SCREENING OF F2 POPULATIONS AND MOLECULAR CHARACTERIZATION OF UFRA RESISTANT GENOTYPES OF RICE

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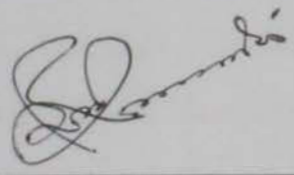

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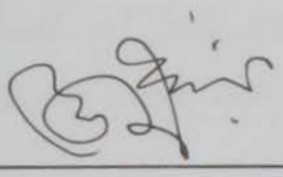

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This is to certify that thesis entitled, ***"SCREENING OF F2 POPULATIONS AND MOLECULAR CHARACTERIZATION OF UFRA RESISTANT GENOTYPES OF RICE"*** Submitted to the faculty of Agriculture, Sher- e- Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY**, embodies the result of a piece of bonafide research work carried out by **Md. Rabiul Basar Talukdar, Roll No. 00716, Registration No. 27750/00716,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or sources of information, received during the course of this investigation have been duly acknowledged by him

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Dedicated To

My Beloved Parents

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BY

MD. RABIUL BASAR TALUKDAR

ABSTRACT

The experiments were conducted in the experimental field and laboratory of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur during 2006-2007. Thirteen F2 lines of rice were tested against ufra. Among the 1146 plants of F2 population under 13 lines, 406 were found highly resistant, 216 moderately resistant, 157 resistant, 94 moderate susceptible, 61 susceptible and 212 highly susceptible. The highly resistant and resistant plants were selected for crossing program for the development of ufra resistant varieties. Genetic diversity was assessed by analyzing 6 SSR and 3VNTR markers for ufra resistant genotypes. All the markers were found polymorphic. Cluster analysis according to the UPGMA method illustrated the genetic relationships among the 12 ufra resistant and susceptible genotypes. These rice genotypes had been differentiated into three main clusters. To develop ufra resistant varieties and broaden the genetic base of rice varieties new breeding program should be initiated preferably using the parents (having greater genetic distances) BR11 and Aokazi, BR3 and Aokazi, Rayeda and BR3, Rayeda and BR11 as they are expected to produce good segregants.

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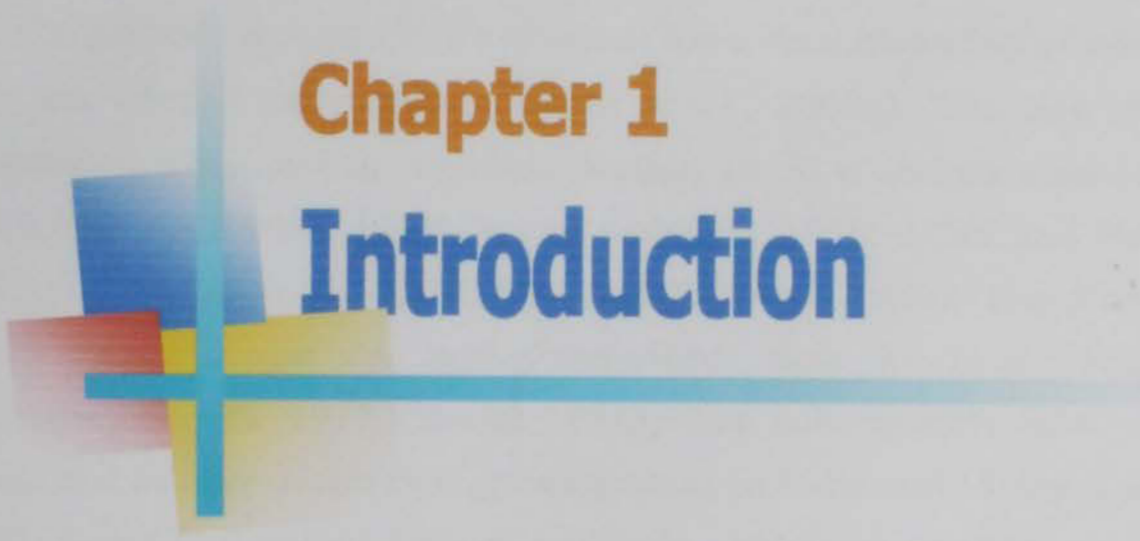
ABBREVIATIONS USED

AFLP	=	Amplified fragment Length Polymorphism
BRRI	=	Bangladesh Rice Research Institute
BAU	=	Bangladesh Agricultural University
bp	=	Base pair
cm	=	Centimeter
cv	=	Cultivar(s)
DAI	=	Days after inoculation
dH_2O	=	Distilled water
DNA	=	Deoxyribo Nucleic Acid
dNTP	=	Deoxynucleotide Triphosphates
EDTA	=	Ethylene diamine tetra acetic acid
et al.	=	And others
ed	=	Edited
FAO	=	Food and Agriculture Organization
Fig.	=	Figure
g	=	Gram
HR	=	Highly Resistant
HS	=	Highly Susceptible
IRRI	=	International Rice Research Institute
Kb	=	Kilo base pair
MR	=	Moderately Resistant
MS	=	Moderately Susceptible
M	=	Molar
mg	=	Miligram
min	=	Minute
ml	=	Milliliter
mM	=	Milimolar

ng	=	Nano gram
nm	=	Nano meter
Newsl	=	Newsletter
PCR	=	Polymerase Chain Reaction
R	=	Resistant
RAPD	=	Random Amplified Polymorphic DNA
RFLP	=	Restriction Fragment Length Polymorphism
rpm	=	Rotation per Minute
SAU	=	Sher-e-Bangla Agricultural University
S	=	Susceptible
SSR	=	Simple Sequence Repeats
SNP	=	Single Nucleotide Polymorphism
STS	=	Sequence Tagged Sites
TBE	=	Tris Boric Acid EDTA
UPGMA	=	Unweighted Pair Group Method with Arithmetic Means
UV	=	Ultra violate
var.	=	Variety
Viz.	=	Videlicet
VNTR	=	Variable Number Tandem Repeats
µl	=	Micro liter
µM	=	Micro molar
%	=	Percent
^{0}C	=	Degree Celsius



Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa)* is the most important cereal crop as well as staple food for about 135 million people of Bangladesh. Rice also comprises the staple food of 60% of the world population. It occupies about 75% of the total cropped area covering 26.6 million acres and is the only source of income for many farmers in our country (BBS, 2004).

In Bangladesh around 32 rice diseases have been identified of which ten are considered as major (Latif *et al.*, 2007a). The rice stem nematode, *Ditylenchus angustus* (Butler, 1913) is serious disease in Bangladesh (Butler, 1919; Watts padwick, 1950; Miah and Bakr, 1977a; Catling *et al.,* 1979; Cox and Rahman, 1980). The disease and causal organism were described first in East Bengal (Bangladesh) in 1913 (Butler, 1913) but subsequently ufra was recorded in deep water rice growing areas in India and Malay (Ling, 1951), the Philippines (Rayes and Palo, 1956), Egypt (Sasser and Jenkins, 1960), Thailand (Hashioka, 1963), Burma (Seth, 1939), Madagaskar (Vuong, 1969) and Vietnam (Kinh, 1981).

D. angustus is mainly associated with deep-water rice. However, with the development of intensive rice cropping and irrigation, it has been reported in irrigated rice adjacent to deep-water rice field. *Ditylenchus angustus* is an obligate ectoparasite. Reproduction is amphimictic, and at least three generations occur in one growing season. The nematode is able to survive from one crop to the next by remaining coiled in rice stubble and debris and in soil. *Ditylenchus angustus* can survive desiccation for at least 6 months,

but the number of survivors' declines over time, with an average half-life of about 2 weeks. The nematode becomes active when the fields are flooded. Initially, the nematode parasitizes the terminal buds of newly sprouted seeds. It then migrates upwards as the rice plant grows and feeding on new tissue. *Ditylenchus angustus* is found mainly on the stem just above the nodes, on the peduncles, and inside the glumes. The nematode becomes inactive when the plant matures. Hashioka (1963) tested several varieties of rice against this nematode in Thailand and found only the variety, "Khao Tah Haeng 17" to be resistant with an infection rate of 42.9%. According to Miah and Bakr (1977a) *Oryza sabulata* (wild rice) and the cultivars R16-06 showed some resistance to this nematode. A large number of wild rices, modern rice varieties, and breeding lines have been screened for resistance to *D. angustus*. Khao Tah Ooh in Thailand (Hashioka, 1963), B-69-1 in Myanrnar (Sein, 1977), BKN 6986-8 in Vietnam (Kinh and Nghiem, 1982), RDI6-06 and *Oryza subulata* (Miah and Bakr, 1977a) and nine Rayeda lines (Rahman, 1987) are resistant or moderately resistant. In addition, the early maturing cultivars Digha and Padmapani escaped post infection damage (Mondal and Miah, 1987; Rathaiah and Das, 1987). There is a need for ufra resistant varieties, but no resistant variety has been found in high yielding varieties (Miah and Bakr, 1977b; Hashioka, 1963). However, by screening large numbers of varieties from the germplasm collection it may be possible to locate source of ufra resistance.

In Bangladesh rice varieties have been developed by selection, hybridization and back crossing with locally adapted high yielding lines. The number of parental lines used in the breeding programmes is however quite small, resulting in a narrow genetic base. Genetic uniformity in crops can be undesirable in terms of

vulnerability to epidemics and environmental disasters. Conventional breeding is time consuming and depends on environmental conditions. It takes 8-12 years to develop a variety through conventional breeding approach. Molecular marker technology offers a possibility by adopting a wide range of novel approaches to improve the selection strategies in rice breeding. Molecular markers provide 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).

A large number of polymorphic markers enable precise classification of the cultivars, several molecular markers viz. RFLP (Becker *et al.,* 1995; Paran and Michelmore, 1993), RAPD (Tingey and Deltufo, 1993; Wiiliams *et al.*, 1990), SSRs (Levinson and Gutman, 1987), ISSRs (Albani and Wilkinson, 1998; Blair *et al.,* 1999), AFLP (Mackill *et al.*, 1996; Zhu *et al.,* 1998) and SNPs (Vieux *et al.*, 2002) are presently available to assess the variability and diversity at molecular level (Joshi *et al.*, 2000). Information regarding genetic variability and diversity at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars. Thus the present study was conducted with the following objectives:

1. To find out the rice genotypes resistant to ufra.
2. To characterize ufra resistant genotypes at molecular level.

Chapter 2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

Symptom of ufra disease

The ufra nematode, *Ditylenchus angustus*, feed ectoparasitically on the younger tissues of leaf, leaf sheath, peduncle and spikelets. It produces different symptoms at different growth stages of the plant. Splash patterned chlorosis is the first visible symptoms on the leaf and leaf sheath of a seedling. At the advanced stages of the disease, the chlorotic portion becomes brown to dark brown in color. In severe cases the margin of the leaf becomes corrugated and the leaf tip is twisted. Some times several branches are produced from the infested node and make the plant bushy.

Butler (1919) described two types of symptoms in the reproductive phase of the plant: "Thor (meaning swollen) ufra" and "pucca (meaning ripe) ufra''. A spindle shaped enclosed panicle within the leaf sheath is the main symptom of thor ufra while an emergence panicle with sterile florets at the base and some normal grains on the tips is the characteristic symptom of pucca ufra.

Cox (1980) recategorized ufra symptoms at the reproductive phase as ufra I, II and III under Bangladesh conditions. In case of ufra I, the panicles fail to emerge and remain completely enclosed by the leaf sheath. Here the grains remain unfilled and turn dark brown in color. Partial emergence of the panicle with few filled grains at the tip of the panicle and completely empty wrinkled grains inside the leaf sheath are the diagnostic symptoms of ufra II while properly emerged panicles with brown colored unfilled grains considered as the symptom for ufra III.

Epidemiology of ufra disease

Generally ufra appears in patches in the field and spreads wider and wider as the season advances. The central plants of a patch are more severely infested than those at the edges. Humidity, temperature, rainfall and surface moisture are the important environmental factors for ufra disease development. A high humidity of above 85% and temperature from 28-30^{0}c have been reported as favorable for severe infestation by the nematode (Butler, 1919; Miah and Bakr, 1977a and Ou, 1985)

Rahman and Evans (1987) also found a significant difference in infestation level and reduction of plant height when nematodes were inoculated at different ages of plants. The highest infestation (73%) occurred when nematode infested material was mixed with soil at sowing time. It was 60% and 13% when the seedlings were inoculated at 4 weeks and 6 weeks after sowing respectively. Plants grown in infested soil were stunted more than in other treatments.

Varietals screening against ufra

Sarma *et al.,* (1999) evaluated nineteen advanced breeding lines from Bangladesh for resistance to ufra disease (*Ditylenchus angustus*) in North Lakhimpur during 1995-97. Ufra-infested panicles ranged form 3.6% (IR63142-J8-2-1) to 81.8% (Rangabao).Three resistant lines were recommended for deeply flooded areas of Assam, while the remaining lines may be more suitable for less flooded areas.

Twenty nine F2 Populations and their progenies were tested for ufra (*Ditylenchus angustus*) resistance during 1990-93. In 1993, 124 selected lines of F5 populations were further evaluated. Some 64 of the lines survived and flowered successfully. Of these, 10 deep

water lines had mild or no resistance symptoms, while 7 other resistant entries were short stemmed and may be used as resistance sources for rainfed and irrigated rice. These entries had either Bazail 65 or Rayeda 16-06 as resistant parent. Seedlings with primary infestations and resistance symptoms did not survive to maturity, but 91-96% of the secondary tillers that emerged from them survived and flowered successfully. In contrast, neither seedlings with primary infestation nor secondary tillers of susceptible varieties survived. Nematode numbers were 0-101/plant in resistant entries and 141- 3280/plant in the susceptible ones. Rayeda 16-06-1 did not have any symptoms or nematodes in this experiment (Rahman, 1994).

Rice cv. Rangabao was sown in the field in Assam on 20 Mar., 4 Apr., 19 Apr., and 4 May in the 1990 and 1991 wet seasons. The late sown crop suffered lowest infestation by *Ditylenchus angustus*. In a separate experiment, the effects of soaking seeds and/or spraying plants with Hostathion [triazophos] or monocrotophos and growing resistant var. Rayeda 16-06 or the early var. Padmapani on disease severity were compared. Padmapani completely escaped infestation while Rayeda 16-06 had the lowest disease severity compared with other treatments (Bhagawati and Bora, 1993).

In field trials during the 1989 and 1990 wet seasons with 100 deep water rice varieties and breeding lines screened for resistance to ufra disease, (caused by nematode *Ditylenchus angustus*), only one variety (Rayeda 16-06) was completely resistant, 5 were moderate resistant, 6 were susceptible, 2 escaped disease because of early crop maturity (September) and the remaining lines were highly susceptible (Bora and Medhi, 1992).

In a series of trails during 1981-85 growing seasons, 1358 deep water varieties were screened in pots or deep water tanks for resistance to *Ditylenchus angustus*. In the pot trials 3 and 4 week old seedlings were inoculated by releasing 100 active nematodes/seedling into 8-10 cm of water, on the basis of percentage infestation and number of nematodes/ plant, 9 varieties were resistant and 9 moderately resistant. These varieties were retested in tanks, each being sown in three 1×1 m plots and inoculated. At harvest, panicles were evaluated for disease and healthy panicles were assessed for yield on the basis of 14% grain moisture content. The 9 resistant and the 9 moderately resistant varieties had 1-20 and 21-40% infestation, respectively, and grain yields ranging form 4.1 to 5.0 and 1.6 to 3.6 t/ha, respectively. The 9 resistant varieties belonged to the Rayeda 16 series (Rahman, 1987).

During 1972-74, when 151 varieties and 76 crosses were screened for resistance to *Ditylenchus angustus* in infected plots. 60 varieties and twelve crossed died including C4-63, IR5, IR24 and many varieties grown in the Irrawaddy Delta. Only B69-1 (Tha-baung-mee-gok), an Irrawaddy selection, proved to be tolerant, having more than 72% fertile tillers in all three seasons (Sein, 1977).

Tested of 8 species of wild rice and one cultivar were inoculated with *Ditylenchus angustus*, only *Oryza sabulata* and the cultivar R16-06 showed resistance. Infection started on R16-06 but did not develop whereas there was no infection at all on *O. sabulata*. The species that became infected were *O. glaberrima*, *O. nivara*, *O. officinalis*, *O. rufipogon*, *O. sativa* var. *fatua* and *O. spontanea* (Miah and Bakr, 1977b).

Molecular characterization of rice

Plant genetic diversity is a key component of any agricultural production system. This genetic diversity or similarity may be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera or high taxonomic categories (Paterson *et al.*, 1991).

In recent years, different marker systems such as Restriction Fragment Length Polymorphisms (RFLPS), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs). Simple Sequence Repeats (SSRs) or microsatellites, Single Nucleotide Polymorphisms (SNPs) and others have been developed and applied to a range of crop species including cereals.

The experiments were conducted to determine genetic variability and relationship among seven Egyptian rice genotypes namely Giza 178, Giza 177, Giza 175, Giza 171, Giza 172, Sakha 102 and sakha 101, by using eight RAPD primers, six SSR primers pairs, eight AFLP Primers, combinations RAPD, SSR and AFLP techniques characterized the seven rice varieties by a large number of unique markers, which revealed 17.4 and 65 unique markers respectively. The level of polymorphism as revaled by RAPD, SSR and AFLP was 72.2, 90 and 67.9% respectively. The highest genetic relationship as revealed by combined RAPD, SSR and AFLP was detected between Giza 175 and Giza 177 (83.4%), while the lowest similarity was found between Giza 178 and sakha 107 (61.5%). Dendrograms derived from different techniques include minor differences in Clustering pattern but did not affect the main grouping of the different genotypes (Mahmoud *et al.*, 2005).

The genetic relationship of popular Iranian rice cultivars were determined by using SSR markers. 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 relationships among the cultivars were 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 programme. Local Iranian cultivars showed high degree of polymorphism (Ali *et al.*, 2003).

To detect genetic diversity among 23 accessions of rice germplasm form 5 countries (China, Brazil, Japan, Korea, and Pakistan), 16 simple sequence repeats (SSR) primers of functional genes were used. The average number of alleles per SSR locus was 5.2 with a range from 2 to 10. Genetic similarities among the 23 rice accessions ranged from 0.13 to 0.64. The Japonicas from Brazil, Japan and China were classified into class 1, along with upland rice from Brazil. The indicas from Pakistan and Korea were classified into class II (Zhao *et al.*, 2002).

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).

SSR markers were used for estimatation of genetic diversity in traditional and evolved Basmati (EB) and semi dwarf non-Basmati (NB) rice varieties. A subset of three rice groups was analyzed by using 19 simple sequence repeat (SSR) loci and 12 inter-SSR-PCR Primes. A total of 70 SSR alleles and 481 inter-SSR-PCR markers were revealed in 24 varieties from the three groups. The lowest genetic diversity was observed among the traditional Basmati rice. The evolved Basmati varieties showed the highest genetic diversity by both markers assays. The results indicated that the subset of aromatic rice varieties is probably derived from single landrace (Nagaraju *et al.*, 2002).

Analyzed with 15 microsatellite markers in comparison to a representative collection of 145 Asian rice varieties from France. Diversity of weedy rice is in agreement with the genetic structure of *Oryza sativa* separated in two main indica and japonica groups of varieties, Weeds from France showed a greater diversity than local Mediterranean varieties (temperate japonica) as well as original alleles or alleles more typical of indica varieties. Results suggest that distant crosses between indica and japonica varieties and their possible effects on genome expression could be also a relevant explanation to weed origin (Bres *et al.*, 2002).

To evaluate the genetic diversity molecular markers were used within a diverse collection of rice (*Oryza sativa* L.) accessions and to determine differences in the patterns of diversity within the two rice subspecies indica and japonica. Thirty eight rice cultivars of particular interest to U.S breeding Programs and two wild species accessions (*O. rufipogon Griffith* and *O. nirvara sharma et. shastry*) were analyzed with 111 micro satellite markers. A total of 753 alleles were detected and the number of

alleles per marker ranged from 1 to 17 with an average of 6.8. A positive correlation was found between the number of alleles per locus and the maximum number of repeats within a microsatellite marker. Compared to indica cultivars, the japonica group showed significantly higher genetic diversity on chromosomes 6 and 7 and considerably lower diversity on chromosome 2 (Junjian *et.al.*, 2002).

Genetic diversity and population genetic structure of natural *Oryza rufipogon* populations in China was determined by ten microsatellite loci. For a total of 237 individuals of 12 populations collected from four regions, a moderate to high level of genetic diversity was observed at population levels with the number of alleles per locus (A) ranging from 2 to 18 (average 10.6) and polymorphic loci (p) from 40.0% to 100% (average 83.3%). The observed heterozygosity (HO) varied from 0.163 to 0.550 with the mean of 0.332, and the expected heterozygosity (HE) from 0.164 to 0.648 with the mean of 0.413. The level of genetic diversity for Guangxi was the highest. These results are in good agreement with previous allozyme and RAPD studies (Zhou *et al.*, 2003).

The extent of genetic diversity has been studied among 27 rice accessions from diverse hydrological habitats using microsatellite markers. A total of 61 SSR primers were screened and 26 primers produced scorable bands in the rice genotypes. Cluster analysis was carried out to group the 27 genotypes based on the marker data from these 26 SSR primers. The average polymorphism information content value was 0.58. A dendrogram was constructed based on the similarity index. The accessions were grouped into 2 major clusters. Cluster A compared of 15 accessions contained all improved

cultivars, except IR62266. Cluster B was composed of 12 accessions. Clustering represented the genetic similarity among the accessions as well as their hydrological habitat adaptation. In cluster A, Azucena was most distantly related to IR36 and IR64 (Chandra *et al.*, 2004).

Eighty one SSR markers were used to estimate genetic diversity of 94 japonica rice using 81 SSR has been assessed. All 81 SSR markers generated a total of 351 alleles. The number of alleles ranged from 1 to 16 with an average of 4.3 alleles per SSR marker. 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 (Suh-Jung *et al.*, 2004).

Genetic diversity has been assessed by analyzing SSR markers and prepared a DNA fingerprint database of 24 rice genotypes. A total of 229 alleles were detected at the 50 SSR loci and 49 alleles were present in only one of the 24 cultivars. The size difference between the smallest and largest allele varied from I (RM333) to 82 (RM206). Multiple alleles were observed in 13 loci. Polymorphism information content (PIC) values ranged from 0.0 (RMK.167) to 0.78 (RMI70), with an average of 0.62 per marker. At 15 of the SSR loci, traditional and cross-bred Basmati rice cultivars amplified a higher number of diverse alleles compared to other rice genotype (Priyanka *et al.*, 2004).

The 18 rice genotypes were analyzed to estimate genetic diversity by using AFLP, ISSR and SSR markers. All the three marker systems generated higher levels of polymorphism and could distinguish between all the 18 rice cultivars. The minimum number

of assay-units per system needed to distinguish between all the cultivars was one for AFLP, two for ISSR and five for SSR. A total of 171 (110 polymorphic), 240 (188 polymorphic) and 160 (159 polymorphic) bands were detected using five primer combination of AFLP, 25 UBC ISSR primers and 30 well distributed, mapped SSR markers, respectively. The study emphasizes the need for using a combination of different marker systems for a comprehensive genetic analysis of Basmati rice germplasm (Navinder *et al.*, 2004).

The genetic diversity of 236 rice accessions were investigated on the basis of genotypic evaluation at 113 RFLP and 60 SSR loci. A total of 274 RFLP and 714 SSR alleles were detected in the entire dataset. The average PIC values were 0.36 for the RFLP and 0.66 for the SSR markers. The accessions from the world collection represented 96% of the SSR diversity, while the cultivars from the USA contained 56% of SSR alleles. Significant differences in allele frequencies were observed between the world and U.S. collections and between older U.S. cultivars and their modern derivatives. A diverse subset of 31 rice cultivars (13% of the 236 cultivars) was identified that embodied 74% of SSR alleles (Yunbi *et al.*, 2004).

The genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers were studied. Salt tolerance was then analyzed among molecularly characterized genotypes. A total of 123 alleles were generated at 25 microsatellite loci among the 33 genotypes. Their results indicated that the adaptation of rice to saline soils is different among genotypes with diverse genetic backgrounds (Zeng *et al.*, 2004).

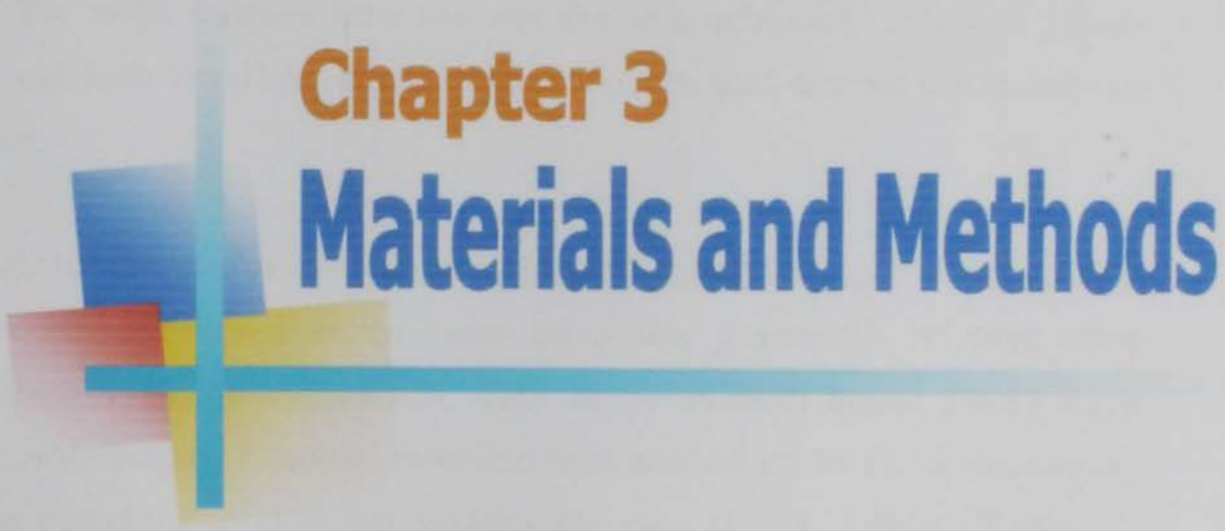
AFLP and SSR analyses were performed using 95 cultivars of local and modern sake-brewing rice together with 76 cultivars of local

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).

The variation among 23 rice cultivars (including Milyang and its relatives) for the presence of blast (*Helminthosporium maydis* [*Cochliobolus heterostropshus*]) resistance genes were investigated. A total of 54 SSK markers representing 57 loci in the rice genome detected polymorphism among the cultivars. The cultivars were classified into 4 groups by cluster analysis and cultivars with the same parents were clustered together. The high quality cultivars Daesanbyeo, Donganbyeo and Milyang 95 belonged to one cluster (Hwang *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 variations 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).

The genetic diversity and DNA fingerprinting of 15 elite rice genotypes using 30 SSR primers on chromosome numbers 7-12 has been investigated. The results revealed that all the primers showed distinct polymorphism among the cultivars indicating the robust nature of microsatellites in revealing polymorphism. Cluster analysis grouped the rice genotypes into 10 classes in which japonica types D11-1 (Azucena) and Moroborekan clustered separately from indica types. Principal component analysis was done to visualize genetic relationships among the elite breeding lines. The larger range of similarity values for related cultivars using microsatellites provides greater confidence for the assessment of genetic diversity and relationships (Chakravarthi *et al.*, 2006).



Chapter 3
Materials and Methods

CHAPTER 3

MATERIAIS AND METHODS

Experiments were conducted in the research field and laboratory of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur during 2006-2007.

Screening of F2 populations against ufra disease

A total of 13 lines of F2 populations were tested against urfa. Different populations were sown in the pot following completely randomized design with three replications. After 10 days, seedlings were inoculated by spreading nematode infested plant materials from the BR3 culture into the pot for this purpose. Infested plants were cut into small pieces of about 2-3 cm and spread uniformly in the pot.

Chlorotic discoloration (diagnostic ufra symptom at the vegetative stage) was observed at the base on a few plants 25-30 days after inoculation. Some symptoms were more evident about 50-55 days after inoculation. Disease reaction was scored at 55 DAS according to the standard evaluation system for rice (IRRI, 1996) (Table 1). The number of nematodes/plant under different categories of ufra resistant and susceptible genotypes was counted under microscope. In each category, 5 plants were taken and cut into small pieces and immerged in the water of petridishes for this purpose.

Table 1. Disease reaction scored according to the standard evaluation system for rice.

Infected tiller (%)	Scale	Symptoms
0	0	May or may not be visible
1-20	1	Visible symptoms
21-40	3	Visible symptoms
41-60	5	Visible symptoms
61-80	7	Visible symptoms
81-100	9	Visible symptoms

Source: IRRI (1996).

Based on the percentage of infestation, test entries were classified into six broad groups, highly resistant, resistant, moderately resistant, susceptible, moderately susceptible, and highly susceptible (Table 2).

Table 2. Six broad groups of test entries based on percentage of plants infested with urfa disease.

Group	Infestation (%)
Highly resistant (HR)	0
Resistant (R)	1-20
Moderate resistant (MR)	21-40
Susceptible (S)	41-60
Moderate susceptible (MS)	61-80
Highly susceptible (HS)	81-100

Source: Rahman (1993).

Data recording

Data on following characters were recorded:

1. Total number of tiller.
2. Total number of infested tiller.
3. Percentage of infested tiller

Molecular characterization of ufra resistant genotypes

A total of 12 consisting of two highly resistant, seven resistant, two highly susceptible and one susceptible genotypes were used in this study (Table 3). The seeds for all the genotypes were obtained from Genetic Resources and Seed Division, BRRI, Gazipur.

Table 3. List of ufra resistant and susceptible genotypes

SL. No	Variety	Accession No.	Source of origin	Type[a]
1.	Fukuhonami	3342	BRRI	Highly Resistant (Local type)
2.	Matsuhonami	3352	BRRI	Highly Resistant (Local type)
3.	Rayeda	4851	BRRI	Resistant(Local type)
4.	Bazail	165	BRRI	Resistant(Local type)
5.	Hunenwase	3348	BRRI	Resistant(Local type)
6.	Shinanokogane	3359	BRRI	Resistant(Local type)
7.	Kinonishiki	3362	BRRI	Resistant(Local type)
8.	Aokazi	3395	BRRI	Resistant(Local type)
9.	Koshinishini	3397	BRRI	Resistant(Local type)
10.	Reihou	3370	BRRI	Susceptible(Local type)
11.	BR3	-	BRRI	Highly Susceptible(HYV)
12.	BR11	-	BRRI	Highly Susceptible(HYV)

BRRI= Bangladesh Rice Research Institute (BRRI)

[a]Source: Ullah (2006).

In total nine primers including 6 SSR and 3 VNTR were studied. Out of six, five SSR markers along with another three VNTR markers showed polymorphism and used in this study (Table 4).

Table 4. SSR and VNTR primers used in the present study

Primers	Oligonucleotide sequence
SSR	
RM21	ACAGTATTCCGTAGGCACGG R GCTCCATGAGGGTGGTAGAG F
RM222	CTTAAATGGGCCACATGCG R CAAAGCTTCCGGCCAAAAG F
RM80	TTGAAGGCGCTGAAGGAG R CATCAACCTCGTCTTCACCG F
RM108	TCTCTTGCGCGCACACTGGCAC R CGTGCACCACCACCACCACCAC F
RM23	CATTGGAGTGGAGGCTGG R GTCAGGCTTCTGCCATTCTC F
VNTR or Mini satellites	
RY	CAGCAGCAGCAGCAG
MR	GAGGGTGGCGGTTCT
GF	TCCTCCTCCTCCTCC

F=Forward; R=Reverse

Genotypic data

The following steps were followed for genotypic analysis

Collection of leaf sample

To extract genomic DNA, young and fresh leaves were collected from different pots of 12 varieties.

Reagents preparation for DNA extraction (stock solution)

DNA Extraction Buffer

Stock solution	Concentration enquired	Volume added for 100ml
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.

1M Tris. HCI, 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 500ml with distilled water. After that it was placed in a bottle and was sterilized by autoclaving.

5M NaCl

For 100ml: 29.22g of sodium chloride was weighed out and was added about 75ml 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 100ml, mixed 70ml of ethanol with 30ml of sterile distilled water.

Sterile Distilled water

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

DNA Extraction

Genomic DNA from each genotype was isolated from fresh leaf tissue of 21 day-old plants. One gram of fresh living leaves was taken from the plant, cut into small pieces (about 0.5cm), wrapped in aluminium foil and quickly placed in freezer at -20°C. Samples were kept in freezer for one hour. Leaves were ground to powder by a pestle and mortar that had been pre-cooled to -20°C.

Approximately 200 mg of powdered leaf was put into each micro centrifuge tube (about 1/3 full) and was replaced into freezer until further use. All tubes were taken out from the freezer and quickly added 800 µl of extraction buffer into each and mixed thoroughly. Tubes were placed in 65°C water baths for 10-15 minutes mixing by inversion every few minutes. Then tubes were centrifuged at 13000 rpm for 5 min. Supernatant was added to fresh tube with 500 µl of chloroform, mixed well and spun 13000 rpm for 5 min. Aqueous layer was transferred to new tube (careful to avoid interface). To each tube one volume of isopropanol and 0.1 volumes of 3M sodium acetate (pH 5.2) was added and mixed by inversion. Tubes were kept on ice up to 30 minutes and spun at 13000 rpm for 5 minutes. After that supernatant was removed from the tubes. DNA was washed in 400 µl 70% ethanol and air dried for 15 minutes. Again DNA was resuspended in 150 µl TE buffer depending on pellet size.

Confirmation and Quantification of DNA concentration

It was important to know the concentration of genomic DNA before PCR (Polymerase Chain Reaction) amplification. Because different DNA extraction methods produced DNA of widely different purities. It was necessary to optimize the amount of DNA for achieving reproducibility and strong signal in PCR assay. Excessive genomic DNA may result smears or lack of clearly defined bands in the gel. On the other hand, too little DNA will give non-reproducible patterns (Williams *et al.*, 1993).

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 25 ng/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.

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 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.

VNTR-PCR Protocols

PCR components were used per reaction (15.0 µl volume) for VNTR analysis: 6 ng of DNA template (1 µl), 0.2 µl of 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_20), 2.0 µl of 3 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°C for 20 seconds 45°C for 30 seconds, 72°C for 2 minutes with a final extension step of 5 minutes at 72°C.

The PCR was carried out in an Appendrof thermocycler using 25 well plates. Amplification products were resolved by agarose (2%) gel electrophoresis using in TBE buffer and 8 µl samples were loaded in gel. Each gel was run for 2-3.5 hrs at 100 volts and stained in ethidium bromide.

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 saved in the computer.

Molecular weight for each band was measured by using Alfa Imager software version 5.5 Polymorphism information content (PIC) values were calculated with the following formula (Botstein *et al.*, 1980):

$$PICi = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where n is the number of marker alleles for marker i and P_{ij} is the frequency of the jth allele for marker i.

Data analysis

SSR marker alleles and VNTR marker loci were scored as present (1) or absent (0). Pair wise comparisons between genotypes were calculated using the Nei's genetic distance using the program Free Tree (Pavlicek *et al.,* 1999). A dendrogram representing the genetic relationships between genotypes, based on the un-weighted pair group method with arithmetic averages (UPGMA), was constructed using the web-based version of the Drawgram program which is part of the Phylogeny Inference Package (PHYLIP 3.5) (Felsenstein, 1993) available on the Pasteur Institute server (http://bioweb-pasfeur.fr/seqanal/interfaces/drawgram-simple.html).

Chapter 4
Results and Discussion

CHAPTER 4

RESULTS AND DISCUSSION

Screening of F2 population against ufra disease

Based on percentage of infested plants, F2 populations showed varying degrees of susceptibility to ufra (Table 5). The populations of 13 F2 lines were found highly resistant (HR), resistant (R), moderate susceptible (MS), susceptible (S) and highly susceptible (HS). Among the 1146 plants of F2 population under 13 entries, 406 were found highly resistant, 216 moderately resistant, 157 resistant, 94 moderate susceptible, 61 susceptible and 212 highly susceptible. Assessment of individual plants also indicated a variation of development of symptom both between and within the test entries. The resistant symptom which was being recorded in preliminary separation of resistant entries. This symptom appeared in whitish patches of varying sizes on the affected leaf blades which subsequently changed to brown or black in color. The mid rib of the affected leaf also became brown or black. The symptom was seemed hypersensitive reaction of the leaf tissues in response of nematode feeding. The highest percentage of highly resistant plant was observed in entry BR7750 followed by BR7758, BR7760, BR7755, BR7769 and BR7756. The highest percentage of resistant plant was observed in entry BR7751 followed by BR7754, BR7761, BR7769 and BR 7750. The highest percentage of highly susceptible plant was found in entry BR7765 followed by BR BR7760, BR7761, BR7766 and BR7757.

This type of screening method was followed in Thailand (Hashioka, 1963), Myanmar (Sein, 1977), Vietnam (Kinh and Nghiem, 1982) and India (Bora and Medhi, 1992). The highest number of nematode/plant was recorded in HS followed by S, MS, MR, R and HR (Fig.1). Similar findings are reported by Rahman (1993). The highly resistant and resistant plants were selected for hybridization program for the development of ufra resistant varieties.

Table 5. Categorization of ufra symptom in 13 selected lines of F2 generation at 55 days after inoculation (DAI).

Entry Name	DAI	Categorization of ufra symptoms (%) (Number of the plants showing)					
		HR	R	MR	MS	S	HS
BR7750	55	67	16	7	1	1	1
BR7751	55	23	25	21	6	4	10
BR7752	55	10	9	25	18	5	18
BR7754	55	36	24	21	4	2	5
BR7755	55	39	9	25	7	6	6
BR7756	55	31	2	24	6	6	10
BR7757	55	20	10	24	8	9	20
BR7758	55	48	6	9	9	5	11
BR7760	55	48	5	6	5	2	30
BR7761	55	21	20	12	5	7	30
BR7765	55	12	5	6	11	1	36
BR7766	55	12	13	26	8	6	28
BR7769	55	39	18	10	6	7	7
Total		406	157	216	94	61	212

DAI= Days after inoculation; HR=Highly resistant (0% stem infested), R=Resistant (1-20% stem infested), MR=Moderate resistant (21-40% stem infested), MS=Moderate susceptible (41-60% stem infested), S=Susceptible (61-80% stem infested), HS =Highly susceptible (81-100% stem infested).

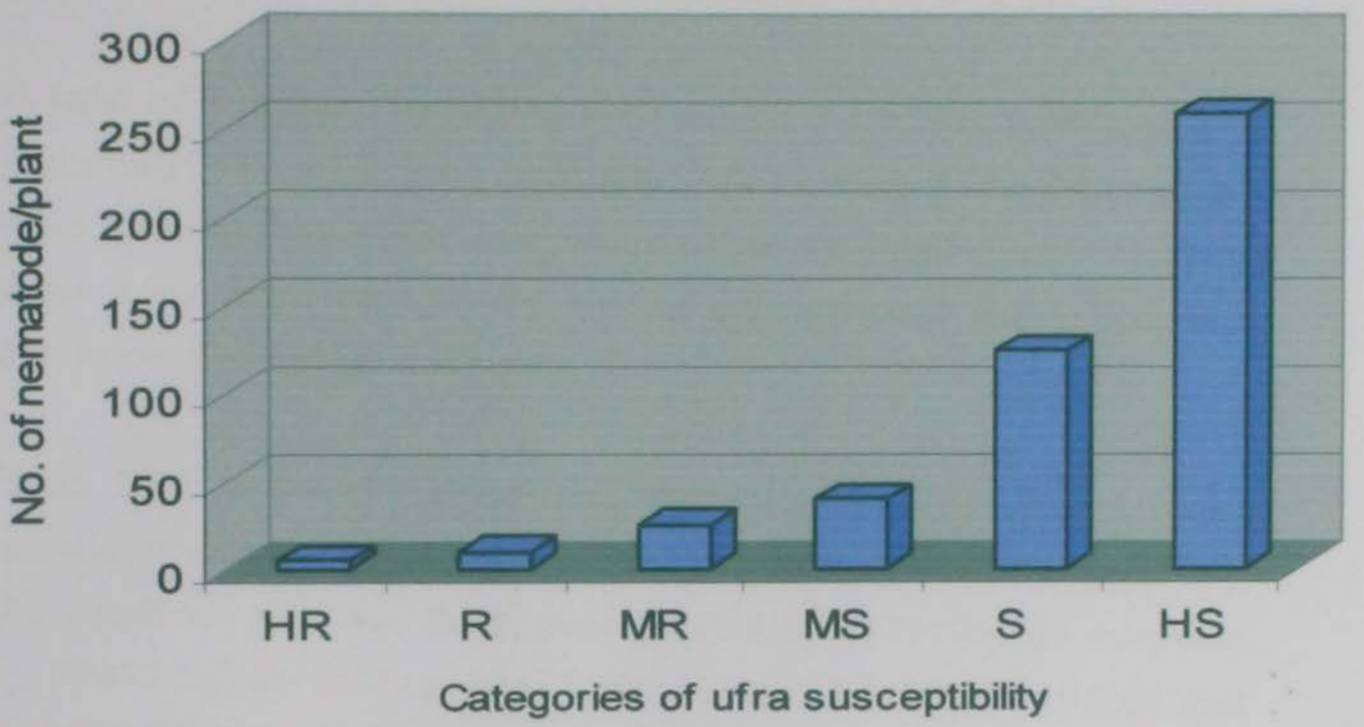


Fig.1.Showing nematode population in different categories of ufra susceptible and resistant genotypes (HR-Highly resistant, R-Resistant, MR-Moderate resistant, MS-Moderate susceptible, S-Susceptible and HS-Highly susceptible).

Molecular characterization of ufra resistant genotypes

Isolated DNA of 12 genotypes showed nearly sickle shaped bands indicated good quality DNA and was quantified by λ DNA marker (Fig.2). DNA for each genotype was quantified as 6ng/µl.

A total of 9 primers including 6 SSRs and 3 VNTRs were tested in this study. Out of 6 SSR markers 5 along with 3 VNTR markers were found polymorphic and produced strong bands (Table 4). Only these 8 markers were scored among ufra resistant and susceptible genotypes for further analysis.

Each SSR marker's allele and different loci of VNTR were measured and are presented in Table 6. Among the SSR markers, the amplicon size was the highest in RM 80 (129-165bp) followed by RM222 (148-157bp), RM21 (90-120bp), RM23 (56-105bp) and RM108 (41-54bp). Range of amplicon size also higher in RM80 (129-165bp) compared to other SSR markers. These results indicated that RM80 (129-165bp) may produce more alleles. But here marker RM108 was produced the highest alleles (6). This information could be used in further study of molecular characterization with other genotypes.

RM108 (41-54bp) SSR marker produced 6 alleles while RM21, RM23 and RM80 produced 4 alleles each and RM222 produced 3 alleles (Table 6).The PIC values for SSR markers was from 0.2652-0.7193. The highest PIC values (0.7193) was for RM108 followed by RM21 (0.4793), RM23 (0.4148), RM80 (0.3409) and RM222 (0.2652).

Table 6. Amplicon size, number of allele/locus and PIC value of SSR and VNTR markers for 12 ufra resistant and susceptible genotypes

Primers	Chr .No.	Position on chr. (cM)	Highly resistant		Resistant							Susceptible	Highly susceptible		No. of Allele/ Locus	PIC
			Fukuhonami	Matsuhonami	Rayeda	Bazail	Hunenwase	Shinanokogane	Kinonishiki	Aokazi	Koshinihsini	Reihou	BR3	BR11		
RM21	11	85.7	100	100	100	120	100	95	95	100	100	95	90	90	4	0.4793
RM222	10	11.3	148	148	157	157	152	152	148	152	148	148	148	148	3	0.2652
RM80	8	103.7	140	165	129	152	140	140	140	140	140	140	165	165	4	0.3409
RM108	9	73.3	43	46	52	52	46	54	54	50	50	50	46	41	6	0.7193
RM23	1	72.6	88	105	105	88	88	88	88	56	56	105	62	56	4	0.4148
RY1	-	-	355	355	-	355	355	-	-	-	-	355	355	355	3(locus)	-
RY2	-	-	406	-	-	406	406	406	406	-	-	-	-	-	3(locus)	-
RY3	-	-	-	545	-	545	545	-	545	-	545	-	545	545	3(locus)	-
MR1	-	-	240	240	-	240	240	-	240	-	-	-	-	-	7(locus)	-
MR2	-	-	298	-	298	298	-	298	298	-	298	-	298	298	7(locus)	-
MR3	-	-	402	402	402	402	-	-	402	-	402	-	402	402	7(locus)	-
MR4	-	-	758	758	-	758	758	-	-	-	-	758	758	758	7(locus)	-
MR5	-	-	1061	1061	1061	1061	1061	-	1061	-	-	1061	-	-	7(locus)	-
MR6	-	-	-	-	1320	1320	-	1320	1320	-	-	-	-	-	7(locus)	-
MR7	-	-	1401	1401	-	-	-	1401	1401	-	-	1401	1401	1401	7(locus)	-
GF1	-	-	-	-	342	-	342	-	342	342	-	342	-	342	3(locus)	-
GF2	-	-	454	-	454	454	454	-	-	454	454	-	-	-	3(locus)	-
GF3	-	-	578	578	578	578	578	-	-	578	578	-	578	578	3(locus)	-

PIC=Polymorphism information content; Chro=Chromosome; - = Absent

Therefore, RM108 was considered as the best marker for characterizing the 12 genotypes and to a lesser extent RM21, RM23, RM80 and RM222 should be useful for molecular characterization of ufra resistant and susceptible genotypes. These reports agree with the results of several authors (Islam *et al.*, 2007; Priyanka *et al.*, 2004; Yunbi *et al.*, 2004). As the primers were random so, more than on loci were present in VNTR (Table 6). In each locus present or absent band was identified and no allele was considered. So, therefore no PIC value was observed in VNTR primers.

The amplicons size of RM21 (90bp) was associated with highly susceptible varieties, BR3 and BR11 while that was absent in highly resistant (Fukuhonami, Matsuhonami) and resistant (Rayeda, Bazail, Hunnenwase, Shinokogane, Koshinishini, Kinonishiki and Aokazi) genotypes. The band size 88bp and 105bp of RM23 were found in highly resistant and resistant genotypes. But those were absent in highly susceptible genotypes (Table 6).

The amplicon size of RY1 the (355bp) was absent in the varieties namely Rayeda, Shinanokogane Kinonishiki Aokazi, Koshinishini while that was found in Fukuhonami, Matsuhonami, Bazail, Hunnenwase, Reihou, BR3 and BR11 (Table 6).

Three amplicons, MR1 (240bp), MR5 (1061bp) and MR6 (1320bp) were absent in highly susceptible varieties (BR3 and BR11) and those were found in some entries of highly resistant and resistant genotypes. MR3 (402bp) band was present in Fukuhonami, Matsuhonami, Rayeda, Bazail, Kinonishiki, Koshinishini, BR3 and BR11 while that was absent in Hunnenwase, Shinokogane, Aokazi and Reihou.

The band size of GF2 (454bp) was present in Fukuhonami, Rayeda, Bazail, Hunnenwase, Aokazi, Koshinishini and was absent in Matsuhonami, Shinanokogane, Kinonishiki, Reihou, BR3 and BR11. GF3 (578bp) band was present in Fukuhonami, Matsuhonami, Rayeda, Bazail, Hunnenwase, Aokazi, Koshinishini, BR3 and BR11 and that was absent in Shinanokogane, Kinonishiki and Reihou (Table 6). Fig. 3, 4, 5 6 and 7 showed gel pictures of some SSR and VNTR markers.

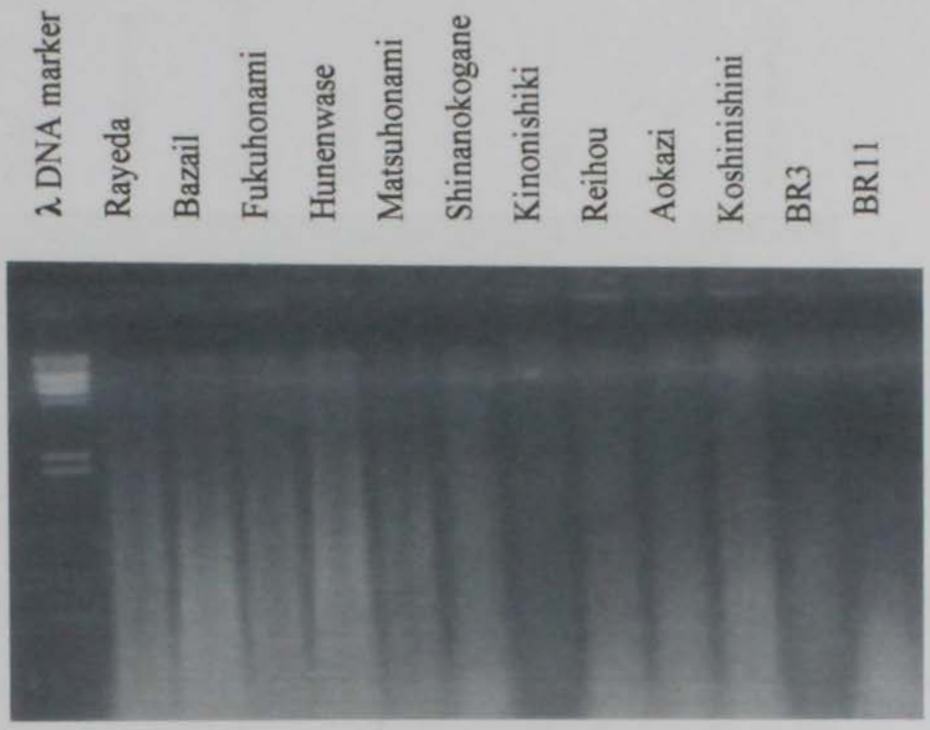


Fig.2. Quantification of DNA of ufra resistant and susceptible varieties through lamda (λ) DNA marker

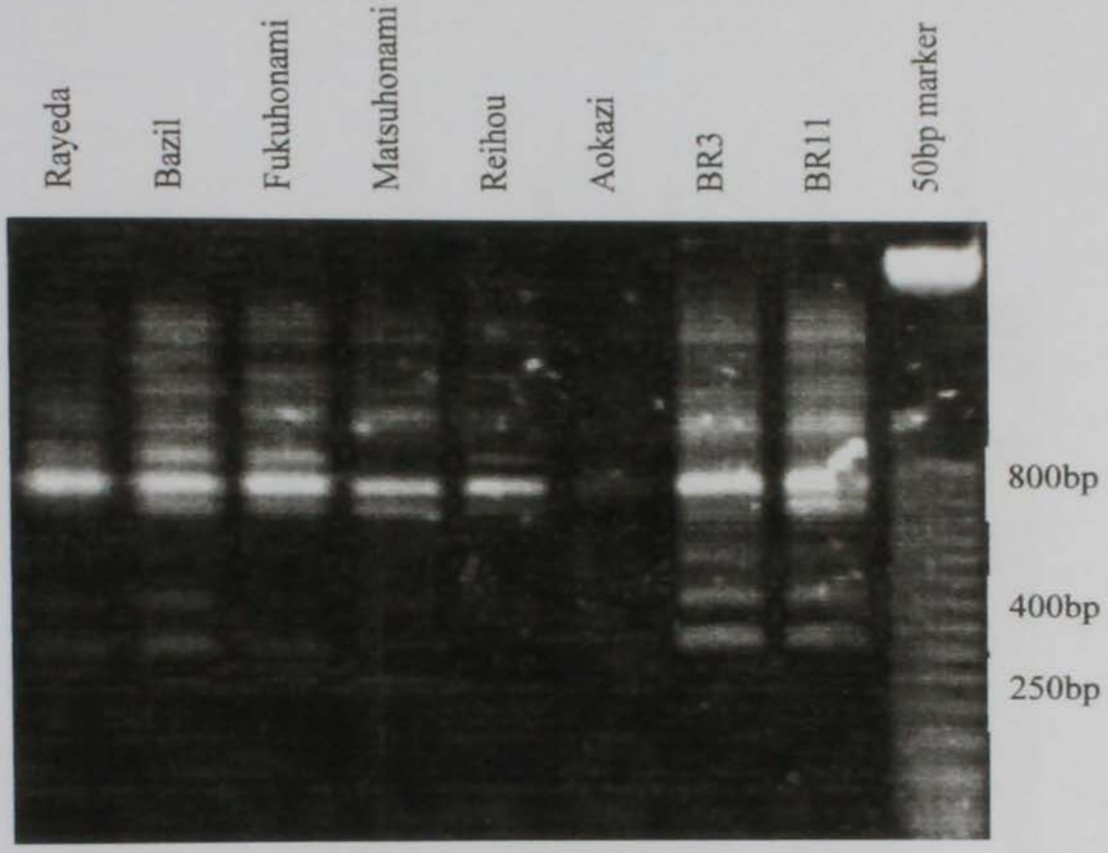


Fig.3. VNTR banding patterns obtained from ufra resistant and susceptible genotypes using MR primer.

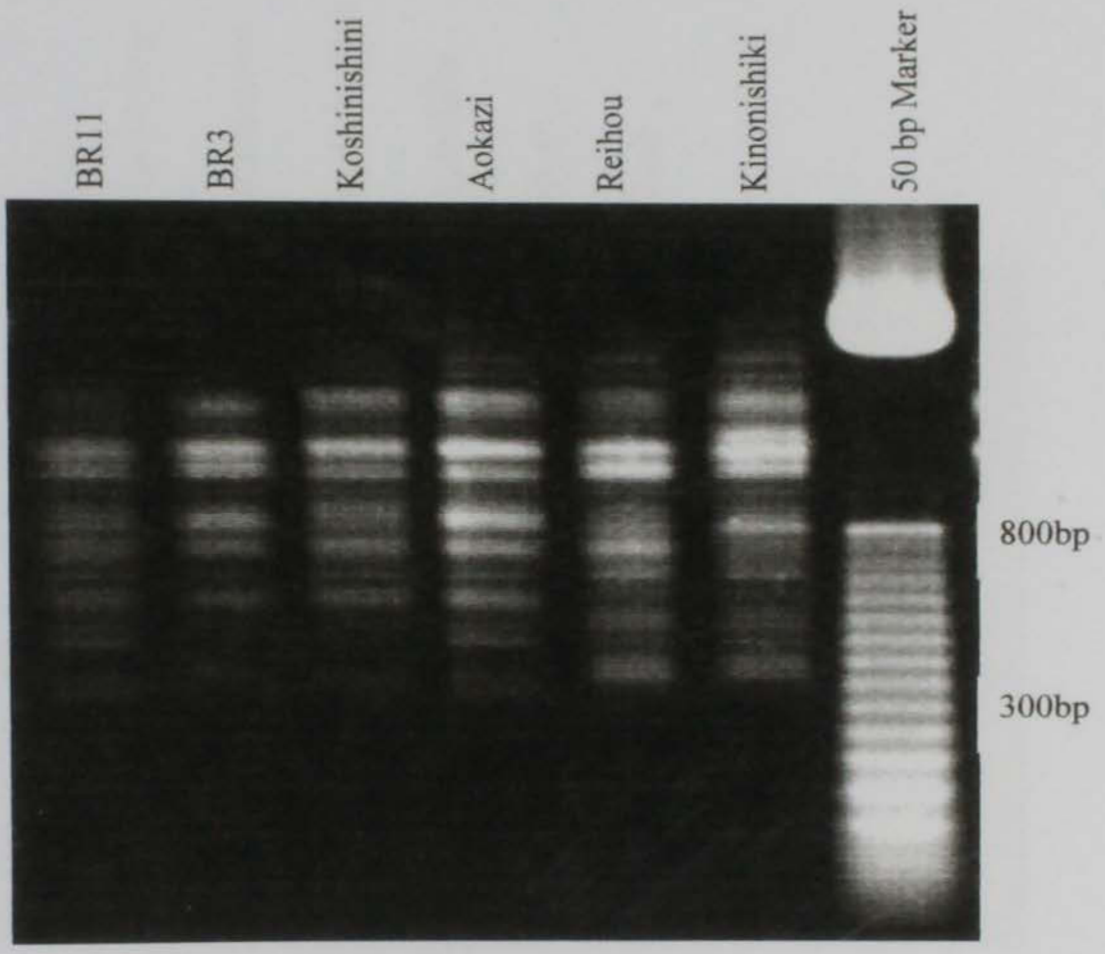


Fig.4. VNTR banding patterns obtained from ufra resistant and susceptible genotypes using GF primer.

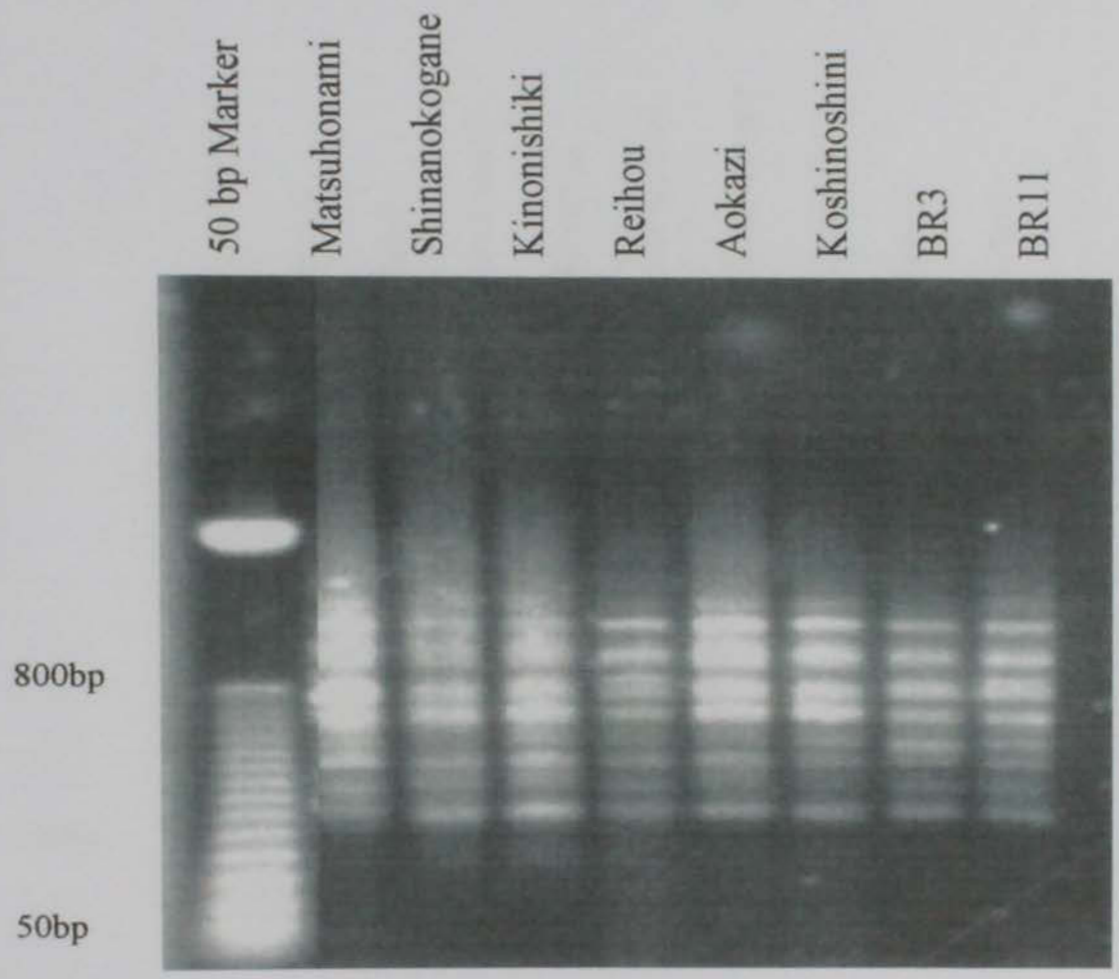


Fig.5. VNTR banding patterns obtained from ufra resistant and susceptible genotypes using RY primer.

Fig.6. SSR banding patterns obtained from ufra resistant and susceptible genotypes using RM 21 primer.

Fig.7. SSR banding patterns obtained from ufra resistant and susceptible genotypes using RM 80 primer.

For diversity analysis pair wise genetic distances were computed between all genotypes. Genetic distance was the smallest between Hunenwase and Shinanokogane (0.2500) followed by Fukuhonami and Bazail (0.2857), Aokazi and Koshinishini (0.2857), Shinanokogane and Kinonishiki (0.3000), Hunenwase and Matsuhonami (0.3333), Kinonishiki and Bazail (0.3333), Koshinishini and Hunenwase (0.3333), Rayeda and Bazail (0.3636), Hunenwase and Aokazi (0.3750) and Kinonishiki and Koshinishini (0.3750) (Table 7). The result suggested that these varieties might have been developed from common ancestor. On the other hand genetic distance was the highest between Aokazi and Reihou (0.8333), Aokazi and BR11 (0.8333), Shinanokogane and Matsuhonami (0.8333) followed by Shinanokogane and Aokazi (0.8000), Aokazi and BR3 (0.8000), Matsuhonami and Rayeda (0.7143), Aokazi and Bazail (0.7143), Reihou and Rayeda (0.7143), BR3 and Rayeda (0.7143), Aokazi and Matsuhonami (0.6667), Reihou and Koshinishini (0.6667), Kinonishiki and Aokazi (0.6667), Shinanokogane and Rayeda (0.6250), BR11 and Rayeda (0.6250), Shinanokogane and BR3 (0.6250) and Reihou and BR3 (0.6250). In order to develop ufra resistant varieties and broaden the genetic base of rice varieties new breeding program should initiated preferably using the parents, BR11 and Aokazi, BR3 and Aokazi, Rayeda and BR3 and Rayeda and BR11. Although the genetic distances between the parents Shinanokogane and Aokazi, Matsuhonami and Rayeda, Aokazi and Bazail, Reihou and Rayeda, Aokazi and Matsuhonami, Reihou and Koshinishini, Kinonishiki and Aokazi and Shinanokogane and Rayeda were higher but these parents were local types. So the hybridization program would not be suggested among them. Allelic variation and genetic diversity of genotypes with abiotic and biotic stress tolerlence has been studied by the several scientists in whom and abroad (Latif *et al.*, 2007b; Hwang *et al.*, 2004; Zeng *et al.*, 2004).

Table 7. Distance matrix based on Nei's genetic distance among ufra resistant and susceptible genotypes.

	Resistant							Susceptible	Highly Susceptible		Highly Resistant	
Genotypes	Rayeda	Bazail	Hunen wase	Shinano kogane	Kinoni shiki	Aokazi	Koshin ishini	Reihou	BR3	BR11	Fukuhon ami	Matsuhona mi
Rayeda	0.0000											
Bazail	0.3636	0.0000										
Hunenwase	0.5556	0.3846	0.0000									
Shinanokogane	0.6250	0.5000	0.2500	0.0000								
Kinonishiki	0.5556	0.3333	0.4000	0.3000	0.0000							
Aokazi	0.5000	0.7143	0.3750	0.8000	0.6667	0.0000						
Koshinishini	0.5000	0.4545	0.3333	0.5714	0.3750	0.2857	0.0000					
Reihou	0.7143	0.5556	0.4000	0.4444	0.4444	0.8333	0.6667	0.0000				
BR3	0.7143	0.4000	0.5000	0.6250	0.4000	0.8000	0.5000	0.6250	0.0000			
BR11	0.6250	0.4167	0.5000	0.5556	0.4545	0.8333	0.5556	0.4444	0.4545	0.0000		
Fukuhonami	0.5000	0.2857	0.4167	0.5000	0.4545	0.5714	0.4000	0.5000	0.5000	0.4167	0.0000	
Matsuhonami	0.7143	0.4000	0.3333	0.8333	0.4444	0.6667	0.5000	0.5714	0.4444	0.5000	0.5000	0.0000

Twelve genotypes including ufra resistant and susceptible were used to make of dendrogram based on Nei's (1972) genetic distance using Unweight Pair Group of Arithmetic Means (UPGMA). Clustering represented the genetic similarity among the genotypes as well as their susceptibility to ufra disease. In this study twelve rice genotypes had been differentiated into three main clusters; Bazail was grouped in cluster 1 (Fig.8).The genotypes BR11, Mutsuhonami, BR3, Kinonishiki, Reihou, Shinanokogane and Hunenwase formed cluster 2, Fukuhonami, Rayeda, Koshinishini, and Aokazi formed cluster 3. In cluster 2, it was divided into two sub-clusters, such as sub-cluster A and B. Sub-cluster A consisted of three genotypes i.e. BR11, BR3 and Mutsuhonami. This sub-cluster was unexpected because Mutsuhonami hignly resistant. So, using more markers this relationship could be confirmed. These results are with the agreement of Islam *et al.*, (2007). Another sub-cluster B consisted of four genotypes Kinonishiki, Reihou, Shinanokogane and Hunenwase. Reihou was susceptible variety and this relationship should also be confirmed by using sufficient SSR markers. In cluster 3 consisted of four genotypes i.e. Fukuhonami, Rayeda, Koshinishini and Aokazi. 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 genotypes belonging to cluster 1st, 2nd and 3rd having greater cluster distance are recommended for inclusion in a hybridization program as they are expected to produce good segregants.

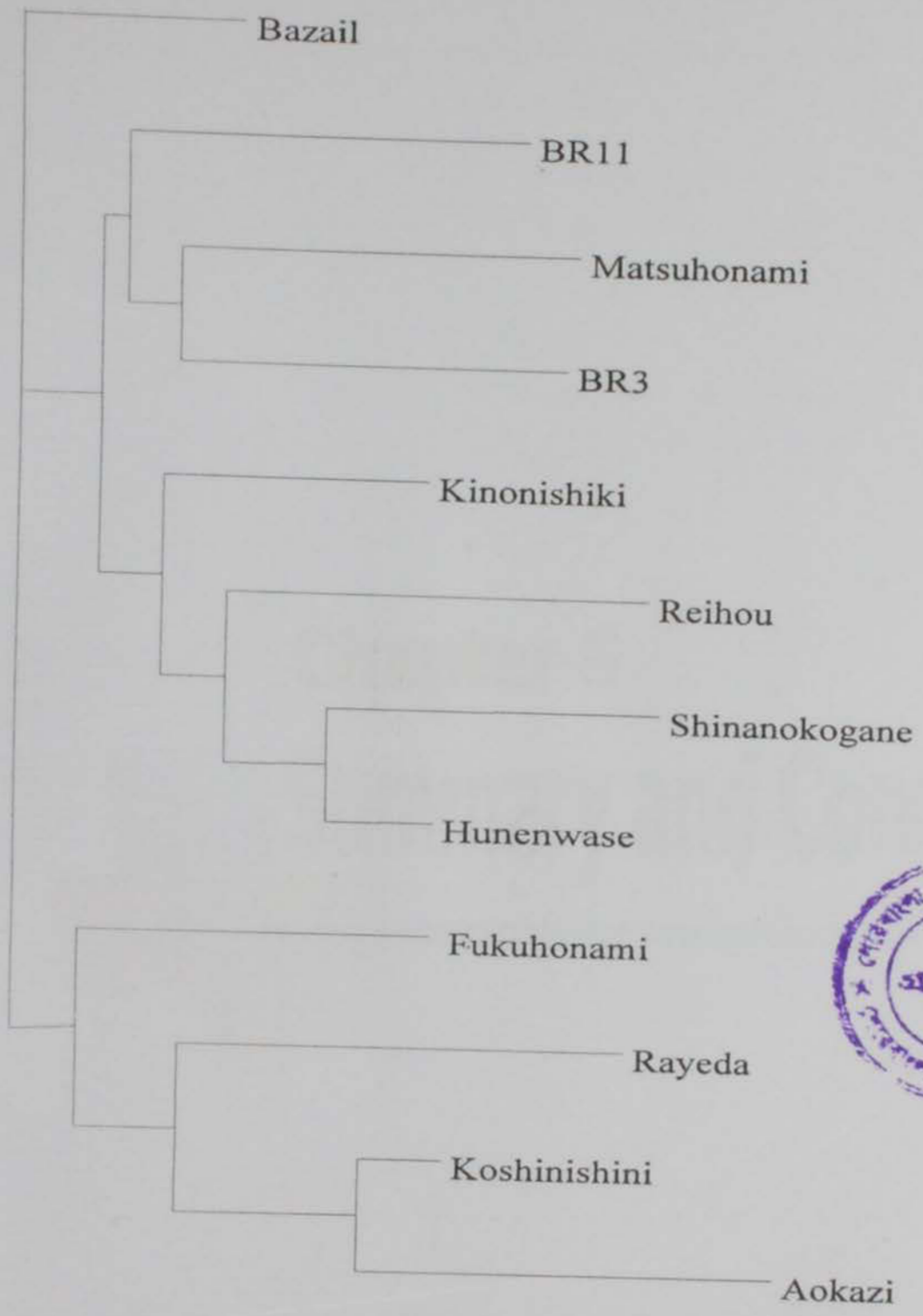


Fig.8. Dendrogram of genetic relationship of 12 ufra resistant and susceptible genotypes

Chapter 5

Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

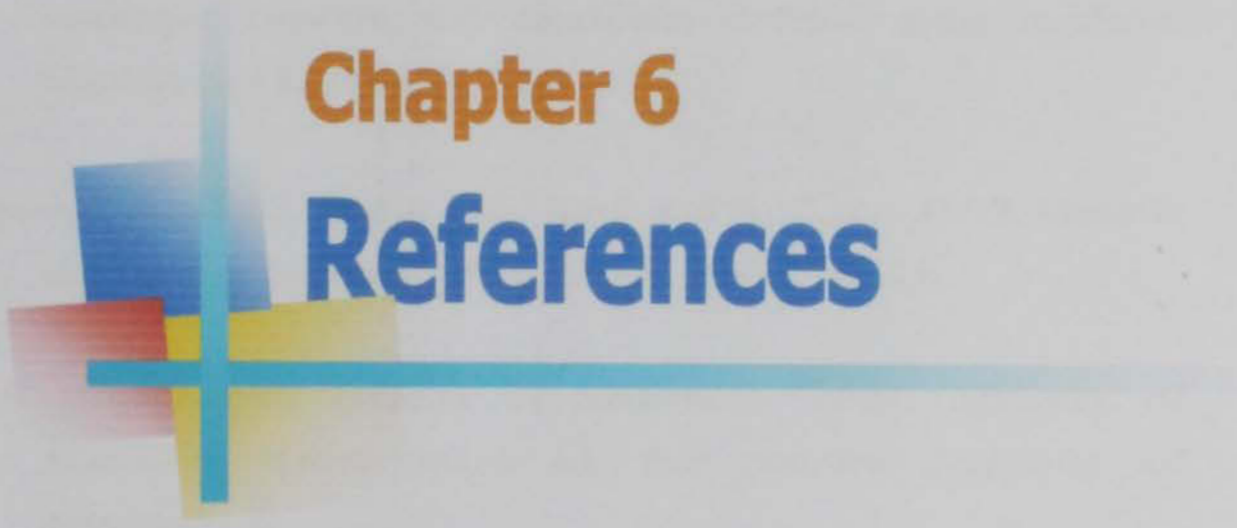
The experiments were conducted in the experimental field and laboratory of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur during 2006-2007. Thirteen F2 lines were tested against ufra. Among the 1146 plants of F2 population under 13 lines, 406 were found highly resistant, 216 moderately resistant, 157 resistant, 94 moderate susceptible, 61 susceptible and 212 highly susceptible. The highest percentage of highly resistant plant was observed in entry BR7750 followed by BR7758, BR7760, BR7755, BR7769 and BR7756. The highest percentage of resistant plant was observed in entry BR7751 followed by BR7754, BR7761, BR7769 and BR 7750. The lowest number of nematode/plant was recorded in HR followed by R, MR, MS, S and HS. The highly resistant and resistant plants were selected for crossing program for the development of ufra resistant varieties.

Out of 6 SSR markers 5 along with 3 VNTR markers were found polymorphic and produced strong bands. The amplicons size of RM21 (90bp) was associated with highly susceptible varieties, BR3 and BR11 while that was absent in highly resistant (Fukuhonami, Matsuhonami) and resistant (Rayeda, Bazail, Hunnenwase, Shinokogane, Kinonishiki, Koshinishini and Aokazi) genotypes. The band size 88bp and 105bp of RM23 were found in highly resistant and resistant genotypes. But those were absent in highly susceptible genotypes. The amplicon size of RY1 the (355bp) was absent in resistant varieties namely Fukuhonami, Matsuhonami, Rayeda, Bazail, Hunnenwase, Shinanokogane, Kinonishiki, Aokazi, Koshinishini, Reihou, BR3 and BR11 showed that band.

The amplicons of MR1 (240bp), MR5 (1061bp) and MR6 (1320bp) were absent in highly susceptible varieties (BR3 and BR11) and those were found in some entries of highly resistant and resistant genotypes. MR3 (402bp) band was present in Fukuhonami, Matsuhonami, Rayeda, Bazail, Kinonishiki, Koshinishini, BR3 and BR11 while that was absent in Hunnenwase, Shinokogane, Aokazi and Reihou. The band size of GF2 (454bp) was present in Fukuhonami, Rayeda, Bazail, Hunnenwase, Aokazi, Koshinishini and was absent in Matsuhonami, Shinanokogane, Kinonishiki, Reihou, BR3 and BR11.

The highest genetic distance was found between Aokazi and Reihou, Aokazi and BR11, Shinanokogane and Matsuhonami followed by Shinanokogane and Aokazi, Aokazi and BR3, Matsuhonami and Rayeda, Aokazi and Bazail, Reihou and Rayeda, BR3 and Rayeda, Aokazi and Matsuhonami, Reihou and Koshinishini, Kinonishiki and Aokazi, Shinanokogane and Rayeda, BR11 and Rayeda, Shinanokogane and BR3 and Reihou and BR3. In order to develop ufra resistant varieties and broaden the genetic base of rice varieties new breeding program should initiated preferably using the parents having greater genetic distances.

Twelve ufra resistant and susceptible genotypes had been differentiated into three main clusters. The genotypes belonging to cluster 1^{st}, 2^{nd} and 3^{rd} having greater genetic distance are recommended for inclusion in a hybridization program as they are expected to produce good segregants. So, the parents Rayeda and BR3, Rayeda and BR11, BR3 and Aokazi, BR11 and Aokazi (having greater genetic distances) could be used for the development of ufra resistant varieties.



Chapter 6
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