under preparation)**

1. Md. Mahfujur Rahman. Resistance Induced by Plant Growth Promoting Bacteria in Rice against Bacterial Blight.

**Master's thesis**

**Completed**

1. Mst. Papiya Sharmin Juthy. Evaluation of field efficacy of formulated rice phylloplane and rhizospheric bacteria in controlling bacterial leaf blight of rice.
2. Md. Nazmul Islam. Possibility of field application of formulated *Trichoderma* in controlling bacterial leaf blight of rice

**Under preparation**

3. Noor-E-Tajkia Islam. Molecular characterization of rice plant growth promoting bacteria antagonistic to *Xanthomonas oryzae* pv. *oryzae*.
4. Md. Abdullah-Al Zobaer. Formulation of plant growth promoting bacteria in controlling bacterial blight of rice.
5. Afia Maliqa. Effect of some selected bacterial bio-agents against bacterial blight of rice.
6. Chanchol Kumar Das. Identification of plant growth promoting bacteria in controlling bacterial blight of rice
7. Arpita Saha Roy. Molecular based identification of plant growth promoting fungi in controlling bacterial blight of rice.
8. Robin Chowdhury. Trichoderma mediated induced resistance in rice against *Xanthomonas oryzae* pv. *oryzae*.

