GENETIC AND MOLECULAR CHARACTERIZATION OF ADVANCED BREEDING LINES OF RICE (*Oryza sativa* L.) FOR BACTERIAL LEAF BLIGHT RESISTANCE

by

Malik Mehraj U Din

(J-21-M-823)

A Thesis submitted to
Faculty of Agriculture
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE IN AGRICULTURE
GENETICS AND PLANT BREEDING

Division of Plant Breeding and Genetics
Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu - 180009
2023

CERTIFICATE - I

This is to certify that the thesis entitled **"Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)** to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further, the level of minor similarities has been declared after checking the manuscript with **URKUND** software provided by the University.

The work has been carried out by **Mr. Malik Mehraj U Din**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

Dr. R.S. Sudan
Professor cum Chief Scientist, PBG
Major Advisor

Place: Jammu
Date: 18-08-2023

Tuhina Dey.
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CERTIFICATE-II

We, the members of Advisory committee of **Mr. Malik Mehraj U Din,** Registration No. **J-21-M-823**, a candidate for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)**, have gone through the manuscript of thesis entitled **"Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance"** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

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This is to certify that the thesis entitled **"Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance"**, submitted by **Mr. Malik Mehraj U Din**, Registration No. **J-21-M-823**, to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)**, was examined and approved by the advisory committee and external examiner(s) on 20-10-2023

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Acknowledgements

No Scientific endeavour is a result of individual efforts and so come the time to look back the path which I travelled during this endeavour and to remember the faces behind the action with due respect. I take this opportunity to acknowledge the persons who inspired me to fulfil the goal.

I am profoundly grateful to my Major Advisor, **Dr. R. S. Sudan**, Professor cum Chief Scientist, Plant Breeding and Genetics, SKUAST-Jammu, for his unwavering support and invaluable guidance. His expertise has been instrumental in shaping my research journey.

With utmost reverence, I extend my sincere thanks to dignified and esteemed members of my Advisory Committee, **Dr.Bupesh Kumar,** Associate Professor, Division of Plant Breeding and Genetics, **Dr. Manmohan Sharma,** Professor, School of Biotechnology and **Dr. Anuradha Saha,** Professor cum Chief Scientist(Agronomy)& incharge AICRP on Rice, Division of Plant Breeding and Genetics , SKUAST-J for their precious suggestions, keen co-operation and kind help rendered as and when needed.

I emphatically and gratefully extend my loyal and venerable thanks to **Dr. Tuhina Dey,** Professor and Head, Division of Plant Breeding and Genetics, **Dr. Parveen Singh,** Professor, Division of Plant Breeding and Genetics, **Dr. Sanjeev Kumar,** Professor, Division of Plant Breeding and Genetics, **Dr. S.K. Rai,** Professor , Division of Plant Breeding and Genetics and **Dr. Subhash C. Khashyap,** Assistant Professor, Division of Plant Breeding and Genetics for their scholarly advice, able co-operation and kind assistance.

I am highly grateful to the supporting staff of the Division of Plant Breeding and Genetics, **Mr. Naresh Choudhary, Mr. Darshan Lal, Mr. Latif, Mr. Vijay, Mr. Zakir** and farm labourers **Mr. Janak** and **Mr. Bikaram** for their cooperation and timely assistance during my degree program.

I am indebted to the love, care and support extended by my friends, **Mr. Aamir Hussain Mr. Aamir Naseer Khan, Mr vibhore khojuria, Mr. Khalid , Mr. Younis , Mr. Tabrez Roshan,** and **Mr. Sajid Ahmad.** Life here would have been difficult without their company and assistance.

I am thankful to the everlasting support, cheerful company, timely co-operation and needful motivation provided by my deary friends and batchmates, **Mr. Rahul Saini, Miss. Mahpara Bashhir, Miss.Binatul Mukhtar, Miss Sehroz sharief , Miss Ragini Padha,** and **Miss Lalita kumari** I am indebted to valuable care and guidance provided by my seniors, **Mr. Danish Mushtaq Khanday, K, Priyatham, Dr. Ashish Sheera** and **Shivam raina .**

This note of acknowledgement will be incomplete without a profound delineation of the serene commitment, enlightening love and path showing inspiration, which I was rewarded by my parents, **Mr Mohd. Yousuf Malik and Mrs Nuzhat Parveen.** I also extend my heartfelt thanks to all my brothers and sisters for being a constant source of encouragement throughout my journey. I reserve a special note of thanks for **Miss Gowhar Jabeen** for her unconditional love affection and support.

I offer my sincere thanks and profound regards with deep sense of gratitude to all who have helped me directly and indirectly to accomplish this destination. Everyone is not adverted but none is forgotten.

Place: **Jammu**

Dated: 27-10-2023

Malik Mehraj u din

ABSTRACT

Title of Thesis : **Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance**
Name of the Student : Malik Mehraj U Din
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ABSTRACT

Present study entitled, **"Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance"** was carried out during *kharif* 2022 at Research Farm and Molecular Laboratory of Division of Plant Breeding and Genetics. The aim of the study was to characterize advanced breeding lines of rice for yield and yield attributing traits and to screen these lines for bacterial leaf blight resistance. Thirty advanced breeding lines were evaluated in Randomized Complete Block Design (RCBD) in three replications having a plot size of 2 m^2. Screening for bacterial leaf blight was conducted under natural field conditions using SES scale (IRRI.1996) and simultaneously with the help of gene specific primers targeting three genes *xa5, xa13* and *Xa21.*

Analysis of variance revealed significant variation among the advanced breeding lines for all the traits recorded. Estimates of genetic parameters revealed that traits like days to 50 per cent flowering, days to maturity, plant height, 1000 grain weight and grain yield per plant were found to have high heritability. Plant height was found to have high heritability coupled with high genetic advance indicating effectiveness of selection in improving this trait. Phenotypic screening for bacterial leaf blight showed significant difference among the advanced breeding lines while, molecular characterization revealed that genes *Xa21* and *xa13* were found to be present in four advanced breeding lines, *xa13* and *xa5* in three advanced breeding lines. While only *xa13* or its close allelic variants were found in 16 advanced breeding lines. Seven lines were found to have none of the BLB resistance genes. Among all the advanced breeding lines ABL-2, 5, and 6 were found to have both *Xa21* and *xa5* genes for bacterial leaf blight resistance and the lines ABL-8,9,10 and 11 were found to have combination of *xa13* and *Xa21* genes and hence can be exploited in hybridization programme.

Keywords: Advanced breeding lines, Bacterial leaf blight, molecular characterization

Signature of Major Advisor

Signature of the Student

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

CHAPTER-1

INTRODUCTION

Rice (*Oryza sativa* L.*)* (2n = 24) belonging to the family Poaceae is one of the leading cereal crops, feeding over half of the world's population (Ricepedia, 2020; USDA, 2020). It is the major food crop in India and holds a prominent place in Indian agriculture. It is the primary staple food for Asia, where approximately 89 per cent of the world's rice was produced and consumed (Milovanovic and Smutka, 2017). Globally, it is reported to provide 27 per cent of dietary energy, 20 per cent of the dietary protein and 3 per cent of dietary fat (Pathak *et al*., 2019). Globally rice is cultivated over an area of about 165 million hectares with a total production of around 518.4 million tonnes of milled rice (FAO, 2021). In India rice was cultivated over an area of about 45.07 million hectares with a production and productivity of 122.27 million tonnes and 2.71 tonnes per hectare respectively (Anonymous, 2022 a), while, in the Union Territory of Jammu and Kashmir it was cultivated over an area of 267.58 thousand hectares with production and productivity of 5186 thousand quintals and 21.74 quintals per hectare respectively (Anonymous, 2022 b).

Rice is cultivated in various climates in the Jammu region of the Union Territory of Jammu and Kashmir. The crop is grown in the subtropical belt of Jammu and the intermediate belts of Rajouri, Poonch, and Bani, as well as the temperate regions of Mandi (Poonch), Doda, Kishtwar and Ramban. The area adjoining R.S. Pura is known as the Basmati Bowl of Jammu. Basmati rice is grown on 62.25 thousand hectares of land in this region, with a total production of 12960 tonnes (Anonymous, 2019). Despite the release of new varieties, traditional varieties such as Basmati 370 remain popular among the farming community because of its excellent quality and aroma. Basmati rice cultivars are known for their extra-long, superfine slender grains and pleasant aroma after cooking (Bhattacharjee *et al*., 2002). Basmati rice is mainly cultivated in the foothills of the Himalayas in Uttarakhand and the Jammu region of Jammu and Kashmir, as well as in Haryana, Punjab, and Uttar Pradesh (Nagaraju *et al*., 2002). India is the world's largest producer of basmati rice, with about 70 per cent of global production, and more than half of it is exported to other countries (Verma *et al*., 2012). The Jammu region is renowned for its high-quality basmati rice, which has enormous export potential and can help increase the income of farmers.

Rice is a staple food for over half of the world's population, and more than 90 per cent of it is grown and consumed in Asia (Khush, 2005). The demand for rice in Asia is still increasing, with a consumption rate of at least 90 per cent. It is globally projected that the demand for rice will rise to 650 million tonnes by 2050 (Chukwu *et al*., 2019). As the world's population grows, so does the demand for staple foods like rice. However, two factors are adversely affecting rice production: biotic and abiotic stress factors. Biotic stress factors include fungi, bacteria, viruses, weeds, nematodes, insects, parasites, rodents, and birds present in the environment. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. Oryzae (*Xoo*) is one of the major diseases affecting the rice crop, resulting in yield losses ranging from 10-20 per cent at medium level of disease incidence in moderate resistant cultivars. While the disease causes up to 50 per cent yield loss in conducive condition in the susceptible cultivars (Ou 1985, Mew *et al*., 1993). In this study, we focused on bacterial leaf blight, which is among the most destructive of the sixty-odd diseases attacking the rice crop (Ou, 1972).

Bacterial Leaf Blight (BLB) is a common disease of rice caused by *Xanthomonas* oryzae pv. *oryzae* (*Xoo*) that affects different growth stages of the plant. The symptoms of the disease include leaf blight and wilting, known as kresek. The initial symptoms appear as water-soaked lesions at the tip of the leaf, which rapidly enlarge in length and width and produce yellow wavy lesions along the leaf margins. The affected portions of the leaves show small droplets of bacterial ooze, which is pale amber in color. The most destructive phase of the disease is kresek, which is caused by systemic infection that multiplies the bacteria and blocks the translocation system during severe infection. The plant leaves wilt, roll up, turn grey, wither, and eventually die. The development of the disease is favored by rainy weather, dull windy days, and an atmospheric temperature of 22-26°C, and relative humidity >70 per cent During monsoon season, the BLB pathogen spreads rapidly and can cause yield losses ranging from 6 per cent to 84 per cent, depending on climate, habitat, and variety or cultivar used (Liu, 2006). Host-plant resistance is the most effective, economical, and environmentally safe method for managing BLB disease, while chemical control is not effective (Devadath, 1989). Researchers are developing resistant cultivars and searching for available resistant genes against BLB disease, which is prevalent throughout the world. Currently, 42 genes that confer resistance to various *Xoo* strains have been designated in a series from *Xa1* to *Xa42*, among which nine genes have been cloned, namely *Xa1*, *Xa3*, *Xa26*, *xa5*, *Xa10*, *xa13*, *Xa21*, *Xa23*, and *xa25* (Vikal and Bhatia, 2017). A rice cultivar carrying many resistant genes can show a broader spectrum and higher level of resistance to pathogens than a cultivar with a single resistant gene, as this may lead to the cultivar being susceptible to pathogen mutation (Rajpurohit *et al*., 2011). The bacterial races continuously vary due to artificial and natural selection of genes resistant to bacterial blight; therefore, it is essential to explore and identify new resistant sources to control the changing races (Xia *et al*., 2012). Indian isolates have been found to be different in pathogenicity compared to Japanese and Philippine races, against which *xa* genes have been identified. As considerable variability is found both in the host and pathogen. No variety can remain resistant to a pathogen for long. Therefore, breeding for resistance to BLB must be a continuous process.

Morphological and molecular screening is widely used approaches in the recent scenario for identifying BLB resistant cultivars. Development of resistant varieties had always been target of rice breeders. Screening of plants is common practice to develop resistant varieties against a particular stress (Ashfaq *et al*., 2016). Host-plant resistance would serve as a beneficial tool in managing BLB in rice crop (Banito *et al.*, 2012). Host-plant resistance should be practiced in combination with other integrated practices to defend crop against menace of this disease (Singh *et al*., 2015).

Keeping in view above facts present study is made to identify lines resistant to bacterial leaf blight for their potential use in breeding programme, with following objectives.

I. To evaluate advanced breeding lines for yield and yield related traits.

II. To screen these lines against BLB resistance using morphological and molecular markers.

CHAPTER-2

REVIEW OF LITERATURE

Rice, scientifically known as *Oryza sativa* L., is a cereal grain that belongs to the family Poaceae and is classified under the division Angiospermae, class Monocotyledoneae, order Glumiflorae, and genus *Oryza*. Originally, only two species of rice were grown, *Oryza sativa* and *Oryza glaberrima*, with *Oryza sativa* being the more important one and having three subspecies: *Indica*, *Japonica*, and *Javanica*. *Oryza sativa* is indigenous to tropical and subtropical Southeast Asia, while *Oryza glaberrima* is native to Africa and is less widely grown.

Rice is the most significant crop in India, with rice-based agricultural systems occupying about 80 per cent of agricultural land in South Asia. The rice-wheat system, covering almost 11 million hectares in India, is the most crucial cropping system in the Indo-Gangetic plains. Basmati rice, known for its quality characteristics, such as its pleasant aroma, fluffy texture of cooked grains, and high-volume expansion during cooking, is a highly desirable rice type. Over 50 per cent of the basmati rice produced in India is exported, mainly to countries such as Saudi Arabia, UAE, UK, European Union countries, Kuwait, and Bahrain. However, traditional Basmati cultivars are superior in terms of grain quality, cooking, and eating quality when compared to recently developed evolved Basmati cultivars. Traditional basmati cultivars are tall, photoperiod and temperature-sensitive, prone to lodging, very low yielding, and highly susceptible to Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). To address this issue, researchers have identified and designed at least 38 BLB resistance genes (both dominant and recessive), from *Xa1* to *Xa38*. Out of these 38 genes, six have been cloned and six have been physically mapped. These genes follow a Mendelian pattern of major gene inheritance and express resistance to diverse groups of *Xo*o pathogens. Regular screening of genotypes under natural and artificial conditions to identify BLB-resistant lines has been carried out, and resistant materials have been used in breeding programmes to confer resistance to susceptible genotypes. Major resistance genes, including *Xa4*, *xa5*, *Xa7*, *xa13*, and *Xa21*, have been incorporated into rice cultivars to develop new resistant varieties.

The present investigation, "Genetic and Molecular Characterization of Advanced Breeding lines of Rice for Bacterial Leaf Blight Resistance," is reviewed with relevant literature pertaining to this topic under the following headings:

2.1 Yield and Yield Related Traits

2.2 Morphological and Molecular Marker Screening for Bacterial Leaf Blight Resistance

2.1. Yield and Yield Related Traits

Nayak *et al*. (2002) evaluated 200 scented rice genotypes to study genetic variability for grain yield and nine yield contributing characteristics, and reported that number of spikelets per panicle, number of panicles per plant, number of grains per panicle, and the grain yield per plant, were found to have high estimates of the genetic and phenotypic coefficients of variation. In addition, they reported high heritability and high genetic advance for the number of spikelet’s per panicle, number of grains per panicle, and the yield of grains per plant.

Singh *et al*. (2002) in a study conducted at Meghalaya on 52 genotypes of Lowland rice for 15 characters reported high genotypic and phenotypic variances for grain yield per plant, panicle weight, number of grains per panicle, and number of branches per panicle, medium for panicle length, 1000-seed weight and low for milling per cent and panicle length with heritability in broad sense ranging from 3.61 for number of effective tillers per plant to 99.55 for grain length. Further they also observed high heritability with high genetic advance for number of grains per panicle followed by panicle weight and grain yield per plant.

Yadav *et al.* (2002) in a study on genetic variability for yield and yield contributing traits in 15 genotypes of rice observed appreciable amount of genotypic variation, heritability, and genetic advance for total grains per panicle, total number of grains per plant and grain yield per plant.

Satish *et al*. (2003) conducted study on 200 scented rice genotypes including one non-scented check, Ratna to assess genetic variability, heritability, and genetic advance for yield and its nine attributing characters. They observed high GCV and PCV values for number of spikelets/panicles, number of grains/panicles, grain yield per plant followed by other characters.

Sinha *et al*. (2004) conducted a study on the variability, heritability, and genetic advance estimates of 19 local midland rice landraces in Ambikapur, Chattisgarh, during the *kharif* seasons of 1998-2000. The study aimed to determine the correlation coefficient for yield and its attributing characters (plant height, tillers per hill, panicles per hill, panicle length, days to 50 per cent flowering, days to maturity, test weight, and grain yield), with IR-36 serving as the standard control. The results showed that grain yield had the highest genotypic coefficient of variation, followed by test weight and panicles per plant. Grain yield also had high heritability with high genetic advance, followed by test weight and panicles per plant. Furthermore, the study found a significant positive genetic correlation between panicles per plant, test weight, and grain yield.

Sabu *et al.* (2009) conducted a study on the genetic information of grain yield and related traits *viz.*, heritability, genetic and environmental factors in the F_1 progenies of a cross between *O. sativa* and *O. rufipogon*. Their study revealed the presence of a considerable amount of additive genetic variation in these families. The researchers identified the traits with high heritability, significant phenotypic correlation, and low seasonal variability, which could be further improved in the F1 progenies. The results were promising and they could aid in the development of new rice cultivars.

Yadav *et al*. (2009) conducted a study on 20 different rice genotypes and found that there was significant differences in plant height, days to 50 per cent flowering, days to maturity, number of tillers per plant, number of spikelet's per panicle, and grain yield per plant. They also observed high values of genotypic and phenotypic coefficient of variation for plant height, number of tillers per plant, and grain yield per plant.

Akinwale *et al*. (2011) conducted study on phenotypic and genotypic coefficients of variation, broad sense heritability, genetic gain and correlations in rice (*Oryza sativa* L.). Their study showed that the genotypes exhibited significant differences ($p>0.001$) in all the traits studied, indicating that they represent a pool of germplasm with ample genetic variability. The genotypic coefficients of variation were lower than the corresponding phenotypic coefficients in all traits studied, implying that the environment had a significant impact on trait expression. Furthermore, high to medium heritability and genetic gain were observed for the number of grains per panicle, grain yield, panicle weight, and the number of panicles per plant, indicating they are primarily under genetic control and selection for these traits can be achieved through their phenotypic performance.

Subbaiah *et al*. (2011) conducted a study to determine the extent of variability and genetic parameters with 16 parents and 48 hybrids for nine yield and its components and 25 quality characters. The difference between PCV and GCV was relatively low for all traits, indicating less environmental influence. Harvest index, total number of productive tillers per plant, and gelatinization temperature in parents, and total number of productive tillers per plant, number of grains per panicle, gelatinization temperature, and amylose content in hybrids exhibited high GCV and PCV. High heritability coupled with high genetic advance as a percentage of the mean were found for gelatinization temperature, harvest index, total number of productive tillers per plant, number of grains per panicle, kernel length, kernel L/B ratio, and grain yield per plant in parents and for gelatinization temperature, amylose content, total number of productive tillers per plant, number of grains per panicle, and harvest index in hybrids. These results suggested that these traits are primarily controlled by additive gene effects and could be improved through simple selection in the present breeding material.

Babu *et al*. (2012) conducted a study on twenty-one rice hybrids to examine the genetic parameters for yield, yield attributing, quality, and nutritional traits. The analysis of variance showed significant differences for all the traits studied. Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were high for number of filled grains per panicle, number of chaffy grains per panicle, and iron content. Small differences between GCV and PCV were found for all the traits, indicating a lesser influence of the environment on these traits. Number of filled grains per panicle and water uptake exhibited high heritability coupled with high genetic advance, suggesting that simple selection could be an effective approach to enhance these traits.

Yadav *et al*. (2013) conducted a study to evaluate the pattern of genetic diversity of in rice using both phenotypic and genotypic variability, molecular markers such as microsatellite or simple sequence repeat markers are used. The study involved 88 rice accessions, including landraces, farmer's varieties, and popular Basmati lines. They used 50 diversity panel markers developed by IRRI's Generation Challenge Programme to evaluate the yield and related components. Significant variability was observed for agronomic traits, with grains and panicle length showing the maximum and minimum differences, respectively. They found that varieties were genetically similar from a common center by molecular diversity grouping. The trait-linked markers showed higher genetic dissimilarity (average value of 0.45) than random markers (average value of 0.37), with average polymorphic information constant values of 0.48 and 0.41, respectively. Furthermore, there was a higher correlation (0.29) between the kinship matrix generated by trait-linked markers and the phenotypes-based distance matrix, compared to random markers (0.19). These results indicated that trait-linked markers are more robust in estimating the genetic diversity of rice germplasm.

Gampala *et al*. (2015) conducted a study on 80 rice genotypes to assess the genetic variability, heritability and genetic advance. The study showed that grain yield per hill and number of spikelets per panicle had the highest PCV, while number of spikelets per panicle had the highest GCV. Traits such as number of spikelets per panicle, test weight, plant height and days to 50 per cent flowering had high heritability estimates. Number of spikelets per panicle and plant height had high genetic advance. Additionally, plant height, panicle length and grain yield per plant had high heritability coupled with high genetic advance.

Sameera *et al*. (2015) conducted study on 25 rice genotypes to examine their variability, heritability and genetic advance. The results indicated that the traits, such as number of productive tillers per plant, number of grains per panicle and number of filled grains per panicle showed high values of both genotypic and phenotypic coefficient of variation. Moreover, these traits exhibited high heritability coupled with high genetic advance as per cent of mean.

Sindhumole *et al*. (2015) conducted study on 64 rice genotypes to assess the genetic variability and heritability for seven biometrical traits. They found that all of the characters showed high genotypic and phenotypic coefficients of variation, except for days to 50 per cent flowering and panicle length. The highest heritability was observed for plant height, whereas grain yield exhibited the lowest. The highest genetic advance was observed for panicles per square meter followed by plant height.

Mohan *et al*. (2016) conducted a study to assess the variability in yield parameters across 44 different rice genotypes. They found that the traits of days to 50 per cent flowering, plant height, and panicle length showed low levels of both phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), suggesting limited variation among these traits. In contrast, the number of grains per panicle and 1000 grain weight showed high estimates of both PCV and GCV, indicating a significant degree of variation in these traits among the genotypes. The study also observed high heritability and genetic advance for the number of grains per panicle and 1000 grain weight.

Nayak *et al*. (2016) conducted a study to assess the genetic variability, heritability and genetic advance for grain yield and yield traits in 25 rice genotypes. The study found that effective tillers per plant, filled grains per panicle, total grains per panicle, and grain yield per plant showed high estimates of both genotypic and phenotypic coefficient of variation, as well as heritability and genetic advance.

Gour *et al*. (2017) conducted a study to estimate the genetic parameters, including GCV, PCV, heritability, and genetic advance (GA), using data collected from 83 rice genotypes. The findings of the study indicated that grain yield per plant had high GCV and PCV values, coupled with high heritability and genetic advance as a percentage of the mean.

Jan *et al*. (2017) conducted a study to analyze the genetic variability of 14 agro-morphological traits among 35 rice genotypes. The results of the research showed that the harvest index had the highest GCV and PCV values, whereas the 1000 grain weight showed the lowest. Furthermore, the number of spikelets per panicle and the number of filled grains per panicle exhibited high broad sense heritability and high genetic advance.

Adhikari *et al*. (2018) conducted a study to analyze the genetic parameters of 26 rice genotypes for grain yield and yield-related traits. The study found that grain yield had high values of PCV, GCV, and genetic advance as a percentage of the mean. While, days to 50 per cent flowering, 1000 grain weight, and plant height exhibited high heritability.

Anyaoha *et al*. (2018) conducted a study to assess the genetic variability, heritability, and genetic advance of 77 genotypes for 10 traits. The study revealed that grain yield had high estimates of both GCV and PCV, while 1000 grain weight had the highest heritability. Moreover, the genetic advance was reported to be highest for grain yield among all the traits studied.

Pratap *et al*. (2018) conducted a study to determine the genetic variability, heritability, and genetic advance for grain yield and yield-contributing traits on 38 rice genotypes. The results showed that traits like grain yield per plant, filled grains per panicle, and effective tillers per plant had high estimates of GCV and PCV. Additionally, the traits filled grain per panicle and spikelet fertility percentage had high heritability coupled with high expected genetic advance as a percentage of the mean.

Sandeep *et al*. (2018) conducted a study to assess the genetic variability, heritability, and genetic advance of 200 rice genotypes. The results showed that single plant yield, number of grains per panicle, number of tillers per hill and spikelet fertility had high genotypic and phenotypic coefficients of variability. Moreover, the traits spikelet fertility, plant height, single plant yield, number of grains per panicle, number of tillers per hill, number of productive tillers per hill, panicle length and 1000 grain weight exhibited high heritability estimates along with high genetic advance as a percentage of the mean.

Sharifi (2019) conducted a study to estimate the heritability of grain yield, agro-morphological, and quality traits in 65 rice genotypes, including four local landraces. The study found that the number of unfilled grains and grain yield exhibited higher values of genotypic and phenotypic coefficient of variation. Furthermore, the traits panicles per plant, number of filled grains, and grain yield exhibited high broad sense heritability and genetic advance over the mean.

Manohara *et al*. (2020) conducted an experiment to determine the genetic variability in ten traits of 82 rice genotypes. The study found that among the traits evaluated, grains per panicle and number of productive tillers per plant exhibited the highest values of both genotypic and phenotypic coefficients of variation. Additionally, heritability in the broad sense was highest for grain yield, followed by panicle length, per cent fertility, and days to 50 per cent flowering. The study also observed high heritability and high genetic advance as a percentage of mean for grain yield and per cent fertility.

Nithya *et al*. (2020) conducted an experiment to assess the genetic variability, heritability, and genetic advance in 81 rice genotypes. Results showed that yield per plant had high values of genotypic and phenotypic coefficient of variation. Heritability estimates ranged from high to medium, with high genetic advance observed for plant height, days to 50% flowering, and grain yield per plant.

Parimala *et al*. (2020) conducted an experiment aimed at exploring genetic variability among 77 rice genotypes for grain yield and its component traits, it was found that the characters, number of filled grains per panicle and 1000 seed weight showed higher values of both phenotypic and genotypic coefficient of variation. Further, 1000 seed weight exhibited high heritability and genetic advance.

Rao *et al*. (2020) conducted a study to investigate the genetic variability, heritability, and genetic advance of 30 rice genotypes for yield and nutritional traits. The study found that the number of productive tillers per plant, yield per plant, and 100-grain weight exhibited high values of both phenotypic and genotypic coefficient of variation. Furthermore, the traits plant height, number of productive tillers per plant, and panicle length exhibited high heritability coupled with high genetic advance as a percentage of the mean.

Sharma *et al*. (2020) Conducted a study to investigate the variability, heritability, and genetic advance of F1s resulting from the crossbreeding of nine basmati rice varieties using a half-diallel mating design. The study found that the genotypic and phenotypic coefficient of variation were high in the number of panicles per plant, number of grains per panicle, and yield per plant. Furthermore, the study recorded high heritability with high genetic advance in plant height, numbers of panicles per plant, numbers of grains per panicle, 100-grain weight, and yield per plant.

Bhargava *et al*. (2021) performed a study to assess the genetic variability of eight yield-related traits in the segregating F2 population. The findings revealed that productive tiller number, grain number per panicle, and plant yield exhibited high phenotypic and genotypic coefficients of variation. Height of plant, productive tiller number, grain number per panicle, and plant yield displayed high heritability combined with a high genetic advance as a percentage of mean.

Chamar *et al*. (2021) conducted a study to determine the genetic variability for yield and yield attributing characters in rice, using 72 germplasm accessions and three local checks. The study found that traits such as the number of tillers per plant, number of productive tillers per plant, number of grains per panicle, and grain yield per plant showed high estimates of phenotypic and genotypic coefficients of variation. Additionally, these traits exhibited high heritability and high genetic advance as a percentage of mean.

Gupta *et al*. (2021) examined 46 types of exotic rice germplasm and four reference samples to determine the genetic variability parameters of yield and yield-contributing characteristics. According to their findings, the traits of grain yield per plant, effective tillers per plant, and 1000 seed weight exhibited high GCV and PCV values. Additionally, the researchers found that grain yield per plant, effective tillers per plant, days to 50% flowering, and the number of filled grains per panicle had high heritability and genetic advancement as a percentage of mean.

Okoye *et al*. (2021) conducted a study to estimate variance components for 11 phenological and yield parameters in 25 rice varieties. Their findings revealed that grain yield per stand had the highest PCV and GCV values, while days to 50 per cent flowering had the lowest. The broad sense heritability estimate was moderate for the majority of the parameters studied, except for tiller number, which exhibited low heritability. Number of grains per panicle and grain yield per stand showed high genetic advance. Panicle length and spikelet fertility exhibited moderate genetic advance, while plant height, days to 50 per cent flowering, tiller number, and 100-grain weight had low genetic advance.

Parimala *et al*. (2021) conducted a study to examine the genetic variability of 34 rice genotypes. Their findings indicated that grain yield per plant had higher estimates of PCV and GCV. High broad sense heritability, combined with high genetic advance as a percentage of the mean, was observed in plant height, number of effective tillers per plant, test weight, and grain yield per plant, which suggests the role of additive gene action.

2.2.1 Morphological Screening for BLB Resistance

Screening for bacterial leaf blight disease resistance in rice, specifically done to confirm the reactions of different genotypes against *Xanthomonas oryzae* pv. *oryzae*, to determine the rate of infection or disease development, and to compare disease development among different genotypes. Screening of various lines/genotypes will be required to identify the resistant sources against the BLB pathogens

Rafi *et al*. (2015) conducted a study to assess the genetic potentiality of 23 rice genotypes against bacterial leaf blight through artificial screening. Their findings indicated that none of the genotypes were resistant, while 13 were moderately resistant, five were moderately susceptible, and six were susceptible. Kashmir Basmati showed a highly susceptible response.

Singh *et al*. (2015a) carried out screening of 34 rice cultivars against *Xanthomonas oryzae* pv. *oryzae* and reported that seven cultivars exhibited resistance, four showed moderate resistance, six were moderately susceptible, and 17 were susceptible to bacterial leaf blight based on mean lesion length 15 days after inoculation.

Fred *et al*. (2016) conducted a screening for resistance to BLB in 32 rice cultivars across two different seasons and locations, namely in a greenhouse during winter and in natural field conditions during summer. Their findings revealed that five cultivars, namely Hanareum, Namcheon, 6 Samgdeok, Samgang, and Yangjo, exhibited resistance to BLB under both greenhouse and open-field conditions.

Islam and awais (2017) carried out screening for BLB disease resistance in ten rice genotypes by estimating their disease severity after artificial inoculation. Their findings revealed that none of the tested genotypes were found to be resistant against the disease, except for KSK133, which exhibited a moderately resistant reaction. Among the remaining genotypes, six, namely Basmati-515, Basmati-385, Basmati-370, Basmati-198, KSK-282, and IRRI-6, were categorized as moderately susceptible. Basmati Pak was found to be susceptible, while Basmati Super and Basmati-2000 were highly susceptible to the disease.

Rahman *et al*. (2017) evaluated 142 indigenous rice accessions and two check cultivars (Super-Basmati and IR-6) for resistance to bacterial leaf blight disease through artificial inoculation with *Xanthomonas oryzae* pv. *oryzae*. The majority of rice accessions and the two commercial cultivars exhibited susceptibility to BLB, while seven accessions displayed moderate levels of resistance and 11 accessions showed strong resistance to the disease.

Kumar *et al*. (2018) conducted an evaluation of 17 local rice varieties, ten near isogenic lines (NILs), and ten pyramided lines with single or combined *Xa* genes for resistance to three different isolates in Chhattisgarh. The study found that IRBB-10 carrying the *Xa10* resistant gene for Xoo was resistant to the Raipur and Kanker isolates and moderately resistant to the Dhamtari isolate. Additionally, IRBB-8 and IRBB-11 were resistant to only the Raipur isolate. The remaining NILs exhibited susceptible to moderately susceptible reactions to all isolates. Local varieties were observed to be more susceptible to leaf blight disease than improved varieties. The study suggests that resistance genes have differential effects against different isolates.

Sutrisno *et al*. (2018) conducted screening for BLB resistance in five black rice cultivars, including Cempo Ireng, Pari Ireng, Melik, Pendek, and Indmira, by inoculating them with with Xoo and reported that Cempo Ireng was the most resistant cultivar against BLB disease based on the lowest disease intensity and Area Under Disease Progress Curve (AUDPC) value.

Majumder *et al*. (2019) conducted a field study to screen a total of 61 rice genotypes including resistant (IRBB 60, IRBB 7) and susceptible (IR 24) checks against bacterial leaf blight disease through artificial inoculation using IX020 strain for two years. The study found that seven cultivars, namely IR-64, IR-68144-2b-2-2-3-1-127, Ratna, Surjamukhi, Kalinga-2, Azucena, and Zheshan-2 exhibited resistance, while 27 were found to be moderately resistant.

Qudsia *et al*. (2019) conducted a study to screen 31 near-isogenic lines (NILs) possessing BLB-tolerant genes *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*, along with 34 locally developed rice lines, for BLB resistance under natural field conditions at three different locations with high disease occurrence records. Results showed that 31 lines were resistant, 28 were moderately resistant, six were moderately susceptible, and one susceptible check was classified as susceptible. The clustering of different lines/varieties with similar responses to BLB disease in corresponding environments indicated their similar genetic resistance against the disease.

2.2.2 Molecular Marker Assisted Screening for BLB Resistance

Ronald *et al*. (1992) conducted studies on genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21* with 123 DNA markers and 985 random primers using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) analysis and reported that the three *Xa21*-linked markers are physically close to each other, with one copy of the RAPD818 sequences located within 60 kb of RAPD248 and the other copy within 270 kb of RG103.

Yoshimura *et al*. (1995) in a study on tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers, tagged four genes of rice, *Oryza sativa* L., conditioning resistance to the bacterial blight pathogen *Xanthomonas* *oryzae* pv. *oryzae* (*X. o.* pv. *oryzae*), by restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers and reported that combinations of resistance genes provide broader spectra of resistance through both ordinary gene action expected and quantitative complementation.

Zang *et al. (*1996) conducted study on RAPD and RFLP mapping of the bacterial blight resistance gene xa-13 in rice and reported that one primer (OPAC05) amplify specifically a 0.9-kb band from the DNA of susceptible plants. The distance between the RAPD marker OPAC05-900 and *xa-13* was estimated to be 5.3 cM. The RAPD marker was then mapped on chromosome 8 using a mapping population of doubled haploid lines derived from the cross of IR64/Azucena. The linkage between RFLP markers and the RAPD marker was analyzed using an F_2 population of 135 plants derived from a cross between a near-isogenic line for *xa-13*, IR66699-5-5-4-2, and IR24.

Sanchez *et al.* (2000) conducted a study on sequence tagged site marker-Assisted selection for three bacterial blight resistance genes in rice. And demonstrate the usefulness of MAS in gene pyramiding for BB resistance, particularly for recessive genes, such as *xa5* and *xa13* that are difficult to select through conventional breeding in the presence of a dominant gene such as *Xa21*.

Singh *et al.* (2001) conducted study on Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106 and reported that the combination of genes provided a wider spectrum of resistance to the pathogen population prevalent in the region; *Xa21* was the most effective, followed by *xa*5. Resistance gene *xa13* was the least effective against *Xoo*.

Shanti *et al.* (2001) conducted a study to identify resistance genes effective against Rice Bacterial Blight Pathogen in Eastern India and reported that the Gene combinations *Xa4* + *xa5*, *xa5* + *Xa21*, and *Xa4* + *xa5* + *Xa21* conferred a broad spectrum of resistance to all the strains evaluated, supporting the strategy of pyramiding appropriate resistance genes.

Porter *et al*. (2003) conducted a study on development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7* and reported that *Xa7* could lie within 40 kilo bases (kb) of M5, a distance suitable for gene pyramiding efforts and Xa7 cloning strategies

Sundaram *et al*. (2008) conducted a study on marker assisted introgression of bacterial blight resistance in Samba Mahsuri, and introgressed three major BB resistance genes (*Xa21*, *xa13* and *xa5*) into Samba Mahsuri from a donor line (SS1113) in which all the three genes were present in a homozygous condition. And reported that the three-gene pyramid and two-gene pyramid lines exhibited high levels of resistance against the BB pathogen.

Singh *et al. (*2015) conducted an experiment for identification of bacterial leaf blight resistance genes in wild rice of eastern India using 35 wild rice accessions against the BX043 strain of *Xanthomonas oryzae* pv. *Oryzae* and identify the presence of bacterial blight resistance genes *Xa21*, *xa13*, *xa5*, *Xa4*, and *Xa2*. The study revealed that the accession NKSWR-25 harbored 3 resistance genes, *xa5*, *Xa4*, and *Xa2*, while accessions NKSWR-16, NKSWR-32, NKSWR-36, NKSWR-41, NKSWR-42, NKSWR-53, NKSWR-64, NKSWR-97, and NKSWR-99 each possessed 2 resistance genes of those 3 (*xa5*, *Xa4*, and *Xa2*). On the basis of disease severity 11 accessions showed moderate resistance, 21 were moderately susceptible, and 3 accessions showed susceptible response to the BX043 strain of *Xanthomonas oryzae* pv. *Oryzae*, while none of the accessions were found to be resistant.

Sabar *et al*. (2016) conducted the study to explore the genetic resources regarding presence and absence of BLB resistance genes *Xa4*, *xa5* and *Xa21* through DNA marker technology on eighty (80) rice genotypes comprising of diverse origin including three isogenic lines *viz*., IRBB4 (carrying *Xa4*), IRBB5 (carrying *xa5*) and IRBB21 (carrying *Xa21*) as positive resistant gene checks and IR24 (carrying none of these) as negative gene check was genotyped. And reported the presence of *Xa4* gene in 41 entries, while 14 lines were positive for *xa5* gene. Only one local line was carrying *Xa21* gene along with *Xa4*.

Baliyan *et al*. (2017) introgressed three BB resistance genes viz., *Xa21*, *xa13* and *xa5* from IRBB-60 into Basmati variety CSR-30 through marker-assisted selection (MAS) exercised with stringent phenotypic selection without compromising the Basmati traits and reported that, based on agronomic evaluation, BB reaction, aroma, percentage recovery of recurrent parent genome, and grain quality evaluation, four genotypes, *viz.,* IC-R28, IC-R68, IC-R32, and IC-R42, were found promising and advanced to BC_3F_2 generation

Dilla-Ermita *et al*. (2017) carried genome-wide association study of bacterial blight resistance using a diverse panel of 285 rice accessions to identify loci that are associated with resistance to nine *Xoo* strains from the Philippines. The results of the genome-wide association study validated known genes underlying resistance and identified novel loci that provide useful targets for further investigation.

Aljumaili *et al*. (2018) quantified genetic divergence of aromatic rice accessions using SSR markers and identified potential accessions for introgression into elite rice lines. Analysis of molecular variance (AMOVA) revealed that 89 per cent of the total variation observed in this germplasm came from within the populations, while 11 per cent of the variation emanated among the populations. Using all these criteria and indices, seven accessions from three populations have been identified and selected for further evaluation before introgression into the existing breeding program and for future aromatic rice varietal development.

Yugander *et al*. (2018) added a novel BB-resistant gene, *Xa38*, into ISM through marker-assisted backcross breeding (MABB), using PR 114 (*Xa38*) as the donor for *Xa38*, and ISM as the recurrent parent. Eighteen homozygous BC_3F_2 plants possessing all four BB-resistant genes in the homozygous state and with a recurrent parent genome (RPG) recovery of more than 92% were identified and advanced to the BC_3F_6 generation. These 18 backcross-derived lines (BDLs) exhibited very high level of resistance against multiple *Xoo* strains and displayed agro-morphological traits, grain qualities and yield levels similar to or better than those of the recurrent parent ISM.

Singh *et al.* (2018) examined the mode of inheritance against bacterial leaf blight disease resistance in rice cultivars, TN-1 (susceptible check), HUBR-10-9 (susceptible parent), PB-1460 (resistant parent) with six populations of cross HUBR-10-9 × PB-1460 against the bacterial strain *BXO1* and *BX043* (wild type) of pathogen *Xanthomonas oryzaepv. oryzae (Xoo)* and observed Mendelian pattern of inheritance for resistance to BLB in backcross generations and modification of Mendelian ratio *i.e.,* 13:3 inhibitory gene action in F_2 segregating population.

Neelam *et al.* (2020) conducted high resolation genetic mapping of novel bacterial blight resistance locus designated as a *xa-45(t)*, identified from *Oryza glaberrima* accession IRGC 102600B, transferred to *O. sativa* and mapped to the long arm of chromosome 8 using ddRAD sequencing approach. And found that An STS marker developed from the locus LOC_Os08g42410 was co-segregating with the trait and will be useful for marker-assisted transfer of this recessive resistance gene in breeding programs**.**

Khannetah *et al.* (2021) assessed the potentiality of BB resistance and molecular characterization of 100 rice accessions for four major BB resistance genes, *viz., Xa4, xa5, xa13* and *Xa21*. They further studied the presence of single, two, three and four-gene combinations and identified four accessions as resistant, 34 accessions as moderately resistant, 49 accessions as moderately susceptible and 13 accessions as susceptible.

Das *et al*. (2014) conducted a study where they developed 34 pairs of primers targeting conserved regions of six BLB resistance genes: *Xa1, xa5, Xa21, Xa21*(A1), *xa26,* and *Xa27*. These primer pairs were utilized to perform PCR-based analyses to investigate the genetic diversity of these six genes in 22 distinct rice accessions, each with a known disease phenotype. Through their investigation, the researchers identified a total of 140 alleles, comprising 41 rare alleles and 26 null alleles. The average polymorphic information content (PIC) value obtained per primer pair was reported to be 0.56. Furthermore, the genetic similarity among the rice accessions varied significantly, ranging from 18% to 89%. Based on their findings, the researchers constructed a dendrogram, which revealed the presence of two major clusters among the tested rice accessions.

Islam *et al*. (2015) conducted a study to assess BLB resistance gene *xa5* in 12 aromatic rice cultivars using two genetic markers, namely RM 122 and RM 390. A BLB-resistant genotype, BR 14, was employed as the control for comparison. The two markers, RM 122 and RM 390, revealed the presence of 6 and 10 alleles, respectively. In terms of gene diversity, locus RM 390 exhibited the highest value of 0.8889, while locus RM 122 displayed the lowest value of 0.7361, resulting in a mean diversity of 0.8125 across the markers. The PIC values varied from 0.7007 (for RM 122) to 0.8785 (for RM 390), with an average PIC value of 0.7896. Among the cultivars tested, Basmati, BRRI dhan50, Kalizira Atasail, Bina dhan9, Uknimodhu, BR 34, BR 37, and Zira Katari demonstrated partial resistance to BLB diseases. However, BRRI dhan38 was found to be either partially or completely susceptible to BLB.

Khan *et al*. (2015) examined 17 rice germplasms using two SSR markers, RM122 and RM390, linked to rice bacterial leaf blight resistance genes. Results showed an average of 10 alleles per locus, ranging from 7 for RM122 to 13 for RM390. The gene diversity was highest at locus RM390 (0.9135) and lowest at locus RM122 (0.7059), with an overall mean diversity of 0.8097. PIC values ranged from 0.6715 (RM122) to 0.9069 (RM390), with an average of 0.7892. Furthermore, the researchers constructed a dendrogram based on genetic distance using the UPGMA method. This dendrogram allowed them to group the 17 rice varieties into six major clusters, with cluster IV containing the maximum number of genotypes.

Acharya *et al*. (2018) examined a total of 60 rice genotypes to identify the presence of *Xa4*, *xa5*, *Xa7*, and *Xa21* genes. To accomplish this, they utilized specific markers, namely MP, RM122, M5, and pTA248, respectively. As controls, they included the resistant genotype IRBB 60 and the susceptible genotype Jumli marshi. Their findings indicated that the genes *Xa4*, *xa5*, and *Xa7* were detected in 25, 24, and 14 rice genotypes, respectively. Among all the genotypes tested, 24 of them did not exhibit any of the targeted genes, while 24 others displayed the presence of more than one gene.

Banerjee *et al*. (2018) conducted a screening of a collection of 70 rice genotypes to investigate the presence of 10 genes associated with resistance to bacterial leaf blight (BLB). The results revealed that among the 70 genotypes, 35 of them exhibited resistance, 21 showed moderate resistance, and 14 were susceptible to BLB. The study found variations in the frequency of R genes within each genotype, ranging from 0 to 5 genes per genotype. Notably, the most commonly occurring gene was *Xa1*, followed by *Xa7, Xa4, Xa10,* and *Xa11*.

Panwar *et al.* (2018) conducted a screening of 25 rice genotypes to identify the major resistance genes against bacterial leaf blight (BLB), namely *Xa4*, *xa5*, and *Xa21*. The results revealed that ten genotypes, namely IR20, IR64, IR72, NWGR1095, NWGR2014, IET14726, GR7, GR102, Pankhali 203, and Ratna, tested positive for the *Xa4* gene. However, only the genotype IET18483 was found to be positive for the *Xa21* gene, and none of the tested genotypes were positive for the *xa5* gene, which confers resistance against BLB.

Ashiba *et al*. (2020) conducted an assessment of 100 rice genotypes using seven SSR markers linked to bacterial leaf blight (BLB) resistance. In their study, they reported a total of 38 alleles, with an average of 5.42 alleles per polymorphic marker. Among the markers used, RM21, RM122, and RM224 exhibited the highest allelic diversity, producing up to 6 alleles per locus. To further characterize the markers, the researchers calculated polymorphic information content (PIC) values, which ranged from 0.345 to 0.688. Interestingly, marker RM-21 displayed the highest PIC value of 0.688, making it the most suitable marker for distinguishing BLB-resistant rice genotypes.

Fordjour *et al.* (2020) conducted a screening of six rice cultivars for resistance against bacterial leaf blight (BLB) using five markers: RM317, RM224, RM13, xa-13prom, and pTA248, associated with *Xa2*, *Xa4*, *xa5*, *xa13*, and *Xa21* genes, respectively. The results revealed that Ghanaian cultivars Tinsibe, AGRIC1, and Krampa White possessed the *Xa2* gene, while Kabre and Krampa White had the *Xa4* gene, and Popa and IRAT10 carried the xa5 gene. However, none of the ten cultivars showed any indication of having xa13 or *Xa21* genes. The resistant control, Tetep, exhibited both the *Xa2* and *xa5* genes, while the susceptible control, IR661, only had the *xa5* gene.

Hasan *et al*. (2020) examined ten cultivated Malaysian rice varieties for BLB resistance genes *xa13*, *xa5*, *Xa2*, *Xa4*, and *Xa21* using markers *xa13*, RM122, RM317, RM224, and pTA-248, respectively. The findings showed that all genotypes possessed the *xa2* gene. Among them, four genotypes had two resistance genes closely linked to specific markers: Mahsuri Mutant carried *Xa4* and *Xa2*, while NMR152 and Tongkat Ali mutant exhibited *Xa21* and *xa2*. However, none of these genotypes showed any presence of *xa13* or *xa5* resistance genes.

Kotasthane and Gaikwad (2021) used marker-assisted breeding to transfer three resistance genes (*xa5*, *xa13*, and *Xa21*) from the indica donor IRBB 59 into the high-yielding but susceptible rice cultivar Karma Mahsuri. They employed specific and linked markers for each gene during the breeding process: *xa5* (RG556, RM122, RM390, RM13, xa5R, and xa5S), *xa13* (RG136, RM230, and xa13Pro), and *Xa21* (RM21 and PT248). The results showed that in the Karma Mahsuri X IRBB 59 cross, three lines had all three genes (*xa5*, *xa13*, and *Xa21*), 23 lines had both *xa5* and *xa13*, and only one line possessed *xa5* and *Xa21* together. Additionally, eight lines carried the *xa5* gene, and 17 lines carried the *xa13* gene.

CHAPTER-3

MATERIALS AND METHODS

The present study entitled, "Genetic and Molecular Characterization of Advanced Breeding Lines of Rice for Bacterial Leaf Blight Resistance" was conducted at the Experimental area of Division of Plant Breeding and Genetics, FoA, Chatha during *Kharif* 2022.

The details of materials used and methodology employed for carrying out the study has been embodied in this chapter under different sub headings as below:

3.1 Experimental Material

3.1.1 Plant material: The study utilized experimental materials obtained from the Division of Plant Breeding and Genetics SKUAST Jammu. The material consisted of a total of twenty-nine advanced breeding lines along with 1 check variety (Basmati 370). These samples encompassed a diverse range of bacterial blight resistant genes, morpho-physiological traits, and quality characteristics. The primary objective of the study was to identify germplasm with basmati quality traits and improved resistance to bacterial blight. The study was conducted in Randomized complete Block Design having 3 replications in which 25 days old seedlings were transplanted in 2 row plot of area $2m^2$ with a spacing of 20x15 cm along with 1 check replicated in each replication. All the agronomic and plant protection practices as applicable for commercial basmati crop were adopted. For each genotype, 5 plants were selected for recording various morpho-physiological observations while molecular markers were used to assess the prevalent diversity for bacterial leaf blight (BLB).

Table 3.1: Experimental material used in the present investigation

S. No.	Advanced breeding line	Source	Cross combination
1	ABL-1	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
2	ABL-2	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
3	ABL-3	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
4	ABL-4	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
5	ABL-5	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
6	ABL-6	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
7	ABL-7	PBG, SKUAST-J	BASMATI 370 X PUSA 1612
8	ABL-8	PBG, SKUAST-J	BASMATI 370 X PB-3
9	ABL-9	PBG, SKUAST-J	BASMATI 370 X PB-3
10	ABL-10	PBG, SKUAST-J	BASMATI 370 X PB-3
11	ABL-11	PBG, SKUAST-J	BASMATI 370 X PB-3
12	ABL-12	PBG, SKUAST-J	BASMATI 370 X PB-3
13	ABL-13	PBG, SKUAST-J	BASMATI 370 X PB-3
14	ABL-14	PBG, SKUAST-J	BASMATI 370 X PB-3
15	ABL-15	PBG, SKUAST-J	BASMATI 370 X PUSA BASMATI 1121
16	ABL-16	PBG, SKUAST-J	BASMATI 370 X JAMMU BASMATI 129
17	ABL-17	PBG, SKUAST-J	BASMATI 370 X JAMMU BASMATI 129

18	ABL-18	PBG, SKUAST-J	BASMATI 370 X JAMMU BASMATI 129
19	ABL-19	PBG, SKUAST-J	BASMATI 370 X JAMMU BASMATI 129
20	ABL-20	PBG, SKUAST-J	BASMATI 370 X JAMMU BASMATI 129
21	ABL-21	PBG, SKUAST-J	BASMATI 370 X TAROAI BASMATI
22	ABL-22	PBG, SKUAST-J	BASMATI 370 X TAROAI BASMATI
23	ABL-23	PBG, SKUAST-J	BASMATI 370 X TAROAI BASMATI
24	ABL-24	PBG, SKUAST-J	BASMATI 370 X TAROAI BASMATI
25	ABL-25	PBG, SKUAST-J	BASMATI 370 X TAROAI BASMATI
26	ABL-26	PBG, SKUAST-J	SAANWAL BASMATI X PUSA 2511
27	ABL-27	PBG, SKUAST-J	SAANWAL BASMATI X PUSA 2511
28	ABL-28	PBG, SKUAST-J	SAANWAL BASMATI X PUSA 2511
29	ABL-29	PBG, SKUAST-J	SAANWAL BASMATI X PUSA 2511
30	BASMATI 370 (Check)	PBG, SKUAST-J	It is a traditional variety

3.2 Recording of Observations

3.2.1 Morphological Characters

3.2.1.1 Plant height (cm): At maturity, the height of each plant was determined by measuring from the ground level up to the tip of the spikelet on the main ear, while disregarding any awns present. A measuring scale was used to determine the height in centimetres, with the measurement taken from the soil surface to the top of the spikelet.

3.2.2 Phenological Characters

3.2.2.1 Days to 50 percent flowering (no.): The duration of time from seed sowing to the emergence of 50 per cent of the panicles was recorded as the "Days to 50 per cent flowering".

3.2.2.2 Days to maturity (no.): The number of days taken for the plants to reach maturity was determined by calculating the interval between the date of seed sowing and the date when the colour change that indicates maturity started to appear.

3.2.3 Yield attributing traits

3.2.3.1 1000-grain weight (g): The weight of one thousand grains was determined in grams (g) using a precision weighing balance.

3.2.3.2 Grain yield per plant (g): After harvesting, the panicles were manually threshed, cleaned, dried, and weighed in grams (g) per hill. To calculate the grain yield per plant in grams (g), the average weight of five hills was taken into account.

3.2.3.3 Panicle length (cm): The length of the panicle for each chosen plant was determined in centimetres (cm) by measuring from the base of the panicle to the tip of the topmost spikelet.

3.2.4 Grain quality parameters

Following the seed harvest, the grains of each genotype were dehulled to assess their quality with respect to attributes such as grain size (length), shape (length-to-width ratio), appearance, and aroma. The grains were categorized into different types based on their dimensions, which were measured using a Digimatic Caliper (Mitutoyo) Model CD-8 CSX that has a range of 0-200 mm/0-8inch.

3.2.4.1 Grain length (mm): Random samples of the bulk produce of each entry were taken and the de husked grains were measured. The length of the de husked grains was measured in milli meters (mm) using a vernier scale, from the base of the sterile lemma to the tip of the apiculus of the fertile lemma.

3.2.4.2 Grain breadth (mm): Random samples were taken from the bulk produce of each entry and the dehusked grains were measured. The breadth of the grains was measured in milli meters (mm) using a vernier scale, during the maturity stage.

3.2.4.3 Grain Length/Grain breadth ratio (mm): To determine the length to breadth ratio (L/B) of the grain, the grain length was divided by the grain breadth.

3.2.4.4 Aroma: First, the grains were dehusked. Then, 10 grams of rice grains were taken and placed into petri plates. Next, 10 ml of 1.7 per cent KOH was added to each petri plate and the plates were covered with a lid. After waiting for 10 minutes, a panel of 5 members determined the presence or absence of aroma using a scale of 0, 1, 2, or 3, where 0 represented no aroma and 3 represented a very strong aroma (Sood and Siddiq 1978).

3.3 Screening of the Germplasm for Disease Scoring in Natural Field Conditions

In this study, all rice genotypes were subjected to screening for bacterial blight (BB) under natural field conditions using the leaf clipping technique, (Kauffman *et al*. 1973). Specifically, the leaf clipping technique was employed for the inoculation of rice genotypes with *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*). This method involved the collection of BB-infected leaves, which were subsequently cut into small pieces measuring 2mm or smaller and submerged in water within a flask. The scissor blades were dipped in a suspension for 20-30 minutes prior to clipping the tops of the leaves simultaneously for five randomly selected plants in each entry, with 20-30 leaves grasped in one hand. Scoring was carried out 14 days post-inoculation, in accordance with the Standard Evaluation System for Rice (SES) established by the International Rice Research Institute (IRRI, 1996).

Table 3.2 Scale for BLB disease IRRI,1996

Infection per cent	**Score**	**Host response**
0 per cent	**0**	**Highly resistant (HR)**
>1-10 per cent	**1**	**Resistant(R)**
>10-30 per cent	**3**	**Moderately resistant (MR)**
>30-50 per cent	**5**	**Moderately susceptible (MS)**
>50-75 per cent	**7**	**Susceptible(S)**
>75-100 per cent	**9**	**Highly susceptible (HS)**

3.4 Statistical Analysis

The statistical analysis was applied to the observations that were made for the thirteen morpho-physiological traits.

3.4.1 Analysis of Variance

Data with respect to check variety was subjected to analysis of variance as per randomized block design analysis and is given below:

Table 3.3: Analysis of variance

Source of variation	Degrees of freedom (d.f.)	Mean sum of squares (MSS)	F value
Replications (r)	r-1	Mr	Mr/Me
Germplasm lines(g)	g-1	Mg	Mg/Me
Error	(r-1) (g-1)	Me	
Total	rg-1		

Where,

r: No. of replications

g: No. of germplasm lines

Mr: MSS due to replications

Mg: MSS due to germplasm lines

Me: MSS due to error

3.4.2 Genetic Variability

3.4.2.1 Mean: To obtain the mean value for each character, the total of observations for that character in each replication was divided by the corresponding number of observations. This was calculated using the following formula:

$$\text{Mean } (\overline{X}) = \frac{\sum x}{N}$$

Where,

∑x represents the sum of all observations for each character in each replication, and N represents the corresponding number of observations.

3.4.2.2 Range: It was taken as the difference between the highest and lowest mean value for each character

Range = Xn – X_1

Where,

Xn = Highest mean value of character

X_1 = Lowest mean value of character

3.4.3 Estimation of Variance Components

- **Genotypic Variance**

The inherent variation that is present in the genotype and has no effect of environment is the genotypic variance. It is estimated using the formula suggested by Burton (1952),

$$\sigma^2_g = [(MS_g) - (MS_e)] / r$$

Where,

σ^2_g = genotypic variance

MS_g = Mean sum of squares due to germplasm lines

MS_e = Error mean sum of squares

- **Phenotypic Variance**

Phenotypic variance is the total variance present in the population. It includes both genotypic variance and variance due to environment and calculated as the sum of genotypic and environmental (error) variance.

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where,

$\sigma^2{}_p$ = Phenotypic variance

$\sigma^2{}_g$ = Genotypic variance

$\sigma^2{}_e$ = Error variance

3.4.4 Coefficient of Variation

Coefficient of variation (CV) can be estimated as the per cent ratio of standard deviation of a sample over its mean. The phenotypic coefficient variation and genotypic coefficient variation was estimated by the formula given by Burton (1952).

3.4.4.1 Genotypic Coefficient of Variability (GCV)

GCV= (Genotypic standard deviation / General mean) ×100

GCV= $[(\sigma^2{}_g)^{½}$ / Mean] × 100

3.4.4.2 Phenotypic Coefficient of Variability (PCV)

PCV= (Phenotypic standard deviation/General mean) ×100

PCV = $[(\sigma^2{}_p)^{½}$ / Mean] × 100

3.4.4.3 Heritability

Heritability in broad sense was estimated as the ratio of genotypic to the phenotypic variance and was expressed in percentage.

Heritability= (Genotypic variance/Phenotypic variance) × 100

$h^2 = [\sigma^2{}_g / \sigma^2{}_p] \times 100$

Where,

h^2 = Heritability

$\sigma^2{}_p$ = Phenotypic variance

$\sigma^2{}_g$= Genotypic variance

3.4.4.4 Genetic Advance (GA)

Improvement of mean genotypic value of selected plants over base population refers to genetic gain. The expected genetic advance for the population understudy was estimated using the formula given by Johnson *et al.*, (1955),

Expected genetic advance = $[\sigma^2_g / \sigma^2_p] \times k \times \sigma_p = h^2 \times k \times \sigma_p$

Where,

h^2= Heritability

k= Selection differential (k= 2.06 at 5 per cent selection intensity)

σ_p = Phenotypic standard deviation

σ^2_p = Phenotypic variance

σ^2_g= Genotypic variance

3.4.4.5 Genetic Advance as Per cent of Mean

Genetic advance as per cent of mean was estimated using the formula given by Johnson *et al.* (1955).

$$\text{GAM (per cent)} = \frac{\text{Genetic Advance}}{\text{Mean}} \times 100$$

3.5 Molecular Analysis

In this study molecular analysis was carried out under following different stages.

3.5.1 Genomic DNA Isolation

Genomic DNA was extracted from each genotype using the cetyl tri-methyl ammonium bromide (CTAB) method, (Clarke 2009) with certain modifications. The genotypes were grown at the Experimental Farm of the Division of Plant Breeding and Genetics, SKUAST-J, Chatha for 2-3 weeks, and 15-day-old fresh leaves were used for the extraction of genomic DNA. Specifically, 5g of plant tissue was ground to a fine powder using a pestle and mortar with liquid nitrogen, and the powder was transferred to a 2ml Eppendorf tube containing 800µl of CTAB buffer. The tube was placed in a water bath set at 65˚C and shaken after 10 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the tube, which was then mixed slowly by inverting it for 10 minutes. The samples were then centrifuged at 8,000 rpm for 10 minutes, and the supernatant (upper phase) was transferred to fresh tubes and again treated with chloroform: isoamyl alcohol (24:1), mixed slowly for 10 minutes, and centrifuged. Next, 0.6 volume of chilled isopropanol was added to the supernatant and stored at 4˚C for 1-2 hours. The samples were then centrifuged at 10,000 rpm for 10 minutes at 4˚C, and the supernatant was discarded. The pellets were washed with 70 per cent ethanol, centrifuged at 10,000 rpm for 5 minutes, and air-dried. To dissolve the pellet, 200µl of 1xTE buffer was added and stored at 4˚C. The samples were treated with RNase by adding 2µl of RNase (10mg/ml) and incubating at 37˚C for 1 hour in a water bath. For the purification of gDNA, an equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and mixed gently for 10 minutes, followed by centrifugation at 13,000 rpm for 10 minutes. The supernatant was collected in another tube, and an equal volume of chloroform: isoamyl alcohol was added. The tube was then spun for 10 minutes at 13,000 rpm, and 0.6 volume of ice-chilled pure ethanol was added and kept in the refrigerator for 10 minutes. The samples were then centrifuged at 7,000 rpm for 5 minutes to pellet the DNA. The DNA pellet was washed with 70 per cent ethanol, spun at 7,000 rpm for 5 minutes, air-dried, dissolved in 100µl of 1xTE buffer, and stored at 4˚C for further use.

3.5.2 DNA Quantification

DNA was quantified by using nanodrop and agarose gel electrophoresis.

3.5.2.1 Nanodrop Method

To quantify the extracted DNA, a thermo scientific Nano Drop spectrophotometer was used. Prior to use, the instrument was blanked with DEPC water. A 2µl DNA sample was then placed onto the receiving fibre of the instrument, and the source fibre was brought into contact with the end of the receiving fibre. The reading was then taken, and the concentration (ng/µl) at the OD260/280 value of the sample was recorded. A pure DNA sample should have an OD value between 1.8-2.0, while an OD value above 2 indicates RNA contamination and a value below 1.8 indicates protein contamination.

3.5.2.2 Agarose Gel Electrophoresis

To quantify the DNA, 2µl of DNA was mixed with 2µl of loading dye (Bromophenol blue) and 6µl of ddH2O, and the mixture was loaded into separate wells on a 0.8 per cent agarose gel. To prepare the agarose gel, 0.8 gm of agarose was weighed and added to 100 ml TBE (Tris, Borate EDTA, 1x) buffer, which was heated in a microwave for 3 minutes. After cooling for a few minutes, 6 µl of ethidium bromide was added for visualisation. The gel was poured into the casting tray with combs and allowed to polymerise at room temperature for 20-25 minutes. To estimate the DNA concentration in each sample, DNA ladder was loaded. Electrophoresis was performed at 75V for 1 hour, and the gel was viewed under Biometra gel documentation system. The concentration of DNA was determined by comparing the band intensity with that of the 1000 bp molecular ladder, and the quality of the DNA was indicated by the presence of intact bands.

3.5.3 Reagents and solutions used for DNA extraction

A. Stock solutions for DNA extraction

1. **1M Tris HCl pH 8.0:** To achieve this, 24.22 grams of 1M Trizma with a molecular weight of 121.1 grams/mole was dissolved in 150 milliliters of purified water. The pH was set to 8.0 using 1N NaOH. The total volume of the initial solution was increased to 200 milliliters by introducing additional purified water. Subsequently, this prepared solution underwent autoclaving and was preserved at ambient temperature.
2. **0.5 M EDTA:** In order to formulate this, 37.22 grams of ethyl diamine tetra acetic acid (EDTA), with a molecular weight of 372.24 grams/mole, were dissolved in 150 milliliters of purified water. The pH was tuned to 8.0, and the volume was extended to 200 milliliters. The 0.5 M EDTA solution was subjected to autoclaving and subsequently conserved at ambient temperature (24-26°C)
3. **5 M NaCl:** To made this, 29.22 grams of sodium chloride (NaCl) with a molecular weight of 58.44 grams/mole were dissolved in 100 milliliters of purified water. The mixture underwent autoclaving and was subsequently retained at ambient temperature.
4. **TE Buffer pH 8.0**: The buffer was prepared with the following constituents:

Tris-HCl pH8.0=2.0 ml
EDTA pH8.0=0.4 ml

They were mixed properly and the volume was made upto 200 ml by adding distilled water.

5. **DNA extraction buffer:** The extraction buffer was prepared with the following constituents:

1 M Tris = 15.0ml
0.5 M EDTA = 6.0 ml
5 M NaCl = 42.0 ml
CTAB = 2.0 g

They were mixed and dissolved properly and then the volume was made upto 200 ml by adding distilled water to it. ß-merceptoethanol 0.2 per cent in 100ml of extraction buffer was added freshly and extraction buffer was pre-warmed before use.

6. **TBE Buffer (10X):** It was prepared with the following constituents:

Tris Base = 108.0 g
Boric acid = 55.0 g
0.5 M EDTA = 40.0 ml

They were dissolved properly and the volume was made upto 100 ml of distilled water with final concentration 10X.

7. **Chloroform: Isoamyl alcohol (C: I):** For To create a 100 ml stock solution of C:I, 96 ml of chloroform was meticulously combined with 4 ml of isoamyl alcohol and thoroughly blended.
8. **RNase:** It was formulated by dissolution of 10mg RNase powder in 1ml of 10mM Tris- HCl and 15mM NaCl and heated to 100°C for 15min. It was then cooled slowly to room temperature and stored at - 20°C.
9. **Working stocks of Primers:** The primers were provided in a freeze-dried state. Stock solutions were generated by reconstituting them with double-distilled Mili-Q water, and then, from these stocks, a functional concentration of 10 picomoles for each primer set was created.

3.5.4 SSR amplification

3.5.4.1 Primers Used for DNA Amplification

Two sets of primers (STS and SSR) were optimized to establish the genetic diversity for BLB resistance among genotypes used for present study.

Primer Dilution

To dilute the primers at a working concentration of 10 pmol for each primer set, autoclaved water was used.

3.5.4.2 Components used for PCR reaction

The PCR reaction was conducted in 0.2ml PCR tubes with a 25 µl reaction mixture. The components included 2 µl of template DNA (50ng/ µl), 2.5 µl of 10x PCR buffer with $MgCl_2$, 0.2 mM of each dNTPS (dCTPs, dGTPs, dTTPs, dATPs), 0.1 mM primer concentration, and 5 units of Taq DNA polymerase. The specific quantities of these components used in the reaction can be found in the table.

Table 3.4: Reagents with their concentration and quantity used for single PCR reaction

S. No.	Reagents	Concentration	Quantity
1.	DNA template	50 ng/µl	2 µl
2.	Sterile water		17.4 µl
3.	PCR Buffer	10 X	2.5 µl
4.	dNTPs	2.5mM/ µl	2 µl
5.	Primer	10pmole	1 µl
6.	*Taq* polymerase	5U/ µl	0.1 µl
	Total		**25 µl**

3.5.4.3 PCR Amplification Program

To confirm the presence of resistance genes, previously reported markers RG136 Pta248 RM13 and RG556 which are closely linked to BB resistance genes *xa13, Xa21* and *xa5* respectively were utilized. The specific details of the markers used in the assessment of BLB resistance can be found in the table. PCR reaction was initiated by initial denaturation of DNA at 94 ^{0}C for 2 minutes (for *xa13*) and for 5 minutes (for

Xa21 and *xa5*), followed by 35 cycles of PCR amplification with the following steps: 30 seconds of denaturation at 94^0C, 40 seconds at 58^0C for primer annealing for *xa13*, 30 seconds at 52^0C for *Xa21*, 30 seconds at 55^0C for *xa5* and 30 seconds of primer extension at 72^0C. A final 5-minute incubation at 72^0C was allowed for the completion of primer extensions.

3.5.4.4 Visualization of amplification products

The amplified DNA fragments through SSR primers were resolved through electrophoresis in 2.5 per cent agarose gel in TAE buffer. Ethidium bromide at a final concentration of 0.03 ng / µg was added to the agarose solution.

For electrophoresis, 15 µl of the PCR mixed was added with 2 µl 6X loading dye (0.25 per cent bromophenol blue in 30 per cent glycerol) and loaded on an ethidium bromide stained 2.5 per cent in the slot of agarose gel in 1X TBE buffer to separate the amplified fragments. In order to determine the molecular size of amplified products or compare the molecular weights of amplified products, each gel was also loaded with 1 µg DNA of a 50 bp DNA size marker (Fermentas, USA) in first well of the Agarose gel. Gel electrophoresis was performed at a constant voltage of 65 ± 20 V for about 3.0 to 4.0 hrs. Finally, after electrophoresis the gels were visualized under a UV light source in a photographed gel documentation (Gel-Doc) system (Gel Doc^{TM} XR+, BIO-RAD, USA or Alpha Innotech Corporation, USA) and the images of amplification products were captured and stored in a computer for further analysis and use

3.5.4.5 PCR analysis

The PCR amplification using SSR and STS /gene specific primers was carried out using sterilized thin-walled PCR tubes (0.2 ml). 2 µl of diluted DNA (100ng/µl) of each line was dispensed at the bottom of the PCR tube. Master mix was prepared separately in an epp endorf tube for each primer as described in the Table 3.4 and 8 µl of cocktail was dispensed in each tube. Amplification was carried out in a thermal cycler (Eppendorf) for 35 cycles. The annealing temperature of primers were standardized using the reported temperature of 57°C.

Table 3.5: Markers used for evaluation of bacterial blight genes

Gene	Location on chromosome	Marker	Type of marker	Primer Sequence
xa13	8	RG 136F RG 136R	STS functional marker	5'- TCCCAGAAAGCTACTACAGC-3' 5'-GCAGACTCCAGTTTGACTTC-3'
Xa21	11	pTA 248F pTA 248R	Gene specific STS marker	5'-AGACGCGGAAGGGTGGTTCCCGGA-3' 5'-AGACGCGGTAATCGAAGATGAAA-3'
xa5	5	a) RM13F RM13R b) RG 556F RG556F	Gene specific SSR and STS markers respectively	5'-TCCAACATGGCAAGAGAGAG-3' 5'-GGTGGCATTCGATTCCAG-3' 5'-TAGCTGCTGCCGTGCTGTGC-3' 5'-AATATTTCAGTGTGCATCTC-3'

CHAPTER-4

RESULTS

The present investigation entitled “Genetic and Molecular Characterization of Advanced Breeding Lines of rice for Bacterial Leaf Blight Resistance” comprised of 30 lines collected and maintained at Division of PBG SKUAST Jammu having different BB resistance genes with the aim of identifying lines having basmati quality traits with enhanced resistance to bacterial blight during crop season *Kharif* 2022. The research was conducted with an aim to screen the various advanced breeding lines for yield and bacterial leaf blight resistance using morphological and molecular markers.

The presence of an adequate amount of variability is essential when selecting lines for further manipulation. Therefore, in the current investigation, the germplasm lines were evaluated to determine their yield performance and characterized based on their morphological, phonological, grain quality features, and the presence of the bacterial blight resistance genes. The morphological, phenological, and yield components that were assessed included plant height (PH), flowering days (number of days to reach 50 per cent flowering), panicle length, days to maturity, grain yield per plant, and plot yield. Additionally, the quality traits such as kernal length (KL), kernal breadth (KB), kernal length/breadth ratio (L/B), and aroma were also analysed. The quantitative data obtained from these assessments were subjected to statistical analysis using the Statistical Analysis System (SAS) to draw meaningful conclusions.

The experimental results of the present study have been presented below:

4.1 Analysis of variance

4.1.1 Morphological, phonological and yield components

4.1.1.1 Plant height: The analysis of variance (Table 4.1) revealed a significant variation in plant height among the various lines. The plant height varied between 103.86 cm and 159.42 cm. Among the lines, ABL-6 exhibited the shortest plant height, while ABL-26 had the tallest plant height, with an average height of 135.73 cm.

4.1.1.2 Days to 50 per cent flowering: The analysis of variance revealed a significant amount of genetic variation among the different genotypes in terms of the time it took for 50% flowering to occur (Table 4.1). The range for the days to 50% flowering was between 116 and 132 days. Among the lines, ABL-19 had the shortest flowering time, taking only 116.67 days. On the other hand, ABL-26 and ABL-27 had the longest flowering time of 132 days, with an average of 124.93 days.

4.1.1.3 Days to maturity: The number of days to reach maturity is crucial, particularly in basmati varieties. Cultivars that mature early allow for earlier preparation of the land for the subsequent crop and also reduce vulnerability to insect pests. Substantial variability was observed among the genotypes tested in terms of the duration needed to reach maturity (Table 4.1). The maturity period ranged from 158 to 171 days. ABL-10 exhibited the shortest maturity period, whereas ABL14 had the longest, with an average of 163.37 days.

4.1.1.4 1000 grain weight: The variation in 1000 grain weight among different types of rice genotypes was found to be substantial, as indicated (Table 4.1). The weight of one thousand grains ranged from 18.00 to 29.06 grams. The breeding line ABL-6 had the lowest recorded weight for 1000 grains, whereas ABL-19 exhibited the highest weight, with an average of 23.26 grams.

4.1.1.5 Panicle length: The average measurements for panicle length are provided (Table 4.2). The analysis in Table 4.1 demonstrates that there were notable genetic distinctions among the various rice genotypes for this particular trait. Panicle length varied from 23.28 to 34.79 centimetres, with ALB-18 having the shortest recorded length (23.28) and ABL-28 having the longest (34.79), resulting in an overall average of 28.42 centimetres.

4.1.1.6 Grain yield per plant: The analysis of variance revealed significant genetic variation among the genotypes for grain yield per plant, as presented in Table 4.1. Grain yield per plant ranged from 10.93 to 25.90 grams, as shown (Table 4.2). The breeding line ABL-3 had the lowest recorded grain yield per plant, while ABL-14 had the highest, resulting in an overall average of 17.95 grams.

4.2 Genetic parameters estimated in the present study

The genetic parameters, comprising genotypic and phenotypic variances, genotypic and phenotypic coefficients of variance, broad-sense heritability, genetic advance, and genetic advance as a percentage of the mean, were calculated and are presented in Table 4.3. A comprehensive overview of these parameters is provided under the subsequent headings.

Table 4.1: Analysis of variance for yield and yield attributing traits

Source of variation	Df	Number of days to 50% flowering	Plant height (cm)	Total no. of tillers per plant	No of effective tillers per plant	Days to maturity	Panicle length (cm)	1000 grain weight (g)	Kernal length (mm)	Kernal breadth (mm)	Kernal L/B ratio	Yield per plant (g)
Replications	2	0.58	304.63	7.46	3.94	3.90	53.77	5.59	0.22	0.04	0.38	5.89
Treatments	29	55.09*	755.12*	41.05*	39.04*	32.93*	20.24*	27.65*	2.34 *	0.03*	0.62*	55.16*
Error	58	6.30	111.58	2.73	1.42	4.28	1.55	2.69	0.28	0.01	0.15	2.92

* represents significance level at 5 per cent.

Table 4.2: Mean performance of advanced breeding lines of rice for yield and yield related traits

Advanced breeding lines	Number of days to 50% flowering	Plant height (cm)	Total no. of tillers per plant	No of effective tillers per plant	Days to maturity	Panicle length (cm)	1000 grain weight	Kernal length (mm)	Kernal breadth (mm)	Kernal L/B ratio	Yield per plant (g)	Aroma (0-3)
ABL-1	120.33	106.40	9.87	7.80	161.00	27.28	23.90	7.24	1.45	4.80	16.73	2
ABL-2	129.33	133.42	15.20	13.00	165.33	30.76	20.85	6.31	1.75	3.65	25.60	2
ABL-3	126.33	116.42	12.20	9.60	162.00	26.54	20.60	8.50	1.47	5.80	10.93	2
ABL-4	117.00	127.37	20.40	17.80	162.00	28.06	19.70	6.61	1.73	4.02	15.05	2
ABL-5	126.00	119.35	15.00	12.00	163.00	28.14	25.67	8.91	1.73	5.16	12.00	2
ABL-6	126.33	103.86	13.00	9.20	169.67	33.52	18.00	8.04	1.59	4.40	21.81	2
ABL-7	128.00	116.81	20.80	17.80	159.67	23.88	18.57	7.61	1.59	4.77	15.51	2
ABL-8	126.00	132.30	16.80	14.00	164.00	27.19	19.88	7.68	1.57	4.88	13.40	2
ABL-9	127.00	134.26	17.80	14.20	164.33	28.66	23.48	8.13	1.74	4.68	13.49	2
ABL-10	120.00	125.10	9.40	7.00	158.00	30.72	22.15	8.49	1.75	4.84	19.64	2
ABL-11	125.33	132.57	13.00	9.60	165.33	32.52	22.80	8.00	1.62	4.95	17.00	2
ABL-12	125.00	132.55	14.80	12.20	160.00	26.80	23.36	7.94	1.74	4.57	13.54	2
ABL-13	126.00	134.25	12.20	9.00	160.33	30.56	23.62	7.91	1.62	4.89	19.36	2
ABL-14	127.33	134.25	10.80	8.20	171.00	30.82	26.80	7.76	1.69	4.33	25.90	2
ABL-15	120.33	139.41	17.00	13.40	161.33	27.70	27.10	8.17	1.68	5.18	19.78	3

ABL-16	124.67	137.32	14.20	10.40	159.00	30.90	20.94	7.92	1.66	4.76	22.79	2
ABL-17	118.33	141.40	20.80	17.60	158.33	25.49	21.80	7.69	1.58	4.85	20.20	2
ABL-18	120.67	134.03	21.15	17.80	159.67	23.28	20.80	7.51	1.65	4.56	11.23	2
ABL-19	116.67	134.69	20.80	17.80	162.33	24.62	29.06	7.80	1.89	4.12	21.60	2
ABL-20	118.67	142.70	15.60	13.00	162.67	30.34	28.25	7.44	1.83	4.06	15.23	2
ABL-22	127.33	143.41	14.60	11.80	167.33	24.91	24.55	7.65	1.70	4.70	23.20	2
ABL-22	126.00	155.74	14.60	11.80	161.33	28.78	22.41	8.03	1.64	4.69	14.74	2
ABL-23	130.00	146.89	12.60	9.60	168.67	34.79	25.75	7.51	1.66	4.53	19.86	2
ABL-24	124.67	154.92	13.00	10.00	164.33	33.84	19.66	7.95	1.58	4.65	20.78	2
ABL-25	122.67	106.78	15.40	12.00	164.00	23.46	23.33	7.47	1.63	4.01	19.90	2
ABL-26	132.00	159.42	16.67	14.20	167.67	28.84	22.01	5.61	1.69	4.64	24.91	2
ABL-27	132.00	157.98	15.80	12.80	165.00	27.72	27.32	7.97	1.74	4.49	19.47	2
ABL-28	130.33	155.61	23.20	19.20	165.00	26.15	29.00	6.94	1.70	3.94	14.53	2
ABL-29	129.67	155.22	21.80	19.80	164.33	28.12	22.51	5.09	1.70	4.56	14.61	2
Basmati370 (Check)	124.00	157.53	13.40	10.93	164.33	28.18	23.78	5.55	1.57	3.78	15.59	3
MEAN	**124.93**	**135.73**	**15.73**	**12.78**	**163.37**	**28.42**	**23.26**	**7.51**	**1.67**	**4.58**	**17.95**	
CV	**2.01**	**8.106**	**10.49**	**9.34**	**1.27**	**6.45**	**7.05**	**7.04**	**7.51**	**8.44**	**9.53**	
CD	**4.11**	**17.97**	**2.70**	**1.96**	**3.39**	**3.00**	**2.69**	**0.87**	**0.20**	**0.63**	**2.80**	

Table 4.3: Estimates of genetic parameters among advance breeding lines of rice

Characters	Genotypic variance (σ^2_g)	Phenotypic variance (σ^2_p)	Genotypic coefficient of variance (per cent)	phenotypic coefficient of variance per cent)	Heritability (bs) (per cent)	Genetic advance	Genetic advance as percent of mean (Per cent)
Days to 50 per cent flowering	16.26	22.96	3.21	3.84	69.96	6.90	5.53
Plant height (cm)	214.51	326.09	10.79	13.30	65.78	24.47	18.03
Total number of tillers per plant	12.77	15.50	22.72	25.03	82.42	6.68	42.49
Number of effective tillers per plant	12.54	13.96	27.69	29.23	89.79	6.91	54.07
Days to maturity	9.55	13.83	1.89	2.28	69.05	0.69	3.24
Panicle length (cm)	8.07	11.43	9.99	11.89	70.57	4.91	17.29
1000 grain weight (g)	8.32	11.01	12.40	14.27	75.57	5.16	22.21
Kernal length	0.69	0.97	11.03	13.09	70.99	1.44	19.14
Kernal breadth	0.0037	0.02	3.6528	8.36	19.07	0.05	3.28
Kernal L/B ratio	0.16	0.30	8.64	12.07	51.18	0.58	12.73
Grain yield per plant (g)	17.41	20.34	23.25	25.13	85.62	7.95	44.32

4.2.1 Phenotypic and genotypic variance

The range of phenotypic variance was found to be between 0.02 and 326.09, whereas the range of genotypic variance was observed to be between 0.0037 and 214.51. Among the traits studied, plant height exhibited the highest phenotypic and genotypic variance, followed by the number of days to 50 per cent flowering, grain yield per plant, total number of tillers per plant, number of effective tillers per plant, number of days to maturity, panicle length, 1000 grain weight, kernel length, and length/breadth ratio. Conversely, the least variance was observed in kernel breadth.

4.2.2 Genotypic coefficient of variance

The genotypic coefficient of variance ranged from 1.89 to 27.69 per cent. The highest genotypic coefficient of variance was observed for the number of effective tillers per plant, followed by grain yield per plant, total number of tillers per plant, 1000 grains weight, kernel length, plant height, panicle length, length/breadth ratio, kernel breadth, and days to 50 per cent flowering. While, the least genotypic coefficient of variance was observed for days to maturity.

4.2.3 Phenotypic coefficient of variance

Phenotypic: The phenotypic coefficient of variance ranged from 29.23 per cent to 2.28 per cent. The highest phenotypic coefficient of variance was observed for the number of effective tillers per plant, followed by grain yield per plant, total number of tillers per plant, 1000 grain weight, plant height, kernel length, length/breadth ratio of the kernel, panicle length, kernel breadth, and days to 50 per cent flowering. On the other hand, the lowest phenotypic coefficient of variance was observed for the number of days to maturity.

4.2.4 Heritability (bs)

The examined characters showed heritability ranging from 19.07 per cent to 89.79 per cent. The highest per cent heritability was observed in no. of effective tillers per plant followed by grain yield per plant, total no. of tillers per plant, 1000 grain weight, kernel length, panicle length, days to 50% flowering, number of days to maturity, plant height, kernel L/B ratio, while lowest heritability was observed in kernel breadth.

4.2.5 Genetic advance

Genetic advance was observed to be ranging from 0.05 to 24.47. The highest estimate of genetic advance was observed in plant height followed by grain yield per plant, no of effective tillers per plant, days to 50 per cent flowering, total number of tillers per plant, 1000 grain weight, panicle length, kernel length, number of days to maturity, kernel L/B ratio, while least was observed in case of kernel breadth.

4.2.6 Genetic advance as per cent of mean

Genetic advance as per cent of mean was observed to be ranging from 3.24 to 54.07. The highest genetic advance as percentage mean was observed in number of effective tillers per plant followed by total number of tillers per plant, grain yield per plant,1000 grain weight, kernel length, plant height, panicle length, kernel L/B ratio, days to 50 % flowering, kernel breadth while least was observed in number of days to maturity.

4.3 Basmati quality parameters

Mean values of various quality parameters are presented (Table 4.2). The results obtained are detailed as under

4.3.1 Kernal length: Grain length holds great importance, particularly in basmati cultivars, where the Government of India has established a minimum acceptable range for export purposes. Upon examination, considerable variation was observed among the genotypes tested for grain length, as indicated (Table 4.1). The range of grain length spanned from 5.09 to 8.91 centimetres. The breeding line ABL-29 exhibited the shortest grain length, while ABL-5 demonstrated the longest grain length, resulting in an average of 7.51 centimetres.

4.3.2 Kernal breadth: Grain breadth also plays a significant role in the basmati cultivars especially in deciding the grain type. Significant variability was found among the tested lines for kernel breadth (Table 4.2). It ranged between 1.45-1.89 cm. Minimum grain breadth was found for ABL-1 while, ABL-19 recorded maximum grain breadth with an overall mean of 1.67 cm.

4.3.3 Kernal Length/ Kernal Breadth ratio: Grain types are categorized as long slender, short slender, medium slender, long bold, and short bold based on grain length and length breadth ratio. Significant variation was observed among the tested genotypes for the length breadth ratio, as indicated (Table 4.1). The length breadth ratio ranged from 3.65 to 5.80 centimetres. ABL-2 exhibited the minimum length breadth ratio, while ABL3 demonstrated the maximum, resulting in an overall average of 4.58 centimetres.

4.3.4 Aroma: Basmati rice is known for its unique flavour reminiscent of pandan leaves (Pandanus amaryllifolius), which is attributed to the presence of 2-acetyl-1-pyrroline, an aromatic compound. Basmati grains naturally contain approximately 0.09 parts per million (ppm) of this aromatic compound, which is about 12 times higher than that found in non-basmati rice varieties. This high concentration contributes to the distinct spicy fragrance and flavour of basmati rice. Among the genotypes examined, ABL-1, ABL-2, ABL-3, ABL- 4, ABL -5, and several others exhibited a strong aroma, while ABL-15 and Basmati 370, possessed a very strong aroma, presented in Table 4.2.

4.4 Screening of the Advanced Breeding Lines for BLB Under Natural Field Conditions

Disease scoring serves as a valuable tool for evaluating the extent of disease. It involves assessing the severity of lesions through a comprehensive evaluation. By employing scoring systems, disease severity can be quantified in a consistent and objective manner. These scoring systems aim to establish standardized values that accurately represent the severity of the disease. The quantification of disease severity enables comparisons between different patients and within the same patient over time, facilitating the evaluation of the efficacy of various treatment approaches. It is important to note that the accuracy of scoring systems may vary depending not only on the scoring system employed but also on the specific disease being evaluated.

Disease scoring was conducted under natural field conditions. The highest disease severity was observed in ABL-26, Basmati 370, and in advanced breeding lines, ABL-18, ABL-19, ABL-20, ABL-1, ABL-3, ABL-7, ABL-12, ABL-13, ABL-14, ABL-15, ABL-16, ABL-17, ABL-21, ABL-22, ABL-23, ABL-24, ABL-25, ABL-27, ABL-28, ABL-29 with respective disease scores of 9,7, 7, 7, 7, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, and 5 respectively.

4.5 Marker Assisted Selection

4.5.1 Screening of the germplasm lines for bacterial blight resistance

In this study, the genetic diversity of 29 advanced breeding lines, along with 1 check was assessed using PCR-based STS and SSR markers. The genotypes were screened using gene specific primers, RG136, RG556 (*xa*13), RM13(*xa*5) and pTA248 (*Xa*21) respectively. Genomic DNA extraction was performed for molecular characterization of the germplasm. Subsequently, PCR amplification was conducted using the four markers. The bands specific to the target genes (*xa13, xa5* and *Xa21*) were analysed and recorded. Detailed results from the primer analysis of all the advanced breeding lines are presented below:

Among these advanced breeding lines, 4 lines were found to have both *Xa21 and xa13* genes with band size 950 bp for *xa21*gene (Plate 1) and with band size of 1000 bp for *xa13* gene (Plate 2), 3 advanced breeding lines were found to have both *xa13* and *xa5* genes with band size of 1000bp for *xa13* gene (plate 2) and with band size of 1500bp (RG136) and 150 bp(RM13) for *xa5* gene respectively (Plate 2 and 3). While 16 lines were found to have only *xa*13 gene with band size of 1000 bp (plate 2). The distribution of *Xa21/xa5/xa13* combinations is presented in table 4.6. ABL-1, 3, 4, 7, 12, 13, 14, 15, 21,22,23, 24, 25, 27, 28, and 29 possessed only one gene i.e., *xa13*, whereas ABL-8, 9, 10 and11 possessed both *Xa21* and *xa13* resistance genes and ABL-2, 5, 6, possesses a combination of *xa5* and *xa21* genes. The ABL-16, 17, 18, 19, 20, 26 and Basmati 370 lack all the resistance genes i.e., *Xa21*, *xa13* and *xa5*.

Table 4.4: Phenotypic screening of advanced breeding lines of rice for bacterial leaf blight resistance

S. No	Advanced breeding line	Score	Disease reaction
1	ABL-1	5	Moderately susceptible (MS)
2	ABL-2	3	Moderately Resistant (MR)
3	ABL-3	5	Moderately susceptible (MS)
4	ABL-4	3	Moderately resistant (MR)
5	ABL-5	3	Moderately resistant (MR)
6	ABL-6	3	Moderately resistant (MR)
7	ABL-7	5	Moderately susceptible (MS)
8	ABL-8	3	Moderately Resistant (MR)
9	ABL-9	3	Moderately Resistant (MR)
10	ABL-10	3	Moderately Resistant (MR)
11	ABL-11	3	Moderately Resistant (MR)
12	ABL-12	5	Moderately susceptible (MS)
13	ABL-13	5	Moderately susceptible (MS)
14	ABL-14	5	Moderately susceptible (MS)
15	ABL-15	5	Moderately susceptible (MS)
16	ABL-16	5	Moderately susceptible (MS)
17	ABL-17	5	Moderately susceptible (MS)
18	ABL-18	7	Susceptible(S)
19	ABL-19	7	Susceptible (S)
20	ABL-20	7	Susceptible (S)
21	ABL-21	5	Moderately susceptible (MS)
22	ABL-22	5	Moderately susceptible (MS)
23	ABL-23	5	Moderately susceptible (MS)
24	ABL-24	5	Moderately susceptible(MS)
25	ABL-25	5	Moderately susceptible (MS)
26	ABL-26	9	Highly susceptible (HS)
27	ABL-27	5	Moderately susceptible (MS)
28	ABL-28	5	Moderately susceptible (MS)
29	ABL-29	5	Moderately susceptible (MS)
30	Basmati 370(check)	7	Susceptible (S)

Table 4.5: Details of excepted product size obtained using SSR/ STS markers in advanced breeding lines of rice

S. No.	Marker	Associated Gene	Expected Product size(bp)	Expected product size amplified in rice genotypes
1	Pta248	*Xa21*	950	ABL-8, ABL-9, ABL-10, ABL-11
2	RG-136	*Xa13*	1000	ABL-1, ABL-2, ABL-3, ABL-4, ABL-5, ABL-6, ABL-7, ABL-8, ABL-9, ABL-10, ABL-11, ABL-12, ABL-13, ABL-14, ABL-15, ABL-21, ABL-22, ABL-23, ABL-24, ABL-25, ABL-27, ABL-28, ABL-29
3	RM-13	*Xa5*	150	ABL-2, ABL-5, ABL-6,
4	RG-556	*Xa5*	1500	ABL-2

Table 4.6: Genotyping of advanced breeding lines of rice for presence /absence of genes

S. No.	Genotypes	Pta-248 (*XA21*)	RG-136 (*xa13*)	RM-13 (*xa*5)	RG556 (*xa*5)	No. of genes present
1	ABL-1	Absent	Present	Absent	Absent	1
2	ABL-2	Absent	Present	Present	Present	2
3	ABL-3	Absent	Present	Absent	Absent	1
4	ABL-4	Absent	Present	Absent	Absent	1
5	ABL-5	Absent	Present	Present	Absent	2
6	ABL-6	Absent	Present	Present	Absent	2
7	ABL-7	Absent	Present	Absent	Absent	1
8	ABL-8	Present	Present	Absent	Absent	2
9	ABL-9	Present	Present	Absent	Absent	2
10	ABL-10	Present	Present	Absent	Absent	2
11	ABL-11	Present	Present	Absent	Absent	2
12	ABL-12	Absent	Present	Absent	Absent	1
13	ABL-13	Absent	Present	Absent	Absent	1
14	ABL-14	Absent	Present	Absent	Absent	1
15	ABL-15	Absent	Present	Absent	Absent	1
16	ABL-16	Absent	Absent	Absent	Absent	0
17	ABL-17	Absent	Absent	Absent	Absent	0
18	ABL-18	Absent	Absent	Absent	Absent	0
19	ABL-19	Absent	Absent	Absent	Absent	0
20	ABL-20	Absent	Absent	Absent	Absent	0
21	ABL-21	Absent	Present	Absent	Absent	1
22	ABL-22	Absent	Present	Absent	Absent	1
23	ABL-23	Absent	Present	Absent	Absent	1
24	ABL-24	Absent	Present	Absent	Absent	1
25	ABL-25	Absent	Present	Absent	Absent	1
26	ABL-26	Absent	Absent	Absent	Absent	0
27	ABL-27	Absent	Present	Absent	Absent	1
28	ABL-28	Absent	Present	Absent	Absent	1
29	ABL-29	Absent	Present	Absent	Absent	1
30	Basmati370 (check)	Absent	Absent	Absent	Absent	0

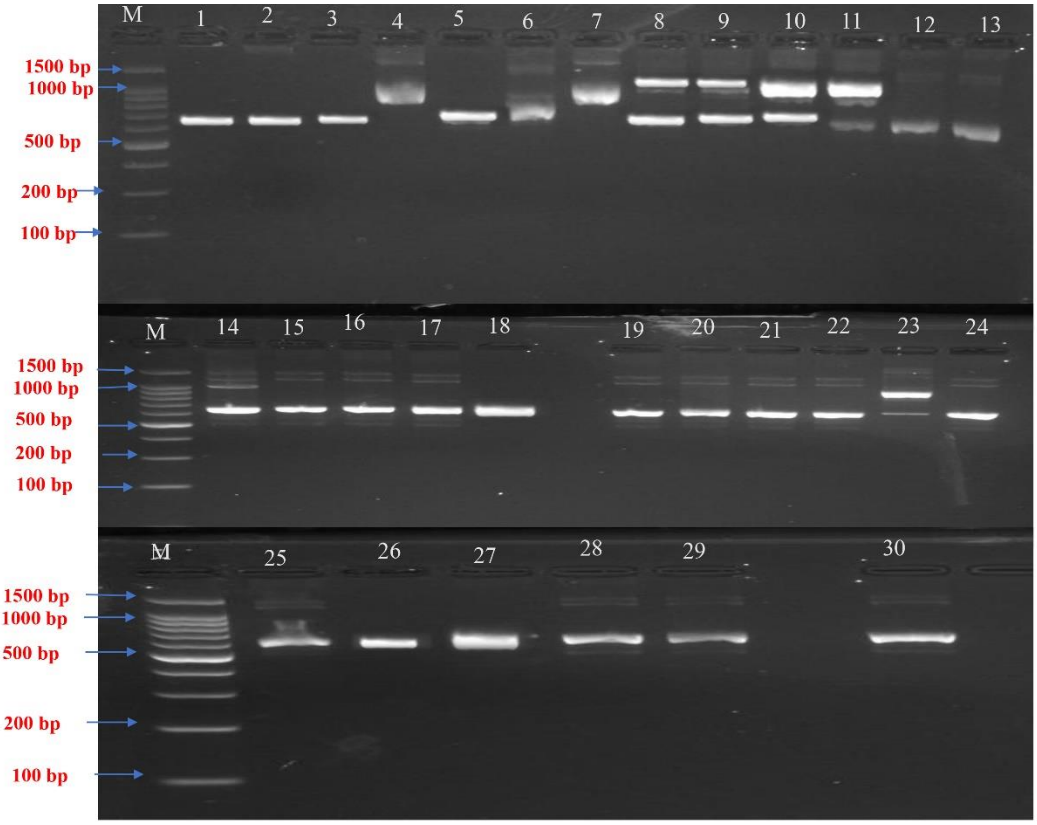

Plate 4.1: PCR amplification of *xa21F*, *xa21R* primer (pTA 248)

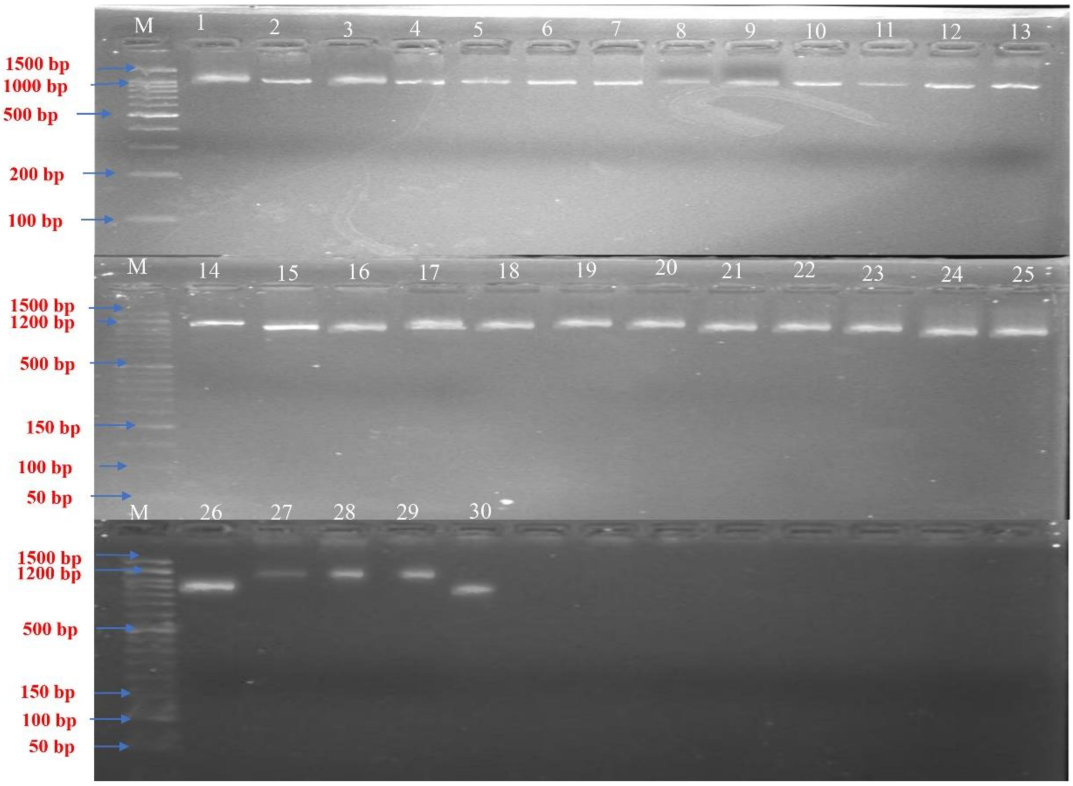

Plate 4.2: PCR amplification of *xa13*F, *xa13*R primer (RG 136)

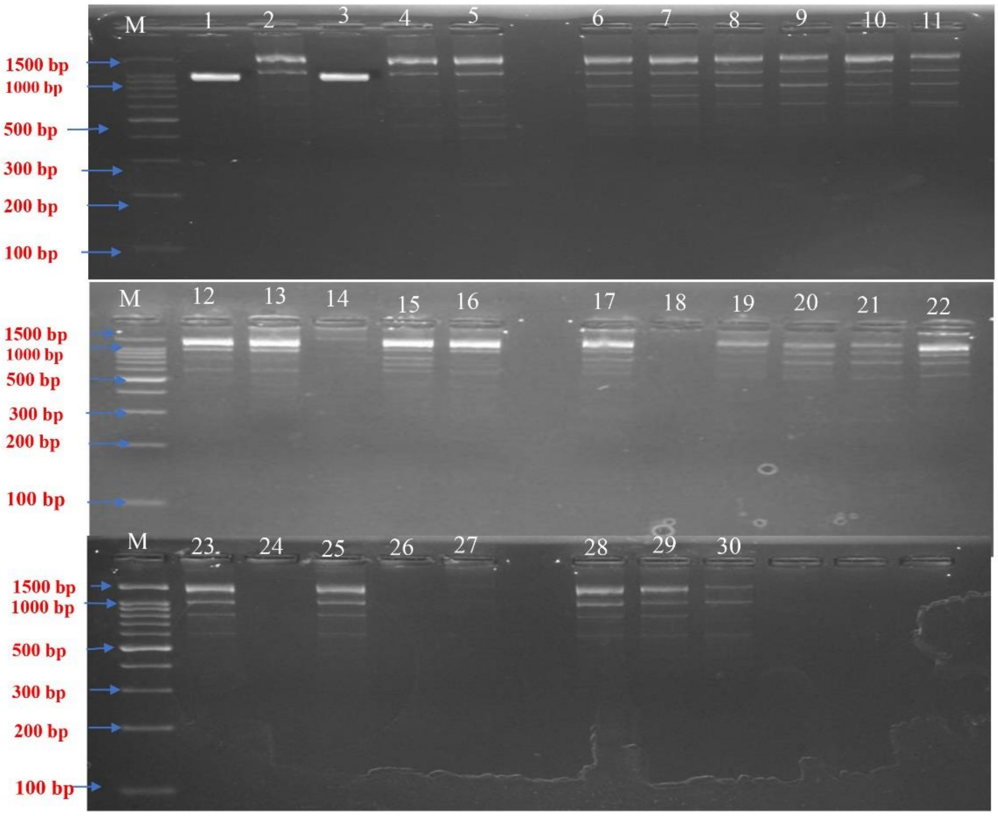

Plate 4.3: PCR amplification of *xa5F*, *xa5* R primer (RG 556)

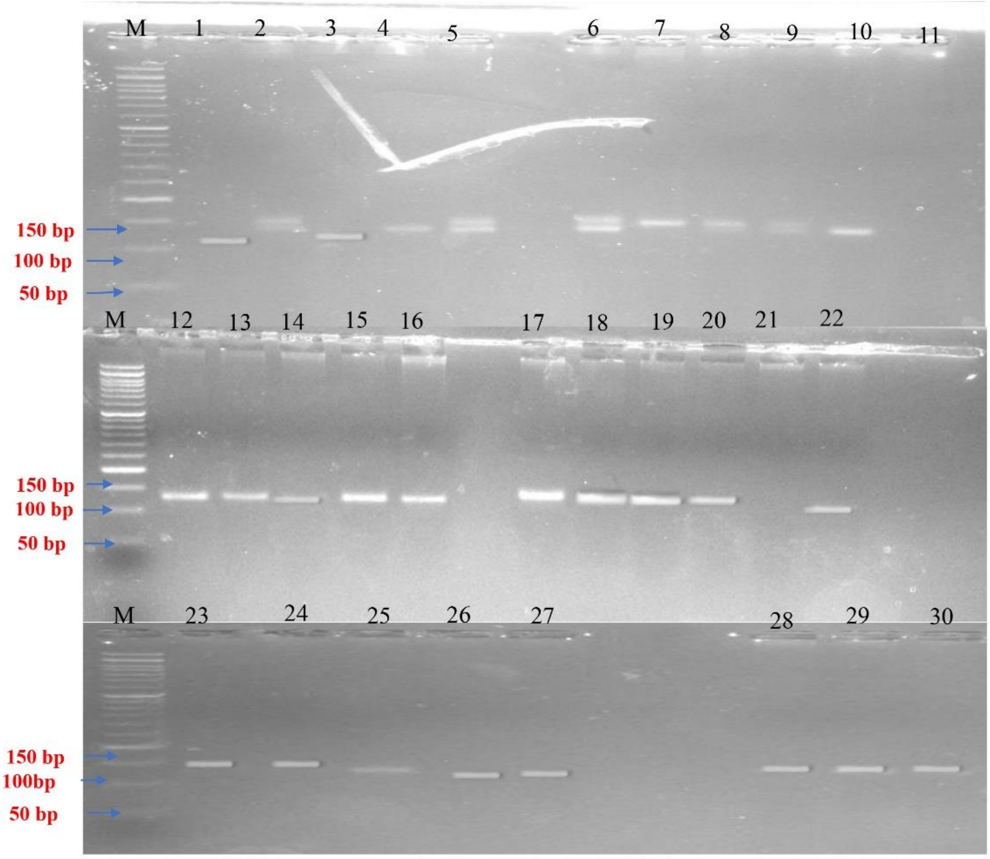

Plate 4.4: PCR amplification of *xa5F*, *xa5* R primer (RM 13)

CHAPTER-5

DISCUSSION

Basmati rice stands as a natural treasure bestowed upon the Indian subcontinent, cultivated by farmers for centuries. These scented grains have earned a distinguished position in both domestic and international markets, commanding a premium due to their exceptional characteristics. However, traditional basmati rice cultivars, while revered for their quality, also exhibit certain limitations and agronomically less desirable traits that result in reduced yield potential. Consequently, it becomes imperative to conduct thorough studies for the characterization of quality attributes in basmati rice cultivars. Such research endeavours are essential to enhance rice quality and cater to the ever-evolving demands of consumers worldwide. One of the significant challenges faced by rice growers is Bacterial blight (BB), a highly consequential disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This detrimental condition leads to leaf wilting, disrupts photosynthesis, and ultimately leads to considerable yield reductions of approximately 20-30% (Ou, 1985) and up to as high as 80% (Singh *et al*., 1997) and even up to 100% under very severe conditions (Zhai and Zhu, 1999). Due to the systemic nature of the disease, there are currently no viable methods to safeguard rice varieties other than focusing on the development of resistant cultivars (Sundram *et al*., 2008). A significant number of Indian Basmati varieties is susceptible to this disease. However, due to the limited availability of resistance sources within the Basmati gene pool, attempts to genetically enhance the cultivars through recombination breeding with solely Basmati varieties have not proven feasible. Consequently, there arises a necessity to identify alternative sources housing resistance genes and introduce them into locally adapted cultivars. Nevertheless, transferring a single resistance gene is unlikely to be successful, as the extensive and prolonged cultivation of such resistant varieties has led to notable shifts in the pathogen's virulence pattern, ultimately resulting in the breakdown of resistance (Mew *et al*., 1992). Gene transfer can be effectively utilized by employing hybridization techniques among the identified lines. This facilitates the assessment of genetic variability and characterization, leading to the development and deployment of superior rice cultivars (Kumar *et al*., 2013). The implementation of rice cultivars with multiple BB resistance genes is anticipated to confer more enduring resistance. Incorporating more than one gene while preserving the aroma in Basmati poses challenges, especially if the genes are recessive, necessitating multiple rounds of progeny testing. Nevertheless, both phenotypic and biochemical characterizations suffer from environmental constraints, labour-intensive requirements, and limitations in terms of quantity and timing. In contrast, DNA-based molecular markers offer widespread applicability, reproducibility, stability, and a high level of reliability (Ford-Lloyd *et al*., 1997, Virk *et al*., 2000, and Song *et al*., 2003). Marker-assisted selection is widely recognized as an efficient breeding method, valued for its swift identification of target genes. Conventional breeding alone often faces challenges in successfully achieving gene pyramiding due to linkages with undesirable traits. Among various DNA markers, microsatellite or simple sequence repeats (SSR) markers are considered highly suitable for their multiallelic nature, high reproducibility, co-dominant inheritance, abundance, and broad genome coverage. A large number of SSR markers have been developed and mapped in rice. (Temnykh *et al*., 2000, McCouch *et al*., 2002), which vary in the degree of polymorphism. The advent of molecular markers has introduced novel approaches for detecting molecular-level variations. In this context, molecular markers linked to target genes prove valuable in identifying lines carrying bacterial blight resistant genes, while preserving aroma and other quality traits. These identified lines hold promise as potential donors for future Basmati breeding programs, exhibiting a broader spectrum and higher level of resistance at all stages of plant growth. A systematic study and characterization of high-quality germplasm become imperative not only for selecting appropriate attribute-based donors but also for safeguarding the uniqueness of rice in the current era. The current study, titled “Genetic and Molecular Characterization of Advanced Breeding Lines of Rice for Bacterial leaf Blight Resistance”, aimed to identify genotypes with favourable qualitative and quantitative traits inherent to Basmati rice, while also possessing enhanced resistance against bacterial blight. The findings of this research are presented in comparison to the work conducted by various scientists and breeders, discussed under the following sections:

5.1 Morphological, Phenological and Yield Components

The presence of genetic variability in a crop is a fundamental requirement for enhancing desirable traits, overall productivity, and production. This variability is often established through hybridization, and its assessment is vital in segregating generations to select favourable segregants. Kikkawa (1912) classified rice varieties based on agronomical and physiological characteristics. Analysis of the mean values of yield and quality traits among different genotypes, along with the mean sum of squares, reveals the existence of significant genetic variability that holds promise for utilization in rice improvement programmes. An analysis of variance was conducted on various morphological traits, encompassing 29 advanced breeding lines and one check variety. The results revealed significant differences among all the traits concerning plant height, days to 50% flowering, days to maturity, panicle length, yield per plant, 1000-grain weight, grain length, grain breadth, and length-breadth ratio. Previous studies by Kumar *et al.* (1999), Yadav *et al.* (2009), and Nascimento *et al.* (2011) also reported similar findings. Mulugeta and Firew (2015) also documented variations in genotypes concerning days to 50% flowering. The panicle length in the genotypes exhibited a range from very short (<11 cm) to very long (~40 cm), according to the classification by Biodiversity International. These findings reveal a wide range of panicle length variability among the genotypes. Similar observations have been reported by Bhat *et al.* (2015).

The 1000 grain weight ranged between 18.00 g to 29.06 g, this is in agreement with the results obtained by Karim *et al.* (2014) in which, they reported a range of mean values for 1000 grain weight from 5.9 g to 30.72 g.

5.2 Basmati quality parameters

The average values for quality traits revealed that grain length varied from 5.09 mm to 8.91 mm, with an overall mean of 7.51 mm. Similarly, grain breadth ranged between 1.45 mm to 1.89 mm, with a general mean of 1.67 mm. The length-to-breadth ratio spanned from 3.65 to 5.80, with an average value of 4.58. ABL-15, Basmati 370 expressed strong aroma while ABL-1, ABL-2, ABL-3, ABL-4, ABL-5 and all other lines expressed mild aroma.

5.3 Molecular Markers for BB Resistance

Basmati rice is an exquisite combination of desirable kernel dimensions, alluring aroma, significant linear kernel elongation with minimal breadth-wise swelling upon cooking, fluffy texture, delectability, easy digestibility, and extended shelf-life, making it highly valuable in the commercial market. However, traditional basmati cultivars have some drawbacks, such as tall stature, susceptibility to lodging, sensitivity to photoperiod and temperature, low yield potential, and high vulnerability to bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). BB is a significant disease that leads to substantial yield losses in rice and is prevalent in various rice-growing states of India. Furthermore, this disease severely affects the grain and cooking quality of basmati rice. To address these challenges, researchers have identified and characterized several BB resistance genes in non-aromatic rice varieties. These genes have been incorporated and combined using Marker-Assisted Selection (MAS) techniques to develop resistant cultivars (Perumalsamy *et al*., 2010; Rajpurohit *et al*., 2010). However, integrating these BB-resistance genes into the basmati rice background negatively affects the superb grain quality and unique aroma of basmati rice. A more effective approach to combat BB disease without compromising the aroma and grain quality of basmati rice is to identify and utilize genes conferring BB-resistance from basmati to high-yielding susceptible commercial basmati cultivars. This strategy will also contribute to expanding the genetic diversity of Basmati varieties.

Various gene pyramiding programmes for BB resistance have reported an increased level of resistance in pyramid lines, leading to reduced lesion length (Huang *et al*., 1997; Sanchez *et al*., 2000; Singh *et al*., 2001; Sundaram *et al*., 2008). However, certain two-gene pyramid lines showed an increased lesion length after 21 days of inoculation, consistent with the findings of Sundaram *et al*. (2008). This suggests that a "critical mass" of genetic resistance might be inherent in such systems, indicating the significance of employing more than two genes to achieve durable resistance against pathogens like *Xoo*. The higher resistance levels observed could be attributed to gene interactions or quantitative complementation between the resistant genes (Huang *et al*., 1997; Sanchez *et al*., 2000). In the current study, breeding lines carrying both *xa5* and *Xa21* or *xa13* and *xa5* genes predominantly displayed disease scores of “3”, whereas those carrying only one gene (either *xa5*, *Xa21* or *xa13*) mostly showed disease scores of “5”, and the lines carrying none of the genes showed the disease score of “7” and “9”. The study indicates that plant lines carrying two genes have the potential to provide more comprehensive and long-lasting resistance against bacterial blight compared to those with only a single gene. The reason for this lies in the fact that a single gene for disease resistance is more susceptible to breakdown when new races or strains of pathogens emerge. In contrast, when two or more effective genes are present, the likelihood of the pathogen overcoming this resistance through mutation is significantly reduced. Researchers Joseph *et al*. (2004) and Gopalakrishnan *et al*. (2008) have highlighted the high effectiveness of the BB resistance gene combination xa13 + *Xa21* throughout India.

In this study, a total of 29 advanced breeding lines, along with 1 standard check, underwent screening for bacterial blight resistance using gene-specific primers, RM13, RG556 (*xa*5) RG 136 (*xa13*) and pTA 248 (*Xa21).* The presence of *xa13* gene with a band size of 1000bp (plate2) was detected in 16 of the advanced breeding lines. Interestingly, 4 of the lines were found to carry both resistance genes *xa13* and *Xa21*, and 3 of the lines were found to carry the resistance genes *xa5* and *xa21*, whereas 7 lines were found to lack all the 3 resistance genes i.e. *xa5*, *Xa21* and *xa13*. Similar findings have been documented by Sabar *et al.* (2016) and Sombunjitt *et al.* (2017). These genotypes, which carry resistant genes, hold the potential to serve as valuable donors for incorporating bacterial blight resistance genes into high-quality basmati cultivars. This would enable rice breeders to introduce known resistance genes from these donor sources into high-yielding basmati varieties and advanced lines using crossing and marker-assisted selection techniques. Huang *et al.* (1997) previously identified lines containing multiple BB-resistant genes with the help of gene-specific PCR markers. Sodhi *et al.* (2003) found that combinations of *Xa21* with *xa13* and *Xa5* resistance genes are effective against prevalent strains of *Xanthomonas* (*Xoo*). However, it is worth noting that pathogen populations in different countries and regions can vary, possibly due to slow pathogen movement or limited genetic mixing among host genotypes, as highlighted by Adhikari *et al.* (1995). As a result, there is an urgent requirement to discover additional BLB resistance genes in rice. Additionally, it is crucial to assess the effectiveness of already identified genes against the prevailing *Xoo* strains. This valuable information can be used to supplement the existing pool of bacterial leaf blight-resistant genes. The study revealed the presence of *Xa21*, *xa13* and *xa5* genes in certain advanced breeding lines, making them potential donor parents for hybridization breeding programs. Utilizing these genes could expedite the development of bacterial leaf blight-resistant cultivars through MAS-based approaches without compromising yield and grain quality.

Pusa basmati 1612 which is used for developing advanced breeding lines , ABL-1, ABL-2, ABL-3, ABL-4, ABL-5, ABL-6, ABL-7 used in the present study, is derived from Pusa Sugandh 5, in which BLB resistant genes *xa5* and *Xa13* has been incorporated from Pusa 1460 (Gopalakrishnan *et al.* 2008; Singh *et al.* 2019). In the advanced breeding lines ABL-2, ABL-5, ABL-6 similar gene combination of *xa5* and *xa13* have been found. The advanced breeding lines ABL-8, ABL-9, ABL-10, ABL-11 possesses a combination of *xa21* and *xa13* genes, these lines have been derived from the sister lines of the cross combination containing PB-3 as one of the parents, which possesses similar combination of both the *xa13* and *xa21* genes (Singh *et al*., 2014). Pusa basmati 1121 used as parent in some of the cross combinations contains both *xa13* and *Xa21* genes (Ellur *et al*., 2016). Pusa 2511 and Taroai basmati which have been used as the parents in different cross combinations contain *xa13* resistance gene (Mishra *et al.,* 2016). Similar results were found in the advanced breeding lines, ABL-1, ABL-3, ABL-4, ABL-7, ABL-12, ABL-13, ABL-14, ABL-15, ABL21, ABL-22, ABL-23, ABL-24, ABL-25, ABL-27, ABL-28, ABL-29 which contain only *xa13* gene. It gives further confirmation that these genes are present in the advanced breeding lines evaluated in the present study.

CHAPTER-6

SUMMARY AND CONCLUSIONS

The present study entitled “Genetic and Molecular Characterization of Advanced Breeding Lines of rice for Bacterial Leaf Blight Resistance” was carried out at the Experimental Area and Molecular Breeding Laboratory of the Division of Plant Breeding & Genetics at Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu. The main goal of study was to identify the lines having good basmati traits and enhanced resistance to bacterial leaf blight. For the current study, material consisted of a total of 29 advance breeding lines along with Basmati 370 as a check procured from the Division of Plant Breeding & Genetics. These advanced breeding lines were subjected to evaluation using a Randomized Complete Block Design during the *Kharif* season of 2022. The evaluation involved transplanting 25-day-old seedlings into two-row plots with a spacing of 15 x 20 cm. Throughout the experiment, standard agronomic and plant protection practices suitable for Basmati rice were implemented. Data was collected for various parameters including plant height (in centimetres), days to reach 50% flowering, days to maturity, panicle length (in centimetres), 1000 grain weight (in grams), and grain yield per plant (in grams). From each genotype, data was recorded from five randomly selected plants.

After harvesting, seeds from the selected plants of each genotype were dehulled, and the grain quality assessment was carried out. The evaluation focused on grain length, grain breadth, and the length-to-breadth ratio based on their dimensions using a Digi Matic Calliper (Mitutoyo) Model CD-8// CSX, which has a range of 0-200mm/0-8 inches. The presence of aroma was ascertained by a panel with the help of scale 0, 1, 2 and 3 for no, mild, strong and very strong (Sood and Siddiq, 1978).

An analysis of variance was conducted using the Randomized block design. To verify the presence of resistance genes, previously reported markers RG136, RG556, RM13 and Pta248 which are closely associated with the BB resistance genes *xa5, xa13* and *Xa21* respectively, were employed. Bacterial blight screening was performed on all advanced breeding lines using the leaf clipping technique, in natural field conditions. Scoring was carried out during the flowering stage under natural field conditions 14 days after inoculation in the natural field conditions, following the Standard Evaluation

System for Rice (SES) established by IRRI in 1996. The experimental results obtained are summarized as:

- Significant variation was observed in all the characters when conducting an analysis of variance for various morphological, phenological, yield attributes, and quality parameters.
- ABL-6 recorded the lowest plant height, whereas ABL-26 recorded the highest height, resulting in an overall mean of 135.73 cm.
- Minimum days to 50% flowering were recorded for ABL-19 with 116.67 days while ABL-26 followed by ABL-27 took maximum days to flower with an overall mean of 124.93 days.
- Minimum days to mature were recorded for ABL-10 while ABL-14 recorded maximum days to mature with overall mean of 163.37 days
- One thousand (1000) grain weight ranged from 18.00 to 29.06 g. Minimum 1000 grain weight was recorded for ABL-6 while, ABL-19 recorded maximum 1000 grain weight with an overall mean of 23.26 g.
- Minimum panicle length was recorded for ABL-18 while ABL-23 recorded maximum panicle length with an overall mean of 28.42 cm.
- Minimum grain yield per plant was recorded for ABL-3 while, ABL-14 recorded maximum grain yield plant with an overall mean of 17.95 g.
- Minimum kernal length was recorded for ABL-29 while maximum was recorded for ABL-5 with an overall mean of 7.51 cm.
- Minimum kernal breadth was found for ABL-1 while, ABL-19 recorded maximum grain breadth with an overall mean of 1.67 cm.
- Minimum length breadth ratio was found for ABL-2 while ABL-3 recorded maximum length breadth ratio with an overall mean of 4.58
- Advanced breeding lines such as ABL-1, ABL-2, ABL-3, ABL4, ABL5, and several others exhibited a mild aroma. On the other hand, ABL-15 and Basmati 370, showcased a distinctly strong aroma.
- Out of the total lines, 4 lines were identified to carry both *xa13* and *Xa21* gene with a band size of 1000 bp for *xa13* and 950 bp for *Xa21*, 3 lines were identified

to carry both *xa5* and *xa13* gene. While 16 lines were found to contain only *xa13* gene with band size of 1000 bp.

- The advanced breeding line ABL-8, 9, 10, and 11 possessed two resistance genes i.e., *Xa21* and *xa13*. The lines ABL-2, 5,6, possesses two resistance genes *xa13* and *xa5*. The advanced breeding lines ABL-1, 3, 4, 7, 12, 13, 14, 15, 21, 22, 23, 24, 25, 27, 28 and 29 possesses only *xa13* gene. While the lines ABL-16, ABL-17, ABL-18, ABL-19, ABL-20, ABL-26 and Basmati 370 does not contain any of the genes.
- In field conditions, maximum disease severity was found for ABL-26, ABL-18, ABL-19, ABL-20, BASMATI 370, ABL-1, ABL-3, ABL-12, ABL-13, ABL-14, ABL-15, ABL-16, ABL-17, ABL-21, ABL-22, ABL-23, ABL-24, ABL-25, ABL-27 and ABL-29 with a disease score of 9, 7, 7, 7, 7, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5 and 5, respectively.
- While evaluating both disease resistance and yield performance, ABL-2, ABL-6 and ABL-10 emerged as the top-performing germplasm lines. These lines demonstrated superior resistance to the disease while also exhibiting high yield potential. Furthermore, ABL-5, ABL-3 and ABL-15 displayed favourable basmati quality traits, making them potential candidates for further evaluation and testing in yield trials.
- ABL-2, 4, 5 6, 8, 9, 10, 11, 23 and 28 can serve as potential donor for the transfer of bacterial blight resistance genes into elite basmati varieties.
- It was observed that germplasm lines containing two genes exhibited a higher yield potential and displayed long-lasting resistance against the bacterial blight pathogen compared to lines with a single gene.
- The identified lines have the potential to serve as valuable donors for incorporating bacterial blight resistance genes into elite basmati varieties through introgression.

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CERTIFICATE – IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled“**Genetic and Molecular Characterization of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Bacterial Leaf Blight Resistance”**, submitted by **Mr. Malik Mehraj U Din**, Registration No. **J-21-M-823**.

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