"STUDY OF HOST PLANTS RESISTANCE GENES AND GENE COMBINATIONS AGAINST *Xanthomonas oryzae* pv. *oryzae* MODEL SYSTEM "

M.Sc. (Ag.) THESIS

by

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DEPARTMENT OF BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

INDIRA GANDHI AGRICULTURAL UNIVERSITY

RAIPUR (C.G.)

2003

"STUDY OF HOST PLANTS RESISTANCE GENES AND GENE COMBINATIONS AGAINST *Xanthomonas oryzae* pv. *oryzae* MODEL SYSTEM "

Thesis

Submitted to the

Indira Gandhi Agricultural University, Raipur

by

Tirlochan Singh

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

Master of Science

In

Agriculture
(Biotechnology)

Roll No. 2080 **ID** No. PG/AG/2000/67

OCTOBER, 2003

CERTIFICATE -1

This is to certify that the thesis entitled **"Study of Host Plants Resistance Genes and gene combinations against *Xanthomonas oryzae* pv. *oryzae* model system "** submitted in partial fulfillment of the requirements for the degree of **"M.Sc.** (Ag.) **Biotechnology"** of the Indira Gandhi Agricultural University, Raipur, is a record of the bonafide research work carried out by Shri. **Tirlochan Singh** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published/Published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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ACKNOWLEDGEMENT

I take this opportunity to express my deepest sense of gratitude to Dr. A.S. Kotasthane, Senior Scientist and Head, Department of Biotechnology, IGAU, Raipur and Chairman of my Advisory Committee whose guidance, fruitful criticism and up-to-the mark recommendations proved to be the flambeau showing me right way towards my destination. I shall ever remain grateful to him for his painstaking efforts, unique supervision and knowledge, which he provided me throughout my post graduation and research investigation despite his heavy schedule of work.

I am highly indebted to members of my Advisory Committee Dr. S.K. Kattiyar (Associate Professor), Department of Biotechnology, Dr.S.B. Verulkar (Associate Professor), Dr. M.L .Lakhera (Assistant Professor), Department of Statistics, Dr. N. Khare (Assistant Professor), Department of Plant Pathology for their valuable suggestions towards completion of my thesis work.

I wish to extend my sincere thanks to Hon'ble Vice Chancellor, Dr. R.P. Bagai, IGAU, Raipur (C.G.) and Dean College of Agriculture, Dr. A.S.R.A.S. Shashtri, Raipur (C.G.) for providing necessary facilities during the course of my research work.

My wholehearted thanks to Dr. S.B. Vereulkar (Associate Professor), Shri G. Chandel (Assistant Professor), Dr. Manorama (Assistant Professor), Shri CM. Sharma (ADO), Shri M.S. Dudhare (SRF) and Miss Aditi Sharma for their kind cooperation.

I emphatically extend my loyal and venerable thanks to my family members - brothers Ranveer, Sukhvinder, and nephew Simron, Mindu, Jashan for special cooperation, affection, care, inspiration, encouragement and moral support which had always enlightened my path to success during the entire period of my research work.

I specially thank to my seniors Ms. Roohi, Ms. Lekha and Surinder Chodharyfor their help and care, it would be ingeniune if I forgot to extend my deepest sense of gratitude towards my friends Ramesh, Anil, Srikant, Amit, Rajesh, as their continuous encouragement and creative criticism kept me on right path enlightened with

affection and care. I would like to thank my friend electrician Balkar Singh and Gurmeet Singh for financially support.

My thanks are also due to my juniors Rajveer, Ashok, Nagar, Mukesh, Bheereu, Mukta, Archana, Mini, Ratnakaran, Jawahar, Humira, Ravindra, Krishna, for their help.

My thanks are also due to Mr.Maneesh Dandekar and Devengan for preparation of this manuscript.

Lastly I would like to convey my wholehearted thanks to all those who helped/intended to help me.

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CHAPTER I
INTRODUCTION

Rice is arguably the world's most important food. Almost two billion people- one third of the world's population- depend primarily on rice for basic nourishment. Rice fields cover more than 360 million acres of land around the globe and yield 560 million tons of grain every year. Rice (*Oryzasativa* L.) is one of the important staple cereal crop of the world, with an area of ~147.3 million hectare and production of ~518.4 million tonnes. In Asia, 90% of the world's rice is grown and consumed and. about 2.8 million people derive 35-39% of the total calorie intake from rice (Anonymous, 1995). India has the largest area under rice, producing 100 million tons of paddy from over 42 million hectare. The present yield of milled rice in India is about 1.5 tons/hectare compared to 5.00 tons/hectare of Korea, Japan, Australia and USA and 4.00 tons/hectare in China. In Madhya Pradesh, rice occupies an average of 5344.4 thousand hectare with a production of 5839.1 thousand tons and is the major *Kharif* cropof the state (Annonymous, 1997). However, the farmers plant much more rice than they harvest, because insect, bacteria, viruses and fungi often claim a substantial portion of each crop. One of the most devastating of these pestline is blight. caused by bacteria common throughout Asia and Africa. Plant pathogens include a very large and heterogeneous group of organisms that occupy positions of great importance in both agriculture and natural plant communities. They show an enormous diversity in life history strategies and the way in which they interact with their hosts. Bacterial populations are notoriously "shifty enemies" and through mutation, recombination, migration, complemented with random drift and selection pressure, they often circumvent disease management strategies.

The bacteria- ***Xanthomonas oryzae*** **pv.** ***oryzae*** **(*Xoo*)**-spreadrapidly from rice plant to rice plant and from field to field in water droplets. In severely infected fields, bacterial blight can wipe out half of a farmer's rice crop. Eight races of the bacterium have been identified in the Philippines (Finckh and Nelson, 1999) and two major pathotypes in India (Reddy and Reddy, 1992). Each race has specific virulence to varieties with different resistance genes, showing a gene for gene relationship in the

host pathogen interaction (Mew, 1987; Veracruz and Mew, 1989). Variation in the virulence of *Xoo* from one location to other location and from one country to other (particularly in Asia) has long been recognised (Noda et al, 1990; Gopinath et al, 1991; Nayak and Reddy, 1993). Isolates from India have been reported more virulent than the isolates from Japan (Nayak and Reddy, 1993).

Host plant resistance has been used extensively for disease control in many crop species. Many types of resistance, however, are not long lasting because of rapid changes in pathogen populations. Two types of resistance to *Xoo*, vertical and horizontal have been recognized in rice (Zhang and Mew 1985). Qualitative resistance to *Xoo* is controlled by 24 major genes Kinoshita 1995; Lin *el al.,* 1998) conferring resistance to various races of the pathogen have been identified and utilized in rice breeding programs. Large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race towards virulence (Mew, 1987; Eamchit and Mew, 1982; Mew and Vera Cruz, 1979; Mew *et al.* 1982; Mew *et al.,* 1992) with consequent breakdown of resistance in these cultivars.

In contrast, horizontal resistance is quantitative, presumably non-race specific, and controlled by polygenes (Van der Plank 1968; Nelson 1972), though these assumptions have not been actively tested. An effective approach for studying complex and ploygenic forms of disease resistance is known as "Quantitative Trait Locus" (QTL) mapping, which is based on the use of DNA marker (Tanksley SD.1993.). With QTL mapping, the roles of specific loci in genetically complex traits can be described, and fundamental questions that have vexed researchers in the field of plant pathology for decades can be addressed. Are genes that control race-nonspecific resistance the same as "defeated" race-specifics genes, and are partial resistance genes race specific? What sort of interactions exists between resistance genes, plant development, and the environment? Are any cloned defense response genes (Bowles DJ.1990.) the same as partial resistance genes? Moreover, genetic mapping the DNA markers makes it possible to ask whether homologous resistance genes exist in related plant taxa. Finally, QTL mapping will eventually provide as entry point for the most

ambitious goal of all-cloning partial resistance genes known only by small and continuous effects.

Durability of resistance *(R)* genes is thus central to sustainable disease management. To date, it has been possible to assess the durability of an *R* gene only after its deployment for a long time over a large area (Johnson, R. 1984). The capacity to predict the durability of *R* genes would be highly desirable for making sound investments in breeding and genetic engineering. In the genetics of host pathogen interaction, a long-standing controversial issue is the nature of the genetic basis of "Stabilizing selection" which largely determines the co-evolution of many plant host-pathogen relationship (Van der Plank 1963, 1968; Leonard and Czochor 1980). This theory states that the pathogenic race(s) will suffer a loss in general fitness when they acquire new virulence genes by mutation. Genetically, this implies that conversion of avirulence genes into virulence genes by mutation is expected to result in lower fitness of the pathogen. However, resistance of a segregating rice population to specific *Xoo* strain often shows both qualitative and quantitative components (Koch and Parlevliet 1991). These characteristics of the relationship between *O. sativa* and *Xoo* offer a unique opportunity to study the genetics of the interaction between host plants and their pathogens.

The *R* genes used in plant disease management are largely single dominant genes that direct the recognition of pathogen components encoded by avirulence (*avr*) genes; this relationship is referred to as a gene-for-gene interaction (Flor, 1971). The mechanisms by which these plant and pathogen genes interact to initiate resistance are becoming clearer because of the isolation and characterization of several plant *R* genes and pathogen *avr* genes (Bent, 1996; Hammond-Kosack, and Jones, 1997). Functional analyses of pathogen *avr* genes have provided evidence that prediction of the durability of disease *R* genes may lie in an understanding of the fitness cost in pathogen evolution to overcome the resistance. In addition to their role in avirulence, several *avr* genes, possess a function in aggressiveness (amount of disease) or disease symptom expression, both of which are components of pathogenic fitness. The dual functions (avirulence and fitness) associated with these genes has led to speculation

that the *R* genes corresponding to these *avr* genes may be more durable in the field (Kearney, & Staskawicz,1990; Swords, *et al.,* 1996). Currently, two observations are generally taken as supportive of this hypothesis: (*i*) the measurable fitness functions of specific *avr* genes, and (*ii*) the (Leach, & White, 1996; Gabriel, 1999). Rice (*Oryza sativa* L.) and its bacterial blight (BB) pathogen *Xoo* present an excellent opportunity for Virulence analysis and host plant resistance. Therefore, the present study is proposed with the following major objectives:

1) Virulence analysis of the bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) isolates
2) Efficacy of different gene and gene combinations against *Xanthomonas oryzae* pv. *oryzae*.isolates.
3) Identification of QTLs against *Xanthomonas oryzae* pv. *oryzae*
4) Study of inheritance of different *Xa* gene(s) in breeding populations for resistance to bacterial leaf blight of rice..

Review of Literature

CHAPTER II
REVIEW OF LITERATURE

Asia accounts for 90 per cent of the world's production and consumption of rice because of its favorable hot and humid climate. In the humid and sub-humid Asia, rice is the principal staple food providing 50 to 80 per cent of the calories consumed by the people. It is also the single most important source of employment and incomes for the rural people (Hossain and Fischer, 1995). Rice is grown in numerous tiny farms primarily to meet family needs. In countries with a per capita income of US$500 or less, rice accounts for one-third to one-half of the value added, and one-fifth to one-third of the gross domestic product. By the year 2050, 90 % of the world's projected 11 billion people will reside in the developing countries (Krattigar, 1996). Since rice is considered the most important crop in tropical and subtropical regions, increasing its yield is an essential factor in securing sufficient food supplies for the increasing human population. With the green revolution of the 1960s, and 1970s changes in rice (*Oryza sativa* L.) varieties and management practice lead to emergence of new pest and disease. The loss of the yield in all crops due to biotic stress is approximately $500 billion and due to Bacterial Blight of rice it is as high as 6 to 60 % depending on location, season, weather condition and cultivars (Srivastava *et.al.*, 1967). Bacterial Blight (BB) has been known for a century and is one of the most serious diseases of rice. It is also one of the oldest recorded rice disease, having been known for a century. The farmers of the fukuoka area, Kyushu, Japan, first noted the disease in 1884 (Tagami and Mizukami, 1962). The disease occurs through out Asia, in several Latin American countries, and in northern Australia as well. Recently, it was reported in Sahelian region and other parts of Africa and in the United states (Texas and Louisiana). BB occurs at all stage of the rice crop and shows either Kresek or leaf blight symptoms. The occurrence of bacterial leaf blight in India was first noted by Kaul, 1959 and Bhopkar et. al., 1960 from Maharashtra.

Bacterial blight caused by *Xoo* is one of the most destructive diseases of rice throughout the world. In some areas of Asia, the disease has the potential to reduce

yield by more than 50%. Eight races of the bacterium have been identified in the Philippines (Finckh and Nelson, 1999) and two major pathotypes in India (Reddy and Reddy, 1992). Each race has specific virulence to varieties with different resistance genes, showing a gene for gene relationship in the host pathogen interaction (Mew, 1987; Veracruz and Mew, 1989). So far, 24 genes conferring resistance to specific races or clusters of races of *Xoo* have been identified through classical genetic analysis. As of now, 10 major genes have been mapped using RFLP, RAPD and Microsatellite markers (McCouch *et al.,* 1991; Ronald *et al.,* 1992; Yoshimura *el al.,* 1992; Borines *et al.,* 2000). The advent of molecular markers tagged to different resistance genes enabled convergence breeding and pyramiding of more than two different genes into an agronomic variety. Marker-assisted breeding (MAS) has been successfully used by Huang *et al.,* (1997) for pyramiding four resistance genes into IR-24 background. Resistance gene deployment in hybrid rice parents is much more complicated and needs a different strategy. MAS has been successfully used to transfer resistance genes into seed parents (Borines *et al.,* 2000) and restorer lines (Chen *et al.,* 2000).

2.1 Causal organism

The bacterial origin of the disease was reported by Ishiyama in 1922. The disease bacterial leaf blight is caused by the bacterium *Xoo.* The isolates and pathogenesity test of Abo was first reported in India by Srinivasan *et. al.* (1959).

2.2 Taxonomy

The nomenclature of causal bacteria has changed over the years as under :-

Bacillus oryzae Hori and Bokura, 1911

Pseudomanas oryzae Uyeda and Ishiyama, 1992

Bacterium oryzae (Uyeda and Ishiyama,) Nakata, 1927

Phytomonas oryzae (Uyeda and Ishiyama,) Dowson,1939

Xanthomonas campestris pv. *Oryzae* (Uyeda and Ishiyama,) Dyeet,al 1980

Xanthomonas oryzae pv. *oryzae* (Ishiyama) Dye 1988

2.3 Symptoms

BB is a vascular disease. The infection is, therefore, systemic. The pathogen normally enters the host through wounds or natural openings such as the water pores. In either case, the bacterium ends up in the xylem tissues, here it multiplies and moves throughout the plant (Mew, 1987).

There are two symptoms on rice: Kresek and leaf blight. Kresek is the more destructive manifestation of the disease. Leaves of entire plants turn pale yellow and wilt during the seedling to early tillering stages.

Leaf blight is the more common disease syndrome. Lesions on the leaf blades may extend to the leaf sheath. The lesion enlarges in length and width, and may have wavy margins. It turns a whitish-straw colour from its initial water-soaked grayish or yellowish hue in 1-2 weeks. Bacterial ooze may be observed in humid and warm conditions. Leaf blight may occur at all growth stages, but it is common from maximum tillering until maturity. "Yellow leaf is also considered a syndrome of BB (Ou, 1985).

Host Range

The weed hosts of *X. oryzae* - Japan were first found by artificial inoculation (Goto et.al., 1953), infected weeds were later found in nature. Among them, Leersia sayanuka ohwi is the most important as it serves as a common over-wintering host .*L .oryzoides* (L) *Sw*., *L. japanica* and *Zizania itifolia*are also naturally infected (Tagami and mizukami ,1962). In the tropics, Leptochloa chinensis (L), *Ness L. flliformis and L. panacea* have been found as weed host in the Philippines. *Cyperusrotundus* L. and *C. deffornis*are also reported from India as being alternative host found infected in nature (Chattopadhyay and mukherjee, 1968)

2.4 Variability

For many year it was unclear whether there was differential interaction between rice and Abo. Many scientist doubt that there are "races" of the bacterial pathogen. The confusion is centered on the question of a system that clearly differentiates pathogenicity of bacterial pathogen. With two different cultivars and to different two

isolates, scientists often look for the host pathogen association when the disease is either present or absent in the cultivar isolates combinations. Resistance in rice to *X. oryzae* pv. *oryzae* is therefore often viewed in the absolute , rather than in the relative terms .neither pathogenic specialization nor specific resistance occurs alone.

2.4.1 Variation in pathogenecity

The degree of specialization differs in various host-pathogen Associations (Browder and Eversmeyer, 1986). Variation in the virulence of *Xoo* from one location to another location and from one country to another (particularly in Asia) has long been recognized (Buddehhagen *et al.*, 1969; Buddenhagen & Reddy, 1972; Kauffman and Rao, 1972; Reddy and Ou, 1976; Mew andVera Cruz, 1977, 1979; Srinivassan and singh, 1982; Durgapal, 1982; Gupta *et al.*, 1986; Noda *et al.*, 1990; Gopinath *et al.*, 1991; Nayank and reddy, 1993). Isolates from India have been reported more virulent than the isolates from Japan (Wakimoto, 1967;AICRIP, 1974; Reddy, 1974; Nayak and Reddy, 1993).

Since the breakdown of Zsakaze resistance, scientist from Japan made attempts to classify rice varieties and bacterial isolates in Japan (Kuhara *et al,* 1965; Washio *et al.*, 1966; Ezuka and horino, 1974). Kozaka (1969) presented a system of varietal classification. On the basis of differential reaction rice varieties were derived into 4 groups viz., Kinmaze, Kogyoku, Rantjemas and Wase Aikoku. The bacterial isolates were put into three groups I II and III. Devdath and padmanabhan (1969) tested 9 isolates on 20 cultivars and found differences in virulence. They noticed that cultivars resistant in Japan or in Philippines became susceptible in India and found differential interaction between isolates and cultivars. Xia and Fu (1983) studied variability and strain separation of *Xoo* in 1979-83. Nearly 200 isolates of the bacterium from rice in each major epidemic region at 46 countries and cities in Fujian Province were tested on the set of differentials in China and some differential cultivars from Japan and IRRI with known resistance genes. Results showed that the pathogen in Fujian differed in virulence from strains in Japan and Philippines. Some cultivars including Jiggang 30, Zaiyequing 8, Honken 57, Zhike, Nan 15, IR 26 and 76 C-Fan 7 could be used as a set of differentials in Fujian to classify 5 strain groups (0,I, II, III, IV) and 9 sub groups.

Noda *et al.,* (1990) distinguished 7 races in Japan using 5 Japanese differential, but when they used 18 differential varieties, from several Asian countries including Japan, races I, II and III could be subdivided further into 10, 17 and 13 subgroups respectively. 5 cultivars (Chugoku 45, IR 20, Tongil, Milyang 42 and DV 85) were resistants to all the isolates tested. Studies at All India Co-ordinated Rice Improvement Projects (AICRIP) have showed the existence of only two pathotypes. Pathotype I is prevalent in all the bacterial leaf blight epidemic regions of the country whereas distribution of pathotype II is restricted to areas in Orissa and West Bengal (Reddy, 1980). Durgapal (1986) obtained the isolates from rice in this epiphytic year. On the basis of reaction BJ-1, TKM-6, T65, and T(M)-1 the isolates were differentiated into 5 pathogenic groups designated pathotypes I, II, III, Iv and V. Reddy and Reddy(1990) collected, 130 isolates of *Xoo* from 25 locations in regions of India were bacterial leaf blight of rice is endemic and grouped into pathotyes Ia, Ib and II based on the reactions of 6 differential cultivars. Gopinath *et al.* (1991) tested the pathogenecity of 12 of 37 isolates of *Xanothomonas oryzae* on 17 rice cultivars. All 12 isolates were virulent on all cultivars tested. Resistant varieties DV85 and Kogyoku were only relatively less susceptible than the rest. Isolates, 2, 26 and 36 were relatively highly virulent on all cultivars. Pathotype studies conducted under the All India Coordinated Rice Improvement Project (A I C R I P) have identified two major pathotypes within the *Xoo* populations in India (Reddy and Reddy, 1992). Das et al. (1994) reported isolates HC44, which was most virulent of 4 different isolates of the bacterial blight pathogen tested. This isolate produces early infection and large lesions on the leaves. Nayak and Reddy (1993) constituted a new set off differentials comprising in rice varieties IR 8, IET 8585, PN13, TKM 6 and DV 85 and/or Malagkit sungsong to classify the isolates of bacterial blight pathogen occurring in India into virulence groups. Based upon the reactions. 36 bacterial isolates (CRXC01 - CRXCO36) were distinguished into 6 groups or pathotypes. DV 85 shows resistance against Indian group I, III, IV. IR 8 shows susceptibility against all isolate groups (I - VI). Thri Murthy *et al.* (1993) isolated, six isolates (6, 6r, 6 - 4R, 6 - 5R, 6 - 6R and 6 - 7R) of *Xanthomonas oryzae* from rice in different parts of Chhattisgarh region. These isolates showed variations in their reaction of isogenic lines, differential varieties and MP rice

germplasm genotypes. They found that isolate 6-5R and 6-5R and 6-4R were most virulent. Among the isogenic lines, IRBB lh(t) was resistant against isolate 6R, 6-5R and 6-7. Among the differential varieties, BJ1, IR 20, IR 13427, and IR 1543-338 were resistant against three isolates. Isolate 6-5R showed virulent reaction on maximum number of differentials followed by 6-4R. All the isolates showed variable disease reaction on the Madhya Pradesh germplasm rice genotypes. BJ 400-1 and RP 2151-33-2 were found resistant against maximum no. of isolates(4 isolates). The overall reaction of the isolates on the 48 varieties (10 isogenic + 24 differential + 14 MP germplasm) showed that isolate 6-5R was virulent on only 2 varieties (IR BB lh(t) and S 971) followed by 6-4 R on 4 varieties (BJ 400-1, RP-2151-33-2, BJ1 and IR 24). IRBB-1 was resistant against isolate 6R.

2.4.2 Variation at DNA level

Plant pathogens are variable. Although plants an pathogens have co-evolved over long periods, human interference through breeding and management practices has disturbed this balance condition. When pressure is exerted on the pathogens to change, they may do so rapidly and dramatically, either by mutation or by genetic recombination.(Vera Cruz and Mew, 1989).

Programs for resistance breeding and disease control of bacterial blight in rice depend on the reliable identification of bacterial pathotypes. Bacterial pathotypes can be identified by using a set of differential cultivars, by morphological, physiological, biochemical characters, serotyping and by molecular techniques. Classical pathotyping using a set of differential cultivars is laborious and time-consuming. Morphological, physiological and biochemical characters do not reveal differences that could be used to delineate pathogenicity/virulence groups of Indian isolates (Reddy and Reddy, 1990). Serotyping, however, can differentiate pathotypes I and II (Reddy and Reddy, 1989) and provide some information on pathogen diversity (Gnanamanickam et al. 1992). In contrasts, molecular approaches provide reliable and useful information on the genetic make up of different isolates of bacteria, including the extent of variability in the pathogen population. BLB can only be effectively controlled by developing resistance variety which needs clear understanding of inheritance of resistance,

availability of various genes and population structure of the pathogen. The reaction of the resistance genes to different strains of the pathogen and their monogenic inheritance make them among attractive the most attractive systems for studying the molecular and genetic bases of host plant-pathogen relationships.

Molecular techniques such as Restriction Fragment Length Polymorphism (RFLP) have provided new tools to more accurately asses the genetic diversity of pathogen populations. RFLP analyses have been successfully used to detect genetic variability within populations of *Xoo* in the Philippines (Leach et al. 1990) and in a broad survey of strains from different countries in Asia (Adhikari et al. 1995).

Sridhar *et al.,* 1996 employed RFLP (Restriction Fragment Length Polymorphism) analysis for differentiating the strains of bacterial blight pathogen *Xoo* to better understand the genetic diversity and population structure of the pathogens. Pathotyping of a set of 241 isolates *of Xoo* originating mainly from eastern India employing a new set of differential varieties consisting of near isogenic lines each carrying a single known resistance gene revealed the presence of 20 different pathotypes. Presence of this diverse group of pathotypes in the country has been brought for the first time. Several groups have demonstrated the utility of RFLP analysis for understanding the population biology and structure of pathogen populations such as *Xoo.*

William *et al.,* 1991 converted RFLP markers into sequences characterized amplified regions (SCRRs) by sequencing the two ends of genomic DNA clones and designing oligonucleotide primers based on the sequences. Ghareyazie et al,(1996) evaluated the usefulness of converting mapped RFLP markers to polymerase chain reaction (PCR) based markers such as Amplicon Length Poly morphism(ALP) and PLR-based RFLP. A repetitive element is III2 isolated from *Xoo* were used to fingerprint DNA from a set of 71 bacterial blight pathogen strains using PCR, PCR-based restriction analysis, and ligation-mediated PCR (George et al, 1996) to compare strains of bacterial blight pathogen from Indonesian and Philippines and found that while there is regional differentiation of the pathogen populations, the predominant strains in the pathogen collections from both countries are closely related. This

indicates the occurrence of regional migration, perhaps as a consequence of germplasm exchange.

A high level of genetic polymorphism was detected among Indian isolates of *Xoo* using hyper variable such as a micro satellite oligonucleotide, probe (Teflo, a human mini satellite probe, an avirulence gene probe, *Avr Xa-10* and a repeat clone, PBS 101. These probes generated complex DNA fingerprints and differentiated all the bacterial isolates (Rajebhosale et al. 1997).

Rajabhosale et al. (1997) and Yoshitala et al. (1997) studied the extent of diversity in the pathogen. They analyzed 67 *Xoo* isolates from 18 locations in India by DNA fingerprinting using two separate repeat element probes from the *X. oryzae* pv. *oryzae* genomes. The results indicated that isolates from 12 out of 18 locations sampled are related and can be grouped into a single pathogen lineage. Pathotype analysis revealed that isolates in the dominant lineage belong to pathotype Ia and Ib. A repetitive DNA sequence PJEL101, isolated from the *Xoo* genome was used to assess the genetic variability and population structure *of Xoo.* From Philippines and Asian countries (Adhikari *et al.,* 1995). They analyzed 308 strains of *Xoo* from several rice growing regions in Asia using RFLPs and virulence typing and grouped all the pathotypes into five major clusters. Of the five clusters, three consisted of strains from a single country. However, strains within clusters were found in different countries suggesting the dissemination pattern of the pathogen. The regional differentiation of clusters of *Xoo* in Asia and the association of some pathotypes of *Xoo* with single cluster suggest that regional resistance breeding and gene deployment strategies are most feasible. Finckh and Nelson (1999) reported the presence of eight races *of Xoo* which are widely used to represent the diversity of the pathogen in IRRI, Philippines and are useful for resistance breeding.

2.5 Rice Resistance Against *Xanthomonas oryzae* pv. *oryzae*

Disease resistance loci have been identified in virtually every plant species examined. Genetic analysis of many plant-pathogen interaction has demonstrated that resistant plants often contain single loci that specify resistance against pathogen with corresponding avirulence genes: the 'gene for gene' interaction (Flor 1971). Despite

extensive genetic studies of many host pathogen interactions, only two disease resistance genes, the maize *Hm 1* locus and the tomato *Pto* locus, have been cloned (Johal and Briggs, 1992; Martin *et al.,* 1993). Elucidation of the molecular basis of disease resistance and susceptibility ultimately will involve the cloning and characterization of a resistance / virulence gene pair. In the bacterial disease of rice, both the host pathogens are amenable to molecular genetic analysis, making it is an attractive system for studies of disease resistance. Genetic resistance is the most economical and effective control for bacterial blight, which is the most destructive bacterial disease of rice in Asia and Africa.

Rice BB, caused by *Xoo* is a devastating disease in Asia. Two types of resistance to *Xoo* i.e. vertical (VR, complete whole-life resistance) and horizontal (HR, quantitative resistance), have been recognized in rice (Zhang and Mew, 1985). Resistance of a segregating rice population to specific *Xoo* strains on ten lines shows both qualitative and quantitative components (Koch and Parlevliet, 1991). These characteristics of the relationship between *O. sativa* and *Xoo* offer a unique opportunity to study the genetics of the intersection between host plant and their pathogens.

Nishimura (1961) reported that BB resistance in variety kogyoku and Kogenemaru was controlled by one dominant gene and was located on chromosome II. In extensive studies on the genetics of BB resistance using 27 resistant varieties, Sakaguchi (1967) concluded that resistance to Japanese bacterial group I was conditioned by gene *Xa-1* while resistance to Japanese bacterial group II was controlled mainly by *Xa-2.* Ezuka et al. (1975) found that resistance in the Wase Aikoku group to Japanese bacterial groups I, II and III was conditioned by one dominant gene, which they designed *Xa-w* (later changed to *Xa-3* according to nomenclature rules for rice genes). Petpisit et al. (1977) concluded that resistance in 1R 20, IR 22, and IR 1529-680-3 showed incomplete dominance, and these cultivars carried the same gene for resistance, which they designated *Xa-4.* They also reported that IR 1545-284 and RP 291 - 7, both carried the recessive gene for resistance, designated as *Xa-5.* Sidhu and Khush (1978) identified another gene, *Xa-6*, in IR 944 -

102. IR 1698 - 241, Zenith, Malagkit sansong, Nagkayat, and Dayaggot. *Xa-6* was reported to be linked with *Xa-4*, with a recombination value of 26% but to be independent of *Xa-5*. Sidhu *et al.,* (1978) analyzed additional BB-resistance of 74 cultivars. 19 cultivars were found to have *Xa-4a,* 20 had *Xa-4b*, and 32 had *Xa-5* (Sidhu *et al.,* 1978). In addition, three cultivars - DV 85, DV 86 and DZ 78 were found to carry one recessive gene, *xa-5,* and one dominant gene that was independent *of Xa-4, Xa-5* and *Xa-6.* Therefore, they designated the dominant resistance gene of DZ 78 as *Xa-7* and conjectured, from morphological similarity and perhaps because they come from common stock that both DV 85 and DV 86 also carried *Xa-7.* Gene *Xa-7* confers resistance only at flowering. In addition to *Xa-7*, they identified one recessive gene in PI231129 and designated it as *xa-8;* it is nonalleic *of Xa-4, xa-5* and *Xa-6.* On the basis of genetic analysis of 58 resistant varieties for BB resistance, Singh et al. (1983) identified a new recessive gene and designated it as *xa-9.* Gene *xa-9* was found to be linked with *Xa-6,* with a recombination value of 5.9%, but no linkage was detected between *xa-9 and Xa-4.* Gene *xa-9* was also independent *of Xa-3, xa-5, Xa-7* and *xa-8.* Yoshimura et al (1983) reported that the resistance of Cas 209 was controlled by a single dominant gene that was independent of *xa-5* but was linked with *Xa-4,* with a recombination value of 27.4%. They designated the gene *Xa-10.* In Japan, Ogawa and Yamamoto (1986) genetically analyzed three breeding lines - IR1529-680-3-2, IR944-102-2-3 and RP 9-3- for BB resistance using Japanese races. They reported that one of the two genes of IR944-102-2-3 that confer resistance to Japanese races IA, II and IIIA is either the same as the *Xa-3* of Chagoku 45 or very closely linked to it, and the second dominant gene of IR 944-102-2-3 for resistance to Japanese races II and IIIA is independent of *Xa-1* of Kogyoku and *Xa-3* of Chugoku 45. This they designated the second dominant gene of IR944-102-2-3 as *Xa-11*, and postulated from their pedigree records and reaction patterns that RP 9-3 and IR8 also carry *Xa-11.* Ogawa *et al.,*(1978) reported that the resistance of Kogyoku and Java 14 to race V was controlled by one dominant gene, which is closely linked with *Xa-1.* This *Xa-* kg gene was recently redesigned as *Xa-2* following the rules of rice gene symbolization (Ogawa, 1987). Ogawa et al.(1987) reported that the five varieties of the BJI group (ACB-I-I, Aus 274, Chinsurah Boro II, Kalimekri 77-5 and BJ I) have

two recessive genes, one is *xa-5* and th other is th new recessive gene. They designated this gene *Xa-13.* Gene *xa-13* conveys resistance only to race 6 but has a complementary effect with *xa-5* in imparting resistance to race 4. The *xa-5* locus was mapped on rice chromosome 5 (Yashimura *et al.,* 1995). The x*a-13* gene was found closely linked to a RAPD marker, AC5-900, and three RFLP markers, RG 136, RZ 28 and CDO 116, in rice chromosome 8 (Zhang *et al.,* 1996). The identified major genes for resistance to BB are given in Table 2.2 .

The story of bacterial blight disease resistance gene *Xa 21* epitomizes nearly all the IPRB program. *Oryza longistaminata* lines originating from Mali, Africa, noted to carry broad spectrum resistance to bacterial blight (Ikeda *et al.,* 1990).

- *Xa 21* is linked to three other bacterial blight resistance gene *(Xa3, Xa 4, Xa 10)* on chromosome 11 (Yoshimura *et al.,* 1984, Ikeda *et al,* 1990, 1991)
- *Xa 21* was transferred into *O. sativa* background through interspecific hybridization (Khush *et al.,* 1991).
- * *Xa 21* locus RFLP mapped (Ronald *et al,* 1992) Map-based cloning via bacterial artificial chromosome library construction (Wang *et al,* 1995).
- *Xa 21* sequencing and demonstration of engineered resistance of a susceptible genotype (song *et al,* 1995, Wang *et al,* 1996).
- *Xa 21* pyramided with other *Xa* Resistance genes via PCR based marker-assisted selection (Huang et al 1997. Reddy *et al,* 1997).
- *Xa 21* experimentally transferred into elite rice varieties (Tu *et al,* 1998, Zhang *et al,* 1998).
- Field trials of pyramided *Xa* genes, including *Xa 21*, reported in China, India, Indonasia, and Philippines (Rockefeller Foundation 1999).
- Commercial hybrid restorer line genetically improved by marker-assisted selection of *Xa21* and resulting hybrid rice demonstrates field-level efficiency (Chen *et al,* 2000)

Several quantitative trait loci (QTL) that provide resistance against *x. oryzae* pv. *oryzae* have also been tentatively localized, using molecular markers to certain regions of rice chromosomes.

2.5.1 Gene **Pyramiding**

A process of incorporation of multiple resistance genes into cultivar is gene pyramiding. Knott (1989) described gene pyramiding as important resistance breeding strategy that was applied worldwide for control of rust disease of wheat.

The genes may act independently when combined in a single genotype (Dyck and Karber 1985, Roelfs 1988, Kloppers and pretorius 1997). However, interaction between genes may also occur such that resistance of a combinations is better (Additive) then that conditioned by any of the genes individually (Dyck and Samborski 1982) and this has often been demonstrated in wheat - rust patho-systems (Samborski and Dyck 1982, Ezzahiri and Roelfs 1989, German and Kolmer 1989, German and Kolmer 1992, Kolmer 1992, Kolmer et al. 1993) It is also possible that the two resistance genes do not interact in duplicate manner and could lead to the lower degree (non-additive)of resistance then the individual gene or even to the susceptibility. When bacterial blight resistance genes were pyramided in different combinations, there were examples of both additive and non-additive phenotypes (Huang *et al.,* 1997).

Sridhar *et al,* 1999 evaluated the effectiveness of known bacterial blight resistance genes Singly and combination under field condition against local population of the pathogen in a trap nursery under irrigated condition during the 1998 wheat season by taking twenty near-isogenic lines in the IR24 background, 11 of them carrying single known resistance genes and the remaining nine possessing various combination of these genes , along with an *Oryzae minuta* derivative line (WHD-IS-78-1-5) and Malagkit Sungsong were tested . Bacterial blight incidence was uniform in the fields. Line possessing *Xa-1, Xa-3 , Xa-4, Xa-7, Xa-10, Xa-11* and *Xa-14* individually were all distinctly susceptible to the disease , whereas all the gene combination were resistance presumably because of interaction are quantitative complementation between resistance genes (Yashimura et al 1995, Huang et al 1997). This study point out that, even though a particular gene ineffective singly against bacterial blight (for example *Xa-4, xa-5* and *Xa-13*) Its combination with another assistance genes *(Xa-4+xa-5 ,Xa-4+xa-13, xa-5+xa-13, Xa-4+xa-5+xa-13*) confers resistance .

Molecular approaches offer new opportunity for the manipulation (by marker - added - selection or MAS) and the understanding of resistance genes (Micheal more 1995) .In theory and practice marker -added- selection allows the target genes the

identified and trap in a segregating population at any plant growth stage based on linked DNA markers (Tanksley et al. 1989, Yasimura et al. 1995 , Zhang et al. 1996). Conventional procedures for gene pyramiding or not practical for plant breeders because of epistatic interaction between resistance genes. The process of pyramiding genes can be made more efficient thought market - added- selection (MAS), if molecular markers linked tightly to the resistance genes are available, since the expression of the molecular markers is not mark by epistatic interactions.

DNA marker-assisted was used to pyramid four bacterial blight resistance genes *Xa-4, xa-5, xa-13* and *Xa-21*. Breeding lines with two, three and four resistance genes were developed and tested for resistance to the bacterial blight pathogen (*Xoo*). The pyramid lines showed a wider spectrum and a higher level of resistance than lines with only a single gene. MAS was applied for pyramiding four genes for BB resistance e.g. *Xa4*, *xa-5*, *xa13*, and *Xa-21*. Breeding lines with two or three genes were also developed. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Huang et al. 1997). Yoshimura et al. (1995) developed lines carrying *Xa4 + xa-5* and *Xa4 + Xa10* using RFLP and RAPD markers linked to the BB resistant genes. These lines were evaluated for reaction to 8 strains of BB, representing eight pathotypes and three genetic lineage. Lines carrying *Xa4 + xa-5* were more resistant to isolates of race 4 than were either of the parental lines. Sanchez et al. (2000) used sequence-tagged site (STS) markers to pyramid three genes for BB resistance in an elite breeding line of rice. The pyramided lines having three or four genes in combination showed an increased and wider spectrum of resistance to bacterial blight than those having a single resistance gene. Singh et al. (2001) also used MAS to pyramid genes for BB resistance into a high-yielding Indica rice cultivar, PR106 that is susceptible to BB.

2.6 Quantitative trait loci

The presence of two major types of disease resistance to plant pathogens- vertical resistance and horizontal resistance- has long been recognized in interaction between plant hosts and their pathogens (Van der Plankl968; Nelson 1972; Simmonds 1979). Vertical resistance in many plant pathogen relationships is hypersensitive, races

specific and is governed by interaction between avirulence genes in pathogens and resistance genes in plant hosts (Van der Plank 1968). In contrast, horizontal resistance is quantitative, presumably nonspecific, and controlled by polygenes (Van der Plankl968; Nelson 1972), though this assumption has not been actively tested. In the genetics of host pathogen interaction, a long-standing controversial issue is the nature of the genetic basis of " Stabilizing selection" which largely determines the co-evolution of many plant host -pathogen relationship (Van der Plank 1963, 1968; Leonard and Czochor 1980). This theory states that the pathogenic race(s) will suffer a loss in general fitness when they acquire new virulence genes by mutation. Genetically, this implies that conversion of avirulence genes into corresponding virulence genes by mutation is expected to result in lower fitness of the pathogen. Unfortunately, direct evidence to support this theory has been difficult to obtain. Rice *(Oryza sativa* L.) bacterial blight caused by *Xoo* is a devastating disease in Asia. Two types of resistance to *Xoo*, vertical and horizontal have been recognized in rice (Zhang and Mew 1985). Vertical resistance to *Xoo* is controlled by at least 20 major genes that are usually race specific (Zhang 1991; Causse *et al.,* 1994; G. Zhang *et al.,* 1996; Q Zhang et al 1996; Lin *et al,* 1996).However , resistance of a segregating rice population to specific *Xoo* strain often shows both qualitative and quantitative components (Koch and Parlevliet 1991). These characteristics of the relationship between *O. sativa* and *Xoo* offer a unique opportunity to study the genetics of the interaction between host plants and their pathogens.

An effective approach for studying complex and ploygenic forms of disease resistance is known as "Quantitative Trait Locus" (QTL) mapping, which is based on the use of DNA marker (Tanksley, 1993). With QTL mapping, the roles of specific loci in genetically complex traits can be described, and fundamental questions that have vexed researchers in the field of plant pathology for decades can be addressed. Are genes that control race-nonspecific resistance the same as "defeated" race-specifics genes, and are partial resistance genes race specific? What sort of interactions exists between resistance genes, plant development, and the environment? Are any cloned defense response genes (Bowles, 1990) the same as partial resistance genes?

Moreover, genetic mapping the DNA markers makes it possible to ask whether homologous resistance genes exist in related plant taxa. Finally, QTL mapping will eventually provide as entry point for the most ambitious goal of all-cloning partial resistance genes known only by small and continuous effects on phenotype.

Wang et al. (1994) mapped *Pi5* and *Pi7* for blast resistance on chromosomes 4 and 11 respectively. Nine QTL having quantitative resistance to isolate PO6-6 of blast were also identified. Li et al. (1995) identified 6 QTL contributing to resistance to *Rhizoctonia solani*. These QTL are located on 6 of the 12 rice chromosomes and accounted for 60% of the genotypic variation in Lemont x Tequing cross.

2.6.1 Relation to Race-Specific, Race-Nonspecific, and Durable Resistance

QTL mapping sheds light on the question of quantitative resistance and its relationships to race-specific, race-nonspecific, and durable resistance in two ways. First QTL mapping helps to determine whether individual QRLs are race-specific or not, and when there is an indication of specificity, the degree to which partial resistance different between races. Second, QTL mapping helps to test the hypothesis that QRLs are simply variants of qualitative resistance loci that have been (partially) overcome by then respective pathogen.

2.6.2 Race-Specificaity Of Partial Resistance Loci

While many complete resistance genes are race-specific (Flor, 1955.; Hulbert and Michelmore, 1985.; Jorgensen and Moseman, 1972; Nelson and Ullstrup, 1964., Pitblado *et al.,* 1984) it is conceivable that partial resistance genes might generally be race-nonspecific. Indeed, distinctions between horizontal and vertical resistance have previously been argued to result, in part, from race-nonspecific resistance genes (Nelson, 1978). With QTL mapping, this question can be asked about partial resistance loci. Quantitative resistance to *P. infestans* in potato has previously been described as race-nonspecific (Vanderplank,1982.). Dissecting the contributions of individual QRLs, however. clearly demonstrated that loci show distinctly different resistance effects against different pathogen races (Leonards-Schippers, *et al,* 1994.). Indeed. only 5 of the 11 statistically significant genomic regions showed no specificity against just two races tested, while the others were significant against just one. Against

bacterial wilt of tomato, one of two major resistance loci was highly race specific, which a second showed no specificity in tests against three distinct strains of P. solanacearum (Young, 1994). Of four partial SCN resistance loci uncovered in soybean, two were clearly race-specific (Concibido *et al.,* 1994).

Table: 2.1 Summary of BB resistance genes originally identified

Genes Identified	Cultivars Analysed	Isolate Used	Reference
Japanese Isolates			
Xl	Kogyoku	Giken 44	Washio *et al.,*
X2, X3	Shimotsuki	Nara Himaeji	
Xa-1	Kogyku, Koganemaru, PiNo.1	X-17	Sakaguchi(1967)
Xa-2	Rantai Emes2	X-17.X-I4	Sakaguchi (1967)
Xa-3	Lease Aikoku-3, Java 14, Koentoelan Nagomasari	Q 6808(1), Q 7102(1) T 7174(1), 11 5809(11) T 7147(11), Q 6809(111) T 7133(111)	Ezuka *et al.,* (1975)
Xa-Ih	IR 28, IR 29, IR 30	T 7141(1)	Yamada (1984)
Xa-Kgh	IR28, IR29, IR30	H75304(V)	
Xa-11	IR 944-102-2-3 RP 9-3	T 7174(1), T 7147(11) T 7133(IIIA), IV A 7505 (IV)	Ogawa and Yamamoto(1986)
Philippine Isolates			
Xa-4	IR 20, IR 22 IR 1529-680-3	PXO 25(l)	Pctpisil et al (1977)
xa-S	IR 1545-339 RP 291-7	PXO 25 (I)	Pelpisit et al (1977)
Xa-4a	IR 22, Siggadis TKM6, etc	PXO 61(1)	Librojo *et al.,* (1976)
Xa-4b	Semoro Mangga	PXO 61(1)	Librojo et al (1976)
Xa-6	Malagkit Zenith , etc.	PXO 61(1). Sungsong	Sidhu et al.(1976)
xa8	PI 23 1129	PXO 61(1)	Sidhu et. Al.(1978)
Xa-9	Khio Lay Nhay Sateng	PXO 61(1)	Sidhu et al (1983)
Xa-10	CAS 209	PXO 61(1), PXO 86(2), PXO 79(3), PXO 71(4)	Yoshimura et al (1983)
Sri Lankan isolates			
Xa-a	Wase Aikoku 3	CAR 1	Watanablr (1976)
Xa-K			
Xa-1	PI 209938		
Zenith			
Xa-b	Blue bonnat / Rexark		
Indonesian Isolates			
Xa-Kg	Kogyoku Java 14	Xo-7306 (v)	Ogawa, et al. (1978)
Indian Isolates			
Xl	Bj1	H14	Jayaraj, et al.(1972)
X2		H89	
X3		H146	
I-X1	IR8	H14, H89, H146	Jayaraj, et al. (1972)
A	Malagkit	X010	Moses, et al (1974)
Sungsong	X032		
Pi	Lacrosse / Zenith Nira		
R	IRR69/469 IRRI70/470		
Ip	IR8		
Chinese Isolates			
Xa-a	IR28		Zhao, et al. (1985)
Xa-h			Yu, et al (1986)

Table: 2.2 Genes conferring resistance to different races of bacterial blight pathogen

Gene	Cultivar	Isolate/race	Reference
Xa-1* and *Xa-2	Kogyoku	Japanese race I and II	Sakaguchi (1967)
Xa-3	Wase Aikoku, Chukogu-45	Japanese race II and III	Ezuka et al. (1975), Ogawa et al. (1991)
Xa-4	IR20, IR22, IR1529-680-3	Philippine race I	Petpisit et al. (1977), Sidhu et al. (1978)
xa-5	IR1545-248, BJ-1, IR291-7, DV85	Japanese races	Petpisit et al. (1977), Sidhu et al. (1978), Singh et al. (1983), Blair and McCouch (1995)
Xa-6	Malaget sunsong, IR994-102, IR1698-241, Zenith	Philippine race I	Sidhu et al. (1978)
Xa-7	DV85, DV87	Philippine race I	Sidhu et al. (1978, 1979)
xa-8	P1231129	Philippine isolates	Sidhu etal. (1978, 1979)
Xa-9	Sateng	Philippine isolates	Singh et al. (1983)
Xa-10	Cas209	Philippine and Japanese isolates	Yoshimura et al. (1983)
Xa-11	IR8, RP9-3	Japanese isolates	Ogawa and Yamamoto (1986), Ogawa et al. (1991)
Xa-12	Kogyoku and Java 14	Japanese and Indonesian isolates	Ogawa et al. (1978a,b)
xa-13	Long grain	Philippine isolates	Zhang et al. (1996a,b)
Xa-14	TN(1)	Japanese isolates	Taura et al. (1989)
Xa-15	M41	Japanese isolates	Anonymous (1989)
Xa-16	Tetep and IR24	Japanese isolates	Noda and Ohuchi (1987)
Xa-17	Asominori	Japanese isolates	Ogawa et al. (1989)
Xa-18	Toyonishiki	Burmese isolates	Ogawa and Yamamoto (1986)
Xa-19	XM5	Japanese isolates	Taura et al. (1991)
Xa-20	XM6	Japanese isolates	Taura et al. (1992)
Xa-21	O. longistaminata	Philippine and Japanese isolates	Khush et al. (1990)
Xa-22	Zhachanglong	Chinese isolates	Lin et al. (1996a,b)
Xa-23	O. nivara	Indian isolates	Kumar (1999)
Xa-24	DV85, DV86, Aus295	Philippine race 6	Lee et al. (2000)
Xa25 t	Somaclonal mutant HX-3		

Materials & Methods

CHAPTER III
MATERIALS AND METHODS

The present investigation was carried out at the Department of Biotechnology and at the research farm, Indira Gandhi Agricultural University, (IGAU), Raipur (C. G.), located at 21-16°N, 81-36°E and 286.56 meter above sea level, during wet season, 2001, and 2002.

3.1 Materials •

3.1.1 Plant Materials

The experimental materials consisted of 26 lines of *Oryza sativa* L. (Table 3.1), out of which 21 lines (ARBN-1 to ARBN-26) were obtain from Central Rice Research Institute, Cuttack.. Three lines namely Mahsuri ; Swarna and Pant 4 were included as test material. All the lines included in the present study, carried different known gene or gene combinations for resistance against bacterial leaf blight, except Mahsuri, Swarna, and Pant 4 , ARBN 6(IR 64), ARBN 31 (Karuna).

Table - 3.1: Lines of *Oryza sativa* with there gene(s) or gene combinations for resistance to bacterial blight

S.No.	Designation	Line	Gene/Gene pyramids
1.	ARBN-6	IR-64	?*
2.	ARBN-31	Karuna	?
3.	ARBN-96	*O. minuta* der	*Xa-23*
4.	ARBN-107	MSS	*Xa-3*
5.	ARBN-118	IRBB 1	*Xa-1*
6.	ARBN-120	IRBB-4	*Xa-4*
7.	ARBN-121	IRBB 5	*xa-5*
8.	ARBN-122	IRBB-7	*Xa-1*
9.	ARBN-123	IRBB-8	*xa-8*
10.	ARBN-124	IRBB-10	*Xa-10*
11.	ARBN-125	IRBB-11	*Xa-11*
12.	ARBN-126	IRBB-13	*xa-13*
13.	ARBN-127	IRBB-14	*Xa-14*
14.	ARBN-128	IRBB-21	*Xa-21*
15.	ARBN-129	AY445-2	*Xa-4 + xa-5*
16.	ARBN-130	NH8-15-1-5	*Xa-4 + xa-13*
17.	ARBN-131	NH9-52-1-4	*Xa-4+Xa-21*
18.	ARBN-132	NH11-21-1-3	*xa-5+xa-13*
19.	ARBN-133	NH12-17-2-4	*xa-5+Xa-21*
20.	ARBN-134	NH15-51-1-3	*xa-13+Xa-21*

S.No.	Designation	Line	Gene/Gene pyramids
21.	ARBN-135	NH21-37-1-1	*Xa-4+xa-5+xa-13*
22.	ARBN-136	NH24-10-1-3	*Xa-4+xa-5+Xa-21*
23.	ARBN-137	NH56-1-44-4	*Xa-4+xa-5+xa-13+Xa-21*
24.	Mahsuri	Mahsuri	?
25.	Swarna	Swarna	?
26.	Pant 4	Pant 4	-

*? - Gene for resistance not known.

3.1.2 Bacterial Culture

The experimental material also consisted of 14 bacterial cultures isolated from different host genotypes with/without known genes for resistance.

3.1.2.1 Isolation of the bacteria (*Xanthomonasoryzae* pv. *oryzae*)

The bacteria was isolated from different cultivars by the following procedure as developed by Kotasthane (Personal communication)

The procedure takes the advantage of this natural phenomenon of oozing which are observed early in the morning. To induce similar conditions, the procedure involves the use of moist chamber in the petri plates, which is prepared by lining them with moderately thick layer of wet blotter paper discs (led and bottom). Bacterial blight infected leaf samples showing guttation droplets were collected from the field early in the morning. Using the sterilized scalpel blade the infected leaves with the advancing lesions together with adjacent healthy tissue (without surface sterilization) were then cut into small pieces (3-4 cm) and were placed in the moist chamber. The plates were then incubated at 22 ±1° C for 5-6 hours. The infected cut ends oozed out bacterial cells present in the xylem vessels which was seen as viscous yellow and opaque mass against transmitted light and could easily be observed using the dissecting stereo binocular microscope.

In the laminar flow using a dissecting stereo binocular microscope and a fine point sterilized inoculation needle the ooze from the cut ends were lifted, and transferred to the medium (Wakimoto's) slants. Care was taken so that the needle did not touch the other parts of the leaf tissue as this may cause an accidental contamination. The inoculated slants were then incubate at 27 ± 1^{0}C, which showed the growth of the bacterium the next day or the day after as smooth, convex, butyrous,

whitish yellow to straw yellow later, and opaque against transmitted light, from which transfer can readily made in the plates to produce the pure cultures. The isolated bacterial blight pathogen was then confirmed by inoculating them on the susceptible variety of rice T N(1).

Table 3.2 : Composition of Wakimoto media

S. No.	Component	Quantity/lit
1.	Peeled Potato	300 gm
2.	Sucrose	20 gms
3.	Peptone (Bacteriological grade)	5 gms
4.	Sodium dihydrogen phosphate	1.87 gms
5.	Calcium nitrate	0.5 gms
6.	Agar-Agar (Bacteriological grade)	17 gms
7.	Distilled water	1000 ml
8.	pH	6.8

3.1.2.2 Purification and Maintenance of Cultures

For purification of bacterial isolates single colony were picked and subcultured on Wakimoto medium repeatedly and were maintained at 4°C. Fourteen cultures were isolated, purified and maintained. These cultures were subsequently used for inoculation on host for (1) pathotyping and reaction against different resistant genes and (2) a subset of which was used for inoculation on CT/IR double haploid population for the identification of QTLs. Pathotyping was done by inoculating the bacterial cultures on 26 different lines (Table 3.1) .

3.2 Methods

The experimental materials (26 lines) were sown on July in wet season 2001 & 2002 respectively with 20 cm. Plant to plant and row to row spacing. Fertilizers were applied @ 120 Kg N:60 Kg P:40 Kg K following normal package of practice to raise a good crop. The lines were tested for their reaction against the bacterial isolates used in the present investigation.

3.2.1 Inoculation on Host

3.2.1.2 Isolates

During the wet season 2001 the set of 26 lines were inoculated with eight different isolates. For inoculations the isolates were revived from the stock cultures maintained at 4°C. The cultures were grown on slants of potato semi-synthetic agar medium for 3 days at 30 °C. The inoculum was prepared by suspending the bacterial mass with sterilized distilled water to a concentration of about 10^9 cells / ml, and was immediately used for inoculations.

3.3 Pathotype analysis

The experimental plant material was inoculated at maximum tillering stage with natural as well as isolated bacterial cultures following the clip inoculation technique (Kauffman*et al.,* 1973). An average of 20-25 leaves (three plants) were inoculated for each isolate-cultivar/line combination.

Disease score was evaluated 21 days after inoculation. The lesion length and total leaf length were recorded on 10 leaves and were further catagorized based on 0-9 score (IRRI 1998). 0= No visible lesion; 1= Lesion restricted to 0.5 to 1.0 mm.; 3= lesion elongated but less then one-fourth of the leaf blade; 5= lesion extended to half of the leaf blade; and 9= lesion completely destroyed the leaf blade and sheath. The disease score were rated as HR;R;MS;S;HS as detailed in table 3.4.

Table :3.3 Disease scoring & the reactions

Score	% of infected leaf area	Reaction
0	0	Highly Resistant (HR)
1	1-5%	Resistant (R)
3	6-12%	
5	13-25%	Moderately Susceptible (MS)
7	26-50%	Susceptible (S)
9	75%	Highly Susceptible (HS)

Quantitative trait Loci (QTL) Analysis

A double haploid population consisting of 154 lines (Table3.4), derived from a cross between CT9993-5-10-1-M / IR62266-42-6-2 was used in the present investigation for the identification of putative QTLs associated with resistance to

bacterial blight. All the 156 lines were replicated twice in RBD during the wet season 2002. A subset of four different isolates was used for inoculation. Observation on reactions to bacterial blight was recorded by physical measurement of lesion length to that of the leaf length and percent-infected leaf area was worked out. The continuity and distribution of data were graphically assessed. The data in terms of percentage of leaf area infected were analyzed for QTL identification associated with bacterial blight. Mapmaker / QTL 1.1 was used for interval mapping (locating the QTLs between flanking molecular markers by maximum-likely-hood estimation) (Lander *et al.*, 1987) and to estimate the percentage of total phenotypic variance explained by each QTL. A threshold of LOD > 1.5 was used per test to claim the presence of a QTL.

Table 3.4 : Lines of CT9993-5-10-1-M / IR62266-42-6-2 double haloid population.

S. No.	DH line No	DESIGNATION	S. No.	DH line No	DESIGNATION
1	42	IR 68586-CA-2	46	101	IR68586-F2-CA-29
2	43	IR 68586-CA-3	47	102	IR68586-F2-CA-30
3	44	IR 68586-CA-4	48	103	IR 68586-F2-CA-31
4	45	IR 68586-CA-5	49	104	IR 68586-F2-CA-32
5	46	IR68586-CA-6	50	105	IR 68586-F2-CA-33
6	48	IR68586-CA-8	51	106	IR68586-F2-CA-34
7	50	IR68586-CA-10	52	107	IR68586-F2-CA-35
8	51	IR68586-CA-11	53	108	IR 68586-F2-CA-36
9	53	IR68586-CA-13	54	109	IR 68586-F2-CA-37
10	56	IR68586-CA-16	55	111	IR 68586-F2-CA-39
11	57	IR 68586-CA-17	56	113	IR 68586-F2-CA-41
12	58	IR 68586-CA-18	57	114	IR 68586-F2-CA-42
13	59	IR 68586-CA-19	58	115	IR 68586-F2-CA-43
14	61	IR68586-CA-21	59	116	IR 68586-F2-CA-44
15	63	IR 68586-CA-23	60	118	IR 68586-F2-CA-46
16	65	IR 68586-CA-25	61	119	IR 68586-F2-CA-47
17	67	IR 68586-CA-27	62	120	IR 68586-F2-CA-48
18	68	IR 68586-CA-28	63	121	IR 68586-F2-CA-49
19	70	IR 68586-CA-30	64	122	IR 68586-F2-CA-50
20	71	IR 68586-CA-31	65	123	IR 68586-F2-CA-51
21	73	IR68586-F2-CA-1	66	124	IR68586-F2-CA-52
22	74	IR 68586-F2-CA-2	67	125	IR68586-F2-CA-53
23	75	IR 68586-F2-CA-3	68	126	IR 68586-F2-CA-54
24	76	IR 68586-F2-CA-4	69	127	IR 68586-F2-CA-55
25	77	IR 68586-F2-CA-5	70	128	IR68586-F2-CA-56
26	78	IR 68586-F2-CA-6	71	129	IR68586-F2-CA-57
27	79	IR68586-F2-CA-7	72	130	IR68586-F2-CA-58
28	80	IR68586-F2-CA-8	73	132	IR68586-F2-CA-60
29	81	IR68586-F2-CA-9	74	133	IR 68586-F2-CA-61
30	82	IR 68586-F2-CA-10	75	135	IR 68586-F2-CA-63
31	83	IR 68586-F2-CA-11	76	136	IR 68586-F2-CA-64
32	85	IR 68586-F2-CA-13	77	137	IR 68586-F2-CA-65
33	86	IR68586-F2-CA-14	78	138	IR 68586-F2-CA-66
34	87	IR68586-F2-CA-15	79	139	IR 68586-F2-CA-67
35	88	IR68586-F2-CA-16	80	140	IR 68586-F2-CA-68
36	89	IR 68586-F2-CA-17	81	141	IR68586-F2-CA-69
37	91	IR68586-F2-CA-19	82	142	IR 68586-F2-CA-70
38	93	IR 68586-F2-CA-21	83	143	IR68586-F2-CA-71
39	94	IR 68586-F2-CA-22	84	144	IR68586-F2-CA-72
40	95	IR68586-F2-CA-23	85	145	IR 68586-F2-CA-73
41	96	IR 68586-F2-CA-24	86	146	IR68586-F2-CA-74
42	97	IR 68586-F2-CA-25	87	147	IR 68586-F2-CA-75
43	98	IR 68586-F2-CA-26	88	148	IR 68586-F2-CA-76
44	99	IR68586-F2-CA-27	89	149	IR 68586-F2-CA-77
45	100	IR 68586-F2-CA-28	90	150	IR 68586-F2-CA-78

S. No.	DH line No	DESIGNATION	S. No.	DH line No	DESIGNATION
91	151	IR68586-F2-CA-79	136	198	IR68586-F2-CA-129
92	152	IR 68586-F2-CA-80	137	199	IR68586-F2-CA-130
93	153	IR 68586-F2-CA-81	138	200	IR 68586-F2-CA-131
94	154	IR68586-F2-CA-82	139	202	IR 68586-F2-CA-133
95	155	IR 68586-F2-CA-83	140	203	IR68586-F2-CA-134
96	156	IR 68586-F2-CA-84	141	204	IR 68586-F2-CA-135
97	157	IR 68586-F2-CA-85	142	205	1R68586-F2-CA-136
98	158	IR68586-F2-CA-87	143	206	IR 68586-F2-CA-137
99	159	IR 68586-F2-CA-88	144	207	IR 68586-F2-CA-138
100	160	IR 68586-F2-CA-89	145	209	IR68586-F2-CA-140
101	161	IR 68586-F2-CA-90	146	210	IR68586-F2-CA-141
102	162	IR 68586-F2-CA-91	147	211	IR 68586-F2-CA-142
103	163	IR 68586-F2-CA-92	148	212	IR 68586-F2-CA-143
104	164	IR 68586-F2-CA-93	149	213	IR 68586-F2-CA-144
105	165	IR 68586-F2-CA-94	150	214	IR 68586-F2-CA-145
106	166	IR 68586-F2-CA-95	151	215	IR 68586-F2-CA-146
107	167	IR 68586-F2-CA-96	152	216	IR 68586-F2-CA-147
108	168	IR 68586-F2-CA-97	153	217	IR68586-F2-CA-148
109	169	IR 68586-F2-CA-98	154	220	IR68586-F2-CA-151
110	170	IR 68586-F2-CA-99			
111	171	1R68586-F2-CA-100	Parents		
112	172	IR68586-F2-CA-101	155	221	CT9993-5-10-1-M
113	173	IR 68586-F2-CA-102	156	222	IR62266-42-6-2
114	174	IR68586-F2-CA-103			
115	175	IR68586-F2-CA-104			
116	176	IR68586-F2-CA-105			
117	177	IR68586-F2-CA-106			
118	178	IR 68586-F2-CA-107			
119	179	IR68586-F2-CA-108			
120	180	IR68586-F2-CA-109			
121	181	IR 68586-F2-CA-110			
122	183	IR 68586-F2-CA-112			
123	184	IR 68586-F2-CA-113			
124	185	IR 68586-F2-CA-114			
125	186	IR 68586-F2-CA-115			
126	187	IR 68586-F2-CA-117			
127	189	IR68586-F2-CA-120			
128	190	IR68586-F2-CA-121			
129	191	IR 68586-F2-CA-122			
130	192	IR68586-F2-CA-123			
131	193	IR 68586-F2-CA-124			
132	194	IR68586-F2-CA-125			
133	195	IR68586-F2-CA-126			
134	196	IR68586-F2-CA-127			
135	197	IR 68586-F2-CA-128			

Results

CHAPTER IV
RESULTS

Host plant resistance has been used extensively for disease control in many crop species. Many types of resistance, however, are not long lasting because of rapid changes in pathogen populations. Durability of resistance *(R)* genes is thus central to sustainable disease management. The capacity to predict the durability of *R* genes would be highly desirable for making sound investments in breeding and genetic engineering. The *R* genes used in plant disease management are largely single dominant genes that direct the recognition of pathogen components encoded by avirulence *(avr)* genes; this relationship is referred to as a gene-for-gene interaction. The direct interaction of the R protein and Avr ligand results in activation of the plant defense response, which often involves a hypersensitive response (HR). However, a clear-cut resistant phenotype like hypersensitivity does not always exist in many other cases and plant resistance often shows both qualitative and quantitative components. The qualitative resistance in many plant-pathogen relationships is hypersensitive, race specific, and governed by interactions between avirulence genes in pathogens and resistance genes in hosts, while the quantitative resistance is non-hypersensitive, presumably non-race specific, and controlled by polygenes.

This characteristic of the relationship between *O. sativa* and *Xoo* offers a unique opportunity to study the genetics of the interaction between host plants and their pathogens. In this study, we analyze the responses of near isogenic line, sets carrying two, three and four R genes and gene combinations to 14 *Xoo* isolates (Table 4.1) and the response of double haploid population to a sub set of selected isolates collected from Raipur and adjoining areas.

Table :4.1 Reaction of gene and gene combinations to *Xanthomonas oryzae* pv. *oryzae* isolates

S. No.	Lines	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"	6"	7"	R	S
1	ARBN-6	?	HS	MS	S	MS	S	R	R	MS	S	MS	MS	R	MS	MS	3	11
2	ARBN-31	?	MS	MS	R	MS	R	MS	R	R	R	R	R	R	MS	S	8	6
3	Mahsuri	?	MS	R	R	R	MS	MS	S	S	R	R	HR	R	MS	S	7	7
4	Swarna	?	MS	MS	R	MS	HR	S	HR	MS	MS	R	R	MS	MS	MS	5	9
5	Pant 4		MS	R	R	MS	R	MS	HR	R	R	HR	MS	MS	MS	S	7	7
Near Isogenic Lines																		
1	ARBN-96	Xa23	MS	MS	R	R	HR	MS	MS	S	S	MS	MS	MS	MS	MS	3	11
2	ARBN-107	Xa-3	MS	HR	HR	R	MS	MS	R	R	HR	HR	R	MS	MS	MS	8	6
3	ARBN-118	Xa-1	S	MS	R	S	HR	S	R	MS	MS	R	R	MS	MS	R	6	8
4	ARBN-120	Xa-4	S	R	R	S	R	S	R	MS	MS	R	R	MS	MS	S	6	8
5	ARBN-121	xa-5	MS	R	R	MS	R	S	R	R	R	R	MS	R	MS	S	8	6
6	ARBN-122	Xa-7	S	MS	R	S	R	S	R	MS	MS	R	S	MS	S	MS	4	10
7	ARBN-123	xa-8	R	R	R	MS	R	MS	R	MS	R	R	R	R	MS	S	9	5
8	ARBN-124	Xa-10	S	MS	R	S	MS	S	R	MS	MS	R	MS	MS	MS	S	3	11
9	ARBN-125	Xa-11	S	MS	R	S	MS	S	R	MS	MS	R	MS	MS	MS	MS	3	11
10	ARBN-126	xa-13	R	R	R	MS	R	R	HR	R	R	R	MS	R	S	MS	10	4
11	ARBN-127	Xa-14	MS	R	R	S	R	MS	R	MS	R	MS	MS	R	S	MS	6	8
12	ARBN-128	Xa-21	R	R	HR	R	R	R	HR	R	MS	R	MS	MS	S	MS	9	5
Two gene pyramid																		
1	ARBN-129	Xa4+xa-5	R	R	R	MS	R	R	R	R	R	R	MS	R	MS	MS	10	4
2	ARBN-130	Xa-4+xa-13	MS	HR	MS	R	R	R	MS	R	MS	R	MS	MS	MS	MS	6	8
3	ARBN-131	Xa-4+ Xa-21	HR	HR	HR	S	R	R	R	R	HR	R	MS	R	MS	MS	10	4
4	ARBN-132	xa-5+xa-13	R	HR	HR	MS	R	R	R	R	R	R	MS	MS	MS	MS	9	5
5	ARBN-133	xa-5+Xa-21	MS	R	R	MS	HR	R	R	R	MS	R	R	R	MS	MS	9	5
6	ARBN-134	Xa-13+ Xa-21	R	HR	HR	HR	HR	HR	R	HR	R	HR	MS	R	MS	MS	11	3
Three gene pyramid																		
1	ARBN-135	Xa-4+xa-5 + xa-13	R	R	R	HR	HR	R	R	R	R	R	MS	MS	MS	S	10	4
2	ARBN-136	Xa-4+xa-5 + Xa-21	MS	R	HR	R	R	MS	R	R	R	R	MS	R	MS	MS	9	5
Four gene pyramid																		
1	ARBN-137	Xa-4+xa-5 + xa-13+xa-21	R	R	R	R	R	R	R	R	R	R	R	R	MS	S	12	2
		Resistant	**9**	**18**	**24**	**9**	**21**	**11**	**23**	**15**	**15**	**23**	**9**	**13**	**0**	**1**		
		Susceptible	**17**	**8**	**2**	**17**	**5**	**15**	**3**	**11**	**11**	**3**	**17**	**13**	**26**	**25**		

1) HS, S, and MS were considered as Susceptible Reaction 2) HR and R were considered as Resistance Reaction

4.1 Virulence analysis of the bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae)* isolates

The virulence spectrum and aggressiveness of 14 isolates was tested against 26 genotypes with different one, two, three and four gene combinations, along with Mahsuri, Swarna and Shyamala with unidentified genes for resistance.

Table: 4.2 Hypothetical presence of *R-Avr* gene combinations in a characteristic highly resistant / hypersensitive relationship between *O. sativa* and *Xoo* isolates

S. No.	Line No.	12	AK 14-2	1	11	14-1	4	7	4-1	29-1	1-3
1	ARBN-118 (Xa-1)					*Avr Xa1*					
2	ARBN-107 (Xa-3)		*AvrXa3*						*AvrXa-3*	*AvrXa-3*	*AvrXa-3*
3	ARBN-126 (xa-13)			*AvrXa-13*							
4	ARBN-128 *(Xa-21)*		*AvrXa-21*	*AvrXa-21*							
5	ARBN-96 (Xa23)					*AvrXa23*					
6	ARBN-130 (Xa-4+xa-13)									*AvrXa-4 Avrxa-13*	
7	ARBN-132 (*Xa-5*+xa-13)		*AvrXa-5 Avrxa13*							*AvrXa-5 Avrxa-13*	
8	ARBN-133 *(xa-5+Xa-21)*										
9	ARBN-131 (Xa-4+*Xa-21)*	*AvrXa4 Avr Xa-21*	*AvrXa4 Avr Xa-21*							*AvrXa-4 Avr Xa-21*	*AvrXa-4 Avr Xa-21*
10	ARBN-134 (Xa-*tt+Xa-21)*		*Avrxa13 Avr Xa-21*		*AvrXa-13 Avr Xa-21*	*avrxa-13 Avr Xa-21*	*Avrxa-13 Avr Xa-21*	*Avrxa-13 Avr Xa-21*	*Avrxa-13 Avr Xa-21*	*Avrxa-13 Avr Xa-21*	
11	ARBN-135 (Xa-4+*xa-5* +xa-13)				*AvrXa-4 avr xa-5 Avr xa-13*	*Avr Xa-4 Avr xa-5 Avr xa-13*					
12	ARBN-136 (Xa-4+*xa-5+Xa-21)*		*AvrXa4 Avrxa-5 AvrXa-21*								

The qualitative component of the R genes is reflected by their large effects against the corresponding avirulent *Xoo* races and reflects the out come of the interactions between alleles at all avirulence loci in the pathogen and alleles at all R loci of the plant. Based on the highly resistance response corresponding *avr* gene in *Xoo* were spaculated (Table 4.2). The Isolates #14-1, #AK 14-2, #29-1, were grouped as the most avirulent with a speculated 6, 5, 5 number of avirulence genes

Typical advancing lesion
of
bacterial blight

Bacterial **blight** disease
in its sever form

respectively. Based on host reaction, the isolates were categorized in the order of increasing virulence as Isolate #11, #1-3, #4-1, #12, #3, #4, #7, #4", #5". #6", #7". The isolates #6"& #7" were the most virulent isolates and were able to overcome all lines with different genes and gene combinations respectively. Based on the host reactions of the 14 isolates, qualitative and quantitative behaviour of the isolates was reflected by the differential effects of the genotypes having R gene and gene combinations. Highly resistance response observed in the present investigation indicates that some sort interplay between the host *(O. sativa)* and the pathogen *Xoo* leads to recognition which is relected in the form of hypersensitive / highly resistance response. The presence of avirulence genes was spaculated in the individul isolate based on the inability of the individual isolate to cause infection. Highly avirulent isolate indicated the presence of several *avr genes* where as few in the virulent isolates of *Xoo.* The isoltes which were able to show specificity against the genotypes with different gene and gene combinations were thus inferred as verticle pathotypes (Isoltates # 14-1, AK 142, 29-1) (Table 4.2 & 4.3).

Table: 4.3 Hypothetical presence of *avr* gene(s) in *Xoo* isolates showing avirulence reaction

			14-2								
1	*Avr genes*	*AvrXa1 Avr Xa-4 Avr xa-5 Avr xa-13 Avr Xa-21 AvrXa23*	*AvrXa-3 AvrXa-4 Avrxa-5 Avrxa-13 AvrXa-21*	*AvrXa-3 AvrXa-4 Avrxa-5 Avrxa-13 Avr Xa-21*	*AvrXa-4 Avrxa-5 Avrxa-13 Avr Xa-21*	*AvrXa-3 AvrXa-4 Avr Xa-21*	*AvrXa-3 Avrxa-13 Avr Xa-21*	*AvrXa4 Avr Xa-21*	*Avrxa-13 AvrXa-21*	*Avrxa-13 Avr Xa-21*	*avrxa-13 avr Xa-21*
2	***Speculated Number of Avr genes***	***6***	***5***	***5***	***4***	***3***	***3***	***2***	***2***	***2***	***2***

4.2 Efficacy of different gene and gene combinations against *Xanthomonas oryzae* pv. *oryzae* isolates

This characteristic of the relationship between *O. sativa* and *Xoo* offers a unique opportunity to study efficacy of different gene and gene combinations against *Xanthomonas oryzae* pv. *oryzae* isolates. In this study, we analyze the responses of near isogenic line, sets carrying two, three and four R genes and gene combinations to 14 *Xoo* isolates (Table 4.1)

4.2.1 Behavior of lines with no genes for resistance

Among the 26 genotypes, six lines (Mahsuri, Swarna, and Pant 4 , ARBN 6(IR 64), ARBN 31 (Karuna) were either with no gene or carried unidentified gene(s) for resistance against *Xoo.* ARBN 31, Mahsuri and pant 4 showed resistace response against 8,7 and 7 isolates tested. Resistant phenotype could be speculated for the presence of some resistant genes.

4.2.2 Behavior of lines with one gene for resistance

The resistance of rice plants to *Xoo* measured by leaf- clipping inoculation should refer strictly to inhibition of pathogen growth, which has been known to have both qualitative and quantitative components. The lines with known single gene for resistance were evaluated against all the fourteen cultures. Amongst the 26 genotypes studied, there were twelve lines with one known gene for resistance (Table 4.4). The genotypes ARBN-107(*Xa3*), 121(*xa-5*), 128(*Xa 21*), 123(*xa-8*), 126(*xa-13*), showed resistance against 8,8,9,9,10 number of isolates respectively, out of 14 isolates tested. ARBN- 121(*xa-5*), 128(*Xa-21*), 126(x*a-13*), although with recessive gene showed a wide spectrum of resistance against a set of bacterial blight isolates having variable range of virulence spectrum, and thus seem to be highly effective genes. But the genotypes ARBN-118(*Xa-1*), ARBN-120(*Xa-4*), ARBN-127(*Xa-14*) showed resistance against less then 50% of the isolates, while ARBN-96(*Xa23*), ARBN-124(*Xa-10*),ARBN-125(*Xa-11*), ARBN-122(*Xa-7*) showed resistance reaction against few isolates thus indicating that these R genes can provide protection against a narrow spectrum of virulent isolates.

Table : 4.4 Response of Near Isogenic Lines (with one gene)against *Xoo* isolates

S. No.	Line No.	Gene	AK 14-2	3	4-1	14-1	29-1	1-3	7	4"	5"	11	12	4	7"
1	ARBN-96	*Xa23*	R	-*		HR		-	-	-	-	R			
2	ARBN-124	*Xa-10*	R	R	R		-						-		
3	ARBN-125	*Xa-11*	R	R	R										
4	ARBN-122	*Xa-7*	R	R	R	R	-						-		
5	ARBN-118	*Xa-1*	R	R	R	HR	-	-	-	R	-	-	-	-	R
6	ARBN-120	*Xa-4*	R	R	R	R	R	-	-	R	-	-	-		
7	ARBN-127	*Xa-14*	R	R	-	R	R	R	-	-	R	-	-		
8	ARBN-107	*Xa-3*	HR	R	HR	-	HR	HR	R	R	-	R	-		
9	ARBN-121	*xa-5*	R	R	R	R	R	R	R	-	R	-	-		
10	ARBN-128	*Xa-21*	HR	HR	R	R	R	-	R	-	-	R	R	R	-
11	ARBN-123	*xa-8*	R	R	R	R	R	R	-	R	R	-	R	-	-
12	ARBN-126	*xa-13*	R	HR	R	R	R	R	R	-	R	-	R	R	-

***- line indicates a susceptible reaction**

4.2.3 Behavior of lines with two genes for resistance

The genotype ARBN-129, 130, 131, 132, 133 and 134 were with two gene combinations. All the six lines with two gene combinations exhibited broader spectrum of resistance compared to individual genes(Table 4.1 & 4.5). ARBN-129, 131 and 134 (*Xa-4+xa-5*,*xa-5+xa-13* and *xa-13+Xa-21*) showed a similar response against the isolates and were the best two gene pyramid. The genotypes ARBN-130, 132 and 133 showed a differential response against the isolates.

Individually these genes responded differentially against the different isolates. The genotypes with *Xa-4* and *xa-5* showed susceptible and moderately susceptible reaction against isolates 12,11,4. *Xa-4* showed moderately susceptible reaction against isolates # 7,1-3 and 5". The combination of*Xa-4* and a recessive gene *xa-5* responded in additive manner against these isolates and showed resistance to highly resistance response. All the six genotypes with two gene combination showed moderately susceptible reaction against isolate # 6" and 7".

Table: 4.5 Response of two gene pyramids against *Xoo* isolates

		12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"
1	ARBN-130 *Xa-4+xa-13*	-	HR	-	R	R	R	-	R	-	R	-	-
2	ARBN-132 *Xa-5+xa-13*	R	HR	HR	-	R	R	R	R	R	R	-	-
3	ARBN-133 *xa-5+Xa-21*	-	R	R	-	HR	R	R	R	-	R	R	R
4	ARBN-129 Xa4+*xa-5*	R	R	R	-	R	R	R	R	R	R	-	R
5	ARBN-131 *Xa-4+Xa-21*	HR	HR	HR	-	R	R	R	R	HR	R	-	R
6	ARBN-134 *xa-13+Xa-21*	R	HR	HR	HR	HR	HR	R	HR	R	HR	-	R

***- line indicates a susceptible reaction**

4.2.4 Behavior of lines with three genes for resistance

The three gene combination in ARBN 135(*Xa-4+xa-5+xa-13*)was found to be most effective and showed resistant reaction against 10 isolates except isolate #4 #5 #6 and #7. Similarly *Xa-4+xa-5+Xa-21* in ARBN-136 imparted resistance against most of the isolates except isolate # 12,4,4",6",7" (Table 4.6). Individually the three genes present in ARBN-135 and ARBN-136 showed differential reactions against isolates (Table 4.1).

Table: 4.6 Response of three gene pyramids against *Xoo* isolates

S. No.	Line No.	Gene combintions	12	29-1	AK 14_2	11	14-1	4	3	7	1-3	4-1	5"
1	ARBN-135	*Xa-4+xa-5+xa-13*	R	R	R	HR	HR	R	R	R	R	R	-
2	ARBN-136	*Xa-4+xa-5+Xa-21*	-	R	HR	R	R	-	R	R	R	R	R

***- line indicates a susceptible reaction**

4.2.5 Behavior of lines with four genes for resistance

The four gene pyramid *Xa-4+xa-5+xa-13+Xa-21* (ARBN-137) showed resistance to all except isolate #6 and isolate #7. No highly resistance/ hypersensitive response was observed (Table 4.7)

Table: 4.7 Response of four gene pyramids against *Xoo* isolates

S. No.	Line No.	Gene combintions	12	29-1	AK 14-2	11	14-	4	3	7	1-3	4-1	4"	5"
1	ARBN-137	*Xa-4+xa-5+xa-13+Xa-21*	R	R	R	R	R	R	R	R	R	R	R	R

4.2.6 Genotypes with highly resistance /hypersensitive response

The qualitative resistance in many plant-pathogen relationships is hypersensitive, race specific, and governed by interactions between avirulence genes in pathogens and resistance genes in hosts. The genotypes with single gene for resistance viz., ARBN-96(Xa23), ARBN-107(Xa-3), ARBN-118(Xa-1), ARBN-126(*Xa-13*), ARBN-128(*Xa-21*), with pyramided two genes ARBN-130 (*Xa-4+Xa-*73), ARBN-132 (*xa-5*+x*a-13*), ARBN-133 (*xa-5+Xa-21*), ARBN-134 (*xa-13+ Xa-*27), ARBN-131 *(Xa-4+ Xa-21*), ARBN-135 (*Xa-4+xa-5*+x*a-13*), ARBN-136 *(Xa-4+xa-5+Xa-21*) and pyramided three genes ARBN 135 (*Xa-4+xa-5 + xa-13*) and ARBN 136(*Xa-4+xa-5+Xa-21*) showed highly resistance/ hypersensitive reactions against different isolates (Table 4.8). Thus suggestive of the fact that hypersensitive response is a result of interaction between respective avr genes in *Xoo* with that of resistance genes in the host. '

Table: 4.8 Highly resistance/ hypersensitive reactions of NILs and pyramids against different *Xoo* isolates.

S. No.	Line No.	Gene(s)	12	AK 14-2	3	11	14-1	4	7	4-1	29-1	1-3
1	ARBN-96	*Xa23*					HR					
2	ARBN-107	*Xa-3*		HR						HR	HR	HR
3	ARBN-118	*Xa-1*					HR					
4	ARBN-126	*xa-13*			HR							
5	ARBN-128	*Xa-21*		HR	HR							
6	ARBN-130	*Xa-4+xa-13*									HR	
7	ARBN-131	*Xa-4+ Xa-21*	HR	HR							HR	HR
8	ARBN-132	*xa-5+xa-13*		HR							HR	
9	ARBN-133	*xa-5+Xa-21*										
10	ARBN-134	*xa-13+ Xa-21*		HR		HR	HR	HR	HR	HR	HR	
11	ARBN-135	*Xa-4+xa-5 +xa-13*				HR	HR					
12	ARBN-136	*Xa-4+xa-5+Xa-21*		HR								

4.2.7 **Epistatic effects of gene(s) in combinations**

4.2.7.1 ***Xa-4*** **vs.** ***Xa-21 & xa-13***

A negative epistatic effect (for increasd resistance) was observed due to *Xa-21,* individually Xa-4 was susceptible to isolate # 12. Against isolate # 29-1 *Xa-4* showed resistance and thus *xa-13 & Xa-21* had a negative epistatic effect (for increased resistance) in two gene pyramids. Xa-4 showed resistance against the isolate AK 14-2 against which *Xa-21* individually as well as in combination with *Xa-4* it showed highly resistance response. Similarly against 1-3 the two gene pyramid showed highly resistance response.*xa-13* in combination with *Xa-4* showed resistance response against isolate 11, but individually both the genes showed susceptibility. The genotypre ARBN 120 (*Xa-4*) was susceptible against isolate # 4 & 7 *but Xa-21* and *xa-13* individually as well as in combination with *Xa-4* showed resistance. In contrast, the positive epistatic effect for increased lesion length was detected almost exclusively in incompitable cases against the isolate 4" against which the *Xa-4* individually showed resistance response. *Xa-21* and *xa-13* or its corresponding gene in the isolate # 4" contributed additively towerds disease developmen (Table 4.9).

Table: 4.9 Epistatic effects of gene(s) in combinations.

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"	7"
1	ARBN-120	*Xa-4*	-	R	R	-	R	-	R	-		R	R	-	-
2	ARBN-128	*Xa-21*	R	R	HR	R	R	R	HR	R	-	R	-	-	-
3	ARBN-126	*xa-13*	R	R	R	-	R	R	HR	R	R	R	-	R	-
4	ARBN-130	*Xa-4+xa-13*	-	HR	-	R	R	R	-	R	-	R	S	-	-
5	ARBN-131	*Xa-4+Xa-21*	HR	HR	HR	-	R	R	R	R	HR	R	S	R	-

***- line indicates a susceptible reaction**

Overall gene combination of *Xa-4+Xa-21* was effective against a wide spectrum of isolate with a variable virlance spectrum.

4.2.7.2 **xa-5 vs.** Xa 21 & xa **13**

xa-5 individually showed susceptibility against isolate #12 & 4, but in combination with *xa-13* showed a resistance response. Against isolate # 29-, and AK 14-2 the two gene pyramid showed highly resistance response. Hypersensitive /

Highly resistance response was observed against isolate # 14-1, against which the individual gene and the two-gene pyramid (ARBN 132) showed resistance. *xa-5* in combination with *Xa-21* showed resistance response against isolate # 4", against which the individual gene and the two gene combination (ARBN 132) showed susceptible reaction (Table 4.10).

Table: 4.10 Epistatic effects of gene(s) in combinations.

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"	7"
1	ARBN-121	*xa-5*	-	R	R	-	R	-	R	R	R	R	-	R	-
2	ARBN-128	*Xa-21*	R	R	HR	R	R	R	HR	R	-	R	-	-	-
3	ARBN-126	*xa-13*	R	R	R	-	R	R	HR	R	R	R	-	R	-
4	**ARBN-132**	***xa-5+\a-13***	**R**	**HR**	**HR**	**-**	**R**	**R**	**R**	**R**	**R**	**R**	**-**	**-**	**-**
5	**ARBN-133**	***xa-5+Xa-21***	**-**	***R***	***R***	**-**	**HR**	**R**	**R**	**R**	**-**	**R**	**R**	**R**	**-**

***- line indicates a susceptible reaction**

4.2.7.3 *Xa-4+xa-13 vs. Xa-4+xa 5+xa-13*

The response of genotype ARBN-130(*Xa-4+xa-13*) and ARBN-135(*Xa-4+xa-5+xa-13*) when compared for its interaction with different *Xoo* isolates it was observed that *xa-5* a recessive resistant gene had a negative epistatic effect (for increased resistance) against the isolate #12, #AK14-2, *#3* against which ARBN-130 *(Xa-4+xa-13*) showed moderately susceptible to susceptible response. Thus suggestive of the fact that incompatibility between the three gene pyramid and the isolates 12,AK14-2, 3 was due to *xa-5* and its corresponding avirulence gene in the pathogen. (Table 4.11)

Table: 4.11 Epistatic effects of gene(s) in combinations.

S. No.	**Line No.**	**Gene(s)**	**12**	**29-1**	**AK 14-2**	**11**	**14-1**	**4**	**3**	**7**	**1-3**	**4-1**	**4"**	**5"**
1	**ARBN-130**	***Xa-4+xa-13***	**-**	HR	**-**	R	R	R	**-**	R	**-**	R	**-**	**-**
2	**ARBN-135**	***Xa-4+xa-5+xa-13***	R	R	R	HR	HR	R	R	R	R	R	**-**	**-**

***- line indicates a susceptible reaction**

4.2.7.4 *xa-5+xa-13* vs. *Xa-4+xa-5+xa-13*

The response of genotype ARBN-132(*xa-5+xa-13*) and ARBN-135(*Xa-4+xa-5+xa-13*) when compared for its interaction with different *Xoo* isolates it was observed that *xa-4* a recessive resistant gene had a negative epistatic effect (for increased resistance) against the isolate 11 against which ARBN-132(*xa-5+xa-13*) showed susceptible response. Thus suggestive of the fact that incompatibility between the three gene pyramid and the isolates 11 was due to *Xa-4* and its corresponding avirulence gene in the pathogen. (Table 4.12)

Table: 4.12 Epistatic effects of gene(s) in combinations.

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"
1	ARBN-132	*xa-5+xa-13*	R	HR	HR	-	R	R	R	R	R	R	-	-
2	ARBN-135	*Xa-4+xa-5+xa-13*	R	R	R	HR	HR	R	R	R	R	R	-	-

***- line indicates a susceptible reaction**

4.2.7.5 xa-5+Xa-21 vs. *Xa-4+xa-5+Xa-21*

The response of genotype ARBN-133 (*xa-5+Xa-21*) and ARBN-136(*Xa-4+xa-5+Xa-21*)when compared for its interaction with different *Xoo* isolates it was observed that *xa-4* a recessive resistant gene had a negative epistatic effect (for increased resistance) against the isolate 11,1-3 against which ARBN-133(*xa-5+Xa-21*) showed susceptible response. Thus suggestive of the fact that incompatibility between the three gene pyramid and the isolates 11was due to *xa-4* and its corresponding avirulence gene in the pathogen. A positive epistatic effect (for decreased resistance) was observed against isolate No 4" thus a corresponding avr gene of *Xa-4* in the pathogen contriibuted additively towards disease development(Table 4.13).

Table: 4.13 Epistatic effects of gene(s) in combinations

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"
1	ARBN-133	*xa-5+Xa-21*	-	R	R	-	HR	R	R	R	-	R	R	R
2	ARBN-136	*Xa-4+xa-5+Xa-21*	-	R	HR	R	R	S	R	R	R	R	-	R

***- line indicates a susceptible reaction**

4.2.7.6 ***Xa-4+ Xa-21*** vs. ***Xa-4+xa-5+Xa-21***

The response of genotype ARBN-131(*xa-4+Xa-21*) and ARBN-136*(Xa-4+xa-5+Xa-21*) when compared for its interaction with different *Xoo* isolates it was observed that *xa-5* a recessive resistant gene had a negative epistatic effect (for increased resistance) against the isolate 11 against which ARBN-131(*xa-4+Xa-21*) showed susceptible response. Thus suggestive of the fact that incompatibility between the three gene pyramid and the isolates 11was due to *xa-5* and its corresponding avirulence gene in the pathogen. Where as a positive epistatic interaction(for decreased resistance) was observed against isolate # 12, and 4 against which the two gene combination ARBN-131(*xa-4+Xa-21*)showed resistance response, suggesting that the compatibility between the three gene pyramid and the isolate # 12 and 4 was due to the presence of corresponding *avr* gene in the pathogen and is thus contriibuted additively towards disease development (Table 4.14).

Table: 4.14 Epistatic effects of gene(s) in combinations

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"
1	**ARBN-131**	***Xa-4+ Xa-21***	**HR**	**HR**	HR	-	R	R	R	R	HR	R	-	R
2	**ARBN-136**	*Xa-4+xa-5+Xa-21*	S	R	HR	R	R	S	R	R	R	R	-	R

***- line indicates a susceptible reaction**

4.2.7.7 *Xa-4+* ***xa-5*** *vs.* ***Xa-4+xa-5 +xa-13 / Xa-4+xa-5+Xa 21***

The response of genotype ARBN-129 (*xa-4+xa-5*), ARBN-135(*Xa-4+xa-5*+xa-13) and ARBN-136(*Xa-4+xa-5+Xa-21*) when compared for its interaction with different *Xoo* isolates. The genotypes with three gene combination differ with the genotype with two gene combination only with a single gene difference i.e. *xa-13* and *Xa-21*. It was observed that *xa-13* and *Xa-21* had a negative epistatic effect (for increased resistance) against the isolate 11 against which ARBN-129(*xa-4+xa-5*) showed susceptible response. The corresponding avr gene of *xa-13* in the pathogen was contributing towards the incompatibility. Where as a positive epistatic interaction (for decreased resistance) was observed against isolate # 12, and 4 in the presence of *Xa-21* against which the two gene combination ARBN-129(*xa-4+xa-5*) showed

resistance response thus suggesting that the corresponding avr gene of *Xa-21* in the pathogen may be contributing additively towards increased lesion development (Table 4.15).

Table: 4.15 Epistatic effects of gene(s) in combinations

S. No.	Line No.	Gene(s)	12	29-1	AK 14-2	11	14-1	4	3	7	1-3	4-1	4"	5"
1	ARBN-129	Xa4+*xa-5*	R	R	R	-	R	R	R	R	R	R	-	R
2	ARBN-135	*Xa-4+xa-5* +x*a-13*	R	R	R	HR	HR	R	R	R	R	R	-	-
3	ARBN-136	*Xa-4+xa-5+Xa-21*	S/MS	R	HR	R	R	S/MS	R	R	R	R	-	*R*

***- line indicates a susceptible reaction**

The overall results indicated that resistance gene(s) behave differentially against different inoculum. The combination of genes some time exhibited additive resistance while nonadditive in some combination and against different *Xoo* cultures

4.3 Identification of QTLs against *Xanthomonas oryzae* pv. *oryza.*

4.3.1 Parental differences and segregation of lesion length in CT 9993-5-10-1-M / IR 62266-42-6-2 double haploids

A high level of resistnce to *Xanthmonas oryzae pv. oryzae* is race specific and controlled by single major genes, the reaction of a segregating rice population to different*Xoo* strains virtually show both qualitative and quantitative components. Both Qualitative and quantitative derrminants contrivute to race specificity. Nine, 12, 1 and 3 QTL's were mapped against 4", 5", 6", 7" respectively, which were largely responsible for segregation of the resistant phenotype in the double haploid population Both the parent showed resistance phenotype against all the isolates, however the segregating populatin exhibited resistance as well as susceptibility, it indicates that both the parents had resistance as well as susceptibe.

- Parents lines CT 9993-5-10-1-M and IR 62266-42-6-2 were resistant but showed differences in the lesion length on inoculation with Isolates 4",5"& 6" *of Xoo.* Isolate 7" showed susceptible reaction on CT 9993-5-10-1-M and resistance reaction on IR 62266-42-6-2. On inoculation with isolate No. 4", 5", 6", 7" the double haploid

Fig: 4.1 The frequency distribution of bacterial blight response of 117 CT 9993-5-10-1-M and IR 62266-42-6-2 double haploid population

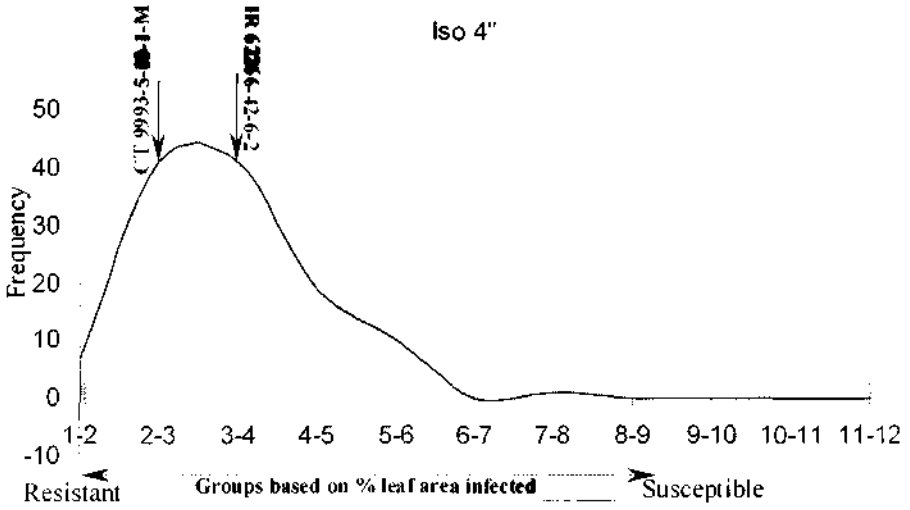


Fig:4.2 The frequency distribution of bacterial blight response of 117 CT 9993-5-10-1-M and IR 62266-42-6-2 double haploid population

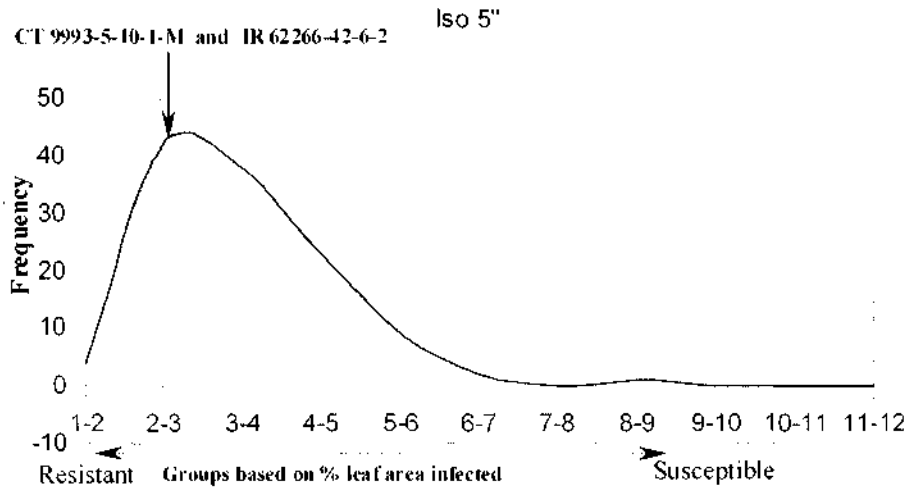


Fig:4.3 The frequency distribution of bacterial blight response of 117 CT 9993-5-10-1-M and IR 62266-42-6-2 double haploid population

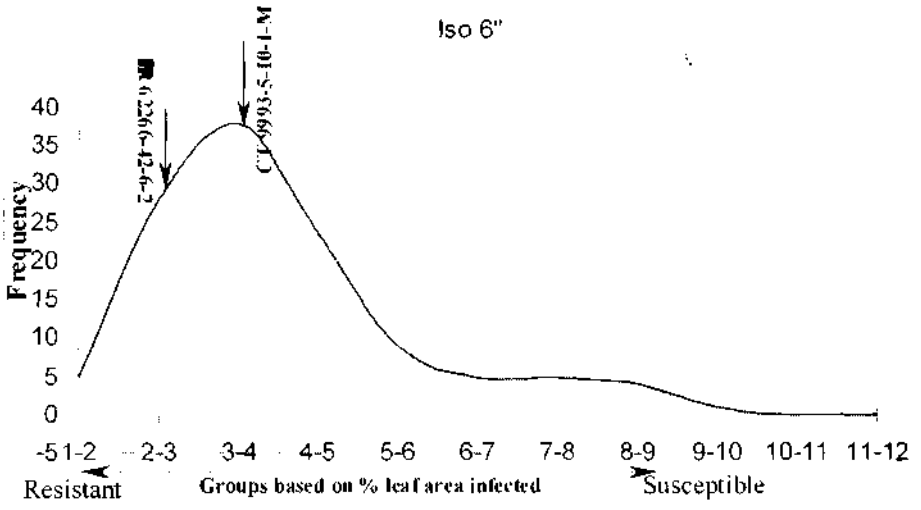


Fig: 4.4 The frequency distribution of bacterial blight response of 117 CT 9993-5-10-1-M and IR 62266-42-6-2 double haploid population

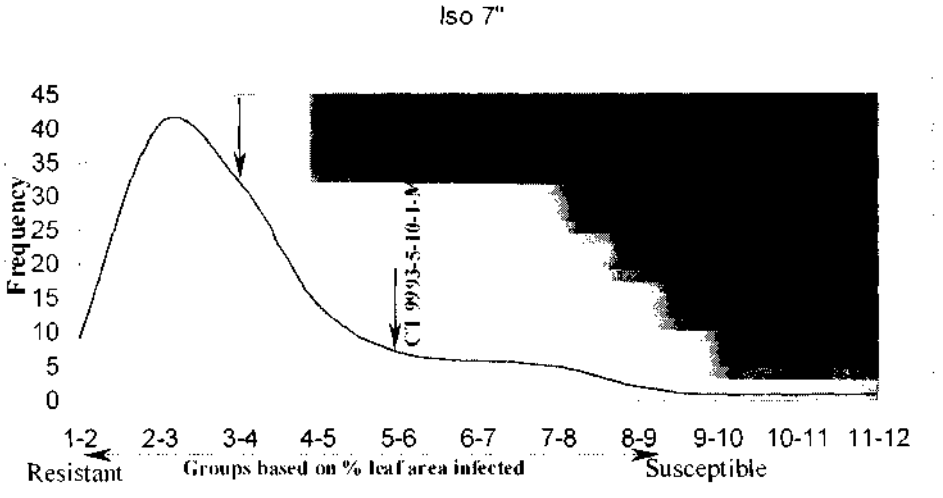
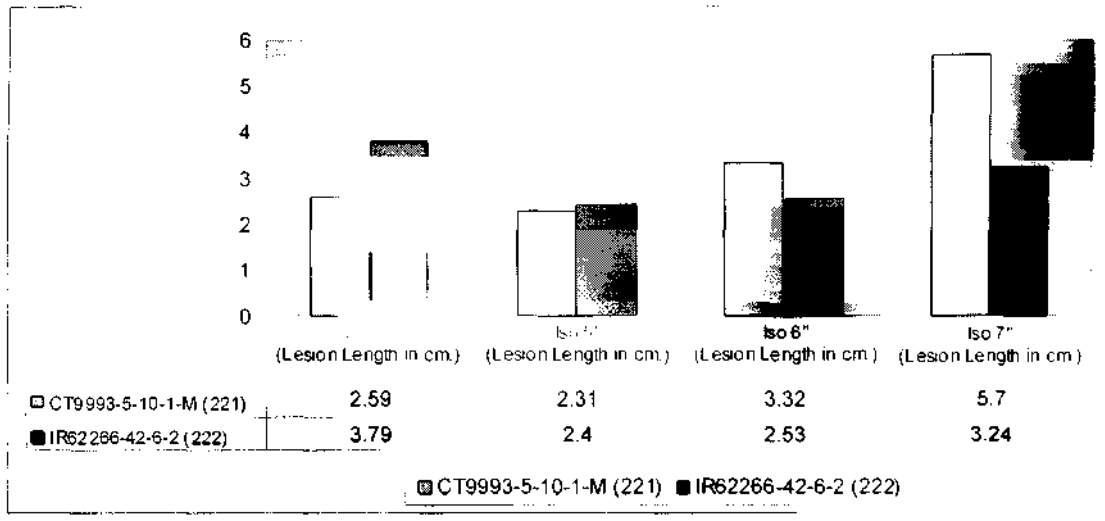


Fig:4.5 Comparison of the average lesion length (in cm) caused by the four *Xoo* isolates on the CT 9993-5-10-1-M and IR 62266-42-6-2 prental lines.



population exhibited continuous variation and transgressive segregation in both the direction, showing typical polygenic inheritance(Fig. 4.1, 4.2, 43, 4.4). A significant portion of the double haploid population had shorter lesion length than either parent. A thresh hold level of LOD >=1.5 was used to claim the presence of QTL.

Isolate No. 4": Nine QTLs were identified on chromosome No. 1,3,7,8, and 9 (Fig., 4.6 & 4.7, Table 4.16). QTLs on 1,3,7,8 and 9 were present between the molecular markers RG1028-ME7_4; C 63-RG 369; ME6_8- RG678; EAAM17_5 - ME 10_6; EM 11_1-BCD 855; ME9_1 - ME2_1; RG 141-RM 205 and explained 7.60, 8.00, 6.70,9.90, 6.90, 7.00 percent of the total phenotypic variaton in lesion length caused by isolate No4 with a LOD value of 3.53, 1.957, 2.03, 1.55, 1.684, 1.776, 1.806, respectively. All the seven QTLs had additive effect on the reducing lesion length. The two QTLs on chromosome No 9, present between the molecular markers G103-ME5_9; RM 219-RG553 explained 8.50% and 8.00% of the total phenotypic variaton in lesion length caused by isolate #4" with LOD value of 2.20 and 2.004 respectively and had an additive effect on increasing lesion development.

Table:4.16 Putative QTLs detected using interval mapping associated with bacterial blight resistance against isolate # 4"

S. No.	Molecular Marker Interval	Weight	Variance explained (%)	LOD
Chromosome No.1				
1	RG1028-ME7_4	-0.3874	13.2%	3.53
Chromosome No.3				
2.	C 63-RG 369	-0.316	7.60	1.957
Chromosome No.7				
3.	ME6 8-RG678	-0.3268	8.00	2.03
4.	EAAM17 5 - ME 10 6	-0.330	6.7	1.55
5.	EM 11_1-BCD 855	-0.420	9.90	1.684
Chromosome No.8				
6.	ME9_1 - ME2_1	-0.285	6.90	1.776
Chromosome No.9				
7.	G103-ME5 9	0.3711	8.50	2.20
8.	RM219-RG553	0.365	8.0	2.004
9.	RG 141-RM 205	-0.349	7.0	1.806

Isolate No 5": Twelve QTLs were identified on chromosome No. 1,2,3,7,8,10 and 11 ((Fig., 4.8 & 4.9). QTLs on 1,2,3,7,8, and 11 were present between the molecular markers C955-R2630; RG 1028- ME 7_4; ME 2_12- RG 532; WG 110-RG

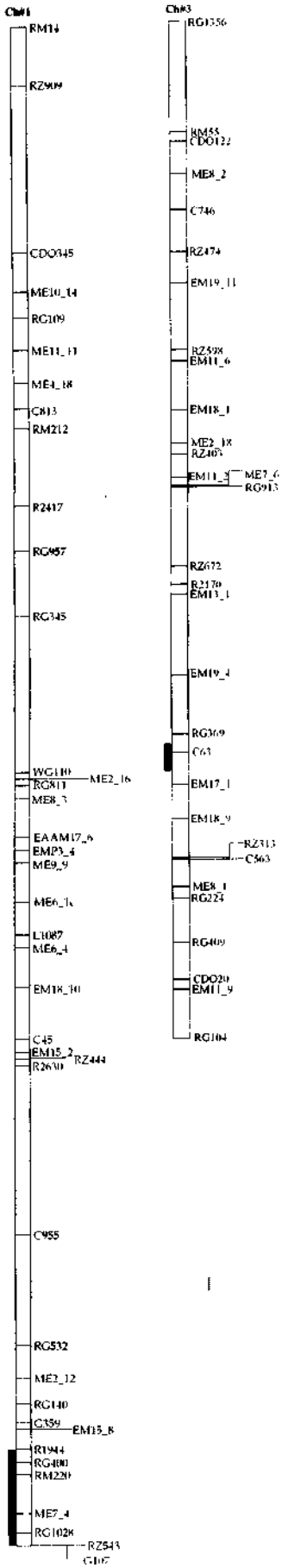


Fig: 4.6 Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 4

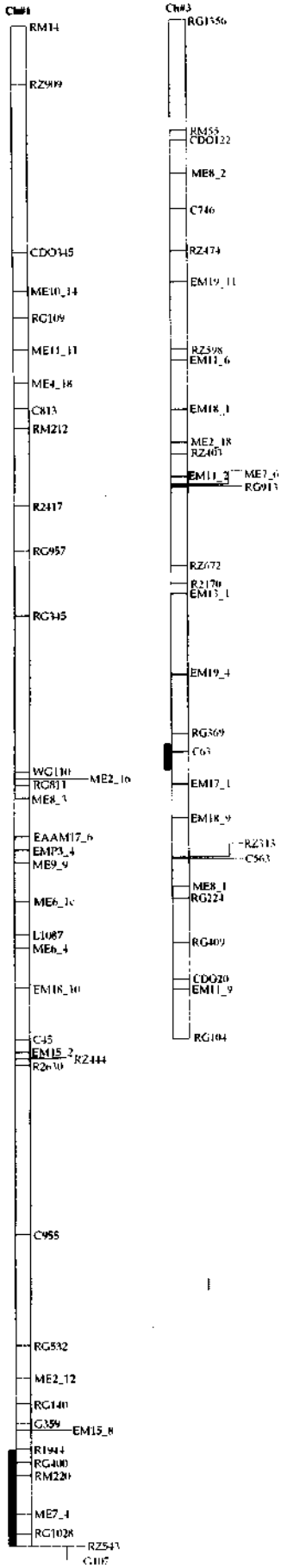


Fig: 4.6 Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 4

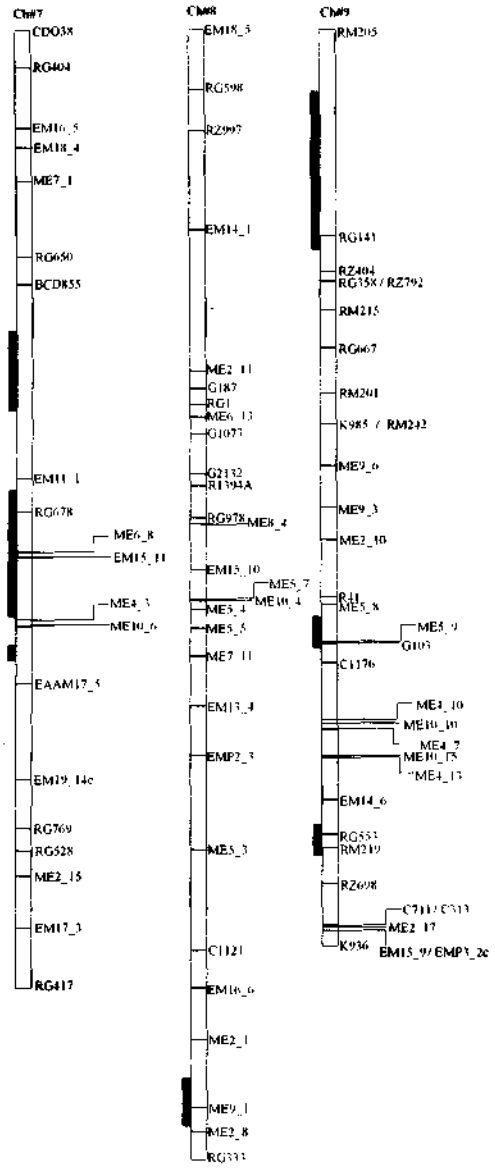


Fig.: 4.7Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 4

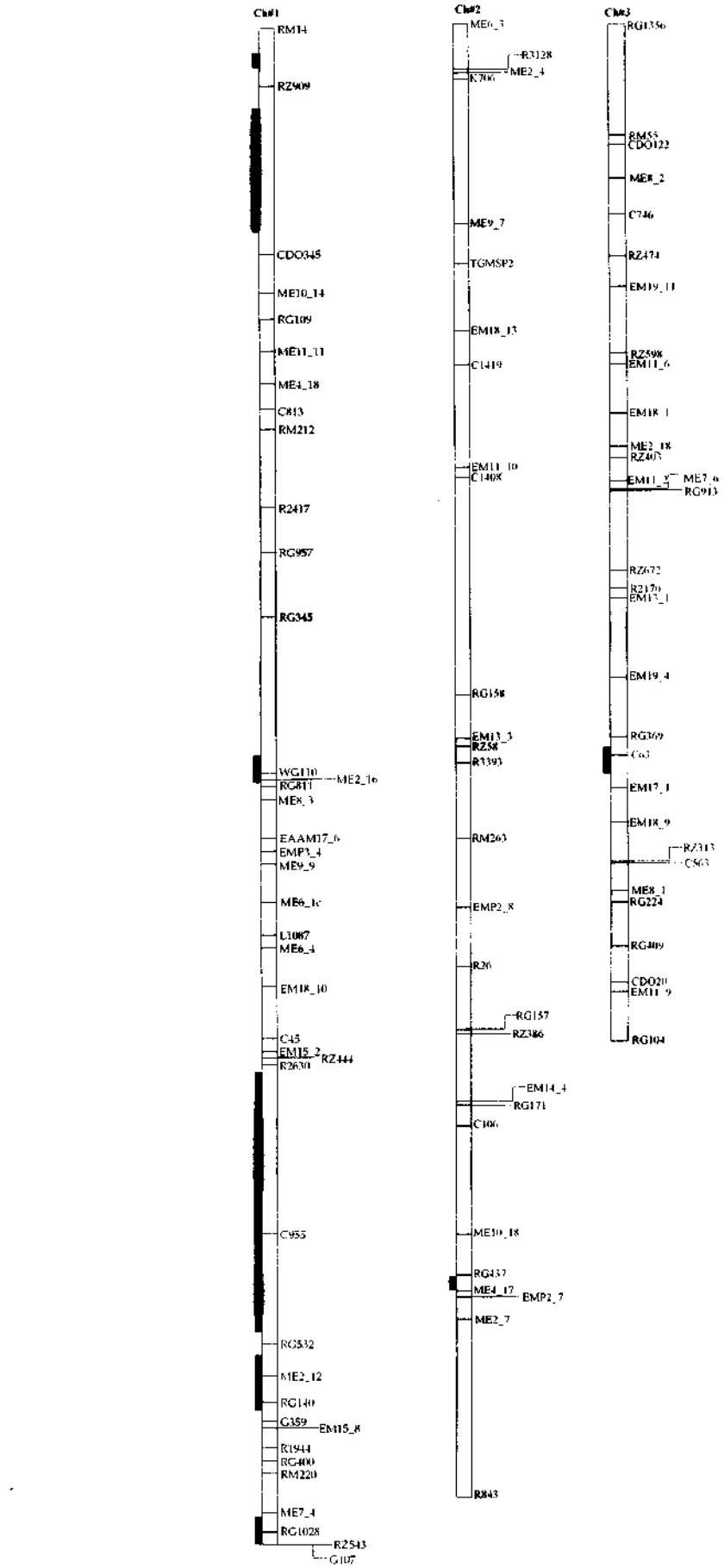


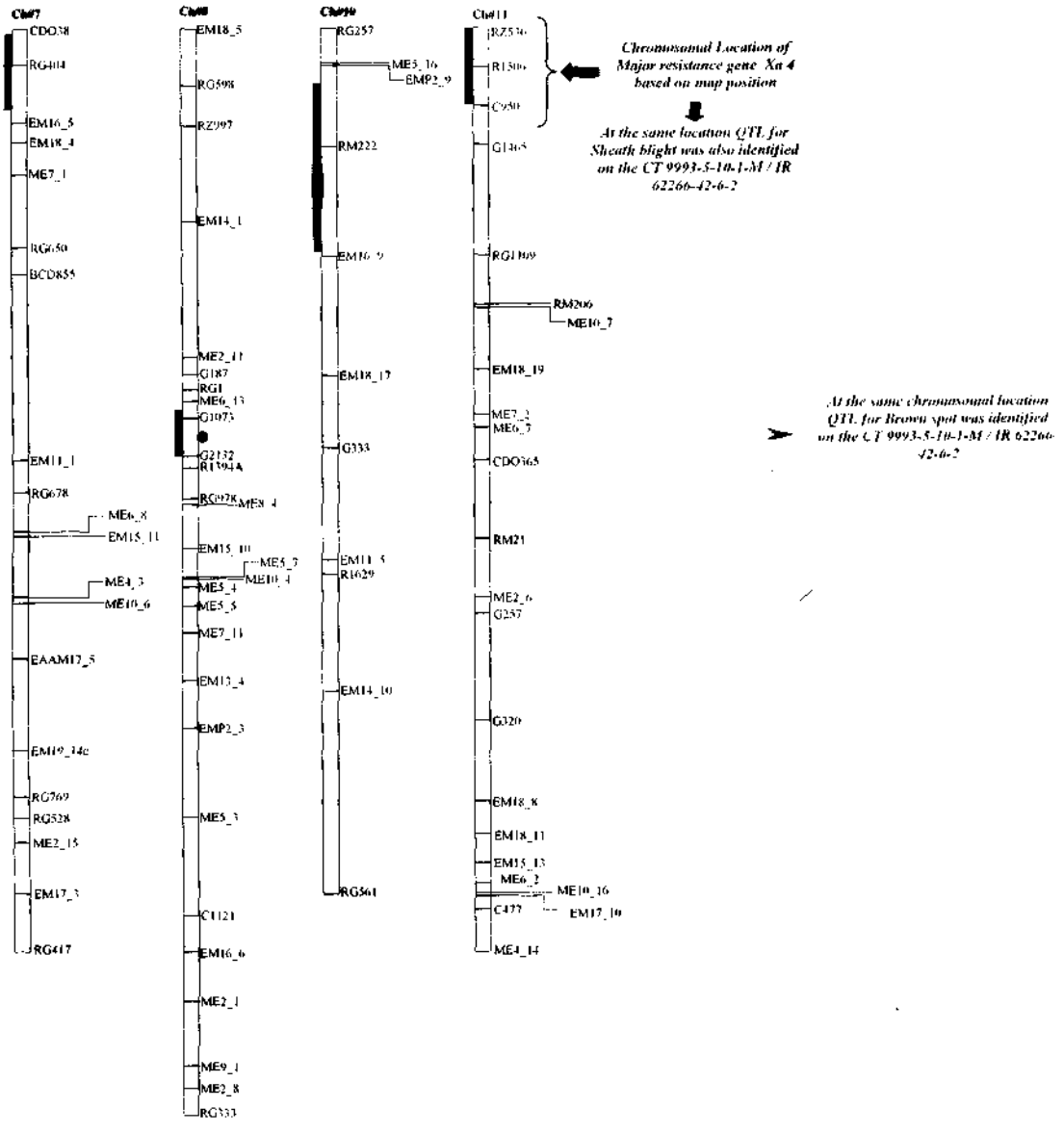
Fig: 4.8 Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 5

345; CDO 345-RZ909; RZ 909-RM 14; ME4_17-RG 437; C63-RG 369; RG404 - CDO 38; G 1073-ME6_13;R1506-RZ536 which explained 5.9; 8.2; 6.3; 7.1; 12.3; 6.6; 61; 7.9; 8.2; 6.5; 18.4 Percent of the total phenotypic variaton in lesion length caused by isolate No5" with an LOD value of 3.87; 2.127; 1.616; 1.817; 1.954; 1.532; 1.571; 2.05; 1.675; 5.00. All the ten QTLs had additive effect on the reducing lesion length caused by isolate No 5". The QTL identified on chromosome No. 10 was present between the molecular marker EM16_9-RM222 and explained 11.7% of the total phenotypic variaton in lesion length caused by isolate #5" with LOD value of 2.83% an additive effect for increasing lesion length was explained (Table 4.17).

Table:4.17 Putative QTLs detected using interval mapping associated with bacterial blight resistance against isolate # 5"

S. No.	Molecular Marker Interval	Weight	Variance explained (%)	LOD
Chromosome No. 1				
1	C955-R2630	-0.4921	15.9	3.87
2	RG 1028- ME 7 4	-0.338	8.2	2.127
3	ME 2 12- RG 532	-0.295	6.3	1.616
4	WG 110-RG 345	-0.316	7.1	1.817
5	CDO 345-RZ909	-0.503	12.3	1.954
6	RZ 909-RM 14	-0.383	6.6	1.532
Chromosome No. 2				
7	ME4_17-RG 437	-0.340	6.1	1.571
Chromosome No. 3				
8	C63-RG369	-0.3567	7.9	2.05
Chromosome No. 7				
9	RG404-CDO38	-0.4171	8.2	2.05
Chromosome No. 8				
10	G 1073-ME6_13	-0.301	6.5	1.675
Chromosome No. 10				
11	EM16_9-RM222	0.4882	11.7	2.83
Chromosome No. 11				
12	R1506-RZ536	-0.6151	18.4	5.00

Isolate No **6"**: On challenge inoculation with isolate 6" on the double haploid population QTL analysis revealed a QTL which was present between the molecular marker RG 4561-EM 14-10 which explained 10.5% of the total phenotypic variaton in lesion length caused by isolate # 6" with a LOD value of 1.647. An additive effect for increasing lesion lengt was explained (Fig. 4. 10, Table 4.18).



p position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 5

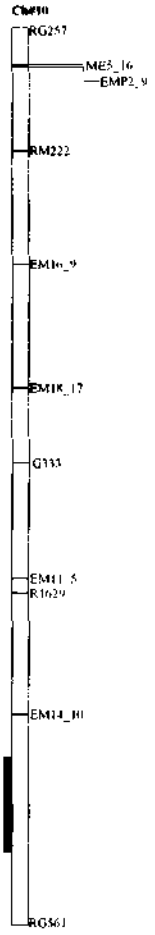


Fig: 4.10 Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 6

Table:4.18 Putative QTL detected using interval mapping associated with bacterial blight resistance against isolate # 6"

S. No.	Molecular Marker Interval	Weight	Variance explained (%)	LOD
Chromosome No. 10				
1	RG4561-EM 14-10	0.684	10.5	1.647

Isolate No 7: Three QTLs were identified, two on chromosome No. 6 and one on Chromosome 7. On Ch. 6 the QTLs were present between the molecilar markers R 2549-RG 172, RZ 682- R 2450 and explained 8.00 and 6.4 % of the total phenotypic variaton in lesion length caused by isolate 7" with an LOD value of 1.921 and 1.588. The QTLs identified explained an additive effect for increasing lesion length. The QTL identified on chromosome No 7 was present between the molecular markers EM 17_3 - ME 2_15 and explained 9.7% of the total phenotypic variaton in lesion length caused by isolate No7" with an LOD value of 2.40. The QTL identified explained additive effect for reducing lesion length (Fig. 4. 11, Table 4.19)

Table: 4.19 Putative QTLs detected using interval mapping associated with bacterial blight resistance against isolate # 7"

S. No.	Molecular Marker Interval	Weight	Variance explained (%)	LOD
Chromosome No. 6				
1	R 2549-RG 172	0.547	8.0	1.921
2	RZ 682- R 2450	0.447	6.4	1.588
Chromosome No. 7				
3	EM 17 3 - ME 2 15	-0.4507	9.7	2.40

4.3.2 Race specificity of resistance QTLs

All the QTLs identified were identified as isolate specific (Table 4.20). Some of the QTLs were present on the common chromosomes but with a different chromosomal location. QTLs identified on Ch 5,6 were specific for isolate No 7".

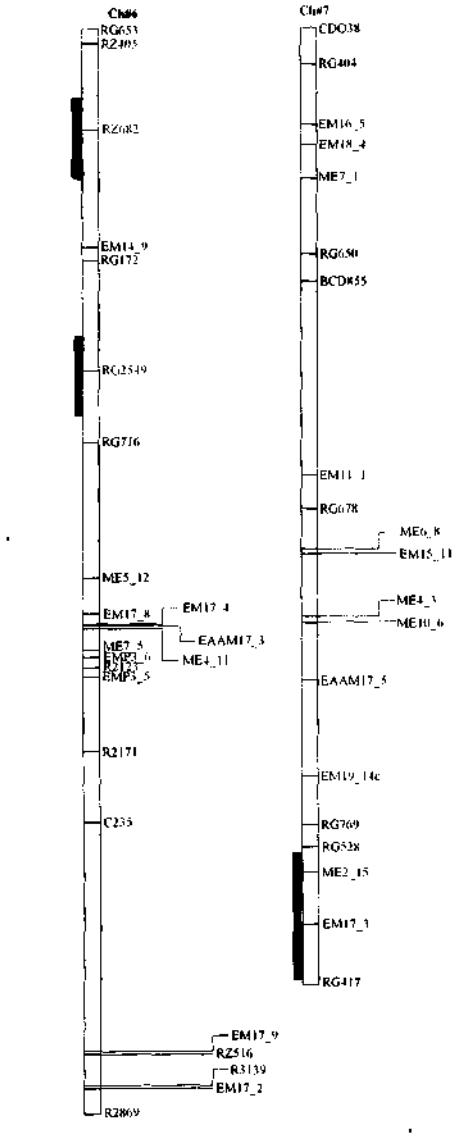


Fig: 4.11 Map position of QTLs for bacterial blight resistance in rice against *Xanthomonas oryzae* pv. *oryzae* Isolate No. 7

Table: 4.20 Race specificity of resistance Quantitative Trait Loci against isolate No. 4",5",6",7"

S. No.	Molecular Marker Interval	Weight	Variance explained (%)	LOD	Chromosome NO.
Isolate No 4"					
1	RG1028-ME7 4	-0.3874	13.2%	3.53	1
2	C 63-RG 369	-0.316	7.60	1.957	3
3	ME6 8-RG678	-0.3268	8.00	2.03	7
4	EAAM17 5 - ME 10 6	-0.330	6.7	1.55	7
5	EM 11 1-BCD 855	-0.420	9.90	1.684	7
6	ME9 1 – ME2 1	-0.285	6.90	1.776	8
7	G103-ME5 9	0.3711	8.50	2.20	9
8	RM 219-RG553	0.365	8.0	2.004	9
9	RG 141-RM 205	-0.349	7.0	1.806	9
Isolate No 5"					
10	C955-R2630	-0.4921	15.9	3.87	1
11	RG 1028- ME 7_4	-0.338	8.2	2.127	1
12	ME 2_12- RG 532	-0.295	6.3	1.616	1
13	WG 110-RG 345	-0.316	7.1	1.817	1
14	CDO 345-RZ909	-0.503	12.3	1.954	1
15	RZ 909-RM 14	-0.383	6.6	1.532	1
16	ME4_17-RG 437	-0.340	6.1	1.571	2
17	C63-RG369	-0.3567	7.9	2.05	3
18	RG404-CDO38	-0.4171	8.2	2.05	7
19	G 1073-ME6_13	-0.301	6.5	1.675	8
20	EM16_9-RM222	0.4882	11.7	2.83	10
21	R1506-RZ536	-0.6151	18.4	5.00	11
Isolate No 6"					
22	RG 4561-EM 14-10	0.684	10.5	1.647	10
Isolate No 7"					
23	R 2549-RG 172	0.547	8.0	1.921	6
24	RZ 682- R 2450	0.447	6.4	1.588	6
25	EM 17 3 - ME 2 15	-0.4507	9.7	2.40	7

4.3.3 Differences in the fitness of *Xoo* strains on resistant and susceptible double haploid population

The race specificity of rice resistance to different *Xoo* strains has both qualitative and quantitative components. Different *Xa* genes and gene combinations are used in many rice breeding programmes and confer durable resistance in many rice commercial cultivars. The present observation indicate that different *Xoo* strains show variable reaction against NILSs and Pyramids. Resistnce reaction against NILs wil indicate the presence of a corresponding avr gene in the pathogen. In other words mutaton at avr locus, in many *Xoo* may result in both quantitative and qulitative

changes in resistance phenotype when they interact with allels at rsistance loci in the host plant.

Our present observation indicates that isolate 4" & 5" showed resistance reaction against a number NILs and Pyramids (Table 4.21) where as isolate 6"&7" showed virulance reaction against all the NILs and Pyamids thus indicative of the fact that 6" & 7" are result of several genenetic changes at different avr loci.

Table: 4.21 Resistance reactions of Iso4",5",6",7" on NILs and Pyramids

S. No.	ARBN Number	Gene(s)	Iso 5"	Iso 4"	Iso 7"	ISO; 6"
1	Swarna	9	-	R	-	-
2	ARBN-107	*Xa-3*	-	R	-	-
3	ARBN-120	*Xa-4*	-	R	-	-
4	ARBN-118	*Xa-1*	-	R	R	-
5	ARBN-127	*Xa-14*	R	-		-
6	ARBN-31	?	R	R	-	-
7	Mahsuri	9	R	HR	-	-
8	ARBN-121	*xa-5*	R	-		-
9	ARBN-126	*xa-13*	R	-		-
10	ARBN-129	*Xa4+xa-5*	R	-		-
11	ARBN-134	*xa-13+ Xa-21*	R	-		-
12	ARBN-131	*Xa-4+ Xa-21*	R	-		-
13	ARBN-136	*Xa-4+xa-5 + Xa-21*	R	-		-
14	ARBN-123	*xa-8*	R	R	-	-
15	ARBN-133	*xa-5+Xa-21*	R	R	-	-
16	ARBN-137	*Xa-4+xa-5+xa-13+Xa-21*	R	R	-	-

Mine indicates susceptible reaction

4.3.4 Differences in the fitness of *Xoo* strains on resistant and susceptible double haploid population

Table: 4.22 Fitness *of Xoo* strains on resistant and susceptible double haploid population

Isolate No.	Number of Resistant Lines	Number of Susceptible Lines	Resistant lines (Range of Lesion Length)	Susceptible lines (Range of Lesion Length)
4"	68	47	1.92-3.54	3.46-7.54
5"	54	61	1.39-2.95	3.82-8.46
6"	38	77	1.97-3.32	3.42-6.28
7"	57	58	1.41-3.18	3.91-11.98

The QTLs with a significant additive effect on reducing lesion length against the avirulent strains (4" & 5") and virulent strains (6"&7") explains, at least in part a durable resistance (Table 4.22). Most resistance loci identified in this study do show some degree of strain specificity but this specificity accounts for only a small portion (6.3 - 18.4%) of the total variation in resistance. Because the number of identified resistance QTLs is fairly large and none of the four *Xoo* strains interact consistantly with all the QTLs, it is expected that cumulative effect of multiple resistance QTLs may result in significant level of durable quantitative resistance to Abo.

4.3.5 QTLs with similar genomic positions for different diseases

In the present investigation one of the QTL was identified at the same chromosomal location at which the *Xa-4* is mapped based on its position (Fig 4.9). On the chromosome 11 where the *Xa-4* a major gene for BLB resistance is inferred based on the map position QTL for sheath blight was also identified(Fig 4.9) using the double haploid population (Personal Communication). Similarly on chromosome 8 at the same chromosomal location QTL for Brown spot was also identified using the same population (Fig 4.9)(Anil kotasthane Personal Communication).

4.3.6 Study of inheritance of different *Xa* gene(s) in breeding populations for resistance to bacterial leaf blight of rice.

Incorporation of bacterial blight resistance is one of the important objectives of rice improvement programs. In the present investigation, F2 and F3 progenies derived from different crosses were evaluated to Study the inheritance of different *Xa* gene(s)

for resistance to bacterial leaf blight of rice. High yielding cultivars viz. Poornima, Swarna, and Mahamaya were crossed with NILs and pyramids with known genes for resistance (**ARBN 96**(*Xa-23*); ARBN **128**(*Xa-21*); ARBN 136(*Xa-4+xa-5+Xa21)* **ARBN** 137 (*Xa-4+xa-5+ xa-13+Xa-21*). For inheritance study, hybrid population (F2 and F3) were grown in field under standard management conditions were tested for bacterial blight resistance by using the most virulent isolate #6". The analysis of F2 and F3 population from different crosses (Table 4.23) did not segregated as per the Mendelian ratio.

Table:4.23 Study of inheritance of different *Xa* gene(s) in breeding populations for resistance to bacterial leaf blight of rice

S. No	Cross combination	Generation	Resistant	Susceptible	Highly Susceptible	Total
1	Poornima X ARBN 136	F2	0	8	103	111
2	Poornima X ARBN 128	F2	0	1	98	99
3	Poornima X ARBN 128	F2	0	3	78	81
4	Poornima X ARBN 128	F2	0	0	103	103
5	Poornima X ARBN 128	F2	0	2	131	133
6	Poornima X ARBN 96	F2	2	9	124	133
7	Poornima X ARBN 96	F2	1	6	128	134
8	Poornima X ARBN 96	F2	0	3	35	38
9	Poornima X ARBN 96	F2	0	5	114	119
10	Swarna X ARBN 96	F2	0	5	130	135
11	Swarna X ARBN 96	F2	1	7	123	130
12	Swarna X ARBN 96	F2	1	6	128	134
13	Swarna X ARBN 96	F2	0	4	131	135
14	Swarna X ARBN 96	F2	1	3	131	134
15	Swarna X ARBN 96	F2	0	19	112	131
16	Swarna X ARBN 128	F2	5	18	119	137
17	Swarna X ARBN 128	F2	11	16	16	32
18	Swarna X Bambaleshwari	F2	3	5	102	107
19	ARBN 137 X Swarna	F2	2	21	275	296
20	ARBN 137X Mahamaya	F2	13	26	110	136
21	ARBN 137X Mahamaya	F2	0	14	117	131
22	ARBN 137X Mahamaya	F2	2	21	201	222
23	ARBN 137X Mahamaya	F2	4	33	286	319
24	ARBN 137X Mahamaya	F2	4	14	338	352
25	ARBN 137X Mahamaya	F2	2	26	285	311
26	ARBN 137X Mahamaya	F2	2	24	289	313
27	ARBN 137X Mahamaya	F2	12	26	231	257
28	ARBN 137X Mahamaya	F2	6	10	275	285
29	Mahamaya X ARBN 136	F2	5	6	121	127
30	Swarna X ARBN 137	F3	2	38	224	262
31	Swarna X ARBN 137	F3	2	16	245	261
32	Swarna X ARBN 137	F3	2	53	219	272
33	Swarna X ARBN 137	F3	19	68	166	234
34	Swarna X ARBN 137	F3	13	103	119	222
35	Swarna X ARBN 137	F3	15	103	147	250
36	Swarna X ARBN 137	F3	22	117	123	240
37	Swarna XIET 14444	F3	44	33	93	126
38	Swarna XIET 14444	F3	21	123	118	241
39	Swarna X IET 14444	F3	38	109	113	222
40	Swarna X IET 14444	F3	96	105	48	153
41	Swarna X IET 14444	F3	0	27	216	243
42	Swarna X IET 14444	F3	4	12	222	234
43	Swarna XIET 14444	F3	0	23	108	131
44	Swarna XIET 14444	F3	1	10	119	129
45	Swarna XIET 14444	F3	1	26	233	259
46	Swarna X IET 14444	F3	2	222	28	250

ARBN 96(Xa- 23); **ARBN 128**(*Xa-21*); ARBN 136(*Xa 4+xa-5+Xa21)* **ARBN 137** *(Xa-4+xa-5 + xa-13+Xa-21*)

Discussion

CHAPTER V

DISCUSSION

Plant disease resistance is often controlled by Mendelian genes and follows a gene-for-gene relationship in many plant species and their pathogens (Flor 1971). The qualitative resistance in many plant-pathogen relationships is hypersensitive, race specific, and governed by interactions between virulence genes in pathogens and resistance genes in hosts, while the quantitative resistance is non-hypersensitive, presumably non-race specific, and controlled by polygenes (Nelson 1972). Resistance in rice to *Xoo* is known to have both qualitative and quantitative components (Mew 1987; Zhang and Mew 1988; Koch and Parlevliet 1991; Li *et al.* 1999). Qualitative resistance to *Xoo* is controlled by 24 major genes (Kinoshita 1995; Lin *et al,* 1998) conferring resistance to various races of the pathogen. Most of these R genes are dominant and have been recently mapped to respective genomic locations (Ronald *et al.* 1992; Yoshimura *et al.* 1992; Causse *et al.* 1994; Lin *et al.* 1996; Ogawa 1996; Zhang *et al.* 1996). Two of these genes (*Xa-21* and *Xa1*) have been cloned and molecularly characterized (Song *et al.* 1995; Yoshimura *et al.* 1998). Recently, near-isogenic lines (NILs) with pyramids of the R genes have been developed through molecular marker-aided backcross breeding, providing valuable materials for detailed characterization of the R genes (Huang *et al.* 1997; Sanchez *et al.* 2000). However, many important questions remain to be answered regarding various R genes. For instance, do different R genes act independently or synergistically? Are genes conferring qualitative effects different from genes conferring quantitative effects? What are the differences between dominant and recessive R genes? Rice *(Oryza sativa* L.) and its bacterial blight (BB)pathogen. *Xoo* present an excellent opportunity for addressing many of the above questions. Nevertheless, the issue prompted us to investigate qualitative resistance using near isogenic lines with pyramids of R genes as a valuable mterial for detailed chracterization of the R genes and the use of double haploid population to analyze the quantitativecomponent.

5.1 Virulence analysis of the bacterial leaf blight *(Xoo)* isolates

Plants and pathogen co-evolve over long periods, human interference through breeding and management practices disturbed this balance and exerted on the pathogen to change, either by mutation or by genetic recombination. Over 30 avirulence genes have been cloned from bacterial plant pathogens. Before cloning, the existence of the genes was inferred by the differences in interactions (compatible or incompatible) of individual pathogen strains with a host plant. The genes are detected in members of a single pathogen species or pathovar by inoculation to sets of *host differentials* (host plant lines or cultivars with different resistance genes). An individual pathogen strain may have multiple avirulence genes, and the combination of *avr genes* within a particular strain specifies the physiologic *race* of the strain (Stakman and Piemeisel, 1917.). Thus, the *avr* genes impose race-specificity on a pathogen that is otherwise compatible in association with a given plant species. Within a species or pathovar, avirulence genes hypothetically are the most recent within a species or pathovar, avirulence genes hypothetically are the most recent genetic variations in the evolutionary adaptation process between the host and pathogen. However, plant pathogenic bacteria generally exist as complexes of related species or pathovars that are adapted to different plant species. Presence of avirulence genes was spaculated in the individul isolate based on the inability of the individual isolate to cause infection. Host specific isolates were referred to as verticle pathotypes. Some sort interplay between the host *(O. sativa)* and the pathogen *(Xoo]* leading to recognition was inferred from the hypersensitive / highly resistance response of the host genotypes with known genes for resistance. As consequence, avirulence genes may be involved in the recognition of related pathogens in non-host plant species.

ıbility of plant disease resistance *(R)* genes may be predicted if the cost of iaptation to overcome resistance is understood. Adaptation of the bacterial ogen, *Xoo,* to virulence in rice is the result of the loss of pathogen gene function. In the present investigation it was observed that the isolates ılitative and quantitative behaviour in their pathogenic capabilities.

Hopkins *et al.,*(1992) have reported Races of *Xoo,*the causal agent of bacterial blight of rice, interact with cultivars of rice in a gene-for-gene specific manner. Multiple DNA fragments of various sizes from all strains of *X. o.* pv. *oryzae* hybridized with avrBs3, an avirulence gene from *Xanthomonas campestris pv. vesicatoria*, in Southern blots; which suggests the presence of several homologs and possibly a gene family. They further suggested that *avrXa7* and *avrXa10* are members of an avirulence gene family from xanthomonads that control the elicitation of resistance in mono- and dicotyledonous plants.

Bai *et al.* (2000) studied the role of avr genes in one component of pathogen fitness, i.e., aggressiveness or the amount of disease *X. oryzae* pv. *oryzae* causes in susceptible rice lines and reported that some, but not all, avr family members contribute to pathogen aggressiveness and that the contributions are quantitatively different. Furthermore, despite their sequence similarity, the aggressiveness functions of these gene family members are not interchangeable. The results suggest that selection and pyramiding resistance genes can be guided by the degree of fitness penalty that is empirically determined in avr gene mutations.

In agreement to our findings, variation in the spectrum of virulence among different strains has been reported has been reported by many workers (Bhopkar *et al.,* 1960; Denny *et al.,* 1988; Kauffman and Pantulu, 1972; Reddy and Ou, 1976). Reddy and Reddy, 1989; Trimurthy *et al.,* 1993 has also reported the pathotypes variation among the Indian isolates.

5.2 Efficacy of different gene and gene combinations against *Xoo.* isolates.

5.2.1 Genotypes with no known gene for resistance

In the present investigation Mahsuri, Swarna, and Pant 4 , ARBN 6(IR 64), ARBN 31 (Karuna) were either with no gene or carried unidentified gene(s) for resistance against Abo out of which ARBN 31, Mahsuri and Pant 4 showed resistace response against 8,7 and 7 isolates tested and showed susceptibility against rest of the isolates. The two varieties, Swarna and Mahsuri exhibited resistance against 5 and 6 cultures, respectively, out of 10 cultivars tested. These two lines are very popular in

Andhra Pradesh, Orissa and Madhya Pradesh and were released for cultivation in 1982 as blight resistant varieties. (Khush *et al.,* 1989). The reaction of these two varieties (Swarna and Mahsuri) against the different isolates were different from each other and different from rest of the other genes. Mahsuri is one of the parents of Swarna, however differential reaction of these two lines indicate the presence of at least one other resistant gene. Their unique reaction compared to other known genes and gene combination indicates the presence of different gene than other included in the study but further needs to be confirmed. Resistance response presented by Pant -4 still needs to be further evaluated for reconfirmation.

5.2.2 **Near isogenic lines (one gene for resistance)**

With the use of improved varieties with major gene resistance increases, and intensive management and changed carrying pattern, breakdown of existing resistance will become a major problem. Vertical resistance to *Xoo* is controlled by at least 20 major genes that are usually race specific (Zhang, 1991; Causse *et al*., 1994; Kinoshita, 1995; Zhang *et al.,* 1996; Lin *et al*., 1996) and some of these have been incorporated into modern rice varieties. For example the exploitation of gene *Xa-4*, present in IR-20, has played an important role in protecting rice from *Xoo* (Khush *et al*., 1989). However, extensive cultivation of varieties with *Xa-4* has resulted in significant shifts in race frequency of *Xoo* (Mew *et al.,* 1992), and as an outcome in many areas of Indonesia, India, China and Philippines, *Xa-4* can no longer impart resistance against *Xoo* (Huang *et al.,* 1997). It is therefore imperative to constantly test the effectiveness of individual genes of a certain location.

Our experimental material consisted of 26 genotypes, each carrying different gene(s) for resistance to *Xoo.* Among the twelve single genes studied, ***Xa-23***, ***Xa-7***, ***Xa-10***, and ***Xa-11*** showed resistance response against only few isolates out of fourteen isolates of *Xoo* tested. Similar results of susceptibility of ***Xa-10***, and *Xa-11* have also been reported by Thri Murty *et al,* 1993; Sridhar *et al,* 1999. These three genes i.e. *Xa-10, Xa-11* and ***Xa-14*** convey high resistance to a few Japanese or Philippines races of BB pathogen. These genes are considered to confer typical qualitative resistance. (Ogawa and Khush, 1989).

Similarly the two genes *Xa-23* and *Xa-7* exhibited compatible reaction with 11 and 10 different isolates of *Xoo,* which indicated the overall ineffectiveness of these resistance genes. Where as *Xa-1*, *Xa-4* and *Xa-14* showed compatible reaction against 8 out of 14 isolates and showed differential interaction against the *Xoo* isolates.

Resistance in Varich Kogyku, Tetap and Java 14 with *Xa-1* exhibited of differential reaction to Philippines and Japanese races (Ogawa and Khush, 1989). *Xa-1* was reported to be resistant to one of the isolate used by Thri Murty *et al.,* 1993, Saify 2000 at Raipur. In agreement to our findings Li *et al,* 1999, has also reported the susceptibility of *Xa-4.* However, the level of infestation was different for different genes. This is expected if these defeated resistance gene acts as QTL against the virulent strain *of Xoo.* Li *et al.* 1999 has reported that *Xa-4* is susceptible to three isolates tested, but nevertheless this gene acted as a recessive QTL with a significant residual effect against CR-6 isolate. The mutant avirulent locus of isolate (pathogen) leads to the breakdown of resistant gene however this is apparently accompanied by corresponding penalties for their fitness.

The present investigation indicates that the genotypes ARBN-107(*Xa-3*); ARBN-121(*xa-5*); ARBN-128(*Xa-21*); ARBN-123(*Xa-8*); ARBN-126(*xa-13*) showed resistance/ incompatibility against a number of isolates indicating a wider spectrum of resistance. The resistance of xa-8, originally derived from PI 231129, also confirmed the resistance to Philippines race 1-3 and to all the Japanese races (Ogawa and Khush, 1989). However, this gene exhibited differential reaction at Raipur (Thri Murty *et al.,* 1993, Saify 2000). The multi-locational trials of different cultivars have confirmed the presence of more virulent strains in India as compared to other Asian countries. (Sheshu, 1988). *xa-13*, another recessive gene derived from BJ-1 confirm resistance against more number of isolates tested. The two recessive genes xa-8 and *xa-13* were reported to be resistant by Sridhar *et al.,* 1999, Saify 2000. A contradictory to result to the generalized report that *Xa-21* derived from *O. longisteminata* against all the races worldwide, very surprisingly was defeated by some of the isolates and has also been reported by Saify 2000.

The recessive gene *xa-5* seems to be bent among the single resistance genes tested. It imparts resistance to 7 cultures of*Xoo,* in one background. This has also been reported to be resistant by a number of other works (Ogawa and Khush, 1989; Trimurti *et al.* 1993; Sridhar *et al.,* 1999, Saify 2000) against a number of Philippine, Japanese and Indian races.

5.2.3 **Two gene pyramids**

Near isogenic lines with pyrimided R genes were used in the present investigation which included 6 lines with two gene combination, 2 lines with three gene combinations and one line with four gene combinations. All the gene combinations showed resistance response against most of the isolates. The near isogenic lines with two gene pyramids **ARBN-129(*Xa-4+xa-5),* ARBN-131(*Xa-4+ Xa-21),* ARBN-134(x*a-13+ Xa-21)*** were identified as the most effective gene combination against the isolates tested. The pyramid line **ARBN-130 (*Xa-4+Xa-13),* ARBN-132 (*Xa-5+xa-13),* ARBN-133 (*xa-5+Xa-21)*** showed resistance response against 6, 9 and 9 isolates respectively out of 14 isolates tested. The potential value of gene combination has often been demonstrated (Schafer *et al.,* 1963; Samborshi and Dych, 1982, Ezzahini and Roelfs, 1989; Kolmer *et al*., 1991; Huang *et al.,* 1997; Sridhar *et al,* 1999).

5.2.4 Three gene pyramids

The three gene combination in ARBN 135(*Xa-4+xa-5+xa-13*)was found to be most effective and showed resistant reaction against most of the isolates except isolate #4" *#5"* #6"and #7". Similarly *Xa-4+xa-5+Xa-21* in ARBN-136 imparted resistance against most of the isolates except isolate # 12,4,4",6",7". Individually the three genes present in ARBN-135 and ARBN-136 showed differential reactions against isolates This combination was also reported to be resistant (Huang *et al,* 1997 and Sridhar *et al,* 1999, Saify 2000).

5.2.5 **Four gene pyramids**

The four genes combination in ARBN-137 (*Xa-4+xa-5+xa-13+Xa-21*)showed resistance response except isolate # 6 and 7. No highly resistance response or

hypersensitive response was observed as expected. Two and three gene combinations were more effective and showed increased level of resistance as compared to the resistance response evaluated individually. Li *et al.,* 2001 The observed susceptibility of pyramid lines might be due to the more virulent strain used for testing the material. The Indian races are more virulent than the races found in rest of Asia. (Sheshu, 1988).

5.2.6 Epistatic effects of gene(s) in combinations

First, the reaction of a plant to a race of its pathogen reflects the outcome of the interactions between alleles at all avirulence loci in the pathogen and alleles at all R loci of the plant, even if there is a one-to-one relationship between each of the interacting *R-avirulence* gene pairs. Also, the reaction is determined by the rate in which the plant defensive responses are triggered through interactions between alleles at plant R loci and alleles at the corresponding avirulence loci in the pathogen. The faster the response rate is, the more likely a resistant phenotype is to arise. *Thus, it was not surprising that more pronounced genotype (rice) _ race Xoo interactions were observed at digenic or trigenic levels than that at the monogenic level in the present study.* Our results indicated that the two components of rice resistance to *Xoo* are associated with the properties of all the R genes studied. *The qualitative component of the R genes was reflected by their large effects against the corresponding avirulent Xoo races and secondly their effects may vary to some extent depending on the pathogen races and the host genetic backgrounds, even in the incompatible cases.* The variation in the resistance response i.e. from HR to R even in incompatible interactions reflects the quatitative behaviour of those R genes which do not happen to show large / main effects resulting into hypersensitive responses against some of the virulent isolates.

The quantitative component of the R genes *(Xa-4, xa-5,* and *xa-13*) appeared to have two elements, the main effects and epistatic effects. Li *et al.* (1999) reported that the breakdown of *Xa-4* caused by a Chinese *Xoo* race, CR6, involves loss of its dominance and a 50% reduction in gene effect.

The results suggest some important differences among the *R* genes. For instance, the fact that the two dominant *R* genes act independently and additively

implies that they might function in different pathways of the rice defensive system. Their larger residual effects against the virulent pathogen races suggest a more complex structure of these dominant *R* genes. This is consistent with the recent findings that most dominant plant *R* genes (including *Xa-21* and *Xa-1*) represent complex gene families, each consisting of several member genes of potentially different functions (Song *et al.* 1995; Wang *et al.* 1996; Yu *et al.* 1996; Yoshimura *et al.* 1998). For instance, member D (*Xa-21D*) of the *Xa-21* gene family is known to confer partial resistance to *Xoo* (Wang *et al.* 1996). In contrast, the more pronounced epistatic role and race specificity of *xa-13* may suggest a relatively simple structure and possible regulatory role of this recessive *R* gene in the more upstream of the rice defensive system. Thus, molecular cloning of *R genes* would greatly enhance our understanding of the defensive system of rice plants.

5.3 Identification of QTLs contributing resistance towards bacterial blight of rice

Some forms of plant disease resistance are genetically simple and have been analysed extensively by traditional methods of plant pathology breeding, and genetics (Flor 1955, Hulbert & Michelmore 1985., Jorgensen & Moseman 1972, Nelson & Ullstrup 1964., Pitblado *et al.*, 1984.). Horizontal resistance is quantitative, presumably nonspecific, and controlled by polygenes (Van der Plank1968; Nelson 1972), though this assumption has not been actively tested. Genetically complex forms of disease resistance by contrast, are more poorly understood (Geiger & Heun1989). In the past, classical quantitative genetics provided the tools for studying complex disease resistance (Falconer1989.). However, quantitative genetics is unsuited for dissecting polygenic resistance characters into discrete genetic loci or defining the roles of individual genes in disease resistance. An effective approach for studying complex and ploygenic forms of disease resistance is known as "Quantitative Trait Locus" (QTL) mapping, which is based on the use of DNA marker (Tanksley SD.1993.). With QTL mapping, the roles of specific loci in genetically complex traits can be described, and fundamental questions that have vexed researchers in the field of plant pathology for decades can be addressed.

Two types of resistance to *Xoo,* vertical and horizontal have been recognized in rice (Zhang and Mew 1985). Vertical resistance to *Xoo* is controlled by at least 24 major genes that are usually race specific (Kinoshita 1995; Lin *et al.,* 1998). However, resistance of a segregating rice population to specific *Xoo* strain often shows both qualitative and quantitative components (Koch and Parlevliet 1991). This characteristic of the relationship between *O. sativa* and *Xoo* offers a unique opportunity to study the genetics of the interaction between host plants and their pathogens.

Both the parent showed resistance phenotype against all the isolates, however the segregating populatin exhibited resistance as well as susceptibility, it indicates that both the parents had resistance as well as susceptibe. Nine, 12, 1 and 3 QTL's were mapped against 4", 5", 6", 7" respectively, which were largely responsible for segregation of the resistant phenotype in the double haploid population All the QTLs identified were identified as isolate specific. QTLs identified on Ch 5,6" were specific for isolate No 7". The QTLs with a significant additive effect on reducing lesion length against the avirulent strains (4"&5") and virulent strains (6"&7") explains, at least in part a durable resistance. Most resistance loci identified in this study do show some degree of strain specificity. The number of identified resistance QTLs is fairly large and none of the *four Xoo* strains interact consistantly with all the QTLs, it is expected that cumulative effect of multiple resistance QTLs may result in significant level of durable quantitative resistance to *Xoo.* Present observation on QTL analysis indicate that with isolate 4 and 5 more number of QTLs were identified where as with 6"&7" few QTLs were identified suggesting for a stabilizing selection may be acting on the rice-*Xoo* relationship.

5.3.1 Evidence for "stabilizing Selection"

The Co-evolution of many plant pathogen relationship may be governed by stabilizing selection(Van der Plank 1963, 1968; Leonard and Czochor 1980); and these author state that new pathogenic race(s) will suffer a loss in general fitness when they acquire new virulance genes by mutation. As a result, pathogenic strains with more virulance genes tend to have lower fitness where as isolate 4"&5" showed resistance

rection against several of the gene(s) and their combination and regarded as more fit in terms of having more number of *avr genes.* Where as genetic changes at different *avr* locus in isolate 6"&7" leads to the reduced effect or "breakdown of different resistance loci which are accompanied by corresponding panalties to their fitnes. (Li *et al.,* 1999). This together with the similar results for the avirulance genes *avrXa-7* and *avrXa-10* in *Xoo* reported by White *et al.* (1995), appears to lend support to Van der Plank's theory and suggest that "stabilizing selection" may be operating on the rice-*Xoo* relations. The present observation suggests that many double haploid populations that are highly resistant to virulent strain can be recovered from the cross between two susceptible parents. It is further speculated that cumulative effect of QTLs along with residual effect of defeated major resistance genes can lead to high level of durable resistance.

Race specific QTLs might be playing an important role in determining Host-Pathogen interaction to become fully compatible from the initial compatible state to the fully compatible state; just like the subsidiary notches of the key fitting into a lock, thus further strengthening the high level of resistance to *Xoo.*

In the genetics of host pathogen interaction, a long-standing controversial issue is the nature of the genetic basis of " Stabilizing selection" which largely determines the co-evolution of many plant host -pathogen relationship (Van der Plank 1963, 1968; Leonard and Czochor 1980). This theory states that the pathogenic race(s) will suffer a loss in general fitness when they acquire new virulence genes by mutation. Genetically, this implies that conversion of avirulence genes to corresponding virulence genes by mutation is expected to result in lower fitness of the pathogen. Unfortunately, direct evidence to support this theory has been difficult to obtain.

5.3.2 **The relationship between QTLs with similar genomic positions for different diseases**

In the present investigation one of the QTL was identified at the same chromosomal location at which the *Xa-4* is mapped based on its position (Table---- Fig____). On the chromosome 11 where the *Xa4* a major gene for BLB resistance is inferred based on the map position QTL for sheath blight was also identified (Fig____) using the double haploid population (Personal Communication). Similarly on

chromosome 8 at the same chromosomal location QTL for Brown spot was also identified using the same population (Fig_____)(Personal Communication).

Xa-4, a major gene confering resistance was mapped between RZ 536 and G 2132b on choromosome 11 with extremely high LOD value (Li *et al.,* 1999). This gene was inferred to be *Xa-4,* based on its map position (Causse et al. 1994) and previous studies on the resistance of several lines related to Teqing (Lin and Ming 1990; Wu *et al,* 1991).Identification of QTLs and a close correspondance in both genomic location for resistance against differet diseases in other populations has also been reported by (Li et al. 1995, Tabien et al. 1998; Tabien et al. 1998; Causse et al. 1994). Such close correspondance in both genomic location and gene action between genes / QTLs conferring resistance to different pathogens is unlikely to be due to chance. Thus, all these results appear to lend support to the postulate that major genes resistance may have a residual effects against different races of the same pathogens, or different pathogens (Martin and Ellingboe 1976; Royer et al. 1984; Li et al. 1999. Secondly disease resistance loci in tomato, rice Soybean tend to exist in clustered multigene family (Thilmony et al. 1995; Song et al. 1996; White et al. 1995; Yu et al. 1996; Kanazn et al. 1996). It can therefore be speculated that major resistance genes or QTLs at similar genomic location may reflect functional differences in member genes within clusters of rsistance gene families. Some QTLs not associated with known major resistance genes may represent loci of unidentified resistance gene families or their regulatory loci. Detailed molecular characterization of these resistance gene families will certainly shed light on some un answered questions like are genes that control race-nonspecific resistance the same as "defeated" race-specifics genes, and are partial resistance genes race specific? What sort of interactions exists between resistance genes, plant development, and the environment? Are any cloned defense response genes (Bowles DJ.1990.) the same as partial resistance genes? Moreover, genetic mapping the DNA markers makes it possible to ask whether homologous resistance genes exist in related plant taxa.

Finally, QTL mapping will eventually provide as entry point for the most ambitious goal of all-cloning partial resistance genes known only by small and continuous effects on phenotype.

5.4 Study of inheritance of different *Xa* gene(s) in breeding populations for resistance to bacterial leaf blight of rice

Control of plant disease by the development of disease-resistant plant varieties is the most efficient and environmentally friendly way to control disease, as long as sources of resistance are available. Disease resistance controlled by *R* genes will continue to be used far into the future. Resistance genes are unique in that they have evolved to control many different defense responses, but to trigger these defenses only where and when they are necessary, minimizing the physiological costs to the plant. Qualitative resistance to *Xoo* is controlled by 24 major genes Kinoshita 1995; Lin et al., 1998) conferring resistance to various races of the pathogen have been identified and utilized in rice breeding programs. Screening of F2 and F3 population derived from various crosses did not segregated as per the Mendelian ratio and the crosses were inferred as failure. In view of the present investigation more number of crosses needs to be attempted and screened.

Summary, Conclusions & Suggestion for furture Research work

CHAPTER VI

SUMMARY, CONCLUSIONS AND SUGGESTION FOR FURTURE RESEARCH WORK

Resistance in rice to *Xoo* is known to have both qualitative and quantitative components. Recently, near-isogenic lines (NILs) with pyramids of the *R genes* have been developed through molecular marker-aided backcross breeding, providing valuable materials for detailed characterization of the *R genes* (Huang *et al.* 1997; Sanchez *et al.* 2000). Rice (*Oryza sativa* L.) and its bacterial blight (BB) pathogen. *Xanthomonas oryzae* pv. *oryzae (Xoo)* present an excellent opportunity to address questions related to to have both qualitative and quantitative resistance. The present investigation was carried out with the identification of virulence spectrum of the isolates used and speculating for the presence of avr genes in the isolates, followed by analysing the behavior of the *R genes* in the NILs and Pyramids. Epistatic behavior of the genes in combination were analyzed by comparing the responses of the genotypes have gene and gene combinations against *Xoo.* The genes that control quantitative disease resistance in plants have long been too difficult to identify or characterize precisely. Still, the need for better forms of disease resistance in agriculture, especially those that hold the promise of long-term durability, calls out to plant pathologists, breeders, geneticists, and molecular biologists to turn their attention to polygenic resistance phenotypes. Before the advent of QTL mapping, analyzing the genes that control complex disease resistance traits was an overwhelming task. With DNA markers and QTL mapping, complex forms of disease resistance and their underlying genes are now far more accessible. The double haploid population was evaluated to identify the quantitative component of resistance in rice. Someday soon, the distinction between qualitative and quantitative disease resistance may finally disappear.

6.1 Virulence analysis of the bacterial leaf blight **(*Xanthomonas oryzae*** pv. *oryzae)* isolates

Within a species or pathovar, avirulence genes hypothetically are the most recent genetic variations in the evolutionary adaptation process between the host and pathogen in the present investigation.

Variation in the spectrum of virulence among different strains was observed.Presence of avirulence genes was speculated in the individual isolate based on the inability of the individual isolate to cause infection. It was spaculated that some sort interplay exists between the host *(O. sativa)* and the pathogen *(Xoo)* which determines the host specificity. Host specific isolates were referred to as verticle pathotypes. In the present investigation it was observed that the isolates showed qualitative and quantitative behaviour in their pathogenic capabilities.

6.2 Efficacy of different gene and gene combinations against *Xanthomonas oryzae* pv. *oryzae*. isolates.

The present investigation indicates that the genotypes :- Mahsuri, Swarna, and Pant 4, ARBN 31 (Karuna), ARBN-107(*Xa-3*); ARBN-121(*xa-5*); ARBN-128 *(Xa-21);* ARBN-123(*Xa-8*); ARBN-126 (*xa-13*) **ARBN-129 (*Xa-4+xa-5),*** ARBN - 131 *(Xa-4+* ***Xa-21),*** **ARBN-134 (*Xa-13+Xa-21)*** ARBN 135 *(Xa-4+xa-5+xa-13*) to be most effective gene and gene combinations showing wider spectrum of resistance The four genes combination in ARBN-137 (*Xa-4+xa-5+*x*a-13+Xa-21*) showed resistance response except isolate # *6* and 7. No highly resistance response or hypersensitive response was observed as expected.

The faster the response rate is, the more likely a resistant phenotype is to arise. Thus, it was not surprising that more pronounced genotype (rice) _ race (Xoo) interactions were observed at digenic or trigenic levels than that at the monogenic level in the present study.

- Our results indicated that the two components of rice resistance to *Xoo* are associated with the properties of all the R genes studied. *The qualitative component of the R genes was reflected by their large effects against the corresponding avirulent Xoo races and secondly their effects may vary to some*

extent depending on the pathogen races and the host genetic backgrounds, even in the incompatible cases.

- The results suggest some important differences among the *R genes.* For instance, the fact that the two dominant *R genes* act independently and additively implies that they might function in different pathways of the rice defensive system. Their larger residual effects against the virulent pathogen races suggest a more complex structure of these dominant *R genes.*

6.3 Identification of QTLs against *Xanthomonas oryzae* pv. *oryzae*

Characteristic of the relationship between *O. sativa* and *Xoo* offers a unique opportunity to study the genetics of the interaction between host plants and their pathogens both qualitatively and quantitatively.

Segregation of the resistant phenotype in the double haploid population was inferred due to the presence of Nine, 12, 1 and 3 isolate specific QTL's. The QTLs with a significant additive effect on reducing lesion length against the avirulent strains (4"&5") and virulent strains (6"&7") explains, at least in part a durable resistance.

Present observation on QTL analysis indicate that with isolate 4 and 5 more number of QTLs were identified where as with 6"&7" few QTLs were identified suggesting for a stabilizing selection may be acting on the *rice-Xoo* relationship. It was spaculated that race specific QTLs might be playing an important role in determining Host-Pathogen interaction to become fully compatible from the initial compatible state.

In the present investigation one of the QTL was identified at the same chromosomal location at which the *Xa-4* is mapped based on its position (chromosome11) Similarly on chromosome 8 at the same chromosomal location QTL for Brown spot was also identified using the same population It was therefore speculated that major resistance genes or QTLs at similar genomic location may reflect functional differences in member genes within clusters of rsistance gene families. Some QTLs not associated with known major resistance genes may represent loci of unidentified resistance gene families or their regulatory loci.

6.4 Study of inheritance of different *Xa* gene(s) in breeding populations for resistance to bacterial leaf blight of rice

Screening of F2 and F3 population derived from various crosses did not segregated as per the Mendelian ratio and the crosses were inferred as failure. In view of the present investigation more number of crosses needs to be attempted and screened .

Suggestion for furture Research work

Durability of plant disease resistance *(R)* genes may be predicted if the cost of pathogen adaptation to overcome resistance is under-stood. Adaptation of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), to virulence in rice is the result of the loss of pathogen avirulence gene function, but little is known about its effect on aggressiveness under field conditions.

In view of the above statement it will be more precise to answer some of the question whch still remains un answered and thus requires a thorough investigation

Are genes that control race-nonspecific resistance the same as "defeated" race-specific genes? Is partial resistance genes race specific? What sort of interactions exist between resistance genes, plant development, and the environment? Are defense response genes the same as partial resistance genes? Moreover, genetic mapping with DNA markers makes it possible to ask whether homologous resistance genes exist in related plant taxa. **The reaction of a plant to a race of its pathogen reflects the outcome of the interactions between alleles at all avirulence loci in the pathogen and alleles at all R loci of the plant. How this interplay determines the qualitative and quantitative relationship between rice *(O. sativa)* and the pathogen (*Xoo*)?.**

Abstract

"Study of Host Plants Resistance Genes and gene combinations against *Xanthomonas oryzae* pv. *oryzae* model system "

By

Tirlochan Singh

ABSTRACT

Resistance in rice to *Xoo* is known to have both qualitative and quantitative components. There are at least 24 major genes that confer resistance to *Xoo* and follow a gene-for-gene relationship in rice (Kinoshita 1995; Lin *et al.,* 1998). The qualitative resistance in many plant-pathogen relationships is hypersensitive, race specific, and governed by interactions between virulence genes in pathogens and resistance genes in hosts, while the quantitative resistance is non-hypersensitive, presumably non-race specific, and controlled by polygenes (Nelson 1972). The salient features of the current strategies in breeding for bacterial leaf blight resistance are to monitor and anticipate pathotype changes, to locate new resistance genes in rice and related genera, and to deploy pathotype-specific resistance genes in new cultivars. It is important to manage known resistance genes to maximize their effectiveness and durability.

Rice *(Oryza sativa* L.) and its bacterial blight (BB) pathogen *Xanthomonas oryzae* pv. *oryzae (Xoo)* present an excellent opportunity to address many important questions regarding various components of the genes involved in contributing resistance phenomenon in rice against *Xoo*.. For instance, do different R genes act independently or synergistically? Are genes conferring qualitative effects different from genes conferring quantitative effects? What are the differences between dominant and recessive R genes? In the present investigation variation in the virulence spectrum was observed and was speculated due to the presence or absence of avr genes in the pathogen. The behavior of the R genes in the NILs and Pyramids indicated that the faster the response rate is, the more likely a resistant phenotype is to arise. *Thus, it was not surprising that more pronounced genotype (rice) _ race (Xoo) interactions were*

observed at digenic or trigenic levels than that at the monogenic level in the present study.

The genes that control quantitative disease resistance in plants have long been too difficult to identify or characterize precisely. With DNA markers and QTL mapping, complex forms of disease resistance and their underlying genes are now far more accessible. The double haploid population which were evaluated to identify the quantitative component of resistance in rice indicated the presence of isolate specific QTLs which may determine the stabilizing selection in rice-*Xoo* relationship. Screening of F2 and F3 population derived from various crosses did not segregated as per the Mendelian ratio and requires further investigation.

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