PATHOBIOLOGY OF *XANTHOMONAS ORYZAE PV. ORYZAE*, THE CAUSAL ORGANISM OF BACTERIAL BLIGHT OF RICE

By

ABDUL RAFI

DEPARTMENT OF MICROBIOLOGY
FACULTY OF BIOLOGICAL SCIENCES
QUAID-I-AZAM UNIVERSITY
ISLAMABAD
2012

PATHOBIOLOGY OF *XANTHOMONAS ORYZAE PV. ORYZAE*, THE CAUSAL ORGANISM OF BACTERIAL BLIGHT OF RICE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

By

Dr. Abdul Rafi

DEPARTMENT OF MICROBIOLOGY
FACULTY OF BIOLOGICAL SCIENCES
QUAID-I-AZAM UNIVERSITY
ISLAMABAD
2012

CERTIFICATE

The Department of Microbiology, Quaid-i-Azam University, Islamabad, accepts this dissertation by Dr. Abdul Rafi in its present form as satisfying the dissertation requirements for the degree of Doctor of Philosophy in Microbiology.

EXTERNAL EXAMINER:

(Prof. Dr. Ikram-ul-Haq)

EXTERNAL EXAMINER:

(Prof. Dr. Arshad Pervez)

SUPERVISOR:

(Prof. Dr. Abdul Hameed)

CO-SUPERVISOR:

(Dr. M. Afzal Akhtar)

CHAIRPERSON:

(Prof. Dr. Safia Ahmad)

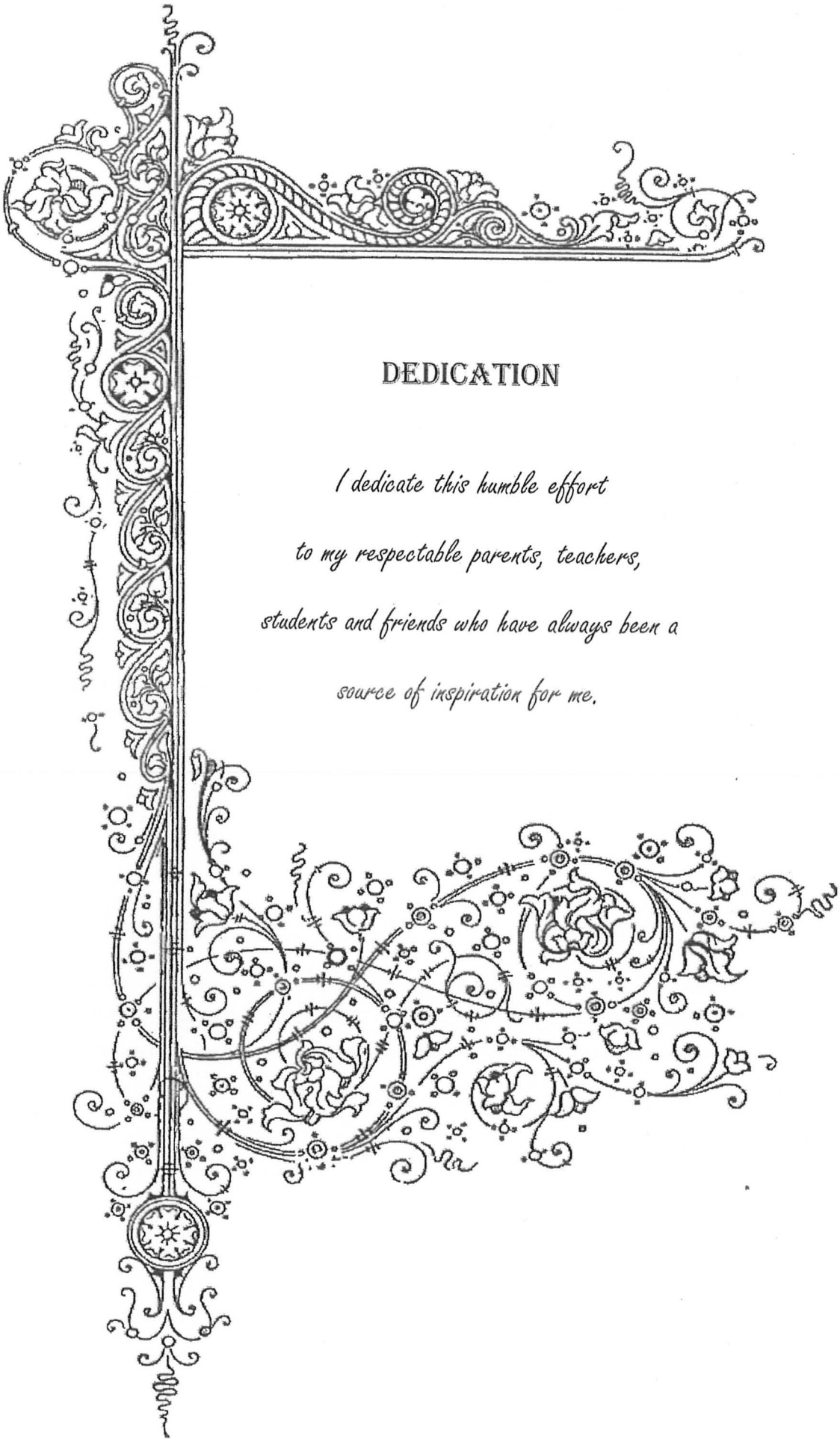
Dated 22/09/2012

FOREIGN REFEREES

1. **Lorna Dawson**
   Professor
   Environmental Sciences,
   The Macaulay Institute, Craigiebuckler,
   Aberdeen AB 15 8QH
   Phone: 01224 498200
   Fax: 01224 498206
   Email: lorna.dawson@hutton.ac.uk

2. **Prof Ken Killham, FRSE, FAAM,**
   Remedies Limited,
   University of Aberdeen,
   Balgownie Technology Centre,
   Aberdeen Science and Technology Park,
   Campus 3, Balgownie Drive,
   Aberdeen, Aberdeenshire
   AB22 8GW
   United Kingdom.
   Emails: kskillham@googlemail.com
   knowehead@killham.freeserve.co.uk

3. **Yalcin Kaya**
   Professor
   Trakya Agricultural Research Institute
   P.O. Box 16, 22100 Edime, Turkey
   Telephone: +90 284 235 8182
   Fax: +90 284 235 8210
   Email: yalcinkaya@uniud.it



DEDICATION

I dedicate this humble effort

to my respectable parents, teachers,

students and friends who have always been a

source of inspiration for me.

Contents

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

%	Percent
Agri	Agriculture
Appl	Applied
BB	Bacterial Blight
BLS	Bacterial Leaf Spot
Bot	Botany
bp	Base Pair
Cfu	Colony Forming Units
cm	Centimeter
cv	Cultivar
cv	Cultivar
DAI	Days After Inoculation
DNA	De-oxy ribose Nucleic Acid
Environ	Environment/Environmental
EPS	Extra Cellular Polysaccharide
FAO	Food and Agriculture Organization
g	Gram
G^-/G^+	Gram Negative/Positive
Genet	Genetics
Gov	Government
ha	Hater
HR	Highly Resistant
HS	Highly Susceptible
Ins	Institute
IRBB	International Rice Bacterial Blight
IRRI	International Rice Research Institute
IS	Insertion Sequence
J	Journal
kg	Kilogram
KOH	Potassium Hydroxide
KP	Khyber Pakhtunkhwa

Ks	Kashmir
LAI	Leaf Area Index
LSD	Least Significant Difference
m	Meter
Microbiol	Microbiology
Min	Minutes
MINFAL	Ministry of Food ,Agriculture and Livestock
ml	Milli Litter
mm	Millimeter
Mol	Molecular
MPMI	Molecular Plant Microbe Interaction
MR	Moderately Resistant
MS	Moderately Susceptible
MT	Metric tons
NA	Nutrient Agar
NARC	National Agricultural Research Center
NIL	Near Isogonic lines
No	Number
NWFP	North West Frontier Province
Pak	Pakistan
PARC	Pakistan Agriculture Research Council
Pathol.	Pathology
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PK	Pakistan
PSA	Potato Semi Synthetic Agar
pv	Pathovar
R	Resistant
RCBD	Randomized complete Block Design
Res	Research
Rev	Review
RFLP	Random Fragment Length Polymorphism
RH	Relative Humidity

S	Susceptible
Sci	Science
SDW	Sterile Distilled Water
Sec	Second
STAT	Statistics
Stat pak	Statistic of Pakistan
TBE	Tris Borate EDITA
TTC	Triphenyl Tetrazolium Chloride
ul	Micro Litter
um	Micrometer
Univ	University
USA	United State of America
w/v	Weight /Volume
WWW	World Wide Web
XOO	*Xanthomonas Oryzae pv Oryzae*
YDC	Yeast Extract Dextrose Calcium Carbonate

Acknowledgments

The author is grateful to almighty ALLAH, the Creator of the Universe for His blessings enabling him to complete this challenging job. The author feels virtuous to afford himself this opportunity to send Durood-o-Salaams on His beloved Prophet Muhammad (Peace Be Upon Him) whose surreal personality is a source of peace for cynical mind and restless soul.

The author wishes to express his deepest gratitude to his supervisor, Professor Dr. Abdul Hameed, Dean, Faculty of Biological Sciences, Quaid-e-Azam University, Islamabad, who has been a constant source of guidance with his valuable comments, positive criticism and encouragement throughout the study. Without his support it would have been difficult to complete the work.

The author expresses very special thanks to Dr. Muhammad Afzal Akhtar, Principal Scientific Officer (Retd.), Crop Disease Research Program (CDRP), NARC, Islamabad for his unforgettable support and contribution all the way throughout the study which made this research work more valuable. Certainly, without his support and assistance achievement of this goal would have been impossible.

The author is very much thankful to Prof. Dr. Hidayat ur Rehman, Dr. Meher Ali Shah, Department of Plant Breeding and Genetics, and Prof. Dr. Shaukat Hussain Department of Plant Pathology, Agricultural University Peshawar for their kind guidance and facilitation during the studies and for outlining and write up of this manuscript.

Sincere thanks for the constant reminders, support and appreciation of Prof. Dr. Khan Bahadar Marwat and Prof. Dr. Farhatullah who inspired me to complete this manuscript. Their concern and support shall always be remembered.

The author also appreciates his colleagues, Dr. Saifullah, Dr. Muhammad Arif, Dr. Fazli Raziq, Dr. Musharaf Ahmad, Dr. Asad Ali, Mr. Hakeem Khan, and Dr. Sartaj Alam for their cooperation and help during research work.

The author pay profound gratitude to Prof. Dr. Akmal, Fazli Munsif, Muhammad Junaid and Syed Fahad Shah for helping in data analysis and composing of thesis.

The author has no words to thank his wonderful students especially Ayesha Bibi, Murad Ali, Muhammad Shahid, Dr. Hussain Shah, Dr. Muhammad Fahim, Dr. Syed Nafees Uddin Gillani, Amjad Ali and Fazli Wahid who have always been his helping hands whenever they were needed.

Heartfelt thanks are due to my wife, daughters especially little Malaika and son for their love, patience and sacrifices during the study period

Abdul Rafi

Abstract

A series of experiments were conducted to study the pathobiology of *Xanthomonas oryzae pv. oryzae* (*Xoo*) the causal organism of bacterial blight of rice. Studies were carried out in field based assessment of Bacterial Leaf Blight (BLB) incidence and severity in all rice growing zones of Pakistan, biochemical characterization and molecular confirmation of KP isolates, determination of best method of inoculation, KP races using International Rice Bacterial Blight (IRBB) lines, exploitation of rice cultivars and land races for resistance to BLB. Survival mechanism and source of primary inoculum of *Xoo* and comparison of the effect of planting dates on BLB incidence and yield performance of selected rice cultivars in multi location trails were also undertaken.

In KP, disease incidence ranged between 35-80%, whereas severity was between 3 to 7% during cropping season of 2005-2007. In Punjab, Sindh and Balochistan, incidence ranged between 37-75%, 12-47% and 12-22%, respectively. While severity ranged between 3-6%, 0.7-3% and 0.3-1%, respectively, in these areas.

One hundred and twentyfive *Xoo* isolates were collected from twelve districts of different agro-ecological zones of KP for characterization through a number of biochemical tests. Some of the isolates did deviate from the normal pattern and showed over lapping results. Therefore, identity of all the candidate *Xoo* isolates was verified through Polymerase Chain Reaction (PCR) with specific primers.

To determine the best method of inoculation of *Xoo*, a bacterial suspension containing 10^{-8} colony forming units (cfu) was used for inoculating six commercial rice cultivars *in-vitro* and *in-vivo*. Three methods of inoculation i.e. clipping, pinprick and brush were evaluated. Clipping method resulted in much more lesion length than either the pinprick or brush method.

Eighty isolates obtained during 2005 to 2007 from various rice growing zones of PK, were grouped using IRBB lines into six races by scheme adopted by Habgood (1970) and modified by Limpert and Muller (1994) nomenclature procedure. These races were

distributed at almost all locations of PK. However, Race-1 and Race-6 showed its dominance at all locations and were found the most prevailing races of PK. Race-3 was confined only to seven locations of rice growing areas of PK. Race-6 represented three isolates of Peshawar district alone.

A total of thirty four rice cultivars were evaluated against KP races for resistance. Seventeen showed resistance or moderate resistance to race-1, while 18, 13, 14, 15 and 09 exhibited resistance or moderate resistance to race-2, race-3, race-4, race-5 and race-6, respectively. Varieties, Bas-2000, Shaheen Basmati, Malhar-346, Khushboo-95, Bas-2008 and Jajai-77 proved to be resistant or moderately resistant to all the six races under study. Race-6 was the most virulent amongst all the KP races as 15 cultivars exhibited susceptible reaction. Conversely, race-1 was found to be the least virulent in terms of response to artificial inoculation.

For post-harvest survival of *Xoo*, 50 samples of field water, seven most common weeds of rice, plant residues and seed were assayed for recovery of the pathogen. Field water, plant residues and seed samples yielded 25%, 37% and 31% of the causal bacterium, respectively. On the other hand, recovery of *Xoo* from all tested weeds was 7%. Both *Cyperus difformis* and *Dactyloctenium aegyptium*, collected from Charsadda, tested positive for isolation of pathogen. Similarly, successful recovery of *Xoo* was recorded from *Echinochloa crus-galli* and *Leersia hexandra*, both were collected from Peshawar. Additionally, *Cyperus rotundus* sampled from Swat also tested positive for recovery of the causal bacterium.

Two years (2007 and 2008) data regarding the effect of planting dates on BLB revealed that sites and varieties significantly affected bacterial leaf blight, tillers plant^{-1}, grains panicle^{-1}, 1000 grains weight, panicle length (cm) and grain yield at both locations. A similar trend of high BLB was recorded for variety Fakhre Malakand followed by variety Basmati-385 during 2007 and 2008. Interaction of sowing dates x varieties indicated increased BLB Dilrosh-97 and Swat-1 till 15th June but further delay did not increase disease incidence.

Site and variety interaction showed that tillers plant^{-1} was reduced for all varieties at Mingora. Interaction between sowing dates and varieties revealed that rice varieties i.e. Dilrosh-97, Fakhre Malakand, JP-5 and Basmati-385 showed decreased tillers plant^{-1} when sowing was delayed at both locations and years. The interaction between sowing dates and variety for grain panicle^{-1} was significant. Maximum grains panicle^{-1} were observed in Baffa during both the years. Sites, sowing dates and varieties, significantly influenced 1000 grain weight (g). Mean value for this trait was greater at Baffa. Variety Fakhre Malakand produced heavier grains than all other varieties. Sowing dates x varietal interaction indicated that 1000 grain weight was enhanced till 15th June in each variety tested but further delay in sowing resulted in decline in 1000 grain weight of all varieties at both location and years. Long panicles (cm) were observed at Baffa as compared to Mingora. Panicle length was drastically decreased when sowing was delayed for almost all tested varieties except JP-5 and Swat-1 during both the years. Site, sowing dates and varieties significantly affected the grain yield. Mean values for rice varieties showed that high grain yield was achieved for Fakhre Malakand, whereas, both Swat-1 and JP-5 resulted in the lowest grain yield during both the years and locations. Higher grain yield was recorded, when planting was done on 15th June.

In short, disease incidence of BLB ranged between 12-80%, whereas severity was between 0.3-7% in Pakistan. Identification of *Xoo* isolates was initially determined by biochemical tests and was later confirmed through PCR. *Xoo* isolates were frequently isolated from field water, plant residues, seed samples and weeds. The isolates were categorized into 6 groups using IRBB lines. Of these, race 1 and race 6 were predominantly present at all the locations of KP, with race 6 being the most virulent one. Among methods of inoculation clipping method proved effective in producing disease as compared to pinprick and brush methods. Cultivars tested for resistance against KP races showed variable results. A significant interactive effect of sites and varieties as well as sowing dates and varieties was evident on yield and BLB.

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa*), a member of family Poacea or the grass family, is cultivated extensively in warm climates (Ezuka and Kaku 2000). Although native to Southeast Asia, it is widely grown throughout the world under variable conditions. It has been cultivated since at least 3500 BC in the Indus Valley and for Neolithic times (5th millennium BC) in the lower Yangtze region of China. Similarly, African rice (*Oryza glaberrima*) seems to have been domesticated in West Africa extending from the central delta of the Niger River to Senegal. The rest of rice species are wild (Khush, 1997; Brar *et al.*, 1996).

As a main source of nourishment for over half the world's population, rice is by far one of the most important commercial food crops. Over 300 million acres of Asian land is used for growing rice. Its annual yield worldwide is approximately 535 million tons. Of the 50 rice producing countries, China and India contribute upto 50% of the total world production. Likewise, Southeast Asian countries alone contribute from 9 to 23 million metric tons annually. Production of rice in million metric tons by countries in year 2006-2007 is given in Figure 1. Rice is an immensely important food crop, produced under greatly diverse environments. It is estimated that rice production must increase by 65% (1.7% per year) in the next 30 years to meet world food demand.

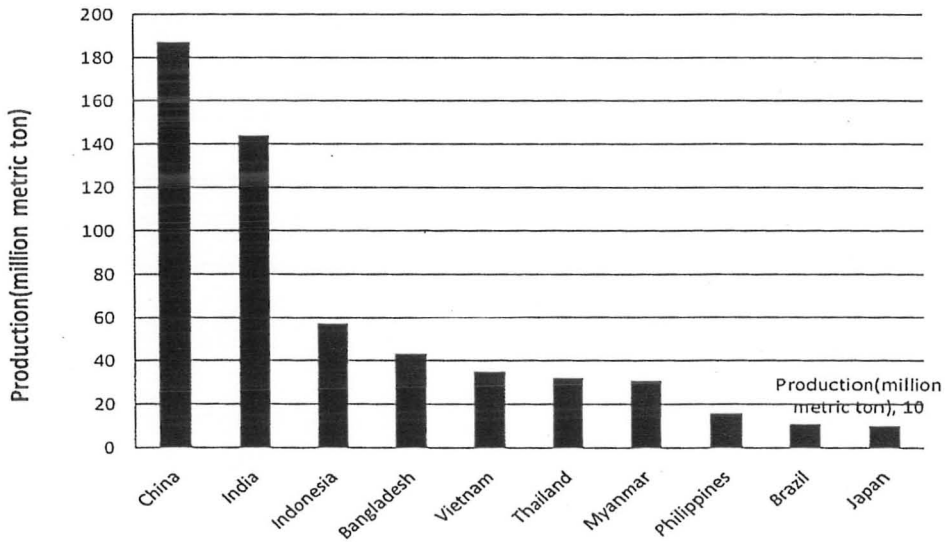


Figure1: (FAOSTAT, 2007, htt://faostat.fao.org)

This means that in the primary rice-producing countries of South and Southeast Asia, production must increase by 100% (2.1% per year) (IRRI, 1989).

Pakistan is the 12th largest rice producing country in the world. Being the third largest crop after wheat and cotton, rice occupies 2.96 million hectare that is more than 11% of the total cultivated area with a production of 6.95 million tons of milled rice in Pakistan (Agri. Statistics of Pakistan, 2008-2009).

In the past few years there has been an increase of 18% in cultivated area from the previous cropping which is attributed to attractive incentives offered by the government. The provincial break up of rice cultivation in the country; includes 68% in the Pujnab, 23% in Sindh, 6% in Baluchistan and 3% in Khyber Pakhtunkhwa (KP) formerly known as North-West Frontier Province or NWFP (MINFAL, 2009).

Rice plays multifarious roles in Pakistan's agrarian economy. Firstly, it is an additional staple food besides wheat and contributes more than 2 million tons to our national food requirement. Secondly, rice industry is an important source of employment and income for rural population. Thirdly, it contributes to the country's foreign exchange. Pakistan became the third largest rice exporting country in the world in 2009, with rice being the second largest foreign exchange earner for the country after cotton. The country's rice exports are expected to cross $2.1 billion mark by the end of the fiscal year 2010 (Daily, The Nation, 10th July, 2009).

The overall situation regarding rice in Pakistan for the year 2003-04 to 2006-07 is shown in Table-1. There was a quantum leap in rice production in the sixties due to large scale adoption of high yielding semi-dwarf varieties. Since then there is marginal increase in its production. Alongside, rice area is also gradually increasing.

Table 1. Year-wise Area, production and yield of rice in Pakistan

Year	Area (000ha)	Production (000tons)	Yield(kg/ha)
2003-04	2225	4.85	1971
2004-05	2461	5.03	1994
2005-06	2621	5.55	2116
2006-07	2581	5.44	2107

Source: www.statpak.gov.pk

1.1 Rice zones in Pakistan

Pakistan has been divided into various agro-ecological zones with respect to rice cultivation.

Zone-1

It consists of northern mountainous areas of KP mainly of Malakand and Hazara divisions where irrigated rice is grown either in flat valleys or terraced valley-sides. The climate is sub-humid with 750 to 1000 mm average rainfall. Besides sticky type Begamai or Swati rice, favorite of the locals, cold tolerant rice cultivars such as KS-282, Basmati-385, Pakhal, Irri-6, Fakhre Mlakand and Dilrosh-97 are also grown. Rice is also cultivated in the plains of Peshawar, Mardan, Swabi, Charsadda, Nowshera and Bannu districts of KP.

Zone-2

It lies in the broad strip of land between rivers Ravi and Chenab where both canal and sub-soil water are used for irrigation. The climate is sub-humid, sub-tropical type with 400 to 700 mm of rainfall mostly in July-August. Rice growing season is fairly long in this zone and suitable for cultivating fine aromatic as well as some IRRI varieties. The "Kalar" tract which is abode of the world famous aroma Basmati rice is located in this zone. Basmati rice such as Basmati 2000, Basmati Super, Super Kernel Basmati, PK-385 Basmati, PK-198 Basmati, Supra Basmati, Kiran Basmati, Punjab Riceland and Super Fine Basmati rice predominate the kalar belt of Zone-2 (Punjab).

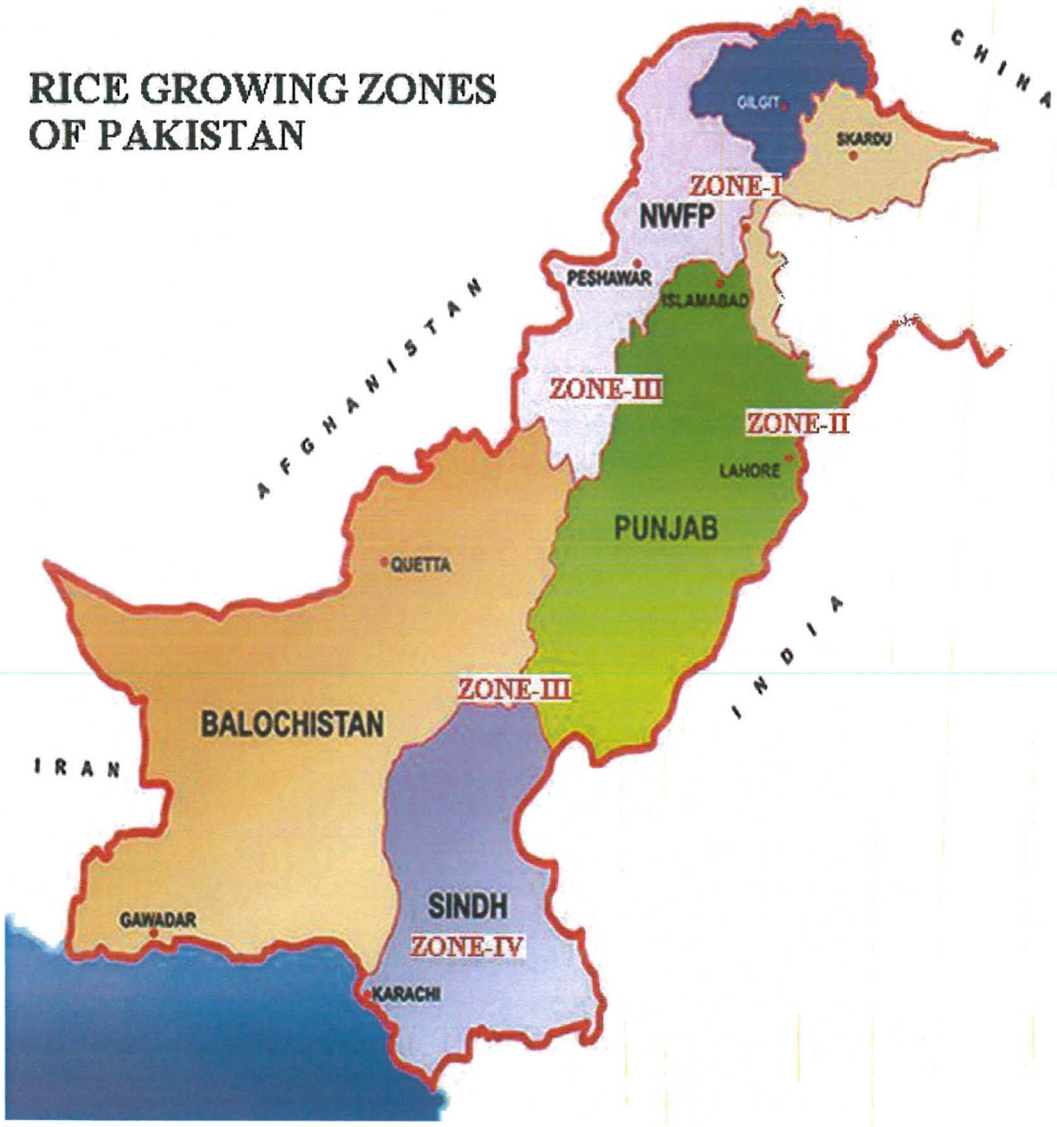


Figure 2. Rice Growing Zones of Pakistan

Zone-3

It comprises of the large tract of land on the west bank of the river Indus. It has an arid sub-tropical climate with 100 mm of average rainfall and maximum temperature higher than both zone 1 and 2. The long, extremely hot summers are well suited for growing coarse rice varieties.

Zone-4

It is the Indus delta which consists of vast spill flats and basins; the latter are mostly irrigated. The climate is arid tropical marine with no marked season and is highly suited to coarse varieties. Wheat, berseem and pulses are grown in rotation with rice in this zone. In the south of KP, Sindh and Balochistan (zones 3 and 4) IRRI type long grain, heat tolerant tropical rice such as Dehradun Basmati, Hansraj, Supri as well as some landraces dominate (Salim *et al.*, 2003).

1.2 Rice diseases

Diseases have always had a significant impact on sustainable rice production. Historically, severe epidemics have led to serious food shortages. The Bengal famine of 1942 was, in part, attributed to brown spot (Padmanabhan, 1973). Similarly, rice blast epidemics caused a major food crisis in Korea in the 1970s (Ou, 1985) where yield losses ranged from 10 to 50%. Considering the large rice production area in the world, even a conservative estimate of 1 to 5 % annual loss would translate into thousands of tons of rice and billions of dollars lost. Thus, minimizing the occurrence of disease epidemics and reducing yearly losses are central to sustaining rice productivity. To achieve this goal, it is important to determine the extent of damage caused by diseases and to be able to identify shifting disease problems associated with technological changes.

Annually, more than 40% of the world's rice crop is lost to multitude of biotic stresses such as insects, pests, pathogens, and weeds (Hossain, 1996). Among several diseases caused by bacterial, fungal, and viral plant pathogens that devastate rice yields all over the world, bacterial leaf blight (BB) caused by *Xanthomonas oryzae pv. oryzae*, blast caused by *Magnaporthe grisea*, sheath blight caused by *Rhizoctonia solani*, sheath rot caused by *Sarocladium oryzae*, and tungro virus are the most important.

These have resulted in huge losses and could be potentially fatal under suitable environmental conditions.

1.3 Bacterial leaf blight

Among the eight bacterial rice diseases in tropical Asia, BB is one of the most important and oldest known diseases of rice. Bacterial blight has received most of the attention because of its high epidemic potential and destructive nature.

1.4 Losses

Accurate estimates of average yield losses are difficult to obtain, since these vary extensively according to geographic region and season. Globally, its incidence has been reported from different parts of Asia, northern Australia, Africa, and the United States. Crop losses of 10 – 20 % in moderate conditions and severe losses of up to 50% in highly conducive conditions have been recorded in several Asian and Southeast Asian countries. In India losses of 15 to 20% have been reported to be common occasionally reaching upto 40% (Ou, 1985; Mew, 1987).

1.5 Etiology

The bacterial nature of leaf blight in rice was established by Mew *et al.*, (1993) in the early 1920s. The causal bacterium is *Xanthomonas oryzae pv. oryzae* (*Xoo)* which was considered as one of the pathovars of *Xanthomonas campestris* till 1992. However, it has now been reclassified as *Xanthomonas oryzae pv. oryzae.*

Xoo is short, rod shaped, with round ends, 0.8-1.0×1.7 μm in host, with monotrichous flagellum of 6-8 μm, Gram negative, aerobic and non-spore forming (Ishiyama, 1922). Its genome consists of a single, circular chromosome of approximately 4941439 bp. Various strains of *Xoo* have been reported to carry one or more indigenous plasmids with molecular weights of about 20.3×10^6 to 21×10^6 Dalton.

The bacterium is surrounded by electron transparent cell wall and a cytoplasm membrane composed of a continuous unit membrane present along the inside of this cell wall. Nuclear material of fibrillar appearance, ribosome, and polysome like structures were observed in the cytoplasm, but no mitochondria, endoplasmic reticula or mesosomes were found (Horino, 1973). Surface of the bacteria is covered by a viscous, capsule-like material composed of hetro-polysaccharide. This material was

assumed to serve as a protectant from unfavorable conditions such as drought (Mizukami, 1959). The extracellular polysaccharide (EPS) was reported to be composed of glucose, manose, glucuronic acid, glucuronolactone, and arabitol. Spontaneous loss of virulence associated with reduction of EPS production has been observed in long-term storage and culture. Therefore, EPS is regarded as a virulence determinant. (Shen and Ronald, 2002)

Xoo neither liquefies gelatin, nor does it reduce nitrate or methylene-blue. It produces a small amount of hydrogen sulfide but no ammonia or indole, digests milk without coagulation, reddens litmus milk, and does not produce gas from sugar (Ishiyama, 1922).

The bacterium develops yellow, circular, smooth and viscous colonies on potato semi synthetic agar medium. Where its growth requires carbon and nitrogen sources (Wakimoto 1955). Sucrose is the most favorable carbon source, followed by glucose, manose, galactose and maltose. Succinic acid is also good carbon source among the organic acids tested (Watanabe, 1963).

The pH range for bacterial growth is 4.0-8.8, with the optimum of 6.0-7.0 while temperature range is 5-40 °C (optimum 26-30 °C) though 20 °C has been shown to be favorable for the growth from the extremely diluted condition. The thermal death point for multiplying cells is 10 min at 53 °C under wet condition, while that for dried cells is 10 min at 56 °C (Tagami and Mizukami, 1962).

1.6 Occurrence and distribution of Bacterial Leaf Blight

For the first time the disease was observed in Japan in 1884-1885. However, it has been reported from many countries of the world including Srilanka, India, Bangladesh, Indonesia, Malaysia, Cambodia, Korea, China, Thailand, Vietnam, The Philippines, USA, West Africa and Australia (Ezuka and Kaku, 2000). Mew and Majid (1977) reported the disease for the first time from Pakistan and its occurrence was confirmed from all the provinces in a later study (Akhtar and Akram, 1987, Akhtar, *et al.*, 2003). It has been observed during recent years that bacterial leaf blight (BB) incidence is on the rise in Pakistan especially in "Kaller" belt which is famous for cultivation of world famous aroma rice (Khan *et al.*, 2000). In a survey conducted by Akhtar *et al.*, (2003) reported 20-25% disease incidence in KP.

1.7 Symptoms

Generally, rice plants are more susceptible to bacterial blight infection at the seedling than at the adult stage. The bacterial blight syndrome has three types of symptoms in the tropics including the leaf blight, the kresek, and the pale-yellow plant. The relationship among these showed that *Xoo* is responsible for all three symptoms, but kresek and leaf blight seem distinct and independent of each other. The kresek-infected plants may serve as a source of inoculum for secondary infection leading to leaf blight. Alternatively, leaf blight infection may return to kresek if infection occurs in the early growth stage or else the variety is susceptible to bacterial blight. Therefore, kresek and leaf blight are the primary effects of infection. Pale yellow appearance is a secondary effect of either leaf blight or kresek. (IRRI, 1978)

The leaf blight phase occurs in tropical and temperate regions, whereas, the kresek phase is confined to temperate regions (Ezuka and Kaku, 2000).

The leaf blight phase appears commonly on leaf blades, and sometimes on leaf sheaths and glumes. It usually appears after the maximum tillering stage and spreads rapidly during and after the heading stage (Goto *et al.*, 1955).

The kresek phase is characterized by systemic infection and acute wilting of young plants. Symptoms usually appear one to two weeks following transplanting. When leaves turn grayish green they suddenly wither and roll up, while some float on the water (Reitsma and Schure 1950). The kresek phase is the most destructive phase and causes severe damage to the crop.

The pale yellow leaves are prominent in mature plants. Contrary to older leaves which are normally green, the younger leaves remain pale yellow in a uniform pattern or broad stripe on the blade (Ezuka and Kaku, 2000).

When a leaf is injured naturally or artificially the pathogen invades through the wound to develop a lesion from that portion (Tagami and Mizukami, 1962). Lesions usually start from the leaf margin near the top. At first a tiny water-soaked lesion appears on the leaf margin then turns yellow while enlarging both in length and width to develop an elongated irregular lesion. The border of the lesion adjoining the healthy part shows a wavy margin. Lesions may start at one or both margins of the leaf. Under moist conditions the infected leaves exude bacterial ooze on the

margins or veins. As the disease advances lesions may cover the entire blade, turn white and later become grayish due to the growth of various saprophytic fungi.

1.8 Disease epidemiology

Xanthomonas oryzae pv. *oryzae* enters rice leaves through wounds, stomata, or hydathodes (Mew, 1987). Bacteria multiply in the epitheme to which they subsequently move and further multiply to infect the plant (Shen and Ronald, 2002). BB is most severe in highly managed systems such as irrigated paddies or those with high N fertilizer applications. The disease is aggravated by warm, humid and wet conditions.

The bacterium overwinters either in perennial weeds or in soil. Grains, straw and rice stubble are other possible sites of overwintering of the pathogen. During growing season, the bacterium remains and multiplies in vascular system of plant where it gives rise peculiar leaf blight symptoms.

It is natural that the disease is hardly seen in upland rice unless the land is irrigated or flooded by heavy rainfall (Kuwatsuka, 1942).

1.9 Role of environment in disease development

The incidence and severity of bacterial blight are influenced by various environmental factors. Which are divided into topographic and soil conditions, climatic conditions, and cultural practices. Among these, rainfall and nitrogen fertilization are the most important factors affecting the occurrence of the disease.

Soil type appears to be a factor affecting the incidence as the disease was severe in sandy loam, clay, or clay loam alluvium soils, but less in the sandy soil adjacent to dune areas in the Hokuriku area of Japan (Yoshimura, 1959). Similarly longer lesions were repeatedly produced in lateritic than sandy soil (Dath *et al.,* 1978).

Climatic conditions also influence disease incidence and development. The most important factors for disease epidemics are rainfall, humidity, worm temperature and flood during the rice-growing season (Bokura, 1911).

The optimum temperature for lesion development was reported to be 25-30 °C, while the symptoms hardly appear at a low temperature of 17 °C (Mukoo and Iida, 1957). In

temperate regions, temperature limits the period of bacterial blight incidence to warmer seasons. In tropical regions, however, temperature is high enough for disease development throughout the year. Instead, there is remarkable alteration of wet and dry seasons and the disease is generally more severe in the wet season than in the dry season (Ezuka and Kaku, 2000).

Fertilization is another important cultural practice affecting the development of bacterial blight in rice. Among the three elements of fertilizers, nitrogen is the most important for encouraging disease development (Bokura, 1911).

Bacterial blight is a typical water-born disease. The causal organism is disseminated by irrigation water (Kuwatsuka, 1942).

1.10 Physiology of affected plants

Rice plants infected with bacterial blight show various changes in their physiology, such as increase or decrease of component substances, change in enzymatic activities, increase in respiratory rate and increase in water permeability of leaf cells.

Reducing and non-reducing sugars, crude starch, ammonium nitrogen, total phosphorus, lipid phosphorus, nucleic acid phosphorus and insoluble phosphates are increased, whereas total nitrogen, water soluble protein, and water insoluble protein-nitrogen are decreased. Among these, the increase in crude starch, lipid phosphorus and nucleic acid phosphorus is assumed to be mostly due to the bacterial body of the pathogen (Misawa and Miyazaki 1973).

1.11 Disease cycle

In temperate regions, soil, seed, rice straw, rice stubble and perennial weeds are assumed to be the possible sites of the pathogen survival during the long and cold winter.

In tropical regions, on the other hand the temperature is sufficiently high for the disease to occur throughout the year, but there is a distinct alternation of dry and wet seasons. It is a critical problem for the pathogen how to survive the dry season that hinders its activity. In irrigated areas, rice is grown also in the dry season and inoculum may be alive on living rice plants or stubble or on weeds such as wild rice, thus the disease cycle is easily completed. In single cropping areas, however, the

pathogen cannot survive the dry season on living plants, as such seed transmission becomes important (Mohan and Rao, 1974).

The pathogen is thought to be carried to the nursery by irrigation water by applying infected straw to soil or by sowing infected seeds (Mizukami and Wakimoto, 1969). The pathogen in field water, survive longer at low (15-25 °C) temperature than at high (30-45 °C) temperatures. The longevity of the pathogen in unsterilized field water was estimated to be 30, 30, 20 and 10 days at 15, 20, 25 and 30 °C (Murty and Devadath, 1982b).

The pathogen that reaches rice seedlings crowds together on root surface of the plant and moves upwards to the crown using metabolites of root for its multiplication (Mizukami, 1957). The pathogen thus multiplies on the root system and may be liberated into the irrigation water. The pathogen from the roots may immediately invade the basal part of leaf sheaths through the wounds made by the emergence of crown roots (Mizukami, 1961) leaf infection also occur when lower leaves come in contact with (contaminated) water (Dath and Devadath, 1983).

The pathogen enters into stomata on coleoptiles and leaf sheaths of rice seedlings, and multiplies in the intercellular space of parenchyma without showing any symptoms (Tabei, 1967).

Thus the pathogen while increasing its population on the surface of the rice plant is transferred upwards by rain splashes or by contact of leaves and invades through water pores or wounds. The pathogen invades more easily through new wounds than older ones (Ezuka and Kaku 2000). It then spreads rapidly through the vascular systems to the tissues around the infected site and the symptoms start appearing. The population density of the bacterium on the leaf surface at the time of appearance of the symptoms is estimated to be about 10^4-10^5 cells per leaf (Yoshimura, 1963)

The bacterium is sometimes transmitted by insects as well. A rice bug, *Leptocorisa acuta*, mechanically transmits the disease (Mohiuddin *et al.*, 1977). A leafhopper *(Nephotettix virescence)* and grasshopper (*Oxya japonica*) have also been implicated to transmit the pathogen mechanically (Murty and Devadath, 1981).

1.12 Disease management

For control of bacterial blight disease, cultural, physical and biological methods have been investigated by some researchers. The cultural methods include use of healthy seed, removing diseased stubble and straw from field, separating the irrigation system from the drainage system and avoiding excess application of nitrogenous fertilizers. Physical control has been limited mainly to seed disinfection. In order to avoid the negative effect of pesticides on the environment and human health, biological control has been investigated in recent years. Most of them however have been limited to attempts to screen microorganisms from phylloplane or other sources for antibacterial activity *in vitro* or *in vivo*. However, growing bacterial blight resistant rice cultivars is the safest and feasible control measure against the disease. (Ezuka and Kaku, 2000)

Bacterial blight has the potential to become a destructive bacterial disease of rice in Pakistan and can cause huge losses as information regarding the survival of the pathogen; mechanism of disease development and its effective control measures are sketchy. Therefore, comprehensive study of the pathogen and pathogenesis is necessary to gain extensive knowledge about the disease in order to properly address the problem and ultimately protect the rice crop from yield losses. The purpose of the present study was, therefore, to document the present status of the disease incidence and severity at national level and characterize the causal agent through biochemical tests to study the pathogenicity of KP isolates to evaluate rice cultivars and landraces as well as wild rice for bacterial blight resistance, to study the source of inoculum of BLB in KP, to asses diversity among *Xoo* isolates of KP (pathotypes) using near isogenic lines (NIL) or IRBB lines and to study the effect of planting dates on BB incidence and yield performance of rice cultivars at different locations of KP. The study is expected to provide first hand information on the host pathogen interaction. The database thus developed, could be useful in developing cultivars with durable resistance against the pathogen in future studies.

Research objectives:

1. Field based assessment of bacterial leaf blight of rice in all rice growing zones of Pakistan
2. Biochemical and diagnostic characterization of isolates from KP
3. Determination of best method of inoculation for evaluation of host resistance
4. Evaluation of Pakistani rice cultivars and landraces for resistance to bacterial leaf blight
5. Determination of *Xanthomonas oryzae* pv *oryzae* pathotypes of isolates from KP using IRBB lines
6. Effect of planting dates on bacterial leaf blight incidence and yield performance of selected rice cultivars in different location of KP.
7. Study of survival and source of primary inoculum of *Xanthomonas oryzae* pv *oryzae*

CHAPTER 2

REVIEW OF LITERATURE

2.1 Bacterial leaf blight, incidence severity, isolation, and characterization

Bangura and John (1991) observed that bacterial leaf streak, caused by *Xanthomonas campestris* pv. *oryzicola,* and bacterial leaf blight, caused by *Xanthomonas campestris* pv. *oryzae*, were more prevalent and severe in the Sahelian than in the forest zone but found rarely in the more humid zone of West Africa. Bacterial leaf blight has been found to be more widely distributed in the Sahelian region than the bacterial leaf streak. The incidence of bacterial leaf blight was aggravated by high dosage of nitrogen. Losses ranging from 2.7% to 41% in grain yield were commonly inflicted by bacterial leaf blight.

Akhtar and Zakria (2003) reported disease incidence in various rice growing districts of the Punjab. Similarly, varying levels of disease severity ranging from 0-9 were observed during the survey of the same regions. A low level of disease incidence 0-5 was observed in various districts of sindh and various regions of KP showed a high incidence of disease 0-100 %. Disease severity ratings ranged between 0-7 in the same areas. A few treatments in Baluchistan showed a low % age of disease 0-5 with a maximum severity of 1. While in Azad Jammu and Kashmir no disease was observed. The causal agent of bacterial blight of rice was confirmed through biochemical, physiological, hypersensitive reaction and pathogenicity.

During another survey Akhtar *et al.*, (2003) reported alarming situation of BB in rice wheat system of Punjab. Surveys conducted during 1999 and 2002 showed that mean incidence (percentage) of BB was 25 in Hafizabad, 28 in Sheikhupura, 15 in Gujranwala, and 29 in Gujrat districts during1999. Mean incidence (percentage) during 2002 was recorded as 64 in Sargodha, 43 in Hafizabad, 36 in Sheikhupura, 34 in Sialkot, 28 in Narwal, 41 in Gujranwala, 55 in Gujrat, 45 in Lahore, 55 in Kasur and 47 in Okara. The highest severity of BB in the range of 1-3 % was in Hafizabad and the lowest 0–1 % was in Gujranwala during 1999 while during 2002 the highest rate 0–9 % in Gujranwala and the lowest rate 0–3 % in Gujrat, Lahore, and Kasur. It was predicted that BB might create havoc in the next couple of years as super basmati is mainly grown in this area, which is severely susceptible to the disease. Secondly, as

the area under zero tillage cultivation is increasing, there are chances of inoculum buildup on the stubble in the zero tillage fields.

Dinh *et al.*, (2008) isolated *Xanthomonas oryzae* pv. *oryzae* from leaf samples which were cleaned with tap water, and air dried. These leaves were excised into small 5-7 cm pieces and surface sterilized with 1% sodium hypochlorite, then washed in sterilized distilled water. These pieces were placed into the test tube containing 1ml of sterilized distilled water for about 5 to 10 minutes, to allow the bacteria to ooze out from the leaf tissue. A sterilized loop bacterial suspension was streaked onto Petri dishes containing Wakimoto's medium.

2.2 Virulence and pathogenicity

Jyufuku *et al.*, (2009) grouped *Xoo* isolates into 13 races using polymerase chain reaction fingerprinting and virulence analysis on the basis of their pathogenicity to international differential lines. Distribution of races was quite specific to the region. Three strains from India and two from Indonesia were virulent to cultivars containing bacterial blight resistance gene *xa5*, whereas most strains from other countries were avirulent to xa5. The strains from India exhibited high virulence and broad range of pathogenicity, in contrast to those from Malaysia. Two varieties containing *Xa21* and *Xa5* gene, respectively, were resistant to almost all Asian *Xoo* strains. *Xoo* collections from Asian countries were also divided into four genetic groups by clustering statistics on the basis of the results from PCR based RFLP with IS1112 primers. A partial relationship was found among the genetic groups, countries and races, thereby suggesting that strategies targeting regional resistance breeding and gene deployment are feasible.

Satya *et al.*, (2005) screened five isolates for variability in virulence. Isolate 5 was the most virulent against aromatic lines, whereas; isolate 4 was the least virulent. The most virulent isolate was then used to screen 48 rice genotypes to identify resistant sources. All aromatic genotypes were moderately or highly susceptible against all the isolates with significant differences in disease progress. Isolate 5 was used to screen 48 genotypes. Thirteen lines showed high resistance against the highly virulent isolate.

Noor *et al.,* (2006) determined virulence of *Xoo* on three basmati rice varieties and showed that all the three rice varieties were susceptible with Super basmati being highly susceptible to all the exotic strains of *Xoo.* The maximum percent disease incidence (89.5%) against PXO 340 was at seedling stage (4 week), with 84.54% at maximum tillering stage and with 56.21% against PXO 61 at leaf flag stage. On the other hand, Basmati 2000 was the most resistant variety at two growth stages and susceptible at maximum tillering stage against PXO 280, with maximum percent disease incidence (75.96%) and PXO 340 with 71.53%. The reaction of eight different strains of *Xoo* was variable against Basmati 385. At seedling stage, it showed susceptibility against PXO 61 with maximum percent disease incidence (65.33%), against PXO, 339 with 58.18% at maximum tillering stage and with the highest rate of maximum percent disease incidence i.e. 75.68% for PXO 341 at leaf flag stage.

Dinh *et al.,* (2008) used 41 isolates on 10 differential rice varieties containing different single resistant genes and demonstrated that the Infection responses were clearly compatible and incompatible reactions on differential rice varieties.

Lai Van *et al.,* (1999) divided isolates of *Xoo* into seven groups based on their pathogenicity on IRBB 1, IRBB 2, IRBB 3, IRBB 4, IRBB 5, IRBB 7, IRBB 8, IRBB 10, IRBB 11, IR 24, IR 20, Kinmaze, TN1 and BJ1. Three materials IRBB5, IRBB 7 and BJ1 however showed complete resistance to all isolates. IRBB 8 and IRBB 3 were resistant to 37 and 36 isolates, respectively. IRBB1, IRBB 2, IRBB 11, Kinmaze and TN 1 were susceptible to all isolates. The remaining near isogenic lines (NILs) were resistant to some isolates. Isolates belonging to dominant pathogenic group were widely distributed at different places from North to the South Vietnam. Almost all Vietnamese cultivars were susceptible to all isolates tested. However, some varieties in Mekong Delta (North Vietnam) were resistant to few isolates.

Mew and Cruz (1979) detected three different virulence groups or strains. A major group included those that infected IR8 i.e., rice that carries no genes for resistance to bacterial blight. Specificity of the isolates in differential infection of rice was also shown in response to infection of rice of different ages planted in the greenhouse or in the growth room under conditions most favorable for plant development.

Goto and Okabe (1967) obtained various colony type variants of *Xoo*, due to natural or induced mutation in artificial culture. In most cases, the mutant types showed

attenuated virulence or avirulence as compared with the wild type. Tsuchiya *et al.*, (1982) found most of the general bacteriological properties to be similar to those of the wild type strains, though phage sensitivity of some mutants had changed. Choi *et al.*, (1981) observed a close relationship between colony types and serotypes of the mutant strains of *Xoo*.

2.3 Evaluation of rice genotypes for bacterial leaf blight resistance

Akhtar and Akram (1987) tested 19 cultivars for reaction to BB at ten sites in different ecological zones under natural epiphytotic conditions in Pakistan. Cultivars Lateefy and IR161-1-1-1 showed the highest mean disease score, and DM16-15-1 the lowest.

Tika and Adekari (1993), evaluated seven, near-isogenic lines of rice in the glasshouse for resistance to Nepalese strains of *Xanthomonas oryzae* pv. *oryzae,* the causative agent of bacterial blight in rice.. Considerable variation in reactions among near-isogenic rice lines was observed. IR-BB3 was highly susceptible to more than 80% of *X. o.* pv. *oryzae* strains, while IR-BB1, IR-BB7, and IR-BB11 were resistant to one-third of the strains. IR-BB4 and IR-BB5 were moderately to highly resistant to nearly 50% of the strains. IR-BB8 exhibited a high level of resistance to nearly two-thirds of the strains of *X. o.* pv. *oryzae* which were evaluated. No specificity of infection was observed, thereby suggesting that these near-isogenic lines are of little value in discriminating amongst pathogenic races of *Xoo*.

Kabir *et al.,* (2007) found 2241 entries as resistant to moderately resistance to BB. Seven entries were consistently resistant whereas 20 entries were reconfirmed to act as a donor source for developing BB resistant variety. In the segregating population, 142 and 79 moderately resistant progenies were selected from F2 and F3 generation respectively in Boro 2007, 142 progenies from F2 populations in T. Aman 2006. Among Near Isogenic Lines (NILs), IRBB21 showed moderately resistant and stable reactions in IRBB lines. In phenotypic analyses of *Xanthomonas oryzae* pv. *oryzae* based on 66 morphological, biochemical and physiological traits of 56 strains, no variation was detected among the isolates and showing existence of homogeneous populations. Conversely, most of the isolates exhibited different groupings in different varieties based on the incubation period which did not correspond with the place of origin except a few avirulent strains. Incubation period was always negatively

correlated with disease development. Variable number of races was noticed when tested on differential varieties.

Shah *et al.,* (2009) compared 14 species of wild rice (*Oryza* sp.) and three widely used cultivated varieties of rice in Pakistan during 2005 to determine resistant sources against virulent isolates of bacterial blight. Amongst wild relatives of rice, *O. nivara*, *O. longistaminata* and *O. grandiglumis* showed resistance against all isolates. *O. nivara* didn't even show lesion against any isolate. The remaining wild species showed differential response to the isolates tested. These species were resistant to one or few isolates but expressed susceptibility to others surprisingly. Bas-385, IR-6 and KSK-282, the cultivated varieties of Pakistan used in this study were found susceptible to most of the isolates.

Ali *et al.*, (2009) screened 15 genotypes against BB and revealed that, Kashmir Basmati was highly resistant showing ≥ 75% resistance to all the tested strains/isolates. Only isolate YR6W14D3 infected this genotype but the severity was not divesting. IR-6, Basmati-370, JP-5 and KSK-370 were ≥ 50% resistant to all the tested strains, while the remaining genotypes were susceptible to all the strains/isolates of *X. oryzae* pv. *oryzae*. Among the four strains/isolates, LKA4 showed 38% severity.

Waheed *et al.*, (2009) conducted some experiments to determine losses in yield and its components in 11 rice genotypes including PARC-291, PARC-292, PARC-293, PARC-294, PARC-295, PARC-296, PARC- 297, PARC-298, PARC-299, PARC-300, PARC-301 under natural field conditions of Mansehra. Significant differences were observed for all the traits studied. The lowest infection was exhibited by PARC-301 with a disease scoring of 42.5, minimum and statistically equivalent number of days to 50% heading were observed in PARC-294 (79 days), PARC-296 (81.3 days) and PARC-299 (81.3 days), maximum plant height 68.5 cm in PARC-298, maximum 25 tillers in PARC-292, maximum value for spike length was exhibited by PARC-296 (21.6 cm), maximum number of grains in PARC-292 (161.3), PARC-292 and PARC-298 out yielded in straw yield and grain yield with a value of 5.6 kg/plot and 2.567 kg/plot, respectively. The genotypes PARC-298, PARC-299 and PARC-301 showed resistance to bacterial leaf blight and out yielded others in grain yield..

2.4 Diversity among strains of *Xanthomonax oryzae* pv. *oryzae*

BB is characterized by a high degree of race cultivar specificity. There are over 30 reported races of isolates from several countries (Mew, 1987, Noda *et al.*, 1996, 2001, Adhikari *et al.*, 1999). A set of races identified in Philippines using five differential rice cultivars (Mew, 1987) has been used widely for identifying and classifying resistance to BB in other cultivars (Ogawa *et al.*, 1991.; Lee *et al.*, 2003). It has been noted, however, that screening for resistance to pathogen populations specific to particular geographical locations and tailoring regional breeding programmes accordingly are important (Mew, 1987).

Xoo also has a high degree of genetic diversity among different isolates, based on RFLP and Pathotype analysis of more than 300 strains from different parts of Asia, using a repetitive insertion sequence (IS) element as the RFLP probe. According to Adhikari *et al.*, (1995) isolates formed five clusters, each with more than one Pathotype. Some correlation of clusters with geographical distribution and specific pathotypes (races) was observed, thereby indicating that tailoring breeding programs for specific regions is indeed a tenable approach for disease control. There was also evidence of movement of strains among regions, which could complicate matters.

Shazia *et al.*, (2009) evaluated local isolates of *Xanthomonas oryzae* pv. *oryzae,* collected from different rice producing areas of Pakistan for virulence on 12 rice lines viz., IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21. Five virulence groups (races) were identified on the basis of virulence of these bacterial isolates on tested lines. No single gene tested was resistant against any virulence group prevalent in Pakistan.

Jyufuku *et al.*, (2009) characterized 57 strains of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from India, Indonesia, Malaysia, Thailand, Taiwan and The Philippines by using PCR fingerprinting and virulence analysis. The strains of *Xoo* were grouped into 13 races on the basis of their pathogenicity to international differential lines. Distribution of races was quite specific to the country. Three strains from India and two from Indonesia were virulent to cultivars containing the bacterial blight resistance gene xa5, whereas most strains from other countries were avirulent to Xa5. Strains from India showed high virulence and broad range of pathogenicity, in contrast to those from Malaysia. Two varieties containing Xa21 and xa5 gene,

respectively, were resistant to almost all Asian *Xoo* strains. *Xoo* collections from Asian countries were also divided into four genetic groups by clustering on the basis of results from PCR based RFLP with IS1112 primers. A partial relationship was found among the genetic groups, countries and races, thereby suggesting that strategies targeting regional resistance breeding and gene deployment are feasible.

Aye *et al.*, (2007) tested 40 Myanmar rice varieties, 10 international differentials of near isogenic lines and 17 varieties of pyramided lines for the resistance to three different pathotypes isolated in Myanmar to identify genetic resources of resistance against the disease. In the inoculation test using international differentials of near isogenic lines, the resistance genes, *Xa21* and *Xa3*, would be effective resistant resources to the diseases. Although bacterial isolate MKM39 was avirulent to IR24, some varieties were susceptible to this isolate. Rice varieties cultivated in Myanmar were thus classified into four groups based on their reactions to three isolates. Group I, which contained one variety, was resistant to all the three isolates of pv. *oryzae* and group II, containing 14 varieties, was susceptible. Group III, containing 23 varieties, were resistant to bacterial isolate MKM39 but susceptible to MKM13 and M3–1. Group IV, containing two varieties, was susceptible to bacterial isolate MKM13 but resistant to M3–1 and MKM39. Furthermore, gene combinations *Xa7*, *Xa3*, *Xa10*, *Xa4*, *xa5* and *Xa4*, *Xa5*, *Xa13*, *Xa21* conferred a broad spectrum of resistance to all three isolates evaluated, supporting the strategy of pyramiding appropriate resistance genes.

2.5 Effect of planting dates on the performance of rice cultivars

Ahonsi *et al.*, (2002) evaluated the effect of plant variety and sowing date on false smut incidence. Varieties reacted differently but followed the same trend at both experimental sites. Rice sown with early rains, between 2 April and 3 May, was virtually free from the disease but rice sown between 16 May and 3 June was the most infected. Varietal differences occurred in false smut infection and planting dates affect disease incidence.

Ghosh *et al.*, (1988) determined the effect of two planting dates and four fertilizer levels on different aromatic rice cultivars, and another nine cultivars during the wet seasons. Thermal and photoperiodic conditions significantly influenced the vegetative (leaf area index and light extinction co-efficient) and reproductive (filled

spikelets/panicle) growth of the crop. Delayed planting significantly reduced grain yield by 0.88 t/ha, amylose content by 0.5% and maturity duration by 10 days. Relative availability of NH4+-N from urea and Azolla influenced the crop growth (leaf area index [LAI], tiller production and leaf chlorophyll content) and nutrient uptake. Supply of inorganic N either alone or in conjunction with Azolla significantly increased grain yield (18-41%) and protein content. These studies suggest that the severity of disease in commercial crops may be reduced by delaying sowing until after mid-June, thus avoiding exposure of young plants to high levels of primary inoculum.

2.6 Survival and source of inoculum of *Xanthomonax oryzae* pv. *oryzae*

Outbreaks of both BB and BLS are more likely to occur during the monsoon season of the south-east Asian and Indian oceans (from June to September) than at other times of the year (Mew *et al.*, 1993). Wind and rain disseminate bacteria from infected rice plants and other hosts, as well as contaminated rice stubble from previous crop seasons which is regarded as the most important sources of primary inoculum. Severe epidemics often occur following typhoons, the fierce winds, wind-blown rain and hail of which both injure rice plants and disperse bacteria. Other agencies of dissemination include irrigation water (Nyvall, 1999), as well as humans, insects and birds (Ou, 1985; Nyvall, 1999). Other hosts of *Xoo* include several species of wild rice (*O. sativa, O. rufipogon, O. australiensis*) and a number of gramineous weeds (*Leersia oryzoides* and *Zizania latifolia* in temperate regions and *Leptochloa* spp. and *Cyperus* spp. in the tropics). Virtually all species of wild rice can serve as hosts for *Xoo* (Moffett and Croft, 1983), but other alternative hosts have not been identified.

Chattopadhyay and Mukherjee (1974) reported that Infection was low (av. 1.2%) in dry leaf tissues, inner tissues of dry stubbles, and sheath tissues on stubbles and dead roots. In irrigated plots and after pre-harvest showers, live tissues of leaves of self-sown plants, ratoon plants, rooted stubbles and roots showed high infection, (av. 14.3%). Two days after harvest infection was 1.5% in dead and 16.8% in live tissues. After 17 days infection disappeared from dead tissues, but fell by only 0.7% in live tissues. In unsterilized soil the bacterium survived for 7 days, while in sterilized soil

up to 30 days. In general it survived in soil with 50% moisture. Pure cultures lasted longer than leaf inoculum in the soil.

Mohiuddin *et al.*, (1977) showed that 10-13% of rice plants grown over wheat debris developed bacterial blight symptoms at 6-8 weeks, whereas those grown in pots after the other crops did not. Similar results were obtained in vitro using a nutrient solution. They hypothesised that pathogen may overwinter in the rhizosphere of wheat

None of several gramineous weeds and crop species inoculated with *Xoo* developed disease (Ou, 1985). In temperate regions, *Xoo* can survive the winter in the rhizosphere of weeds of the genera Leersia and Zizania as well as in the base of the stem and the roots of rice stubble (Mizukami and Wakimoto, 1969). Additionally, *Xoo* can survive in the soil for 1–3 months depending on the soil moisture in temperate regions.

Murty and Devadath (1984) exhibited that the bacterium survived longer (170–180 days) in kharif than rabi (120–130 days) harvested seed. The percentage of infected seeds was higher in kharif than rabi. The infected seed on sowing failed to produce the symptoms on respective seedlings due to the low number of bacterial population.

Akhtar *et al.*, (2008) reported that thirteen isolates showed typical colony characteristics of *Xoo* on Nutrient agar medium and were positive for their hypersensitive reaction on tobacco leaves. It was concluded that *X oryzae pv. oryzae* can survive on rice leaves for more than three years; indicating rice leaf debris could serve as source of inoculum for the spread of BB. Out of six rice husk samples collected from different rice mills, five isolates were isolated showing positive hypersensitive reaction on tobacco. It showed the bacterium can also survive on rice husk in the field. The bacteria can only move short distances in infected crops. The only means of long-distance dispersal is the infected rice seeds. The bacteria are usually found in the glumes, but may also penetrate the endosperm. Seed transmission is not considered to be a particularly important means of carry-over of *X. oryzae* pv. *oryzae* in infested countries. It is; however, sufficiently frequent to present a quarantine risk. For *X. oryzae* pv. *oryzicola*, planting of disease-free seed is considered of utmost importance for disease control.

Mary *et al.*, (2001) revealed that survival in infected plant material definitely will play a critical role in the recurrence of bacterial blight in Kuttanad, if infected seed material was used for raising next crop especially in an area having immediate past history of bacterial blight. It was observed that many farmers habitually used seeds from a previous crop without knowing whether such seeds were infected with the pathogen or not. If such infected seeds were used before the end of the apparent period of viability of the pathogen in the seed material, these could serve as a potential source of inoculum for the subsequent crop.

Singh *et al.*, (1980) observed that the bacterium could survive for about ten months at room temperature which was sufficient to cause an epidemic under favorable conditions. In a continuous cropping pattern of Kuttanad, the interval between two successive seasons was only around 30 days; the infected seeds could thus serve as a potential source of primary inoculum for the recurrence of the disease in the region. It was found that due to various reasons in Kuttanad there seems to be continuity in cultivation between two cropping seasons. As such fields were not fully cleared of infected stubbles thereby providing another source of inoculum for infection to the subsequent crop. The pathogen could survive upto 28 days under field condition. It is therefore to let the field exposed to sunlight or under flood fallowing after harvest for a minimum of one month to ensure complete destruction of the pathogen.

Singh and Rao (1975) revealed that the bacterium diffuses into water during imbibitions prior to germination, but bacterial cells are killed before the first leaf develops, thereby failing to infect seedlings (Goto *et al.*, 1988). It is not clear why the bacterial cells in seed hulls decline so rapidly. It could be due to lower tolerance for adverse soil environment including antibiotics, or lower competitiveness than other soil microorganisms. Bacteriophages may also be involved (Kauffman and Reddy, 1975) but may not be critical. *Xoo* may also be present as an epiphyte on the plant surface. The resident phase of the bacterial pathogen could also result from germination of infected seed, and the bacterial cells carried by seed might "migrate" during imbibition to the surface of the seedlings and eventually to the main field during transplanting. Although, no evidence has confirmed this speculation, thus needs to be substantiated.

Xoo could be detected in seed, especially immature panicles, although their frequency and number are much lower in mature panicles. In temperate environment, the bacteria were detected and shown to survive for about 5 months during winter (Mizukami, 1961). The length of survival in seed is critical to determine the possibility of seedborne transmission. Survival period of 9 months was reported by Singh and Rao (1975) and Singh *et al.*, (1983). Results with phages indicate that free phages could be detected in the immature seed up to 1.5 months after collection. In mature seed, both free and adsorbed phages were detected up to two weeks after harvest.

Approximately 102 cells were estimated in a seed (Wakimoto, 1955). Phages could not be detected which is an indirect means of detecting bacteria, in seed stored at 6 and 28 °C for two weeks. It seems clear that, wherever seed is stored, the bacteria can live only for a short while, i.e., a few weeks.

The disease index of seed lots collected from IRRI fields was correlated with the isolation of adsorbed phages. The adsorbed phages were isolated from seeds collected from severely infected plants. It appears, therefore, that *Xoo* may be seedborne; although, seedborne inoculum may not play an important role in disseminating the disease, especially in areas where disease is prevalent. However in regions where the disease has not been reported, seedborne inoculum may become essential in disease transmission.

Liao *et al.*, (2003) used specific probes to developed real time PCR for detection of *X. oo* and *X.o.oryzicola.* They developed a protocol for detection of the pathogen from seed washings even from little amounts as 0.3g leaf and 10g seeds. The whole process was completed in only in two hours with no chance of contamination and 100 times more sensitive than common PCR method.

Sakthivel *et al.*, (2001) explored some molecular techniques for *Xoo* pathogen in rice seed. Naturally infected seed (of cvs Jaya and TN1) washings were used. They find that pathogen can be recovered 4-9 months naturally infected seeds. The *Xoo* was detected in mature plants, seedlings and seeds naturally infected seed grown plants.

Shoichi *et al.*, (2002) screened and assayed rice plants and weed (Leersia) infected with *X. oryzae pv. oryzae*. Diseased rice plants were found in eight of the 17 areas

surveyed of these diseased and PCR-positive Leersia plants were found in four areas; asymptomatic but PCR-positive Leersia were found in two areas; while two areas were found with Leersia. In the remaining nine areas, all Leersia were asymptomatic and PCR-negative. PCR assay could therefore prove useful for detecting the pathogen and can safely be used as a forecasting method for bacterial blight of rice.

2.7 Yield losses from bacterial leaf blight

Rajarajeswari and Muralidharan (2006) showed that Disease prevalence at levels above the economic threshold (score 45) ranged from 7 to 39%. Studies were made with samples from representational farms taken at the rate of one farm or every 10% disease prevalence out of 100 farms per district. The spatial pattern of BB development within and among rice hills was either random or uniform. On the representational farms, the mean BB severity was 65–71%. Disease injury caused highly significant reductions in the well-filled grains harvested. In the Cramer method, the per cent yield loss was derived by dividing the difference between the means for well filled grains in healthy and diseased by the potential yield, i.e., actual yield from healthy plants to which the difference was added. The yield loss calculated by the Cramer method was 17–31% for BB epidemics in all four districts. Partially filled grains and chaff/hill showed minor variations, but their influence on grain harvest was negligible. In the representational farms investigated, there was a highly significant and negative correlation between well-filled grains/hill and BB severity. The yield loss estimated on the attainable yield using regression models ranged from 31 to 44%. In the linear models, the coefficient of determination ranged from 0.61 to 0.68 on three farms, and was 0.21 and 0.39 on two other farms. The Cramer and regression methods gave similar estimates for loss of potential yield in only one farm, located in Karnal district. In calculating production loss, the actual production in the entire district is usually taken, assuming damage from disease at all farms, and this leads to an over-estimation. A modification was made to consider disease prevalence as a percentage of farms with disease above the economic threshold level in a district. This adjustment enabled the production loss estimate to be tailored to the actual area damaged by the pathogen. By multiplying district production estimates by the disease prevalence (%), production loss in a district was precisely derived. In BB epidemics, the most conservative estimates for production yield losses were 3–16% in surveyed districts. The production losses during the four epidemics were considerably different, ranging from 92,000 to 105,000 tones in Nellore, 30,000 to 36,000 tones in West Godavari, 46,000 tones in Karnal and 22,000 tones in Rangareddy districts of India.

CHAPTER 3

MATERIALS AND METHODS

3.1 Incidence and severity of bacterial leaf blight of rice in Pakistan

Three comprehensive surveys of all rice growing zones of Pakistan (Fig.2) were conducted at the mature grain stage of the crop during 2005-2007, on an annual basis. In KP (zone-1) the Northern mountainous areas as well as the flat or terraced valley-sides including areas such as Chitral, Upper and Lower Dir, Swat, Buner, Shangla, Mansehra, plains of Mardan, Swabi, Nowshera, Peshawar and Charsadda, were surveyed. Similarly in Punjab (zone-2), the broad strip of land between the rivers, Ravi and Chenab including Sargodha, Hafizabad, Sheikhupura, Sialkot, Narowal, Gujranwala, Gujrat, Lahore, Kasur and Okara were surveyed. In Sindh province that includes rice growing zone-3 and 4, the large tract of land on the west bank of the river Indus including the areas of Larkana, Sheikarpur, Thatta, Tando Muhammad Khan, Hyderabad, Badin, Dadu and Nawab Shah were surveyed for BB incidence and severity during the study. The area of Usta Muhammad was surveyed in Baluchistan during cropping season from 2005-2007, due to limited rice cultivation.

3.1.1 Field based disease assessment and sampling

At each location surveyed, rice fields were observed visually for the presence and/or absence of bacterial blight. Incidence of disease was recorded in terms of percentage following the schematic diagram (Table 3.1) showing the percentage of plants infected in a field (IRRI, 1996).

For calculating actual incidence of disease in a field, plants were observed at ten points along a diagonal transect (IRRI, 1996). Points were selected randomly five paces apart, starting ten meter inside the field. At each point, five plants were examined for disease symptoms. Actual incidence of disease in a field was then recorded as percentage of infected plants out of total plants examined as outlined below.

$$\text{Disease Incidence \%} = \frac{\text{Number of bacterial blight infected plants}}{\text{Total number of plants examined}} \times 100$$

Severity of disease in a field was recorded as percentage of tissue area infected out of total leaf area examined. Percentage average lesion area of 15 leaves collected was measured for disease severity in each field. Following scale was used for scoring of BB severity in field (Chaudhary, 1996, Table 3.1).

Table 3.1 Disease severity scale

Disease Rating	Lesion size (% of leaf length)
0	0
1	>1-10 %
3	>11-30 %
5	>31-50 %
7	>51-75 %
9	>76-100 %

For calculating the amount of disease in an area following formula (IRRI, 1996) was applied;

$$\text{Disease Index} = \frac{n(1) + n(3) + n(5) + n(7) + n(9)}{tn}$$

Where: n (1), n (3), n (5), n (7) and n (9) = Number of leaves showing severity score of 1, 3, 5, 7 and 9.

tn = Total number of leaves scored

3.2 Assessment of hypersensitivity response and pathogenicity test

3.2.1 Isolation and characterization of *Xoo* isolates from KP

During the last survey (2007) a randomly selected composite representative sample of ten leaves exhibiting typical symptoms of bacterial blight were collected from each district of KP (zone-1), for pathogen isolation. Samples were placed in plastic bags appropriately labeled and sealed. Samples were then brought to plant pathology laboratory, at Agricultural University Peshawar and stored at 4 °C for further analysis.

Isolation of *Xoo* collected from 125 samples was carried out at National Agricultural Research Centre, Islamabad during 2007. Leaf pieces measuring 3x3 mm in size were excised from the advancing margin of the lesion with a pair of Sesser sterile scalpel

and surface sterilized with 1% Clorox for three minutes under aseptic conditions. Following a quick rinse in sterile distilled water (SDW), seven leaf pieces were blotted dry on sterile blotting papers and transferred to Petri plates containing yeast extract-dextrose-calcium-carbonate (YDC) agar medium (see appendix) using a laminar flow unit. For three days plates were incubated at 27°C (Wilson *et al.*, 1967). Xanthomonads produce mucoid, doomed, yellow colonies on YDC plates which were sub-cultured to obtain pure culture and stored for further use. Cultures were suspended in SDW or grown on YDC slants, and stored at 4 °C for short-term preservation. For long-term use, however, cultures were preserved in soil by pippetting approximately 0.5 ml of inoculated, heavy nutrient broth suspension onto approximately 3 g of sterile loamy garden soil in small, test tubes (75x10 mm) or bijou bottles. The soil was air-dried, passed through a 2 mm mesh sieve to obtain finer particles, autoclaved thrice at 121 °C for 1 h and stored at 4 °C. Bacteria were recovered by plating the infested soil onto YDC agar plates whenever needed (Lelliot and Stead, 1987).

Hypersensitivity Reaction (HR)

Hypersensitivity response of the isolates was assessed on tobacco (*cv. virginia*) plant grown in earthen pots containing loamy soil. Approximately 10^8-10^9 CFU ml^{-1} of freshly cultured bacteria were injected onto the abaxial surface of tobacco leaf with a hypodermic syringe at 5-6-leaf stage. Inoculated plants were then kept in a moist chamber for a few hrs to promote symptom development which was later shifted to the screenhouse. Controls were similarly inoculated with SDW. Complete collapse of tissue after 24 h, followed by necrosis was interpreted as positive reaction (Klement and Goodman, 1967).

Pathogenicity Test

All 80 isolates selected on the basis of colony morphology and strong hypersensitivity reactions were used. For pathogenicity test, inoculum was prepared by streaking a loopful of each isolate in the middle of nutrient agar plates and incubating at 25-27 °C. The bacteria were washed from the plate surface after 24 h with 5 ml of SDW. The inoculum thus prepared was serially diluted and adjusted to a concentration of 10^{-7}- 10^{-8} cfu ml^{-1}.

Based on its susceptible nature to BB, cultivar JP-5 was used for pathogenicity test. Seedlings of the cultivar were grown on moist sterilized filter papers in Petri plates, which were maintained in a growth chamber at 30-35 °C (100% RH). Two weeks old seedling were transplanted to small plastic pots (13 cm diameter) and placed on a screenhouse bench. At pre-tillering stage, plants were transplanted again to bigger plastic pots (27 cm diameter) and inoculated with clip inoculation method at panicle initiation stage (60-70 days old plants) as outlined below.

Plants were inoculated using clip method of inoculation by dipping a pair of scissors in the inoculum and clipping off three leaves approximately 2-3 cm from their tip (Kauffman *et al.*, 1973). Three leaves per isolate were thus test inoculated (Di Ming *et al.*, 1991) whereas control was similarly inoculated with SDW. The plants were wrapped in moist plastic bags to conserve moisture and placed in a screenhouse at 25-27 °C immediately after inoculation (Schaad, 1980) until optimum development. Lesion size was then measured with a scale (Table 3.1) along both the leaf axes, 18 days after inoculation (DAI). The effect of isolates on mean lesion size was determined with two factorial F test. Means showing significant differences were separated by least significant difference (LSD) test. Re-isolation were made from infected plants to fulfill Koch's postulates.

3.3 Biochemical Characterization

3.3.1 Gram staining

Gram staining procedure was performed as described by Gerhardt (1981). Bacteria were heat fixed on a glass slide treated with (0.5%) crystal violet for 30 seconds then washed with tape water. Then treated with Iodine for 1 min, washed again and decolorized with (95%) ethanol for 30 sec, washed again and counter-stained with safranin. Magnifications of X-10 and X-40 were used microscopic observation. G-ive bacteria stained red whereas G+ive retained the color of crystal violet.

3.3.2. 3% KOH (Potassium hydroxide) test

3% KOH test confirmed results of Gram staining (Suslow *et al.*, 1982). The bacterial culture taken tooth pick was vigorous stirred in drop of 3% KOH solution. Thread-like slime formation when picked the toothpick indicated the presence of G-ive bacterium. But no slime or thread formation was the indication of G+ive bacterium (Schaad, 1980; Ryu, 1940).

3.3.3. Starch hydrolysis test

For each hydrolysis test 20 g Nutrient Agar (NA) was added to 80 ml of water and dissolved by successive heating and stirring similarly two gram starch was then thoroughly dissolved in 10 ml distilled water separately and added to hot molten agar with through stirring. Amount of 100 ml of this basal medium was then transferred to conical flask (250 ml) and autoclaved at 115 °C for duration of 10 minutes. The medium was then poured into Petri plates. The plates were then inoculated with individual isolate aseptically, labeled and sealed to avoid chances of contamination. These plates were then incubated in upside down position at 27 °C for 7 days. After scraping bacterial growth to each plate Lugol's iodine was added which was prepared by mixing 1g iodine and 2 g potassium iodide in 300 ml distilled water, stirred for until dissolved completely. The appearance of clear zones around the colonies was indicative of presence or absence of starch hydrolysis as described by Cowan (1974).

3.3.4. Tween 80 hydrolysis test

Sterilized Tween 80 was added to the basal medium [peptone (10 g), $CaCl_2.2H_2O$ (0.1 g), NaCl (5 g), agar (15 g), 1 Lit final volume, pH to 7.2-7.4] to obtain a final concentration of 1%, poured into plates, streak inoculated for each individual isolate and incubated at 27 °C for one week. The reaction was considered positive if milky white precipitate developed around the colonies (Sierra, 1957).

3.3.5 Acid production from carbohydrates

For this assay, 10 ml of medium [$NH_4H_2PO_4$ (0.5 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (5 g), yeast extract (1 g), agar (12 g) water (1 L), bromocresol purple (1.5% alcohol solution 0.7 ml)], was dispensed in test tubes and autoclaved at 121°C for 15 minutes. A 10% (w/v) aqueous solution of glucose, fructose, sucrose and

galactose was prepared, filter-sterilized through Millipore injection and added to the molten base to give a final concentration of 1%. Each isolate was then transferred aseptically into a tube, incubated at 27 °C and checked for acid production from carbohydrates after two, four and six days. Production of yellow color indicated production of acid (Dye, 1968).

3.3.6 Egg yolk reaction

Egg yolk emulsion was prepared from a fresh egg, which was washed well in soap solution, rinsed and surface sterilized with 70% ethanol for 5 minutes. The egg was then flamed, broken aseptically, yolk separated into a sterile graduated cylinder and diluted to 40% v/v with sterile water. An aliquot of 10 ml of the egg yolk was incorporated into 100 ml molten nutrient agar (cooled to 55 °C) prior to pouring into the plates. The medium was spot cultured and incubated for three days at 27 °C. Production of turbid zone of free fats around the colonies was considered positive (M_CClung and Toabe, 1947).

3.3.7 Tetrazolium salt tolerance test

Nutrient agar was prepared and dispensed in 100 ml flasks and sterilized at 121°C for 15 minutes. Aqueous 1% TTC solution was aliquoted (filter-sterilized) to the molten agar at 55°C to give a concentration of 0.02%. Similarly, 0.1% concentration was also prepared. The medium was then dispensed in plates and inoculum was added to the medium held at two different concentrations whereas nutrient agar alone served as control. Presence or absence of growth was recorded since most xanthomonads are inhibited at 0.02% and completely inhibited at 0.1% concentration of TTC.

3.3.8 Anaerobic growth test

5 ml Basal medium [Peptone: 2g; NaCl: 5g; Agar: 0.3g; KH_2PO_4: 0.3g; bromothymol blue: (3 g) in 1% aqueous solution, 1 lit final volume] was poured into test tubes and sterilized at 121°C for 20 minutes. An amount 0.5 ml 10% glucose suspension was added to each tube aseptically. For each isolate two test tubes were inoculated. One of the tubes was sealed with paraffin, and incubated at 27°C. Anaerobic growth was noticed if color change occurred from blue to yellow (Hugh and Leifson, 1953).

3.3.9 Oxidase test

One day old bacterial colony, grown on nutrient agar as described previously, supplemented with 1% glucose was used in this assay. A loopful of the inoculum was rubbed onto a filter paper impregnated with 1% (w/v) freshly prepared aqueous solution of tertramethyl-p-phenylene diamine dihydrochloride. The isolate was rated oxidase-positive if a purple color developed within 10 seconds, delayed positive if coloration developed within 10-60 seconds; and negative if no color developed after 60 seconds (Kovacs, 1956).

3.4 Identification of bacterial leaf blight Pathogen through PCR

Overlapping results in *Xoo* and cross contamination with other identical isolates made it necessitated to confirm the identity of *Xoo* isolates through PCR.

3.4.1 DNA extraction

DNA was extracted from *Xoo* isolates with DNA Zol-direct, commercially available kit (Mole. Res. Center Inc. Cincinnati, Ohio state, USA). The bacteria were grown in a shaking incubator at 27 °C and in 5 ml LB broth. 0.5 ml of the growing culture was poured into eppendorf tube and centrifuged for 10 minutes at 4 °C using 7000 rpm to pellet the DNA. The supernatants were discarded and pellet was mixed with DNA Zol-direct (100 µl). After 15 min template DNA was ready to use.

3.4.2 PCR analysis

For molecular confirmation of the pathogen polymerase chain reaction (PCR) was performed with *Xoo* specific primers, XOR-F 5'-GCA TGA CGT CAT CGT CCT GT-3' and XOR-R2 5'-CTC GGA GCT ATA TGC CGT GC-3' (Adachi and Oku, 2000). 3 µl of the lyesate was directly used in PCR reaction. The PCR master mix included 2 mMol $l^{-1}MgCl_2$, 0.2 mMol l^{-1} dNTPs, 1 µMol l^{-1} each primer, and *Taq* buffer (67mM l^{-1} Tris HCl pH 8.8). In order to prolong the working life of the enzyme, 5 U (1 µl) of Taq DNA polymerase was added to PCR tube after the initial (one time) denaturation (95 °C for 5 minutes) step. DNA amplification was performed in a MJ mini thermocycler (Bio-rad, USA) under the following conditions: 95°C for 5 minutes (once), 94 °C for 30 sec, 47 °C for 30 sec and 72 °C for 50 sec. The number of cycles was kept 40 at the end a final extension of 72 °C for 8 min was

given for completion of the process. PCR master mix was prepared as Primer F and R = 1 µl each, dNTPs = 5 µl, $MgCl_2$ = 4 µl, *Taq* buffer = 4.5 µl and *Taq* polymerase enzyme = 1 µl. 3 µl of template DNA was added for each isolate to PCR tube. Nuclease free water 30.5 µl was added to make 50 µl final volume. 25 µl of PCR product after amplification was electrophoresed through on 2% (w/v) agarose gel as described by Sambrook *et al.,* (1989).

3.4.3 Gel electrophoresis

2% agarose gel was prepared by dissolving 0.66 g in 30 ml TBE buffer, heated in an oven for 2 min and poured into gel tray when it temperature was about 45-50°C and allowed for solidification. The gel was completely submerged in TBE buffer. The PCR products was added by 2 µl blue tracking dye and loaded separately into the wells of the gel. 1 kb DNA ladder was also used as marker for comparison. A negative control was also included (master mix only and extracted DNA). Electrophoresis was performed at 200 volts for 30 min. After completion the gel was stained in 0.5 µg/ml ethedium bromide solution for 15 min, distained in water. Glowing bands were observed under UV light and photographed using UV-tech machine (ESSENTIAL, Model: D-55-20-M-Auto, UK).

3.5 Comparison of Inoculation Methods

Six commercial rice cultivars including, BAS 2000, BAS 385, JP5, IR6, BAS 370 and Super BAS, were used in the experiment. Plastic pots (10 cm diameter) containing screenhouse potting mixture, were used for planting 10 seeds of each cultivar. A culture of *Xoo* was revived on yeast extract dextrose Calcium corbonate (YDC) (Schaad, 1980) at 27 °C for 72 hrs., for inoculation. Bacterial concentration was adjusted to 10^8 colony forming units (cfu/ml). The inoculation methods were as follows:

3.5.1 Detached leaf assay

Detached leaves were thoroughly washed under running tap water to remove dirt and then surface disinfected in 70% Ethanol for one minute and rinsed thrice with sterile distilled water. The leaves were placed abaxial side up on four layers of sterile paper towel on two glass slides in 30 cm glass dishes. The leaves were then inoculated with a multiple pin mount prepared with four insect mounting pins of 0.1 mm diameter in a

25 mm square piece of styrofoam. The pins were initially dipped in the bacterial suspension of the test strain prepared in 0.1 M phosphate buffer saline (PBS) having a concentration of 10^8 cfu/ml as determined with a spectronic 20 (Baush and Limb) adjusted to an absorbance of 0.1 at 590 nm. The leaves were pricked with inoculum laden pins. Following inoculation, three leaves were kept in each glass dish. Similarly, the leaves were inoculated by dipping the inoculating scissors in inoculum kept in a beaker. In the third method the inoculum was applied with a brush. Each treatment was replicated three times. Leaves inoculated with PBS only served as control. The leaves were incubated at 27 ^{0}C under 14 hrs illumination. Disease data were then recorded on lesions length (cm) after 14 days of inoculation.

3.5.2 Pot experiment

Six commercial rice cultivars including, BAS-2000, BAS-385, JP-5, IR-6, BAS-370 and Super-Bas were used in this experiment. Ten seeds per pot were planted in plastic pots (10 cm diameter) containing screenhouse potting mixture. Three leaves/plant and three plant in each pot randomly selected, were inoculated with the bacterial suspension of 10^8 cfu/ml prepared in PBS. Leaves of seedlings were sprayed with sterile water to keep them moist and bacterial suspension was then applied using three methods of inoculation as described previously. One month old plants were inoculated with bacterial suspension of *Xoo*. Plants in control treatment were inoculated with sterile PBS only. Following inoculation, plants were covered with polyethylene bags containing water saturated cotton for 24 hrs to facilitate symptom development. Plants were kept at 27 °C with 70% relative humidity during the course in the experiment. Data on lesion development were recorded 15 days after inoculation

3.6 Screening of rice cultivars for bacterial leaf blight resistance against KP isolates

34 rice cultivars (Table 3.2) obtained from NARC, Islamabad were screened for resistance against KP races of bacterial leaf blight and to observe quantitative traits under field conditions. These experiments were conducted at Agricultural University, Peshawar, during cropping season, 2007.

3.6.1 Preparation of nursery

Nursery of 34 cultivars and line was prepared in earthen pots at screenhouse of the Department of Plant Breeding and Genetics, Agricultural University, Peshawar. The pots filled with puddled soil and with enough organic matter were sown and allowed to germinate. Pots were watered daily and 30 days old plants were then transplanted in the field.

3.6.2 Field preparation

The experiment was conducted in a field measuring 100 m x 18 m at Agricultural University, Peshawar Research Farm. Transplanting of all cultivars and lines were done in well puddled field, keeping row length of 2 m, row to row and plant to plant distance as 30 cm and 20 cm, respectively. All required agronomic practices such as fertilizer, irrigation and weeding were applied per requirement of the crop.

3.6.3 Field layout and inoculation

The experiment was conducted by using RCB experimental design and replicated thrice. The data on BLB and other yield parameters were recorded at different stages of the crop. The plantation of the crop was done manually and routine visits of the field were made daily. 30 days old plants were clip inoculated with each KP race separately. This was done by clipping off five leaves of each individual cultivar with sterilized pair of scissors dipped in inoculum suspension of bacterium.

Table 3.2. Rice varieties obtained from National Agricultural Research Center (NARC) used in the present study

Serial number	Varieties
1	Bas-2000
2	Kangni-27
3	Shadab-31
4	Shaheen Basmati
5	Malhar-346
6	Pakhal
7	Sug Desi
8	Kashmir Basmati
9	Basmati-370
10	JP-5
11	DR-82
12	Swat-1
13	NIAB-IR-9
14	IR-8
15	Basmati-Pak
16	PK-177
17	Sarshar
18	Dilrosh-97
19	Sada Hayat
20	Sathra
21	TN-1
22	DR-83
23	Khusboo-95
24	Basmati-2008
25	Shua-92
26	Dokri Basmati
27	Bas-6129
28	Muskhan
29	Jajai-77
30	DR-92
31	Rachna Basmati
32	Lateefy-98
33	Fakhr-e-Malakand
34	Bas-385

3.6.4 Disease severity (%)

Disease severity data were recorded according to the scale (Table 3.3) of Chaudhry (1996). Data were recorded on five plants selected randomly in each replication block as percent area of the plant infected with KP races and categorized accordingly.

$$\text{Disease severity (\%)} = \frac{\text{Lesion length on leaf (cm)}}{\text{Total length of leaf (cm)}} \times 100$$

Table 3.3. Disease severity scale and category of host resistance

Disease Rating	Lesion size (Percent of leaf length)	Category
0	0	Highly resistant (HR)
1	> 1-10 %	Resistant (R)
3	> 11-30 %	Moderately resistant (MR)
5	> 31-50 %	Moderately susceptible (MS)
7	> 51-75 %	Susceptible (S)
9	> 76-100 %	Highly susceptible (HS)

3.7 Determination of *Xoo* pathotypes of KP isolates using IRBB lines

A set of near isogenic lines was used to differentiate various isolates collected in the present study into different races. Out of total 125 isolates, obtained from 12 rice growing zones of KP, 80 isolates were characterized on set of differential host cultivars i.e. IRBB lines by using methods of naming the races developed by Habgood (1970) and modified by Limpert and Muller (1994). Data recorded on the spectrum of their pathogenicity on these IRBB lines were used to categorize these isolates into defined races. This race coding system contains information for virulence of the race which can be rated on a dichotomous scale, assigning 0 for avirulence on a differential and 1 for virulence (Habgood, 1970). Each pathogen isolate was classified by its disease reactions on a chosen differential set, resulting in a sequence of 0s and 1s, the 'pathotype vector'. These 0s and 1s served as a binary number, whose value is converted into its decimal (decanary) representation, giving a binary/decanary code (Habgood, 1970). The number derived in decanary conversion then serves as a race name for that particular pathotype and translation back to the binary code reveals the

spectrum of virulence. Initially, the pathotypes vector is determined by the following notation by adopting modified formula and adopted by Limpert and Muller (1994).

$$\vec{P} = (1, 0)$$

$$\vec{W} = (2^0, 2^1, \ldots, 2^{n-1})$$

$$\vec{P}.\vec{W} = (1, 0.2^0 + 1, 0.2^1 + \ldots.. + 1, 0.2^{n-1})$$

Where $\vec{P}$ is pathotype vector (Virulence or avirulence reaction on host i.e. 1or 0)

n = number of differential

$\vec{W}$= weight vector

Table 3.4. International differentials with Near-isogenic lines with different Single resistance genes to bacterial leaf blight of rice and developed in the genetic background of IR24, ecotype Indica.

Differentials	**Resistance Gene**
IR-BB 2	Xa-2
IR-BB 3	Xa-3
IR-BB 4	Xa-4
IR-BB 5	Xa-5
IR-BB 7	Xa-7
IR-BB 8	Xa-8
IR-BB 10	Xa-10
IR-BB 11	Xa-11
IR-BB 13	Xa-13
IR-BB 14	Xa-14
IR-BB 21	Xa-21

For this purpose, seeds of 10 near isogenic lines (Table 3.4) with single resistant gene were obtained from NARC, Islamabad. The lines included IRBB 1, IRBB 2, IRBB 3, IRBB 4, IRBB 5, IRBB 7, IRBB 10, IRBB 11, IRBB 14, and IRBB 21 (Huang *et al.*, 1997). Beside these international differential lines, IR 24 was used as susceptible check. Seedling nursery of the IRBB lines were raised in trays filled with puddle soil. Twenty day old seedlings were transplanted to plastic pots (100 cm diameter). Recommended dose of NPK was added in the form of ammonium sulfate, super phosphate and nitrate of potash. Plants were watered daily and then 5g urea was added 30 days after transplantation to each pot. The experiment was arranged in a split plot design where the isogenic lines were the main plot and bacterial isolates as subplots. Three plants of each isogenic line were inoculated with each of the 80 isolates of *X. oryzae* pv. *oryzae* using clip inoculation method as described previously (Section 3.5) 40 days after sowing (Kauffman *et al.*, 1973). The bacterial suspension of each isolate for inoculation was prepared using two days old culture in 10 ml sterilized distilled water and adjusting the inoculum concentration to 10^8 CFU/ml.

For inoculation, 1 to 2 cm of the tips of three fully expanded leaves of each plant in a pot were clipped with a pair of scissors dipped in inoculum prior to inoculation. Control plants were inoculated similarly with sterilized distilled water

3.7.1 Disease assessment

The lesion length (cm) from the cut leaf tip was measured 18 days after inoculation. Disease reactions were categorized into two groups according to lesion length developed after inoculation. Lesion length between 0 to 6 cm was classified as resistant (R) whereas that of more than 6 cm as susceptible (S) (Sanchez *et al.*, 2000).

3.8 Determination of source of over-wintering inoculum of *Xanthomonas oryzae* pv *oryzae* in KP

3.8.1 Isolation of *Xanthomonas oryzae* pv. *Oryzae* from rice field water

Fifty water samples from rice field were collected in sterilized plastic bottles at booting stage. A total of 16 samples were collected from Peshawar and 17 each from Charsadda and Swat. Samples were brought to the laboratory for isolation of the pathogen. In each field of a particular location, 5 representative samples were

randomly selected at several points and then combined to obtain a composite, representative sample.

For isolation, an aliquot of representative water sample was spread on YDC (15 g Agar, 10 g Yeast Extract, 20 g Dextrose and 20 g $CaCo_3$ per litter) medium using serial dilution method to ensure properly isolated growth of the pathogen. The plates were incubated at 29 °C for 48-72 hrs (Wilson *et al.*, 1967).

After incubation single isolated colony showing mucoid, doomed, yellow bacterial growth of *X. oryzae* pv *oryzae* were selected for obtaining pure culture and were maintained for further study. Different biochemical tests, as described previously, were carried out to confirm the pathogen.

3.8.2 Isolation of *Xanthomonas oryzae* pv. *Oryzae* from weeds

A set of 50 samples of 7 weeds prevalent in rice fields were collected from rice growing areas of Peshawar, Charsadda and Swat which included *Cyperus difformis* L, *C. iria* L, *C. rotundus* L, *Dactyloctenium aegyptium*, *Echinochloa crus-galli*, *Paspalum paspalodes* and *Leersia hexandra*. The samples were shipped to the plant pathology laboratory, Agricultural University and subjected to pathogen isolation. Plants were excised into small pieces, surface sterilized by dipping first in 20% Clorox solution for one min and then rinsed twice with sterilized distilled water (SDW). The excised pieces were blotted dry and aseptically placed on Yeast extract-Dextrose-$CaCO_3$ (YDC) medium in petri plates and incubated at 29 °C (Wilson *et al.*, 1967). Plates were assessed daily for growth of the pathogen.

Plates showing characteristic bacterial growth were selected for obtaining pure culture for further study. Different biochemical tests as outlined previously, were carried out to confirm the identity of the pathogen.

3.8.3 Isolation of *Xanthomonas oryzae* pv. *oryzae* from plant residues

Samples of rice plant residues such as leaves, straw and ratoons were collected two weeks after harvesting of the crop and shipped to plant pathology laboratory, Agricultural University, Peshawar for isolation of *Xanthomonas oryzae* pv. *oryzae*.

Samples were washed under running tape water to remove dirt, surface sterilized with 20% Clorox for one minute, rinsed twice with sterilized distilled water (SDS) and

placed on YDC medium in petri plates to be incubated at 29 °C (Wilson *et al.*, 1967). Bacterial colonies thus developed were then sub cultured onto fresh YDC plates for further studies.

3.8.4 Isolation of *Xanthomonas oryzae* pv. *oryzae* from seed

A composite sample of rice seed from Swat, Khyber Pakhtunkhwa was collected. Seeds were treated with 20 % Clorox, and rinsed twice with SDW to remove excess of disinfectant. Using a mortar and pestle the seed was crushed in sterilized distilled water and an aliquot of the resulting suspension was dispensed on YDC plates. Which were incubated at 29 °C for 48 hrs. Single isolated colonies were selected for obtaining pure culture of the pathogen. Different biochemical tests were also carried out to confirm the identity of the bacterium.

3.8.5 Cumulative frequency (%) of *Xanthomonas oryzae* pv. *oryzae* recovery

Pure culture of the causal organism obtained from above sources for physiological characteristics and biochemical tests as described earlier were made to confirm the bacterium as *Xoo*. For determination of frequency percentage of the recovery of the causal pathogen from above sources the following formula was applied.

$$\text{Frequency percentage of recovered pathogen} = \frac{\text{Total recovered isolates}}{\text{Total samples assayed}} \times 100$$

3.9 Effect of planting dates on bacterial leaf blight infection and rice yield components.

A two year multilocation study was conducted at the Agricultural Research Institute Mingora (North) and Agricultural Research Station, Baffa, Mansehra during 2007 and 2008. During each year, five rice cultivars including, Swat-1, Basmati-385, Dilrosh-97, Fakhr-e-Malakand and JP-5 were subjected to three transplanting dates viz., June 5, June 15 and June 25. Trials were replicated four times in a split plot design. The row length was maintained at 2.5 meters with 20 cm plant – plant and 30 cm row – row distance. Planting dates were kept in main plots while cultivars served as sub plots. For each transplanting date, 30 days old seedlings of each variety were used. NPK fertilizer at the rate of 120:60:40 kg/ha was applied to the field in two split

doses. NPK at the rate of 60:60:40 kg/ha was applied at the time of transplanting and 60:0:0 kg/ha NPK after one month of transplanting. Data on BLB severity were recorded 20 days after flowering (Booting). Data on yield and yield parameters were recorded at maturity. Disease severity was recorded according to the scale of Chaudhry (1996).

CHAPTER 4

RESULTS

4.1 Disease incidence and severity of bacterial leaf blight in Pakistan

4.1.1 Disease incidence (%) in Khyber Pakhtunkhwa

Disease incidence in (KP) province during the period 2005-2007 is presented in Table 4.1. During 2005, the highest disease incidence (80.2 %) in KP was recorded in district Shangla, followed by Chitral, Swat and Mansehra districts. Conversely, the lowest disease incidence was recorded in district Charsadda (50.4 %) followed by district Swabi, Lower Dir and Mardan with incidence of 56.8, 61.0, and 61.6%, respectively.

During rice growing season of 2006, the overall disease incidence was more than the year 2005. Yet again, district Shangla excelled with the highest value (75.0 %) of disease incidence, followed by districts Mansehra (74.4%) and Buner (73.0 %). On the other hand, district Charsadda showed the lowest disease incidence (55.4 %) with marginal increase as compaired to the disease incidence in the previous year 2005.

Interestingly, a declining trend of disease incidence in KP was observed during 2007 with maximum value recorded in district Swat (71.8 %), which exhibited almost similar disease incidence in three cropping seasons surveyed. The minimum disease incidence was recorded in district Chitral (35.0 %), which was significantly different from the values recorded in other districts. However, difference in values recorded in districts Buner (69.2 %), Shangla (66.8 %), Upper Dir (63.4 %), Lower Dir (63.0), Peshawar (66.0 %), Mansehra (64.6 %) and Mardan (65.8 %) were not significant.

Table 4.1. Disease incidence (%) in KP during 2005-2007

Locations	Year		
	2005	2006	2007
Chitral	75.8 ab	64.8 bc	35 d
Buner	71.8 b	73 ab	69.2 ab
Shangla	80.2 a	75 a	66.8 ab
Swat	73.8 ab	70.6 abc	71.8 a
Dir(upper)	69.6 b	67.2 abc	63.4 ab
Dir(lower)	61 cd	68 abc	63 ab
Nowshera	68.2 bc	65.4 bc	60.4 b
Mansehra	72.6 ab	74.4 a	64.6 ab
Peshawar	71.6 b	62.6 cd	66 ab
Mardan	61.6 cd	63 cd	65.8 ab
Swabi	56.8 de	64.6 bc	61.4 b
Charsadda	50.4 e	55.4 d	45 c
LSD (P≤0.05)	7.76	8.97	8.81

Means followed by different letters in the same column are significantly different from each others at P≤0.05

Table 4.2. Disease severity (%) in KP during 2005-2007

Locations	Year		
	2005	2006	2007
Chitral	6.83 a	6.33 ab	6 ab
Buner	5.67 bcd	5.67 ab	5.5 b
Shangla	7 a	6.5 a	6.5 a
Swat	6.17 bc	5.5 b	5.33 b
Dir(upper)	6.67 ab	6.17 ab	5.83 ab
Dir(lower)	6.33 ab	6 ab	5.5 b
Nowshera	6.33 ab	5.83 ab	5.33 b
Mansehra	5.17 cde	4 c	4 cd
Peshawar	4.67 de	4.33 c	4.33 c
Mardan	4.17 e	3.83 c	3.33 d
Swabi	4.83 de	4.5 c	3.83 cd
Charsadda	4.67 de	4.5 c	4.17 cd
LSD (P≤0.05)	1.05	0.97	0.86

Means followed by different letters in the same column are significantly different from each others at P≤0.05

4.1.2 Disease severity in KP

Table 4.2 revealed that in 2005, disease severity was hight in district Shangla (7 %), followed by district Chitral (6.83 %) whereas it was the lowest in district Mardan (4.17 %). Disease severity recorded in districts Dir Upper (6.67 %), Dir Lower (6.3 %), and Nowshera (6.3 %) was at par with Shangla and Chitral. Similarly, differences among the disease severity recorded in districts Mardan (4.17 %), Swabi (4.83 %) and Charsadda (4.67 %) were also non-significant.

Disease severity recorded in KP during 2006 was not much different from the previous years. Again maximum disease severity was recorded in distract Shangla (6.5 %) followed by districts, Chitral (6.33 %), Dir upper (6.17 %) and Dir lower (6.0 %). However, the values recorded in distracts Buner (5.67 %), and Nowshera (5.83 %), were not significantly different ($P \leq 0.05$) from the values recorded in Shangla, Chitral, Dir upper and Dir Lower. Similarly, disease severity observed in Mardan district (3.83 %) was the lowest which was statistically at par with the values recorded in Peshawar (4.33%), Swabi (4.5 %) and Charsadda (4.5 %).

In 2007, the highest disease severity was again recorded in Shangla (6.5 %) followed by Chitral (6 %), whereas the lowest disease severity was observed in Mardan (3.33 %). The disease severity observed in Dir upper, Buner, Swat, Dir Lower and Nowshera were statistically non significant when compared with Shangla and Chitral. Similarly, disease severity recorded in district Charsadda (4.17 %), Swabi (3.83 %), Mansehra (4 %) and Peshawar (4.33 %) were non-significant ($P \leq 0.05$) from District Mardan data.

4.1.3 Disease incidence (%) in Punjab

Ten locations namely Lahore, Gujranwala, Kasur, Shekhupura, Hafizabad, Sargodha, Narowal, Sialkot, Okara, Gujrat were surveyed during 2005, 2006 and 2007 for disease incidence and severity of BLB (Table 4.3).

In 2005 the highest incidence was recorded in Gujranwala (71.2 %) followed by Sialkot (68.2 %) and Shekhupura (65.4 %) in Punjab, whereas the lowest disease incidence was observed in Hafizabad (53.8 %). Disease incidence over years in Punjab, showed that the disease during 2006 exceeded when compared with other years at all location as high incidence was recorded during this cropping year. Overall

incidence recorded in 2007 showed a decline among all locations except Lahore where, the value recorded was the highest (67.8 %).

In 2005, the highest disease incidence was recorded in Kasur followed by Narowal and Gujrat while the lowest disease incidence was observed in Hafizabad though non significant differences were found among the incidence recorded in Sialkot, Gujrat and Narowal but significantly different incidence was recorded at Kasur and Hafizabad. Similarly, differences in incidence observed in Lahore Gujranwala and Sargodha were non significant but were significantly different from all other locations.

Maximum disease incidence was observed in Okara (74.6 %) and Gujrat (73 %) during 2006, whereas, the lowest incidence was recorded in Lahore (56 %) and Kasur (58.2 %). However, differences among, Gujranwala (66.2 %), Sargodah (70 %), Shekhupura (68.2 %) Hafizabad (66.8 %) and Sailkot (69.6 %) were statistically similar. However, the differences in disease incidence at narowal (63 %) Gujranwala, Hafizabad, Shekhupura, Sargodah, Sailkot, Kasur, and Lahore were non-significant.

Table 4.3. Disease incidence in Punjab during 2005-2007

Locations	Year		
	2005	2006	2007
Lahore	58 cd	56 d	67.8 ab
Gujranwala	71.2 a	66.2 bc	50.6 c
Kasur	58 cd	58.2 d	42 de
Shekhupura	65.4 abc	68.2 abc	60.2 b
Hafizabad	53.8 d	66.8 bc	50.8 c
Sargodha	56.8 d	70 abc	51.6 c
Narowal	55.8 d	63 cd	36.8 e
Sialkot	68.2 ab	69.6 abc	69.6 a
Okara	61.8 bcd	74.6 a	48.8 cd
Gujrat	59.6 cd	73 ab	46 cd
LSD (P≤0.05)	8.13	7.13	7.85

Means followed by different letters in the same column are significantly different from each others at P≤0.05

Table 4.4. Disease severity (%) in Punjab during 2005-2007

Locations	Year		
	2005	2006	2007
Lahore	5 abc	5.33 a	5.17 a
Gujranwala	5 abc	5.17 a	5 a
Kasur	6 a	5.83 a	5.5 a
Sheikhupura	4.5 bc	4 b	4.5 a
Hafizabad	3 d	3.67 b	4.83 a
Sargodha	5 abc	5.33 a	5.5 a
Narowal	5.67 ab	5.5 a	5 a
Sialkot	5.33 ab	5.33 a	5.33 a
Okara	3.83 cd	6 a	5.67 a
Gujrat	5.5 ab	5.33 a	4.83 a
LSD (P≤0.05)	1.22	1.14	1.18

Means followed by different letters in the same column are significantly different from each others at P≤0.05

During 2007, the differences in disease incidence recorded throughout survey at all locations were non significant. However, the maximum disease incidence was recorded in Sailkot (69.6 %) and Lahore (67.8 %) followed by Shekhupura (60.2 %).

Comparison of data of three years in Punjab revealed that maximum disease incidence prevailed during 2006 while minimum incidence was observed in 2007.

4.1.4 Disease severity (%) in Punjab

Disease severity in Punjab during cropping years 2005, 2006 and 2007 is presented in Table 4.4. The highest disease severity (6 %) was observed at Kasur closely followed by Narowal (5.66 %) and Gujrat (5.5 %) during 2005. The lowest disease severity (3 %) was observed at Hafizabad during the same year. There was no significant difference among locations in 2006 except Sheikhupura and Hafizabad, which showed lower disease severity than the rest of the locations. However, the two locations were statistically at par with each other. Maximum disease severity was observed in Okara (6 %) and the minimum in Hafizabad (3.67 %) during the same year. Results were non-significant during 2007 where maximum value of disease severity was recorded in Okara (5.67 %) and minimum in Shekhupura (4.5 %).

4.1.5 Disease incidence (%) in Sindh

Data regarding bacterial leaf blight incidence (%) in Sindh given in Table 4.5 revealed that the highest incidence (46.67 %) was recorded in Badin followed by Shikarpur (45 %). These were significantly different from the rest of the locations. However, the lowest and similar mean incidence was observed in Thatta (18.33 %) which was statistically similar to the values observed in Sukkar, Tando Muhammad Khan and Badin ($P \leq 0.05$).

In 2006 cropping season, maximum disease incidence was recorded in Badin (41.67 %) and Shikarpur (40 %) which were significantly different from all other locations of sindh. BLB incidence recorded in Dadu, sukkar, Tando Muhammad Khan and Thatta were non significant at 5 % level of probability during 2006 rice cropping season.

Data on BLB incidence recorded during 2007 revealed that mean values of all locations were significantly different. However, maximum disease incidence recorded in Badin (40 %) was at par with Shikarpur. The minimum disease incidence in sindh during 2007 was found in Thatta (11.6 %) which was not significantly different from values recorded in Tando Muhammad Khan, Dadu and Sukkar when tested at 5% level of probability.

4.1.6 Disease severity (%) in Sindh

Bacterial leaf blight severity recorded in sindh during 2005, 2006 and 2007 is outlined in Table 4.6. Predominant cultivars of rice in Sindh were, Dehradun Basmati, Hansraj, Supri, IRRI-6 and IRRI-9. The response of cultivars was different at different locations. During 2005, the highest disease severity was recorded in Shikarpur (3.33 %), followed by Dadu (2.66 %), whereas the lowest value (1 %) of BLB severity was recorded in Thatta and Tando Muhammad Khan which was at par with the BLB severity recorded in Sukkar (1.33 %). BLB severity recorded in Dadu (2.67 %), Larkana (2.33 %), Badin (2 %) and Hyderabad (2.33 %) were not statistically different at 5% level of probability ($P \leq 0.05$).

During the survey of 2006, the highest value of BLB severity (2.33 %) was recorded in Shikarpur followed by Dadu, Larkana and Badin which gave similar value (2 %),

and were not different from Shikarpur at significance level of P≤0.05. BLB severity recorded in Thatta (0.67 %) in the same cropping season was the lowest among all the locations, which was at par with severity observed in Sukhar and Tando Muhammad Khan.

The trend of disease severity during 2007 was quite similar to that observed in 2006. The highest BLB severity were recorded in Dadu and Badin (2 %) followed by Larkana and Shikarpur (1.67 %) with insignificant difference (P≤0.05). In the same cropping season, lowest BLB severity was observed in Tando Muhammad Khan and Thatta (0.67 %) which were statistically similar in values recorded in Sukkar (1 %) and Hyderabad (1.33 %).

4.1.7 Disease incidence in Baluchistan

Bacterial leaf blight incidence recorded in Baluchistan during 2005, 2006 and 2007 is shown in Table 4.7. As mentioned previously, rice in Baluchistan is limited to a few areas including Usta Muhammad, therefore, only this location (Usta Mohammad) was included in the survey.

Disease incidence of BLB recorded in 2005, 2006 and 2007 was 21.67, 16.33 and 12 % respectively. Such a low incidence is attributed to low inoculum level and the unfavorable environmental conditions during critical stage of the rice crop. Comparison of observations with the data previously recorded by Akhtar and Zakria (2003) reveals that the incidence of BLB increased in recent years since in 2002 disease incidence in Usta Mohammad was recorded as only 6-5 % as compared to 21.67 % in 2005.

4.1.8 Disease severity (%) in Baluchistan

Data on BLB severity in Baluchistan were recorded (Table 4.8) only at one location due to limited rice cultivation in the province. Rice cultivation predominates the area compared to other rice zones of the country. It seems that inoculum build up was at the bottom and therefore, BLB severity recorded was lower. In 2005 rice cropping season, BLB severity (1.22 %) was comparatively higher than 2006 (0.67 %) and 2007 (0.33 %).

Table 4.5. Disease incidence in Sindh during 2005-2007

Locations	Year		
	2005	2006	2007
Dadu	25 cd	18.33 c	16.67 de
Larkana	33.3 bc	30 b	26.67 bcd
Shikarpur	45 a	40 a	35 ab
Sukhar	21.67 d	18.33 c	18.33 cde
Tando muhammad khan	21.67 d	18.33 c	15 e
Badin	46.67 a	41.67 a	40 a
Hyderabad	35 b	28.33 b	28.33 bc
Thatta	18.33 d	15 c	11.67 e
LSD (P≤0.05)	8.52	9.53	11.46

Means followed by different letters in the same column are significantly different from each others at P≤0.05

Table 4.6. Disease severity (%) in Sind during 2005-2007

Locations	Year		
	2005	2006	2007
Dadu	2.67 b	2 a	2 a
Larkana	2.33 bc	2 a	1.67 ab
Shikarpur	3.33 a	2.33 a	1.67 ab
Sukhar	1.33 d	1 bc	1 bc
Tando muhammad khan	1 d	1 bc	0.67 c
Badin	2 c	2 a	2 a
Hyderabad	2.33 bc	1.33 b	1.33 abc
Thatta	1 d	0.67 c	0.67 c
LSD (P≤0.05)	0.59	0.56	0.71

Means followed by different letters in the same column are significantly different from each others at P≤0.05

Table 4.7. Disease incidence in Baluchistan during 2005-2007

Location	Years		
	2005	2006	2007
Usta Muhammad	21.67	16.33	12

Table 4.8. Disease severity (%) in Baluchistan during 2005-2007

Location	Year		
	2005	2006	2007
Usta Muhammad	1.23	0.67	0.33

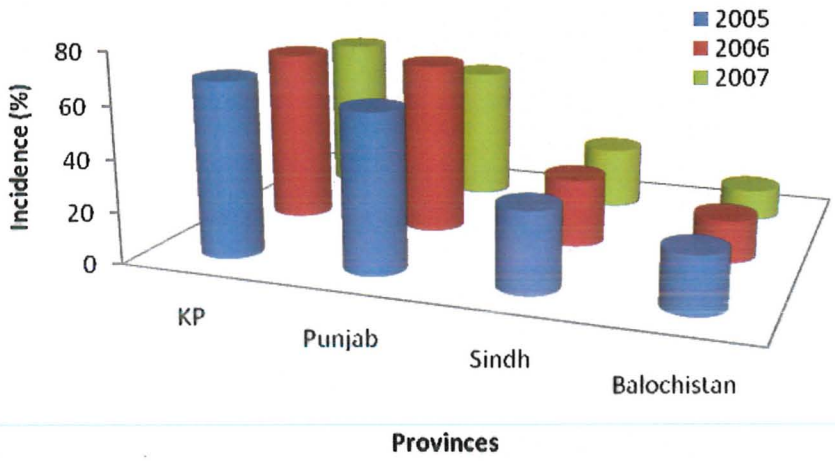


Figure 3. Year and province-wise comparision of bacterial blight incidence (%)

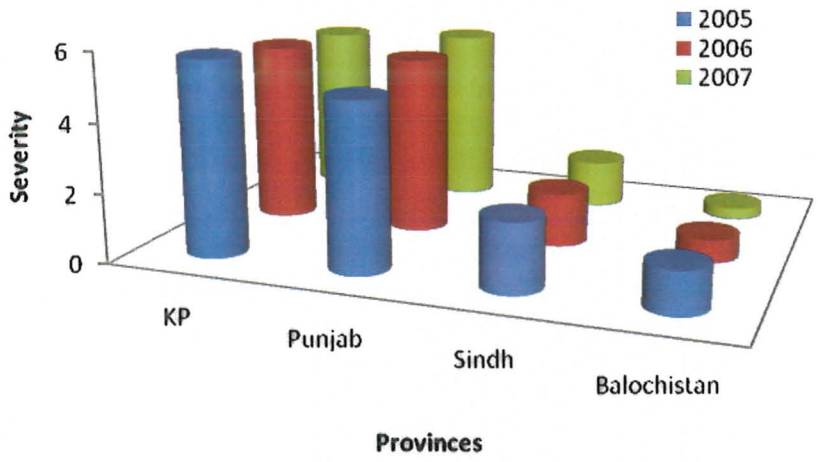


Figure 4. Year and provice-wise comparision of bacterial blight severity

4.2 Isolation, identification and preliminary characterization of *Xanthomonas oryzae* pv *oryzae* in rice fields

A detailed survey of various rice growing areas of KP was conducted during 2007 and 2008. As many as 120 rice representative samples of 12 districts of different agro-ecological zones of KP were assayed for isolation of *Xanthomonas oryzae* pv. *oryzae*. From each district at least ten representative samples were collected. Almost all samples yielded the bacterium (Table 4.9). One hundred and twenty five isolates were eventually recovered from these samples. A couple of isolates were recovered from samples collected from Taimargara (Dir Lower), Lailonai (Shangla), Adina (Swabi), Pir payai (Nowshera) and Naguman (Peshawar). Colonies recovered were mucoid, convex and shinny in texture on YDC agar medium (Ezuka and Kaku, 2000). All samples collected were positively confirmed to be infected with BLB and the pathogen consistently recovered from the infected leaf tissues was the causal agent i.e. *X. oryzae* pv. *oryzae*. Each isolate was considered individual strain until these were characterized through series of biochemical tests as described in Burgey's manual and performing diagnostic tests such as hypersensitivity response and pathogenicity reaction (Table 4.10a).

Table 4.9: List of isolates of *Xanthomonas oryzae* pv. *oryzae* recovered from rice samples, collected from different agro-ecological zones of KP during 2007 and 2008.

CHITRAL				
Isolate No	**Sample**	**Location**	**Cultivar/ germplasm**	**Isolation**
Xo-184	Ch-1	Drosh	Land race	+
Xo-185	Ch-2	Drosh	Butani	+
Xo-186	Ch-3	Ayun	Butani	+
Xo-187	Ch-4	Ayun	Shoga	+
Xo-188	Ch-5	Chitral	Land race	+
Xo-189	Ch-6	Koguzi	Land race	+
Xo-190	Ch-7	Garam chashma	Butani	+
Xo-191	Ch-8	Garam chashma	Land race	+
Xo-192	Ch-9	Bonni	Land race	+
Xo-193	Ch-10	Shagram	Land race	+

Dir Upper				
Isolate No	**Sample**	**Location**	**Cultivar/ germplasm**	**Isolation**
Xo-194	UD-1	Dobandi	Shoga	+
Xo-195	UD-2	Dir Upper	Basmati-385	+
Xo-196	UD-3	Dir Upper	Fakhri Malakand	+
Xo-197	UD-4	Darora	Fakhri Malakand	+
Xo-198	UD-5	Darora	Shoga	+
Xo-199	UD-6	Darora	Basmati-385	+
Xo-200	UD-7	Warai	Shoga	+
Xo-201	UD-8	Sahib abad	Shoga	+
Xo-202	UD-9	Sahib abad	Fakhri Malakand	+
Xo-203	UD-10	shalflam	Shoga/Basmati-385	+

Dir Lower				
Isolate No	**Sample**	**Location**	**Cultivar/ germplasm**	**Isolation**
Xo-204	LD-1	Rabat	Shoga	+
Xo-205	LD-2	Rabat	Basmati-385	+
Xo-206	LD-3	Rabat	Fakhri Malakand	+
Xo-207	LD-4	Khal	Fakhri Malakand	+
Xo-208	LD-5	Khal	Shoga	+
Xo-209	LD-6	Teimargara	Basmati-385	+
Xo-210a	LD-7a	Teimargara	Shoga	+
Xo-210	LD-7	Teimargara	Shoga	+
Xo-211	LD-8	Chakdara	Shoga	+
Xo-212	LD-9	Monda	Fakhri Malakand	+
Xo-213	LD-10	Monda	Basmati-385	+

Swat				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-214	Swt-1	Mingora	Dilrosh-97	+
Xo-215	Swt-2	Mingora	Fakhri Malakand	+
Xo-216	Swt-3	Mingora	Breeding material	+
Xo-217	Swt-4	Kanjo	Shoga	+
Xo-218	Swt-5	Kanjo	Fakhri Malakand	+
Xo-219	Swt-6	Matta	Shoga	+
Xo-220	Swt-7	Charbagh	Fakhri Malakand	+
Xo-221	Swt-8	Khwazakhela	JP-5/ Shoga	+
Xo-222	Swt-9	Ghalegee	Fakhri Malakand	+
Xo-223	Swt-10	Burikot	Fakhri Malakand	+

Buner				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-224	BN-1	Dagar	JP-5	+
Xo-225	BN-2	Dagar	Fakhri Malakand	+
Xo-226	BN-3	Shal bandi	Shoga	+
Xo-227	BN-4	Shal bandi	Basmati-385	+
Xo-228	BN-5	Qadar Nagar	Shoga	+
Xo-229	BN-6	Qadar Nagar	Fakhri Malakand	+
Xo-230	BN-7	Sowarai	Fakhri Malakand	+
Xo-231	BN-8	Mela	Shoga	+
Xo-232	BN-9	Mela	Fakhri Malakand	+
Xo-233	BN-10	Sowarai	Shoga	+

Shangla				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-234	Sh-1	Lailonai	Swat-1	+
Xo-235	Sh-2	Lailonai	Basmati-385	+
Xo-236	Sh-3	Lailonai	Shoga	+
Xo-237	Sh-4	Shangla	Shoga	+
Xo-238	Sh-5	Shangla	Swat-1	+
Xo-239a	Sh-6a	Yakh Tangi	Shoga	+
Xo-239	Sh-6	Yakh Tangi	Shoga	+
Xo-240	Sh-7	Yakh Tangi	Fakhri Malakand	+
Xo-241	Sh-8	Sabir Abad	Fakhri Malakand	+
Xo-242	Sh-9	Shah Pur	Shoga	+
Xo-243	Sh-10	Shah Pur	Dilrosh-97	+

Mardan				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-244	MR-1	Shergarh	Land race	+
Xo-245	MR-2	Shergarh	Swat-1	+
Xo-246	MR-3	Hatyan	Swat-1	+
Xo-247	MR-4	Lund khwar	Land race	+
Xo-248	MR-5	Lund khwar	Basmati-385	+
Xo-249	MR-6	Katlang	Land race	+
Xo-250	MR-7	Amazo garhi	Basmati-385	+
Xo-251	MR-8	Bakhshali	Land race	+
Xo-252	MR-9	Shehbaz garhe	Basmati-385	+
Xo-253	MR-10	Takhtbhai	Shoga	+

Mansehra				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-254	MN-1	Ghazi kot	Pakhal	+
Xo-255	MN-2	Baffa	Dilrosh-97	+
Xo-256	MN-3	Baffa	JP-5	+
Xo-257	MN-4	Baffa	Basmati-385	+
Xo-258	MN-5	Baffa	Kashmiri Basmati	+
Xo-259	MN-6	Baffa	Pakhal	+
Xo-260	MN-7	Dodhial	JP-5	+
Xo-261	MN-8	Shinkiari	JP-5	+
Xo-262	MN-9	Balakot	Pakhal	+
Xo-263	MN-10	Garhi Habibullah	Kashmiri Basmati	+

Swabi				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-264a	SB-1a	Adina	JP-5	+
Xo-264	SB-1	Adina	JP-5	+
Xo-265	SB-2	Adina	Land race	+
Xo-266	SB-3	Turlandi	Land race	+
Xo-267	SB-4	Karnalsher kaly	Land race	+
Xo-268	SB-5	Dobhyan	Land race	+
Xo-269	SB-6	Yar hussain	Land race	+
Xo-270	SB-7	Yar hussain	JP-5	+
Xo-271	SB-8	Zaida	JP-5	+
Xo-272	SB-9	Marghuz	JP-5	+
Xo-273	SB-10	Lahore	Land race	+

Nowshehra				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-274	NR-1	Pabbi	JP-5	+
Xo-275	NR-2	Pabbi	IRRI-6	+
Xo-276	NR-3	Amankot	IRRI-6	+
Xo-277	NR-4	Mohib Banda	Land race	+
Xo-278	NR-5	Azakhail	Land race	+
Xo-279	NR-6	Pir pai	Land race	+
Xo-280	NR-7	Akbar pura	Land race	+
Xo-281	NR-8	Tarkha	Land race	+
Xo-282	NR-9	Tarkha	IRRI-6	+
Xo-283	NR-10	Kurway	IRRI-6	+

Peshawar				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-284	PR-1	Matra	Dawoodzai	+
Xo-285	PR-2	Matra	IRRI-6	+
Xo-286	PR-3	Gara Tajik	Dawoodzai	+
Xo-287	PR-4	Nahki	Dawoodzai	+
Xo-288	PR-5	Pajagi	Dawoodzai	+
Xo-289a	PR-6a	Pirbala	Dawoodzai	+
Xo-289	PR-6	Pirbala	Dawoodzai	+
Xo-280	PR-7	Terahi	Dawoodzai	+
Xo-291	PR-8	Darmangi	Dawoodzai	+
Xo-292	PR-9	Potohar	Dawoodzai	+
Xo-293	PR-10	Shekh kali	Dawoodzai	+

Charsadda				
Isolate No	**Sample**	**Location**	**Cultivar/ Germplasm**	**Isolation**
Xo-294	CA-1	Nisata	IRRI-6	+
Xo-295	CA-2	Nisata	Land race	+
Xo-296	CA-3	Shabara	Land race	+
Xo-297a	CA-4a	Noguman	Land race	+
Xo-297	CA-4	Noguman	Land race	+
Xo-298	CA-5	Gulabad	Land race	+
Xo-299	CA-6	Gulabad	Land race	+
Xo-300	CA-7	Battagram	Land race	+
Xo-301	CA-8	Gonada	Land race	+
Xo-302	CA-9	Mazara	Land race	+
Xo-303	CA-10	Shabkadar	Land race	+

4.2.1 Diagnostic Tests

4.2.1.1 Hypersensitivity response

A set of 125 isolates initially recovered were tested for hypersensitive response. Collapse and water soaking of inoculated tissues in 24-48 hours was followed by a dry, light brown necrosis of water soaked tissues within three days. Isolates exhibiting such response were ranked HR- positive. Out of 125 isolates, 91 tested positive for hypersensitivity reaction when injected through infiltration into the mesophyll of tobacco leaves. The remaining 34 isolates failed to produce such reaction and were therefore designated as HR- negative (Table 4.10 and Appendix 4). These isolates were either avirulent strains of the *Xoo* or were other spp. of the same bacterium.

4.2.1.2 Pathogenicity Test

On the basis of colony morphology, all 125 isolates were tested for pathogenicity using JP-5 as susceptible host of BB. Of the all isolates tested, only 45 did not develop symptoms after 21 days of inoculation (Table 4.10 and Appendix 4). The results indicated that these pathogenic isolates were widely distributed in rice growing areas of KP, Pakistan.

4.2.2 Biochemical Characterization of *Xanthomonas oryzae* pv. *oryzae*

Xanthomonas oryzae pv. *oryzae* are gram negative, aerobic, rods having single polar flagellum. These are KOH positive, and readily hydrolyze Tween-80 and starch, form acid from carbohydrates, their egg yolk (lacithenase test) reaction being negative. Further, these show no tolerance to 0.1% tetrazolium negative, anaerobic test negative and oxidase test negative as described by Burgey's manual of bacteriology as well as by Azuka and Kaku (2000). Similar characteristics of the bacterium were also reported by Akhtar *et al.*, (2004) and Manan *et al.* (2007).

A total of nine biochemical tests were conducted to characterize the pathogen isolated from BLB infected samples collected from different areas of the KP. The tests included Gram staining, potassium hydroxide test, anaerobic growth test, egg yolk

reaction, starch hydrolysis test, oxidase test, acid production from carbohydrates, Tween 80 hydrolysis and Tetrazolium tolerance test.

4.2.2.1 Gram staining

Gram staining is essential for differentiating bacteria into two broad groups G+ and G-. Out of 125 isolates tested for Gram reaction, 102 exhibited Gram negative reactions with pink or red color when studied under light microscope as these isolates retained the color of the counter stain i.e. safranin (Table 4.10 and Appendix 4). The remaining 23 were G+.

4.2.2.2 Potassium hydroxide test

Treatment of bacterium with 3% KOH demonstrated the confirmation of the Gram staining result. Out of 125 isolates tested, 23 showed no thread like slime after vigorous mixing the bacterial growth with 3 % KOH and pulling the toothpick up to observed viscosity of the bacterium (Table 4.10 and Appendix 4).

4.2.2.3 Starch hydrolysis test

Xanthomonas oryzae pv. *oryzae* isolates when incubated for 7 days to determine starch hydrolysis test demonstrated clear zones when plates were stained with Lugol's iodine. Of all isolates tested, 23 demonstrated negative results whereas the remaining 102 were ranked positive (Table 4.10 and Appendix 4).

4.2.2.4 Tween-80 hydrolysis

Out of 125 isolates tested for Tween-hydrolysis, 117 isolates showed positive reaction by production of opaque zones around the colonies. These opaque zones were much clear on the third and fourth day of the addition of the inoculum to Tween-80 (Table 4.10 and Appendix 4). However, 8 isolates failed to produce opaque zones around the colonies

4.2.2.5 Acid production from carbohydrates

The change of color of the inoculated media in the tubes from purple to yellow after 2, 4 and 6 days of incubation, is an indication of positive reaction as well as acid

production from carbohydrates. One hundred and seventeen isolates showed positive results and the remaining 8 were scored negative (Table 4.10 and Appendix 4).

4.2.2.6 Egg yolk reaction / Lecithinase activity

This test is based on the observation that the enzyme lecithinase can break down the phospholipid emulsion of egg yolk, liberating a turbid zone of free fats around the colonies. This zone staint greenish-blue with copper sulfate. No such zones or color was visible for all the isolates tested (Table 4.10 and Appendix 4).

4.2.2.7 Tetrazolium tolerance test

Xanthomonas are extremely sensitive to tetrazolium salts. At 0.1% concentration of this salt no isolate out of 125 was able to grow. The results were similar at 0.02% concentration of TTC salt which proved that all isolates were extremely sensitive to TTC salt (Table 4.10 and Appendix 4).

4.2.2.8 Anaerobic activity

Anaerobic growth test is differential test for aerobic and non-aerobic bacteria. In our experiment none of the 125 isolates tested gave positive anaerobic activity (Table 4.10 and Appendix 4). This test indicated the true aerobic nature of the bacterium.

4.2.2.9 Oxidase test

This test determines whether a bacterium has cytochrome oxidase, a compound that is present in most plant saprophytic bacteria. All the isolates tested, were found negative in the since that these failed to produce the desired color (purple) with oxidase solution and impregnated strips production after one min application (Table 4.10 and Appendix 4).

Table 4.10: Summary of Biochemical Test results

HR- response negative (34)	Xo-187, Xo-188, Xo-191, Xo-192, Xo-193, Xo-194, Xo-196, Xo-201, Xo-205, Xo-209, Xo-212, Xo-227, Xo-230, Xo-236, Xo-239, Xo-243, Xo-248, Xo-252, Xo-257, Xo-259, Xo-261, Xo-265, Xo-266, Xo-268, Xo-272, Xo-275, Xo-279, Xo-284, Xo-288, Xo-290, Xo-292, Xo-295, Xo-297a, Xo-302
Pathogenecity test negative (45)	Xo-187, Xo-188, Xo-190, Xo-196, Xo-197, Xo-201, Xo-202, Xo-205, Xo-206, Xo-209, Xo-212, Xo-213, Xo-216, Xo-223, Xo-227, Xo-228, Xo-230, Xo-236, Xo-237, Xo-239, Xo-243, Xo-245, Xo-248, Xo-251, Xo-252, Xo-256, Xo-259, Xo-261, Xo-265, Xo-266, Xo-267, Xo-268, Xo-272, Xo-275, Xo-279, Xo-280, Xo-284, Xo-287, Xo-288, Xo-290, Xo-292, Xo-295, Xo-297a, Xo-298, Xo-302
Gram reaction positive (23)	Xo-190, Xo-196, Xo-197, Xo-202, Xo-206, Xo-213, Xo-216, Xo-223, Xo-228, Xo-236, Xo-237, Xo-245, Xo-251, Xo-256, Xo-261, Xo-266, Xo-267, Xo-272, Xo-280, Xo-287, Xo-292, Xo-298, Xo-302
KOH test negative (23)	Xo-190, Xo-196, Xo-197, Xo-202, Xo-206, Xo-213, Xo-216, Xo-223, Xo-228, Xo-236, Xo-237, Xo-245, Xo-251, Xo-256, Xo-261, Xo-266, Xo-267, Xo-272, Xo-280, Xo-287, Xo-292, Xo-298, Xo-302
Starch hydrolysis negative (23)	Xo-188, Xo-191, Xo-194, Xo-195, Xo-201, Xo-203, Xo-204, Xo-212, Xo-217, Xo-225, Xo-232, Xo-242, Xo-247, Xo-253, Xo-260, Xo-266, Xo-267, Xo-272, Xo-277, Xo-280, Xo-287, Xo-292, Xo-302
Tween 80 hydrolysis negative (8)	Xo-190, Xo-196, Xo-213, Xo-228, Xo-245, Xo-272, Xo-292, Xo-298
Acid from carbohydrates negative (8)	Xo-190, Xo-196, Xo-213, Xo-228, Xo-245, Xo-272, Xo-292, Xo-298
Egg yolk reaction, Tetrazolium Tolerance test 0.1% 0.02%, Anaerobic growth test, Oxidase Test	These tests showed negative results for all 125 isolates

4.2.3 Identification of pathogen through PCR

All 125 isolates were extensively characterized and identified using standard procedures as mentioned previously. However, some of the key diagnostic tests gave overlapping results regarding the identity of the causal organism of bacterial blight. For these reasons, Polymerase Chain Reaction (PCR) was performed (using *Xoo*-specific primers) for (freshly extracted DNA of) all the isolates to confirm their identity. As clear from Fig.5 the *Xoo*-specific 470 bp band (Adachi and Oku, 2000) was amplified from a total of 80 isolates confirming (pathogencity test results) that these were *Xantomonas oryzae* pv. *oryzae*. However, no such band was amplified from the remaining 45 isolates indicating that these were not *Xoo*.

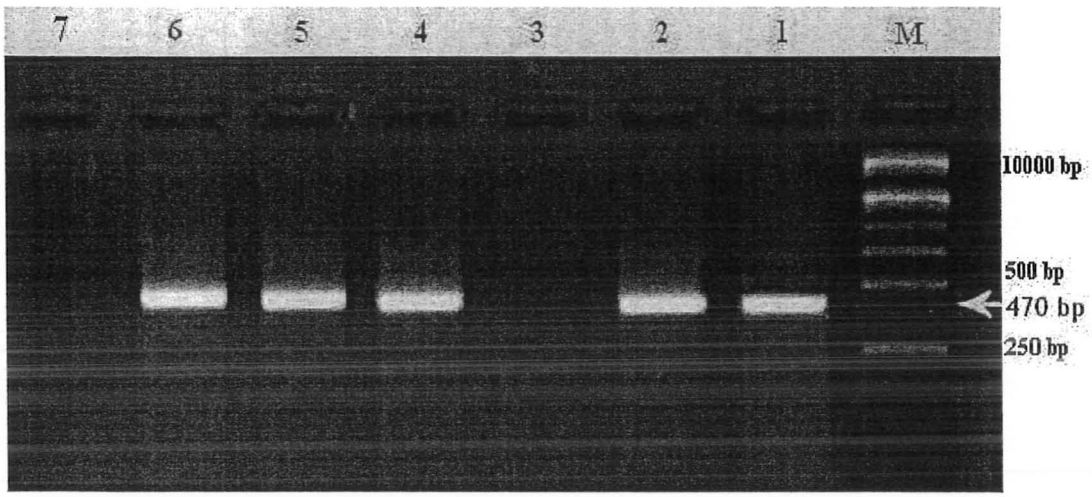


Fig. 5 Gel electrophoreses, PCR Amplified 470 bp bands from *Xoo* isolates (with specific primers), isolate 2, 4, 5 and 6 confirming *Xoo*-specific bands. Where, 1 is positive control (*Xoo*) and M represents I kb DNA ladder used as marker for comparison.

4.3 Comparison of Inoculation Methods

4.3.1 Detached leaf assay

Xanthomonas oryzae pv. *oryzae* isolate exhibited differential response with respect to cultivars and method of inoculation. On detached leaves, lesions appeared within 5 days after inoculation. Cultivar Bas 2000, Bas 385, Super Basmati and Bas 370 were susceptible to bacterial blight (Table 4.11). Rice varieties Super Basmati, Bas 370, Bas 385 and Bas 2000 showed typical disease symptoms with clipping method and exhibited medium susceptibility, but no variety was found resistant. The clipping inoculation method showed typical yellow lesions on cultivars Basmati 2000, Basmati 385 and Super Basmati and was declared as the most effective inoculation method with 14.15 cm lesion size, as compared to other two methods i.e pinprick (11.8 cm) and paint brush (10.15 cm).

4.3.2 Pot experiment

For pot experiment the response was differential with respect to cultivars and method of inoculation and lesions appeared 15 days after inoculation. Cultivar Bas 2000, Bas 385, Super Basmati and Bas 370 were susceptible to the disease (Table 4.12). None of the varieties was found resistant against the disease. The clipping inoculation method showed typical yellow lesions with wavy margin two weeks after inoculation on cultivars Basmati 2000, Basmati 385 and Super Basmati. Here again clipping method was found to be the most effective inoculation technique with 14.48cm lesion size, as compared to other two methods i.e Pinprick (9.92cm) and paint brush (12.3cm).

Table 4.11. Effect of different methods of inoculation on development of rice blight lesion on detached leaves of rice

S. No	Varieties	Rice blight lesion (cm)			Mean (LSD=1.44)	Reaction
		Clipping	Pinprick	Paint brush		
1	BAS-2000	16.9	15.5	14.8	15.73 a	S
2	BAS-385	13.5	10.5	9.7	11.23 b	MS
3	JP-5	10	7	4.9	7.30 c	MR
4	IR-6	9	5.8	3.3	6.03 c	MR
5	BAS-370	17.9	16.5	15.5	16.63 a	S
6	Super BAS	17.6	15.5	12.7	15.27 a	S
	Mean (LSD =1.02)	14.15 a	11.80 b	10.15 c	12.03	

Table 4.12. Effect of different methods of inoculation on development of rice blight lesion on potted plant leaves of rice

S. No	Varieties	Rice blight lesion (cm)			Mean (LSD=1.82)	Reaction
		Clipping	Pinprick	Paint brush		
1	BAS-2000	17	13	15	15 b	S
2	BAS-385	15	8	13	12 c	MS
3	JP-5	8	3	6	5.67 e	MR
4	IR-6	11	6	9	8.67 d	MR
5	BAS-370	17	13	15	15 b	S
6	Super BAS	18.9	16.5	15.8	17.07 a	S
	Mean (LSD=1.28)	14.48 a	9.92 c	12.3 b	12.3	

Means followed by different letters in categories are significantly different at 5% level of probability

Comparison of the methods of inoculation on rice varieties against *X.oryzae* pv. *oryzae* R: (1-5 cm) resistant with browning margin on around lesions, MR: moderately resistant (5-10 cm), MS: moderately susceptible (10-15), S: susceptible (15 cm to above)

4.4 Identification of Races/Pathovars and their virulence on IRBB lines carrying resistance genes against *Xanthomonas oryzae* pv. *oryzae*

Using the nomenclature developed by Habgood (1970) and modified by Limpert and Muller (1994), 80 isolates of KP were tested which resulted in 6 races of BLB (Table 4.13).

Race 1

Out of 8, 16 representatives isolates yielded similar weight vector numbers according to modified Limpert and Muller (1994) adopted formula and as such were assembled into the same race. Isolates included Race 1 were consisted of XO-189 from Chitral, XO-195, XO-200 and XO-203 from Dir Upper, XO-208 from Dir Lower, XO-215 and XO-220 from Swat, XO-229 from Buner, XO-234 from Shangla, XO-253 from Mardan, XO-257 and XO-258 from Mansehra, XO-269 from Swabi, XO-274 XO-283 and from Nowshera, and XO-300 from Charsadda. This race had no representative isolate from Peshawar (Table 4.13 and 4.15).

Race 1 exhibited virulence against all IRBB lines tested except IRBB-5 and IRBB-13 which possessed Xa-5 and Xa-13 resistant genes against *Xanthomonase oryzae*. Race 1 showed diverse distribution among rice growing areas of KP since it prevailed in all locations survived except Peshawar. This race was pre dominantly present at three locations of Dir upper and two locations of Swat, Mansehra and Nowshera.

Race 2

A total of 10 isolates were assembled as Race 2, which included XO-185 and XO-191 from Chitral, XO-199 from Dir Upper, XO-222 from Swat, XO-224 from Buner, XO-240 and XO-242 from Shangla, XO-282 Nowshera, XO-289a from Peshawar and XO-294 from Peshawar. However, none of the isolates from Dir Lower, Mansehra, Mardan and Swabi represented this race (Table 4.13 and 4.15).

Race 2 was widely distributed in rice growing zones of KP. However, it was not found in Dir Lower, Mardan, Mansehra and Swabi. All IRBB lines showed different patterns of resistance to Race 2 since it was virulent to IRBB-2, IRBB-4, IRBB-7, IRBB-8, IRBB-9, IRBB-11, IRBB-13, IRBB-21 and IR-24 (used as check). However,

IRBB-3, IRBB-5 and IRBB-14 were resistance to Race 2 since these lines were decorated with resistance genes Xa-5, Xa-8 and Xa-14 against *Xanthomonas oryzae*. The race predominantly prevailed in Chitral and Shangla, both of which are located in the hilly areas of KP.

Race 3

Table 4.13 and 4.15 revealed that 9 *Xoo* isolates collected from various rice growing areas of KP, comprised Race 3 with isolates; XO-194 and XO-198 from Dir Upper, XO-211 from Dir Lower, XO-218 from Swat, XO-240a from Shangla, XO-249 from Mardan, XO-260 and XO- 263 from Mansehra, and XO-277. It was however absent in Chitral, Buner, Peshawar, Swabi and Charsadda.

Race 3 has been detected in all rice growing locations under survey except Chitral, Buner, Swabi, Peshawar and Charsadda. It represented two isolates each from Dir Upper and Mansehra and one each from Dir lower, Swat, Shangla, Mardan and Nowshera. Race 3 showed avirulence to Xa-3, Xa-5 and Xa-14 resistant genes, while Xa-2, Xa-4, Xa-7, Xa-8, Xa-10, Xa-11 and Xa-13 resistant genes could not tolerate the virulent nature of this race.

Race 4

Race 4 was predominant in Chitral, Buner, Mardan and Swabi and has been detected in 15 locations, but was absent in Shangla, Dir (Upper), Nowshera and Mansehra. This race had XO-186 and XO-193 from Chitral, XO-207 from Dir Lower, XO-217 from Swat, XO-225, XO-226 and XO-232 from Buner, XO-246 and XO-247 from Mardan, XO-264, XO-270 and XO-273 from Swabi, XO-291 from Peshawar and XO-299 and XO-303 represented Charsadda district of KP.

Race 4 was equally distributed in all rice growing areas of KP. However, this race was not found in Shangla and Nowshera. Conversely, in Buner, Swabi, Mardan and Charsadda this race appears to be common and mostly homogeneous in nature. The race was found virulent on Xa-2, Xa-3, Xa-4, Xa-8, Xa-10, Xa-13, Xa-14, Xa-21 and the check IR-24. Xa-5, Xa-7 and Xa-11 showed resistance to this race when evaluated for resistance against 80 *Xoo* isolates of KP.

Race 5

Race 5 had isolates XO-192 from Chitral, XO-204 and XO-210a from Dir Lower, XO-221 from Swat, XO-238 from Shangla, XO-244 from Mardan, XO-255 from Mansehra, XO-276 and XO-281from Nowshera, XO-286 from Peshawar and XO-296, XO-297 and XO-301 were from Charsadda. This race could not been detected in Buner, Dir (Upper) and Swabi. 13 isolates out of 80, showed similar results, and hence were placed in one group (Table 4.13 and 4.15).

Race 5 predominantly appeared in samples from Charsadda, Dir Lower and Nowshera. Swabi, Buner and Dir Upper were the locations where Race 5 was not detected. Race 5 showed virulent trend against resistant genes Xa-2, Xa-4, Xa-7, Xa-8, Xa-11, Xa-14, Xa-21 and the check variety IR-24 during the study. Xa-3, Xa-5, Xa-10 and Xa-13 responded with complete resistance when challenged by artificial inoculation.

Race 6

This group had maximum number of isolates (17) but was not detected in Dir (Upper). Race 6 contained XO- 184 from Chitral, XO-210 from Dir Lower, XO-214 and XO-219 from Swat, XO-231and XO-233 from Buner, XO-235 and XO-241 from Shangla, XO-250 from Mardan, XO-254 and XO-262 from Mansehra, XO-264a and XO-271 from Swabi, XO-278 from Nowshera and XO-285, XO-289 and XO-293 from Peshawar District. It was evident (Tables 4.13 and 4.15) that this race was equally important in terms of its distribution in almost all rice growing areas, including planes as well as mountainous areas of KP.

Race 6 was present in almost all locations of KP except Dir Lower and Charsadda. Out of 80 isolates, this race comprised the maximum isolates tested. Out of 11 IRBB lines and a check cultivar, only three lines having resistant genes Xa-4, Xa-10 and Xa-14 exhibited resistance when inoculated by 80 isolates of KP.

Table 4.13. Identification of Pakistani (PK) Races representing KP, Pakistan based on virulence reactions on IRBB differential rice cultivars with known resistance genes to Bacterial Leaf Blight

ISOLATES	ORIGIN	ISOLATE CODE: NO.	RACES
XO-189	Chitral	PK-1	1
XO-195	Dir upper	PK-2	1
XO-200	Dir upper	PK-3	1
XO-203	Dir upper	PK-4	1
XO-208	Dir lower	PK-5	1
XO-215	Swat	PK-6	1
XO-220	Swat	PK-7	1
XO-229	Buner	PK-8	1
XO-234	Shangla	PK-9	1
XO-253	Mardan	PK-10	1
XO-257	Mansehra	PK-11	1
XO-258	Mansehra	PK-12	1
XO-269	Swabi	PK-13	1
XO-274	Noshehra	PK-14	1
XO-283	Noshehra	PK-15	1
XO-300	Charsadda	PK-16	1
XO-185	Chitral	PK-17	2
XO-191	Chitral	PK-18	2
XO-199	Dir upper	PK-19	2
XO-222	Swat	PK-20	2
XO-224	Buner	PK-21	2
XO-240	Shangla	PK-22	2
XO-242	Shangla	PK-23	2
XO-282	Noshehra	PK-24	2
XO-289A	Peshawar	PK-25	2
XO-294	Charsadda	PK-26	2
XO-194	Dir upper	PK-27	3
XO-198	Dir upper	PK-28	3
XO-211	Dir lower	PK-29	3
XO-218	Swat	PK-30	3
XO-240A	Shangla	PK-31	3

XO-249	Mardan	PK-32	3
XO-260	Mansehra	PK-33	3
XO-263	Mansehra	PK-34	3
XO-277	Noshehra	PK-35	3
XO-186	Chitral	PK-36	4
XO-193	Chitral	PK-37	4
XO-207	Dir lower	PK-38	4
XO-217	Swat	PK-39	4
XO-225	Buner	PK-40	4
XO-226	Buner	PK-41	4
XO-232	Buner	PK-42	4
XO-246	Mardan	PK-43	4
XO-247	Mardan	PK-44	4
XO-264	Swabi	PK-45	4
XO-270	Swabi	PK-46	4
XO-273	Swabi	PK-47	4
XO-291	Peshawar	PK-48	4
XO-299	Charsadda	PK-49	4
XO-303	Charsadda	PK-50	4
XO-192	Chitral	PK-51	5
XO-204	Dir lower	PK-52	5
XO-210A	Dir lower	PK-53	5
XO-221	Swat	PK-54	5
XO-238	Shangla	PK-55	5
XO-244	Mardan	PK-56	5
XO-255	Mansehra	PK-57	5
XO-276	Noshehra	PK-58	5
XO-281	Noshehra	PK-59	5
XO-286	Peshawar	PK-60	5
XO-296	Charsadda	PK-61	5
XO-297	Charsadda	PK-62	5
XO-301	Charsadda	PK-63	5
XO-184	Chitral	PK-64	6
XO-210	Dir lower	PK-65	6

XO-214	Swat	PK-66	6
XO-219	Swat	PK-67	6
XO-231	Buner	PK-68	6
XO-233	Buner	PK-69	6
XO-235	Shangla	PK-70	6
XO-241	Shangla	PK-71	6
XO-250	Mardan	PK-72	6
XO-254	Mansehra	PK-73	6
XO-262	Mansehra	PK-74	6
XO-264A	Swabi	PK-75	6
XO-271	Swabi	PK-76	6
XO-278	Noshehra	PK-77	6
XO-285	Peshawar	PK-78	6
XO-289	Peshawar	PK-79	6
XO-293	Peshawar	PK-80	6

Table 4.14. Ecological distribution of six races in Rice growing areas of KP, Pakistan

Races	Distribution of races in KP, Pakistan
1	Chitral, Dir (upper), Dir (lower), Swat, Buner, Shangla, Mardan, Swabi, Charsadda, Mansehra, Nowshera
2	Chitral, Dir (upper), Swat, Buner, Shangla, Charsadda, Nowshera, Peshawar
3	Dir(upper), Dir (lower), Swat, Shangla, Mardan, Mansehra, Nowshera
4	Chitral, Dir (lower), Swat, Buner, Mardan, Swabi, Charsadda, Peshawar
5	Chitral, Dir (lower), Swat, Shangla, Mardan, Mansehra, Nowshera, Peshawar, Charsadda
6	Chitral, Dir (lower), Swat, Buner, Shangla, Mardan, Mansehra, Swabi, Nowshera, Peshawar

Table 4.15. Pre dominance of races of *Xoo* in rice growing areas of KP, Pakistan

Locations	Race1	Race2	Race3	Race4	Race5	Race6
Chitral	1	2	-	2	1	1
Buner	1	1	-	3	-	2
Shangla	1	2	1	-	1	2
Swat	2	1	1	1	1	2
Dir(upper)	3	1	2	-	-	-
Dir(lower)	1	-	1	1	2	1
Nowshera	2	1	1	-	2	1
Mansehra	2	-	2	-	1	2
Peshawar	-	1	-	1	1	3
Mardan	1	-	1	2	1	1
Swabi	1	-	-	3	-	2
Charsadda	1	1	-	2	3	-
Total	16	10	9	15	13	17

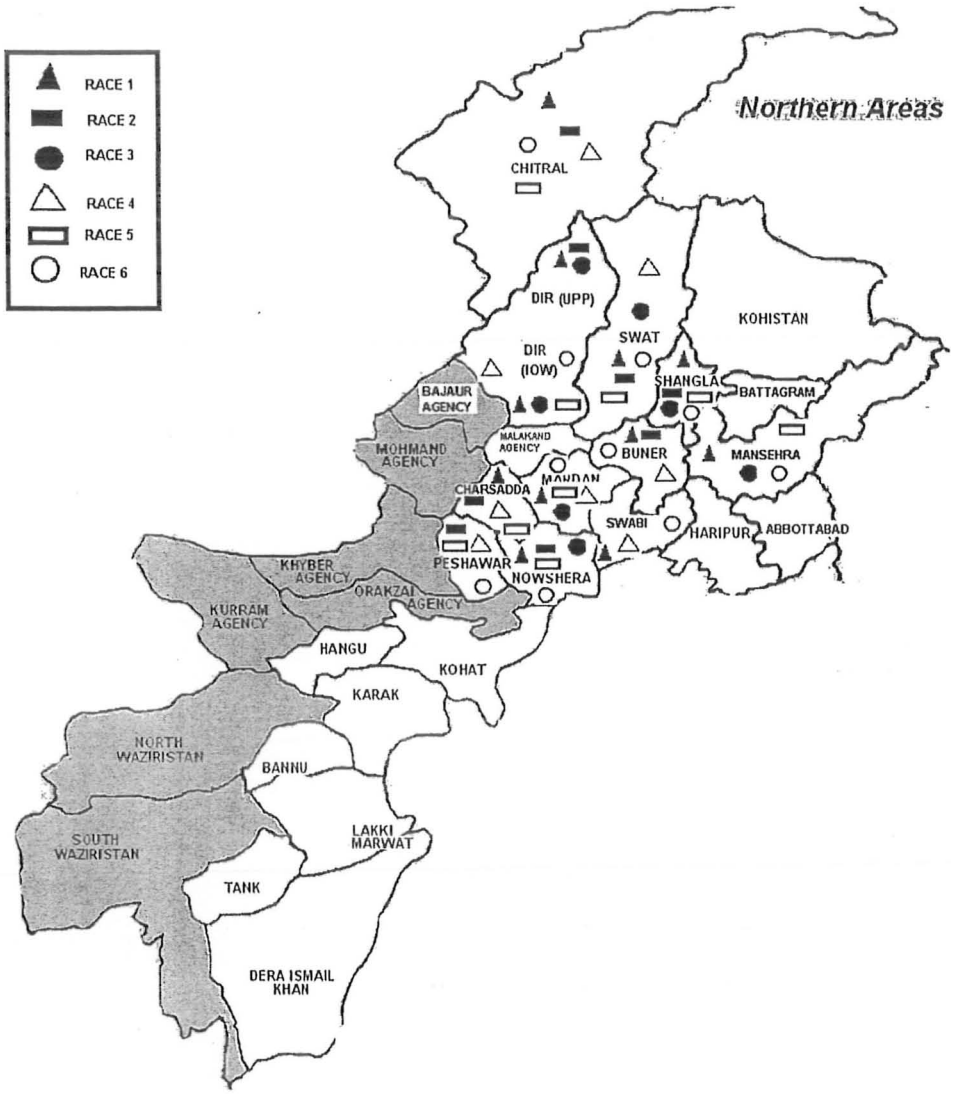


Figure 6: Ecological distribution of 6 races in rice growing areas of Khyber Pakhtunkhwa, Pakistan

4.5 Reaction of rice cultivars to *Xanthomonas oryzae* pv. *oryzae* Races

Disease reaction and lesion length (cm) of rice cultivars for resistance to 6 races of *Xoo* in KP is outlined in Table 4.16 a and b. Seventeen rice cultivars out of 34 were resistant or moderately resistant to KP race1, whereas 18 cultivars were resistant or moderately resistant to KP Race 2. Thirteen tested cultivars were found resistant or moderately resistant to KP Race 3. Similarly, 3 cultivars showed complete resistance whereas 11 were found moderately resistant to KP Race 4 of *Xoo*. A total of 15 varieties exhibited either resistant or moderate resistant response to KP Race 5 when inoculated artificially in a screenhouse experiment. Likewise, 9 out of 34 varieties were found either resistant or moderately resistant to KP Race 6. Varieties Bas-2000, Shaheen Basmati, Malhar-346, Khushbo-95, Basmati-2008, Jajai-77 showed complete or moderately resistant reaction to all the 6 races under study whereas, the reaction of Basmati-370 and Basmati-Pak was resistant or moderately resistant to 5 KP races but susceptible to KP Race6. Similarly, variety PK-177 exhibited complete resistance to all KP races except KP Race 4. Dokri basmati was the only variety showing moderate resistance to KP Race1, KP Race 2, KP Race 4 and KP Race 5 when checked for resistance against 6 KP races of *Xoo*.

Race 6 was the most virulent one amongst all the races under study. As many as 15 cultivars involved with this race showed susceptible reaction and only 1 was found to be resistant. Conversely, Race 1 proved to be the least virulent exhibiting 3 susceptible, 14 moderately susceptible and 6 resistant reactions. All other races were found intermediate within this range.

Table 4.16a: Disease reaction and lesion length of rice varieties to six races of KP

S.No	Varieties	Disease reaction and lesion length (cm) to bacterial races					
		Race1	Race2	Race3	Race4	Race5	Race6
1	Bas-2000	4.1 R	3.2 R	7.7 MR	3.4 R	8.3 MR	3.5 R
2	Kangni-27	14.5 MS	17.1 S	16.2 S	13.5 MS	12.3 MS	18.2 S
3	Shadab-31	7.1 MR	8.3 MR	13.6 MS	6.8 MR	14.2 MS	11.2 MS
4	Shaheen Basmati	6.4 MR	8.3 MR	3.4 R	2.4 R	7.2 MR	8.4 MR
5	Malhar-346	3.5 R	7.2 MR	6.5 MR	7.9 MR	1.7 R	8.8 MR
6	Pakhal	12.5 MS	13.3 MS	12.7 MS	18.8 S	13.4 MS	17.5 S
7	Sug Desi	9.1 MR	8.3 MR	8.6 MR	13.2 MS	8.3 MR	12.3 MS
8	Kashmir Basmati	12.4 MS	12.7 MS	18.9 S	17.2 S	14.5 MS	18.1 S
9	Basmati-370	2.3 R	6.8 MR	1.3 R	8.3 MR	7.1 MR	12.8 MS
10	JP-5	12.2 MS	13.9 MS	14.4 MS	19.9 S	18.5 S	19.2 S
11	DR-82	6.3 MR	14.3 MS	7.9 MR	8.4 MR	14.6 MS	13.2 MS
12	Swat-1	18.2 S	11.2 MS	17.2 S	12.1 MS	13.7 MS	19.1 S
13	NIAB-IR-9	19.4 S	13.4 MS	14.2 MS	11.3 MS	14.1MS	18.6 S
14	IR-8	7.2 MR	7.4 MR	12.1 MS	8.2 MR	13.1MS	17.2 S
15	Basmati-Pak	7.2 MR	3.7 R	3.3 R	1.2 R	7.3 MR	12.2 MS
16	PK-177	2.4 R	7.3 MR	6.6 MR	12.2 MS	7.6 MR	6.1 MR
17	Sarshar	12.3 MS	19.3 S	14.6 MS	18.8 S	14.3 MS	19.2 S
18	Dilrosh-97	13.4 MS	13.2 MS	18.2 S	12.1 MS	14.3 MS	19.1 S
19	Sada Hayat	19.2 S	18.1 S	13.9 MS	18.3 S	19.6 S	18.2 S
20	Sathra	7.2 MR	8.1 MR	13.1 MS	13.3 MS	9.1MR	14.0 MS
21	TN-1	11.2 MS	12.2 MS	14.5 MS	11.2 MS	19.7 S	19.2 S
22	DR-83	14.1MS	14.6 MS	17.3 S	12.7 MS	13.9 MS	12.2 MS
23	Khusboo-95	8.2 MR	3.9 R	2.3 R	6.3 MR	7.9 MR	8.2 MR
24	Basmati-2008	2.9 R	3.8 R	4.2 R	8.5 MR	3.3 R	7.7 MR
25	Shua-92	13.8 MS	14.6 MS	19.2 S	17.2 S	18.6 S	18.1 S
26	Dokri Basmati	8.2 MR	7.2 MR	13.9 MS	8.5 MR	7.9 MR	14.1 MS
27	Bas-6129	14.8 MS	6.4 MR	8.9MR	12.9 MS	6.4MR	13.6 MS
28	Muskhan	8.9 MR	7.2 MR	6.6 MR	7.2 MR	13.5 MS	14.2 MS
29	Jajai-77	3.5 R	4.1 R	3.8 R	7.2 MR	2.3 R	8.5 MR
30	DR-92	13.8 MS	12.4 MS	19.6 S	17.7 S	14.1MS	19.7 S
31	Rachna Basmati	13.6 MS	8.5 MR	12.8 MS	7.2 MR	8.3 MR	6.6 MR
32	Lateefy-98	12.8 MS	18.7 S	12.6 MS	14.1 MS	18.2 S	17.6 S
33	Fakhr-e-Malakand	8.3 MR	7.1 MR	12.8 MS	11.8 MS	7.9 MR	13.9 MS
34	Bas-385	13.9 MS	14.1 MS	19.8 S	13.8 MS	14.3 MS	19.9 S

Table 4.16b: Summarized table for the degree of resistance/susceptibility of rice cultivars to KP races

Races	Reaction				Total
	R	MR	S	MS	
Race-1	06	11	03	14	34
Race-2	05	13	04	12	34
Race-3	06	07	08	13	34
Race-4	03	11	07	13	34
Race-5	03	12	05	14	34
Race-6	01	07	15	11	34

4.6 Post-harvest survival of *Xoo* in the field

4.6.1 Recovery of *Xanthomonas oryzae* pv. *oryzae* from field water samples

Among 50 field water samples (Table 4.17), obtained from three rice growing areas of KP, *Xanthononas oryzae* pv *oryzae* was isolated from 6 samples collected from Peshawar, 5 samples of Charsadda, and 7 samples of Swat. The frequency percentage per location determined was 37.5, 29.4 and 41.17 for Peshawar, Charsadda and Swat, respectively. However, overall frequency percentage calculated for all 3 locations was 25.35 (Table 4.21).

Table 4.17. Recovery of *Xoo* from field water samples

Sample No.	Location	Isolation	Sample No.	Location	Isolation
WS-1	Peshawar	-	WS-26	Charsadda	+
WS-2	Peshawar	-	WS-27	Charsadda	-
WS-3	Peshawar	+	WS-28	Charsadda	-
WS-4	Peshawar	-	WS-29	Charsadda	-
WS-5	Peshawar	-	WS-30	Charsadda	+
WS-6	Peshawar	-	WS-31	Charsadda	-
WS-7	Peshawar	+	WS-32	Charsadda	-
WS-8	Peshawar	+	WS-33	Charsadda	+
WS-9	Peshawar	-	WS-34	Swat	-
WS-10	Peshawar	+	WS-35	Swat	-
WS-11	Peshawar	-	WS-36	Swat	+
WS-12	Peshawar	+	WS-37	Swat	-
WS-13	Peshawar	-	WS-38	Swat	-
WS-14	Peshawar	-	WS-39	Swat	+
WS-15	Peshawar	-	WS-40	Swat	-
WS-16	Peshawar	+	WS-41	Swat	+
WS-17	Charsadda	-	WS-42	Swat	+
WS-18	Charsadda	-	WS-43	Swat	-
WS-19	Charsadda	+	WS-44	Swat	+
WS-20	Charsadda	-	WS-45	Swat	-
WS-21	Charsadda	-	WS-46	Swat	-
WS-22	Charsadda	-	WS-47	Swat	-
WS-23	Charsadda	-	WS-48	Swat	+
WS-24	Charsadda	+	WS-49	Swat	-
WS-25	Charsadda	-f	WS-50	Swat	+

Over all isolation frequency = 25.35%

4.6.2 Isolation *Xoo* from weeds

Isolation of the pathogen (*Xoo*) from 7 selected rice weeds occurring commonly in rice fields are as followed:

***Cyperus difformis* L.** was collected from 3 locations of Charsadda and 2 each of Swat and Peshawar. Among these 7 samples, only 1 obtained from Charsadda, tested positive for *X. oryzae* pv *oryzae* (Table 4.18).

***Cyperus iria* L.** was collected from three locations of Charsadda and two locations each of Swat and Peshawar. However, none of the samples yielded the bacterium (Table 4.18).

***Cyperus rotundus* L.** was collected from 3 locations of Charsadda and 2 locations each of Swat and Peshawar when tested for the isolation of *X. oryzae* pv *oryzae* in the laboratory, only 1 sample representing Swat was positive for the isolation of the bacterium (Table 4.18).

Among 7 samples of *Dactyloctenium aegyptium* collected as above, *X. oryzae* pv *oryzae* was isolated from only 1 sample of Charsadda (Table 4.18).

Seven samples of the weed *Echinochloa crus-galli* collected from three locations of KP, and assayed for the isolation of *X. oryzae* pv *oryzae*. The bacterium was isolated from only 1 sample, representing Peshawar (Table 4.18).

Paspalum paspalodes was also collected from two locations each of Peshawar and swat, and 3 locations of Charsadda. However, none of the samples were found positive for the the bacterium (Table 4.18).

Leersia hexandra weed has frequently been considered as common source of initial inoculum (Ezuka anfd Kaku, 2000). It is prevalent in rice growing areas of KP, and may pose the same problem. After processing for isolation of *X. oryzae* pv *oryzae*, through isolation of causal agent from one sample of Peshawar confirmed the previous findings, while the rest of the samples of the same weed were found negative for the pathogen (Table 4.18).

4.6.3 Isolation of *Xanthomonas oryzae* pv. *oryzae* from plant residues (PR)

Fifty plant residue samples from rice fields comprising of 16 from Peshawar and 17 each from Charsadda and Swat were assayed for isolation of *Xanthomonas oryzae* pv *oryzae*. The frequency (%) of recovery of the pathogen for the three locations ranged between 41-65 % for Peshawar, Charsadda and Swat. This trait accounts for overall frequency of 36.61 % recovery of the causal pathogen for all locations (Table 4.19).

4.6.4 Isolation of *Xoo* from Seeds

Fifty samples of bulk seed comprising 16, from Peshawar and 17 each from Charsadda and Swat were assayed for isolation of *X. oryzae* pv *oryzae*. The frequency of recovery of the pathogen for the 3 locations was 37.5, 52.9 and 41.1 % for Peshawar, Charsadda and Swat, respectively. This trait accounts for overall frequency of 30.98 % recovery of the causal pathogen for all locations (Table 4.20).

Table 4.18. Isolation *Xoo* from weeds

Name of the Weed	Peshawar		Charsadda			Swat		
	L1	L2	L1	L2	L3	L1	L2	L3
Cyperus difformis L.	-	-	-	+	-	-	-	.
Cyperus iria L.	-	-	-	-	-	-	-	.
Cyperus rotundus L.	-	-	-	-	-	-	+	.
Dactyloctenium aegyptium	-	-	+	-	-	-	-	.
Echinochloa crus-galli	+	-	-	-	-	-	-	.
Paspalum paspalodes	-	-	-	-	-	-	-	.
Leersia hexandra	-	+	-	-	-	-	-	-

Overall isolation frequency = 7.06%

Table 4.19. Isolation of *Xoo* from plant residues (PR)

Sample No.	Location	Isolation	Sample No.	Location	Isolation
PR-1	Peshawar	-	PR-26	Charsadda	-
PR-2	Peshawar	+	PR-27	Charsadda	-
PR-3	Peshawar	+	PR-28	Charsadda	+
PR-4	Peshawar	-	PR-29	Charsadda	+
PR-5	Peshawar	-	PR-30	Charsadda	-
PR-6	Peshawar	+	PR-31	Charsadda	+
PR-7	Peshawar	+	PR-32	Charsadda	+
PR-8	Peshawar	+	PR-33	Charsadda	+
PR-9	Peshawar	-	PR-34	Swat	+
PR-10	Peshawar	+	PR-35	Swat	-
PR-11	Peshawar	+	PR-36	Swat	-
PR-12	Peshawar	-	PR-37	Swat	+
PR-13	Peshawar	-	PR-38	Swat	+
PR-14	Peshawar	-	PR-39	Swat	-
PR-15	Peshawar	-	PR-40	Swat	+
PR-16	Peshawar	+	PR-41	Swat	-
PR-17	Charsadda	+	PR-42	Swat	+
PR-18	Charsadda	+	PR-43	Swat	-
PR-19	Charsadda	+	PR-44	Swat	-
PR-20	Charsadda	+	PR-45	Swat	-
PR-21	Charsadda	-	PR-46	Swat	+
PR-22	Charsadda	+	PR-47	Swat	+
PR-23	Charsadda	-	PR-48	Swat	-
PR-24	Charsadda	+	PR-49	Swat	-
PR-25	Charsadda	-	PR-50	Swat	-

Over all isolation frequency = 36.61%

Table 4.20. Isolation of *Xoo* from Seeds

Sample No.	Location	Isolation	Sample No.	Location	Isolation
S-1	Peshawar	+	S-26	Charsadda	+
S-2	Peshawar	-	S-27	Charsadda	+
S-3	Peshawar	-	S-28	Charsadda	-
S-4	Peshawar	+	S-29	Charsadda	+
S-5	Peshawar	-	S-30	Charsadda	+
S-6	Peshawar	+	S-31	Charsadda	-
S-7	Peshawar	-	S-32	Charsadda	-
S-8	Peshawar	-	S-33	Charsadda	-
S-9	Peshawar	+	S-34	Swat	-
S-10	Peshawar	-	S-35	Swat	+
S-11	Peshawar	-	S-36	Swat	+
S-12	Peshawar	+	S-37	Swat	-
S-13	Peshawar	-	S-38	Swat	-
S-14	Peshawar	+	S-39	Swat	+
S-15	Peshawar	-	S-40	Swat	-
S-16	Peshawar	-	S-41	Swat	+
S-17	Charsadda	-	S-42	Swat	-
S-18	Charsadda	-	S-43	Swat	-
S-19	Charsadda	+	S-44	Swat	+
S-20	Charsadda	+	S-45	Swat	-
S-21	Charsadda	+	S-46	Swat	-
S-22	Charsadda	-	S-47	Swat	-
S-23	Charsadda	+	S-48	Swat	-
S-24	Charsadda	-	S-49	Swat	+
S-25	Charsadda	+	S-50	Swat	+

Over all isolation frequency = 30.98%

Table 4.21. Cumulative Frequency (%) of *X. o.o* recovery from different sources of inoculum

Location	Total samples	Isolates			
		Field Water	Plant residues	Seeds	Weeds
Peshawar	16	6	8	6	2
Charsadda	17	5	11	9	2
Swat	17	7	7	7	1
Total isolates	50	18	26	22	5
Frequency %		25.35	36.61	30.98	7.06

4.7 Effect of planting dates on incidence of *Xanthomonas oryzae* pv. *oryzae* and plant biomass

4.7.1 Tillers plant^{-1}

Two years data regarding tillers plant^{-1} are presented in Tables 4.22 and 4.23, for 2007 and 2008, respectively. Statistical analysis of the data revealed that there was an interactive effect of sites and varieties on tillers plant^{-1} during 2007. Conversely, sites during 2008 did not have a significant effect on tillers m^{-2}. on the other hand the effect of sowing dates was not significant on tillers plant^{-1} during both years. Interactions among sowing dates x varieties and sites x varieties were found significant during 2007 for tillers plant^{-1}. However, in 2008, interaction between sites and varieties was found non-significant. Interaction among sowing dates x sites, sites x sowing dates x varieties were not significant for tillers plant^{-1} in both years. Means indicated that higher number of tillers plant^{-1} were recorded in rice variety Dilrosh-97; although, it was at par with F. Malakand and Basmati-385, whereas a lower number of tillers plant^{-1} was recorded in JP-5, which was statistically similar, to Swat-1 during both years. In case of sites, number of tillers plant^{-1} was higher at Baffa as compared to Mingora during both years. Sites and varieties interaction showed that tillers plant^{-1} were decreased for all varieties at Mingora as compared to Baffa and the maximum number of tillers plant^{-1} (29.25) were found for Dilrosh-97 at Baffa while lower number of tillers plant^{-1} (18.43) was recorded for the same variety at Mingora during 2007. Interaction between sowing dates x varieties indicated that rice varieties i.e. Dilrosh-97, F.Malakand, JP-5, and Basmati-385 showed decreased tillers plant^{-1} when sowing was delayed and higher number of tillers plant^{-1} was recorded at 25th June, whereas it was higher at 15th June during both years for the other two varieties.

Table 4.22: Tillers plant^{-1} affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2005.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	33.25	26.25	25.25	23.25	25.50	26.70
	June, 15	27.00	26.25	22.25	22.50	27.50	25.10
	June, 25	27.50	26.75	22.75	23.50	25.50	25.20
Mingora	June, 05	18.12	19.65	22.70	17.70	22.38	20.11
	June, 15	18.38	19.70	18.20	20.45	19.98	19.34
	June, 25	16.13	19.68	14.40	16.62	16.85	16.74
		Site x Varieties					
Baffa		29.25	26.42	23.42	23.08	26.17	25.67
Mingora		17.54	19.67	18.43	18.26	19.73	18.73
		Sowing Dates x Varieties					
June, 05		25.69	22.95	23.97	20.47	23.94	23.40
June, 15		22.69	22.97	20.22	21.47	23.74	22.22
June, 25		21.81	23.21	18.57	20.06	21.17	20.97
Means		23.40 a	23.05 a	20.92 b	20.67 b	22.95 a	

LSD (P≤0.05) for sites = 1.43 (1.43), LSD (P≤0.05) for sowing = 2.50 (2.50)
LSD (P≤0.05) for varieties = 1.531.53, LSD (P≤0.05) for S x SD= 3.06 (3.06
LSD (P≤0.05) for S x V= 2.3, LSD (P≤0.05) for SD x V = 3.33
LSD (P≤0.05) for S x SD x V= 4.43

Table 4.23. Tillers plant^{-1} affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
	June, 05	27.25	20.25	19.25	17.25	19.50	20.70
Baffa	June, 15	21.00	20.25	16.25	16.50	21.50	19.10
	June, 25	21.50	20.75	16.75	17.50	19.50	19.20
	June, 05	16.79	18.31	21.36	16.36	21.04	18.77
Mingora	June, 15	17.04	18.36	16.86	19.11	18.64	18.00
	June, 25	14.79	18.34	13.06	15.29	15.51	15.40
		Site x Varieties					
Baffa		23.25	20.42	17.42	17.08	20.17	19.67
Mingora		16.20	18.34	17.09	16.92	18.39	17.39
		Sowing Dates x Varieties					
June, 05		22.02	19.28	20.31	16.81	20.27	19.74
June, 15		19.02	19.31	16.56	17.81	20.07	18.55
June, 25		18.14	19.54	14.91	16.39	17.51	17.30
Means		19.73 a	19.38 a	17.26 b	17.00 b	19.28 a	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD (P≤0.05) for sites = 1.43, LSD (P≤0.05) for sowing = 2.50,
LSD (P≤0.05) for varieties = 1.53, LSD (P≤0.05) for S x SD= 3.06
LSD (P≤0.05) for S x V= 2.3, LSD (P≤0.05) for SD x V = 3.33
LSD (P≤0.05) for S x SD x V= 4.43

4.7.2 Bacterial leaf blight (%)

Data pertaining to bacterial leaf blight for the year 2007 and 2008 are shown in tables 4.24 and 4.25, respectively. Perusal of the data indicates that bacterial leaf blight was significantly affected by sowing dates and varieties, whereas the effect of sites was found non-significant during both years. All interactions were found non- significant except sowing dates x varieties during both years. Higher bacterial leaf blight was recorded for F. Malakand (17.92 %) followed by basmati-385 (13.33 %), whereas the lower incidence of bacterial leaf blight (7.05 %) was observed in swat-1, although it was statistically similar to JP-5 and Dilrosh-97 during 2007 (Table 4.24). During 2008, a similar trend was observed and F. Malakand was found extremely prone to bacterial leaf blight (21.88), followed by Basmati-385 while the least amount blight was observed on Swat-1 (9.38 %), which was at par with JP-5 and Dilrosh-97. Interaction between sowing dates x varieties indicated that rice varieties i.e. Dilrosh-97, and Swat-1 resulted in increase bacterial leaf blight till 15th June but further delay did not significantly increase.

Table 4.24. Bacterial Leaf Blight (%) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2007.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
	June, 05	5.0	17.5	17.5	7.5	16.3	12.8
Baffa	June, 15	10.0	17.5	6.3	8.8	11.3	10.8
	June, 25	6.3	17.5	5.0	5.0	11.3	9.0
	June, 05	6.3	18.8	16.3	7.5	17.5	13.3
Mingora	June, 15	12.5	17.5	6.3	8.8	11.3	11.3
	June, 25	5.0	21.3	6.3	5.0	13.8	10.3
		Site x Varieties					
Baffa			17.5	9.6	7.1	12.9	10.8
Mingora			19.2	9.6	7.1	14.2	11.6
		Sowing Dates x Varieties					
June, 05			18.1	16.9	7.5	16.9	13.0 a
June, 15			17.5	6.3	8.8	11.3	11.0 ab
June, 25			19.4	5.6	5.0	12.5	9.6 b
Means			18.3 a	9.6 c	7.1 c	13.5 b	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD (P≤0.05) for sites = 3.45, LSD (P≤0.05) for sowing = 2.92
LSD (P≤0.05) for varieties = 3.08, LSD (P≤0.05) for S x SD= 4.42
LSD (P≤0.05) for S x V= 4.88, LSD (P≤0.05) for SD x V = 5.47
LSD (P≤0.05) for S x SD x V= 7.93,

Table 4.25. Bacterial Leaf Blight (%) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	10.00	22.50	22.50	12.50	21.25	17.75
	June, 15	15.00	22.50	11.25	13.75	16.25	15.75
	June, 25	11.25	22.50	10.00	10.00	16.25	14.00
Mingora	June, 05	10.00	25.00	15.00	5.00	25.00	16.00
	June, 15	12.00	17.50	7.50	10.00	12.50	12.00
	June, 25	5.00	21.25	7.50	5.00	13.75	10.50
		Site x Varieties					
Baffa		12.08	22.50	14.58	12.08	17.92	15.83
Mingora		9.17	21.25	10.00	6.67	17.08	12.83
		Sowing Dates x Varieties					
June, 05		10.00	23.75	18.75	8.75	23.12	16.88 a
June, 15		13.75	20.00	9.38	11.88	14.38	13.88 b
June, 25		8.12	21.88	8.75	7.50	15.00	12.25 b
Means		10.62 c	21.88 a	12.29 c	9.38 c	17.50 b	

Means followed by different letters in categories are significantly different at 5% level of probability

LSD (P≤0.05) for sites = 3.14, LSD (P≤0.05) for sowing dates = 2.65

LSD (P≤0.05) for varieties = 2.91, LSD (P≤0.05) for S x SD= 4.02

LSD (P≤0.05) for S x V= 4.54, LSD (P≤0.05) for SD x V = 5.12

LSD (P≤0.05) for S x SD x V= 7.41

4.7.3 Grains panicle^{-1}

Data regarding grains panicle^{-1} during the year 2007 and 2008 are shown in Tables 4.26 and 4.27, respectively. During each year, sites and varieties significantly affected grains panicle^{-1}. The effect of sowing dates was however nonsignificant. None of the interactions was significant except interaction between sowing dates and varieties. Higher grains panicle^{-1} was recorded at Baffa as compared to Mingora. In case of varieties, higher number of grains panicle^{-1} was found in rice variety JP-5, followed by Basmati-385, which were statistically at par. Lower grains panicle^{-1} was counted in Dilrosh-97; which was at par with Swat-1. Interaction between sowing dates and varieties showed that grains panicle^{-1} decreased with delay in sowing for variety Dilrosh-97, whereas rice varieties Basmati-385 and Swat-1 produced maximum number of grains panicle^{-1} by 25th June. Higher number of grains panicle^{-1} in F. Malakand was recorded on 15th June, whereas in case of JP-5 higher number of grains panicle^{-1} was recorded on 5th June.

Table 4.26. Grains panicle^{-1} affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2007

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	186.0	195.2	233.8	188.2	219.5	204.6
	June, 15	184.0	209.0	221.2	179.2	215.5	201.8
	June, 25	181.0	194.5	227.2	204.2	225.2	206.5
Mingora	June, 05	169.3	178.5	217.0	171.5	202.8	187.8
	June, 15	167.3	192.2	204.5	162.5	198.8	185.1
	June, 25	164.2	177.7	210.5	187.5	208.5	189.7
		Site x Varieties					
Baffa		183.7	199.6	227.4	190.6	220.1	204.3
Mingora		166.9	182.8	210.7	173.8	203.3	187.5
		Sowing Dates x Varieties					
June, 05		177.6	186.9	225.4	179.9	211.1	196.2
June, 15		175.6	200.6	212.9	170.9	207.1	193.4
June, 25		172.6	186.1	218.9	195.9	216.9	198.1
Means		175.3 c	191.2 b	219.0 a	182.2 c	211.7 a	

Means followed by different letters in categories are significantly different at 5% level of probability

LSD (P≤0.05) for sites = 11.90, LSD (P≤0.05) for sowing dates = 12.80

LSD (P≤0.05) for varieties = 7.45, LSD (P≤0.05) for S x SD= 17.53

LSD (P≤0.05) for S x V= 14.10, LSD (P≤0.05) for SD x V = 16.66

LSD (P≤0.05) for S x SD x V= 23.42

Table 4.27. Grains panicle^{-1} affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	171.5	180.8	219.2	173.8	205.0	190.1
	June, 15	169.5	194.5	206.8	164.8	201.0	187.3
	June, 25	166.5	180.0	212.8	189.8	210.7	191.9
Mingora	June, 05	147.0	156.2	194.8	149.2	180.5	165.6
	June, 15	145.0	170.0	182.2	140.2	176.5	162.8
	June, 25	142.0	155.5	188.2	165.2	186.2	167.4
		Site x Varieties					
Baffa		169.2	185.1	212.9	176.1	205.6	189.8
Mingora		144.7	160.6	188.4	151.6	181.1	165.3
		Sowing Dates x Varieties					
June, 05		159.2	168.5	207.0	161.5	192.8	177.8
June, 15		157.3	182.2	194.5	152.5	188.8	175.1
June, 25		154.3	167.8	200.5	177.5	198.5	179.7
Means		156.9 c	172.8 b	200.7 a	163.8 c	193.3 a	

Means followed by different letters in categories are significantly different at 5% level of probability

LSD (P≤0.05) for sites = 19.04, LSD (P≤0.05) for sowing dates = 12.80

LSD (P≤0.05) for varieties = 7.45, LSD (P≤0.05) for S x SD= 22.08

LSD (P≤0.05) for S x V= 20.02, LSD (P≤0.05) for SD x V = 16.66

LSD (P≤0.05) for S x SD x V= 26.7

4.7.4 1000 grain weight

Data regarding 1000 grain weights (g) are presented in Tables 4.28 and 4.29, for both the years. Sites, sowing dates and varieties, significantly influenced 1000 grain weight during 2007. All interactions were non-significant except interaction between sowing dates and varieties. Mean values indicated that greater 1000-grain weight was produced at Baffa as compared to Mingora. Comparing different rice varieties, it was observed that heavier grains were recorded in rice variety F. Malakand followed by Dilrosh-97, whereas lighter grains were recorded for Swat-1. Interaction between sowing dates and varieties showed that 1000 grain weight was enhanced till 15th June in each variety but further delay in sowing resulted in decline in 1000 grain weight for all rice varieties, although the increase was higher in both F. Malakand and Dilrosh-97. For the year 2008, on the other hand the effect of varieties was significant whereas sowing dates and sites did not considerably alter 1000 grain weight of rice. None of the interactions was found significant for 1000 grain weight during 2008. Comparing different rice varieties during 2008, revealed that greater 1000-grain weight was recorded for F.Malakand, which was at par with Dilrosh-97, and basmati-385, whereas minimum 1000-grain weight was observed in Swat-1, while it was statistically similar to JP-5.

Table 4.28. 1000 grains weight (g) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2007

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	17.34	18.54	15.59	15.06	16.39	16.58
	June, 15	18.36	19.79	16.31	15.59	17.27	17.46
	June, 25	17.39	18.61	15.63	15.07	16.44	16.63
Mingora	June, 05	14.39	15.59	12.64	12.11	13.69	13.68
	June, 15	15.66	17.09	13.61	12.89	14.58	14.77
	June, 25	14.69	15.91	12.93	12.37	13.74	13.93
		Site x Varieties					
Baffa		17.70	18.98	15.84	15.24	16.70	16.89
Mingora		14.92	16.19	13.06	12.46	14.00	14.13
		Sowing Dates x Varieties					
June, 05		15.86	17.06	14.11	13.59	15.04	15.13 b
June, 15		17.01	18.44	14.96	14.24	15.93	16.12 a
June, 25		16.04	17.26	14.28	13.72	15.09	15.28 b
Means		16.31 b	17.59 a	14.45 d	13.85 e	15.35 c	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD ($P \leq 0.05$) for sites = 1.30, LSD ($P \leq 0.05$) for sowing dates = 0.38
LSD ($P \leq 0.05$) for varieties = 0.06, LSD ($P \leq 0.05$) for S x SD= 1.31
LSD ($P \leq 0.05$) for S x V= 1.36, LSD ($P \leq 0.05$) for SD x V = 0.39
LSD ($P \leq 0.05$) for S x SD x V= 1.31

Table 4.29. 1000 grains weight (g) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	17.15	18.34	15.39	14.87	16.19	16.93
	June, 15	18.17	19.59	16.12	15.39	17.08	17.27
	June, 25	17.20	18.41	15.44	14.87	16.25	16.43
Mingora	June, 05	17.63	18.88	13.87	14.12	17.65	16.43
	June, 15	17.15	16.45	15.95	15.20	16.45	16.24
	June, 25	16.95	13.70	17.70	16.95	15.95	16.25
		Site x Varieties					Means
Baffa		17.50	18.78	15.65	15.04	16.51	16.70
Mingora		17.24	16.34	15.84	15.43	16.68	16.31
		Sowing Dates x Varieties					Means
	June, 05	17.39	18.61	14.63	14.50	16.92	16.41
	June, 15	17.66	18.02	16.03	15.30	16.76	16.75
	June, 25	17.07	16.06	16.57	15.91	16.10	16.34
Varieties		17.37 a	17.56 a	15.75 bc	15.23 c	16.59 ab	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD (P≤0.05) for sites = 2.19, LSD (P≤0.05) for sowing dates = 1.26
LSD (P≤0.05) for varieties = 1.14, LSD (P≤0.05) for S x SD= 2.43
LSD (P≤0.05) for S x V= 2.45, LSD (P≤0.05) for SD x V = 2.12
LSD (P≤0.05) for S x SD x V= 3.38

4.7.5 Panicle length

Perusal of the data exhibited that sites had a significant effect on panicle length, whereas the effect of sowing dates and varieties was nonsignificant during 2007. None of the interactions was found significant except interaction between sowing dates and varieties for panicle length. By comparing both sites, it was obvious that longer panicles were observed at Baffa as compared to Mingora (Tables 4.30 and 4.31). Interaction between sowing dates and varieties showed that panicle length decreased when sowing was delayed for rice varieties Dilrosh-97, F.Malakand and Basmati-385, whereas JP-5 and Swat-1 resulted in a higher panicle length when sowing was delayed during both years.

Table 4.30. Panicle length (cm) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2007.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	27.00	28.00	23.00	23.25	27.25	25.70
	June, 15	26.75	26.25	25.75	25.00	26.25	26.00
	June, 25	26.75	23.50	27.50	26.75	25.75	26.05
Mingora	June, 05	23.73	24.73	19.73	19.98	23.50	22.33
	June, 15	23.00	22.30	21.80	21.05	22.30	22.09
	June, 25	22.80	19.55	23.55	22.80	21.80	22.10
		Site x Varieties					
Baffa		26.83	25.92	25.42	25.00	26.42	25.92
Mingora		23.18	22.19	21.69	21.28	22.53	22.17
		Sowing Dates x Varieties					
June, 05		25.36	26.36	21.36	21.61	25.38	24.02
June, 15		24.88	24.28	23.78	23.03	24.28	24.05
June, 25		24.78	21.53	25.53	24.78	23.78	24.08
Means		25.00	24.05	23.55	23.14	24.48	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD (P≤0.05) for sites = 2.70, LSD (P≤0.05) for sowing dates = 1.74
LSD (P≤0.05) for varieties = 1.62, LSD (P≤0.05) for S x SD= 3.08
LSD (P≤0.05) for S x V= 3.14, LSD (P≤0.05) for SD x V = 2.98
LSD (P≤0.05) for S x SD x V= 4.58

Table 4.31. Panicle length (cm) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	26.25	26.50	23.00	23.25	27.25	25.25
	June, 15	26.25	26.00	25.75	25.00	26.25	25.85
	June, 25	26.25	23.00	27.50	26.75	25.50	25.80
Mingora	June, 05	23.73	24.73	19.73	19.98	23.50	22.33
	June, 15	23.00	22.30	21.80	21.05	22.30	22.09
	June, 25	22.80	19.55	23.55	22.80	21.80	22.10
		Site x Varieties					
Baffa		26.25	25.17	25.42	25.00	26.33	25.63
Mingora		23.18	22.19	21.69	21.28	22.53	22.17
		Sowing Dates x Varieties					
June, 05		24.99	25.61	21.36	21.61	25.38	23.79
June, 15		24.63	24.15	23.78	23.03	24.28	23.97
June, 25		24.53	21.28	25.53	24.78	23.65	23.95
Means		24.71	23.68	23.55	23.14	24.43	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD ($P \leq 0.05$) for sites = 2.61, LSD ($P \leq 0.05$) for sowing dates = 1.72
LSD ($P \leq 0.05$) for varieties = 1.57, LSD ($P \leq 0.05$) for S x SD= 3.01
LSD ($P \leq 0.05$) for S x V= 3.04, LSD ($P \leq 0.05$) for SD x V = 2.89
LSD ($P \leq 0.05$) for S x SD x V= 4.44

4.7.6 Grain yield

Tables 4.32 and 4.33 show grain yield (tones ha^{-1}) during 2007 and 2008, respectively. Sites, sowing dates and varieties significantly affected grain yield. Interactions among sowing dates x varieties, sites x varieties, sites x sowing dates and sites x sowing dates x varieties were found significant during both the years. Means values for rice varieties showed that higher grain yield was achieved for varieties F. Malakand followed by Dilrosh-97, whereas both Swat-1 and JP-5 resulted in the lowest grain yield during both years. Greater grain yield was observed at Baffa as compared to Mingora for both years. In case of sowing dates, higher grain yield was produced when planting was done on 15th June, while lower grain yield was recorded for early sowing (5th June).

Interaction between Sites x varieties indicated that the least grain yield was obtained for all varieties at Mingora as compared to Baffa. Greater grain yield was recorded for Dilrosh-97 at Baffa but lower for Swat-1 at Mingora during both years. Interaction between sowing dates and varieties exhibited that Dilrosh-97, F. Malakand and JP-5 varieties yielded better when sowing was delayed from 5 June to 15th June but further delay in sowing resulted in lower grain yield, whereas delay in sowing upto 25th June for Swat-1 and Basmati-385 resulted in increased grain yield during both years. Sites x sowing dates interaction showed that grain yield was enhanced with delay in sowing from first to third sowing at Baffa, but at Mingora, the increase was noted up to 15th June and further delay upto 25th June resulted in reduced grain yield. Interaction between sites x sowing dates x varieties revealed that grain yield increased with delay in sowing till 15th June for each of varieties Swat-1, Basmati-385 and F.Malakand at Baffa, whereas delay in sowing resulted in decline in grain yield for JP-5 at Baffa. Delay in planting upto 15th June for Dilrosh-97 gave better grain yield at Baffa. At Mingora, all rice varieties planted on 15th June resulted in higher grain yield.

Table 4.32: Grain yield (tones ha^{-1}) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2007.

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
Baffa	June, 05	7.89	7.33	6.93	5.38	4.27	6.36
	June, 15	9.14	7.19	5.66	6.57	5.66	6.85
	June, 25	5.95	8.08	5.38	9.83	8.30	7.51
Mingora	June, 05	3.42	6.54	3.17	2.54	4.96	4.13
	June, 15	7.42	10.75	6.17	4.29	7.08	7.14
	June, 25	5.71	6.79	5.63	3.83	5.25	5.44
		Site x Varieties					
Baffa		7.66	7.53	5.99	7.26	6.08	6.90
Mingora		5.51	8.03	4.99	3.56	5.76	5.57
		Sowing Dates x Varieties					
June, 05		5.65	6.94	5.05	3.96	4.61	5.24 c
June, 15		8.28	8.97	5.92	5.43	6.37	6.99 a
June, 25		5.83	7.43	5.50	6.83	6.78	6.47 b
Means		6.59 b	7.78 a	5.49 d	5.41 d	5.92 c	

Means followed by different letters in categories are significantly different at 5% level of probability
LSD ($P \leq 0.05$) for sites = 0.35, LSD ($P \leq 0.05$) for sowing dates = 0.28
LSD ($P \leq 0.05$) for varieties = 0.33, LSD ($P \leq 0.05$) for S x SD= 0.44
LSD ($P \leq 0.05$) for S x V= 0.51, LSD ($P \leq 0.05$) for SD x V = 0.57
LSD ($P \leq 0.05$) for S x SD x V= 0.82

Table 4.33. Grain yield (tones ha^{-1}) affected by sowing dates and varieties of rice at Mansehra (Baffa) and Mingora during 2008

Sites	Sowing dates	Rice Varieties					S x SD
		Dilrosh-97	F. Malakand	JP-5	Swat-1	Bas-385	
	June, 05	7.63	7.10	6.63	5.06	4.04	6.09
Baffa	**June, 15**	8.85	6.93	5.45	6.36	5.45	6.61
	June, 25	5.74	7.87	5.17	9.62	8.09	7.30
	June, 05	3.29	6.41	3.04	2.41	4.83	4.00
Mingora	**June, 15**	7.29	10.62	6.04	4.16	6.95	7.01
	June, 25	5.58	6.66	5.50	3.70	5.12	5.31
		Site x Varieties					
Baffa		7.41	7.30	5.75	7.02	5.86	6.67
Mingora		5.38	7.90	4.86	3.43	5.63	5.44
		Sowing dates x Varieties					
June, 05		5.46	6.75	4.83	3.74	4.44	5.04 c
June, 15		8.07	8.78	5.75	5.26	6.20	6.81 a
June, 25		5.66	7.26	5.33	6.66	6.61	6.30 b
Means		6.40 b	7.60 a	5.30 d	5.22 d	5.75 c	

Means followed by different letters in categories are significantly different at 5% level of probability.
LSD ($P \leq 0.05$) for sites = 0.40, LSD ($P \leq 0.05$) for sowing dates = 0.29
LSD ($P \leq 0.05$) for varieties = 0.33, LSD ($P \leq 0.05$) for S x SD= 0.48
LSD ($P \leq 0.05$) for S x V= 0.53, LSD ($P \leq 0.05$) for SD x V = 0.57
LSD ($P \leq 0.05$) for S x SD x V= 0.84

CHAPTER 5

DISCUSSION

Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922), is a major limiting factor in rice production in various rice growing zones in Asia. Likewise, in Pakistan rice production in major rice growing zones (I and II) has been hampred due to high susceptibility of rice cultivars *Xoo* grown in this area, especially basmati types (Akhtar *et al.*, 2004).

Disease control through the use of pesticides has been ineffective in reducing the losses due to bacterial leaf blight. Therefore, efforts have been diverted to develop resistant cultivars. Such efforts started in Japan in 1923 and are considered as the most economical option for controlling the disease with concurrent reduction in losses. These efforts have been regularly coordinated by the International Rice Research Institute (IRRI). Consequently, a huge collection of rice germplasm was screened for resistance to the BLB pathogen. Furthermore, the cultivars were genetically analyzed and some 30 major resistant genes have been identified against BB pathogen, *X. oryzae pv. oryzae (Xoo)* (Aye *et al.*, 2007; Jyufuku *et al.*, 2009; Shah *et al.*,2009). These resistance genes now constitute a major foundation block for resistance breeding and have since been regularly incorporated into rice varieties with improved agronomic characteristics and desirable attributes. However, with the emergence of new races of the pathogen, these resistance genes need a re-evaluation from time to time. It is, therefore, of significant value to study the biology of the pathogen, its virulence and survival mechanism; its effect on the available cultivars with and without resistance, pathotyping and race identification to pave way to a successful breeding program against BLB of rice.

Incidence and severity are the two quantitative parameters of disease which are commonly assessed in epidemiological studies (James and Teng, 1979; Campbell and Madden, 1990; Nagarajan and Muralidharan, 1995). Disease incidence is the proportion of visible infected plant units, and is usually expressed as a percentage of the total population (Zadoks and Shein, 1979). While, disease severity is the proportion of tissue infected by disease, and is expressed as a percentage of total

tissue area. It has been shown that yield losses due to BLB may vary from 10% in case of mild infection to 50% in case of severe infection (Ou, 1985; Mew 1987). Similar observations have been recorded in individual studies, where bacterial blight was noted to have attacked rice crop in the country infecting over 50,000 acres in Sindh and Punjab provinces resulting in a total of 2% loss in yield despite 0.2% increase in production land (The News, 3rd October 2007).

In the present studies rice crop was monitored for BLB incidence and severity in 12 rice growing districts of Khyber Pakhtunkhwa (KP), 10 districts of Punjab, 8 districts of Sindh and only 1 location in Baluchistan for the 3 consecutive years during 2005, 2006 and 2007. The results of the surveyed areas indicated a high level of infestation of rice in these rice-growing zones of Pakistan. In the current study, disease incidence varied from 35-80%, 36-74%, 11-46% and 12-21% in KP, Punjab, Sindh and Balochistan, respectively, and was indicative of the seriousness of the situation. These results not only confirmed previous observations (Akhtar *et al.*, 2003) but also shed light on the gradual built up of inoculum in the absence of effective resistance (Akhtar and Akram, 1987). Such differences in % incidence could also be due to rice cultivars and cultural practices which were not taken into consideration during the study. However, it was observed that local rice cultivars such as JP- 5, Shoga and other land races were more susceptible to the disease and yield loss may reach 100% in case of Basmati type rice (Agarwal *et al.*, 2005). Additionally, during the cropping season in 2007, less precipitation and high temperature was observed throughout the country, which also influenced the occurrence of BLB. Furthermore, in Shangla, Chitral, Swat, Dir Lower and Dir Upper which are hilly areas of KP, rice crop has traditionally been grown on the river side within small fields and irrigated by river and spring water for years. Additionally no resistance is available in most of the varieties and land races commercially cultivated. The disease has, therefore, caused tremendous losses in recent years. The evolution of new races as reported by Manan *et al.*, (2007) from this area has further aggravated the situation.

The maximum disease incidence and severity was recorded in district Shangla comprising of mountainous location in northern zones of KP, during all the 3 years was not surprising since susceptible cultivars such as JP-5 and Shogga, which were famous for stickiness and very much preferred by the locals, predominated most of

the rice fields. New rice cultivars have now been introduced by Agriculture Research Institute (North), Mingora, Swat. Which are now gaining popularity among the farmers since these gave high yield besides being resistant and may help reduce the disease incidence in the coming years.

According to Azuka and Kaku (2000) incidence and severity of bacterial leaf blight are influenced by various environmental factors including topographic and soil conditions, metrological conditions, and cultural practices. However, high rainfall and heavy nitrogen fertilization are the most important factors affecting the occurrence of the disease. It is evident from the current study that high incidence of BB was observed in KP province followed by Punjab. Monsoon rains and high humidity served as main factors in disease prevalence. These results are in conformity with the results of above mentioned workers. Furthermore, this problem vastly aggravated, perhaps due to the socio-economic conditions of the rice farmers. In Pakistan, poverty and small land holding are some of the major factors responsible for practicing mono-cropping in major rice growing areas. Such practices have led to gradual build up of the inoculum in the field and subsequently became a limiting factor in rice production. The disease usually occurs in fields following heavy rain and/or cloudy weather with winds which injure the foliage thus providing an entry point to the pathogen along with natural openings in the leaf such as stomata and hydathods.

Similarly, the high incidence of BLB in hilly areas of KP such as Swat and Shangla districts can also be attributed to injudicious application of nitrogen fertilizers and high rainfall during monsoon season (Dera Wadan, personal communication). Rice is extensively grown in these areas, and being the desire of farmers to grow this crop each year due to low land holdings, the amount of inoculum might have increased and became a potential threat to rice. However, areas where a low incidence of the disease was observed it can be attributed either to low relative humidity, low nitrogen fertilizer application or due to crop rotation with wheat, maize and other vegetables crops. However, further investigation is needed to see the effect of crop rotation on disease development.

According to Akhtar and Zakaria (2003), BLB incidence in KP in 2002 ranged 0-95%, 0-100% and 0-85% in Dir lower, Swat and Malakend, respectively. Disease incidence at other locations of the province was reported for the first time and no

previous reports exist. The present study clearly supported the reports of Akhtar and Zakaria (2003) as the disease has increased in recent years and created an alarming situation.

Comparing the data of three years in Punjab indicates that maximum disease incidence prevailed during 2006 while minimum incidence was observed in 2007. This decline is possibly due to prolonged drought in the year 2007. These results substantiated the findings of Khan *et al.*, (2000) who eported increased bacterial blight incidence in recent years especially in Kaller belt of the Punjab which is famous for producing high quality aroma rice. The same findings were also subtantiated by Akhtar *et al.*, (2003) who reported basmati rice which predominates the rice fields of Punjab to be more susceptible and cautioned an alarming situation of BLB in rice wheat system due to inoculum build up. Furthermore, the disease is humidity and temperature dependent especially if prevailed during critical booting stage of the rice crop in monsoon season.

Previous studies conducted in the area (Akhtar *et al.*, 2003) reported an overall less disease in various districts of Punjab when compared with present Sind. There has been a consistent increase in BLB prevalence over time.

The overall BLB situation has thus further aggravated. This is due to the inoculum build up in rice wheat system adopted in these districts.

Sindh is one of the major rice growing provinces of Pakistan, however, the low incidence compared to Punjab and KP provinces can be linked to the fact that a number of different rice cultivars (Dehradun Basmati, Hansraj, Supri, IRRI-6 and IRRI-9) are grown in rice field of Sindh compared with Shogga, JP5 of KP and a highly BB susceptible Basmati in Punjab province. Furthermore, the climates of all three major rice growing provinces are diverse and are an additional factor in differences observed in disease incidence in the present survey.

Akhtar and Zakaria (2003) reported minimal disease in Sind during 2002. However, this inoculum load has increased many folds during 2004, 2005 and 2006. The critical situation of inoculum build up in Sindh rice growing areas warrants to plan sustainable management of BLB to protect the crop against the prevalent infection.

The low incidence of BLB in Balochistan may be due to the fact that rice cultivation is limited to few areas. The only location studied in these studies indicated a similar pattern of disease incidence and severity due to unfavorable environmental conditions for BLB pathogen during critical stage of the rice crop. However, comparison with previous data (Akhtar and Zakria, 2003) revealed that the incidence of BLB has also increased in recent years since in 2002 disease incidence in Usta Mohammad was recorded as 5-6%.

BLB of rice is very much influenced by high relative humidity or moisture during monsoon season. Warm temperature near 34 °C and high humidity favor the development of BLB as reported by Azuka and Kaku (2000). These discrepancies in % incidence could also be due to rice cultivars and cultural practices which were not taken into consideration during the study. However, it was observed that local rice cultivars such as JP- 5, Shoga and other land races were more susceptible to disease. Additionally, during the cropping season in 2007, less precipitation and high temperature was observed throughout the country which also influenced the occurrence of BB.

The maximum disease incidence and severity was recorded in district Shangla comprising mountainous location in northern zones of KP, during all three years are not surprising since, susceptible cultivars such as JP-5 and Shogga which are famous for stickiness and very much preferred by the locals, predominated most of the rice fields. Though new rice cultivars have now been introduced by Agriculture Research Institute (North), Mingora, Swat which are now gaining popularity among the farmers high yield, because of resistance to bacterial leaf blight and other and desired traits.

A total of 125 *Xoo* isolates were collected from rice samples representing 12 districts of different agro-ecological zones of KP. Colonies recovered were mucoid, convex and shinny in texture on YDC agar medium Ezuka and Kaku (2000). Similar characteristics of the *Xoo* were also reported by Akhtar *et al.*, (2004) and Manan *et al.*, (2009). All samples collected were positively confirmed to be infected with BLB and the pathogen consistently recovered from the infected leaf tissues was the causal agent i.e. *X. oryzae* pv. *oryzae*.

However, these isolates were further characterized based on a series of biochemical tests as described in Burgey's manual and some diagnostic tests such as hypersensitivity response and pathogenicity reaction. It was appeared that some of isolates deviated from the normal pattern and had over lapping results. Therefore, identity of the all *Xoo* isolates was verified through *Xoo*-specific primers XOR-R2 and XOR-F with PCR, which amplify a 470 bp band (Adachi and Oku, 2000). However, no such band was amplified in 45 isolates indicating that these were not *Xoo* or these were intermediate form of the pathogen between *Xoo* and *Xooc* (*Xantomonas oryzae* pv. *oryzaecola*). The confirmed isolates were used in the next set of experiments. The results were in line with those of Jyufuku *et al.*, (2009).

The purpose of comparison of inoculation methods was to develop a simple, quick and reliable method of inoculation of different rice cultivars with *Xanthomonas oryzae* pv. *Oryzae*. Results showed that Bas 2000, Bas 385, Super Basmati and Bas 370 were susceptible to Rice bacterial blight disease showing typical disease symptoms with clipping method and no variety was found resistant. The clipping inoculation method showed typical yellow lesions with wavy margin 2 weeks after inoculation on cultivars Basmati 2000, Basmati 385 and Super Basmati. Based on lesion length scoring the susceptible and resistant varieties were assessed. Variation in lesion development was noticed with method of inoculation and with respect to cultivar. However, clipping method remained the best inoculation technique as compared to brush and pin prick methods. These results corroborated previous findings (Kauffman *et al.*, 1973). The brush and pin pricking method resulted in less BLB symptom as compared to clipping method. Brush method was the least effective as bacteria cannot penetrate the intact host surface. In screening for resistance such method can, therefore, cannot be used with confidence. Lesion development is significantly effective with method and variety. Moreover, on detached leaves lesions appeared within 5 days of inoculation with clipping method in contrast to other methods which took about 15 days on intact leaves. This might be due to loss of resistance due to excision shocking of incubation period. These studies suggested that clipping inoculation method provided a reliable method for screening of germ plasm resistance against bacterial blight.

The differential system presented from different countries has an essential significance in the respective region. However, in order to compare the pathogenic differentiation among different countries, an international set of differentials was needed. For this purpose, an international collaborative research project was initiated by IRRI in 1977 with active participation of IRRI, Bangladesh, China, Japan, India, Indonesia, Thailand, Korea, Malaysia, and Nepal (Horino, 1981). Since 1982, the collaboration has been focused on IRRI-Japan collaborative research project to establish international differentials consisting of near-isogenic lines (Ogawa *et al.*, 1991a). The international differentials thus developed are now used for identification of races as well as for various phytopathological studies due to the advantage of their near-isogenic nature. Keeping in view, the variation that existed in KP isolates, an experiment was conducted to categorize the collected isolates into different races.

The *Xoo* isolates confirmed through molecular analysis were further classified into 6 races based on their reaction on IRBB lines which have defined resistant genes. Current studies indicated that Race 1 was prevalent in all rice growing area of KP except Peshawar and showed virulence against all IRBB lines except IRBB-5 and IRBB-13 which possessed Xa-5 and Xa-13 resistant genes against *X. oryzae*. Furthermore, this race was predominantly present at three locations of Dir upper and two locations of Swat, Mansehra and Nowshera. Recently, PCR analysis of cultivars from rice growing areas revealed the presence of *Xa13* gene. In the present studies Seventeen rice cultivars out of a set of 34 were found resistant or moderately resistant to KP race 1. Analysis of the presence of Xa genes in these cultivars would be valuable. It has been shown that approximately 20 of the reported rice resistance genes to *Xoo* such as *Xa5* and *Xa13* are transmitted in a recessive manner (Sanchez *et al.*, 1999). This gene *(Xa13)* also conferred specific resistance to *Xoo* race 6 (Ogawa *et al.*, 1987). However, in the current studies it was found that only Xa-4, Xa-10 and Xa-14 were effective against Race6 of BLB pathogen. Resistance conferred by recessive genes such as *Xa5* and/or *Xa13* may represent very different and largely unknown biochemical pathways in the host defense system (Sanchez *et al.*, 1999). Therefore, cloning and the molecular study of *Xa13* may shed light on their role in the epidemiology of BLB in KP area. This would further lead to more efficient strategies for combating *Xoo* and can be achieved through marker-assisted selection (MAS) for pyramiding the resistance against BLB.

Race 2 broke resistance in Xa-5, Xa-8 and Xa-14 genes. Similarly, Race 3 failed to infect lines carrying Xa-3, Xa-5 and Xa-14 resistant genes alternatively. Race 4 was not pathogenic against Xa-5, Xa-7 and Xa-11, while Race 5 faced complete resistance from Xa-3, Xa-5, Xa-10 and Xa-13 carrying lines. Resistant genes were effective against all isolates of KP.

Similarly, Pakistani basmati varieties were screened by Khan *et al.*, (2000) along with some mutant Basmati lines against the virulent strains of *Xoo* prevalent in Pakistan, and found only Basmati 370 showed some resistance against *Xoo.*

The observations indicated that the strains causing disease in the field were different from each other and also different strains were prevailing in different rice growing areas. Vera Cruz *et al.*, (1996) observed in their study that different races of the same pathogen existed in the same field on the same cultivar.

Sodhi *et al.*, (2003) found high level of diversity in pathogen population collected from different parts of Indian Punjab while studying *Xoo* populations *Xoo* resistance genes *Xa8*, *Xa21*, *Xa5* and *Xa7* were involved. Since the Indian and Pakistani Punjab have the same climatic and eco-ecological conditions, it could be possible that the same genes could be effective in Pakistan's rice growing areas.

The present study revealed that the regionally defined pathogen populations are not alike between the two reigns within the contry, which might be due to slow dispersal of the pathogen and thus slow partitioning of host genotypes (Leach *et al.*, 1992; Nelson *et al.*, 1994; Adhikari *et al.*, 1995). Therefore, it is needed to identify other BLB resistance genes in rice germplasm and Basmati breeding lines. Effectiveness of identified bacterial blight resistance genes against the prevalent strain of *Xoo* also needs to be checked in Pakistan. The knowledge of the effective resistance genes and the pathogen population structure would be helpful in deploying suitable resistance genes in different rice growing areas.

118 rice varieties from PGRI, NARC Islamabad and Agriculture Research Center, Mingora, Swat have already been analyzed genetically for variation and the presence of resistance genes like *Xa13.*

Results of this study are in line with those of Lai-Van *et al.*, (1999) who divided isolates of *Xoo* into seven groups based on their pathogenicity on IRBB lines. In a similar study Dinh *et al.*, (2008) found Gene Xa5, highly effective against the disease. However, the results of Jyufuku *et al.*, (2009) are in conflict with the previous finding.

These results have significant implications for breeding resistance against BB pathogen. Ideally, a rice cultivar carrying Xa-5, Xa-14 and any other resistance genes from the above effective resistances should minimize the losses incurred by BB pathogen.

The epidimiology of bacterial leaf blight has been extensively studied and an outline of its disease cycle is well documented. However, controversy still exists on the probable overwintering, invasion and transmission of the pathogen. In temperate regions, source of primary infections and possible sites for overwintering are assumed to be soil, seed, rice straw, rice stubble, and perennial weeds (Azuka and Kaku, 2000). In the present study, source of primary infection in rice growing areas of Peshawar, Charsadda and Swat districts of KP were investigated during cropping season 2007. Cumulative Frequency (%) of *Xoo* recovery was determined for field water, weeds, plant residues and seeds. Results were in line with the findings of Li *et al.*, (1985) who reported a number of wild hosts (*Leersia* spp., *Leptochloa* spp., *Oryza* spp., *Paspalum scrobiculatum*, *Zizania*, *Zoysia* spp.) including poaceous weeds which may act as carriers. The spread pattern of *Xoo* in a rice field through field water has been analyzed by Nayak and Reddy (1985).

Role of potential inoculum sources including infected planting material, volunteer rice plants etc was determined by Durgapal (1983). Infected straw or chaff was investigated by Devadath and Dath (1985). Present results are in line with the findings of Hsieh *et al.*, (1974) who stated that seed transmission is generally thought to occur to a certain extent, but in contrast with Murty and Devadath (1984) who faced difficulty in demonstrating that infected seeds did not give rise to infected seedlings but did introduce the bacterium into the soil. Singh *et al.*, (1983), however, observed seed transmission regularly in growth chambers when used heavily infested samples.

Singh (1971) found that the bacterium cannot survive in unsterilized soil and only 15-38 days in field and pond water, but Murty and Devadath (1982) argued that this

depends on the soil type. Raj and Pal (1988) failed to obtain overwintering in soil or seed, but found survival only in infected leaves. Reddy (1972) demonstrated that *X. oryzae* pv. *oryzae* survives for 7-8 months in seed, but only for 3-4 months in straw and stubble; Kauffman and Reddy (1975) reported that, although glumes were readily infected, viable bacteria could not be detected on seed stored for 2 months. It is thought that bacteriophages play a role in reducing bacteria in germinating seed. In general, it is clear that the present results on survival and sources of inoculum contradictory to previous studies.

The variation in rice production could be attributed to different biotic and abiotic factors. It has been observed that regardless of providing similar agronomic conditions variation at a particular location and within locations can be observed. However, some of this could be managed through reducing experimental errors. The source of such variations may be due to genotype of the cultivar, existence of different pathogen races, crop husbandry practices and suppressive or concussive environmental conditions. The optimal growing season of popular cultivars has been determined by testing their growth and yields at different sowing dates. There are several reports that indicate that delaying sowing altered qualitative as well as quantitative traits of a crop. In the present studies 2 years data of Baffa, Mansehra and Mingora, Swat, showed similar trends in terms of agronomic traits as well as BB severity percentage. Cultivars attained maximum benefits of prolonged photoperiodic exposure when planted earlier. Both, locations are situated in semi-temperate zones of KP where winter starts earlier than plains and as such rice cultivars sensitivity to low temperature is a problem.

Results of the effect of planting dates on BB infection under natural conditions are at par with the reports of Singh and Parsed (1999), Hari *et al.*, (1999), Pirdash *et al.*, (2000), Habibullah *et al.*, (2007) and Safdar *et al.*, (2008) who found that delayed sowing decreased number of tillers. However, these results were in conflict with the findings of Baloch (2005) who reported that delayed sowing increased the number of tillers. This difference can be attributed to different agro-ecological zones and different cultural practices.

Delay in sowing decreased bacterial leaf blight for cultivars JP-5 and Basmati-385 during 2007. The reason for higher bacterial leaf blight in Baffa may be due to the

conducive environmental conditions compared to Mingora. Similarly, varieties have differential response to bacterial leaf blight due to their different genetic make up. The results are in line with the findings of Kabir *et al.*, (2007); Shah *et al.*, (2009); Ali *et al.*, *(2009)* and Waheed *et al.*, (2009) who also reported losses in yield in rice genotypes as a result of manifestation by bacterial leaf blight.

Maximum number of grains panicle^{-1} in Baffa as apposed to that at Mingora may be due the favorable climatic condition for growth and thus ultimately gave more number of grains panicle^{-1}. The reason for higher number of grains panicle^{-1} in JP-5 and Basmati-385 may be due their difference in genetic potential from other varieties since grains panicle^{-1} is a genetic character. Sherief *et al.*, (2000) showed a marked influence on number of grain panicle^{-1} with delay in seeding. Similarly, Nazir (1994) reported that earlier transplanting in rice causes lower number of grains per panicle due to grain sterility because of high temperature at the time of grain maturation. Transplanting at its optimum time reduces grain sterility. However, present results different from those of Khalifa (2009) who stated that grain panicle^{-1} was significantly decreased with delay in sowing and higher grain panicle^{-1} was recorded at seeding on 20th April.

Guilani *et al.*, (2002) supported the results and Khedikar *et al.*, (2003) and showed that the differences among the rice cultivar were not only due to genetic potential but also due to the influence of environmental conditions.

The results showed that panical length was non-significant for both 2005 and 2006 while changing sowing dates. These results are in line with those of Akram *et al.*, (2007) who reported that panicle length was not significantly influenced by sowing dates. Moreover, it was found that significant differences in various rice varieties did exist.

In both the years 2007 and 2008 results regarding grain yield proved 15th June as the best sowing date. The results are in line with the findings of Shah and Bhurer (2005) who found a significant seeding and variety interaction in rice and reported that rice variety Chaiti gave the highest yield seeding at 15th June. Likewise, Munda *et al.*, (1994) Koirala (1983), Kunwar, reported similar results and Shrestha (1975) and Bhurer *et al.*, (1990) who found that late rice varieties resulted in least grain yield.

The increase in grain yield on 15th June might be associated with lower bacterial leaf light incidence as compared to 5th June during both the years and sites. Similarly, greater grain yield at Baffa than at Mingora may be attributed to the favorable climate at the former than the latter for all the rice varieties during both years. Differences in grain yield of various rice cultivars may be due to their genetic make up and potential under suitable climate of both regions. The second day of sowing (15th June) benifited from improved sunshine and suitable temperature that ultimately gave higher grain yield in both years for all varieties except Swat-1 and Basmati-385, which resulted in greater grain yield with delay in seeding upto 25th June at both sites.

Singh and Parasad (1999), Hari *et al.*, (1999), and Pirdashty *et al.*, (2000) found that delayed sowing decreased the grain and, straw yield, harvest index, tiller number, panicle length, number of grain/panicle and fertility percentage. Sherief *et al.*, (2000) studied the effect of sowing dates (April 25th, May 10th, May 25th and June 10th) on yield and yield components of rice. A remarkable increase was observed in number of panicles per square meter, number of filled grains per panicle, 1000-grain weight, grain and straw yields per hectare by early sowing (May 10th). However, late planting on May 25th or June 10th significantly reduced the above mentioned traits.

Reports of Toth (1978), Ritchie *et al.*, (1989), Penning de Varies *et al.*, (1989), William *et al.*, (1989) and Singh *et al.*, (1977) indicated that the highest grain yield of rice was obtained from the 2nd may of sowing under Philippines conditions.

El-Hity *et al.*, (1987) found that the number of days from sowing to panicle initiation, maximum tillering, heading dates and grain yield (T/ha) were drastically reduced with delay of sowing time. Khalifa (2005) observed that early sowing date on April 20th gave the highest values of leaf area index (LAI), sink capacity [number of spikelets per M2 X1000], spikelets/ leaf area ratio, panicle length, and number of filled grains (%). H5 hybrid rice cultivar surpassed the other cultivars in leaf area index and sink capacity

CONCLUSIONS

1. The higher disease incidence in KP was recorded in Shangla during the first two years of survey. However, Swat excelled in the same parameter when survey was conducted in 2007. Comparatively higher disease severity was recorded throughout the course of the study. In Punjab disease incidence and severity was different at different locations across the year. In Sindh, on the other hand, Badin was the most severely affected area in case of disease incidence and Shikarpur in terms of disease severity. The regional differences could be attributed to inoculum build up and due to meteorological conditions.
2. A total of 125 isolates were recovered from disease samples collected from KP. The identity of the majority of isolates was confirmed with biochemical and molecular traits. Similarly, 91 out of 125 isolates tested positive for hypersensitive response and 80 isolates were pathogenic on JP-5.
3. Among inoculation techniques clipping method remained the best inoculation assay when compared with brush and pinprick methods, since it produced maximum lesion size in tested plants. Brush method proved to be the least effective as the pathogen could not breach the intact plant surface when inoculated by this method.
4. As many as 6 races were identified on the bases of their reaction on IRBB lines. Race-1 is present in all rice growing areas of KP except Peshawar.
5. Studies on epidemiology of *Xoo* revealed that the pathogen survived in irrigation water, weeds, plant residues as well as seed between seasons which must be addressed to manage the pathogen efficiently. This is due to the fact that during overwintering the pathogen could not find a suitable substrate for survival except these sources, though in small quantity.
6. Moreover, early sowing proved better across locations in terms of agronomic traits as well as reduced disease and could be an option for disease management. It is recommended that agriculture department should design a policy guideline for rice growers outlining the role of early sowing in disease suppression and increased production.

FUTURE PROSPECTS

1. Based on results from the present study bacterial blight could be a serious threat to rice crop in future. Therefore, extensive monitoring system based on inoculum build, environmental conditions and crop genotype shall be developed to manage the disease well on time.

2. Six KP races discovered in this study shall be further exploited for molecular diversity and a culture bank of these races may be established on national level.

3. Being a disease of notorious nature extensive search for resistant genes may be continued in exotic, indigenous as well as wild rice and this resistance may be incorporated in rice cultivars through conventional and molecular techniques.

4. Variant response of disease in different locations may be further studied in details and correlations of disease development to environmental regime may be established.

5. Perennial and seasonal rice weeds are considered potential over seasoning site for pathogen survival. All rice weed may be studied and to see that if these are also be encountered for pathogen survival.

6. Furthermore, inoculum density with reference to plant age and prevailing weather conditions in various locations may be established. The information gathered may be helpful in preparing a good disease forecasting system.

REFERENCES

Adachi, N. and Oku, T. **(2000)**. "PCR-mediated detection of Xanthomonas oryzae pv. oryzae by amplification of the 16S-23S rDNA spacer egion sequence." ***J.Gen. Plant Pathol.***, 66, 303–309.

Adhikari, T. B., Vera, C. C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and leach, J. E. **(1995)**. "Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia." ***App.Environ. Microbl.***, 61(3), 966–971

Agarwal, P. K., Sidu, G. S. and Gosal, S. S. **(2005)**. "Induction of Bacterial Blight Resistance in Elite Indian Rice (Oryza Sativa L.) Cultivars using Gamma Irradiation and Ethyl Methane Sulfonate." ***Mutat.Breed.Newsl.Rev.***, 1, 17-18.

Ahonsi, M. O., Adeoti, A. A., Erinle, I. D. and Alegbejo, M. D. **(2002)**, "Effect of variety and sowing date on false smut incidence in upland rice in Edo State, Nigeria." ***Pest Sci.Manag.***, (htt//www.irri.org /publications)

Akhtar, M. A. and Akram, M. **(1987)**. "Evaluation of National Uniform Rice Yield Trial 1985 against bacterial blight (BB) in Pakistan." ***Intl. Rice Res.Newsl.***, 12(6), p. 12.

Akhtar, S., Gilani, S. T. S. and Hasan, N. **(2004)**. "Persistence of Chlorpyrifos and Fenpropathrin alone and in combination with fertilizers in soil and their effect on soil microbes." ***Pak.J.Bot.***, 36(4), 863-870.

Akhtar, M. A., Irfan-ul-Haq, Shahzad, M., and Idrees. **(2008)**. "Survival of *Xanthomonas oryzae* pv *oryzae* on rice leaves and husk." ***Pak.J.Phytopathol.***, 20(1), 77-81.

Akhtar, M. A. and Zakria, M. **(2003)**. "Incidence of bacterial blight of rice in Pakistan during 2002." ***Pak.J.Bot.***, 35(5), 993-997.

Akhtar, M. A., Zakria, M., Abbassi, F. M. and Masod, M. A. **(2003)**. "Incidence of bacterial blight of rice in Pakistan during 2002." ***Pak.J.Bot.***, 35(5), 993-997.

Akhtar, M. A., Zakria, M. and Abasi, F. M. **(2003)**. "Inoculum build up of bacterial blight of rice in rice-wheat cropping areas of Punjab and relation to zero tillage." ***Asia.J.Plant Sci.*** 2, 548-550

Akram, H. M., Ali, A., Nadeem, M. A. and Iqbal, M. S. **(2007)**. "Yield and yield components of rice varieties as affected by transplanting dates." ***J.Agric.Res.***, 45(2), 105-111.

Ali, A., Khan, M. H., Bano, R., Rashid, H., Raja, N. I. and Chaudhry Z. **(2009)**. "Screening of Pakistani rice (*Oryzae sativa*) cultivars against *Xanthomonas oryza* pv. *oryza*." ***Pak.J.Bot.***, 41(5), 2595-2604.

Aye, S. S., Masaru, M., Hisatoshi, K., Takahiro, G., Naruto, F. and Aatsushi, Y. **(2007)**. "Evaluation of Resistance in Rice Plants to Myanmar Isolates." ***J.Fac.Agric.Kyushu Univ.***, 52(1), 17-22.

Bangura, N. and John, V. T. **(1991)**. "Incidence, distribution and severity of bacterial diseases on rice in West Africa." ***Intl.J.Pest Manag.***, 37(2), 113-117.

Baloch, A. W., Soomro, A. M., Naqvi, M. H., Bughio, H. R., Bughio, M. S., Abbasi, S. J. and Mastoi, N. N. **(2005)**. "Sustainable rice production achieved by mutation breeding. PAWEES 2005." ***Intl.Conf.Manag. Paddy Water Environ.Sust. Rice Prod. Kyoto Jpn.***, pp. 163-167.

Bhurer, K. P., Rijal, S. K. and Chaudhary, D. N. **(1990)**. "Influence of date of seeding and age of seedlings on grain yield of rice under rainfed conditions." In: The Fourth Summer Crops Workshop and Research Program. ***Nat.Agric.Res. Cent.Min.Agric.***, Pp. 117-121.

Bokura, U. **(1911)**. "Bacterial leaf blight of rice [in Japanese]." ***Teikoku Nokaiho***, 2, 62-66.

Brar, D. S., Dalmacio, R., Eloloran, R., Aggarwal, R., Angels, R. and Khush, G. S. **(1996)**. "Gene transfer and molecular characterization of introgression from wild Oryza species into rice." **In:** ***Rice Genet. III***, Pp. 477-486.

Campbell, C. L. and Madden, L. V., **(1990)**. "***Introduction to Plant Disease Epidemiology***." Wiley, USA.

Chattopadhyay, S. B. and Mukherjee, N. **(1974)**. "Seed transmission of *Xanthomonas oryzae* (Uyeda and Ishiyama) Downson, the pathogen of bacterial leaf blight of rice." ***Int.Rice Comm.Newsl.***, 20, 41-47.

Chaudhary, R. C. **(1996)**. "Internationalization of elite germplasm for farmers: Collaborative mechanisms to enhance evaluation of rice genetic resources." ***New Approaches for Improved use of Plant Genetic Resources,*** Fukuyi, Japan, 26 p.

Choi, J. E., Matsuyama, N. and Wakimoto, S. **(1981)**. "Colony type variants of *Xanthomonas compistis* pv. *oryzae* and their serological properties." ***Ann. Phytopathol.Soc.Jpn.***, 47, 224-251.

Cowan, S. T. **(1974)**. "***Manual for the Identification of Medical Bacteria.***" Cambridge Univ. Press, Great Britain. 238 p.

Dath, A. P. and Devadath, S. **(1983)**. "Role of inoculum in irrigation water and soil in the incidence of bacterial blight of rice." ***Indian Phytopathol.***, 36, 142–144.

Daily The Nation. (2009). http://www.nation.com.pk/pakistan-news-newspaper-daily-english-online//Business/ 10-Jul-2009/Pakistan-becomes-third-largest-rice-exporting-country-TDAP

Devadath, S. and Dath, A. P. **(1985)**. "Infected chaff as a source of inoculum of *Xanthomonas campestris* pv. *oryzae* to the rice crop." ***Zeitschrift.Pflanzen. Pflanzen.*** 92, 485-488.

Di Ming, Y. H., Schaad, N. W. and Roth, D. A. **(1991)**. "Selective recovery of Xanthomonas spp from rice seed." ***Phytopathol.***, 81, 1358-1363.

Dinh, H. D., Nghi, K. O., Nguyen, D. T., Pham, V. D. and Le C. L. **(2008)**. "Pathotype profile of *Xanthomonas oryzae* pv *oryzae* isolates from the rice ecosystem in cuulong rever delta." ***Omonrice***, 16, 34-40

Durgapal, J. C. **(1983)**. "Management of bacterial blight of rice by nursery treatment preliminary evaluation." ***Indian Phytopatho.***, 36, 146-149.

Dye, D. W. **(1968)**. "A taxonomic study of the genus Erwinia 1. The "amylovora" group." ***New Zealand.J.Sci.***, 11, 590-607.

El-Hity, M. A., El-Keredy, M. S. and AbdelHafez, A. G. **(1987)**. "Yield response of same rice varieties to different planting time. " ***J.Agric.Res.***, 13(14), 1006-1014.

Ezuka, A. and Kaku, H. **(2000)**. "A Historical Review of Bacterial Blight of Rice." ***Bul.Natl.Inst.Agrobiol.Res.***, 15, 211.

FAOSTAT, 2007, htt://faostat.fao.org

Gerhardt, P. (1981). "Manual of Methods of General Bacteriology." ***Amer.Soc.Microbiol Washington,D.C.***

Ghosh, S. K., Iyengar, K. V. K., Rengarajan, T. N., Tandon, S. N., Verma, R. P. and Daniel, R. R. **(1988)**. *App.J.*, 330, 928.

Goto, K., Fuktatzu, R. and Okata, K. **(1955)**. "Overwintering the causal bacteria of rice blight in the rice plant and grasses." ***Agric.Hortic.Japan***, 28, 207-208.

Goto, M., Zeigler, R. S. and John, V. T. **(1988)**. "Progress in seed health research on seedborne and contaminant bacteria, viruses and nematodes." ***Proc.Intl.Rice Res.Inst.Manila***, Pp, 131-150.

Goto, K. and Okabe **(1967)**. "Accumulation of starch around diseased spots." ***Diy.Plant.Breed.Cult.Tokai-Kinki.Agric.Expt.Sta.Bull.***, 2, 41.

Guilani, A., Fathi, G. and Siadat, S. A. **(2002)**. "Effect of planting dates on yield and yield components of seven high quality rice cultivar in Khuzestan." ***J.Sci.Tech.Agric.Natl.Res.***, 6(1), 163-176.

Habgood, R. M. **(1970)**. "Designation of physiological races of plant pathogens." *Nature*, 227, 1268-1269.

Habibullah, N., Shah, H., Ahmad, N. and Iqbal, F. **(2007)**. "Response of rice varieties to various transplanting dates." ***Pak.J.Plant.Sci.***, 13(1), 1- 4.

Hari, O., Katyal, S. K., Dhiman, S. D. and Om, H. **(1999)**. "Response of two rice (*Oryza sativa*) hybrid to graded levels of nitrogen." ***Indian J.Agric.Sci.***, 70(3):140-142.

Horino, O. (1973). "Ultrastructure of *Xanthomonas oryzae* and its morphological changes by chemical treatment." ***Ann.Phytopathol.Soc.Japan.***, 39, 14-26.

Horino, O. **(1981)**. Ultrastructural histopathology of rice leaves infected with *Xanthomonas campestris* pv. *oryzae* on Kogyoku group rice varieties with different levels of resistance at the seeding stage." ***Ann.Phytopathol.Soc. Japan.***, 47, 501-09.

Hossain, M. **(1996)**. "Economic prosperity in Asia: Implication for rice research. IRRI. Rice Genetics III." ***Proc.Intl.Rice Gen.Sym.Manila,Phil.***

Hsieh, S. P. Y., Buddenhagen, I. W. and Kauffman, H. E. **(1974)**. "An improved method for detecting the presence of *Xanthomonas oryzae* in rice seed." ***Phytopathol.***, 64, 273-274.

Hugh, R. and Leifson, E. **(1953)**. "The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates of various Gram-bacteria." ***J.Bact.***, 66, 24-26.

IRRI: International Rice Research Institute. **(1996)**. "Strengthening international partnerships." ***Manila,Phil.Int.Rice Res.Inst.***,143-145.

IRRI: International Rice Research Institute. **(1989)**. "IRRI Toward 2000 and Beyond." ***Manila,Phil.Int.Rice Res.Inst.***, 66 pp.

IRRI: International Rice Research Institute. **(1977)**. "Annual Report for 1976." ***P.O.B. 933.Manila,Phil.***, pp. 42-50.

Ishiyama, S. (1922). "Studies on bacterial leaf blight of rice." ***Rep.Agric.Exp.Sta. Tokyo***, 45, 233-261.

James, W. C. and Teng, P. S., **(1979)**. "The quantification of production constraints associated with plant disease." ***Appl.Biol.***, 4, 20.

Jyufuku S., Furuya, N., Goto, T., Kenichi, T. and Yoshimura, A. **(2009)**. "Pathogenic and Genetic Diversity in Asian Strains of *Xanthomonas oryzae* pv. *Oryzae*." ***J.Fac.Agr.Kyushu.Univ.***, 54(1), 19-23.

Kabir, M. S., Akter,S., Biswas, P. S., Latif, M. A. and Hossain, M. A. **(2007)**. "Screening, phenotypic and physiological structures of *Xanthomonas oryzae* pv. *oryzae* and its races in Bangladesh." ***2nd International Conference on Bacterial Blight of Rice, China,*** 45-59.

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y. and Merca, S. D. **(1973)**. "An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. " ***Pl.Dis.Rep.***, 57, 537-541.

Kauffman, H. E. and Reddy, A. P. K. **(1975)**. "Seed transmission studies of *Xanthomonas oryzae* in rice." ***Phytopathol.***, 65, 663-666.

Khalifa, A. A. B. A. **(2009)**. "Physiological evaluation of some hybrid rice varieties under different sowing dates." ***Aust.J.Crop Sci.***, 3(3), 78-183.

Khalifa, A. A. B. A. (2005). "Physiological behaviour of some rice cultivars under different sowing dates and seedling age. " ***The 11th Conference of Agronomy, Agron.Dept.Assiut.Univ.***, 285-295.

Khan, J. A., Jamil, F. F. and Gill, M. A. **(2000)**. "Screening of rice varieties/lines against Bakanae and Bacterial Leaf Blight (BB)." ***Pak.J. Phytopathol.***, 12, 6-11.

Khedikar, V. P., Bharose, A. A., Sharma, D. and Khedikar, Y. P. **(2003)**. "Studies on genetic parameter in scented rice genotypes." ***J.Soils Crops***, 13(2), 338-342.

Khush, G. S., **(1997)**. "Origin, dispersal, cultivation and variation of rice." ***Plant Mol. Biol.***, 35, 25–34

Klement, A. and Goodman, R. (**1967**). "The hyperensitivity reaction to infection by bacterial plant pathogens." ***Ann. Review Phytopathol.***, 5, 17-44.

Koirala, G. P. (**1983**). "Effect of seeding dates on performances of improved rice varieties." ***11th Summer Crops Workshop at NRIP, Parwanipur.***

Kovacs, N. (**1956**). Identification of *Pseudomonas pyocyaneu* by the oxidase reaction." ***Nature***, 178: 703.

Kuwatsuka, K. (1942). "The intervarietal variation of stainability of rice grains by phenol and its relation to the blast disease." ***Stud.Plant Breed.*** 1, 43-52.

Lai, V. E., Takahito, N. and Pham V. D. (**1999**). "Resistance assessment of rice cultivars to *Xanthomonas oryzae* pv. *oryzae* and pathogenicity testing of bacterial leaf blight isolates in Vietnam." ***OMonRice***, 7, 97-105.

Lai, V. E., Noda, T. O. and Du, P. V. (**1999**). "Resistance assessment of rice cultivars to *Xanthomonas oryzae* pv. *oryzae* and pathogenicity testing of bacterial leaf blight isolates in Vietnam." ***OMonRice***, 7, 120-131.

Leach, J. E., Rhoades, M. L., Vera, C. C. M, White, F. F., Mew, T. W. and Leung, H. (**1992**). "Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element." ***Appl.Environ.Microbiol.***, 58, 2188-2195.

Lee, B. M., Young, J. P., Dong, S. P., Jeong, G. K., Hee, W. K., Tae, H. N., Gilb, L. and Joung, K. A. (**2003**). "PCR-Base sensitive detection and identification of *Xanthomonas oryzae* pv. *oryzae*." ***Korean J.Microbiol.***, 32, 256–264.

Lelliott, R. A. and Stead, D. E. (**1987**). "Method for the Diagnosis of Bacterial Diseases of Plants." Vol. 2. British Soc. for Plant Pathol. 216 p.

Li, Z. Z., Zhao, H. and Ying, X. D. (**1985**). "The weed carriers of bacterial leaf blight of rice." ***Acta Phytopathol. Sinica,*** 15, 246-248.

Limpert, E. and Muller, K. (**1994**). "Designation of pathotypes of plant pathogens." ***J.Phytopathol.***, 140, 346-358.

Liao, K. W., Lin, Z. Y., Pao, H. N., Kam, S. Y., Wang, F. I. and Chu, R. M. **(2003)**. "Identification of canine transmissible venereal tumor cells using in situ polymerase chain reaction and the stable sequence of the long interspersed nuclear element." ***J.Vet.Diagn.Invest.***, 15, 399-406.

Manan, A., Khan, Z., Ahmed, B. and Abdullah. **(2007)**. "Prevalence and identification of ixodid tick genera in frontier region Peshawar." ***J.Agri.Biol.Sci.***, 2(1), 21-25.

Shazia, M., Malik, S., Ahamad, A. I., Mirza, J. I. and Akhtar, M. A. **(2009)**. "Studies on virulence reactions of local isolates of *Xanthomonas oryzae* pv. *oryzae*." ***Pak.J.Bot.***, 41(1), 391-402.

Mary, C. A., Nair, S. K. and Saraswathi, P. **(2001)**. "Survival of *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922, Swings *et al.*, 1990)." ***J.Trop.Agric.***, 39, 76-78.

McClung, L. S. and Toabe, R. **(1947)**. "The egg yolk plate reaction for the presumptive diagnosis of *Clostridiuim sporogenes* and certain species of the Bangrene and Botulinum groups. ***J.Bacteriol.***, 53, 139-147.

Mew, T. W. **(1987)**. "Current status and future prospects of research on bacterial blight of rice." ***Annu.Rev.Phytopathol.***, 25, 359-382.

Mew, T. W., Alvarez, A. M., Leach, J. E. and Swings, J. **(1993)**. "Focus on bacterial blight of rice." ***Plant Dis.***, 77, 5-12.

Mew, T. W., and Vera, C. M. **(1979)**. "Variability of *Xanthomonas oryzae*: Specificity in infection of rice differentials." ***Phytopatholo.***, 69, 152-155.

Mew, T.W. and Majid, A. **(1977)**. "Bacterial Blight of rice in Pakistan." ***Intl.Rice Res.Newsl.***, 2, 5.

MINFA. **(2009)**. "Ministry of Food and Agriculture (Economic wing) Islamabad." Pp 5-6.

Misawa, T. and Miyazaki, E. (1973). "Quantitative analysis of some organic compounds in the cell of *Xanthomonas oryzae* and their relations to the inoculated rice leaf blade." ***Ann.Phytopathol.Soc.Jpn.***, 39, 79-80.

Mizukami, T. **(1957)**. "Relationship between Xanthomonas oryzae and roots of rice seedling." ***Agric.Bull. Saga Univ.***, 6, 87-93.

Mizukami, T. **(1961)**. "Studies on the ecological properties of *Xanthomonas oryzae* Dowson, the causal organism of bacterial blight of rice plant." ***Agric.Bull. Saga Univ.***, 13, 1-85.

Mizukami, T. and Wakimoto, S. **(1969)**. "Epidemiology and control of bacterial leaf blight of rice." ***Annu.Rev.Phytopathol.***, 7, 51-72.

Moffet, M. L. and Croft, B. J. (1983) "Xanthomonas in Plant Bacterial Diseases." ***A Diag. Guide.***, in P. C. Fahy and G. J. Persley (ed.), Acad. Press: New York Lond. pp. 189-228.

Mohan, S. K. and Rao, Y. P. **(1974)**. "Bacterial blight and leaf streak disease of rice, their epidemiology and control." In: (Rachaudhuri, S. P. and Verma, J. P. (ed.). current trends in plant pathology. ***Univ. Lacknow India.***, pp. 129-133.

Mohiuddin, M. S., Rao, Y. P., Mohan, S. K. and Verma, J. P. **(1977)**. "Survival of *Xanthomonas oryzae*, the incitant of bacterial blight of rice in the rhizosphere of wheat." ***Science and Culture***. IARI, New Delhi, India.

Mukoo, H. and Iida, T. **(1957)**. "Report on investigation of white leaf of rice in Cuba." ***Adm.Estabiliz.Arroz.Bol.***, 1, 5-14.

Munda, G. C., Chaudhri, P.B. and Patel, C. S. **(1994)**. "Variety, date of transplanting and space on high altitude rice." ***Indian J. Hill Farming***, 7(1), 96-98.

Murty, V. S. T. and Devadth, S. **(1984)**. "Role of seed in survival and transmission of *Xanthomonas campestris* pv. *oryzae* causing bacterial blight of rice." ***J.Phytopathol. Zeitschrift***, 110, 15-19.

Murty, V. S. T. and Devadath, S. **(1982)**. "Survival of *Xanthomonas campestris* pv. *oryzae* in different soils." ***Indian Phytopatho.***, 35, 32-38.

Nagarajan, S. and Muralidharan, K., **(1995)**. "Dynamics of Plant Diseases. " ***Allied Publishers, Hyderabad, New Delhi.***

Nayak, P. and Reddy, P. R. **(1985)**. "Spread pattern of bacterial blight disease in rice crop." ***Indian Phytopatho.,*** 38, 39-44.

Nazir, M. S. **(1994)**. "Crop Production." (Ed.): E. Bashir and R. Bantel. ***Natl. Book Found. Islamabad,*** pp. 252.

Nelson, R. J. S., Baraoidan, M. R., Vera-Cruz, C. M. S., Yap, I. V., Leach, J. E., Mew, T. W. and Leung, H. **(1994)**. "Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice." ***Appl.Environ.Microbiol.***, 60, 3275-3283.

Noda, T., Yamanmoto, T. and Horino, O. **(1996)**. "Geographical distribution of pathogenic races of *Xanthomonas oryzae* pv. *oryzae* in Japan in 1991 and 1993." ***Ann.Phytopathol.Soc.Jpn.***, 62, 549-553.

Noda, T., Li, C., Li, J., Ochiai, H., Ise, K. and Kaku, H. **(2001)**. "Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* strain from Yunnam province, China." ***J.A.R.Q.***, 35, 97-103.

Noor, A., Chaudhry, Z., Rashid, H. and Mirza, B. **(2006)**. "Evaluation of resistance of rice varieties against bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*." ***Pak.J.Bot.***, 38(1), 193-203.

Nyvall, R. F. **(1999)**. "Field crop diseases. third edition." ***Iowa State Univ. Press. Ames. IA.*** 1021 pp.

Ogawa, T., Yamamoto, Y., Khush, G. S., Mew, T. W. **(1991)**. "Breeding nearisogenic lines of rice with single gene for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv *oryzae*)." ***Jpn.J.Breed.***, 41, 523-529.

Ogawa, T., Busto, G. S., Tabien, R. E., Romero, G. O., Endo, N. and Khush, G. S. **(1991a)**. "Grouping of rice cultivars based on reaction pattern to Philippine races of bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*)." ***Jpn.J.Breed.***, 41, 109-119.

Ogawa, T., Lin, L., Tabien, R. E. and Khush, G. S. **(1987)**. "A new recessive gene for resistance to bacterial blight of rice." ***RGN***, 4, 98-100.

Ou, S. H. **(1985)**. "Bacterial leaf blight." ***In Bacterial Diseases, Rice Disease 2nd edition***, 61-96.

Padmanabhan, S. Y. **(1973)**. "The great Bengal famine." ***Annu.Rev.Phytopathol.***, 11, 11-26.

Penning de Vries, F. W. T., Jansen, D. M., Berge, H. F. M. and Bakema, A. **(1989)**. "Simulation of ecophysiological processes of growth in several annual crops." ***Intl. Rice Res.Inst. Los Banos.***, Simulation Monographs 29, Pudoc, Wageningen, 272 pp.

Pirdashry, H. Z., Sarvestani, T., Narsiri, M. and Fallah, V. **(2000)**. "Effect of transplanting date on yield and yield components of some rice cultivars." ***Seed plant***, 16, 164-158.

Raj, K. and Pal, V. **(1988)**. "Overwintering of *Xanthomonas campestris* pv. *oryzae*." ***Intl.Rice Res.Newsl.***, 13, 22-23.

Rajarajeswari, N. V. L. and Muralidharan, K. **(2006)**. "Assessments of farm yield and district production loss from bacterial leaf blight epidemics in rice." ***Crop Prot.***, 25(3), 244-252.

Reddy, P. R. **(1972)**. "Studies on bacteriophages of *Xanthomonas oryzae* and *Xanthomonas translucens* f.sp. *oryzicola*, the incitants of blight and streak diseases of rice." ***PhD thesis, Banaras Hindu University***. Varanasi, India.

Reitsma, J. and Schure, P. S. J. **(1950)**. "Kresek, a bacterial blight disease of rice." ***Contr.Gen.Agric.Res.St. Bogor***, 117, 1-17.

Ritchie, J. T., Singh, U., Godwin, D. and Hunt, L. **(1989)**. "A User's Guide to CERES-Maize V2.10." Muscle Shoals: ***Intl.Fert.Devel.Centr.***

Ryu, E. **(1940)**. "A simple method of differentiation between Gram-positive and Gram-negative organisms without staining." ***Ixilasato Arch.Hxpcr.Med.***, 17, 58-63.

Safdar, M. E., Ali, A., Sher, M., Ghulam, S. and Tahir, H. A. **(2008)**. "Effect of transplanting dates on paddy yield of fine grain rice genotypes." ***Pak.J.Bot.***, 40(6), 2403-2411.

Sakthivel, N., Mortensen, C. N. and Mathur, S. B. **(2001)**. "Detection of *Xanthomonas oryzae* pv. *oryzae* in artificially inoculated and naturally infected rice seeds and plants by molecular techniques." ***Appl.Microbiol.Biotechnol.***, 56, 435-441.

Salim, M., Akram, M., Akhtar, M. E. and Ashraf, M. **(2003)**. "Rice, A production Hand Book." ***Pak.Agric.Res.Coun.Islamabad.***, pp. 70.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). "Molecular Cloning" A Lab. Manual, ***Euphytica***, 130(1), 107-115.

Sanchez, A.C., Brar, D. S., Huang, N., Li, Z. and Khush, G. S. **(2000)**. "Sequence tagged site markerassisted selection for three bacterial blight resistance genes in rice." ***Crop Sci.***, 40, 792-797.

Sanchez, A. C., Ilag, L. L., Yang, D., Brar, D. S., Ausubel, F., Khush, G. S., Yano, M., Sasaki, T., Li, Z. and Huang, N. **(1999)**. "Genetic and physical mapping of xa13, a recessive bacterial blight resistance gene in rice." ***Theor.Appl.Gen.***, 98(6-7), 1022-1028.

Satya, P., Singh, V. P. and Singh, A. K. **(2005)**. "Genetic analysis of pyramided genes for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and development of resistant lines of basmati rice (*Oryza sativa*)." ***Ind.J.Agric.Sci.***, 275(7), 428-431.

Schaad, N. W. **(1980)**. "Laboratory guide for identification of plant pathogenic bacteria." ***Dept.Plt.Pathol.Univ. Georgia***, pp. 28.

Shah, M. M., Khalid, Q., Khan, U. W., Shah, S. A. H., Shah, S. H., Hassan, A. and Pervez, A. **(2009)**. "Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat." ***Gen.Mol.Res.***, 8(3), 783-793.

Shah, L. M. and Bhurer, K. P. **(2005)**. "Response of wet seeded rice varieties to sowing dates." ***Nepal Agric.Res.J.***, 6, 35-38.

Shah, S. M. A., Rahman, H., Abassi, F. M., Akhtar, M. A., Rafi, A. and Khan, I. A. **(2009)**. "Resistance characterization of wild relatives of rice in response to bacterial blight." ***Pak.J.Bot.***, 41(2): 917-925.

Sherief, A. E., El-Hinidi, M. H., Abd El-Rahman, A. A. and Abdo, G. M. **(2000)**. "Rice productivity as influenced by planting dates and seedling." ***Ages.J.Agric.Sci. Mansoura Univ.***, 3, 1511-1521.

Shen, Y. and Ronald, P. (2002). "Molecular determinants of disease and resistance in interactions of *Xoo* and rice." ***Microb.Infect.***, 4, 1361-1367.

Shoichi, T., Naohito, A. and Koji, T. **(2002)**. "Detection and estimation of *Xanthomonas oryzae* pv. *oryzae* in Leersia by PCR." ***Ann.Phytopathol.Soc.Jpn.***, 68(2), 153-155.

Shrestha, S. L. **(1975)**. "Effect of seedling age, normal transplanting and re-transplanting (Kheruha or Khaur) on yield of local variety." Paper presented: (Third rice improvement workshop held during 1 to 3 March 1976): ***Deptt. Agric. Hariharbhawan Lalitpur Nepal.***

Sierra, G. **(1957)**. "A simple method for the detection of Lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates." ***Antonie Van Leeuwenhoek.***, 23, 15-22.

Singh, D. V., Banerjee, A. K., Kishun, R. and Abidi, A. B. **(1980)**. "Effect of bacterial leaf streak on the quantitative and qualitative characters of rice." ***Ind.J.Mycol. Plant Pathol.***, 10, 67-68.

Singh, U. P. and Prasad, A. **(1999)**. "Response of promising spring sown rice (*Oryza Sativa* L,) varieties to sowing time and N levels under upland rained low-hill valley situation of latter Pradesh." ***Ann.Agric.Res***. 20, 296-300.

Singh, D., Vinther, F. and Mathur, S. B. **(1983)**. "Seed transmission of bacterial leaf blight in rice." ***Seed Pathol. News***, 15, 11.

Singh, R. N. **(1971)**. "Perpetuation of bacterial blight disease of paddy and preservation of its incitant. I. Survival of *Xanthomonas oryzae* in water. II. Survival of *Xanthomonas oryzae* in soil." ***Indian Phytopathol.***, 24, 140-144.

Singh. R. A. and Rao, M. H. S. **(1975)**. "A simple technique for detecting *Xanthomonas oryzae* in rice seed." ***Rice Pathol.Newsl.***, 75(2), 9-10.

Sodhi, M., Vikal, Y., George, M. L. C., Bala, G. S., Mangat, G. S., Garg, M., Sidhu, J. S. and Dhaliwal, H. S. **(2003)**. "DNA fingerprinting and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* isolates from Punjab, northern India.

Suslow, T. V., Schroth, M. N., and Isaka, M. **(1982)**. "Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining." ***Phytopathol.***, 72, 917-918.

Tabei, H, **(1967)**. "Anatomical studies of rice plant affected with bacterial leaf blight, with special reference to stomatal infection at the coleoptile and the foliage leaf sheath of rice seedlings." ***Ann.Phytopathol.Soc.Jpn.***, 33, 12-16.

Tagami, Y. and Mizukami, T. **(1962)**. " Historical review of the researches on bacterial leaf blight of rice caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson." ***Plant Prot.Div.Minist.Agric.Jpn.***, 10, 1-112.

The News. **(2007)**. "3rd October."

Tika, B. A. **(1993)**. "Assessment of near isogenic rice lines for resistance to Xanthomonas oryzae pv. oryzae in Nepal." ***Intl J. Pest Manag.***, 39 (3), 293-296.

Toth, S. **(1978)**. "Rice yield response to sowing date and sowing rate." ***Novenytermeles***, 27(1)65-75. (C.F. Field Crop Abst., 34(7): 5401, 1981).

Tsuchiya, K., Mew, T. W. and Wakimoto, S. **(1982)**. "Bacteriological and pathological characteristics of wild types and induced mutants of *Xanthomonas campestris* pv. *oryzae*." ***Phytopathol.***, 72, 43-46.

Vera C. C. M., Ardales, E. Y., Skinner, D. Z., Talag, J., Nelson, R. J., Louws, F. J., Leung, H., Mew, T. M. and Leech, J. E. **(1996)**. "Measurement of haplotypic variation in *Xanthomonas oryzae* pv. *oryzae* within a single field by rep-PCR and RFLP analysis." ***Phytopathol.***, 86, 1352-1359.

Waheed, M. A., Inamullah, Ahmad, H., Sirajuddin, Ali, H., Khan, A.Q. and Khan, A. **(2009)**. "Evaluation of rice genotypes for resistance against bacterial leaf blight." ***Pak.J.Bot.***, 41(1), 329-335.

Wakimoto, S. **(1955)**. "Overwintering of *Xanthomonas oryzae* on unhuUed grains of rice." ***Agr.Hort.Jpn.***, 30, 1501 p.

Watanabe, B. **(1963)**. "Epidemiological studies on bacterial leaf blight of rice, Xanthomonas oryzae (Uyeda et Ishiy) Dowson. In: The overwintering of the pathogen." ***Bull. Kyushu Agric.Exp.Stn.***, 9(1), 89-122.

Williams, J. R., Jones, C. A., Kiniry, J. R. and Spanel, D.A. **(1989)**. "The EPIC Crop Growth Model." ***Trans.A.S.A.E.***, 32(2), 497-511.

Wilson, E. E., Zeitoun, P. M. and Fredrickson, F. M. **(1967)**. "Bacterial phloem canker, a new disease of Persian walnut trees." ***Phytopathol.***, 57, 618-621.

www.statpak.gov.pk

Yoshimura, S., Morihashi, T. and Suzuki, Y. **(1959)**. "Major weed hosts for the overwintering of the bacterial leaf blight organism and its distribution and development in Hokuriku District." ***Ann.Phytopathol.Soci.Jpn.***, 24, 6 pp.

Yoshimura, S. **(1963)**. "Diagnostic and ecological studies of rice bacterial leaf blight, caused by *Xanthomonas oryze* Dowbon Balk." ***Hukoriku Agric.Exp.Stn.***, 5, 27, 27-182.

Zadoks, J. C. and Shein, R. D. **(1979)**. "Epidemiology and Plant Disease Management." ***Oxford Univ.Press New York,*** pp. 247-264.

APPENDICES

Appendix 1:
Year and province vise comparison of Bacterial Blight incidence (Figure 3)

	2005	2006	2007	Means
KP	67.78	67	61.03	65.27
Punjab	60.86	66.56	52.42	59.94
Sindh	30.83	26.25	23.96	27.01
Balochistan	21.67	16.33	12	16.66
Means	45.285	44.035	37.3525	42.22

Appendix 2:
Year and province vise comparison of Bacterial Blight severity (Figure 4)

	2005	2006	2007	Means
KP	5.7	5.26	4.97	5.31
Punjab	4.88	5.15	5.13	5.05
Sindh	2	1.54	1.37	1.63
Balochistan	1.22	0.67	0.33	0.74
Means	3.45	3.155	2.95	3.18

Appendix 3:
Average Rainfall and temperature data for the two research stations during the period 2005-2007

Locations	Month	Average low temp. (°C)	Average high temp. (°C)	Average Rain fall (mm)	Rainfall (days)
Baffa (Mansehra)	June	08	11	0	3
	July	08	12	03	4
	August	06	11	27	6
	September	04	13	12	9
	October	08	13	12	3
Mingora (Swat)	June	14	20	0	5
	July	12	16	24	5
	August	14	18	45	8
	September	10	15	0	2
	October	13	19	09	2

http://www.worldweatheronline.com

Appendix 4:

Hypersensitivity reaction and Characterization of various isolates of *Xanthomonas oryzae pv. oryzae*, collected from different rice growing areas of Zone-1,by a range of biochemical tests. (Table 4.10)

Isolates	HR-respose	Pathogenecity test	Gram reaction	KOH test	Starch Hydrolysis	Tween-80 hydrolysis	Acid From Carbs	Egg yolk reaction	Tetrazolium Tolerance test 0.1%	Tetrazolium Tolerance test 0.02%	Anaerobic growth test	Oxidase Test
Xo-184	+	+	-	+	+	+	+	-	-	-	-	-
Xo-185	+	+	-	+	+	+	+	-	-	-	-	-
Xo-186	+	+	-	+	+	+	+	-	-	-	-	-
Xo-187	-	-	-	+	+	+	+	-	-	-	-	-
Xo-188	-	-	-	+	-	+	+	-	-	-	-	-
Xo-189	+	+	-	+	+	+	+	-	-	-	-	-
Xo-190	+	-	+	-	+	-	-	-	-	-	-	-
Xo-191	-	+	-	+	-	+	+	-	-	-	-	-
Xo-192	-	+	-	+	+	+	+	-	-	-	-	-
Xo-193	-	+	-	+	+	+	+	-	-	-	-	-
Xo-194	-	+	-	+	-	+	+	-	-	-	-	-
Xo-195	+	+	-	+	-	+	+	-	-	-	-	-
Xo-196	-	-	+	-	+	-	-	-	-	-	-	-
Xo-197	+	-	+	-	+	+	+	-	-	-	-	-
Xo-198	+	+	-	+	+	+	+	-	-	-	-	-

Xo-199	+	+	-	+	+	+	+	-	-	-	-	-
Xo-200	+	+	-	+	+	+	+	-	-	-	-	-
Xo-201	-	-	-	+	-	+	+	-	-	-	-	-
Xo-202	+	-	+	-	+	+	+	-	-	-	-	-
Xo-203	+	+	-	+	-	+	+	-	-	-	-	-
Xo-204	+	+	-	+	-	+	+	-	-	-	-	-
Xo-205	-	-	-	+	+	+	+	-	-	-	-	-
Xo-206	+	-	+	-	+	+	+	-	-	-	-	-
Xo-207	+	+	-	+	+	+	+	-	-	-	-	-
Xo-208	+	+	-	+	+	+	+	-	-	-	-	-
Xo-209	-	-	-	+	+	+	+	-	-	-	-	-
Xo-210a	+	+	-	+	+	+	+	-	-	-	-	-
Xo-210	+	+	-	+	+	+	+	-	-	-	-	-
Xo-211	+	+	-	+	+	+	+	-	-	-	-	-
Xo-212	-	-	-	+	-	+	+	-	-	-	-	-
Xo-213	+	-	+	-	+	-	-	-	-	-	-	-
Xo-214	+	+	-	+	+	+	+	-	-	-	-	-
Xo-215	+	+	-	+	+	+	+	-	-	-	-	-
Xo-216	+	-	+	-	+	+	+	-	-	-	-	-

Xo-217	+	+	-	+	-	+	+	-	-	-	-	-
Xo-218	+	+	-	+	+	+	+	-	-	-	-	-
Xo-219	+	+	-	+	+	+	+	-	-	-	-	-
Xo-220	+	+	-	+	+	+	+	-	-	-	-	-
Xo-221	+	+	-	+	+	+	+	-	-	-	-	-
Xo-222	+	+	-	+	+	+	+	-	-	-	-	-
Xo-223	+	-	+	-	+	+	+	-	-	-	-	-
Xo-224	+	+	-	+	+	+	+	-	-	-	-	-
Xo-225	+	+	-	+	-	+	+	-	-	-	-	-
Xo-226	+	+	-	+	-	+	+	-	-	-	-	-
Xo-227	-	-	-	+	+	+	+	-	-	-	-	-
Xo-228	+	-	+	-	+	-	-	-	-	-	-	-
Xo-229	+	+	-	+	+	+	+	-	-	-	-	-
Xo-230	-	-	-	+	+	+	+	-	-	-	-	-
Xo-231	+	+	-	+	+	+	+	-	-	-	-	-
Xo-232	+	+	-	+	-	+	+	-	-	-	-	-
Xo-233	+	+	-	+	+	+	+	-	-	-	-	-
Xo-234	+	+	-	+	+	+	+	-	-	-	-	-
Xo-235	+	+	-	+	+	+	+	-	-	-	-	-

Xo-236	-	-	+	-	+	+	+	-	-	-	-	-
Xo-237	+	-	+	-	+	+	+	-	-	-	-	-
Xo-238	+	+	-	+	+	+	+	-	-	-	-	-
Xo-239	-	-	-	+	+	+	+	-	-	-	-	-
Xo-240a	+	+	-	+	+	+	+	-	-	-	-	-
Xo-240	+	+	-	+	+	+	+	-	-	-	-	-
Xo-241	+	+	-	+	+	+	+	-	-	-	-	-
Xo-242	+	+	-	+	-	+	+	-	-	-	-	-
Xo-243	-	-	-	+	+	+	+	-	-	-	-	-
Xo-244	+	+	-	+	+	+	+	-	-	-	-	-
Xo-245	+	-	+	-	+	-	-	-	-	-	-	-
Xo-246	+	+	-	+	+	+	+	-	-	-	-	-
Xo-247	+	+	-	+	-	+	+	-	-	-	-	-
Xo-248	-	-	-	+	+	+	+	-	-	-	-	-
Xo-249	+	+	-	+	+	+	+	-	-	-	-	-
Xo-250	+	+	-	+	+	+	+	-	-	-	-	-
Xo-251	+	-	+	-	+	+	+	-	-	-	-	-
Xo-252	-	-	-	+	+	+	+	-	-	-	-	-
Xo-253	+	+	-	+	-	+	+	-	-	-	-	-

Xo-254	+	+	-	+	+	+	+	-	-	-	-	-
Xo-255	+	+	-	+	+	+	+	-	-	-	-	-
Xo-256	+	-	+	-	+	+	+	-	-	-	-	-
Xo-257	-	-	-	+	+	+	+	-	-	-	-	-
Xo-258	+	+	-	+	+	+	+	-	-	-	-	-
Xo-259	-	-	-	+	+	+	+	-	-	-	-	-
Xo-260	+	+	-	+	-	+	+	-	-	-	-	-
Xo-261	-	-	+	-	+	+	+	-	-	-	-	-
Xo-262	+	+	-	+	+	+	+	-	-	-	-	-
Xo-263	+	+	-	+	+	+	+	-	-	-	-	-
Xo-264a	+	+	-	+	+	+	+	-	-	-	-	-
Xo-264	+	+	-	+	+	+	+	-	-	-	-	-
Xo-265	-	-	-	+	+	+	+	-	-	-	-	-
Xo-266	-	-	+	-	-	+	+	-	-	-	-	-
Xo-267	+	-	+	-	-	+	+	-	-	-	-	-
Xo-268	-	-	-	+	+	+	+	-	-	-	-	-
Xo-269	+	+	-	+	+	+	+	-	-	-	-	-
Xo-270	+	+	-	+	+	+	+	-	-	-	-	-
Xo-271	+	+	-	+	+	+	+	-	-	-	-	-

Xo-272	-	-	+	-	-	-	-	-	-	-	-	-
Xo-273	+	+	-	+	+	+	+	-	-	-	-	-
Xo-274	+	+	-	+	+	+	+	-	-	-	-	-
Xo-275	-	-	-	+	+	+	+	-	-	-	-	-
Xo-276	+	+	-	+	+	+	+	-	-	-	-	-
Xo-277	+	+	-	+	-	+	+	-	-	-	-	-
Xo-278	+	+	-	+	+	+	+	-	-	-	-	-
Xo-279	-	-	-	+	+	+	+	-	-	-	-	-
Xo-280	+	-	+	-	-	+	+	-	-	-	-	-
Xo-281	+	+	-	+	+	+	+	-	-	-	-	-
Xo-282	+	+	-	+	+	+	+	-	-	-	-	-
Xo-283	+	+	-	+	+	+	+	-	-	-	-	-
Xo-284	-	-	-	+	+	+	+	-	-	-	-	-
Xo-285	+	+	-	+	+	+	+	-	-	-	-	-
Xo-286	+	+	-	+	+	+	+	-	-	-	-	-
Xo-287	+	-	+	-	-	+	+	-	-	-	-	-
Xo-288	-	-	-	+	+	+	+	-	-	-	-	-
Xo-289a	+	+	-	+	+	+	+	-	-	-	-	-
Xo-289	+	+	-	+	+	+	+	-	-	-	-	-

Xo-290	-	-	-	+	+	+	+	-	-	-	-	-
Xo-291	+	+	-	+	+	+	+	-	-	-	-	-
Xo-292	-	-	+	-	-	-	-	-	-	-	-	-
Xo-293	+	+	-	+	+	+	+	-	-	-	-	-
Xo-294	+	+	-	+	+	+	+	-	-	-	-	-
Xo-295	-	-	-	+	+	+	+	-	-	-	-	-
Xo-296	+	+	-	+	+	+	+	-	-	-	-	-
Xo-297a	-	-	-	+	+	+	+	-	-	-	-	-
Xo-297	+	+	-	+	+	+	+	-	-	-	-	-
Xo-298	+	-	+	-	+	-	-	-	-	-	-	-
Xo-299	+	+	-	+	+	+	+	-	-	-	-	-
Xo-300	+	+	-	+	+	+	+	-	-	-	-	-
Xo-301	+	+	-	+	+	+	+	-	-	-	-	-
Xo-302	-	-	+	-	-	+	+	-	-	-	-	-
Xo-303	+	+	-	+	+	+	+	-	-	-	-	-

Analyzed data Baffa 2007

Appendix 5
Anlyses of varience table for BB %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	255.00	85.00	1.81	
Sowing	2	140.83	70.42	1.50	0.297
Residual	6	282.50	47.08	1.36	
Varieties	4	941.67	235.42	6.78	<.001
sowing.varieties	8	388.33	48.54	1.40	0.231
Residual	36	1250.00	34.72		
Total	59	3258.33			

Appendix 6
Anlyses of varience table for grain spik^{-1}

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	2415.7	805.2	1.17	
Sowing	2	218.6	109.3	0.16	0.857
Residual	6	4138.2	689.7	4.11	
Varieties	4	17035.4	4258.8	25.40	<.001
sowing.varieties	8	2152.7	269.1	1.61	0.158
Residual	36	6035.1	167.6		
Total	59	31995.7			

Appendix 7
Anlyses of varience table for 1000 grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	11.84082	3.94694	7.58	
Sowing	2	9.87498	4.93749	9.48	0.014
Residual	6	3.12583	0.52097	48.80	
Varieties	4	106.35418	26.58854	2490.72	<.001
sowing.varieties	8	0.76223	0.09528	8.93	<.001
Residual	36	0.38430	0.01068		
Total	59	132.34234			

Appendix 8
Anlyses of varience table for spilke length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	32.983	10.994	0.87	
Sowing	2	1.433	0.717	0.06	0.946
Residual	6	76.167	12.694	1.59	
Varieties	4	26.167	6.542	0.82	0.522
sowing.varieties	8	110.233	13.779	1.72	0.126
Residual	36	287.600	7.989		
Total	59	534.583			

Appendix 9
Anlyses of varience table for Tillers plant^{-1}

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	52.267	17.422	0.58	
Sowing	2	32.133	16.067	0.54	0.611
Residual	6	180.133	30.022	3.28	
Varieties	4	304.667	76.167	8.32	<.001
sowing.varieties	8	98.533	12.317	1.35	0.254
Residual	36	329.600	9.156		
Total	59	997.333			

Appendix 10
Anlyses of varience table for yield (tones hac^{-1})

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.26722	0.08907	1.68	
Sowing	2	13.27880	6.63940	125.30	<.001
Residual	6	0.31792	0.05299	1.44	
Varieties	4	31.29637	7.82409	212.16	<.001
sowing.varieties	8	90.87540	11.35942	308.03	<.001
Residual	36	1.32759	0.03688		
Total	59	137.36330			

Analyzed data Baffa 2008

Appendix 11
Anlyses of varience table for BB %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	255.00	85.00	1.81	
Sowing	2	140.83	70.42	1.50	0.297
Residual	6	282.50	47.08	1.36	
Varities	4	941.67	235.42	6.78	<.001
Sowing.Varities	8	388.33	48.54	1.40	0.231
Residual	36	1250.00	34.72		
Total	59	3258.33			

Appendix 12
Anlyses of varience table for grain spik^{-1}

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	7142.7	2380.9	3.45	
Sowing	2	218.6	109.3	0.16	0.857
Residual	6	4138.2	689.7	4.11	
Varities	4	17035.4	4258.9	25.40	<.001
Sowing.Varities	8	2152.7	269.1	1.61	0.158
Residual	36	6035.1	167.6		
Total	59	36722.7			

Appendix 13
Anlyses of varience table for 1000 grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	12.36747	4.12249	7.92	
Sowing	2	9.86262	4.93131	9.47	0.014
Residual	6	3.12281	0.52047	48.73	
Varities	4	106.38645	26.59661	2490.35	<.001
Sowing.Varities	8	0.76024	0.09503	8.90	<.001
Residual	36	0.38448	0.01068		
Total	59	132.88407			

Appendix 14
Anlyses of varience table for spilke length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	12.36747	4.12249	7.92	
Sowing	2	9.86262	4.93131	9.47	0.014
Residual	6	3.12281	0.52047	48.73	
Varities	4	106.38645	26.59661	2490.35	<.001
Sowing.Varities	8	0.76024	0.09503	8.90	<.001
Residual	36	0.38448	0.01068		
Total	59	132.88407			

Appendix 15
Anlyses of varience table for Tillers plant^{-1}

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	52.267	17.422	0.58	
Sowing	2	32.133	16.067	0.54	0.611
Residual	6	180.133	30.022	3.28	
Varities	4	304.667	76.167	8.32	<.001
Sowing.Varities	8	98.533	12.317	1.35	0.254
Residual	36	329.600	9.156		
Total	59	997.333			

Appendix 16
Anlyses of varience table for yield (tones hac^{-1})

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.51467	0.17156	1.86	
Sowing	2	14.60397	7.30198	79.33	<.001
Residual	6	0.55224	0.09204	2.28	
Varities	4	30.69684	7.67421	190.36	<.001
Sowing.Varities	8	89.85865	11.23233	278.62	<.001
Residual	36	1.45131	0.04031		
Total	59	137.67768			

Appendix 17

Three way interaction for Sites, Sowing dates and Varieties

SOV	DF	SS	MS	Fcal	P
Sites	1	1444.214	1444.214	141.62	<.001
Replication over sites	6	61.188	10.198	0.39	
Sowing	2	118.858	59.429	2.27	0.146
Sites.sowing	2	38.405	19.203	0.73	0.501
errorI	12	314.695	26.225	3.72	
Varities	4	160.126	40.031	5.67	<.001
Sites.varieties	4	188.009	47.002	6.66	<.001
sowing.varieties	8	116.489	14.561	2.06	0.051
Sites.sowing.variety	8	99.726	12.466	1.77	0.098
ErrorII	72	508.079	7.057		
Total	119	3049.789			