MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF TUNGRO RESISTANT GENOTYPES OF RICE

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This is to certify that the thesis entitled, "**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF TUNGRO RESISTANT GENOTYPES OF RICE"** submitted to the Department of Plant Pathology, 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 Mohammad Mahfuzur Rahman, Registration No. 26226/00781 under my supervision and my guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, has been availed during the course of this investigation have been duly acknowledged.

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Dedicated To

My Beloved Parents

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ABSTRACT

Genetic divergence analysis through Principal Component, Principal Coordinate and Canonical Vector analyses were done for tungro resistant and susceptible genotypes using 13 morphological characters. The genotypes were grouped into six clusters according to D^2 statistic and Canonical Vector analysis. Plant height, days to flowering, days to maturity, panicle length, number of spikelet per panicle, number of unfilled grain per panicle and yield were indicated as important contributiors to genetic divergence in 14 rice genotypes. On the basis of cluster distances, cluster I (BR10, BRRI dhan44) with cluster III (Sonahidemota, Kumragoir, Nakuchimota) and cluster VI (Bazail Acc 252, bazail Acc171, Rayeda 4849) or cluster II (BR11, BRRI dhan31, BRRI dhan32) with cluster III were identified for utilization in hybridization programme. A total of six primers including three SSRs and three VNTRs were used to characterize tungro resistant genotypes. PIC values of SSR primers, RM20, RM21 and RM23 ranged from 0.65-0.84. So these primers were considered as very much useful for characterizing tungro resistant genotypes. The amplicons size of RM21 (186 bp) was found in tungro susceptible, BR10 and moderately resistant genotypes while that was absent in highly resistant and susceptible, BR11 genotypes. In VNTR primer GF, two amplicons (543 bp and 1003 bp) were fixed with susceptible and moderately resistant varieties while those were absent in highly tungro resistant varieties. Based on Nei's genetic distance, seven clusters were formed among the tungro resistant genotypes. Diversity analysis of genotypes using morphological and molecular data indicated that new breeding program could be initiated preferably using the parents, BRRI dhan44 and Kachamota, BRRI dhan32 and Kachamota, BR10 and Kachamota, BRRI dhan31 and Kachamota, BRRI dhan32 and Sonahidemota for the development of tungro resistant varieties.

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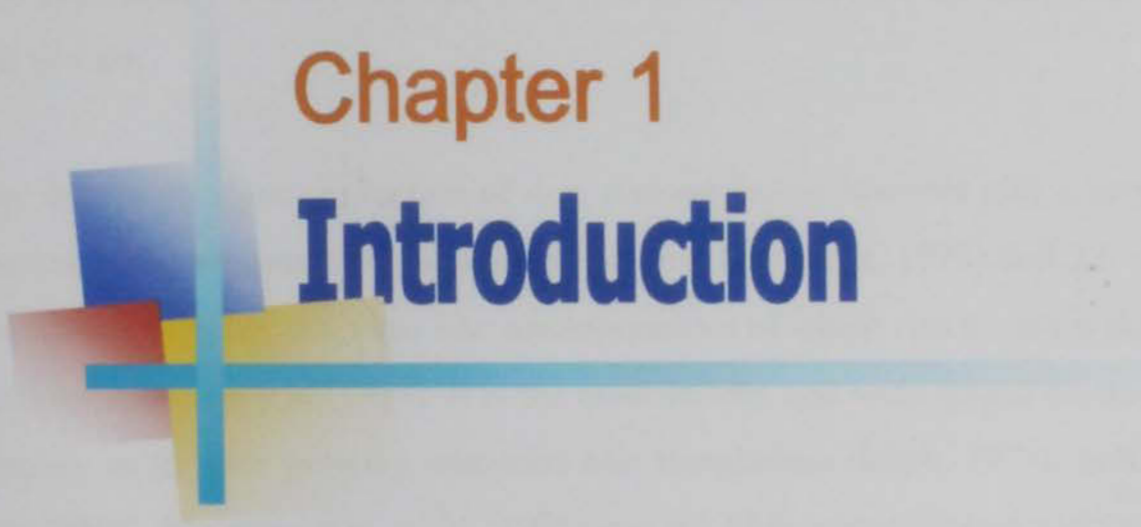
LIST OF ABBREVIATIONS

BRRI	=	Bangladesh Rice Research Institute
BAU	=	Bangladesh Agricultural University
IRRI	=	International Rice Research Institute
T. aman	=	Transplant aman
DAT	=	Date After Transplanting
Wt	=	Weight
Kg	=	Kilogram
PCA	=	Principal Component Analysis
PCR	=	Polymerase chain reaction
PCO	=	Principal Coordinate Analysis
CVA	=	Canonical Vector Analysis
no.	=	Number
AFLP	=	Amplified Fragment Length Polymorphism
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
S	=	Susceptible
IRRI	=	International Rice Research Institute
Kb	=	Kilo base pair
MR	=	Moderate Resistant

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
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
hr	=	Hour
PIC	=	Polymorphism Information Content
Chro	=	Chromosome



Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

Rice is the foremost food of the developing countries of the world. It provides about four fifth of the calories for more than two billion people of Asia and one third calories intake of nearly one billion in Africa and Latin America (Khush *et al.*, 1994). In Bangladesh annual production is 25157 MT of rice growing from about 25,621 acreage of land (BBS, 2005). About half of the world populations live on rice and over 90% of the world rice are grown and eaten in Asia.

Many factors decrease production of rice. Among factors diseases play a very important role. Seventy two diseases affect rice (Bergonia, 1978) and 22 of them caused by virus and virus like microorganism of which rice tungro is the most important one (Ou, 1984). It is the most serious and wide spread disease occurring in the rice growing countries like Bangladesh (Miah, 1973), India (John, 1968), Malaysia (Ou *et al.*, 1965) and the Philippines (Rivara and Ou, 1965).

In Bangladesh, tungro was identified in 1969 (Nuque and Miah 1969). Since then it has been one of the major constraints in rice production particularly in the Aus and T. Aman seasons (Miah, 1984, Miah *et al.*, 1985). It is the severest and most widespread being in 1990 when both Aus and T. Aman crops were affected in almost all over the country (Shahjahan *et al.* 1992).

Tungro is caused by tungro virus (RTV) which is a complex of two morphologically different virus particles namely, the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino *et al.*, 1978;

Omura *et al.*, 1983). The viruses are transmitted by green leafhopper, *Nephotettix virescens* (Ling, 1967).

The disease can attack rice plants at all growth stages, but the symptoms and yield losses are severe when infection takes place at seedling or early growth stages (Miah, 1973; Nahar *et al.*, 1985). For a susceptible variety without any recovery ability RTV may cause 100% infection and resulting in total yield loss under favourable condition (BRRI, 1983).

There is no efficient method to cure tungro infected plants in the field. To control the vector serves as the indirect measure to control the disease. The use of pesticide provides good control measures for the vector but the vector may have already transmitted the virus before being killed. The use of resistant variety is one of the most effective ways to control rice disease. This concept is applicable to tungro disease (Rivera and Ou, 1965; Ling, 1967) because tungro control by killing the vector with insecticides is often not effective. Resistance of a variety to tungro may be due to its resistance to the insect vector, or to the virus or to both (Karim, 1978; Ling, 1979).

Many vector resistant modern rice varieties developed by International Rice Research Institute (IRRI) and National Agricultural Research Centers (NARC) are now being cultivated by farmers in different tungro epidemic countries like Indonesia, Philippines (Tiongco *et al.*, 1986; Heinrichs and Rapusas, 1983). At IRRI, with the same GLH resistance gene (Glh 1) Pankhari 203 showed high resistance to tungro, while Jhingasail showed intermediate reaction. Similarly, TAPL 769 (GLH 6) showed tungro resistance, while IR 36 (Glh 6) became susceptible (Flores *et. al.*, 1987). On the other hand Kachamota and ARC 11554 were found resistant to RTV in Malaysia and Indonesia. ARC 11554 was found resistant to RTV in many countries.

Varietals resistance to tungro may not be stable. Observation in the Philippines revealed that IR 54 and IR 64 which had been highly resistant to GLH and also tungro virus but in Indonesia became susceptible at 2-3 years of intensive cultivation (Cabunagan *et al.*, 1987).

Genetic divergence is one of the criteria of parent selection. A knowledge of genetic diversity among plant populations and its quantitative assessment usually helps a breeder in choosing desirable parents for breeding programmes as selection of parents on the basis of divergence analysis would be more effective. Genetic diversity is the essential to meet the diverse goals of plant breeding such as producing cultivars with increased yield (Joshi and Dhawan 1966), 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 genotype based on minimum divergence between them (Sharma, 1997). Multivariate analysis is a useful tool in quantifying the degree of divergence between biological populations at genotypic level and assessing the relative contribution of different components to the total divergence both at intra and inter cluster levels (Murthy, 1965).

To evaluate the genetic diversity, molecular markers are used within a diverse collection of rice (*Oryza sativa*) accession and to determine differences in the patterns of diversity within the two rice subspecies *Indica* and *Japonica* (Junjian *et al.*, 2002). 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). Present studies were undertaken with the following objectives:

1. To know the genetic diversity using morphological traits of tungro resistant genotypes.
2. To characterize tungro resistant genotypes at molecular level.

Chapter 2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

2.1 Occurrence and distribution

Tungro is a serious virus disease in South and Southeast Asia since 1960. Devastating outbreaks of the disease caused tremendous yield losses in the Philippines and elsewhere during 1960-1971 (Bergonia, 1978; Ling, 1979; Siwi *et al.*, 1987). In Indonesia alone, rice crops worth more than US $ 89 million were damaged by tungro during 1967-1972 (Tantera, 1985). The disease has been epidemic rather than endemic because it has often struck suddenly and destroyed large areas of rice fields (Ling, 1977).

Tungro disease was observed in 1963 on many rice varieties at IRRI experimental farms and characterized and identified as virus disease (IRRI, 1967). Ling and Tiongco (1979) mentioned that rice tungro disease (RTD) was first studied in Japan and the Philippines before 1950. It is prevalent in Southeast Asian countries under different names. However, based on symptomatology, vector species, virus vector interaction, particle morphology of the virus and serological aspects the different names represent the same disease tungro (Ang *et al.*, 1983; Hibino *et al.*, 1978; Ling 1972 and Saito, 1977).

RTV can attack rice plant at all growth stages but most critical period of infection is the seedling stage of the plant and can cause 100% yield loss in case of susceptible variety (BRRI, 1983).

2.2 Symptoms

Different rice varieties express symptoms differently. In some moderately resistant varieties, the symptoms on the infected plants are conspicuous at one stage and may gradually disappear at later stages of growth. This is often termed as recovery from the disease (Ling, 1979). Ou and Ling (1966) mentioned that the degree of stunting and discoloration varies with rice cultivar, environmental condition, age of the plant and strain of the virus.

Tungro infected plants express various symptom which is dependent on the susceptibility of the rice variety, age of the plant, the time of infection (Palomar and Ling, 1966), virus strain (Rivera and Ou 1965; Rivera and Ling 1971; Anjaneyulu and Vaktavatasalam, 1986) and the type of virus particle (Hibino *et al.*, 1978). The general symptoms are yellowing of leaves, stunting of plants, twisting of young leaves, reduction of tiller number, incomplete and delayed panicle emergence and delayed flowering (Anjaneyulu and John 1972; Ling and Tiongco, 1981). The symptom may vary from slight to severe stunting and yellow-orange to orange-red discoloration of leaves usually starting from the tips and gradually moving downwards (Saito *et al.*, 1986; Rao and Anjaneyulu, 1976).When rice plants are infected by tungro then leaves are slightly rolled outward and some what spirally twisted. Stunting is due both to shortening of the leaf sheath and the leaf blade. Tillering is significantly reduced if the plants are affected at the early growth stage. Root development is poor, panicles are small and spikelets are sterile (Siwi *et al.*, 1987).

2.3 Diagnosis of tungro disease

Ling (1969) and Omura (1986) reported that for management of tungro disease prerequisite is a proper diagnosis of the disease. The firsthand identification is generally done by visual observation of the external symptom.

Visual symptom may not always be reliable because viruses other than RTV may cause tungro like symptoms. Moreover, many other factors like soil nutrition, drought, weeds, and insect injury may cause tungro like symptoms.

2.4 The causal agent

Hibino *et al.* (1978) mentioned that tungro caused by two kinds of morphologically different virus particles have been identified from tungro infected plants: the rice tungro spherical virus (RTSV) and the rice tungro bacilliform virus (RTBV).

Rice tungro bacilliform virus (RTBV) alone causes only mild symptom and rice tungro spherical virus (RTSV) causes only mild stunting of the plants. The symptoms are severe when RTBV and RTSV are present in the same infected plant (Hibino and Mariappan, 1983). Both the particles are restricted in phloem cells (Favali *et al.*, 1975; Saito, 1977). Plant cells are infected with both RTBV and RTSV particles together and either RTBV or RTSV alone (Hibino, 1987).

2.5 The vector

The principal vector of the tungro virus is *Nephotettix virescens*. *Nephotettix nigropictus* and *Recilia dorsalis* have also been reported as vector of RTV (IRRI, 1969). Hsieh (1972) explained that the vector causes damage to rice plants directly by feeding on the growing plants thus greatly reducing its vigor, tiller number and ultimately its yield potential.

According to Miah (1976) there are two groups of the vector *Nephotettix viresccens*, the transmitter group and non transmitter group. The transmitter group can transmit the virus in to the host plant, whereas non transmitter group can not transmit.

Ling (1975) mentioned that the adult insect vector *Nephotettix viresccens* appears to be efficient than the nymph in transmitting RTV in rice plants. The vector *Nephotettix virescens* carries more viruses from the susceptible cultivar than from the resistant cultivar during acquisition feeding (Mohana and Anjaneyulu 1984).

2.6 Varietal resistance:

Karim (1978) reported that the resistance of a rice variety to tungro may be due to its resistance to the insect vector or to the virus or to both. The mechanism of resistance of a rice variety may either be due to the inactivation of the virus or the inhibition of the virus multiplication by substance or substances present in the rice plant.The resistance of the rice plant to rice tungro infection increase with age of the plant (Palomar and Ling 1966).

Mohana and Anjaneyulu (1984) mentioned that the resistance of a rice variety to tungro virus is not a permanent character of the plant but it changes with the certain factor. At low temperature (15° to 28°c), IR 20 loss its resistance against rice tungro virus in winter season but it maintains resistance during the summer season.

Flores *et al.* (1987) mentioned that at IRRI with the same GLH resistance gene (GLH 1) Pankhari 203 showed high resistance to tungro, while Jhingasail showed intermediate reaction. Similarly, TAPL 796 (GLH 6) showed tungro resistance, while IR 36 (GIH 6) became susceptible.

Vidhyasekaran *et al.* (1987) reported that by rearing the vector for several generations on resistant variety, GLH resistant ADT 16 and IR 43 became susceptible to tungro. By rearing the vector for several generations on resistant

IR 34 it was demonstrated that GLH can adapt and transmit the virus successfully on the resistant variety reported by Cabunagan and Ling (1982).

Varietal resistance to tungro may not be stable. Observation in the Philippines revealed that IR50, IR54 and IR64 which had been highly resistant to GLH and also to tungro infection in the field succumbed to tungro infection in south Cotabato. The same trend was reported from Indonesia where rice varieties were resistant to tungro during release, but susceptible after 2- 3 of years intensive cultivation (Cabunagan *et al.*, 1987).

Miah (1984) mentioned that Ptb18 showed resistant reaction to rice tungro virus under the environmental condition of Bangladesh and India, while it showed intermediate to resistant reaction at IRRI under the environmental condition of the Philippines. Rahman (1983) found that Variety Ptb18 was resistant to the vector but susceptible to the virus.

Variety IR 56 was found to be Moderately reaction to RTV in the Philippines but resistant in Malaysia (IRRI, 1985). Varieties Kachamota and ARC 11554 were found resistant to RTV in Malaysia and Indonesia. ARC 11554 was showed resistant to RTV in many countries (Miah, 1984)

2.7 Morphological characterization

Rather *et al.* (2001) reported the genetic divergence in 56 rice cultivars in Jammu and Kashmir, India during the rainy season of 1997 and 1998. Significant variations for days to 50% flowering, leaf length, leaf breadth, productive tillers per plant, plant height, days to maturity, total and sterile grains per panicle, panicle length, harvest index, grain yield, length, breadth ratio of the grain and 1000 grain weight were observed among cultivars. The grouping of cultivars from various regions into the same cluster (as apparent

in clusters I, II and VI) indicated that the geographical distribution did not necessarily indicate genetic divergence. The highest mean value for harvest index and the lowest spikelet sterility were observed for K332. Based on the mean performance for plant height, maturity, spikelet fertility, grain yield and intercluster distance, cultivars from clusters II and IV may be used for initiating hybridization.

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/panicle, number of filled grain/panicle, number of unfilled grains panicle, 100 grain weight, grain length/ breadth ratio 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/breadth ratio contributed maximum towards the genetic divergence followed by 100 grain weight and grain yield/plant accounting to 87% 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 find out variability and genetic association for grain yield and its component characters was studied by Iftekharuddaula *et al.*(2001). All the characters tested showed significant variation among the varieties. The highest genetic variability was obtained in spikelets/panicle and grains/panicle. High heritability together with high genetic advance in percentage of mean was observed in plant height, 1000 grain weight, grains/panicle and

spikelets/panicle. 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 weight 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. Moreover, days to maturity, days to flowering, plant height and spikelets/panicle had positive and higher indirect effect on grain yield through grains/panicle.

Roy and Panwar (1993) mentioned that ninety nine genotypes of rice were studied for their genetic divergence by D^2 analysis of a set of 11 characters related to bacterial blight, severity, yield and its contributing characters. The genotypes were grouped into 16 clusters. The genetic diversity was not related to geographic diversity. Yield/plant, panicles/plant, spikelets/panicle, grains/panicle and bacterial blight severity were mainly responsible for genetic divergence. The assessment of genetic diversity is likely to be more fruitful could be grouped into three clusters, exclusively of variants of specific species, without any overlap. Cluster I represented *Oryza nivara* with fourteen accessions, while cluster II comprised of five accessions of *Oryza sativa* var. spontanceae and cluster III with only accessions of *Oriza rufipogon.*

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 was 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.

Kanwal *et al.* (1983) studied 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.

Vivekanandan and Subramaniam (1993) used the Mahalanobis's D^2 statistics to assess the nature and magnitude of genetic divergence of 28 genotypes of rain fed 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.

Kaw (1995) evaluated ninety four genotypes for genetic divergence, comprising 74 F_1s and 20 parents, grown over three cold stress environments, based of five reproductive stage cold tolerances and adaptability related characters. Using D^2 statistic and canonical analysis, the genotypes were grouped into 18 clusters. The clustering pattern suggested that genetic and geographic diversity were necessarily related. Plant height, days to flowering, fertile spikelets/panicle and percent fertility were indicated as important contributors to genetic divergence in cold rices.

The nature of genetic divergence in the 82 local rice varieties of Tripura using D^2 statistics based on 15 agro-morphological characters was reported by Sardana *et al.* (1997). The cultivars were grouped into 18 clusters, of which cluster 1 and 15 genotypes was the largest. The genetic diversity was due to genetic drift, selection in pecuiliar topography and diverse agro-climatic condition of Tripura. Number of grains/panicle, leaf area, grain

weight/panicle, effective tillers/plant and grain yield/plant were the major components which contributing to the cluster VIII.

Anandakumar and Subramaniam (1989) used the D^2 analysis to estimate the genetic divergence existing in 23 drought tolerant varieties and to know the contribution of characters to the genetic divergence. Using Tocher's clustering technique genotypes were grouped into 6 clusters. Clustering patterns failed to reveal any relationship between geographic divergence and genetic variability.

Rahman *et al.* (1997) evaluated 52 lowland rice cultivars under 40-50 cm water depth situation to estimate the nature and magnitude of genetic divergence under 40-50 cm water depth situation. The cultivars were grouped into eight clusters showing no relationship between geographic distribution and genetic divergence. Cluster exhibited relatively high mean value for spikelets/panicle and grain yield/plant while cluster VI scored the highest value for ear- bearing tillers/plant and panicle length.

Mishra *et al.* (1994) reported that the multivariate analysis of divergence for 7 quantitative traits among 37 strains of *Oryza sativa* grouped the genotypes into 5 clusters. The first cluster contained 31 genotypes, the second 3, while the third, fourth and fifth cluster each contained 1 genotype. Numbers of fertile grains/ panicle, number of sterile grains/ panicle and 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 programme.

2.8 Molecular characterization

Genetic diversity is commonly measured by genetic distance 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 (Becker *et al.*, 1995; Paran and Michelmore, 1993), RAPD (Tingey and Deltufo, 1993; Williams *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).

Paterson *et al.* (1991) reported that 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 evolution any relationship within and between species, genera or higher taxonomic categories.

An experiment was conducted to design and application of micro satellite markers panels for semi automated genotyping of 12 rice. The objective of this study was to develop a systematic and flexible method for assembling multiplex simple sequence repeat (SSR) marker panels for high throughout genome analysis in rice and to test these panels on a set of cultivated rice germplasm. On a standard set of 13 genetically diverse cultivars of markers detected an average of five alleles per locus and had a mean PIC value of 0.67(Coburn *et al.*, 2002).

An experiment was conducted to determine genetic variability and relationships among seven Egyptian rice genotypes (Mahmoud *et al.*, 2005). Rice genotypes namely, Giza 178, Giza 177, Giza 175, Giza 172, Sakha 102 and Sakha 101 by using eight RAPD primers, Six SSR primer pairs, eight AFLP primer 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 revealed 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 101 (61.5%). Dendrograms derived from different techniques include minor differences in clustering pattern but did not affect the main grouping of the different genotypes.

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 programme. Local Iranian cultivars showed high degree of polymorphism.

Zhao *et al.* (2002) used 16 simple sequences repeats (SSR) primers of functional to detect genetic diversity among 23 accessions of rice germplasm from 15 countries (China, Brazil, Japan, Korea and Pakistan). 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 I along with upland rice from Brazil. The *indicas* from Pakistan and Korea was classified into class II.

To estimate genetic diversity in traditional and evolved Basmati (EB) and semi dwarf non Basmati (NB) rice varieties, an experiment was conducted by Nagaraju *et al.* (2002) 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.

Chandra *et al.* (2004) reported the extent of genetic diversity among 27 rice accessions from diverse hydrological habitats by using micro satellite 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 composed of 15 accessions contained all improved cultivars, except TR62266. 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 IR 36 and IR 64.

Eighty one SSR markers were used to estimate genetic diversity of 94 *japonica* rice (Suh-Jung P *et al.*, 2004). 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 markers. 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 and Naravaneni (2006) were investigated the genetic diversity and DNA fingerprinting of 15 elite rice genotypes using 30 SSR primers on 7-12 chromosomes numbers. The results revealed that all the primers showed distinct polymorphism among the cultivars indicating the robust nature of micro satellites in revealing polymorphism. Cluster analysis grouped the rice genotypes in to 10 classes in which *japonica* types D11-I (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 micro satellite provides greater confidence for the assessment of genetic diversity and relationships.

Chapter 3
Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

The studies were conducted at the experimental field and laboratory of plant pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, during the period of 2006-2007.

3.2 Genotypes

A total of 14 including six highly resistant, three resistant, three moderately resistant and two susceptible genotypes were used in this study (Table 1). The seeds for all the genotypes were obtained from Genetic Resources and Seed Division, BRRI, Gazipur.

Table 1. List of tungro resistant and susceptible genotypes

SL.No	Variety	Type
1.	BR10	Susceptible (HYV type)
2.	BR11	Susceptible (HYV type)
3.	BRRI dhan31	Moderately resistant (HYV type)
4.	BRRI dhan32	Moderately resistant (HYV type)
5.	BRRI dhan44	Moderately resistant (HYV type)
6.	Sonahidemota	Highly Resistant (local type)
7.	Kaiyamota	Highly Resistant (local type)
8.	Khairymota	Highly Resistant (local type)
9.	Nakuchimota	Highly Resistant (local type)
10.	Kachamota	Highly Resistant (local type)
11.	Kumragoir	Highly Resistant (local type)
12.	Bazail Acc 171	Resistant (local type)
13.	Bazail Acc 252	Resistant (local type)
14.	Rayeda Acc 4849	Resistant (local type)

BRRI=Bangladesh Rice Research Institute, **Source**: Latif *et al.*, (2007)

3.3 Germination of Seed

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

3.4 Phenotypic Data

The seedlings of 14 genotypes were raised in pots. Then thirty day 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 quantitative characters namely, plant height (cm), number of tiller per hill, tillering ability, phenotypic acceptability, days to flowering, days to maturity, panicle length (cm), number of spikelet per panicle, number of filled grain/panicle, number of unfilled grain/panicle, 1000 grain weight (g), yield per hill (g) and disease index.

3.5 Analysis of data

Mean data of the character was analyzed by multivariate analysis using GENSTAT 5.13 software program (copyright 1987, Lawes agricultural Trust, Rothamasted Experimental station, UK). 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 techniques are required to represent the results more clearly and it is obvious from the results of many researches (Bashar 2002, Uddin 2001, Junned *et al.*, 1988, Ariyo 1987, Patil *et al.*, 1987, Dani and Murthy 1985 and Balasch *et al.*, 1984). In the analysis of genetic diversity in rice following multivariate techniques were used and mean data for each character were subjected to use.

3.6 Principal component analysis (PCA)

Principal components were computed from the correlation matrix and genotype scores obtained from first components (which has the properly 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 coordinates 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

The following steps were followed for genotypic analysis

3.11 Collection of leaf sample

To extract genomic DNA, young and fresh leaves were collected from different pots of 14 varieties.

3.12 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 about 200 ml of distilled water was added. Again about 5g of NaOH was added and stirred for 10 minutes and 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, 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. 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 hydrochloric acid was added slowly 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 a bottle and was sterilized by autoclaving.

70% ethanol

Ethanol and sterile distilled water was 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

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

3.13 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 (about1/3 full) and was replaced into freezer until further use. All tubes were taken from the freezer and quickly added 800 µl of extraction buffer into each. Tubes were mixed thoroughly and then placed in 65°C water bath for 10-15minutes mixing by inversion every few minutes. The 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 (pH5.2) 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.

3.14 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 for 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).

3.15 Use of λ (Lambda) DNA marker

λ (lambda) DNA was used for quantification of DNA concentration as 5µl. It is 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.

3.16 Use of primers

In total twelve primers including 9 SSR (Simple sequence repeat) and 3 VNTR (Variable number tandem repeat) studied. Out of nine, three SSR markers along with another three VNTR markers showed polymorphism and used in this study (Table 8).

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 (1 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.

3.18 VNTR-PCR Protocols

PCR components were used per reaction (15.0 µl volume) for VNTR analysis: 6 ng of DNA template (1.5 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%) and polyacrylamide (8 %) gel electrophoresis using in TBE buffer. For agarose gel, 8 µl samples were loaded while 3 µl samples loaded in polyacrylamide gel. Each gel was run for 2-3.5 hrs at 100 volts. Gels were stained in ethidium bromide.

3.19 Documentation of the DNA samples

After electrophoresis the gel was taken out carefully from the gel chamber and 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.

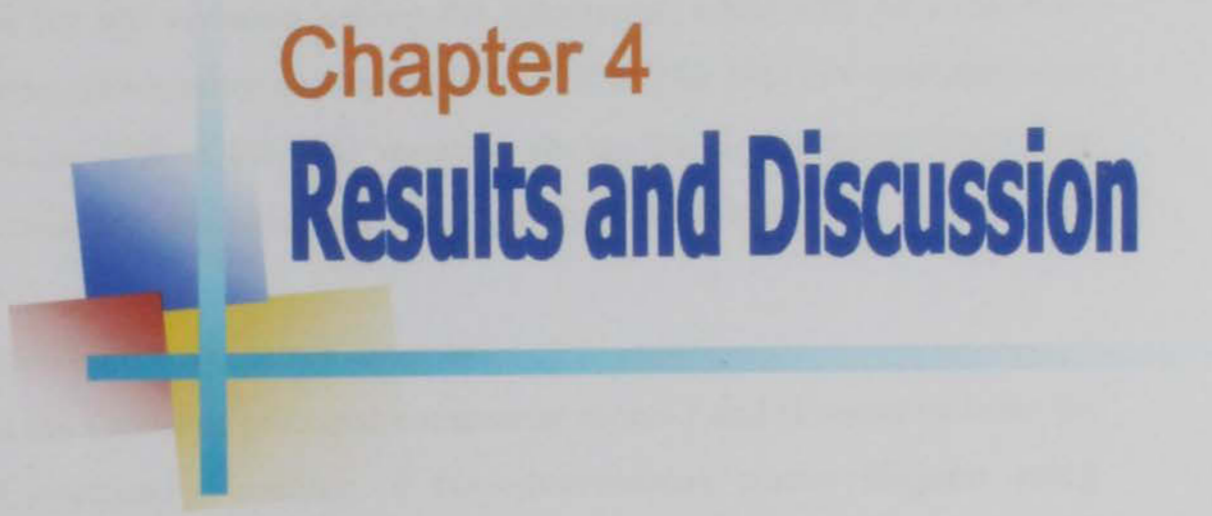
3.20 Data analysis

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):

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

Where n is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i.

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 theasteur Institute sever (http:/bioweb-pasfeur. Fr/sequanal/ interfaces/drawgram-simple.html).



Chapter 4
Results and Discussion

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Morphological characterization

The results of the genetic diversity of 14 tungro resistant and susceptible rice genotypes are represented in table 2 to 7 and figure 1.

4.1.1 Principal component analysis

The principal component analysis yielded eigen values of each principal component axes of ordination of genotypes with the first axes totally accounted for the variation among the genotypes, while four of these with eigen values above unity accounted for 90.68%. The first two principal exes accounted for 75% of the total variation among the 13 characters describing 14 tungro resistant and susceptible rice genotypes (Table 2).

4.1.2 Construction of scatter diagram

Based on the values of principal component scores I and II obtained from the principal component analysis, a two dimensional scatter diagram using component score 1 as X axis and component score 2 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 six groups, which indicated that there exists considerable diversity among the genotypes. The scattered diagram for tungro resistant and susceptible rice genotypes of different clusters revealed that the genotype number 14, 13, 12, 11, 10, 9, 8, 7 and 6 were distantly located which suggesting more diverged from rest of the genotypes.

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

Principal component characters	Eigen values	Percentage of total variation accounted for	Cumulative percentage
Number of tiller/hill	8.0233	61.72	61.72
Tillering ability	1.7077	13.14	74.86
Plant height (cm)	1.2299	9.46	84.32
Phenotypic acceptability	0.8265	6.36	90.68
Days to flowering	0.4293	3.30	93.98
Days to maturity	0.3204	2.46	96.44
Panicle length (cm)	0.2205	1.70	98.14
Number of spikelet/panicle	0.1114	0.86	99.00
Number of filled grain/panicle	0.0828	0.64	99.64
Number of unfilled grain/panicle	0.0288	0.22	99.86
1000 grain wt (g)	0.0106	0.08	99.94
Yield (g/hill)	0.0085	0.07	100.01
Disease index	0.0003	0.00	100.01

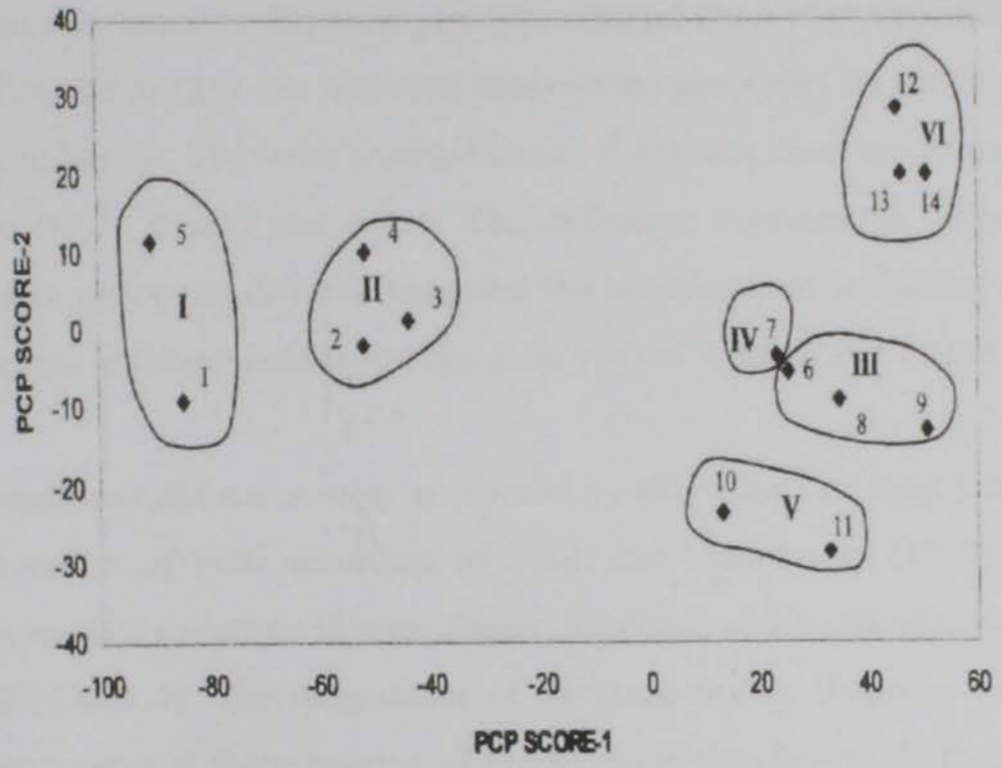


Fig. 1 Scattered distribution of 14 T.aman rice genotypes based on their principal component scores superimposed with clustering

4.1.3 Principal co-ordinate analysis

Principal coordinate analysis (PCO) was performed on auxiliary of principal component analysis. This analysis helps in estimating distances (D^2) for all possible 91 combinations between pairs of genotypes. The highest inter genotype distance 2.472 was observed between the genotypes Nakuchimota and BR10 followed by Kumragoir and BR10 (2.354), Nakuchimota and BR11 (2.349), Kachamota and BR10 (2.323). The tenth highest pair distance was 2.162 that was observed between genotypes Bazail (Acc 171) and BR10. The lowest distance (0.125) was observed between the genotypes Bazail (Acc 171) and Sonahidemota. The tenth lowest distance 0.476 was observed between the genotype BRRI dhan31 and BR10. The difference between the highest and lowest inter genotypes distance indicated the prevalence of variability among the 14 tungro resistant and susceptible genotypes of T. aman rice (Table 3).

The intra-cluster distances were computed by the values of inter genotypic distance matrix of PCO according to Singh and Chaudhaury (1985). There were not marked variations in intra-cluster distances, which ranged from 0.661 to 1.992 (Table 4). The magnitudes of the intra-cluster distances were not always proportional to the number of genotypes in the clusters. In the present study it was found that clusters II, III and VI composed of largest number of genotypes (3) (Table 5). The intra-cluster distances in all the 6 clusters were more or less low indicated the genotypes within the same cluster were closely related. The highest intra-cluster distance was computed for cluster III (1.992) composed of 3 genotypes followed by the cluster V (1.719) composed of 2 genotypes. The lowest intra-cluster distance was in cluster II (0.661) followed by the cluster I (0.831) consisting of 3 and 2 respectively. However, the higher value (1.992) of intra-cluster distance in cluster III indicated that the genotypes (3) 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 tungro resistant genotypes

10 lower D^2 values	Genotypic combination	10 higher D^2 values	Genotypic combination
0.125	Bazail(Acc-171) and Sonahidemota	2.472	Nakuchimota and BR10
0.328	BRRI dhan31 and BR11	2.354	Kumragoir and BR10
0.334	Kachamota and BRII dhan32	2.349	Nakuchimota and BR11
0.341	Nakuchimota and BR11	2.323	Kachamota and BR10
0.370	BR11 and BR10	2.262	Kumragoir and BR11
0.438	Khairymota and BR11	2.208	Kachamota and BR11
0.453	BRRI dhan44 and BRRI dhan32	2.206	Khairymota and BR10
0.472	Kachamota and BR10	2.195	Rayeda(Acc-4849) and BR10
0.473	Khairymota and BR10	2.163	Bazail(Acc-252) and BR10
0.476	BRRI dhan31 and BR10	2.162	Bazail(Acc-171) and BR10

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

Cluster	I.	II.	III.	IV.	V.	VI.
I.	0.830					
II.	7.456	0.661				
III.	17.898	17.490	1.992			
IV.	16.254	13.422	9.412	1.544		
V.	16.063	16.535	5.126	9.883	1.719	
VI.	18.693	15.872	7.019	4.434	9.355	1.20

Table 5. Distribution of 14 tungro resistant rice genotypes in six clusters

Cluster	Number of genotypes	Name of genotypes
I.	2	BR10, BRRI dhan44
II.	3	BR11, BRRI dhan31 and BRRI dhan32
III.	3	Sonahidemota, Kumragoir and Nakuchimota
IV.	1	Khaiyamota
V.	2	Khairymota and Kachamota
VI.	3	Bazail (Acc-252), Bazail (Acc-171) and Rayeda (Acc-4849)

4.1.4 Canonical variate analysis

Canonical variate analysis was performed to obtain the inter-cluster distances (Mahalanobis's D^2 values). These values of inter-cluster distance (D^2) are presented in Table 4. Statistical distances 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 genotype 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 I and VI (18.693) followed by the distance between cluster I and III (17.898), cluster II and III (17.490) while the distance was minimum between cluster VI and IV (4.434) followed by the distance between cluster V and III (5.126). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster I and II was far diverged from those of clusters VI and III. Similarly the higher inter-cluster values between cluster I and III, clusters III and II. Clusters IV and I, clusters V and II, clusters V and I, clusters II and VI indicated the genotypes belonging to each pair of cluster were far diverged. These relationships were also reflected in the scatter diagram (Fig. 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 reports were also made by Bansal *et al.* (1999), Mokate *et al.*, (1998), Kumari and Rangasamy (1997) and Singh *et al.*, (1996). The genotypes belonging to clusters VI and I, clusters III and I, clusters II and III having grater cluster distance are recommended for inclusion in a hybridization programme for the development of tungro resistant varieties as they are expected to produce good segregants. 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 V, clusters I and IV, clusters I and V.

4.1.5 Non hierarchical clustering

Non hierarchical clustering using co-variance matrix grouped 14 tungro resistant and susceptible genotypes into six different clusters. These results confirmed the clustering pattern of the genotypes obtained through principal component analysis and 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 Table 5. The distribution pattern indicated that the maximum numbers of genotypes (3) were found in clusters II, III and VI. Clusters I and V consisted of two genotypes while cluster IV consisted of one genotype.

4.1.6 Intra-cluster mean

Intra-cluster mean for 13 characters are presented in Table 6. In case of number of tiller per hill, the highest intra cluster mean (19.00) was recorded in cluster II followed by cluster I and cluster IV. The lowest intra-cluster mean for this trait was observed in cluster III (8.33). Intra cluster mean of tillering ability was the highest (7.00) in cluster III and the lowest mean for this trait was observed in cluster I, II, IV and V (5.00). Intra-cluster means for plant height was the highest in cluster VI (173.50) followed by cluster III (172.47).

The lowest cluster mean for this trait was observed in cluster II followed by cluster I (7.00). Phenotypic acceptability had the highest group mean in cluster V and VI followed by cluster III (6.33) and cluster IV (5.00). The lowest intra-cluster mean for this trait was observed in cluster II (1.67) followed by cluster I (2.00). Intra-cluster means for days to flowering was the highest in cluster IV (115.00) followed by III (111), V (110) and VI (109). The lowest intra-cluster means for this trait was observed in cluster I (97) followed by II (101). It was observed that days to maturity had the highest intra-cluster mean in cluster V (177) followed by cluster III (160). The lowest intra-cluster means for this trait was observed in cluster I (136) followed by cluster II and cluster VI. Panicles length had the highest group mean in cluster VI (26) followed by cluster (25.64). The lowest cluster mean for this trait was found in cluster IV (23.80) followed by cluster III, cluster II and cluster V. Intra-cluster mean for number of spikelet per panicle was the highest in cluster I (165.32) followed by II (128.32). The lowest cluster mean was found in cluster III (77.48) followed by cluster IV and cluster V. The highest intra-cluster mean for number of filled grain per panicle was observed in cluster I (141.4) followed by cluster II. The lowest cluster mean was found in cluster VI (48.30) followed by cluster IV, cluster III and cluster V.

The highest intra-cluster mean for number of unfilled grain per panicle was observed in cluster IV (30.60) followed by cluster VI, cluster III and cluster V. The lowest intra-cluster mean was observed in cluster II (17.60) followed by cluster I. Intra-cluster means for 1000 grain weight was highest in cluster V (32.45) followed by cluster III and IV. The lowest cluster mean was found in cluster VI 20.13) followed by clusters II and I. Yield showed the highest in cluster I (43.99) followed by cluster II. This character had the lowest mean in cluster IV followed by cluster VI, cluster III and cluster V. The highest intra-cluster mean for disease index was found in cluster I (6.50) followed by

cluster II (5.67). The lowest cluster mean was observed in cluster III, IV and V followed by cluster VI.

The inter-cluster distances of cluster I and II with other clusters were higher than the inter-cluster distances between the remaining cluster combinations. The cluster mean of these two clusters for number of tiller per hill, tillering ability, panicle length, number of spikelet per panicle, number of filled grain per panicle, yield and disease index were also divergent. These indicated that the genotypes included in cluster I and II were very important to contribute into the total divergence among 14 tungro resistant and susceptible genotypes for these characters.

Genotypes of cluster I produced the highest mean for number of spikelet per panicle, number of filled grain per panicle, yield and disease index. In cluster I the lowest cluster mean were observed for days to flowering, days to maturity and number of unfilled grain per panicle.

The lowest and the highest cluster means for different characters in cluster I indicated the genotypes included in this cluster would offer good scope for improvement of rice through rational selection for these characters. The lowest cluster mean for days to flowering and days to maturity indicated that the genotypes in this cluster could be used to improve short duration variety. The highest value for number of spikelet per panicle, number of filled grain per panicle and yield revealed that the genotypes in this cluster could be used to improve high yielding variety.

The genotypes of cluster II produced the higher mean for number of tiller per hill, second highest for panicle length, no. of spikelets per panicle, no. of filled grain per panicle, yield and disease index. The lowest cluster mean for plant

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

Characters	I	II	III	IV	V	VI
Number of tiller/hill	13.00	19.00	8.33	13.00	10.00	10.00
Tillering ability	5.00	5.00	7.00	5.00	5.00	6.33
Plant height	123.50	118.66	172.47	149.20	154.90	173.50
Phenotypic acceptability	2.00	1.67	6.33	5.00	7.00	7.00
Days to flowering	97.00	101.00	111.00	115.00	110.00	109.00
Days to maturity	136.00	137.00	160.00	153.00	177.00	137.67
Panicle length	25.64	24.25	24.10	23.80	24.83	26.00
Number of spikelet/panicle	165.32	128.32	77.48	84.95	86.65	80.32
Number of filled grain/panicle	141.40	114.03	73.77	71.80	78.50	48.30
Number of unfilled grain/panicle	18.60	17.36	26.50	30.60	27.00	28.73
1000 grain wt	24.03	22.71	30.66	29.10	32.45	20.13
Yield	43.99	41.33	32.80	30.80	34.80	31.09
Disease index	6.50	5.67	1.00	1.00	1.00	3.00

height, tillering ability, phenotypic acceptability and number of unfilled grain per panicle. The result indicated that the genotypes in this cluster could be used to improve the variety with higher number of tiller per plant, short stature, good phenotypic acceptability and high yielding characters.

The genotypes of clusters III, IV and V gave the higher mean for plant height, days to flowering, days to maturity and number of unfilled grain per panicle. The lower mean was observed in number of tiller per hill and disease index. The result indicated that the genotypes in these cluster could be used to develop tungro resistant varieties.

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 plant height, days to flowering, days to maturity, panicle length, number of spikelet per panicle, number of unfilled grain per panicle and yield. These results indicated that seven characters had the highest contribution towards the divergence among the 14 tungro resistant and susceptible genotypes. In vector 1, the important characters responsible for the genetic divergence in the major axis of differentiation were tillering ability, plant height, days to flowering, days to maturity, panicle length, number of spikelet per panicle, number of unfilled grain per panicle, 1000 grain wt and yield having positive vector values while in vector 2 (the second axis of differentiation) panicle length, number of spikelet per panicle, number of filled grain per panicle, number of unfilled grain per panicle and yield were important. Negative values in both vectors for

number of tiller per hill and disease index indicated this character had the lowest contribution to the divergence.

From the above results it appeared that contribution of panicle length was the highest followed by plant height, number of unfilled grain per panicle, tillering ability, yield, days to maturity, 1000 grain wt, number of spikelet per panicle to the total divergence in 14 genotypes. Variavan *et al.* (1973) reported in that 1000 grain weight and plant height of rice were responsible for primary as well as secondary axis of differentiation. Julfiquar *et al.* (1985) also reported similar response for yield, 1000 grain weight, days to maturity and plant height in rice. Plant height and grain yield considerably contributed to the total divergence reported by Vivekanondan and Subramaniam (1993). On the other hand Chauhan and Chauhan (1994) reported that the contribution of 1000 grain was the highest followed by days to 50% flowering, panicle length and spikelet per panicle. Sing *et al.* (1999) also reported that harvest index and number of filled grains per panicle contributed maximum to the divergence in rice.

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

Character	Vector 1	Vector 2
	-0.2610	-1.3223
Number of tiller/hill		
Tillering ability	0.0635	-0.0847
Plant height	0.1344	0.0178
Phenotypic acceptability	-0.1393	0.4370
Days to flowering	0.0263	0.1450
Days to maturity	0.0556	0.1225
Panicle length	0.2438	0.6120
Number of spikelet/panicle	0.0066	0.0954
Number of filled grain/panicle	-0.0746	0.0985
Number of unfilled grain/panicle	0.1322	0.0978
1000 grain wt.	0.0075	-0.1482
Yield	0.0631	0.3510
Disease index	-0.8352	-0.9752

4.1.8 Comparison of result based on different multivariate techniques

Results obtained 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 character 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) provided the information regarding the contribution of characters towards divergence of 14 tungro 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; Moll *et al.*, 1962; Ramanujam *et al.*, 1974; Ghaderi *et al.*, 1984). Considering magnitude of genetic distance, contribution of different characters towards the total divergence, magnitude of genetic cluster means for different characters and performance of the genotypes were considered for hybridization programme. The genotypes of cluster I could be selected for earliest flowering and maturity, higher number of spikelet per panicle, higher number of filled grain per panicle and higher yield. The genotype of cluster II could be selected for higher no. of tiller per hill, lower plant height, high phenotypic acceptability and the lowest number of unfilled grain per panicle. The genotypes of cluster III, IV and V selected for higher 1000 grain weight and lower disease index. Therefore, high yielding along with susceptible (BR10 and BR11) and

moderately resistant (BRRI dhan31, BRRI dhan32 and BRRI dhan44) genotypes could be crossed with local low yielding but highly resistant genotypes (Sonahydemota, Kumragoir, Nakuchimota, Khaiyamota, Khairymota and Kachamota) for the development of tungro resistant varieties.

4.2 Molecular characterization

Isolated DNA for 14 genotypes showed nearly sickle shaped bands indicated good quality DNA and was quantified by λ DNA marker (Plate 1). DNA for each genotype was quantified as 6 ng/µl.

Three SSR along with 3 VNTR markers produced polymorphic bands. Only these 6 markers were scored among tungro resistant and susceptible genotypes for further analysis (Table 8).

Table 8. SSR and VNTR primers used in the present study

Primers	Oligonucleotide sequence
SSR	
RM20	ATCTTGTCCCTGCAGGTCAT F
	GAAACAGAGGCACATTTCATTG R
RM21	ACAGTATTCCGTAGGCACGG F
	GCTCCATGAGGGTGGTAGAG R
RM23	CATTGGAGTGGAGGCTGG F
	GTCAGGCTTCTGCCATTCTC R
VNTR or Mini satellites	
RY	CAGCAGCAGCAGCAG
MR	GAGGGTGGCGGTTCT
GF	TCCTCCTCCTCCTCC

F-Forward; R-Reverse

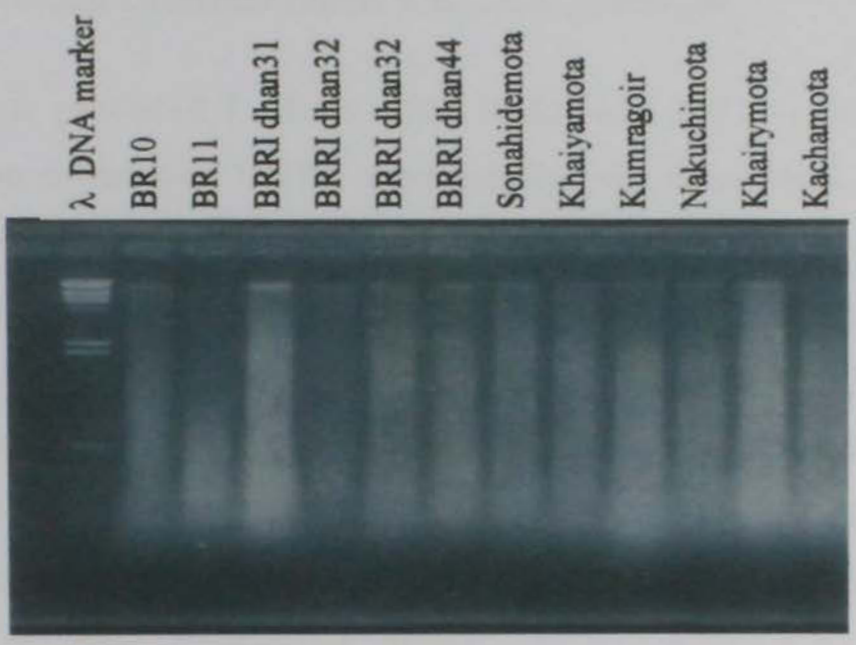


Plate 1.Quantification of DNA of tungro resistant genotypes

Each SSR markers allele and different loci of VNTR were measured and were present in table 9. Among the SSR markers, the amplicon size was the highest in RM 20 (199bp) followed by RM 21 (186bp) and RM23 (150bp). The range of amplicon size was also higher in RM20 (127-199bp) compared to RM 21(130-186bp) and RM 23(109-150bp). This result indicated that RM20 and RM23 might produce more alleles. This information could be used in further study of molecular characterization with other genotypes.

RM21 marker produced 7 alleles while RM20 and RM23 produced 8 alleles each. As the primers of VNTR were random so, more than one loci were present in VNTR. In each locus present or absent band (locus) was identified and no allele was considered. So, therefore PIC values were absent in VNTR primers (Table 9). The highest PIC values (0.8415) was for RM21 followed by RM23 (0.8406), RM20 (0.8058). All primers were considered as good markers for characterizing the 14 tungro resistant and susceptible genotypes. These findings agree with the results of several authors (Islam *et al.*, 2007; Priyanka *et al.*, 2004; Yunbi *et al.*, 2004).

The amplicons size of RM21 (186 bp) was found in tungro susceptible, BR10 and moderately resistant genotypes (BRRI dhan31, BRRI dhan32 and BRRI dhan44) while that was absent in highly resistant (Kachamota, Khairymota, Khaiyamota, Kumragoir, Nakuchimota and Sonahidemota), resistant (Bazail-Acc171, Bazail-Acc252, Rayeda- Acc4849) genotypes along with susceptible BR11. In locus RY2 of VNTR primer, the band size 390 bp was absent in resistant varieties namely Bazail and Rayeda while two susceptible (BR10 and BR11), highly resistant and moderately resistant varieties showed that band. Two amplicons one from GF2 (543 bp) and the other from GF5 (1003 bp) locus were fixed with susceptible and moderately resistant varieties while those were absent in highly tungro resistant varieties (Table 9). Plates 2, 3 and 4 showed gel pictures of some SSR and VNTR markers.

Table 9. Amplicon size, number of allele/locus and PIC values of SSR and VNTR markers for 14 tungro resistant and susceptible genotypes.

Primers	Chro. No.	Position on chro. (cM)	Tungro susceptible		Moderately resistant to tungro			Highly resistant to tungro						Tungro resistant			Allele / locus no	PIC
			BR10	BR11	BRRI dhan31	BRRI dhan32	BRRI dhan44	Sonahide mota	Khaiya mota	Kumrag oir	Nakuchi mota	Khairy mota	Kacha mota	Bazail Acc252	Bazail Acc171	Rayeda 4849		
RM20	12	32.0	189	170	183	183	183	176	199	170	127	170	170	197	143	143	8	0.8058
RM21	11	85.7	186	160	190	189	190	165	165	186	155	155	130	146	155	136	7	0.8415
RM23	1	72.6	144	129	125	125	129	113	121	121	109	109	117	121	150	150	8	0.8406
RY1	-	-	237	237	237	237	237	-	237	237	237	237	-	-	-	-	2 locus	-
RY2	-	-	390	390	390	390	390	390	390	390	390	390	390	-	-	-	2 „	-
MR1	-	-	323	323	-	-	-	323	323	323	329	-	-	-	-	323	5 „	-
MR2	-	-	-	-	371	371	371	-	-	-	371	371	-	371	371	371	5 „	-
MR3	-	-	773	-	773	773	773	773	773	773	773	773	-	-	-	-	5 „	-
MR4	-	-	-	-	1126	1126	1126	-	1126	1126	-	1126	-	1126	1126	-	5 „	-
MR5	-	-	2003	2003	-	-	-	2003	2003	2003	2003	2003	2003	2003	-	-	5 „	-
GF1	-	-	389	389	389	389	389	389	389	389	-	-	-	389	389	-	6 „	-
GF2	-	-	543	543	543	543	543	-	-	-	-	-	-	-	-	543	6 „	-
GF3	-	-	-	-	-	-	-	647	-	647	-	-	647	-	-	-	6 „	-
GF4	-	-	-	-	872	-	-	872	872	872	872	872	872	872	872	-	6 „	-
GF5	-	-	1003	1003	1003	1003	1003	-	-	-	-	-	-	-	-	1003	6 „	-
GF6	-	-	-	-	-	-	1114	1114	1114	-	-	-	-	1114	1114	-	6 „	-

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

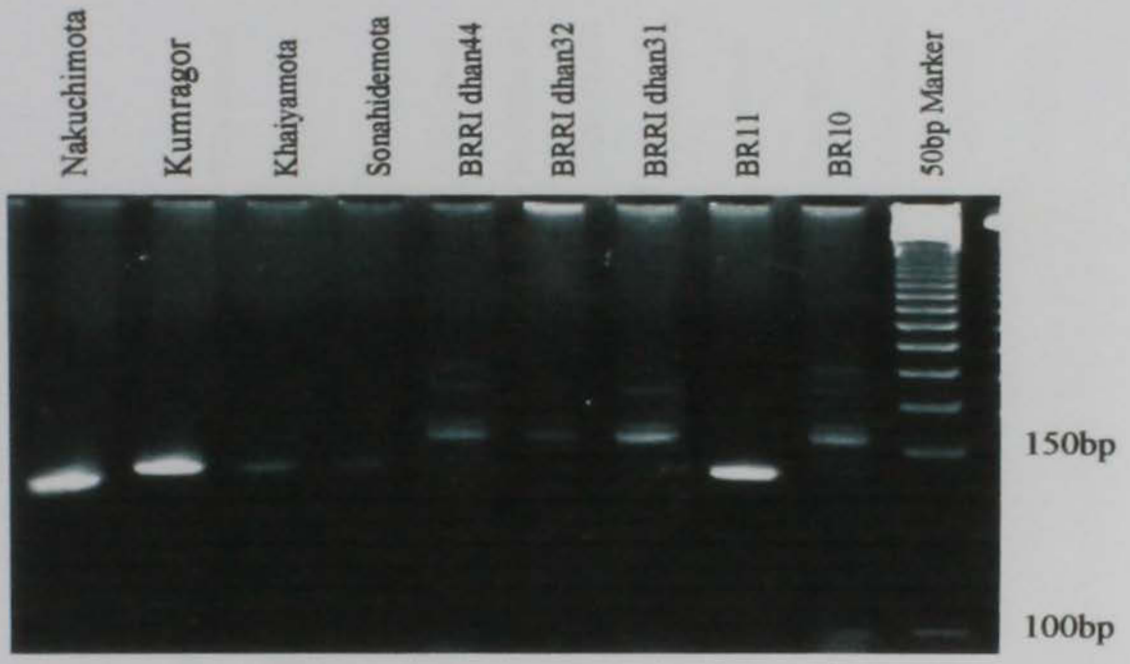


Plate 2. SSR banding patterns obtained from tungro resistant and susceptible genotypes using RM21 primer.

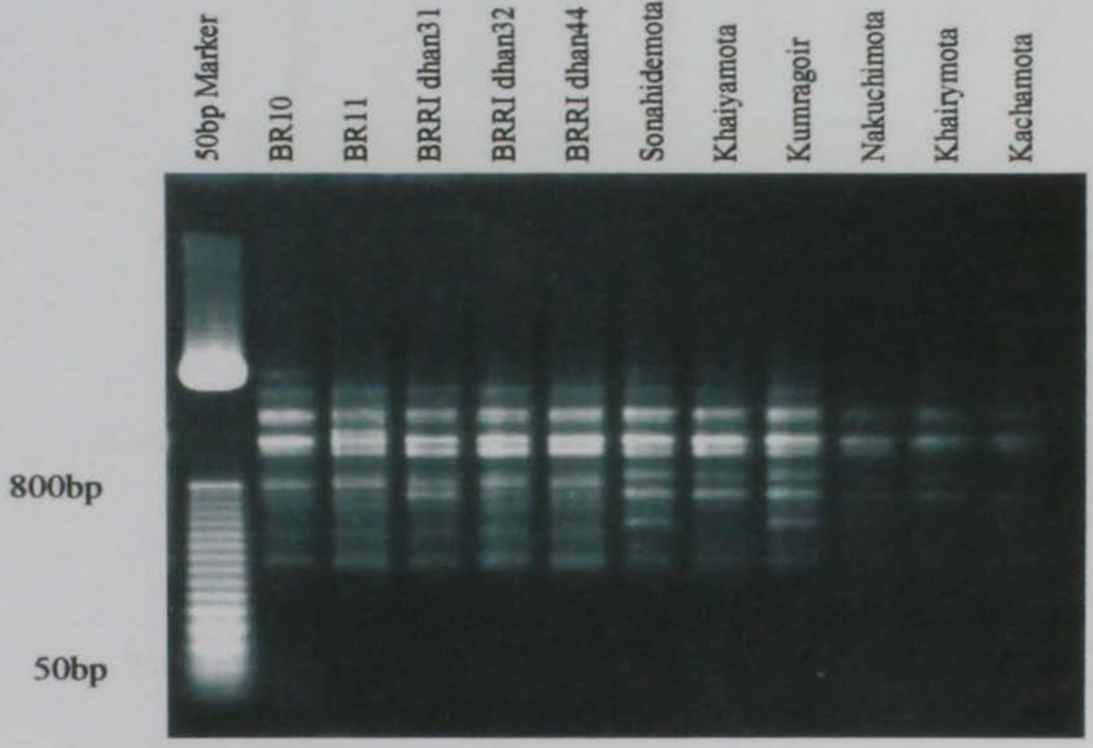


Plate 3. VNTR banding patterns obtained from tungro resistant and susceptible genotypes using GF primer.

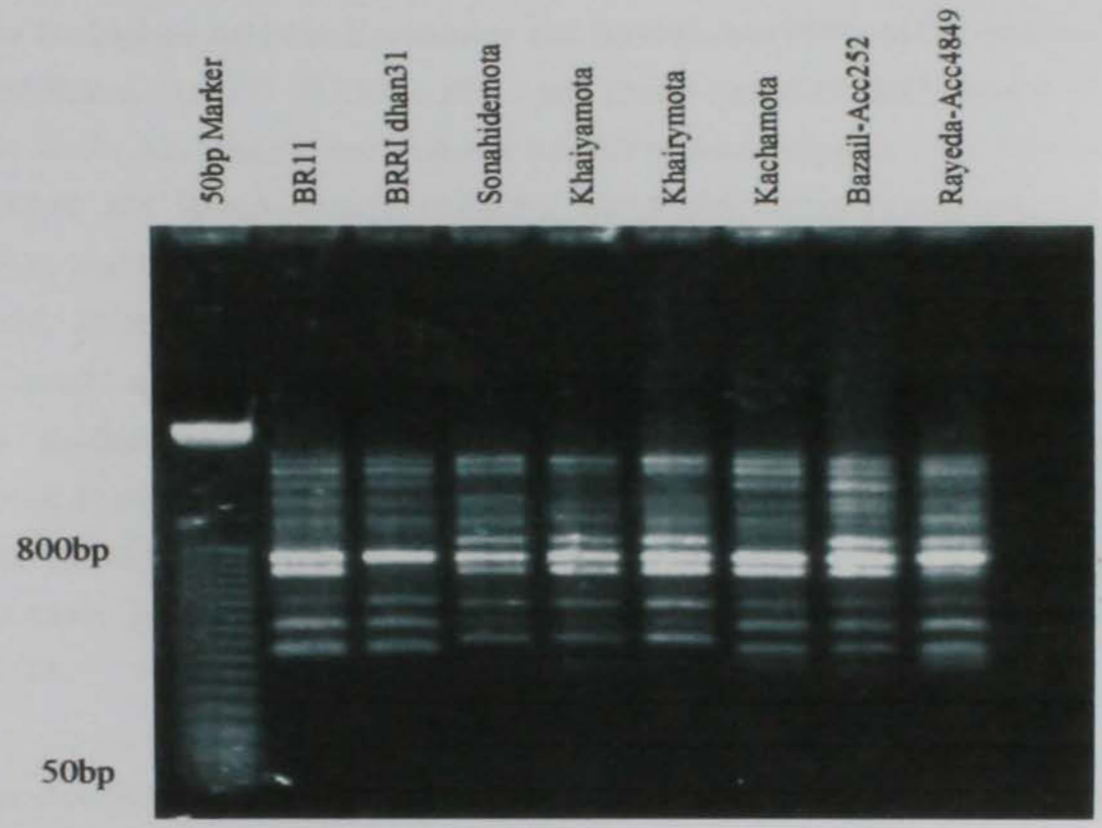


Plate 4. VNTR banding patterns obtained from tungro resistant and susceptible genotypes using MR primer.

For diversity analysis pair wise genetic distances were computed between all genotypes. Genetic distance was the smallest between BRRI dhan31 and BRRI dhan44 (0.0909), Khaiyamota and Kumragoir (0.0909) followed by Khairymota and Nakuchiomota (0.1111), BRRI dhan 32 and BRRI dhan 44 (0.1818), Khaiyamota and Sonahidemota (0.2000), Kumragoir and Sonahidemota (0.2000) and Khaiyamota and Khairymota (0.2222) (Table 10). These results suggested that these varieties might have been developed from common ancestors. On the other hand genetic distance was the highest between Kachamota and Rayeda-Acc4849 (1.000) followed by BR10 and Bazail- Acc171 (0.7500), BR11 and Bazail-Acc4849, Khaiyamota and Rayeda-Acc 4849, Kumragoir and Rayeda-Acc4849, Sonahidemota and Rayeda-Acc4849, BR10 and Bazail-Acc252 (0.6000), BR11 and Bazail-Acc252, Bazail-Acc252, BR11 and Bazail-Acc252, Kachamota and Bazail-Acc252, Kachamota and BRRI dhan32, BRRI dhan44 and Kachamota, Nakuchimota and Rayeda-Acc4849 and BRRI dhan32 and Bazail-Acc171 (0.5000), BRRI dhan32 and Bazail-Acc252, BR10 and Kachamota, BRRI dhan31 and Kachamota, BRRI dhan32 and Sonahidemota, Kumragoir and Bazail-Acc171, BR10 and Rayeda-Acc4849, BR11 and Rayeda-Acc4849, BRRI dhan31 and Rayeda-Acc4849, BRRI dhan32 and Rayeda-Acc4849, BRRI dhan44 and Rayeda-Acc4849 and Sonahidemota and Bazail-Acc171.

In order to develop tungro resistant variety and broaden the genetic base of rice new breeding program should be initiated preferably using the parents (highly resistant and susceptible but HYV type), BRRI dhan44 and Kachamota, BRRI dhan32 and Kachamota, BR10 and Kachamota, BRRI dhan31 and Kachamota, BRRI dhan32 and Sonahidemota. Although the genetic distances between some parents were higher but crossing program was not suggested because they were local types.

Table 10. Distance Matrix based on Nei's genetic distance among tungro resistant and susceptible genotypes

Genotypes	Resistant			Susceptible		Moderately resistant			Highly resistant					
	Bazail-ACC171	Bazail-ACC252	Rayeda-ACC4849	BR10	BR11	BRRI dhan31	BRRI dhan32	BRRI dhan44	Kacha mota	Khairy mota	Khaiya mota	Kumragoir	Nakuchi mota	Sonahide mota
Bazail-ACC171	**0.0000**													
Bazail-ACC252	0.3750	**0.0000**												
Rayeda-ACC4849	0.2500	0.7500	**0.0000**											
BR10	0.7500	0.6000	0.5000	**0.0000**										
BR11	0.7500	0.6000	0.5000	0.3000	**0.0000**									
BRRI dhan31	0.4286	0.4286	0.5000	0.3333	0.3750	**0.0000**								
BRRI dhan32	0.5000	0.5000	0.5000	0.3333	0.3750	0.0909	**0.0000**							
BRRI dhan44	0.4286	0.4286	0.5000	0.3333	0.2500	0.0909	0.1818	**0.0000**						
Kachamota	0.7500	0.6000	1.0000	0.5000	0.4000	0.5000	0.6000	0.6000	**0.0000**					
Khairymota	0.3333	0.4286	0.7500	0.4286	0.3333	0.3333	0.3750	0.3750	0.2857	**0.0000**				
Khaiyamota	0.4286	0.2500	0.7500	0.3333	0.3750	0.3333	0.3750	0.3333	0.4286	0.3333	**0.0000**			
Kumragoir	0.5000	0.2857	0.7500	0.3333	0.2500	0.3333	0.3750	0.3750	0.2500	0.2222	0.0909	**0.0000**		
Nakuchimota	0.4000	0.5000	0.6000	0.3750	0.4286	0.3750	0.4286	0.4286	0.4286	0.1111	0.3333	0.3333	**0.0000**	
Sonahidemota	0.5000	0.4286	0.7500	0.3750	0.4286	0.4286	0.5000	0.4286	0.3750	0.4286	0.2000	0.2000	0.3750	**0.0000**

Cluster analysis demonstrated the genetic relationship among the 14 genotypes including tungro resistant and susceptible genotypes. Dendrogram resolved the genotypes into 7 clusters (Fig. 2). The first cluster consisted of 3 genotypes namely Kumragoir, Khaiyamota and Bazail-Acc252. Second cluster consisted of only one genotype, Sonahidemota while 3rd cluster consisted of Kachamota. Khairymota and Nakuchimota grouped into 4th cluster while BR10 and BR11 grouped into 5th cluster. The varieties BR10 and BR11 were genetically similar but tungro susceptible varieties. Although the highly resistant tungro genotypes, Nakuchimota, Khairymota, Kachamota, Kumragoir, Khaiyamota and Sonahidemota grouped into 4 clusters but they were closely related clusters. The tungro registrant genotypes, Bazail-Acc171 and Rayeda-Acc4849 grouped into 6th cluster while moderately resistant genotypes BRRI dhan31, BRRI dhan32 and BRRI dhan44 grouped into 7th cluster. Clustering represented the genetic similarity among the genotypes as well as their susceptibility to tungro disease.

The genotypes belonging to the distant clusters 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 clusters 1st, 2nd, 3rd, 4th and 6th having greater cluster distance are recommended for inclusion in a hybridization program as they are expected to produce good segregants. Thus it could be suggested that crosses should be made between genotypes belonging to the distant clusters for higher heterotic response. Similar findings of genetic divergence are also reported by several authors (Ali *et al.*, 2003; Suh-Jung P *et al.*, 2004; Chakravarthi and Naravaneni, 2006)

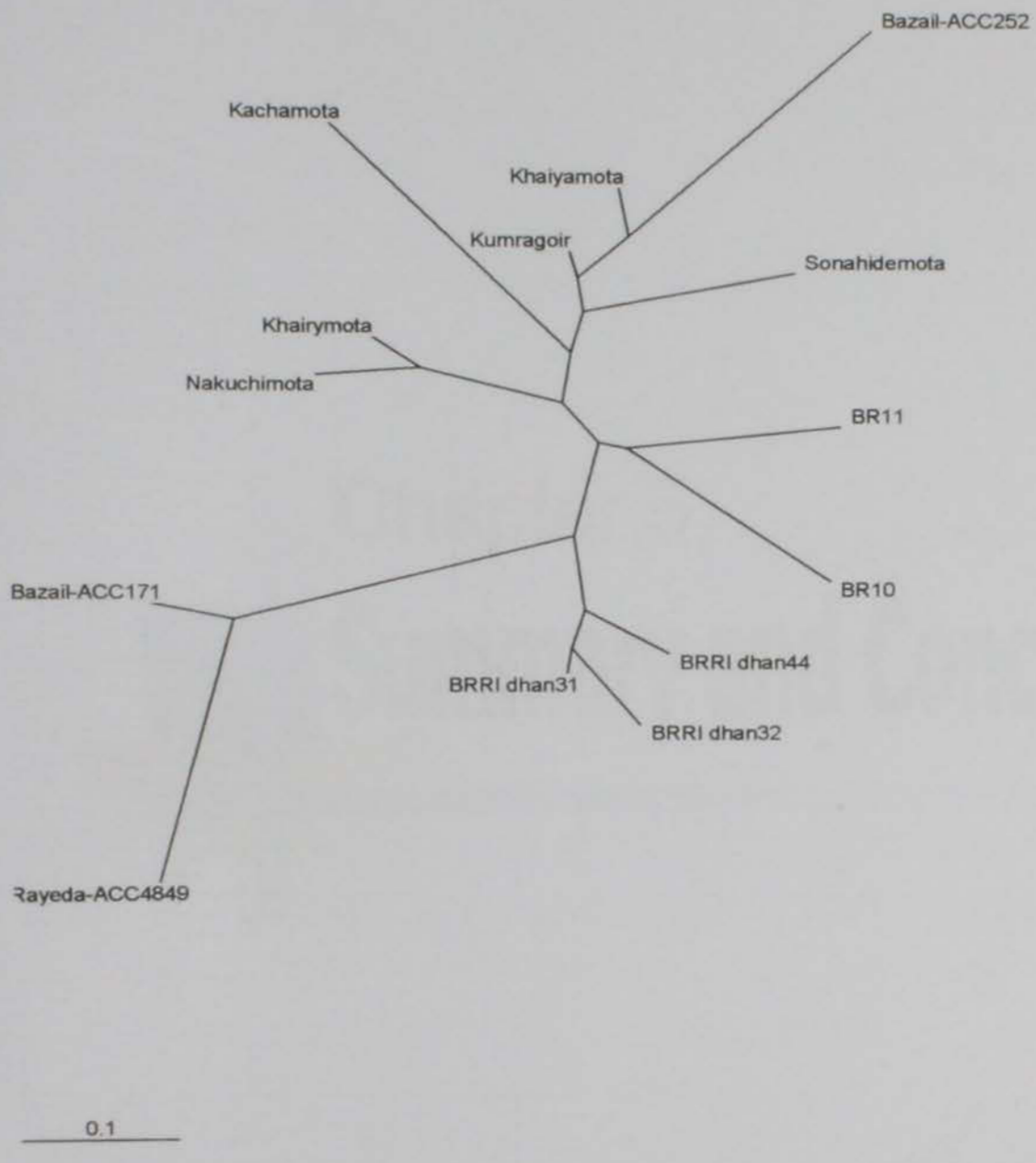


Fig.2. Dendrogram of genetic relationship of 14 tungro resistant and susceptible genotypes

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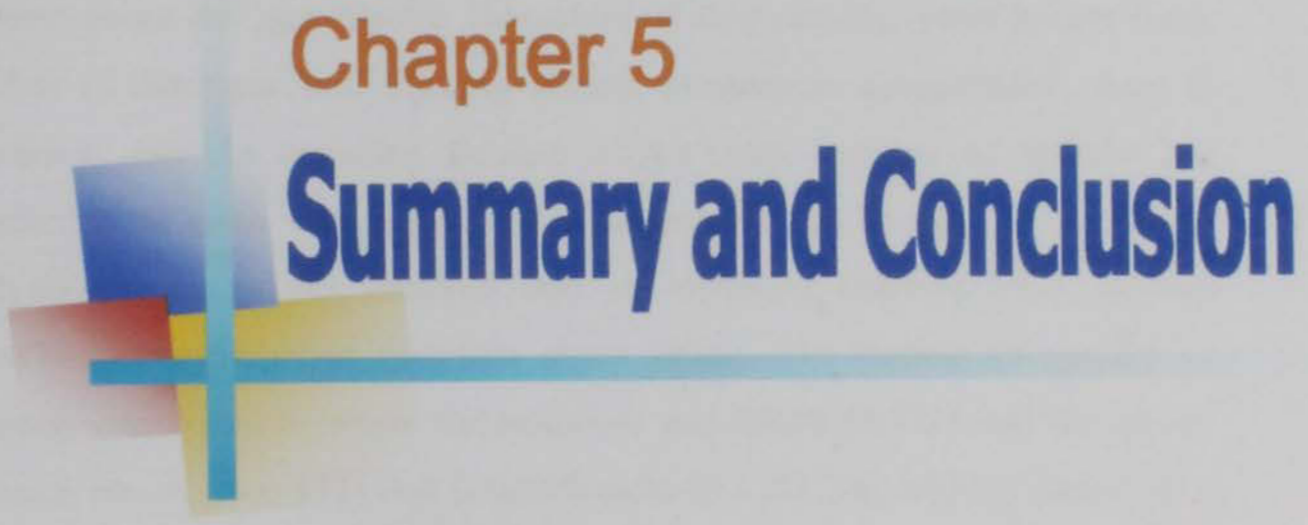


Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

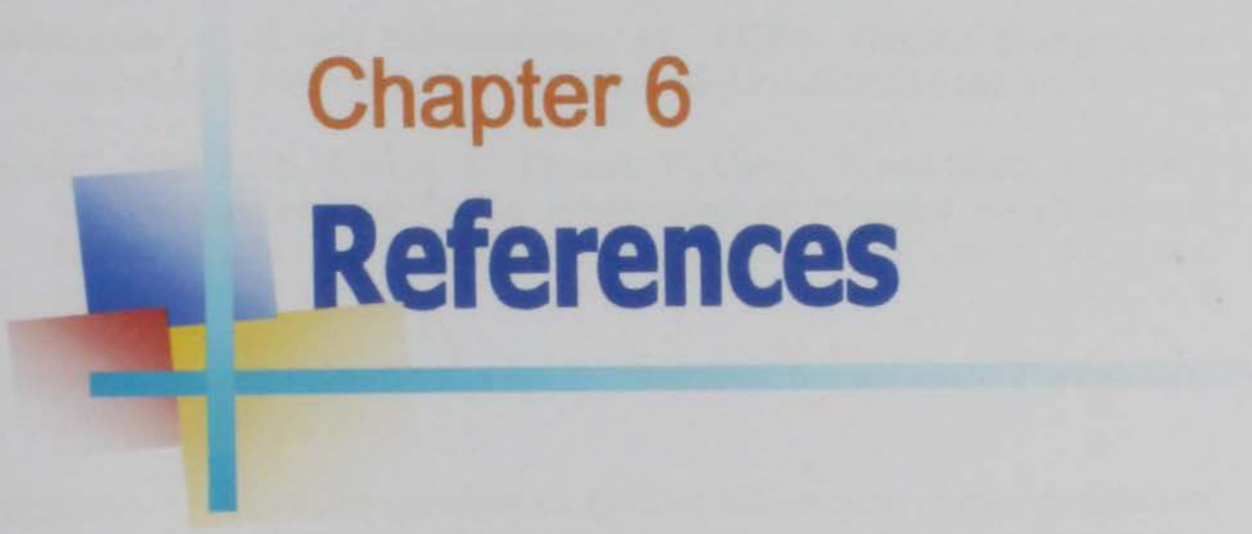
The studies were conducted in the experimental field and laboratory of Plant Pathology, BRRI to characterize tungro resistant and susceptible genotypes morphologically as well as molecular level. A total of 14 including six highly resistant, three resistant, three moderately resistant and two susceptible genotypes were used in this study. For knowing the genetic diversity of rice genotypes, multivariate analyses were performed through Principal Component Analysis, Principal Coordinate Analysis and Canonical vector analysis using the quantitative characters of rice namely, plant height (cm), number of tiller per hill, tillering ability, phenotypic acceptability, days to flowering, days to maturity, panicle length (cm), number of spikelet per panicle, 1000 grain weight (g) and yield per hill (g). As per PCA and D^2 analysis, the genotypes grouped into six different clusters. PCA showed 90.68% variation against first four eigen values. The highest intergenotypic distance was found between Nakuchimota and BR10 (2.472) and the lowest between Bazail (Acc 171) and Sonahidemota (0.125). The highest intra cluster distance was found for cluster III (1.992) composed of 3 genotypes and intra cluster was the lowest (0.661) in cluster II among the six clusters. In CVA the inter cluster distance was maximum between cluster I and VI (18.693) followed by the distance between cluster I and III (17.898), cluster II and III (17.490) while the distance was minimum between cluster VI and IV (4.434) followed by the distance between cluster V and III (5.126). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster I and II were far diverged from those of clusters III and VI.

Considering magnitude 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 I could be selected for earliest flowering and maturity, higher number of spikelet per panicle, higher number of filled grain per panicle, higher yield. The genotype cluster II could be selected for higher number of tiller per hill, lower plant height, high phenotypic acceptability and the lowest number of unfilled grain per panicle. The genotypes of cluster III, IV and V could be selected for higher 1000 grain weight and lower disease index.

The genotypes belonging to clusters VI and I, clusters III and I, clusters II and III having greater cluster distance are recommended for inclusion in a hybridization programme for the development of tungro resistant varieties as they are expected to produce good segregants. Heterosis could also be exploited by crossing between genotypes belonging to clusters with moderate diversity like between genotypes of clusters II and V, clusters I and IV, clusters I and V.

A total of six primers including three SSRs and three VNTRs were used to characterize tungro resistant genotypes. PIC values of SSR primers, RM20, RM21 and RM23 ranged from 0.65-0.84. So these primers were considered as very much useful for characterizing tungro resistant genotypes. The amplicons size of RM21 (186 bp) was found in tungro susceptible, BR10 and moderately resistant genotypes (BRRI dhan31, BRRI dhan32 and BRRI dhan44) while that was absent in highly resistant (Kachamota, Khairymota, Khaiyamota, Kumragoir, Nakuchimota and Sonahidemota), resistant (Bazail-Acc171, Bazail-Acc252, Rayeda- Acc4849) and BR11 genotypes. In VNTR primer RY, the band size 390 bp was absent in resistant varieties while two susceptible, highly resistant and moderately resistant varieties showed that

band. In another VNTR primer GF, two amplicons (543 bp and 1003 bp) were fixed with susceptible and moderately resistant varieties while those were absent in highly tungro resistant varieties. Based on Nei's genetic distance, seven clusters were formed among the tungro resistant genotypes. Susceptible varieties, BR10 and BR11 formed a cluster while moderately resistant varieties, BRRI dhan31, BRRI dhan32 and BRRI dhan44 formed another cluster. The highly resistant genotypes formed four groups but they were closely related clusters. Diversity analysis of tungro resistant genotypes using morphological and molecular data indicated that new breeding program could be initiated preferably using the parents, BRRI dhan44 and Kachamota, BRRI dhan32 and Kachamota, BR10 and Kachamota, BRRI dhan31 and Kachamota, BRRI dhan32 and Sonahidemota.



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References

CHAPTER 6

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Chapter 7
Appendices

CHAPTER 7

APPENDICES

Appendix 1: Mean values of 13 characters of 14 tungro resistant and susceptible genotypes.

enotypes	TPH	TI	PH	PA	DF	DM	PL	SP	FGP	UNFGP	1000GW	GY	DI
R10	14	5	125.1	1	106	146	25	163	143	18	21	42	7
R11	13	5	114	1	104	143	23	132	112	17	23	45	7
RRI dhan31	13	5	124	1	103	140	24	127	111	18	23	40	5
RRI dhan32	12	5	118	3	96	128	24	125	118	16	21	38	5
RRI dhan44	12	5	122	3	88	126	25	167	139	18	26	45	5
bnahidemota	9	7	172	5	110	154	25	87	79	21	36	37	1
haiyamota	13	5	149	5	115	153	23	84	71	30	29	30	1
umragoir	9	7	168	7	110	161	24	79	73	30	27	29	1
akuchimota	7	7	176	7	113	165	22	65	68	27	27	31	1
hairymota	10	5	156	7	112	173	24	95	86	28	30	34	1
achamota	10	5	153	7	108	181	25	78	70	25	34	35	1
azail(Acc-52)	9	7	175	7	107	130	25	81	50	30	24	29	3
azail(Acc-71)	9	7	170	7	109	139	27	80	49	29	24	30	3
ayeda(Acc-849)	12	5	174	7	111	144	25	79	45	26	11	33	3

TI= Tiller per hill

TA= Tillering ability

PA= Phenotypic acceptability

PL= Plant height

DF= Days to flowering

DM= Days to maturity

PL= Panicle length

SP= Spikelet per panicle

UNFGP= No. of unfilled grain per panicle

FGP= No. of filled grain per panicle

1000GW= 1000 grain weight

GY=Grain yield

DI= Disease index

Appendix: 2. Allele frequency of SSR and VNTR primers of 14 tungro resistant and susceptible genotypes

Marker	Allele/locus	Count	Freq	Variance	SD
RM20	127	2	0.0714	0.00473761	0.0688
RM20	143	4	0.1429	0.00874636	0.0935
RM20	170	8	0.2857	0.01457726	0.1207
RM20	176	2	0.0714	0.00473761	0.0688
RM20	183	6	0.2143	0.01202624	0.1097
RM20	189	2	0.0714	0.00473761	0.0688
RM20	197	2	0.0714	0.00473761	0.0688
RM20	199	2	0.0714	0.00473761	0.0688
RM21	130	2	0.0714	0.00473761	0.0688
RM21	136	2	0.0714	0.00473761	0.0688
RM21	146	2	0.0714	0.00473761	0.0688
RM21	155	6	0.2143	0.01202624	0.1097
RM21	160	2	0.0714	0.00473761	0.0688
RM21	165	6	0.2143	0.01202624	0.1097
RM21	186	2	0.0714	0.00473761	0.0688
RM21	189	2	0.0714	0.00473761	0.0688
RM21	190	4	0.1429	0.00874636	0.0935
RM23	109	4	0.1429	0.00874636	0.0935
RM23	113	2	0.0714	0.00473761	0.0688
RM23	117	2	0.0714	0.00473761	0.0688
RM23	121	6	0.2143	0.01202624	0.1097
RM23	125	4	0.1429	0.00874636	0.0935
RM23	129	4	0.1429	0.00874636	0.0935
RM23	144	2	0.0714	0.00473761	0.0688
RM23	150	4	0.1429	0.00874636	0.0935
RY1	237 locus	18	1.0000	0.00000000	0.0000
RY2	390 locus	22	1.0000	0.00000000	0.0000
MR1	323 locus	14	1.0000	0.00000000	0.0000
MR2	371 locus	16	1.0000	0.00000000	0.0000
MR3	773 locus	20	1.0000	0.00000000	0.0000
MR4	1126 locus	16	1.0000	0.00000000	0.0000
MR5	2003 locus	18	1.0000	0.00000000	0.0000
GF1	389 locus	20	1.0000	0.00000000	0.0000
GF2	543 locus	12	1.0000	0.00000000	0.0000
GF3	647 locus	6	1.0000	0.00000000	0.0000
GF4	872 locus	18	1.0000	0.00000000	0.0000
GF5	1003 locus	12	1.0000	0.00000000	0.0000
GF6	1114 locus	10	1.0000	0.00000000	0.0000

Appendix: 2. Allele frequency of SSR and VNTR primers of 14 tungro resistant and susceptible genotypes

Marker	Allele/locus	Count	Freq	Variance	SD
RM20	127	2	0.0714	0.00473761	0.0688
RM20	143	4	0.1429	0.00874636	0.0935
RM20	170	8	0.2857	0.01457726	0.1207
RM20	176	2	0.0714	0.00473761	0.0688
RM20	183	6	0.2143	0.01202624	0.1097
RM20	189	2	0.0714	0.00473761	0.0688
RM20	197	2	0.0714	0.00473761	0.0688
RM20	199	2	0.0714	0.00473761	0.0688
RM21	130	2	0.0714	0.00473761	0.0688
RM21	136	2	0.0714	0.00473761	0.0688
RM21	146	2	0.0714	0.00473761	0.0688
RM21	155	6	0.2143	0.01202624	0.1097
RM21	160	2	0.0714	0.00473761	0.0688
RM21	165	6	0.2143	0.01202624	0.1097
RM21	186	2	0.0714	0.00473761	0.0688
RM21	189	2	0.0714	0.00473761	0.0688
RM21	190	4	0.1429	0.00874636	0.0935
RM23	109	4	0.1429	0.00874636	0.0935
RM23	113	2	0.0714	0.00473761	0.0688
RM23	117	2	0.0714	0.00473761	0.0688
RM23	121	6	0.2143	0.01202624	0.1097
RM23	125	4	0.1429	0.00874636	0.0935
RM23	129	4	0.1429	0.00874636	0.0935
RM23	144	2	0.0714	0.00473761	0.0688
RM23	150	4	0.1429	0.00874636	0.0935
RY1	237 locus	18	1.0000	0.00000000	0.0000
RY2	390 locus	22	1.0000	0.00000000	0.0000
MR1	323 locus	14	1.0000	0.00000000	0.0000
MR2	371 locus	16	1.0000	0.00000000	0.0000
MR3	773 locus	20	1.0000	0.00000000	0.0000
MR4	1126 locus	16	1.0000	0.00000000	0.0000
MR5	2003 locus	18	1.0000	0.00000000	0.0000
GF1	389 locus	20	1.0000	0.00000000	0.0000
GF2	543 locus	12	1.0000	0.00000000	0.0000
GF3	647 locus	6	1.0000	0.00000000	0.0000
GF4	872 locus	18	1.0000	0.00000000	0.0000
GF5	1003 locus	12	1.0000	0.00000000	0.0000
GF6	1114 locus	10	1.0000	0.00000000	0.0000