MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF BLAST RESISTANT GENOTYPES OF RICE

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CERTIFICATE

This is to certify that the thesis entitled *"MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF BLAST RESISTANT GENOTYPES OF RICE"* submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY*, embodies the result of a piece of bona fide research work carried out by *MD. MOTIUR RAHMAN, Registration No. 00923* under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that any help or sources of information as has been availed of during the course of this inquire have been duly acknowledged and the contents & style of the thesis have been approved and recommended for submission.

Dated:
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Dedicated To

My Beloved Parents

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ABSTRACT

Genetic divergence analysis was done for blast resistant and susceptible genotypes using 13 morphological characters. The genotypes were grouped into seven clusters according to D^2 statistic and Canonical Vector analysis. Plant height, days to flowering, days to maturity, panicle length, number of spikelet/ panicle, number of filled grain/ panicle, number of unfilled grain/ panicle, 1000 filled grain weight(gm), yield/hill(gm) were indicated as important contribution to genetic divergence in 14 rice genotypes. On the basis of cluster distances, high yielding along with highly susceptible (BRRI dhan 29) genotypes could be crossed highly resistant genotypes (BR 6017-3-3-4-1, ZHONG-YU 7) and BRRI dhan 28 and BRRI dhan 36 could be croosed with QING LIALI NO1 for the development of blast resistant rice varieties. A total of 8 microsatellite and 3 minisatellite or VNTR markers were used for studying molecular variability across 14 blast resistant and susceptible rice genotypes. A total of 33 alleles were detected at the loci of 8 microsatellite markers across 14 blast resistant and susceptible rice genotypes. The number of alleles per locus ranged from 2 alleles (RM108) to 5 alleles (RM21, RM80, RM531), with an average of 4.13 alleles across the 30 loci. The Polymorphism Information Content (PIC) values ranged from 0.280 (RM108) to 0.726 (RM21). PIC value revealed that RM21 was considered as the best marker for 14 rice genotypes. The two dimensional graphical view of Principal Coordinate Analysis (PCoA) for blast resisitant genotypes showed the genotypes ZhongYu7, OM1207, SIPI692033, BRRI dhan29, BRRI dhan36, QINGLIALI1, IR 60913-42-3-3-2-2 and NJ70507 were found placing far away from the centroid of the cluster and rest of the genotypes were placed more or less around the centroid. The genetic similarity analysis using UPGMA clustering system generated nine genetic clusters with similarity coefficient of 0.66 for the study with blast disease resistance. The pair-wise genetic dissimilarity coefficients indicated that the highest genetic distance was obtained between NJ70507 with BR36 & BR29 for blast disease. In order to develop blast resistant varieties and broaden the genetic base of rice varieties new breeding program should initiated preferably using the parents, BRRIdhan29 and NJ70507 and BRRIdhan36 and NJ70507 and to create genetic variability among these two modern rice varieties.Both molecular and morphological data did not show the similar results. So, use of more molecular markers might be resolved the results of morphological analysis.

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LIST OF ABBRIVIATION TERMS

AFLP = amplified fragment length polymorphism

BRRI = Bangladesh Rice Research Institute

bp = Base Pair

CVA = Canonical Vector Analysis

DNA = Deoxyribo Nucleic Acid

dNTP = Deoxynucleotide Triphosphates

EDTA = Ethylene Diamine Tetraacetic Acid

INGER = International Network for Genetic Evaluation of Rice.

IRBN = International Rice Blast Nursery

Kb = Kilo base pair

mM = Mili molar

PCA = Principal Component Analysis

PCO = Principal Co-ordinate Analysis

PCR = Polymerase Chain Reaction

PIC = Polymorphism Information Content

RAPD = Random Amplified Polymorphism DNA

RFLP = Restriction Amplified Length Polymorphism

rpm = Rotation Per Minutes

SNP = Single Nucleotide Polymorphism

SSR = Simple Sequence Repeats

TBE = Tri Boric Acid EDTA

UPGMA = Unweighted Pair Group Method with Arithmetic Means

USA = United States of America

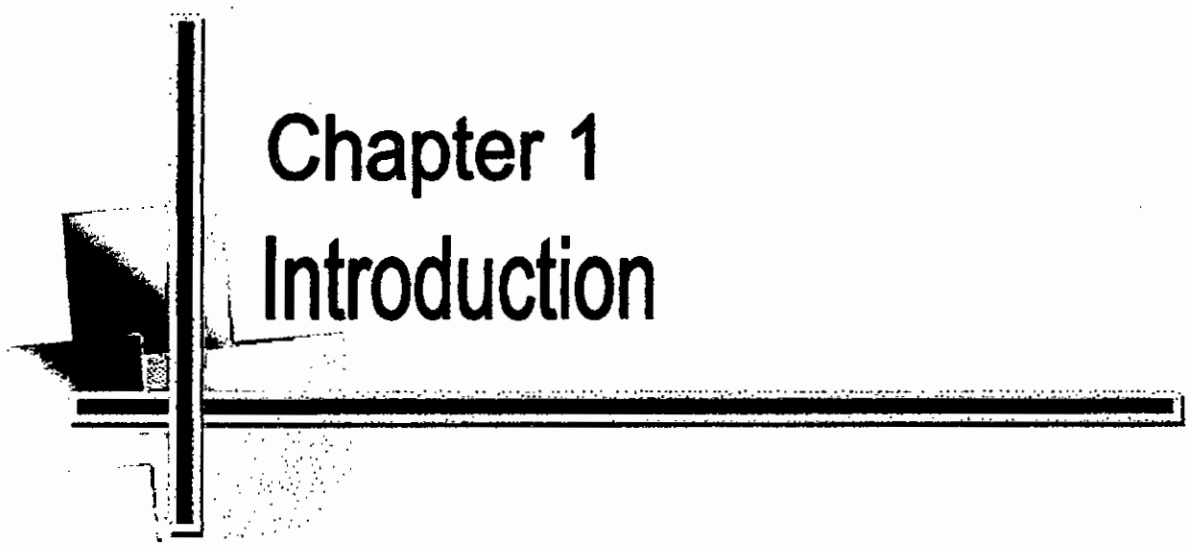
UV = Ultra Violate

UVPRO= Vipro Platinum

VNTR = Variable Number Tandem Repeats

λ = Lambda

μl = Microlitre



Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of more than 50% of the world's population (Zheng *et al.*, 1995). About 40% of the world's population consumes rice as a major source of calorie (Banik, 1999). In Bangladesh the rice is extensively cultivated over a large area and it covers 74% of the total calorie intake of the people of Bangladesh. About 10.77 million hectares (mha) of land is used for rice cultivation, which produces 25.18 million metric tons of rice (BBS, 2003).

Rice crops suffer from a number of diseases. In Bangladesh a total of 32 diseases have been identified of which ten are considered as major (Latif *et al.*, 2007a). Among these diseases, rice blast caused by *Pyricularia grisea* is one of the most important widely distributed plant disease (Ou, 1985). Blast was first reported in Asia more than three centuries ago and is now present in over 85 countries. It is highly adaptable to environmental conditions and can be found in irrigated lowland, rain-fed upland, or deepwater rice fields (Rao *et al.*, 1994).

Blast is a disease of major concern in Bangladesh (Mia *et al.*, 1985). Outbreaks began appearing from 1973, especially on modern varieties. There is a yearly variation in blast incidence in different Agro Ecological Regions (AER) and there is cycle of severe outbreaks of blast in the country every 4-5 years. Two of the most recent outbreaks of blast occurred in Bangladesh in 1980 and 1990. The cultivars grown, such as BR1, BR11, IR8, IR50, Pajam and some local varieties were susceptible in both the leaf and neck blast stages (Shahjahan *et al.*, 1991).

Rice blast causes significant crop losses through out South East Asia and South America. Major epidemics covering vast areas occur on a regular basis causing

severe food storage to entire nations. Yield loss estimates from other areas of the world have ranged from 1-50%. Rice blast causes between 11% and 30% crop losses annually. This represents a loss of 157 million tones of rice. Rice blast is estimated to cause production losses of US$55 million each year in South and Southeast Asia. In India, upto 75% yield loss has been recorded due to blast disease (Padmanabhan, 1965b).

Now-a-days, use of chemicals is discouraged to save the environment. Therefore, emphasis has given on the host plant resistance which is economically viable and environment friendly technique for disease management. In Bangladesh, many resistant sources against blast pathogen have been isolated from many improved varieties and local germplasm at Bangladesh Rice Research Institute (Ahmed *et al*., 1985).

Blast resistant rice varieties are available but this resistant is often either partial or controlled by a dominant single gene which is therefore inherently unstable to an onslaught by a genetically variable pathogen (McCouch *et al.*, 1994). Geographical variability of the blast pathogen has been extensively studied (Chen *et al.*, 1995, Kumar *et al.*, 1999, Mekwatanakern *et al.*, 2000) in various countries and several effective methods have been developed for molecular analysis of population biology using Restriction Fragment Length Polymorphisms (RFLP) in the mitochondrial genome, transposable elements or other repetitive regions (Levy *et al.*, 1991, George *et al.*, 1997, Bridge *et al.*, 1997).

Genetic diversity is the essential to meet the diverse goals of plant breeding such as producing cultivars with increasing yield, genetic adoption, desirable quantity, pest and disease resistant (Nevo *et al.,* 1982). Quantitative classification offers a quantified degree of divergence among genotypes or populations, this serve as a sound basis of grouping any two or more genotypes based on minimum divergence between them (Sharma, 1997).

Although resistance to blast is often short-lived, some cultivars are considered to possess durable resistance (Jhonson, 1981). Durable resistance is thought to be associated with partial resistance that is in many cases under ooligo-or polygenic control (Higashi and Saito 1985; Wang *et al.,* 1994). For example, the rice cultivar Monoberekan displays durable resistance to blast in upland conditions (Bidaux 1978; Ahn 1994; Fomba and Taylor 1994).

The use of molecular markers has facilitated studies of the genetic basis of this durable blast resistance (Wang *et al.*, 1994; Chen *et al.,* 1997; Inakai *et al.,* 1997). Molecular markers technology allows us to dissect the complexity of resistance in durable resistant cultivars (Chen *et al.*, 1999; Wang *et al.,* 1994; Naqvi and Chattoo 1996), and facilitate introgression of genes associated with durable resistance. Several molecular markers such as SSR, RFLP, AFLP and RAPD are presently available to asses the variability and diversity at molecular level (Joshi *et al.*, 2000). These molecular markers are used within a diverse collection of rice (*Oryza sativa*) accessions and to determine the genetic diversity (Junjian *et al.,* 2002).

Molecular marker technology offers a possibility by adopting a wide range of novel approaches to improve the selection strategies in rice breeding. It also provides information that can help to define the distinctiveness of germplasm and their ranking according to the number of close relatives and their phylogenetic position (Khan, 2006).

OBJECTIVES

- To know the genetic diversity and selection of resistant genotypes in rice breeding on the basis of morphological characters against rice blast disease.
- To know the genetic variability of rice blast using molecular markers.

Chapter 2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

2.1 Occurrence and distribution

Rice blast had been causing damage in South Carolina as early as 1876, and regarded as one of the most serious eight rice diseases recorded in the USA in 1907 (Metcaf, 1907). He was perhaps the one who first called the disease 'blast' in the English language.

Hashioka (1950a) postulated that, in eastern Asia, the prevalence of blast in the temperate regions is conditioned solely by the relation of temperature to the growth of blast fungus; in the sub temperate regions it is controlled by temperature and also by the relation of the age of the plant to resistance; in the subtropical and tropical regions, prevalence is governed mostly by factors relating to the resistance of the host.

Padmanabhan (1965b) mentioned that the disease was first recorded in India in 1913 and a devastating epidemic occurred in 1919 in the Tanjore delta area of Tamil Nadu. According to the CMI (1981) blast disease has been reported from almost 85 rice growing countries of the world. Losses due to blast disease may be ranged up to 90%, depending upon the part of the plant infected.

Ahmed and Hossain (1985) reported that 11.84, 4.0, 25.23, 20.01, 10.01 and 5.10% rice plants were infected by blast disease in Dhaka, Jessore, Dinajpur, Rangpur, Comilla and Pabna districts respectively during 1983-84 while 4.13 and 14.67% blast infection were found during 1984-85 in Dhaka and Jessore districts respectively.

2.2 Symptoms and Causal Organism

Rice blast caused by *Pyricularia grisea* is a principal disease of rice due to its worldwide distribution and destructiveness. *P. grisea* infects rice at various stages of growth and inflicts losses in yield. Typical leaf blast symptoms are elliptical leaf sports with more or less pointed ends, gray or whitish centers, and brown or yellow margins. Early infections can lead a complete destruction of seedlings in susceptible cultivars. Lesions remain as pinhead, brown specks on resistant varieties. Nodal blast causes the rotting of the sheath pulvinus and breaking of the culm at the infection node. The area of the panicle can also be infected causing neck or panicle blast through the formation of brown to black lesions on the panicle, panicle branches, and glumes (Ou, 1985; Bhatt and Singh, 1991).

2.3 Favorable condition for blast disease development

Hemmi and Abe (1941) denoted that when the rice seedlings are grown under different soil temperature, infection occurs most severely on those grown at 20^0 C, less at 24^0C and least at 28^0C. On contrary, seedling blight of germinating seedlings increases with soil temperature from 20 to 32^0 C.

Hashioka (1950a) stated that time of planting is an important factor in blast development, early planting in Japan usually having less disease than later plantings. This is explained by the fact that in early planting the air temperature is too low at the tillering stage and too high at the heading stages for vigorous disease development.

Zeigler *et al.* (1994) cited that blast is favored by excessive nitrogen fertilization, aerobic soils and drought stress. High nitrogen rates and nitrate nitrogen increase rice susceptibility to the disease. Ammonium nitrogen is converted to nitrate when fields are drained and aerated. This may explain why rice is more susceptible on non flooded aerated soil. Extended drain periods

encourage the disease by aerating the soil, converting ammonium to nitrate and by causing drought stress to rice.

2.4 Morphological characterization

Mahajan *et al.* (1988) demonstrated the genetic diversity of rice (D^2 statistics) for 11 characters related to yield in 60 cultures of rice developed from 14 crosses involving 23 parents. The 60 cultures were grouped into 18 clusters. Mostly the cultures in a cluster came from the same cross. They stated that geographical diversity was associated with identified genetic diversity. High yield component and multiple resistance can be utilized as parents in future rice breeding program.

Genetic variability simple correlations and path co-efficient of eight quantitative characters in 80 *indica* rice varieties, including HYV and indigenous high quality rice in two environments each at two locations during rainy season were studied by Chauby and Richharia(1993). Statistical analysis was done based on character means on pooled data. A wide range of variation was recorded for most of the characters. Heritability in broad sense was very high for all the characters except harvest index. Spikelets per panicle and plant height exhibited high heritability coupled with high genetic advance. Grain yield/ plant showed significant positive correlation with plant height, panicle length, spiketets per panicle, panicle weight and test weight. Path analysis indicated a greater contribution of panicle weight to grain yield.

Kanwal *et al.* (1993) studies on 100 strains using Mahalanobis's D^2 statistics and canonical analysis revealed that panicle weight number of days to maturity, height and grain size contributed most towards divergence. The strains were grouped into nine characters, which were not correlated with geographical diversity.

Vivekanan and Subramanian (1993) used the Mahalanobis's D^2 statistics to assess the nature and magnitude of genetic divergence in 28 genotypes of rainfed rice. The population was grouped into five clusters. Plant height and grain yield contributed considerably, accounting for 85% if total divergence. The geographical diversity has not been found related to genetic diversity.

Mirsha *et al.* (1994) reported that the multivariate analysis of divergence for 7 quantitative traits among 37 strains of *Oryza sativa* groped the genotypes into 5 clusters. The first cluster contained 31 genotypes, number of fertile grains/ panicle, number of sterile grains/ panicle, plant height were the highest contributors of Mahalanobis's D^2 values. On the basis of cluster distances, UPR 485-10-1-1, Basmati 370 and Kamod were identified for utilization in breeding programmes.

Sing *et al.* (1999) mentioned the genetic divergence in rice which was carried out through multivariate analysis with 42 boro genotypes having 11 quantitative characters including grain yield. The genotypes were grouped into four clusters. No relationship between geographic origin and genetic diversity has observed. The four characters, viz. harvest index, total number of grains/panicle, number of fertile grains/panicle and stand ability accounted 90.61% of the total divergence.

Bharadwaj *et al.* (2001) conducted a field experiment in Andra Pradesh, India during the kharif season to classify 50 rice genotypes. Data were collected on 9 quantitative characters: Plant height, days to 50% flowering, number of ear bearing tillers/ plant, panicle length, number of spikelets/ panicles number of filled grain/panicle, number of unfilled grains panicle, 100 grain weight, grain length/ breath and grain yield/ plant. The analysis of variance for means indicated that the differences among the genotypes were significant for all the characters studied. Multivariate analysis indicated that all the 9 characters studied contributed differently to the genetic divergence amongst the

genotypes, length/breath ratio contributed maximum towards the genetic divergence followed by 100 grain weight and grain yield/plant accounting to 47% of total divergence. Tabulated data on the ANOVA for 9 quantitative characters in 50 genotypes of rice and cluster classification identified from dendogram are presented.

Twenty four modern rice varieties of irrigated ecosystem with a view to finding out variability and genetic association for grain yield and its component characters was studied by Iftekharuddaula *et al.* (2001). All the characters tested were showed significant variation among the varieties. Genotypic correlations co-efficient were higher than the corresponding phenotypic correlation co-efficient in most of the traits. Days to flowering, days to maturity-grains/panicle, 1000 grain weigh and harvest index showed significant positive correlation with grain yield. Path analysis revealed that higher of grains/panicle, bold grains, more panicles/m^2 and higher harvest index had positive and higher direct effect on grain yield.

Hossain and Haque (2003) mentioned that both genotypic and phenotypic variances found highly significant in all the traits with little higher phenotypic variations as usual. Similarly the low differences between the phenotypic and genotypic co-efficient of variations indicated low environmental influences on the expression of the characters. High heritability coupled with high genetic advance of yield, grains per panicles days to flowering and height suggested effective selection for the improvement of these characters could be made. Direct and indirect effects of these characters through path co-efficient analysis supported the significant positive correlation co-efficient at both genotypic and phenotypic levels for of plant height, panicles per hill, panicle length and 1000 grain weight on yield. Thus selection on yield in rice through theses characters will be effective.

2.5 Varietal Resistance

Certain rice cultivars are resistant to blast in the leaf stage and to be susceptible to neck rot at the later stage of growth, and, conversely, cultivars susceptible to leaf blast show little or no neck blast (Hashioka, 1950a). Such observations have led to the believe that resistance to leaf blast and neck rot are not correlated and that different genes are involved. Many experiments have therefore been made in breeding programmes to test for resistance to neck blast as well as leaf blast.

Ou (1985) denoted that the use of resistant cultivars is the most economically viable and effective way of controlling rice blast but the useful life span of many cultivars is only a few years in disease conducive environments (Lee and Cho, 1990) because of the breakdown of resistance in the face of the high *P. oryzae* pathogenic variability (Ou, 1997; Bonman *et al.*, 1986) and the breeding of cultivars with more durable resistance has become a priority in rice improvement programs.

Lee (1989) mentioned that resistance is considered durable when it remains effective in a cultivar despite wide spread cultivation in an environment favoring the disease. Durable resistance may be controlled by a single gene, multiple genes with cumulative effects or poly genes, and the resistance produced may be either complete or incomplete (partial). Several rice cultivars with durable blast resistance have been identified (Bonman and Mackill, 1988), and some up land cultivars such as the traditional African cultivars "Moroberekan" and "OS6" have been cultivated for many years in large areas of West Africa without high losses from blast (Notteghem, 1985). These plants have been used as resistance donors in breeding programs. Major resistance genes have been successfully used for developing blast resistance cultivars (Khus, 1989) and several dominant resistance genes have been identified which confer complete blast resistance (Kiyosawa, 1981).

2.6 Molecular characterization

Bonman (1992) Major gene resistance can be deployed either to prevent the blast fungus from easily adapting or to minimize selection pressure on the blast pathogen (*P. grisea*). These objectives can be accomplished by pyramiding conventional blast resistance genes to generate cultivars with durable blast resistance, but phenotypic selection cannot be used to pyramid resistance because the presence of one major gene obscures the effect of other genes. Molecular markers linked to major blast resistance genes offer a powerful tool for marker-aided indirect selection of resistance loci in gene-pyramiding strategies. Random amplified polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) have been used to construct genetic maps and for the molecular tagging of various agronomic traits in various crop species (O'Brien, 1990; Williams *et al.*, 1993) and a number of blast resistance genes have been mapped relative to tightly linked RAPD and RFLP markers (Naqvi *et al.*, 1995; Naqvi and Chattoo, 1996).

Genetic diversity is commonly measured by genetic distance of genetic similarity both of which imply that there are either differences or similarities at genetic level (Weir, 1990). Availability of a large number of polymorphic markers enables precise classification of the cultivars. Several molecular markers viz. RFLP, RAPD, SSRs, ISSRs and SNPS are presently available to assess the variability and diversity at molecular level (Joshi *et al.*, 2000).

Coburn *et al.*, (2002) were conducted an experiment to design and application of Microsatellite Marker panels for Semi automated genotyping of 12 rice. The objective of this study was to develop a systematic and flexible method for assembling multiples simple sequence repeat (SSR) marker panels for high throughout genome analysis in rice, and to the these panels on a set of cultivated rice germplasm. On a standard set of 13 genetically diverse cultivars of markers detected and average of five alleles per locus and gad a mean PIC value of 0-67.

Ali *et al.*, (2003) used SSR marker to determine the genetic relationship of popular rice cultivars. Fifty SSR markers were used to assess the genetic diversity among popular rice cultivars from Iran. Estimation of gene diversity of the 16 core breeding lines was 0.440-0.028 based on SSR markers. Genetic relationship among the cultivars was determined by cluster analysis using SSR markers. Both molecular and phenotypic data represent a narrow genetic basis in local and improved cultivars in Iran and the need for including more diversity for the breeding program. Local Iranian cultivars showed high degree of polymorphism.

To estimate genetic diversity in traditional and Evolved Basmati (EB) and semi dwarf Non Basmati (NB) rice varieties Nagaraz *et al.*, 2002 assed an experiment by using SSR markers. A subject of three rice groups was analyzed by using 19 simple sequences repeat (SSR) loci and 12 inter SSR- PCR primers. A total of 70 SSR alleles and 48 inter SSR-PCR markers were revealed in 24 varieties from the three groups. The lowest genetic diversity was observed among the traditional Basmati varieties. The EB varieties showed the highest genetic diversity by both the marker assays. The results indicated that the subset of aromatic rice varieties is probably derived from a single land race.

Eighty one SSR markers were used to estimate genetic diversity of 94 japonica rice using 81 SSR has been assessed (Suh-Jung Pil *et al.*, 2004). All 81 SSR markers generated a total of 351 alleles. Six of 81 SSR markers showed monomorphic bands in 94 Japonica rice. But several SSR markers on chromosomes 4, 9 10 and 11 produced many alleles within the japonica rice. Japonica rice was classified into six major groups by the clustering analysis.

Chakravarthi *et al.* (2006) investigated the genetic diversity and DNA fingerprinting of 15 elite rice genotypes using 30 SSP primers on 7-12 chromosome numbers. The results revealed that all the primers showed district polymorphism among the cultivars indicating the robust nature of microsatellites in revealing polymorphism. Cluster analysis grouped the rice

genotypes in to 10 classes in which japonica types D11-1 (Azucena) and Moroborekan clustered separately from indica types. Principal component analysis was done to vizualize genetic relationships among the elite breeding lines. The larger range of similarity values for related cultivars using microsatellite provides greater confidence for the assessment of genetic diversity and relation ships.

Determination of the genetic diversity represented by accessions of rice and to identify DNA markers that might be useful in identifying hybrids between red and cultivated rice. Seventy nine red rice accessions 10 known or putative hybrid derivatives of red rice and cultivated rice (C hybrids) and seven rice cultivars were analyzed using microsatellite DNA markers developed for cultivated rice (Gealy *et al.*, 2002).

AFLP and SSR analyses were performed using 95 cultivars of local and modern sake-brewing rice together with 76 cultivars of local and modern cooking rice. Their analysis showed that the genetic diversity in sake-brewing rice cultivars was much smaller than the diversity found in cooking rice cultivars. Cluster analysis and chloroplast haplotype analysis suggested that the local sake-brewing cultivars originated monophyletically in the western regions of Japan (Hashimoto *et al.*, 2004).

Genetic diversity were assessed among Indian aromatic and quality rice (*Oryza sativa*) germplasms using 30 fluorescently labeled rice microsatellite markers. The 69 rice genotypes used in that study included 52 Basmati and other scented/quality rice varieties from different parts of India and 17 indica and japonica variaties that served as controls. A total number of alleles were 235 at the 30 SSR loci, 62 (26.4%) of which were present only in Basmati and other scented/quality rice germplasm accessions. The results indicate that Indian aromatic and quality germplasm is genetically distinct from other groups within *O. sativa* and is the product of a long independent pattern of evolution (Jain *et al.*, 2004).

Chapter 3

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site:

The study was conducted at the experimental field and molecular laboratory of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, during the period of 2007-2008.

3.2 Genotypes:

A total of 14 genotypes including blast resistant and susceptible genotypes were used in the study. Description of the genotypes is given in Table-1.

Table-1: Name of the genotypes

SL. No	Variety	Source	Reaction Type
1.	BR 6017-3-3-4-1	IRBN, INGER	Resistant
2.	IR 45912-9-1-2-2	IRBN, INGER	Resistant
3.	ZHONG-YU 7	IRBN, INGER	Resistant
4.	OM 1207	IRBN, INGER	Resistant
5.	NR-11	IRBN, INGER	Resistant
6.	IR 60913-42-3-3-2-2	IRBN, INGER	Resistant
7.	SIPI 692033	IRBN, INGER	Resistant
8.	QING LIALI NO1	IRBN, INGER	Resistant
9.	NJ 70507	IRBN, INGER	Resistant
10.	BR 14	BRRI	Resistant
11.	BR 16	BRRI	Resistant
12.	BRRI dhan28 (BR28)	BRRI	Moderately Resistant
13.	BRRI dhan29 (BR29)	BRRI	Highly Susceptible
14.	BRRI dhan36 (BR36)	BRRI	Moderately Susceptible

BRRI= Bangladesh Rice Research Institute

IRBN= International Rice Blast Nursery

INGER=International Network for Genetic Evaluation of Rice.

3.3 Germination of Seed:

Seeds of all collected rice genotypes soaked separately for 48 hours in clothes bag. Soaked seeds were picked out from water and wrapped with straw and gunny bag to increase the temperature for facility.

3.4 Phenotypic Data:

The seedlings of 14 genotypes were raised in earthen pots. Then thirty days old seedlings were transplanted in the field. Recommended doses of fertilizers were applied. Cultural practices were done as and when necessary. Data were recorded on 13 quantitative characters namely, number of tiller/hill, tillering ability, plant height (cm), phenotypic acceptability, days to flowering, days to maturity, panicle length (cm), number of spike let / panicle, number of filled grain/panicle, number of unfilled grain/panicle, 1000 filled grain weight (gm), yield/ hill (gm) and disease index.

3.5 Analysis of data:

Mean data of the characters was analyzed by multivariate analysis using Genstat 5 Fifth Edition (Beta) software program. Genetic diversity analysis involves several steps, i.e., estimation of distance between the varieties clustering and analysis of inter-cluster distance. Therefore, more than one multivariate technique is required to represent the results more clearly and it is obvious from the results of many researches (Bashar 2002, Uddin 2001, Ariyo 1987and Patil *et al.,* 1987).

3.6 Principal Component Analysis (PCA)

Principal components were computed from the correlation matrix and genotype scores obtained from first components (which have the property accounting for maximum variance) and succeeding components with latent roots greater than unity (Jeger *et al.*, 1983) and contribution of the different morphological characters towards divergence are discussed from the latent vectors of the first two principal components. To divide the varieties of a data set into some

number of mutually exclusive groups clustering was done using non-hierarchical classification. The algorithm is used to search for optimum values of chosen criterion. Starting from some initial classification of the varieties into required number of groups, the algorithm repeatedly transfers varieties from one group to another so long as such transfer improve the value of the criterion the algorithm switches to a second stage which examines the effect of swapping two varieties of different classes and so on.

3.7 Principal Coordinate Analysis (PCO)

Principal coordinate analysis is equivalent to PCA but it is used to calculate inter unit distances. Through the use of all dimension of P it gives the minimum distance between each pair of the N point using similarly matrix (Digby *et al.*, 1989).

3.8 Canonical Vector Analysis (CVA)

Canonical vector analysis (CVA) complementary to D^2- statistic is a sort of multivariate analysis where canonical vector and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively and derived. Canonical vector analysis finds linear combination of original variability than maximize the ratio of between groups to within groups variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus in this analysis a series of orthogonal transformation sequentially maximize the ratio of among groups to within group variation.

3.9 Computation of average intra-cluster distances

The average intra cluster distance for each cluster was calculated by taking all possible D^2 values within the members of a cluster obtained from PCO.

The formula used to measure the average intra-cluster distance was:

Intra-cluster distance = $\sum D^2/n$

Where

D^2 is the sum of distances between all possible combinations (n) of the genotypes included in a cluster.

The square root of the D^2 values represents the distance (D) within cluster.

3.10 Genotypic data

Plant materials

Fourteen Blast resistant genotypes including susceptible checks were selected from the rice germplasm collection at the Bangladesh Rice Research Institute (BRRI) gene bank and International Rice Blast Nursery (IRBN), INGER for this study. Five grams of germinated seed from each genotype was sown in the pot. (Table 1)

3.11 Reagents preparation for DNA extraction (stock solution):

DNA extraction Buffer

Stock solution	Concentration required	Volume added for 100 ml
1 M Tris. HCl pH 8.0	50 mM	5 ml
0.5M EDTA,pH 8.0	25 mM	5 ml
5 M Nacl	300 mM	6 ml
10% SDS	1%	10 ml
Sterile distilled water	--	74 ml

0.5M EDTA, pH 8.0

For 250 ml: 46.53 g of EDTA was weighed out into a beaker and was added about 200 ml of distilled water. Again about 5g of NaOH was added and stirred for 10 minutes. pH meter was placed in the solution and continued to slowly add NaOH pellets while stirring until the powder was dissolved and the pH reached 8.0. 250 ml volume was made and stored in bottle and was sterilized by autoclaving

10% SDS

Simply, 10% SDS i.e. 10g SDS was added in 100ml water. It was stirred until dissolved. SDS is hazardous, so the mixture was not autoclaved.

1M Tris. HCl, pH 8.0

For 500ml: 60.6 g of Tris base was weighed out into a beaker. 30 ml of distilled water was added and stirred until dissolved. While stirring and monitoring pH and slowly hydrochloric acid was added until pH fall to 8.0. Volume was made up to 500 ml with distilled water. After that it was placed in a bottle and was sterilized by autoclaving.

5M NaCl

For 100 ml: 29.22g of sodium chloride was weighed out and was added about 75 ml of water and stirred to dissolve. Volume was made up to exactly 100ml. Then it was placed in bottle and was sterilized by autoclaving.

70% ethanol

Ethanol and sterile distilled water were mixed in 70:30 proportions in a clean and sterile bottle e.g. for 100 ml, mixed 70ml of ethanol with 30 ml of sterile distilled water.

Chloroform-Isoamyl alcohol (24:1) (100ml)

Four milliliter of Isoamyl alcohol was added in 96 ml chloroform

Sterile Distilled water

Sterile distilled water was placed in extremely clean bottles and was sterilized by autoclaving.

DNA confirmation using gel electrophoresis

Preparation of 1.5% agarosegel (200 ml)

- 200 ml of 10 x TBE (electrophoresis buffer) was taken in a flask and 3 g of agarose was added to it.
- The mixture was then kept in micro oven cooked for 5 minutes to dissolve it.
- The gel was kept in room temperature for 10-15 min to cool down at tolerable level.
- The gel was then poured into gel tank carefully.
- Meanwhile two combs were placed on the gel.
- Within 30 minutes the gel was solidified.
- The gel was submerged in to 1x TBE buffer
- The combs were removed from the gel
- The gel then ready for loading the DNA samples.

3.14 Use of λ (Lambda) DNA marker

λ (lambda) DNA was used for quantification of DNA concentration as 5µl. It was worth while to mention that 5µl DNA contains 25ng/µl DNA. Three microliter 2x loading dye was mixed with the 7µl DNA sample of each genotype. Then total DNA sample (10µl) was loaded in the 1.5% agarose gel in the gel tank, λ (Lambda) DNA was loaded in a well as known DNA concentration marker. The electrophoresis machine was run for 2 hr at 100 volts. Two colors appeared after few minutes. The separation was monitored by the migration of the dye in the gel when the first dye (bromo-plenol-blue) had reached two third of the gel length, then the power supply was cut off and the gel was stained with ethidium bromide. After staining the gel was taken out carefully from the electrophoresis chamber and placed on high performance ultraviolet light box for checking the DNA bands. The DNA was visualized as band and photographed using a gel Doc.

3.15 Use of Molecular Markers

A total of eight Microsatellite or Simple sequence repeat (SSR) markers and three minisatellite or VNTR (Variable Number Tandem Repeats) markers with clear amplifications were selected for variability study of 14 blast resistant rice genotypes (Table 2).

3.16 VNTR- PCR PROTOCOLS

The Following PCR components were used per reaction (15.0 µl volume) for VNTR analysis:

6ng of DNA template (1.5µl), 0.2 µl 0f 5 U/ul Taq polymerase enzyme, 0.9 µl of 1 pmol/µl primer, 0.15 µl of 0.2 mM dNTPs, 1.6 µl of 1x PCR buffer (200 mM Tris- HCl, 500 mM KCl, Gelatin 0.01% and H_2O), 2.0 µl 0f mM $MgCl_2$ and 8.65 µl nano-pure Sterilized H_2O. PCR was initiated by a denaturation step at 94°C for 2 minutes then the reaction was subjected to 35 cycles of 94°Cfor 20 seconds 45°C for 30 seconds, 72°C for 2minutes with a final extension step of 5 minutes at 72°C.

3.17 SSR-PCR Protocols

The following PCR (Polymerase Chain Reaction) components were used per reaction (15.0 µl volume) for SSR analysis:

18 ng of DNA template (3.0 µl), 0.3 µl of 5 U/ul Taq polymerase enzyme, 0.7 µl of 5 µM forward primers, 0.7 µl of 5 µM reverse primers, 0.3 µl of 0.2 mM dNTPs, 1.5 µl of 1x PCR buffer (200 mM Tris-HCl, 500 mM KCl, Gelatin 0.01% and H_20), 1.8 µl of 3 mM $MgCl_2$ and 6.7 µl nano-pure sterilized H_2O. PCR was initiated by a denaturation step at 94°C for 5 minutes then the reaction was subjected to 35 cycles of 94°C for 30 seconds 55°C for 1 minute, 72°C for 1 minute with a final extension step of 7 minutes at 72°C.

The PCR was carried out in an Appendrof thermocycler using 25 well plates. Amplification products were resolved by polyacrylamide (8%) gel

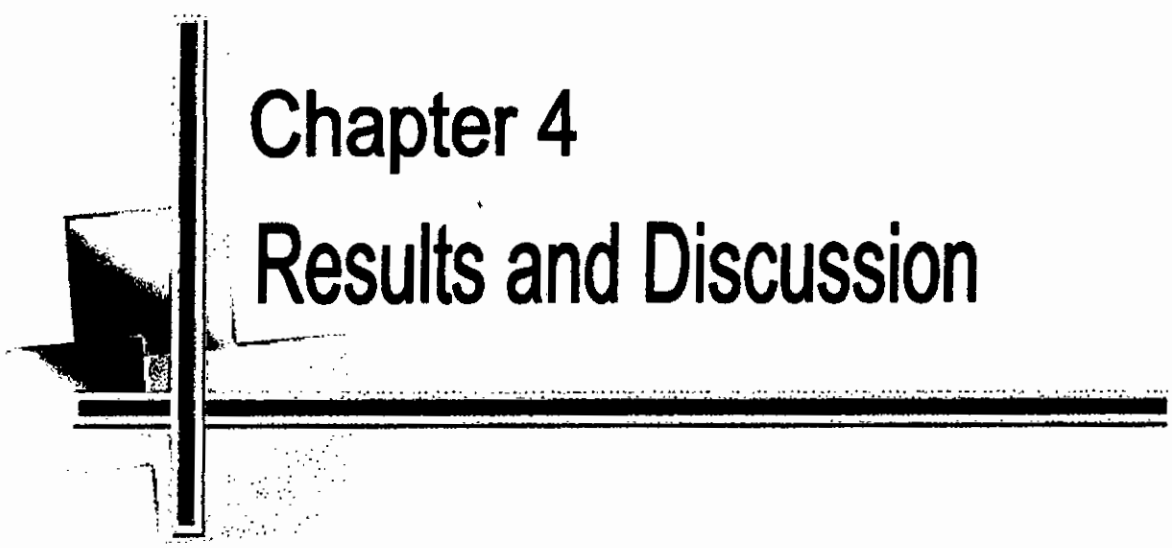
electrophoresis using in 1X TBE buffer. 3 µl samples were loaded in polyacrylamide gel. Each gel was run for 3 hours at 100 volts. Gels were stained in ethidium bromide.

3.18 Documentation of the DNA samples

After staining the gel chamber was placed on the UV Transilluminator in the dark chamber of the Image Documentation System. The UV light of the system was switched on and the image was viewed on the monitor and was documented using UVPRO (Uvipro Platinum, EU) gel documentation unit and saved in the computer.

3.19 Data analysis

Molecular weight for each amplified allele was measured in base pair using Alpha-EaseFC 5.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, Polymorphism Information Content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). The genetic distance was calculated using the "Nei 1983" distance as implemented in Power Marker. The allele frequency data from Power Marker was used to export the data in binary format (allele presence = "1" and allele absence = "0") for analysis with NTSYS-pc version 2.1 (Rohlf, 2002). A similarity matrix was calculated with the Simqual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method as implemented in NTSYS-pc. The similarity matrix was also used for principal coordinate analysis (PCoA) with the D Center, Eigen, Output, and MXP lot subprograms in NTSYS-pc.



Chapter 4
Results and Discussion

CHAPTER 4

RESULT AND DISCUSSION

4.1 Morphological Characterization

The result of the genetic diversity of 14 blast resistant and susceptible rice genotypes are represented in table 2 to 7 and figure 1 on the basis of morphological characters.

4.1.1 Principle component analysis

The principle component analysis was yielded eigen values of each principle component axes of ordination of genotypes with the first axes totally accounted for the variation among the genotypes, while five of these with eigen values above unity accounted for 90.57 %. The first three principal exes accounted for 78.72% of the total variation among the 13 characters describing 14 blast resistant and susceptible rice genotypes (Table 2).

4.1.2. Construction of scatter diagram

Based on the values of principal component score I and II obtained from the principal component analysis, a two dimensional scatter diagram using component score I as X axis and component score II as Y axis was constructed, which has been presented in figure 1. The positions of the genotypes in the scatter diagram were apparently distributed into seven groups, which indicated that there exists considerable diversity among the genotypes. The scattered diagram for blast resistant and susceptible rice genotypes of different clusters revealed that the genotype number 1, 2, 3, 4, 8, 12 and 14 were distantly located which suggesting more diverged from rest of the genotypes.

Table 2. Eigen values and percentage of variation for corresponding 13 component characters in 14 blast resistant and susceptible rice genotypes

principle component characters	Eigen values	Percentage of total variation accounted for	Cumulative percentage
Number of tiller/hill	5.4042	45.03	45.03
Tillering ability	2.5163	20.97	66.00
Plant height (cm)	1.5259	12.72	78.72
Phenotypic acceptability	0.7964	6.64	85.36
Days to flowering	0.6247	5.21	90.57
Days to maturity	0.5000	4.17	94.74
Panicle length(cm)	0.2907	2.42	97.16
Number of spikelet/panicle	0.2331	1.94	99.10
Number of filled grain/ panicle	0.0680	0.57	99.67
Number of unfilled grain/ panicle	0.0249	0.21	99.88
1000 filled grain weight(gm)	0.0139	0.12	100.00
Yield/hill(gm)	0.0018	0.02	100.02
Disease index	0.0000	0.00	100.02

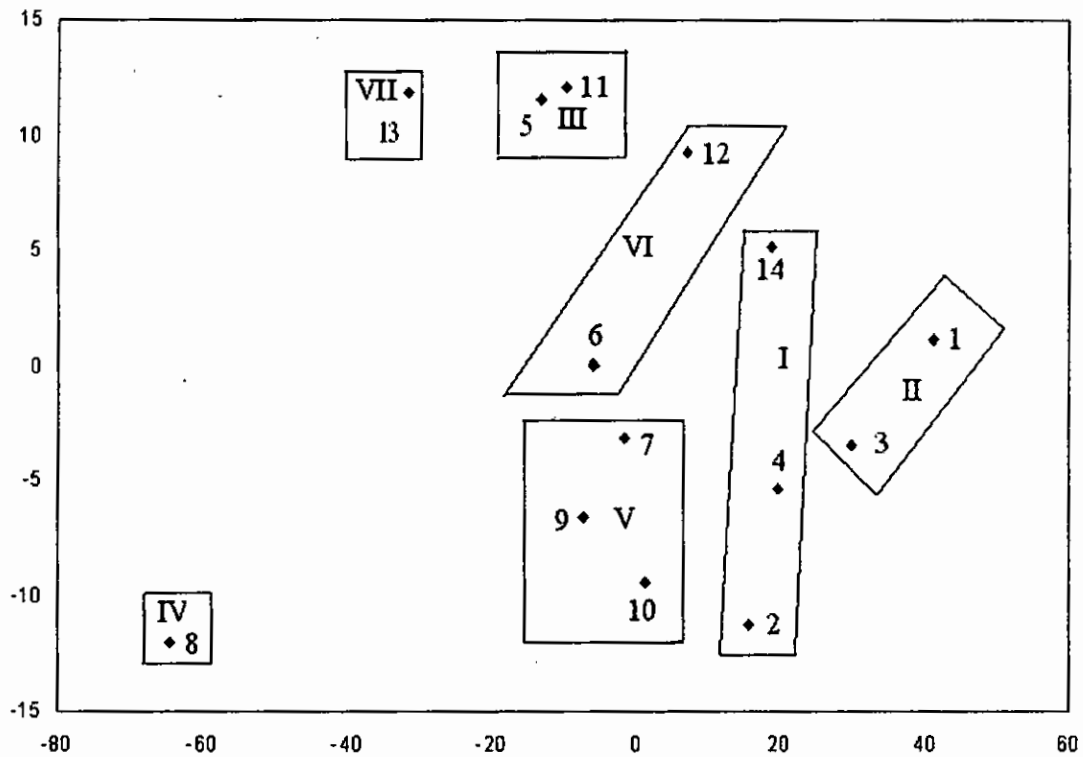


Figure 1. Scattered distribution of 14 Boro rice genotypes based on their principal component scores superimposed with clustering.

4.1.3. Principal Co-ordinate Analysis

Principal co-ordinate analysis (PCO) was performed on auxiliary of principal co-ordinate analysis. This analysis helps in estimating distances (D^2) for all possible 91 combinations between pairs of genotypes. The highest inter genotype distance 106.80 was observed between the genotypes BR 6017-3-3-4-1 and QING LIALI followed by ZHONG-YU 7 and QING LIALI NO1(95.70), QING LIALI NO1 and BRRI dhan36(85.50), OM 1207 and QING LIALI NO1(84.89). The tenth highest pair distance was 67.94 that observed between genotypes QING LIALI NO1 and BR14. The lowest inter genotype distance (12.57) was observed between the genotypes ZHONG-YU 7 and OM 1207. The tenth lowest pair distance 17.78 was observed between the genotypes IR 60913-42-3-3-2-2 and BRRI dhan28.

The difference between the highest and lowest inter genotypes distance indicated the prevalence of variability among the 14 blast resistant and susceptible genotypes of rice (Table 3).

The intra-cluster distances were computed by the values of inter genotypic distance matrix of PCO according to Sing and Chaudhaury (1985). In the present study it was found that clusters I and V composed of largest number of genotypes (3) (Table 5). The intra-clusters distances in all the seven clusters were more or less indicated the genotypes within the same cluster were closely related. The highest intra-cluster distance was computed for cluster II (19.870) composed of two genotypes followed by the cluster I (18.450) composed of three genotypes. The lowest intra-cluster distances were in clusters IV and VII (0.00, 0.00) followed by the cluster V (17.040) consisting of 1, 1 and 3 genotypes respectively. However, the higher value (19.870) of intra-cluster distance in cluster II indicated that the genotypes (2) constituted this cluster might have diverged characters, which contributed to the formation of this cluster (Table 4).

Table 3. Ten of each lower and higher intergenotypic distance (D^2) between pairs of blast resistant genotypes

10 Lower D^2 Values	Genotypic Combination	10 Higher D^2 Values	Genotypic Combination
12.57	ZHONG-YU 7 and OM 1207	106.80	BR 6017-3-3-4-1 and QING LIALI NO1
14.65	SIPI 692033 and NJ 70507	95.70	ZHONG-YU 7 and QING LIALI NO1
15.50	SIPI 692033 and BR14	85.50	QING LIALI NO1 and BRRI dhan36
15.60	OM 1207 and BRRI dhan36	84.89	OM 1207 and QING LIALI NO1
16.18	IR 60913-42-3-3-2-2 and SIPI 692033	80.99	IR45912-9-1-2-2 and QING LIALI NO1
16.77	NR-11 and IR 60913-42-3-3-2-2	75.68	QING LIALI NO1 and BRRI dhan28
17.69	NR-11 and BR16	73.69	BR 6017-3-3-4-1 and BRRI dhan29
17.78	IR 60913-42-3-3-2-2 and BRRI dhan28	67.94	QING LIALI NO1 and BR14

Table 4. Average intra (Diagonal) and inter-cluster distances (D^2) for 14 blast resistant and susceptible rice genotypes

Cluster	I	II	III	IV	V	VI	VII
I	**18.450**						
II	23.095	**19.870**					
III	37.155	50.755	**17.690**				
IV	83.793	108.250	59.585	**0.000**			
V	26.002	41.003	24.555	63.686	**17.040**		
VI	25.492	39.583	22.727	68.380	21.290	**17.780**	
VII	53.843	69.870	27.005	42.300	37.800	37.685	**0.00**

Table 5. Distribution of 14 blast resistant rice genotypes in seven clusters

Clusters	Numbers of Genotypes	Name of Genotypes
I	3	IR45912-9-1-2-2, OM 1207 and BRRI dhan36
II	2	BR 6017-3-3-4-1 and ZHONG-YU 7
III	2	NR-11 and BR16
IV	1	QING LIALI NO1
V	3	SIPI 692033, NJ 70507 and BR14
VI	2	IR 60913-42-3-3-2-2 and BRRI dhan28
VII	1	BRRI dhan29

4.1.4 Canonical variate analysis (CVA)

Canonical variate analysis was performed to obtain the inter-cluster distances (Mahalanobis's D^2 values). These values of inter-cluster distances (D^2) are presented in table 4. Statistical distance represented the index of genetic diversity among the clusters. The inter-cluster distances were bigger than the intra- cluster distances suggesting wider genetic diversity among the genotypes of different clusters. Singh *et al.* (1986) obtained larger inter-cluster distances than the intra- cluster distances in a multivariate analysis in rice.

The inter-cluster distance was maximum between cluster II and IV (108.250) followed by the distance between cluster I and IV (83.793), cluster II and VII (69.870), cluster IV and VI (68.380), while the inter-cluster distance was minimum between cluster V and VI (21.290) followed by the distance between cluster III and VI (22.727)(Table-4). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster IV was far diverged from those of cluster II and I. Similarly, the higher inter-cluster values between

cluster IV and VI, cluster II and VII and cluster IV and cluster V indicated the genotypes belonging to each pair of cluster were far diverged. These relationships were also reflected in the scatter diagram (Figure 1).

The genotypes belonging to the distant cluster could be used in hybridization programme for obtaining a wide spectrum of variation among the segregates. Similar report was also made by Bansal *et al.* (1999), Mokate *et al.* (1998), Kumari and Rangasamy (1997). The genotypes belonging to the clusters II and IV, clusters I and IV having greater cluster distance are recommended for inclusion in hybridization programme for the development of blast resistant varieties as they are expected to produce good segregates. Thus it could be suggested that crosses should be made between genotypes belonging to the distant cluster for higher heterotic response.

Sinha *et al.* (1991) reported that selection of parents from distantly placed clusters exhibited significant high heterosis. Thus heterosis could also be exploited by crossing between genotypes belonging to clusters with moderate diversity like between genotypes of clusters II and VII, clusters IV and VI, clusters IV and V.

4.1.5 Non hierarchical clustering

Non hierarchical clustering using co-variance matrix grouped 14 blast resistant and susceptible rice genotypes into seven different clusters. These results confirmed the clustering pattern of the genotypes obtain through principal component analysis. Cluster analysis in 75 rice genotypes and stated 75 varieties into 10 clusters described by Sawant *et al.* (1995).

The pattern of distribution of genotypes into various clusters is given in Table 5. The distribution pattern indicated that the maximum number of genotypes (3) was found in clusters I and V. Cluster II, III and VI consisted of two genotypes while clusters IV and VII consisted of one genotype.

4.1.6 Intra cluster mean

Intra cluster mean for 13 characters are represented in Table 6. In case of number of tiller per hill, the highest intra- cluster mean (15.00) was recorded in cluster III followed by cluster VI (14.50) and VII (13.00). The lowest intra cluster mean for this trait was observed in cluster V (10.33). Intra cluster mean for tillering ability was 5 in cluster I to cluster VII. Intra cluster mean for plant height was the highest in cluster IV (124.30) followed by cluster V (100.63). The lowest intra cluster mean for this trait was observed in cluster II (86.33) followed by cluster III (87.40). Phenotypic acceptability had the highest group mean in cluster II (4.00) followed by cluster mean (3.00) in cluster I, III, IV, V, VI, and VII. The lowest intra cluster mean for this trait was observed (3.00) in cluster I, III, IV, V, VI, and VII followed by cluster II (4.00). Intra cluster mean for days to flowering was the highest in cluster IV (124.00) followed by cluster VII (121.00). The lowest intra cluster mean for this trait was observed in cluster VI (106.00) followed by cluster II (108.00). Intra cluster mean for days to maturity was the highest in cluster IV (160.00) followed by cluster VII (154.00), cluster V (151.00) and cluster III (150.50). The lowest intra cluster mean for this trait was observed in cluster VI (139.00) followed by cluster II (141.50) and cluster I (142.67). Intra cluster mean for panicle length was the highest in cluster IV (30.00) followed by cluster VII (28.00). The lowest intra cluster mean for this trait was observed in cluster II (20.50) followed by cluster V (21.33), cluster III (22.00). Intra cluster mean for number of spikelet per panicle was the highest in cluster IV (187.00) followed by cluster VII (166.00). The lowest intra cluster mean for this trait was observed in cluster II (116.50) followed by cluster I (129.67). Intra cluster mean for number of filled grain per panicle was the highest in cluster IV (145.00) followed by cluster VII (135.00). The lowest intra cluster mean for this trait was observed in cluster II (93.00) followed by cluster I (103.00). Intra cluster mean for number of unfilled grain per panicle was the highest in cluster IV (42.00) followed by cluster III (37.00). The lowest intra cluster mean for this trait was observed in cluster I (23.33) followed by cluster II (23.50). Intra cluster mean for 1000grain weight was the highest in cluster V (27.00) followed by cluster I (25.65) and cluster IV

(25.20). The lowest intra cluster mean for this trait was observed in cluster VII (23.06) followed by cluster VI (23.20), cluster II (23.26) and cluster III (23.36). Intra cluster mean for yield was the highest in cluster VII (40.47) followed by cluster VI (38.11). The lowest intra cluster mean for this trait was observed in cluster II (31.10) followed by cluster V (31.69). Intra cluster mean for disease index was the highest in cluster VII (7.00) followed by cluster VI (3.75). The lowest intra cluster mean for this trait was observed in cluster II (2.00) followed by cluster III (2.50).

The inter cluster distances of cluster IV and cluster II with other cluster were more or less higher than the inter cluster distances between the remaining cluster combinations (Table-4). The cluster mean of these two clusters for higher tillering ability, higher phenotypic acceptability and lower disease index were divergent. The highest plant height (124.30 cm), days to flowering, days to maturity, panicle length (cm), number of spikelet/panicle, number of filled grain/panicle, third highest for 1000 filled grain weight (gm) was found in cluster IV while in cluster VII had the highest yield/ hill (gm) and disease index. These indicated that the genotypes included in cluster II, cluster IV and VII were very important to contribute into the total divergence among the 14 blast resistant susceptible genotypes for these characters.

Genotypes of cluster II had the highest tillering ability, the highest phenotypic acceptability and lower disease index revealed that the genotypes in cluster could be used to improve blast resistant variety.

The genotypes of cluster IV gave the higher mean for plant height, days to flowering and maturity, panicle length, number of spikelet/ panicle, number of filled grain/ panicle and number of unfilled grain/panicle and lower disease index. The highest cluster means for different characters in cluster IV indicated the genotypes included in this cluster would offer good scope for improvement of rice through rational selection for these characters. The result indicated that the genotypes in this cluster could be used to develop blast resistant variety.

The genotypes of cluster VII produced the higher (third highest) mean for number of tiller per hill, second highest for panicle length, number of spikelets/panicle, number of filled grain/panicle, highest yield/hill (40.47gm) and highest disease index (7.00). The result indicated that the genotypes in this cluster could be used to improve the variety with higher number of tiller per plant, good phenotypic acceptability and high yielding characters.

Table 6. Cluster mean for 13 characters of 14 blast resistant and susceptible rice genotypes

Characters	I	II	III	IV	V	VI	VII
Number of tiller/hill	12.33	12.50	15.00	12.00	10.33	14.50	13.00
Tillering ability	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Plant height (cm)	93.93	86.33	87.40	124.30	100.63	93.30	92.00
Phenotypic acceptability	3.00	4.00	3.00	3.00	3.00	3.00	3.00
Days to flowering	109.33	108.00	111.00	124.00	109.67	106.00	121.00
Days to maturity	142.67	141.50	150.50	160.00	151.00	139.00	154.00
Panicle length(cm)	22.67	20.50	22.00	30.00	21.33	23.00	28.00
Number of spikelet /panicle	129.67	116.50	155.00	187.00	143.67	144.50	166.00
Number of filled grain/ panicle	103.00	93.00	118.00	145.00	113.00	115.00	135.00
Number of unfilled grain/ panicle	23.33	23.50	37.00	42.00	30.33	29.50	31.00
1000 filled grain weight (gm)	25.65	23.26	23.36	25.20	27.00	23.20	23.06
Yield/hill (gm)	32.50	31.10	37.11	36.84	31.69	38.11	40.47
Disease index	3.00	2.00	2.50	3.00	3.00	3.75	7.00

4.1.7 Contribution of characters towards divergence

The characters contributing maximum to the divergence are given greater emphasis for deciding on the cluster for the purpose of further selection and the choice of parents for hybridization (Jagadev *et al.*, 1995).

Contribution of characters towards divergence obtained CVA is presented in Table 7. The values of vectors had positive values for days to maturity, panicle length (cm), number of unfilled grain/ panicle. These result indicated that three characters had the highest contribution towards the divergence among the 14 blast resistant and susceptible genotypes. In vector 1, the important characters responsible for the genetic divergence in the major axis of differentiation were phenotypic acceptability, days to maturity, panicle length (cm), number of unfilled grain/ panicle, disease index having positive vector values while in vector 2 (the second axis of differentiation), plant height (cm), days to flowering, days to maturity, panicle length(cm), number of filled grain/ panicle, number of unfilled grain/ panicle, 1000 filled grain weight(gm) were important. These characters contributed to the total divergence in 14 genotypes of rice.

Variavan *et al.*, (1973) reported that 1000 grain weight and plant height of rice were responsible for primary as well as secondary axis of differentiation. Julfiquar (1985) also reported similar response for yield, 1000 grain weight, days to maturity and plant in rice. Plant height and grain yield considerably contributed to the total divergence reported by Vivcekanondan and Subramaniam (1993). On contrary Chauhan (1994) reported that the contribution of 1000 grain was the highest followed by 50% flowering, panicle length and spikelet per panicle. Singh *et al.* (1999) also reported that harvest index and number of filled grains per panicle contributed maximum to the divergence in rice. Negative values in both vectors for number of tiller per hill, number of spikelet/panicle and yield/hill (gm) indicated this character had the lowest contribution to the divergence.

Table 7. Latent vector for 13 characters of 14 blast resistant and susceptible rice genotypes

Characters	Vectors 1	Vectors 2
Number of tiller/hill	-0.1755	-0.2592
Tillering ability	0.0000	0.0000
Plant height (cm)	-0.0447	0.0551
Phenotypic acceptability	0.7852	-0.5403
Days to flowering	-0.0305	0.0839
Days to maturity	0.1001	0.0602
Panicle length(cm)	0.0050	0.2799
Number of spikelet/panicle	-0.2816	-0.1013
Number of filled grain/ panicle	-0.1201	0.0652
Number of unfilled grain/ panicle	0.0125	0.0073
1000 filled grain weight(gm)	-0.1113	0.0241
Yield/hill(gm)	-0.3363	-0.1846
Disease index	0.0254	-0.0517

4.1.8 Comparison of result based on different multivariate techniques

Results obtain from different multivariate techniques concluded that all techniques gave more or less similar results and one technique supplemented and confirmed the results of the other. The cluster pattern of D^2 analysis through non- hierarchical clustering had been taken care of simultaneous variation in all the characters under study. However the distribution of genotypes in different clusters of the D^2 analysis had followed more or less similar trend of the principal component score 1 and component score 2 of the principal component analysis. The D^2 and principal component analysis were found to be alternative methods in giving the information regarding the clustering pattern of genotypes. Nevertheless the canonical vector analysis

(CVA) provides the information regarding the contribution of characters towards divergence of 14 blast resistant and susceptible rice genotypes.

4.1.9. Selection of genotypes for future hybridization purpose

Genotypes were to be selected on the basis of specific objectives. No common criterion was considered for the selection of genotypes. Genotypically distant parents usually able to produce higher heterosis (Falconer, 1960; Mall *et al*,. 1962; Ramanujam *et al.,* 1974; Ghaderi *et al.,* 1984). Considering magnitudes of genetic distance, contribution of different characters towards the total divergence, magnitude of genetic cluster means for different characters and performance the genotypes were considered for hybridization programme. The genotypes of cluster II could be selected for higher tillering ability higher phenotypic acceptability and lower disease index. The genotypes of cluster IV could be selected for higher plant height, higher panicle length, latest flowering and maturity, higher number of spikelet/panicle, higher number of filled grain/ panicle, higher number of unfilled grain/ panicle, higher (third highest) 1000 filled grain weight. The genotypes of cluster VII could be selected for the highest yield/ hill and higher disease index. The genotypes of clusters I, II and III could be selected for lower number of tillering/hill, earlier flowering and maturity, lower plant height, lower panicle length(cm), lower number of spikelet/panicle, lower number of filled grain/ panicle, lower number of unfilled grain/ panicle, lower yield/hill(gm) and lower disease index. The genotypes of cluster VI could be selected for early flowering and maturity, higher yeild and lower disease index. Genetic distance between cluster II and Cluster IV was higher. So, crosses could be made among the genotypes of above clusters but this is not expected because those genotypes were resistant but lower yielder. Therefore, high yielding along with highly susceptible BRRI dhan 29 genotypes could be crossed with highly resistant genotypes (BR 6017-3-3-4-1, ZHONG-YU 7 and BRRI dhan 28 and BRRI dhan 36 genotypes could be crossed with QING LIALI NO1 for the development of blast resistant along with high yielding rice varieties.

4.2 Molecular characterization

4.2.1 Molecular Markers

A total of eight Microsatellite or Simple sequence repeat (SSR) markers and three minisatellite or VNTR (Variable Number Tandem Repeats) markers with clear amplifications were selected for variability study of 14 blast resistant susceptible rice genotypes (Table 8).

Table 8: SSR and VNTR Primers used for genetic variability study of blast resistant genotypes

Primers	Oligonucleotide sequence
SSR	
RM11	**ATAGCGGGCGAGGCTTAG R** **TCTCCTCTTCCCCCGATC F**
RM17	**GGTGATCCTTTCCCATTTCA R** **TGCCCTGTTATTTTCTTCTCTC F**
RM21	ACAGTATTCCGTAGGCACGG R GCTCCATGAGGGTGGTAGAG F
RM23	CATTGGAGTGGAGGCTGG R GTCAGGCTTCTGCCATTCTC F
RM80	TTGAAGGCGCTGAAGGAG R CATCAACCTCGTCTTCACCG F
RM108	TCTCTTGCGCGCACACTGGCAC R CGTGCACCACCACCACCACCAC F
RM443	**CCAGTCCCAGAATGTCGTTTCG R** **GCGAAGCCCAATCTGAAGAAGC F**
RM531	**CACGTTTCCTTCTTCAGATCATGG R** **GTTCCCACTCATAGTAAACCGATACG F**
VNTR	
MR	GAGGGTGGCGGTTCT
GF	TCCTCCTCCTCCTCC
RY	CAGCAGCAGCAGCAG

F=Forward

R=Reverse

4.2.2 Overall SSR diversity for blast resistant and susceptible genotypes

A total of 33 alleles were detected at the loci of 8 microsatellite markers across 14 rice genotypes. The number of alleles per locus ranged from 2 alleles (RM108) to 5 alleles (RM21, RM80, RM531), with an average of 4.13 alleles across the 30 loci (Table 9). In a study conducted by Hossain *et al.* (2007) with microsatellite markers, the average number of alleles obtained was 4.53 in rice.

The PIC values ranged from 0.280 (RM108) to 0.726 (RM21), were identified at all 8 loci. The frequency of the most common allele at each locus ranged from 28.6% (RM21 and RM531) to 78.6% (RM108). On an average, 46.2% of the 14 rice genotypes shared a common major allele at any given locus. The range of Polymorphism Information Content (PIC) values was from 0.280 to 0.726. The highest PIC value (0.726) was obtained for RM21 followed by for RM11 & RM531 (0.69), RM80 (0.657) and RM23 (0.654), respectively (Table 9). Polymorphism Information Content (PIC) value revealed that RM21 was considered as best marker for 14 genotypes. The results are presented in the figures 2,3,4 and 5 gel pictures of some SSR and VNTR markers for all 14 genotypes.

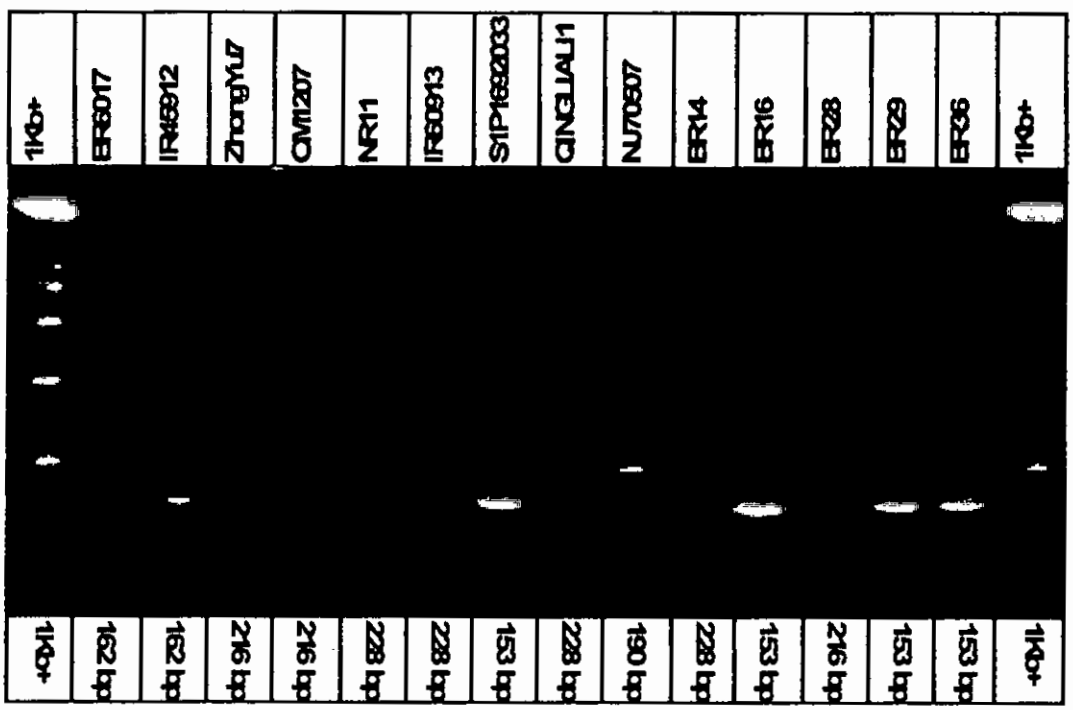


Fig. 2. DNA profile of the 14 blast resistant and susceptible genotypes with the microsatellite marker RM21

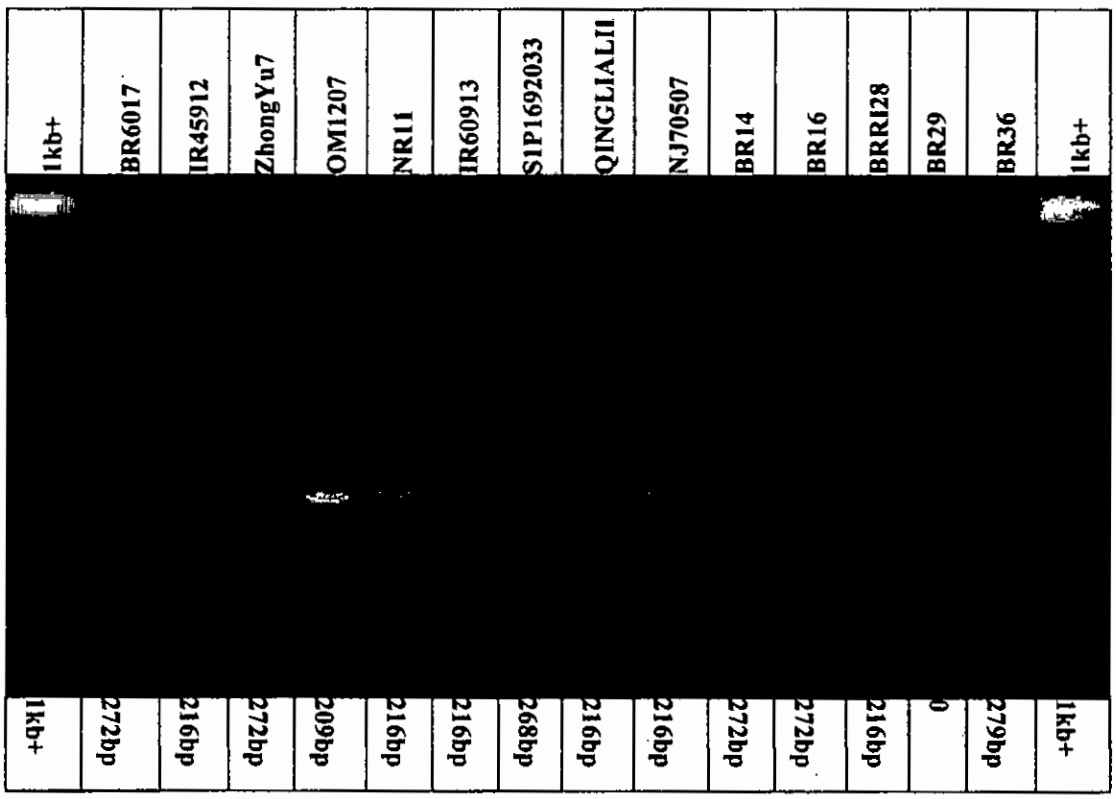


Fig. 3. DNA profile of the 14 blast resistant and susceptible genotypes with the microsatellite marker RM17

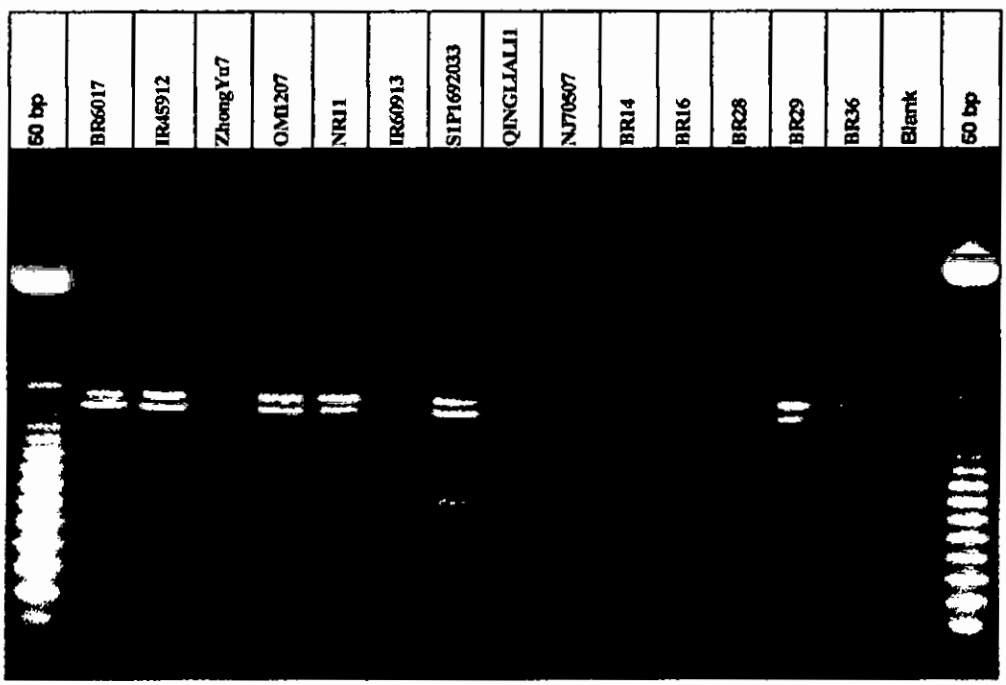


Fig. 4. VNTR banding patterns obtain from blast resistant and susceptible genotypes using MR primer

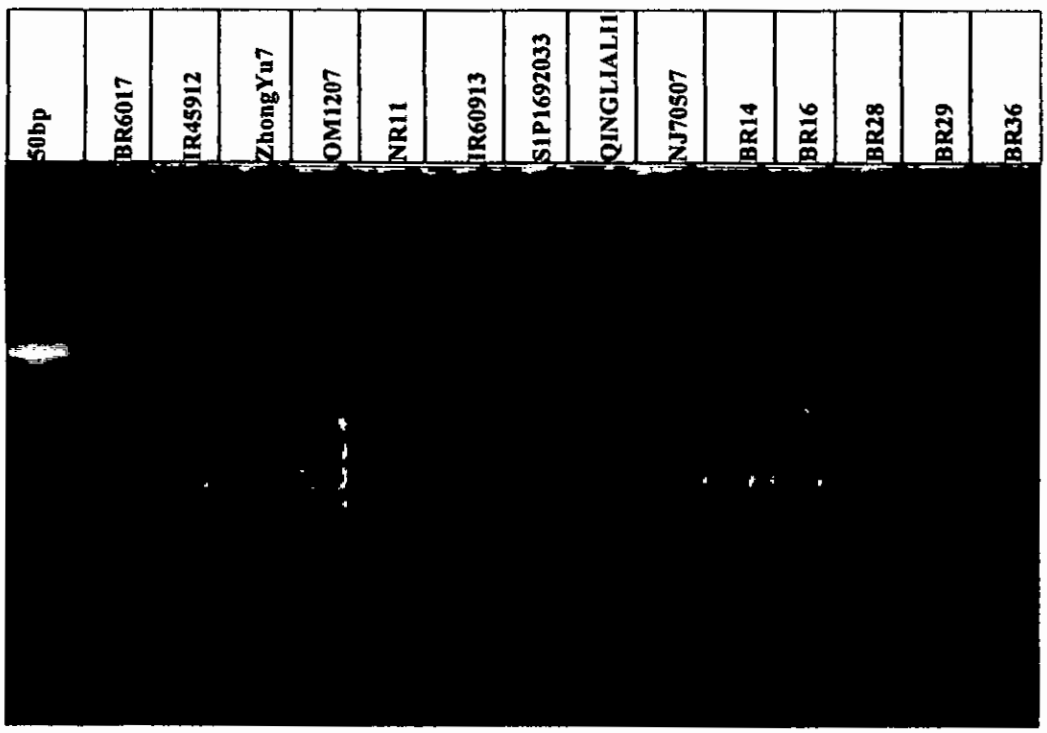


Fig. 5. VNTR banding patterns obtain from blast resistant and susceptible genotypes using RY primer

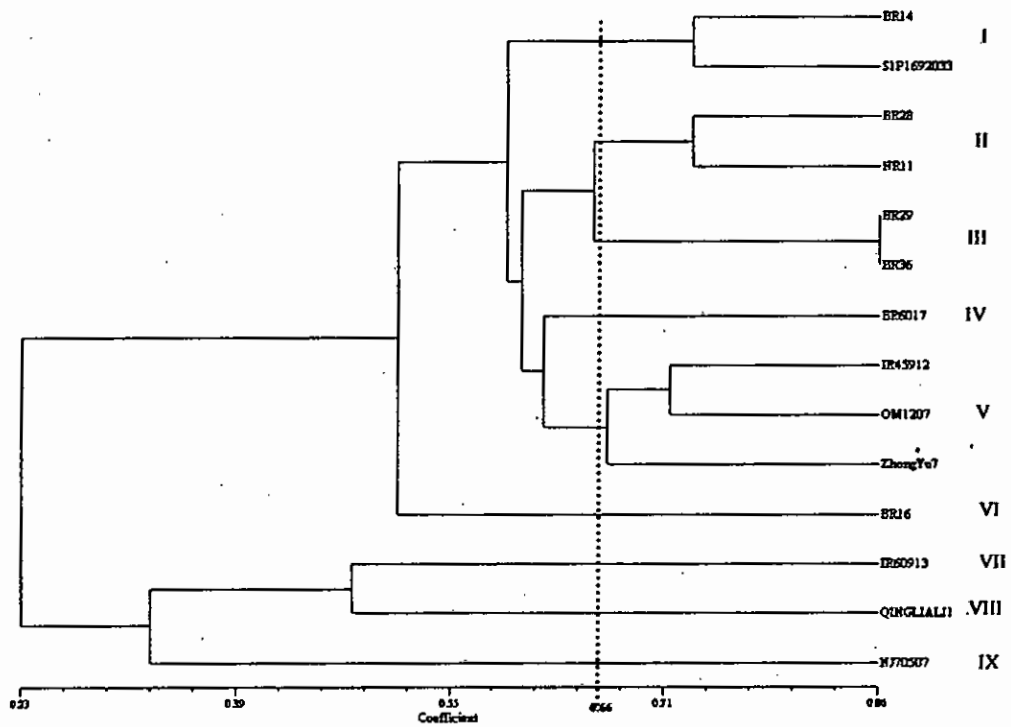


Fig. 6 . A UPGMA dendrogram showing the genetic relationships among 14 blast resistant and susceptible based on the alleles detected by 8 microsatellite and 3 VNTR markers

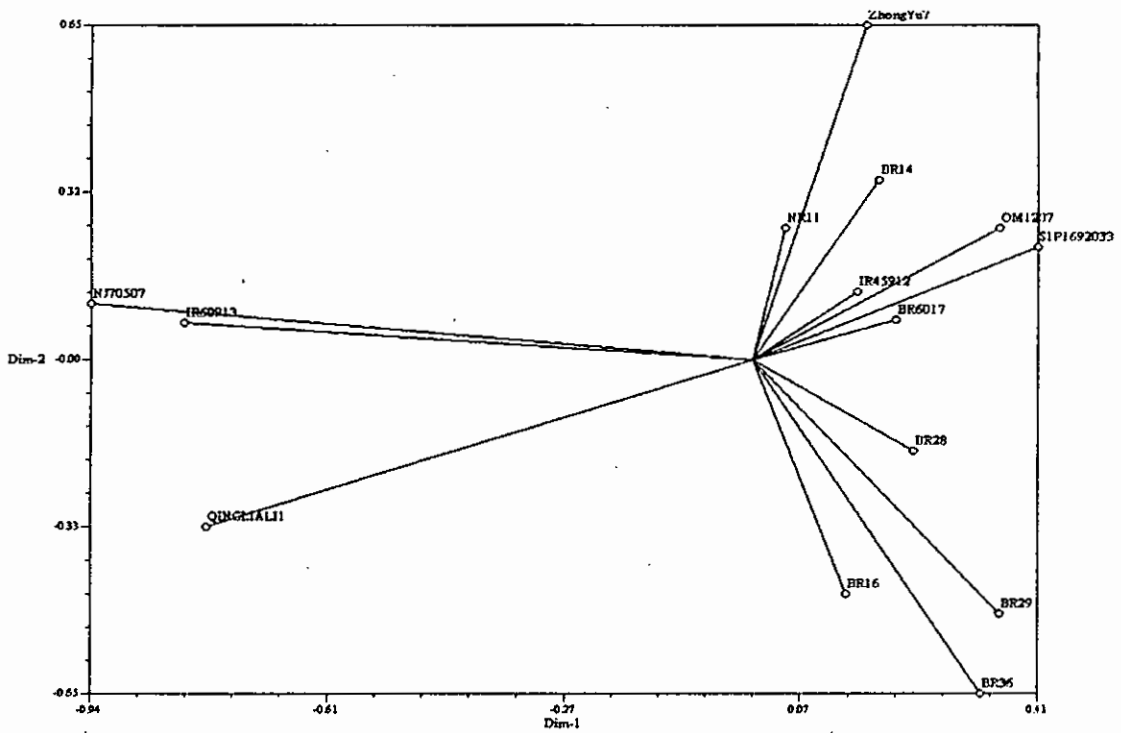


Fig. 7. Two dimensional view of Principal Coordinate Analysis (PCoA) with 8 microsatellite and 3 VNTR markers over 14 genotypes

4.2.3. Genetic distance-based analysis for blast resistant genotypes

In addition to eight SSR markers, three VNTR markers have been added for genetic diversity analysis of 14 blast resistant and susceptible genotypes. As the VNTR primers were random so, more than one locus was present in VNTR (Appendix 1). In each amplified locus, presence or absence of allele was considered and binary outputs were used in the analysis. The genetic similarity analysis using UPGMA clustering system generated nine genetic clusters with similarity coefficient of 0.66 (Fig. 6). A total of five clusters contained single genotype and those were resistant phenotype against blast disease. The clusters were IV, VI, VII, VIII and IX containing the genotypes BR6017, BR16, IR60913, QINGLIALI1, & NJ70507, respectively. Cluster I possessed two genotypes viz. BR14 and SIPI692033 which were resistant to blast. Again, three resistant genotypes against blast viz. IR45912, OM1207 & ZhongYu7 had been grouped in cluster number V. Only the cluster number III possessed the susceptible genotypes which were BRRI dhan29 and BRRI dhan36. The results indicated that in order to improve the blast tolerance along with other genetic variability of the two important boro varieties of BRRI, they required to be crossed with genetically distant blast resistant genotypes of the cluster VII, VIII and IX. The genotypes belonging to the distant cluster could be used in hybridization program for obtaining a wide spectrum of variation among the segregates. Similar reports were also made by Bansal *et al.* (1999), Mokate *et al.* (1998) and Kumari and Rangasamy (1997).

The two dimensional graphical view of Principal Coordinate Analysis (PCoA) showed the spatial distribution of the genotypes along the two principal axes. The genotypes viz. ZhongYu7, OM1207, SIPI692033, BRRI dhan29, BRRI dhan36, QINGLIALI NO.1, IR60913 and NJ70507 were found placing far away from the centroid of the cluster and rest of the genotypes were placed more or less around the centroid (Fig. 7). The results indicated that the genotypes placed far away from the centroid were more genetically diverse

while the genotypes placed near around the centroid possessed more or less similar genetic background. However, centroid may be defined as the vector representing the middle point of the cluster which contained at least one number for each variable. The connecting line between the each genotype and the centroid represented eigen vectors for the respective genotypes.

Quantitative genetic distance between different pairs of different genotypes was calculated using "Nei 1983" coefficient. The pair-wise genetic dissimilarity coefficients indicated that the highest genetic distance was obtained between NJ70507 with BRRI dhan36 & BRRI dhan29 as well as between QINGLIALI NO.1 & BR6017 (85.71%) (Table 4) followed by between IR60913 & BR6017 (83.33%), between NJ70507 & BR14 and between IR60913 & BR16 (77.78%), between IR60913 & BRRI dhan36 and between ZhongYu 7 & QINGLIALI No. 1 (75.0%). The results are in agreement with the findings of principal coordinate analysis and suggested that these genotypes were more genetically diverse. In crop improvement breeding program for blast resistance these genetically diverse genotypes could be chosen as parents in the crossing program to enhance disease resistance capability. Particularly, blast susceptible genotype BRRI dhan29 and BRRI dhan36 could be crossed with NJ70507 to enhance blast tolerance and to create genetic variability among these two genotypes. On the other hand BRRI dhan36 & BRRI dhan29 were the most similar genotype (5.88% dissimilar) followed by BRRI dhan28 & BRRI dhan29 (11.76% dissimilar) and BRRI dhan36 & BRRI dhan28 (12.5% dissimilar). The highest genetic similarities among BRRI dhan36, BRRI dhan28 & BRRI dhan29 might be because all these varieties are boro varieties with similar grain type. Allelic variation and genetic diversity of genotypes with abiotic and biotic stress tolerance has been studied by the several scientists in home and abroad (Latif *et al.*, 2007b; Hwang *et al.*, 2004; Zeng *et al.*, 2004).

Morphological data represented a broad genetic basis in resistant and improved high yielding but susceptible cultivars while molecular data showed narrow genetic basis between them. For getting more diversity between resistant and high yielding but susceptible cultivars more primers should be needed (Ali *et al.*, 2003).

Table 9. Data on the number of alleles, allele size range, highest frequency allele and polymorphism information content (PIC) found among 14 rice genotypes for 8 microsatellite markers

Marker	Chr No	Position (cM)	Motif*	Allele no.	Size range (bp)	Highest frequency allele		PIC Value
						Size (bp)	Freq (%)	
RM11	7	47.0	(GA)11	5	141.9-185.5	175	41.67	0.6990
RM17	12	109.1	(GA)21	4	208.3-278.9	217	46.15	0.5556
RM21	11	85.7	(GA)18	5	151.6-229.8	228	28.57	0.7261
RM108	9	73.3	(GGT)10	2	57.5-68.0	59	78.57	0.2800
RM23	1	71.6	(GA)15	4	105.7-123.0	106	42.86	0.6549
RM80	8	103.7	(TCT)25	5	93.6-114.9	105	42.86	0.6574
RM531	8	90.3	(AT)15	5	203.1-231.1	228	28.57	0.6999
RM443	1	122.7	(GT)10	3	131.6-114.6	116	60.00	0.4662
Mean				**4.13**			**46.20**	**0.5924**

*Motif of the SSR and number of repeats as previously published;

Chr = Chromosome (http://www.gramene.org).

Table 10. Genetic dissimilarity index using "Nei 1983" among 14 blast resistant and susceptible rice genotypes using 8 microsatellite & 3 VNTR Markers

Genotype	BR14	BR16	BRRI dhan28	BRRI dhan29	BRRI dhan36	BR6017	IR45912	IR60913	NJ70507	NR11	OM1207	QING LIALI1	S1P1 692033	ZhongYu7
BR14	0.0000													
BR16	0.2667	0.0000												
BRRI dhan28	0.3333	0.4375	0.0000											
BRRI dhan29	0.2857	0.2667	0.1176	0.0000										
BRRI dhan36	0.3846	0.3333	0.1250	0.0588	0.0000									
BR6017	0.3846	0.3846	0.3333	0.2667	0.3333	0.0000								
IR45912	0.3750	0.4286	0.2941	0.2941	0.3125	0.2500	0.0000							
IR60913	0.4000	0.7778	0.4545	0.6250	0.7500	0.8333	0.5000	0.0000						
NJ70507	0.7778	0.7500	0.6000	0.8571	0.8571	0.5714	0.5556	0.6667	0.0000					
NR11	0.2778	0.4667	0.2000	0.2353	0.3125	0.2000	0.2222	0.1818	0.5000	0.0000				
OM1207	0.2500	0.2941	0.2727	0.2632	0.3333	0.3125	0.2000	0.4545	0.6000	0.2273	0.0000			
QINGLIALI1	0.7143	0.6667	0.4444	0.5556	0.5556	0.8571	0.4444	0.3750	0.5000	0.3333	0.5000	0.0000		
S1P1692033	0.2000	0.3529	0.2727	0.2778	0.2353	0.1875	0.3684	0.6364	0.6000	0.2727	0.2917	0.6667	0.0000	
ZhongYu7	0.2941	0.4286	0.3333	0.3750	0.4000	0.2000	0.2778	0.5556	0.5556	0.2105	0.2000	0.7500	0.3000	0.0000

Chapter 5
Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

A study was conducted to characterize blast resistant and susceptible genotypes morphologically as well as molecular level in the experimental field and Molecular laboratory of Plant Pathology Division, BRRI. A total of 14 including 11 resistant, one moderately resistant, one highly susceptible and one moderately susceptible were used in the study. Multivariate analysis were performed for knowing the genetic of rice genotypes through Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO) and Canonical Vector Analysis(CVA) using 13 quantitative characters namely, number of tiller/hill, tillering ability, plant height (cm), phenotypic acceptability, days to flowering, days to maturity, panicle length (cm), number of spike let / panicle, number of filled grain/panicle, number of unfilled grain/panicle, 1000 filled grain weight (gm), yield/ hill (gm) and disease index according to PCA and D^2 analysis. The genotypes were grouped into seven different clusters. PCA showed 90.57% variation against first five eigen values. The highest inter genotypic distance (106.80) was found between QING LIALI NO1 with BR 6017-3-3-4-1and the lowest (12.57) between ZHONG-YU 7 and OM 1207. The inter-cluster distance was maximum between cluster II and IV (108.250) followed by the distance between cluster I and IV (83.793), cluster II and VII (69.870), cluster IV and VI (68.380), while the inter-cluster distance was minimum between cluster V and VI (21.290) followed by the distance between cluster III and VI (22.727). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster IV was far diverged from those of cluster II and I.

The genotypes of cluster II could be selected for higher tillering ability higher phenotypic acceptability and lower disease index. The genotypes of cluster IV could be selected for higher plant height, higher panicle length, latest flowering

and maturity, higher number of spikelet/panicle, higher number of filled grain/ panicle, higher number of unfilled grain/ panicle, higher (third highest) 1000 filled grain weight. The genotypes of cluster VII could be selected for the highest yield/ hill and higher disease index. The genotypes of cluster VI could be selected for early flowering and maturity, higher yield and lower disease index. Genetic distance between cluster II and Cluster IV was higher. So, crosses could be made among the genotypes of above clusters but this is not expected because those genotypes were resistant but lower yielder. Therefore, high yielding along with highly susceptible BRRI dhan 29 genotypes could be crossed with highly resistant genotypes (BR 6017-3-3-4-1, ZHONG-YU 7 and BRRI dhan 28 and BRRI dhan 36 genotypes could be crossed with QING LIALI NO1 for the development of blast resistant along with high yielding rice varieties.

A total of 8 microsatellite and 3 minisatellite or VNTR markers were used for studying molecular variability across 14 blast resistant and susceptible rice genotypes. A total of 33 alleles were detected at the loci of 8 microsatellite markers across 14 blast resistant and susceptible rice genotypes. The number of alleles per locus ranged from 2 alleles (RM108) to 5 alleles (RM21, RM80, RM531), with an average of 4.13 alleles across the 30 loci. The PIC values ranged from 0.280 (RM108) to 0.726 (RM21). PIC value revealed that RM21 was considered as the best marker for 14 rice genotypes. The two dimensional graphical view of Principal Coordinate Analysis (PCoA) for blast resistant genotypes showed the genotypes ZhongYu7, OM1207, SIPI692033, BRRI dhan29, BRRI dhan36, QINGLIALI1, IR 60913-42-3-3-2-2 and NJ70507 were found placing far away from the centroid of the cluster and rest of the genotypes were placed more or less around the centroid. The genetic similarity analysis using UPGMA clustering system generated nine genetic clusters with similarity coefficient of 0.66 for the study with blast disease resistance.

The pair-wise genetic dissimilarity coefficients indicated that the highest genetic distance was obtained between NJ70507 with BRRI dhan 36 & BRRI dhan 29 for blast disease. In order to develop blast resistant varieties and broaden the genetic base of rice varieties new breeding program should initiated preferably using the parents, BRRI dhan 29 and NJ70507 and BRRI dhan 36 and NJ70507 and to create genetic variability among these two modern rice varieties. Both molecular and morphological data did not show the similar results. So, use of more molecular markers might be resolved the results of morphological analysis.

References

REFERENCES

Ahmed, H.U., Haque, M.A., Shahjahan, A.K.M. and Miah, S.A. 1985. Blast resistance in rice germplasm in Bangladesh. *Bangladesh J. Plant Pathol.* **1**:73-74.

Ahmed, H.U. and M.M. Hossain.1985. Crop disease survey and establishment of a herbarium of Bari plant path. Div.BARI. Joydebpur, Gazipur, Bangladesh.107pp

Ahn, S.W. (1994). International collaboration on breeding for resistance to rice blast, in Zeigler R.S., Leong, S.A. and Teng, P.S. (eds.), Rice Blast Disease, CBA and IRRI, Wallingford, Oxon, UK,pp.137-153.

Ali, B.Y. Wu. H. Leung, Wu. J.I. and II. Leung. 2003. Genetic diversity and relatedness of selected Iranian rice cultivars and disease resistance donors assayed by simple sequence repeats and candidate defense gene markers. *Euphytica.* **131** (3): 275-284.

Ariyo, O.J. (1987). Multivariate analysis and choice of parents for hybridization in Okra. *Thero. Appl. Genet.* **74**:361-363.

Banik, P. 1999. Cold injury problem in Boro rice Proc. Workshop on Modern Rice Cultivation. Rice Research Institute. Joydebpur, Gazipur, Bangladesh. pp. 14-16.

Bansal, U.K, Saini RG, Rani NS & Kaur A. 1999. Genetic divergence in quality rice. *Oryza.* **36**: 20-23.

Bashar, M.K. (2002) Genetic and Morphophysiological basis of heterosis in rice. Ph.D thesis. Department of genetics and plant breeding. BSMRAU, Gazipur.

BBS (Bangladesh Bureau of Statistics). September 2003. Monthly Statistical Bulletin, Bangladesh Stat. Div. Mins. Planning, Govt. People's Repub. Bangladesh, Dhaka. p. 57.

Bharadwaj, C, C.T, Satyavathi and D. Subramanyam. 2001. Evaluation of different classificatory analysis methods in some rice (*Oryza sativa*) collections. *Indian J. Agril. Sci.* **71** (2): 123-125.

Bhatt, J.C., and R.A. Singh .1991. Blast of Rice. In Singh, U.S., A.N. Mukhopadhyay, J. Kumar, and H.S. Chaube. Palnt diseases of Economic Importance: Disease of Cereals and Pulses. Vol.1. Prentice-Hall Inc., New Jersey.pp.80-115.

Bidaux, J.M. (1978). Screening for horizontal resistance to rice blast (*Pyricularia oryzae*), in Buddenhagen, I.W. And Persley, G.J. (eds.), Rice in Africa. Academic press, London, pp. 159-174.

Blair, M.W., O. Panaud, S.R. McCoueh. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingeprinting in rice (*Oryza sativa* L.) *Theor, Appl.Genet.* **98**: 780-792.

Bonman J.M., Vergyl de Dois T.I. and Khin M.M. (1986). Physiologic specialization of *Pyricularia oryzae* in the Philippines. *Plant Dis.* **70**:767-769.

Bonman, J.M. and Mackill, D.J. (1988). Durable resistance to rice blast disease. *Oryza* **25**:103-110.

Bonman, J.M., Khus, G.S. and Nalson, R.J. (1992). Breeding rice for resistant to pests. *Annu. Rev. Phytopathol.* **30**:507-528.

Bridge, P.D., Pearce, D.A., Rivera, A. and Rutherford, M.A. 1997. VNTR-derived oligonucleotides as PCR primers for population studied in filamentous fungi. *Letters in applied microbiol.* **24**: 426-430.

Chakravarthi, B.K and R. Naravaneni. 2006. SSR marker based DNA fingerprinting and diversity study rice (*Oryza sativa*). *African J. Biotech.* **5**: 684-688.

Chaubey P. K. and A. K. Richharia. 1993. Genetic variability, correlations and pathcoefficients in indica rices, *Indian J. Genet.* **54**: 356-360.

Chen, D., Zeigler, R.S., Leung, H. and Nelson, R.J. 1995. Population Structure of *Pyricularia grisea* at two screening sites in the Philippines. *Phytopathol.* **45**: 1011-1019.

Chen D.H., Wang, G.L., and Ronald. P.C. (1997). Location of the rice blast resistance locus *pi-5(t)* in moroberekan by AFLP bulked segregant analysis. *Rice Genetic Newsletter.* **14**: 95-98.

Chen D.H., M. Dela Vina, T. Inakai, D.J. Mackill, P.C. Ronald and R.J. Nelson. (1999). Molecular mapping of blast resistance gene,*pi44(t)* in a line derived from a durable resistant rice cultivar. *Theor. Appl. Gent.* (in press)

Chouhan, J.S. and Chauhan, V.S. (1994). Hybrid performance for panicle exertion and flag leaf angle of rice. *Oryza.* **30**: 352-354.

Coburn, J.R.S.V. Temnykh, E.M. Paul and S.R. McCouch. 2002. design and application of microsatellite market panels for semi automated genotyping of rice (*Oryza sativa L*). *Crop Sci.* **42**: 2092-2099.

Digby, P., Galway, M. and Lane, P. (1989). A second course of Oxford Science Publication, Oxford. pp.103-108.

Falconer, D. S. (1960). Introduction quantitative genetics, Oliver and Bond, London. P. 304.

Fomba, S.N.and Taylor, D.R. (1994). Rice Blast in West Africa: Its Nature and control, in Geigler, R.S., Leong, S.A. and Teng, P.S.(eds.), *Rice Blast Disease*, CAB and IRRI, Wallingford, Oxon, UK, pp. 343-355.

Gealy, D.R., T.H. Tai and C.H. Sneller. 2002. Identification of red rice, rice and hybrid populations using microsatellite markers. *Weed Sci.* **50**: 333-339.

George, M.L.C., Nelson, R.J., Zeigler, R.S. and Leung, H. 1997. Rapid population analysis of Magnaporthe grisea by using rep-PCR and endogenous.

Ghaderi, A., Shishergar, m., Regai, A. and Ehdate, B. (1984). Multivariate analysis of genetic diversity for yield and its components in mungbean. *J. American Soc. Hort. Sci.* **104**: 728-732.

Hashimoto, Z., N. Mori, M. Kawamura, T. Ishii, S. Yoshida, M. Ikegami. S. Takumi and C. Nakamura. 2004. Genetic diversity and phylogeny of Japanese sake-brewing rice as revealed by AFLP, Nuclear and Chloroplast SSR markers. *Theor. Appl. Genet.* **109**: 1586-1596.

Hashioka, Y. (1950a). Studies on the mechanism of prevalence of rice blast disease in tropics. Technical Bulletin, Taiwan Agricultural research institute No. 8, 237pp.

Hemmi,T and Abe, T.(1941). Studies on the rice blast disease. Relation of the environment to the development of blast disease and races of the blast fungus. Ministry of Agriculture and Forest, Japan. 156-232.

Higashi, T. and Saito, S. (1995). Linkage Groups of field resistance genes of upland rice variety Sensho to leaf blast caused by *Pyricularia oryzae. Jap.J.of Breed.* **35**: 438-448.

Hossain, M.A. and M.E. Haque. (2003). Genetic variability and path analysis in rice genotypes. Bangladesh *J. Pl. Breed Genet.* **16** : 33-37.

Hossain MZ, Rasul MG, Ali MS,. Iftekharuddaula KM & Mian MAK. (2007). Molecular Characterization and Genetic Diversity in Fine Grain and Aromatic Landraces of Rice Using Microsatellite Markers. *Bangladesh J. Genet. Pl. Breed.* **20**: 01-10.

Hwang H., Kwon S, Cho C, Ahn S, Suh P & Moon H. 2004. Genetic diversity of high-quality rice cultivars based on SSR markers linked to blast resistance genes. *Korean J. Crop Sci.* **49** : 251-255.

Iftekharuddaula, K. M.A. Badshah. M.S. Hassan, M. k. Bashar and K. Akter. 2001. Genetic variability Character association and path analysis of yield components in irrigated rice *(Oryza sativa L). Bangladesh J. Plant. Breed. Genet.* **14** : 43-49.

Inakai, T. and Aya, S. (1997). Determination of chromosomal location of blast resistance gene in West African upland cultivar Moroberekesn. *Breed. Sci.* **47**: 268.

Jagadev, P. N., Rasul, M. G., Bhowmic, A. and Akbar, A. A. (1995). Genetic divergence in groundnut (*Arachis hypogaea* L.). *Bangladesh J. Plant Breed Genet.* **8** : 35-38.

Jain, S., R.K. Jain and S.R. McCouch. 2004. Genetic analysis of lindian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled mlerosatellite markers. *Theor. Appl. Genet.* **109**: 965-77.

Jeger, M.I.D. Gerethrojones and E. Griffiths. 1983. Components of partial resistance of wheat seedlings to Septoria nodorum. *Euphytica.* **32**: 558-575.

Johnson, R. (1981). Durable resistance: definition of, genetic control, and attainment in plant breeding. *Phytopathol.* **71**: 567-568.

Joshi, S.P., Gupta, R.D. Aggarwal, P.K. Ranjekar and D.S.Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat polymorphism in the genus *Oryza. Theor. Appl. Genet.* **100**: 1311-1320.

Julfiquar, A. W., Virmani, S. S., Kabir, K. A. and Molla, A. H. (1985). Differential heterosis in some growth physiological characters of indica/japonica and japonica/japonica crosses of rice. *Progress. Agric.*. **5** : 671-678.

Junjian, Ni., M. Peter. Colowit. David, and J. Mackill. 2002. Evatuation of Genetic Diversity in Rice Subspecies Using Mierosatellite Markers. *Crop Sci.* **42**: 301-607.

Kanwal, K. S. M. Singh, J. Singh and R. B. Singh. 1993. Divergence gene pools in rice improvement. *Theor. Appl. Genet.* **65**: 263-367.

Khan, A.Q. 2006. Molecular marker based characterization and genetics diversity analysis of rice using SSR and RAPD. M.S. thesis. Department of Plant Pathology. BAU. Mymensingh. Bangladesh. pp. 73

Khus, G. S. (1989). Multiple disease and insect resistance for increased yield stability in rice. Progress in irrigated rice research. International Rice research Institute, P.O. Box 933, Manila, The Philippines.

Kiyosawa, S. (1981). Gene analysis for blast resistance. *Oryza.* **18**: 196-203.

Kumari RU & Rangasamy P. 1997. Studies on genetic diversity in international early rice genotypes. *Annals Agril. Res.* **18**: 29-33.

Kumar, J., Nelson, R.J. and Zeigler, R.S. (1999). Population structures and dynamics of *Magnaporthe grisea* in the Indian Himalayas. *Genetics*. **152**: 972-984.

Latif, M. A., Kabir M. S., Sharma N. R. and Hossain, M. A. (2007a). Integrated management of five major disease of rice (in Bangla): BRRI, Publication No. 170. P.28.

Latif, M. A., Rahman, M. M., Hossain, M. A., Mia, M. A. T. and Ali, M. S. (2007b). Molecular characterization of tungro resistant genotypes of rice. *Intl. J. Bio Res.* **3**: 19-26.

Lee EJ, Zhang Q and Mew T.W. (1989). Durable resistance in rice disease in irrigated environments, pp 93-110. Progress in irrigated rice research. International Rice research Institute, P.O. Box 933, Manila, The Philippines.

Lee E. J. and Cho S.Y. (1990). Variation in races of rice blast disease and varietal resistance in Korea. Paper presented in the focus on irrigated Rice, 27-31 Aug.1990, Seoul, Korea.

Levy, M., Romao, J., Marchetti, M.A. and Hamer, J.E. (1991). DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus *Magnaporthe grisea. Plant Cell.* **3**: 95-102.

Liu K, & Muse SV. (2005). Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics.* **21**: 2128–2129.

Mahajan, R. K., J.R. Rao, Y.R.S. Sharma and A. Ghosh. (1988). Genetic divergence in rice. *Crop Improv.* **8:** 323-64.

Mall, C.N. and D.M. Murya. (1962). Genetic diversity in breeding lines of two crosses in rainfed lowland rice. *Oryza.* **29**: 89-91.

McCouch, S.R., Nelson, R.J., Tohme, J.and Zeigler, R.S. (1994). Mapping of blast resistance genes in rice. International Rice Blast Disease. *CAB International, UK, pp. 167-186.*

Mekwatanakern, P., Kositratana, W., Levy, M. and Zeigler, R.S. (2000). Pathotype and avirulence gene diversity of *Pyricularia grisea* in Thailand as determined by rice lines near-isogenic for major resistance genes. *Plant disease.* **84**: 60-70.

Metcalf, H. (1907). A preliminary report on the blast of rice, with notes on other rice diseases. Bulletin of the South Carolina Agricultural Experiment station No. 121, 43 pp.

Mia, S.A., Shahjahan, A.K.M., Hossain, M.A. and Sharma, N.R. (1985). Survey of rice disease in Bangladesh. *Trop. Pest Management.* **31**: 208-213.

Mishra, S. B., U. K. Singh and S. P. Sahu. (1994). Studies on genetic divergence in fine land races of rice in North Bihar. *Ann. of Agril.* **15**: 217-221.

Mokate AS, Mehetre SS, Bendale VW & Birari SP. (1998). Genetic divergence in rice. *Advances in Plant Sci.* **11**:189-192.

Nagaraz, J., M. Knthirve, R. k. Ramesh. E. A. Siddiq. and E. Hasnain. (2002). Genetic analysis of traditional and evolved Basmati and non- Basmati rice varieties by using fluorescence- based ISSR- PCR and SSR markers. *Science* **99** : 5836-5841.

Naqvi, N.I. and Chatto, B.B. (1996). Molecular genetic analysis and sequence characterized amplified region-assisted selection of blast resistance in rice, in [IRRI] International Rice Research Institute, Rice Genetics III. Proceedings of the third International Rice Genetics Symposium, 16-20 Oct 1995. Manila (Philippines): IRRI. Pp 507-572.

Naqvi N.I., Bonman J.M., Mackill D.J., Nelson R.J. and Chattoo B.B. (1995). Identification of RAPD markers linked to a major blast rsistance gene in rice. *Molecular Breeding.* **1**: 341-348.

Nevo, E.E. Golenberg, A. Beilies, A. H. D. Brown and D. Zohary. (1982). Genetic diversity and environmental association of wild wheat, Triticum diococcoides in Israel. *Thero. Appl. Genet.* **62**: 241-254

Notteghem J.L. (1985). Definition d'une strategic d'utilisation de la resistance par analysis gnetique des relation hoteparasite Cas du Couple siz- *Prricularia oryzae. Agron Trop.* **40**: 129-147.

O'Brien S.J. (1990). Genetic maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Ou, S.H. (1985). Rice Disease (2nd ed.) Commonwealth Mycological Institute, Kew, Surrey, England. pp. 380.

Ou, S.H. (1997). Breeding rice for resistance to Blast, A critical view, pp 79-137. Proceedings of the rice blast workshop. International Rice Research Institute, Manila, Philippines.

Padmanbhan, S.Y. (1965b). Breeding for blast resistance in India. Ibid., 343-359.

Patil, F.V., Thomba, M.B. and Sidde, W.M. (1987). Genetic Divergence in Onion. *J. Maharastra Agril. Univ.* **12**: 252-253.

Ramanujam, S., Tiwari, A. S. and Mehra, R. B. (1974). Genetic divergence and hybrid performance in mungbean. *Thero. Appl. Genet.* **25**: 211-214.

Rao, K. M (1994). *Rice Blast Disease*, Daya Publishing House, Delhi, India, pp.1-2.

Rohlf, F. (2002). NTSYS-pc: Numerical taxonomy and multivariate analysis system, 2.1 edn. Department of Ecology and Evolution, State University of NY, Stony Brook.

Sawant, D. S., Patil, S. L., Jadhav, B. B. and Bhave, S. G. (1995). Genetic divergence, character association and path analysis ion rice. *J. Maharashtra Agril. Univ.* **20**: 412-414.

Shahjahan, A.K.M., Ahmed, H.U., Miah, M.A.T., Hossian,M.A., Sharma, N.R. and Nahar, N.S. (1991). Outbreaks of leaf and neck blast in Boro rice crop in Bangladesh. In R.S. Zeigler, S.A. Leong and P.S. Teng (ed.), Rice Blast Disease. IRRI and CABI Publication. p465-488.

Sharma, A. (1997). Genetic divergence, variability and character association among various agronomic and quality traits in aromatic rice. MS Thesis . CCS Haryana Agricultural University, Hisar, India. 150.p.

Singh, R. K. and Chaudhury, B. D. (1985). Biometrical method in quantitative genetic analysis. Kalayani publishers, New Delhi.

Singh, S. k., Singh, R. S., Maurya, D. M. and Verma, O. P. (1986). Genetic divergence among lowland rice cultivars. *Indian J. Genet.* **47**: 11-14.

Singh, U. K., Mishra, S. B. and. Thakur, R. (1999). Genetic divergence in boro rice. *Oryza.* **36**: 76-77.

Sinha, P. K. Chauhan, V. S., Prosad, K. and Chauhan, J. S. (1991). Genetic divergence in indigenous upland rice varieties. *Indian J. Genet.* **51**: 47-50.

Suh- Jung. P., Choi- Yong H., Kim-Kee, J., Cho- Young, C., Kwon-Soo, J., Jeong – Young, P., Jeung, J. U., Chor- Im, S., Kim- Yeon, G., Choi-Hae, C. and Hwang- Hung, G. (2004). Genetic diversity and QTLs for grain quality in japonica rice. K*orean J. Breed.* **36** : 31-37.

Uddin, M.J. (2001). Morphogenetic diversity and gene action in sesame *(Sesamum indicum* L.*)* Ph.d thesis. Department of Genetics and Plant Breeding, BSMRAU, Gazipur. p.59.

Variavan, S., Siddq, E. A., Arunachalam, V. and Swaminathan, M, S. (1973). A study on the nature of genetic divergence in rice from Asam and North East Himalayan. *Theor. Appl. Genet.* **43**: 213-221.

Vivekanandan, P. and S. Subramaniam. (1993). Genetic divergence in rice. *Oryza* **30**:60-62.

Wang, G.L., Mackill, D.J., Bonman, J.M., McCouch, S.R., Champox, M.C., Nelson R.J. (1994). RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics.* **136**: 1421-1434.

Weir, B.S. (1990). Genetic Data Analysis: Methods for Discrete Population Genetic Data. Sinauer Associates. Inc. Publishers. Sunderland. Massachusetts. 377pp.

Williams, J.G.K., Rafalski J.A. and Tingey S.V. (1993). Genetic analysis using Dmarkers. *Methods Enzymology.* **218**:704-740.

Zeigler, R.S., S.A. Leong and P.S. Teng (ed.). (1994). Rice Blast Disease. CAB International Mycological Institute, Kew, UK

Zeng L.H., Kwon T.R., Liu X, Wilson C., Grieve C.M. & Gregorio G.B. (2004). Genetic diversity analyzed by microsatellite markers among rice (Oryza *sativa* L.) genotypes with different adaptation to saline soils. *Plant Sci.* **166**: 1275-1285.

Zheng, K., N. Huang, J. Bennett and G. S. Khush. (1995). PCR-based marker-assisted selection in rice breeding. International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines, pp. 1-24.

Appendices

Appendix 1: Mean values of thirteen characters of 14 blast resistant and susceptible genotypes.

Genotypes	Number of tiller / hill	Tillering ability	Plant height (cm)	Phenotypic acceptability	Days to flowering	Days to maturity	Panicle length (cm)	Number of Spike let / Panicle	Number of filled grain/ Panicle	Number of unfilled grain/ Panicle	1000 filled grain wt (gm)	Yield/ hill (gm)	Disease index
BR 6017-3-3-4-1	12	5	82.80	3	106	138	20	112	95	17	23.53	32.00	3.0
IR 45912-9-1-2-2	13	5	102.30	3	108	143	23	127	109	18	24.14	34.20	3.0
ZHONG-yu 7	13	5	89.90	5	110	145	21	121	91	30	23..00	30.20	1.0
OM 1207	12	5	94.49	3	110	145	24	128	98	30	27.20	31.98	3.0
NR-11	13	5	88.80	3	108	145	20	158	120	38	21.60	33.69	3.0
IR 60913-42-3-3-2-2	12	5	98.60	3	106	140	23	149	118	31	24.80	35.11	3.0
S_1P_1 692033	10	5	96.60	3	114	150	21	143	115	28	25.40	29.50	3.0
QING Liali NO1	12	5	124.25	3	124	160	30	187	145	42	25.20	36.84	3.0
NJ 70507	11	5	102.80	3	114	152	23	148	110	38	27.40	33.15	3.0
BR 14	10	5	102.50	3	101	151	20	140	115	25	28.20	32.43	3.0
BR 16	17	5	86.00	3	114	156	24	152	116	36	25.12	40.53	2.0
BRRI dhan 28	17	5	88.00	3	106	138	23	140	112	28	21.60	41.12	4.5
BRRI dhan 29	13	5	92.00	3	121	154	28	166	135	31	23.06	40.47	7.0
BRRI dhan 36	12	5	85.00	3	110	140	21	134	102	22	25.60	31.33	3.0

Appendix 2 : Binary outputs of SSR and VNTR markers over 14 blast resistant and susceptible genotypes

Markers	BR14	BR16	BRRI dhan28	BRRI dhan29	BRRI dhan36	BR6017	IR45912	IR60913	NJ70507	NR11	OM1207	QING LIALII	S1P1 692033	ZhongYu7
GF1	1	0	1	0	0	0	0	0	0	1	1	0	1	1
GF2	1	1	1	0	0	0	0	0	0	0	1	0	1	0
GF3	1	0	1	0	0	0	0	1	1	1	1	0	1	0
GF4	1	0	0	0	0	0	1	0	0	1	1	0	1	1
GF5	1	0	1	0	0	0	1	1	1	1	1	0	1	1
GF6	0	0	0	0	0	0	0	0	1	0	0	0	0	1
MR1	1	0	1	1	0	1	1	0	0	1	1	0	1	1
MR2	0	0	1	1	1	1	1	0	0	1	1	0	1	1
MR3	0	0	1	1	1	1	1	0	0	1	1	0	1	1
MR4	0	1	1	1	1	1	1	0	1	1	1	1	1	0
MR5	1	1	1	1	1	1	1	0	0	1	1	0	1	1
RM108	1	1	1	1	1	0	1	1	0	1	1	1	0	1
RM108	0	0	0	0	0	1	0	0	1	0	0	0	1	0
RM11	0	1	0	0	0	0	0	0	0	0	1	0	0	0
RM11	1	0	0	0	0	0	0	0	0	0	0	0	1	0
RM11	0	0	1	1	0	0	0	1	0	1	0	1	0	0
RM11	0	0	0	0	0	0	0	0	1	0	0	0	0	1
RM11	0	0	0	0	0	0	1	0	0	0	0	0	0	0
RM17	0	0	0	0	0	0	0	0	0	0	1	0	0	0
RM17	0	0	1	0	0	0	1	1	1	1	0	1	0	0
RM17	1	1	0	0	0	1	0	0	0	0	0	0	1	1
RM17	0	0	0	0	1	0	0	0	0	0	0	0	0	0
RM21	0	1	0	1	1	0	0	0	0	0	0	0	1	0
RM21	0	0	0	0	0	1	1	0	0	0	0	0	0	0
RM21	0	0	0	0	0	0	0	0	1	0	0	0	0	0
RM21	0	0	1	0	0	0	0	0	0	0	1	0	0	1
RM21	1	0	0	0	0	0	0	1	0	1	0	1	0	0
RM23	0	0	0	0	0	1	1	1	0	1	1	0	0	1
RM23	1	0	0	0	0	0	0	0	0	0	0	0	1	0
RM23	0	0	1	1	1	0	0	0	0	0	0	0	0	0
RM23	0	1	0	0	0	0	0	0	1	0	0	1	0	0
RM443	0	0	0	0	0	0	0	1	0	1	1	1	1	1

Markers	BR14	BR16	BRRI dhan28	BRRI dhan29	BRRI dhan36	BR6017	IR45912	IR60913	NJ70507	NR11	OM1207	QING LIALI1	SIP1 692033	ZhongYu7
RM443	0	0	1	1	1	0	0	0	0	0	0	0	0	0
RM443	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RM531	0	0	0	0	0	0	1	0	0	0	0	0	0	0
RM531	1	1	0	1	0	0	0	0	0	0	1	0	0	0
RM531	0	0	1	0	1	0	0	0	0	0	0	1	1	0
RM531	0	0	0	0	0	1	0	0	1	1	0	0	0	1
RM531	0	0	0	0	0	0	0	1	0	0	0	0	0	0
RM80	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RM80	0	0	1	1	1	1	0	0	0	1	0	0	1	0
RM80	1	0	0	0	0	0	0	1	0	0	0	0	0	0
RM80	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RM80	0	0	0	0	0	0	1	0	1	0	1	1	0	0
RY1	1	1	0	1	1	1	1	0	0	1	1	0	1	1
RY2	1	1	1	1	1	1	1	0	0	1	1	0	1	1
RY3	1	1	1	1	1	1	1	0	0	0	1	0	1	1
RY4	1	1	1	1	1	1	1	0	0	1	1	0	1	1
RY5	1	1	1	1	1	1	1	0	0	1	1	0	1	1
RY6	0	0	0	1	1	0	1	0	0	0	1	1	0	0
RY7	1	1	1	1	1	0	0	1	0	1	1	0	1	0

1= Present; 0= absent band in genotypes