# DNA FINGERPRINTING AND PHYLOGENETIC ANALYSIS OF BASMATI AND OTHER RICE TYPES USING SSR AND TRANSPOSON ELEMENT BASED MARKERS

By

**AMIT KAUSHIK**
**[2001BS62D]**

**Dissertation submitted to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in the partial fulfillment of the requirements for the degree of**

## Doctor of Philosophy
## in
## Biotechnology and Molecular Biology

**COLLEGE OF BASIC SCIENCES AND HUMANITIES**
**CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY**
**HISAR-125004**

**2006**

**To My Parents**

# CERTIFICATE - I

This is to certify that this dissertation entitled, **“DNA fingerprinting and phylogenetic analysis of Basmati and other rice types using SSR and Transposon element based markers”** submitted for the degree of **Doctor of Philosophy** in the subject of **Biotechnology and Molecular Biology** to **Chaudhary Charan Singh Haryana Agricultural University, Hisar,** is a bonafide research work carried out by **Amit Kaushik** under my supervision and guidance and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

**(Dr. R.K. Jain)**
**Major Advisor**
PROFESSOR
Department of Biotechnology and Molecular Biology
CCS Haryana Agricultural University
Hisar-125004, India

# CERTIFICATE - II

This is to certify that this dissertation entitled **"DNA fingerprinting and phylogenetic analysis of Basmati and other rice types using SSR and Transposon element based markers"** submitted by **Amit Kaushik** to **Chaudhary Charan Singh Haryana Agricultural University, Hisar,** in the partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Biotechnology and Molecular Biology**, has been approved by the student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

**MAJOR ADVISOR** **EXTERNAL EXAMINER**

**CO-MAJOR ADVISOR**

**HEAD OF THE DEPARTMENT**

**DEAN, POST GRADUATE STUDIES**

# Acknowledgement

As I submit my thesis, it gives me a sense of immense contentment to express my gratefulness to all those who have contributed in their special way to make the competition of this thesis possible.

With the sense of gratitude and profound privilege, I express my sincere thanks to my major advisor **Dr. Rajinder K Jain**, Senior Scientist, (Department of Biotechnology and molecular biology, for his keen interest, constant motivation, constructive criticism and wise counsel during my entire study. His patience and insight into the subject has always made me realize and understand it in a broader way.

I am highly thankful to **Dr. KS Boora** (Head Department of Biotechnology and Molecular Biology) continuous support and cooperation.

A an highly thankful to the members of Advisory committee, **Dr. V.K. Chowdhury** (Co-Major), Dean, College of Basic Sciences and Humanities, **Dr. OPS Rana** and **Dr. PK Sareen** (Retd.), Professor, Department of Genetics, **Dr. Sunita Jain**, Associate Professor, Department of Biochemistry and **Dr.(Mrs.) Suresh Sharma** (DeanPGS, nominee), for their constant support and cooperation.

I would like to thanks **Dr. Rakesh K Kashyap** (Professor, Department of Seed Science and Technology) for intellectual advice and everlasting support.

**Dr. Sunita Jain** deserves special mention for her immense patience in listening to my troubles, valuable advice, affectionate behavior, everlasting support and helpful suggestions.

Among the staff members, I would like to thanks **Dr RC Yadav**, **Dr (Mrs.) NR Yadav** (Department of Biotechnology and Molecular Biology) and **Dr. Sudhir Sharma** (Professor, Department of Chemistry) for their encouragement and pertinent help. I am also thankful to **Dr. Kamla Chaudhary** (Professor) for her continuous support throughout my degree and to **Dr. Meenakshi** (ABT, COVS, CCSHAU) and **Dr. VK Sikka** for their helping attitude.

I am thankful to **Dr. Kuldeep Singh** and **Dr Naveen Singh Kaliramna** (PAU, Ludhiana) and **Dr. AK Singh** (IARI, N. Delhi) for providing me research material.

I am deeply indebted to **Dr. Navinder Saini** (Scientist, JNKVV, Jabalpur, MP), **Vijai Soni** (Scientist, DFRL, Mysore), **Dr. Seema Bhutani** (Research Associate, Department of Biotechnology and Molecular Biology, CCSHAU), **Munish bhaisahab** and **Sudershan aunty**, **Dr. Poonam Rana** (Scientist, INMAS, N. Delhi), **Dr. Neelu Jain** (Scientist, IARI, N. Delhi), **Dr. Ajay Amar Vashisht** (ICGEB, N. Delhi), **Dr. Sheetal Yadav** (Research Associate, Phytotron, IARI, N. Delhi) and **Mr. Surender Pal Kapoor** (URL, Gurgaon) for their assistance, guidance, encouragement and timely help.

I can not forget to acknowledge my senior **Dr. Mayank Yadav** and my friends: **Manoj Verma, Umesh Dada, Dinesh Shandilay, Sushil Goyat, Vivek Balyan, Satish Nara, Avnish, Krishan Garg, Nawal Sharma, Harpreet Singh, Niranjan** and **Rakesh Kharb** for their everlasting support.

I convey my heartful thanks to my juniors **Dandi, Parminder, Singla, Poria, Mattu, Sheenu, Aarti Sharma, Javid, Suman, Rakesh Sehrawat, Desraj, Ashok Dhankhar, Naveen Kaushik, Parveen Goyal, Ritesh,**

**Bhagwat, Mirchi Seth, Chetak, Supriya Neelam, Sahrdul Deepak** and **Rouchika** for their cheerful support during my Ph.D. I owe thanks to office staff members **Thakral ji, Sarsoot ji, Daya aunty, Praveen Bhaiya, Subhash ji, Pandey ji, Sunil** and **Dharamvir** for their kind help.

Words cannot express the supporting attitude, encouragement and assistance of Garima, who was always there from beginning to end during my Ph. D. programme.

Feeling could not be expressed in words for **Bhabhi, Pitaji, Sanju bhaiya** and my family members for their supportive attitude.

The financial help provided by **CSIR** for pursuing my thesis is duly acknowledged.

Finally I would like to thanks all those persons who knowingly or unknowingly helped me in my endeavor and I wish everyone mentioned here the best of luck for their future.

**(Amit Kaushik)**

2001BS62D

# CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

# LIST OF PLATES

# CHAPTER -I

# Introduction

The history of domesticated plant form and function evolves along a two-tiered track that doubles back on itself, offering panoramic vistas of natural forces intertwined with the creative force of human endeavor. All grasses belong to a single family, *Gramineae*, that contains ~10,000 species, and originated ~65 million years ago (mya; Kellogg, 2000) and was domesticated probably in northeastern India and southern China about 8000 years ago (Khush, 1997).

The rice genus *Oryza*, including two cultivated species and about 21 wild species, is widely distributed in tropical and subtropical regions (Vaughan 1989). Rice is endowed with very rich genetic diversity. They have an invaluable gene pool of wild relatives and primitive landraces/cultivars for the rice genetic improvement (Chang 1984; Oka 1988; Yuan *et al.*, 1989; Jackson, 1994, 1997; Xiao *et al.*, 1996; Tanksley and McCouch, 1997). In the last three decades, wild species and landraces in rice have been successfully utilized for introgression of diverse traits such cytoplasmic male sterility (cms), abiotic and biotic stress, yield and its components and grain quality into the cultivars.

Cultivated rice (*Oryza sativa* L.) holds a unique position among domesticated crop species in that it is both a critical food staple and the first fully sequenced crop genome. Rice is consumed as a grain almost exclusively by humans, supplying 20% of daily calories for the world population (World Rice Statistics http://www.irri.org; FAOSTAT, http://apps.fao.org). The Green Revolution has dramatically increased the food supply in the developing world over the past four decades. Since 1962, the population in Asia has more than doubled from 1.6 to 3.7 billion. Rice production has grown by 170%, whereas the land area used for agriculture has grown by <30% during the same period. Increased production efficiency has reduced the rice price to ~50% in real terms over the past three decades. Population growth is continuing at more than 2% annually in many developing rice growing countries. To feed this growing population, the growth rate of rice production needs to be accelerated.

Besides grain yield, quality has also been an important consideration in rice breeding and gained greater importance with increase in world-wide demand for quality rices (Juliano, 1998; Khush and Juliano, 1991; Singh *et al.*, 2000b). Based on the survey of 11 major rice growing countries, Juliano and Duff (1991) reported that grain quality is second only to yield as the major breeding objective. Among the various rice types, Basmati rice has long been preferred in Indian sub-continent, Middle-east, Europe, United States and Australia for its grain qualities (Bhasin, 2000). Basmati rice constitute a small but special group of rice, which is considered best because of its aroma, and excellent cooking qualities including extra elongation, soft and flaky texture (Singh *et al.*, 2000a; Khush and dela Cruz, 2002; Nene, 2003). Basmati rice has its origin in the foothills of Himalayas and then spread to eastern and western regions of Indian sub-continent and is the result of centuries of selection and cultivation by farmers (Khush, 2000). General characteristics of Basmati rice are aromatic, fragrant, long grain, slender rice, and have a nutty flavor. Length is one; the grain should be long (6.61 - 7.5 mm) or very long (more than 7.50 mm). Shape or length-to-width ratio should over 3.0. The colour of a basmati is translucent, creamy white. Upon cooking, the texture should be firm and tender without splitting, and it should be non-sticky. *Oryza* market report in 2006 reported that India, world's second largest producer of rice had exported 4.7 million tonne last fiscal year and the world's largest exporter of Basmati rice. During 2003-04, India's exports of basmati rice totaled 7.10 lakh tonnes valued at Rs 2,062 crore (Rs 20.62 billion) against 6.67 lakh tonnes valued at Rs 1,842 crore the previous year.

Historically, genetic diversity and intraspecific classification in Asian rice has been studied using morphological, serological and hybrid fertility parameters in combination (Kato *et al.*, 1928), morphogeographical (Matsuo, 1952; Oka, 1958), hybrid sterility (Terao and Mizushima, 1942), isozymatic (Glaszmann, 1987) data and more recently using the DNA markers (Aggarwal *et al.*, 1999; Blair *et al.*, 1999; Jain *et al.*, 2004; McCouch *et al.*, 2001; Blair *et al.*, 2002). Kato (1928) recognized two rice varietal groups, *indica* and *japonica*. Basmati rice varieties of Indian subcontinent have been conventionally classified as aromatic *indica*. Glaszmann (1987, 1988) used the isozyme markers based polymorphism to classify the Asian rice germplasm comprising of 1688 native cultivars into six varietal groups. The Basmati rice varieties were clustered in a distinct Group V different than those for *indica* (Group I) and *japonica* (Group VI) rice varieties. PCR-based molecular markers including micro-satellite DNA markers (simple sequence repeats (SSRs); Bligh *et al.*, 1999; Bligh, 2000; Blair *et al.*, 2002; Nagaraju *et al.*, 2002; Jain *et al.*, 2004), AFLPs (Cho *et al.*, 1996; Maheshwaran *et al.*, 1997; Aggarwal *et al.*, 1999, 2002;) and ISSRs (Blair *et al.*, 1999; Qian *et al.*, 2001; Nagaraju *et al.*, 2002) have been successfully used for genotype identification and diversity analysis in rice including Basmati rice. In most of these studies, Basmati types clustered into a separate group distinct from that of *indica* and *japonica* rice varieties (Bligh *et al.*, 1999; Aggarwal *et al.*, 2002; Nagaraju *et al.*, 2002; Jain *et al.*, 2004). The results in fact indicated that Indian Basmati germplasm may have a long, independent and complex pattern of evolution that distinguishes it from other groups within *Oryza sativa* (Jain *et al.*, 2004).

Rice has been at the forefront of plant genomics because of its small genome size and relatively low amount of repetitive DNA, its diploid nature, and its ease of manipulation in tissue culture. With the rice genome sequence database in place, it will be possible to create a more or less exhaustive inventory of genes of rice and try to assign a function to them, allele mining in large collection of rice varieties and wild species of rice maintained at various national and international agencies, to identify and use an almost unlimited number of DNA markers (SSR, SNP, InDel, etc) for genome analysis, marker-assisted breeding and positional cloning, compare the genome organization, genes and intergenic regions between cereal species, which may provide crucial insights into genome evolution, speciation and domestication and gather information on various biochemical pathways pathways and gene expression. Knowledge of the rice genome has already accelerated the process of gene discovery (functional genomics) and shall be a boon for plant breeders who are trying to increase yield and create new varieties which are resistant to disease, pests, drought and salinity and/or with improved nutritional quality. Thus, rice genomic information shall provide the vital information about the genome of other cereals crops required for genetic improvement programs.

Earlier there has been no reliable way to distinguish ecotypes on the basis of phenotypic evaluation and because information about the varietal groupings is rarely available from genetic resource collections, a genetically based identification of groups is required to fully utilize these resources. Molecular marker technology is playing a vital role in both basic and applied research, such as fingerprinting genotypes, analyzing genetic diversity, determining variety identity, marker-assisted breeding, phylogenetic analysis, and map-based cloning of genes (McCouch *et al.*, 1997; Joshi *et al.*, 2000; Nagaraju *et al.*, 2002; Ni *et al.*, 2002). In rice (*Oryza sativa*), more than 10,000 molecular markers have been developed (Saito *et al.*, 1991; Causse *et al.*, 1994; Kurata *et al.*, 1994; Harushima *et al.*, 1998; Wu *et al.*, 2002). Most of them are expressed sequence tags and RFLPs. In addition to expressed sequence tags and RFLPs, simple sequence repeat (SSR) markers, transposable element based markers and single nucleotide polymorphism (SNP) markers have been developed in recent years.

Simple sequence repeats (SSRs) are highly polymorphic sequences found throughout plant and animal genomes (Chen *et al.*, 1997; Temnykh *et al.*, 2000, 2001; McCouch *et al.*, 2002; Joshi *et al.*, 2000). These markers are based on repeats of short DNA sequences (2-5 bp) that are highly polymorphic, co-dominant and easy to detect. The markers can be detected with relatively low-cost manual gel systems or can be carried out on a large scale using DNA sequencing equipment (Coburn *et al.*, 2002). Approximately 2,740 SSR markers have been genetically mapped in rice (Chen *et al.*, 1997; Temnykh *et al.*, 2000, 2001; McCouch *et al.*, 2002). The rice sequence database shows the presence of one SSR marker for every 157 bp (IRGSP, 2005). Being highly polymorphic, SSRs can be used for the characterization of germplasm collection composed of closely related genotypes (Ni *et al.* 2002, Yang *et al.* 1994). Studies have shown that microsatellite markers developed for cultivated rice, can be successfully used in related wild species, and thus provide powerful tools for phylogenetic analysis (Gao *et al.* 2002a). Microsatellite DNA polymorphisms have also been widely used to study population and conservation genetics (Nevo 1998; Friar *et al.* 2001; King *et al.* 2001; Olsen and Schaal 2001). The use of SSR's to interpret population structure provides much greater resolution than other types of markers (Cho *et al.*, 2000).

Transposable elements (TEs) are mobile DNA sequences that are widely distributed in prokaryotes and eukaryotes. Transposable elements (TEs) are fragments of DNA that can insert into new chromosomal locations and often make duplicate copies of them in the process. With the advent of large-scale DNA sequencing, it has become apparent that, far from being a rare component of some genomes, TEs are the single largest component of the genetic material of most eukaryotes. They account for at least 45% of the human genome (Lander *et al.*, 2001) and 50–80% of some grass genomes (Meyers *et al.*, 2001). The recent availability of large quantities of genomic sequence has led to a shift from the genetic characterization of single elements to genome-wide analysis of enormous transposable-element populations. Nowhere is this shift more evident than in plants, in which transposable elements were first discovered and where they are still actively reshaping genomes.

Despite its small genome, rice is a model organism for the study of transposable elements (Jiang *et al.*, 2002). TE constitute a large portion of rice genome and may have greatly contributed towards the evolution and diversification in rice/cereals. Recently, a new class of markers based on transposable elements (TEs) have been developed (mPing and Dasheng elements), which provides a ubiquitous source of polymorphism and genome size variation in eukaryotes (Zhang and Wessler 2004; Kazazian, 2004). Since, transposable elements are known to play a vital role in speciation/evolution, markers based on such elements might give a better account of genetic relationships between different varieties and/or sub-species. The study of these markers is helpful in studying the evolutionary and phylogenetic studies of the species as under pressure or any other stress, transposon tends to jump form one loci to the other loci in the genome. Such transposable elements may also provide useful markers for varietal differentiation in rice.

In the last decade, a polyphyletic group of class II non-autonomous elements named 'MITEs' (miniature inverted-repeat transposable elements) has been described (Bureau and Wessler, 1992; Zhang *et al.*, 2001b). These elements are of small size (from 150 to 600 bp), high copy number (in the thousands), longer terminal inverted repeats (TIRs) of size range between 15 and 25 bp, and a unique target site preference (most often TA or TAA). Previous estimates of the transposon content in the rice genome range from 10 to 25% (Mao *et al.*, 2000). However, the increased availability of transposon query sequences indicates that the transposon content of the *O. sativa* spp. *japonica* genome is at least 35% (IRGSP, 2005). MITEs are small transposable elements (usually <500 base pairs) containing short terminal repeats (TIR's) that occur in high copy number (approx. 1000-15000) in rice genome.

Dasheng is a very recently amplified family of 800–1300 nonautonomous elements, making it one of the most recently amplified and highest-copy-number families in rice. The family also includes ~16% solo LTRs. Like many other high-copy-number LTR elements, Dasheng elements are concentrated in the gene-poor pericentromeric regions of the chromosomes, which might be the reason for Dasheng's success in the small genome of rice. High levels of polymorphism were also detected within *indica* and *japonica* subspecies, indicating Dasheng elements can serve as a valuable marker system (Waugh *et al.*, 1997; Van den Broeck *et al.*, 1998; Casa *et al.*, 2000).

Public accessibility to the genome sequences of several organisms has enabled the study of sequence variations between individuals, cultivars, and subspecies. These studies revealed that single-nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) are highly abundant and distributed throughout the genome in various species including plants (Garg *et al.*, 1999; Drenkard *et al.*, 2000; Nasu *et al.*, 2002). By comparing sequences from a *japonica* rice cultivar to those from an *indica* cultivar, Yu *et al.* (2002) identified, on average, one SNP every 170 bp and one InDel every 540 bp in rice. The abundance of these markers in plant genomes makes them very attractive for their application in marker-assisted breeding and map-based cloning programs (Gupta *et al.*, 2001; Rafalski, 2002; Batley *et al.*, 2003b).

Adulteration of premium Basmati rice varieties with cheaper varieties is common practices as it fetch more prices in comparison to pure Basmati rice varieties. Due to the recent development of DNA-analysis for evaluation of adulteration the Code of Practice for Rice developed by GAFTA (Grain and Feed Trade Association) permits the presence of up to 7% non-Basmati rice. Basmati is not only nearly twice as expensive as normal rice, but import tax is 250 €/ton lower than for conventional rice (EU-regulation 1503/96). Some of the Basmati rice varieties recommended for export have been exempted from export duty. Varietal identification of Basmati rice supplies has become more important in basmati rice export to Europe due to the existence of this variable duty regime on Basmati rice export. European Union has revised its Basmati-policy. Regulation No. 2294/2003 amending regulation 1503/96 imposes stricter controls including use of DNA-analysis. Only 7 of the approved 15 Basmati-varieties are tax-exempted under this regulation. Zero duty on some Basmati rice *varieties* (Ranbir Basmati, Dehradun Basmati type III, Karnal Local, HBC-19) and other rice varieties (Basmati 217, Basmati 370, Pusa Basmati, Kasturi, Mahi Suganda, Basmati 385) developed from crosses enjoys low export duty. Due to these reasons it has been recommended that DNA markers can be used to check adulteration and varietal identification.

The phylogenetic status of Basmati rice varieties vis-à-vis *indica*/*japonica* is still not clear. While Basmati rice has been classified as aromatic *indica* based on long grain types and geographical location, but both isozymatic and DNA marker based studies showed that Basmati rice types are genetically distinct from *indica* and *japonica* types and may have long history of independent evolution. Transposon element based markers has been used in many cases to trace the evolutionary path of species (*Drosophila melanogaster*, maize, yeast, *E. coli*, *Antirrhinum*, *Caenhorabditis elegansi,* human etc.). TE plays important role in evolution and evolution of TE is an important landmark for a species (Kidwell and Lisch, 1997; Kikuchi *et al.*, 2003; Fescotte *et al.*, 2002). “Master” or limited amplification model given by Deininger *et al.* (1992) concluded that most TE copies (of retro types) in the genome arouse from a few active master copies and that different sub-families were active at different evolutionary periods. Therefore, TE sub-families that are active after the radiation of two sub-species (or two phylogenetic groups) should generate new copies at specific loci not shared between the species.

The present study was undertaken with the following objectives:

1. To prepare DNA fingerprinting database of Basmati and other rice types using SSR and transposon element based markers.
2. To deduce phylogenetic relationship between Basmati and non-Basmati rice types.

# CHAPTER -II

# Review of Literature

Rice (*Oryza sativa*) belongs to the genus *Oryza* which includes more than 25 wild species (diploid or tetraploid) either perennial or annual. Rice is composed of two major subspecies, *indica* and *japonica* and several ecotypes, which are adapted to diverse environmental conditions ranging from upland to lowland and from tropical to temperate zones. With such a wide range of geographic distribution, rice is well-known for its rich genetic resources maintained worldwide with more than 250,000 germplasm collections of more than 20 cultivated and wild species (Evernson *et al.*, 1998). Archeological evidence supports a similar time of domestication for rice, wheat (*Triticum aestivum*), and maize (*Zea mays* ssp. *mays*), 5–10,000 years ago, but evolutionary histories of these cereals differ in several significant ways (Solheim, 1972; Sharma and Manda 1980; Zohary and Hopf, 2000; Piperno and Flannery 2001). Rice was domesticated probably in northeastern India and southern China about 8000 years ago and is the staple food for more than 50% of the world's population (Khush, 1997). Rice cultivars are planted over 150 million hectares with production of about 600 million tonnes in the world. In Asia, rice production would increase 25% by 2010 which means that the current average yield of 5 tonnes per hectare needs to be increased to 8 tonnes per hectare. However, yield of about 17 tonnes per hectare was reported for super hybrids in China (Xiao *et al.*, 1996). Rice is consumed as a grain almost exclusively by humans, supplying 20% of daily calories for the world population (World Rice Statistics, http://www.irri.org; FAOSTAT, http://apps.fao.org).

Rice has one of the largest *ex situ* germplasm collections in the world (Jackson and Juggan, 1993). Rice is predominantly autogamous and, hence, gene flow is restricted. As a result, geographically or ecologically distinct groups of rice are expected to show greater genetic differentiation than would be the case in an out-crossing species. This accessible collection of diverse cultivated and wild rice germplasm has made great contributions to rice breeding. Due to the continuous selection of domesticated wild rice for the development of high yielding and well adapted varieties, which occupied more than half of world's rice growing area, has led to genetic erosion which in-turn narrowed variability in rice.

## 2.1 Classification

Kato *et al.* (1928), the pioneer for rice classification, recognized two main varietal groups, designated as *indica* and *japonica* on the basis of morphological, serological characters and intervarietal hybrid fertility parameters. Although this classification provided high probability of placement of varieties into two varietal groups, a few varieties remained unclassified as a typical. Morinaga (1954) introduced a third group *'javanicas'* based on their geographical distribution. Later on, Oka (1958) demonstrated that *japonica* and *javanica* are the temperate and tropical forms of a single *japonica* group. Chang (1976) gave a revised classification referring to three morphological types, the *indica*, *japonica* and *javanica* because of their differentiation based on characters. Some researchers found peroxidase and acid phosphatase alleles specific for only two types i.e. *indica* and *japonica* (Pai *et al.*, 1975; Fu and Pai, 1979).

Glaszmann (1987) examined 1688 native cultivars from different Asian countries for allelic frequencies at 15 iso-enzyme loci and analyzed the data by multivariate technique (factor analysis of correspondence). The results showed that 95% of the cultivars fell into 6 groups and remaining 5% scattered over intermediate position. Group

I (*indica*) is observed in tropical Asia and South of Indian subcontinent. It comprises most varieties of the Aman ecotypes, Hsien ecotypes and lowland varieties of Southeast Asia, China and Indonesia, respectively. Group II is observed exclusively in Sri Lanka, South India and frequent along Himalayas, from Iran to Assam hills. Group II corresponds to Aus and Boro ecotypes of Bangladesh and India. Group III is found only in Bangladesh and in Manipur state of India. It consists of particular rice types of short cycle, photoperiod insensitive and adapted to deep water conditions. Group IV corresponded to Rayada rice of Bangladesh. These are late maturing, photoperiod sensitive varieties and are able to stand 12 days flooding and to adjust their elongation up to 6 m depth. Group V spreads from Iran to Burma. It consists of very diverse varieties including world famous high quality aromatic rice's called Basmati, from India and Pakistan. It also includes high quality rice's from Iran, Nepal and Burma, which have very high cooking elongation. Group VI is dominant in temperate areas and in high elevation areas in Southeast Asia and South Asia. It includes the Bulu rice's from Java and Bali, most upland rice's from Southeast Asia, the Keng rice's from China and the traditional rice's from Japan and Korea (*japonica*). Group I is considered as '*Indica'* type and Group VI as '*Japonica*' type. Groups II, III, IV and V share common differences from groups I and VI that shows alternative evolutionary history.

## 2.2 Basmati rice

Various scented or aromatic rice varieties have been grown in Indian sub-continent for centuries. All aromatic rice's of Southern and Southeastern Asia originated in the Himalayan foothills of U.P., Bihar and Terai Nepal, and dispersed out of here (Khush, 2000).

Of these, the most talked about today is the Basmati rice, Bas originating from Prakrit Vas which has a sanskrit root-Vasay connoting aroma; and mati from mayup meaning ingrained from the origin. Common usage has changed Vas to Bas while joining bas and mayup the latter changed to mati. Thus the word Basmati "King of Rice and queen of fragrance" originated. The preferential treatment Basmati receives over scented and non-aromatic types is due to its distinguishing characters such as superfine grains with typical aroma in both the raw and cooked state, excellent linear elongation on cooking which is almost double its kernel length, soft and flaky consistency of cooked rice (Siddiq, 1982; Khush and Juliano, 1985; Shoba Rani, 1992). Cooked Basmati rice is non-sticky, and has longer shelf life and is easily digestible. It is concluded that Basmati has been a distinct cultivar of rice grown in India and Pakistan for more than 250 years (Nene, 1998).

General characteristics of Basmati rice are aromatic, fragrant, long grain, slender rice, and have a nutty flavor. Length is one; the grain should be long (6.61 - 7.5 mm) or very long (more than 7.50 mm). Shape or length-to-width ratio should be over 3.0. The colour of a basmati is translucent, creamy white. Upon cooking, the texture should be firm and tender without splitting, and it should be non-sticky. This quality is derived from the amylose content in the rice. Higher the amylose content (more than 20%) the rice will be non-sticky. The rice elongates almost twice upon cooking but does not fatten much. Low-amylose cooked rice tends to be moist, tender and cohesive, while high amylose content is more likely to result in dry, fluffy, and separated grains (Juliano, 1971).

Buttery *et al.* (1983) identified 2-acetyl, 1-pyrroline (AP) as the major component contributing to rice aroma. AP is found in all plant parts except roots (Lorieux *et al.*, 1996) and the concentrations of this component is found up to 100 times lower in non-fragrant rice’s (Grosch and Schieberle, 1997). In addition to 2-acetyl, 1-pyrroline, there are about 100 other volatile compounds which are associated with the aroma development in rice. 86 varieties have been classified as basmati; only 18 of them qualify under the strict basmati standards.

India is the world's second largest producer and exporter of Basmati rice and. Major destinations of Basmati rice export include Saudi Arabia, other middle east countries, Europe and USA. Basmati rice export crossed the milestone of 1 million metric tonne MT for the first time in 2005-06 at 1.15 million metric tonne. The traditional destination, Saudi Arabia happened to be the main destination last year and accounted for nearly 60 per cent of the total export volume. However, the Middle East region accounted for around 72 per cent of the total export share. The European Union followed with a share of around 15 per cent with the United Kingdom accounting for around 10 per cent share (www.basmati.com). The following varieties (**Indian varieties:** Basmati 217, Basmati 370, Basmati 386, Dehradun Type III, Haryana Basmati (HKR 228/IET 10367), Kasturi (IET 8580), Punjab Basmati (Bauni Basmati), Mahi Suganda, Pusa Basmati (IET 10364), Ranbir Basmati (IET 11348), Taraori (Karnal Local, HBC-19). **Pakistani varieties:** Basmati 198, Basmati 370, Basmati 385, Kernel (Basmati Pakistan), Super Basmati) have been approved by the Indian and Pakistani authorities and can use the description "Basmati". They all have at least one parent which is a true-line Basmati variety, and they have been approved on the basis of having the above unique properties as measured by various objective tests such as grain dimension, amylose content, cooking elongation, and aroma.

European Commission has already resolved the issue of duty on Basmati rice. While a tariff of 65 €/tonne would be imposed on husked rice, for four varieties of Basmati rice the import duty would be zero. The import of four varieties of Basmati (Ranbir Basmati, Dehradun Basmati type III, Karnal Local, HBC-19.) and other rice varieties (Basmati 217, Basmati 370, Pusa Basmati, Kasturi, Mahi Suganda, Basmati 385) developed from crosses enjoys low export duty. During 2003-04, India's exports of basmati rice totaled 7.10 lakh tonnes valued at Rs 2,062 crore (Rs 20.62 billion) against 6.67 lakh tonnes valued at Rs 1,842 crore the previous year.

## 2.3 Wild relatives and their relationship with *O sativa*

Wild relatives and early landrace varieties of rice have long been recognized as the essential pool of genetic variation that will drive the future of plant improvement (Bessey, 1906; Burbank, 1914). Early plant collections made by Nikolai Vavilov (1887– 1943) or Jack Harlan

(1917–1998) inspired the international community to establish long-term collections of plant genetic resources that provide modern plant breeders with the material they need to creatively address the challenges of today. The old land races can be considered as populations of genes and genetic variability, which can substantially contribute towards various rice breeding programmes and is absolutely essential for further improvement. In fact, variability is absolutely essential to even hold onto what we already have" (Harlan, 1972a).

Rice is gifted with very rich genetic diversity, including 2 cultivated species and about 21 wild species, is widely distributed in tropical and subtropical regions (Vaughan, 1989). Wild species in *Oryza* are important genetic resources and have been proven an invaluable gene pool for the rice genetic improvement (Chang, 1984; Oka, 1988; Yuan *et al.*, 1989; Jackson, 1994, 1997; Xiao *et al.*, 1996; Tanksley and McCouch, 1997). Wild germplasm have played significant roles in rice breeding by contributing genes valuable for resistance to disease and insect pests, and tolerance to abiotic stress (Chang *et al.*, 1975; Sitch *et al.*, 1989; Khush, 1997). Species in the genus are highly diverse in morphology (Clayton and Renvoize, 1986; Vaughan, 1994), and are represented genetically by ten different genome types, i.e. the AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH and JJKK genomes, providing opportunities to explore variations for rice breeding programs.

The fuller exploitation of the wild-rice gene pool essentially relies on the better understanding of genetic diversity and relationships of the *Oryza* species, which will pose a significant impact on rice production (Sharma, 1983). Therefore, a thorough assessment of genetic diversity and relationships between rice and its wild relatives, particularly those with the AA-genome, has become increasingly important not only for their efficient utilization, but also for their effective conservation (Sun *et al.*, 2001; Lu *et al.*, 2002).

Large genetic variability still remains untapped in the wild relatives and primitive cultivars of rice. Considering the large hidden variability and the rare, agronomically important genes they possibly possess, utilization of the wild species is critical to future crop improvement. Utilization of these exotic species as donors in interspecific crosses is one of the strategies to harness their hidden potential and broaden the genetic diversity of the existing gene pool. Over the last decade, wild species in rice have been successfully utilized for introgression of diverse traits such cytoplasmic male sterility (cms), abiotic and biotic stress, yield and its components and grain quality into the cultivars. A great deal of work in the recent past, on the wild species of rice, concentrated on the utilization of these species for quantitative traits such as yield and its components long with grain quality.

Future rice security depends upon continued access to genetic diversity as a source of genes with useful traits from ~21 wild *Oryza* species distributed in the world (Vaughan, 1994).

## 2.4 Rice genomics

Genomics broadly refers to the mapping, sequencing and functional analysis of genomes, the entire genetic complement of an organism. The rice genomic information is a vital starting point for mining new genes and various pathways (Delseny *et al.*, 2001; Osterlund and Paterson, 2002). This treasure of sequence information helps:

1) Geneticists and breeders to acquire an almost unlimited number of DNA markers and genes for crop improvement,
2) Molecular biologists can use the full set of genes to permit comprehensive characterization of gene expression by several high-throughput approaches,
3) Physiologists, developmental biologists, and biochemists can inspect the gene complements in rice and related species to see which pathways are shared and which are unique, and how these pathways may have been modified,

4) Quantitative geneticists gain instantaneous access to the genes in their segregating population that may be responsible for the traits they have mapped,
5) Population geneticists can survey unlimited rice germplasm through single nucleotide polymorphisms (SNPs) for the study of allelic variability and distribution in rice,
6) Comparative geneticists will have unlimited opportunities to relate specific changes in gene structure and content to identify the differences in different plant, animal and microbial species,
7) Structural biologists can use rice database for complete set of predicted and known peptides to identify those that are of most interest for three-dimensional characterization, and finally
8) Evolutionary biologists can mine rice genome and genes to understand how gene families are created, amplified and diverge to create new biological activities and specificities. A new biological discipline of functional genomics has emerged after the completion of entire rice genome sequencing and large gene sets prediction.

The implementation of new technological platforms also allows us to investigate gene functions and their products at the genome level. Transcriptome profiling will be particularly valuable to investigate the coordinated expression of a suite of genes involved in specific biochemical pathways or in the adaptive response to biotic and abiotic stresses. Consequently, the integration of transcriptome data with proteome and metabolome data will be necessary to obtain a more realistic view of the role of specific genes. Our capacity to improve steadily cereal production and quality will depend increasingly on our ability to identify and manipulate the genes controlling quantitative traits.

In the next decade, the amount of information generated both *in vivo* and *in silico* will increase exponentially. The ultimate challenge will be to manage such a deluge of information properly (Hess *et al.*, 2001) in order to characterize allelic variation (natural and/or artificially induced in cultivated species as well as their wild relatives) and harness its potential to promote and stabilize productivity in a broader range of environments (Miflin, 2000). Clearly, cereal genomics and its applications have a great and unrivalled potential to shape the future of agriculture and its sustainability.

Rice has been at the forefront of plant genomics because of its small genome size and relatively low amount of repetitive DNA, its diploid nature, and its ease of manipulation in tissue culture. In the 1990s, many advances occurred in the application of molecular markers in rice (Mackill and Ni, 2001; Temnykh *et al.*, 2001; Xu, 2002). In fact, progress towards development of molecular techniques has been more rapid with rice than any other cereal. Some of the milestones includes: (i) development of the first saturated restriction fragment length polymorphism (RFLP) map (McCouch *et al.*, 1988); (ii) the application of polymerase chain reaction (PCR) based markers such as simple sequence repeat (SSR) markers (McCouch *et al.*, 2002; Jain *et al.*, 2004), amplified fragment length polymorphism (AFLP) (Cho *et al.*, 1996; Saini *et al.*, 2004); (iii) the identification of genes/quantitative trait loci (QTLs) for many agronomically important traits and marker assisted breeding (Mackill and Li, 2001; Li 2001; Xu 2002; Jain *et al.*, 2003); (iv) development of efficient techniques for genetic transformation which makes rice the easiest cereal to transform (Jain 2003); (v) complete sequencing and annotation of *indica* and *japonica* rice genomes (Goff *et al.*, 2002; Yu *et al.*, 2002; IRGSP, 2005), Development of new generation markers (SNP, indels etc. Rafalski, 2002) and (vii) synteny between genomes of rice and other cereals (Moore *et al.*, 1995) that makes rice a good entry point for characterizing the genes of other cereals, and associating them with various agronomic traits.

The genome of the *japonica* subspecies of rice, an important cereal and model monocot, was sequenced and assembled by whole-genome shotgun sequencing (Goff *et al.*, 2002). The assembled sequence covers 93% of the 420-megabase genome. Of the 30,000 to

50,000 predicted genes in rice, approximately 20,000 have sequence similarity, or homology, to previously discovered genes whose function is known, which allows researchers to predict the role of those genes in rice.

## 2.5 Molecular Markers

Molecular markers are valuable tools in both basic and applied research such as fingerprinting genotypes, analyzing genetic diversity, determining variety identity, marker-assisted breeding, phylogenetic analysis, map-based cloning of genes (McCouch *et al.*, 1997; Joshi *et al.*, 2000; Nagaraju *et al.*, 2002; Ni *et al.*, 2002) and have greatly enhanced the genetic analysis of crop plants. A variety of molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs), have been developed in different crop plants [Philips *et al.*, 2001; Varshney *et al.*, 2004]. In rice (*Oryza sativa*), more than 10,000 molecular markers have been developed (Saito *et al.*, 1991; Causse *et al.*, 1994; Kurata *et al.*, 1994; Harushima *et al.*, 1998; Wu *et al.*, 2002). Approximately 2,740 SSR markers have been genetically mapped in rice, about one SSR marker every 157 bp (Chen *et al.*, 1997; Temnykh *et al.*, 2000, 2001; McCouch *et al.*, 2002). The assessment of genetic diversity and/or differentiation of rice has been attempted in diverse rice samples with many systems of genetic markers such as morphological traits (Oka, 1964; Morishima and Oka, 1981; Cheng, 1985), biochemical markers (Nakagahra, 1978; Second, 1982; Glaszmann, 1987), DNA randomly amplified polymorphisms (RAPDs) (Mackill, 1995), DNA restriction fragment length polymorphisms (RFLPs) (Wang and Tanksley, 1989; Zhang *et al.*, 1992; Sun *et al.*, 2002), amplified fragment length polymorphisms (AFLPs) (Mackill *et al.*, 1996; Zhu *et al.*, 1998) and simple sequence repeats (SSRs) (Yang *et al.*, 1994).

### 2.5.1 Microsatellite Markers

Among different classes of molecular markers, SSR markers are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multi-allelic nature, Mendelian inheritance, co-dominant inheritance, relative abundance and good genome coverage [Squirrell *et al.*, 2003]. SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously have provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variation and proved to be a powerful tool to analyse significantly higher polymorphisms in rice for evolutionary analyses (Wu and Tanksley, 1993; Yang *et al.*, 1994; Nagaraju *et al.*, 2002; Gao *et al.*, 2002).

Microsatellites are tandemly arranged repeats of short DNA motifs (1–6 bp in length) that frequently exhibit variation in the number of repeats at a locus. Because of their abundance and inherent potential for variation, these simple sequence repeats (SSRs) have become a valuable source of genetic markers. Previous studies in rice have contributed to the development of several hundred microsatellite markers and a genetic map consisting of 320 SSRs (Wu and Tanksley 1993; Akagi *et al.*, 1996; Panaud *et al.*, 1996; Chen *et al.*, 1997; Temnykh *et al.*, 2000). These markers have been used to analyze diversity (Yang *et al.*, 1994; Olufowote *et al.*, 1997; Cho *et al.*, 2000; Harrington, 2000) and to locate genes and QTLs on rice chromosomes using both intra- and interspecific crosses (Xiao *et al.*, 1998; Bao *et al.*, 2000; Zou *et al.*, 2000; Bres-Patry *et al.*, 2001; Moncada *et al.*, 2001). SSRs are increasingly useful for integrating the genetic, physical, and sequence-based maps of rice, and they simultaneously provide breeders and geneticists with an efficient tool to link phenotypic and genotypic variation. In the past, the advantages of microsatellite markers were partially offset by the difficulty inherent in marker development, as laborious iterations of genomic DNA library screening with SSR probes were required to isolate microsatellite containing sequences (Panaud *et* *al.*, 1996; Chen *et al.*, 1997). As random rice EST sequences became available, they provided a new source of SSR markers (Akagi *et al.*, 1996; Temnykh *et al.*, 2000) but the chromosomal positions of these markers had to be determined by genetic mapping. More recently, the growing pool of DNA sequence information being generated by the International Rice Genome Sequencing Project (IRGSP) and by other organizations (http:// www.rice-research.org) allows high-throughput *in silico* identification of SSR loci in sequenced regions, often with known map position, providing an excellent starting point for marker development.

Analysis conducted by Goff *et al.* (2002) revealed a total of 48,351 dinucleotide SSRs (eight repeat units minimum), trinucleotide SSRs (five repeat units minimum), and tetranucleotide SSRs (four repeat units minimum), or about one SSR every 8000 bp. Di-, tri-, and tetranucleotide SSRs account for 24%, 59%, and 17%, respectively, of the SSRs found in rice. The frequency of specific SSRs is neither random nor representative of the genome GC content. The most frequent dinucleotide SSR is AG/CT and variants, representing 58% of all dinucleotides. The most frequent trinucleotide is CGG/CCG and variants, at 44% of all trinucleotide SSRs. ATCG/CGAT is the most common tetranucleotide repeat unit. More than 7000 SSRs were found in predicted genes. Most of these SSRs (92%) are trinucleotides, so length changes should maintain the open reading frame. In addition to SSRs, 38 Mbp of long repetitive DNA and 150 Mbp of short repetitive DNA were also identified (Goff *et al.*, 2002)

### 2.5.2 Transposable Element

Mobile or transposable, elements are prevalent in the genomes of all plants and animals (Kidwell and Lisch, 2000). Indeed, in mammals they and their recognizable remnants account for nearly half of the genome (kazazian, 2004), and in some plants they constitute up to 90% of the genome (SanMiguel *et al.*, 1996). Over millions of years of evolution, mobile elements have achieved a balance between detrimental effects on the individual and long-term beneficial effects on a species through genome modification. Indeed, we may soon learn that the shaping of the genome by mobile elements has played an important role in events leading to speciation. Whether these repeated sequences are now “junk DNA” is a complex issue. Some may have had an important function long ago, but have lost that role today. Others may never have had a function, yet the cluttering of our genomes with nonfunctional DNA was a small price to pay for the genome malleability they provided. These elements include (i) DNA transposons, (ii) autonomous retrotransposons, and (iii) nonautonomous retrotransposons

### 2.5.2.1 DNA Transposons

DNA transposons are prevalent in bacteria (where they are called IS, or insertion sequences), but are also found in the genomes of many metazoa, including insects, worms, and humans. These elements are generally excised from one genomic site and integrated into another by a “cut and paste” mechanism. Insertions can occur at a large number of genomic sites. However, daughter insertions for most, but not all, DNA transposons occur in proximity to the parental insertion. This is called “local hopping.”

### 2.5.2.2 LTR Retrotransposons

Retrotransposons are transcribed into RNA, and then reverse transcribed and reintegrated into the genome, thereby duplicating the element. The major classes of retrotransposons either contain long terminal repeats at both ends (LTR retrotransposons) or lack LTRs and possess a polyadenylate sequence at their 3 termini (non-LTR retrotransposons).

### 2.5.2.3 Non-LTR Retrotransposons

Non-LTR retrotransposons are typified by LINE-1 (long interspersed nucleotide elements –1, or L1) elements of mammals. Full length non-LTR retrotransposons are 4 to 6 kb in length and usually have two open reading frames (ORFs), one encoding a nucleic acid binding protein, and the other encoding an endonuclease and a reverse transcriptase.

The rice genome is populated by representatives from all known transposon super families, including elements that cannot be easily classified into either class I or II (Turcotte *et al.*, 2001). Previous estimates of the transposon content in the rice genome range from 10 to 25% (Mao *et al.*, 2000). However, the increased availability of transposon query sequences and the use of profile hidden Markov models allow the identification of more divergent elements and indicate that the transposon content of the *O. sativa* spp. *japonica* is at least 35% (IRGSP, 2005).

Despite its small genome, rice is still a model organism for the study of transposable elements. The LTR elements are classified into two types *O. sativa* contains all of the major types of elements on the basis of the order of their encoded genes found in the larger grass genomes, including retrotransposons, miniature inverted repeat transposable elements (MITEs), and other DNA elements (Bureau *et al.*, 1996; Mao *et al.*, 2000; Tarchini *et al.*, 2000; Turcotte *et al.*, 2001). Furthermore, the availability of several well-characterized wild relatives provides the material necessary to analyze the impact of TEs on genome evolution and speciation. *O. sativa* is composed of two cultivated subspecies (*indica* and *japonica*) with thousands of diverse cultivars distributed worldwide. The genus Oryza has more than 20 species whose evolutionary relationships have been the subject of several phylogenetic analyses (Uozo *et al.*, 1997; Ge *et al.*, 1999; Sharma *et al.*, 2000).

Recently, a new class of markers based on transposable elements (TEs) have been developed, which provides a ubiquitous source of polymorphism and genome size variation in eukaryotes (Zhang and Wessler, 2004; Kazazian, 2004). Since, transposable elements are known to play a vital role in speciation/evolution, markers based on such elements might give a better account of genetic relationships between different varieties and/or sub-species. Such transposable elements may also provide useful markers for varietal differentiation in rice. It must be noted that the nucleotide substitutions and insertions/deletions (InDels), are the most common sources of sequence variation in DNA and transposable elements (TEs) provides a ubiquitous source of InDels in eukaryotes. InDels of transposable element origin are well known sources of polymorphism in the genome of maize (*Zea mays*); retrotransposon insertions have doubled the size of the maize genome in the last three million years (SanMiguel *et al.*, 1998). Recently, the role of InDels as sources of polymorphism has also been established in species with relatively lean genomes such as rice (*Oryza sativa* L.) (Bureau *et al.*, 1996; Mao *et al.*, 2000; Edwards *et al.*, 2004). TE-based markers such as mini-inverted transposable elements (mPing; Jiang *et al.*, 2003; Kikuchi *et al.*, 2003) and non-autonomous retroelements (e.g Dasheng elements; Edwards *et al.*, 2004) have been used for rice genome analysis and provided important clues for rice evolution.

#### 2.5.2.3.1 MITES (Miniature Inverted-repeat Transposable Elements)

In the last decade, a polyphyletic group of class II non-autonomous elements has been described, named 'MITEs' for miniature inverted-repeat transposable elements (Bureau and Wessler, 1992; Zhang *et al.*, 2001b). MITEs were first discovered in the grasses and later found in other flowering plants as well as in animal genomes (Feschotte *et al.*, 2002b), are short, non-autonomous DNA elements that are widespread and abundant in plant genomes. Most of the hundreds of thousands of MITEs identified to date have been divided into two major groups on the basis of shared structural and sequence characteristics: *Tourist*-like and *Stowaway*-like. MITEs share characteristics that distinguish them from other class II non-autonomous elements. These characteristics include their small size (from 150 to 600 bp), high copy number (in the thousands), longer TIRs (commonly ranging between 15 and 25 bp), and target site preference (most often TA or TAA). Many MITES are predicted to form stable secondary structures (Wessler *et al.*, 1995; Braquart and Buhin, 1999). As described for hAT elements, subterminal repeats may also play important structural or functional roles in some MITEs (Tu, 2001). The origination of MITEs from active DNA transposons has been described for several classes of MITEs (Feschotte *et al.*, 2002b). Emigrant- class MITEs from *A. thaliana* show high levels of similarity to the Tc1/Mariner-like transposons Lemi1 (Feschotte and Mouche's, 2000a). The maize Tourist-like MITE, mPIF is closely related to the transposase-encoding PIF (P Instability Factor), based on identical TIRs, similar STRs, and related insertion site preferences (Zhang *et al.*, 2001a). The active rice MITE called mPing is an apparent derivative of the 5.2 kb element Pong that encodes a transposase that is likely to mobilize the mPing elements (Jiang *et al.*, 2003; Kikuchi *et al.*, 2003; Nakazaki *et al.*, 2003). These data suggest that MITEs are defective class II elements possibly mobilized by autonomous transposons. The rice genome harbors 90,000 MITEs: 60,000 *Tourist*-like MITEs and 30,000 *Stowaway*-like MITEs (Feschotte *et al.*, 2003)

### 2.5.2.3.2 Dasheng elements

A very recently amplified family of 800–1300 non-autonomous elements, making it one of the most recently amplified and highest-copy-number families in rice. The family also includes 16% solo LTRs. Like many other high-copy-number LTR elements, Dasheng elements are concentrated in the gene-poor pericentromeric regions of the chromosomes, which might be the reason for Dasheng's success in the small genome of rice. Dasheng markers cluster around the centromeric region of the chromosome. Dasheng is among the rice elements of highest copy number and most recent amplification, it is of great interest to know if members of the Dasheng family are still capable of retrotransposition. To date, activity has not been demonstrated for any of the high-copy number LTR retrotransposons in rice.

Although wide variation in copy number, distribution and type exists from one species to another, TEs comprise a huge fraction of the genomes of many animals and plants; for example, the human genome consists of at least 35% TEs (Smit and Riggs, 1996). In addition, retrotransposons constitute more than 50% of the maize genome. TE insertions have resulted in a doubling of the size of this genome within the past few million years (SanMiguel *et al.*, 1998).

Charlesworth *et al.* (1994) have documented the low frequency of fixed TE sites in *Drosophila melanogaster*. They concluded that, apart from the deleterious consequences of induced mutation and recombination, these elements are of little or no importance to the evolution of their hosts. However, it has not been demonstrated whether these results are generally applicable to a wide range of species. Indeed, a large number of TE insertions are fixed within mammalian species, leaving open the possibility that a subset of insertions has been subject to positive selection (Deininger, 1989).

Henikoff *et al.* (1997) conclude that they 'may be a manifestation of the evolutionary benefits of genomic flexibility'. However, given the huge variation in TE copy number among a wide variety of successful eukaryotic lineages, it is unclear whether increased TE copy number is itself subject to positive selection by virtue of its potential to increase genome flexibility.

Kidwell and Lisch (2000) concluded that TEs might have played an important role in enhancing the evolutionary potential of their hosts. The properties that lead TEs to be labeled 'junk DNA' might have enabled TEs to provide genomes with the plasticity to evolve new tools for generating diversity. Thus, a balance between fidelity and exploration might have evolved through the operation of natural selection and chance on the products of ancient interactions between hosts and transposable elements.

## 2.6 Role of wild genotypes in crop improvement

Plant scientists believe that crop yields have not yet reached their theoretical maximum, but finding ways to achieve that potential increase and to push the yield frontier still further is an ongoing international effort. Tanksley and McCouch have pioneered searches for beneficial alleles in wild varieties that might improve modern crops. Their work has demonstrated the genetic diversity available in wild relatives of domesticated plants. In rice, an estimated

80 percent of each species' total allelic diversity remains untapped. So without the technology to use genes or chromosomal loci as molecular markers, scientists will find identifying some of these desirable traits or moving them into modern crops nearly impossible. Wild/weedy species along with very large number of primitive cultivars and landraces constitute an important reservoir of useful genes. The size of additional variability they can provide would be of great value to the ongoing crop improvement endeavor. Wild genotypes represent a permanent genetic resource that greatly facilitates the utilization of wild and exotic germplasm in a breeding program, and they are also an efficient reagent for the discovery and isolation of genes underlying traits of agricultural importance.

After thousands of years of inbreeding, modern crop varieties are far less genetically diverse than their wild relatives, making the wild plants a rich reservoir for novel alleles. The untapped wealth in wild plants is not always obvious: in experiments with rice ancestor *Oryza rufipogon*, alleles from the wild plant were moved into a modern high-yield Chinese rice variety using marker-assisted breeding. The low-yield wild plant's genes raised the modern variety's yield by 17 to 18 percent.

Marri *et al.* (2005) used advanced backcross method to introgress and map new quantitative trait loci (QTLs) relating to yield and its components from an Indian accession of *Oryza rufipogon*. An interspecific $BC_2$ testcross progeny was evaluated for 13 agronomic traits pertaining to yield and its components. Thirty nine QTLs were identified using interval mapping and composite interval mapping. In spite of it's inferiority for most of the traits studied, *O. rufipogon* alleles contributed positively to 74% of the QTLs. Their study confirms that the progenitor species constitute a prominent source of still unfolded variability for traits of complex inheritance like yield.

Song *et al.* (2003) studied genetic diversity in the northernmost *Oryza rufipogon* populations using SSR markers. The 23 rice SSR primer pairs selected from the Rice Genes Database detected a total of 115 alleles, indicating that all the SSR loci were polymorphic and the total gene diversity was 0.919 in the six *O. rufipogon* populations.

Septiningsih *et al*. (2003) identified QTLs for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. For most of the grain quality traits tested, the alleles coming from *O. rufipogon* were inferior in the 'IR64' background. While other aspects of grain quality remain to be tested, such as protein content, gelatinization temperature, grain length and grain width, it is likely that the reknowned grain quality of IR64 will largely defy efforts to improve it. The information critical for understanding which loci contribute positively to grain quality in an elite cultivar and for efficiently selecting against those introgressions that would diminish it. Further research aimed at fine mapping and cloning these agriculturally important QTLs will accelerate efforts to improve the resolution and accuracy of marker assisted plant improvement as well as provide insights into the molecular mechanisms that govern critical aspects of crop performance.

Thomson *et al*. (2003) mapped QTLs for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and *Oryza sativa* cultivar Jefferson. The *O. rufipogon* allele was favorable for 53% of the yield and yield component QTLs, including loci for yield, grains per panicle, panicle length, and grain weight. Crosses revealed *O. rufipogon* alleles with stable effects in multiple genetic backgrounds and environments, several of which have not been detected in studies between *Oryza sativa* cultivars, indicating potentially novel alleles from *O. rufipogon*. The QTLs detected will provide a rich source of information about the natural genetic variation underlying the evolution, domestication, and breeding of rice. The fine mapping and cloning of selected *O. rufipogon* derived QTLs may ultimately provide the means to explore the molecular mechanisms underlying quantitative variation in rice and the grasses.

### 2.7 Application of PCR based markers in rice

During the last decade, the advent of PCR based markers has revolutionized the entire scenario of biological sciences from localization of a gene to the improvement of plant varieties by marker-assisted selection. Rice is the only cereal crop where molecular map is saturated with >2500 molecular markers mapped on rice genome and these markers have several diverse and extensive applications. Molecular markers have already been shown to facilitate introduction of agronomically useful QTL's from exotic cultivars or wild relatives (Tanksley and McCouch, 1997; Tanksley and Nelson, 1996). Favorable genes or alleles from wild species of rice have been detected after backcrossing to elite cultivars (Moncada *et al.*, 2001; Xiao *et al.*, 1998). Similarly, this approach can identify alleles from exotic cultivars that result in improved phenotype, even though the parent may not possess this trait (Li, 2001). Such approaches, however, can only sample a small number of accessions.

Molecular methods can be used to screen a large number of accessions through a pooling strategy. This can be used to screen germplasm collections for alleles of candidate genes that are involved in important processes of the plant, even though known variants for these genes have not been observed through genetic studies.

### 2.7.1 Genetic Diversity and phylogenetic studies using SSRs

Assessment of genetic diversity and phylogeny is important in plant breeding if there is to be improvement by selection. For the assessment of genetic diversity and phylogenetic studies molecular markers have been generally superior to morphological, pedigree, heterosis and biochemical data (Melchinger *et al.*, 1991). Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duwick, 1984). Published reports on MMGDA in rice are enormous and reflect potential applications in rice breeding.

SSRs also provide highly informative and polymorphic markers for plant, fungal, and animal fingerprinting (Weising *et al.*, 1991). Synthetic oligonucleotide SSRs have been used for in situ hybridization to chromosomes, revealing that microsatellite sequences vary widely with regard to genomic organization, raising implications for amplification and dispersion mechanisms and hence evolution. In genomes of eukaryotic organisms, microsatellites are often found in proximity to dispersed repetitive elements such as *Alu* sequences in primates (Arcot *et al.*, 1995; Jurka and Pethiyagoda, 1995) and long terminal repeats of retrotransposons in barley (Ramsay *et al.*, 1999). These associations have immediate practical implications for the success of SSR marker development.

SSRs are particularly attractive for distinguishing between cultivars because the level of polymorphism detected at SSR loci is higher than that detected with any other molecular marker assay (Saghai-Maroof *et al.*, 1994; Powell *et al.*, 1996b). Mackill *et al.* (1996) found that microsatellites have average polymorphism levels at least 1.5 times higher than AFLP and RAPD markers in a comparison of 12 *japonica* cultivars. Provan *et al.* (1997) using SSR detected intra and inter cultivar polymorphism between the cultivated and wild rice and the extent of chloroplast genomic differentiation was quantified.

### 2.7.2 DNA fingerprinting and varietal identification

Different types of markers including RAPD (Ko *et al.*, 1994), SSR (Panaud *et al.*, 1996), AFLP (Mackill *et al.*, 1996) and ISSRs (Blair *et al.*, 1999; Saini *et al.*, 2004) have been used for DNA fingerprinting and cultivar identification in rice. AFLP and SSLP analysis were found to be more reliable methods for rice genome analysis both in terms of reproducibility and amount of polymorphism detected between cultivars (Akagi *et al.*, 1996; Mackill *et al.*, 1996). Akagi *et al.* (1997) found 5 to 10 alleles among 59 closely related *japonica* cultivars.

Panaud *et al.* (1996) found 2 to 9 alleles for microsatellite markers in 22 *japonica* and *indica* cultivars and Yang *et al.* (1994) found 3 to 25 alleles for 10 microsatellite markers among 238 accessions of *indica* and *japonica* cultivars and landraces.

A study by Olufowote *et al.* (1997) showed that a selection of six SSR markers was sufficient to discriminate between 71 related lines of rice. Polymorphic RAPD and SSR markers were used for distinguishing commercial barley cultivars and for comparison of differentiation capability of RAPD and SSR techniques (Kraic *et al.*, 1998). All 23 cultivars were distinguished from each other by SSR markers. The differentiation effectiveness of SSRs was more than seven times higher in comparison with RAPDs.

Blair *et al.* (1999) reported the use of ISSR amplification for analysis of microsatellite motif frequency and fingerprinting in rice. A higher percentage of polymorphic bands were produced with the ISSR technique than the AFLP method. ISSR pattern was used to differentiate the genotypes belonging to either *japonica* or *indica* subspecies of cultivated rice and to dissect finer levels of diversity in each sub-species.

Bligh *et al.* (1999) used SSR polymorphisms to identify cultivars of brown and white milled rice. SSRs showed sufficient variation to distinguish all seven cultivars with only a small number of markers. Our study demonstrates that, using white milled grains, SSRs are a more robust and efficient method for the identification of rice cultivars. Because of the existence of a large number of commercially available primers sets, SSRs have the advantage of being easier to perform, as well as being cheaper, faster, and considerably more robust with regard to the quality of DNA needed for successful application. We would therefore recommend SSR as the method of choice for identification of white milled rice.

Use of microsatellite polymorphisms for the identification of Australian breeding lines of rice was investigated (Garland *et al.*, 1999) and most of the cultivars could be uniquely identified by at least one microsatellite marker. The results of MMGDA in rice were used to establish pedigree relationship (Akagi *et al.*, 1997), predict hybrid performance (Xiao *et al.*, 1996; Liu and Wu, 1998; Zhao *et al.*, 1999) and identify varieties (Olufowote *et al.*, 1997; Bligh, 2000; Virk *et al.*, 2000). Considering the potential applications of MMGDA, a study was made involving a set of 40 cultivated and five wild relatives of rice to assess the level of genetic diversity among the rice cultivars released over a period of 65 years.

Mackill and Li (2000) have considered microsatellite markers (SSR markers) as the best for DNA fingerprinting as they are highly polymorphic, reproducible and co-dominant.

The potential of microsatellites $(GATA)_n$ from rice for inter- and intraspecific variability was analysed by Davierwala *et al.* (2001). The microsatellite, $(GATA)_n$ has been frequently used for DNA fingerprinting. However, very few attempts have been made to analyze $(GATA)_n$ containing loci in rice. The large allele number obtained reveals the potential of $(GATA)_n$ containing loci as powerful tools to detect simple sequence length polymorphism (SSLP). The $(GATA)_n$ flanking primers were not only useful in distinguishing between closely related genotypes, but could also be used for cross-species amplification and are also conserved across different cereal genera. These loci could also cluster the bacterial blight resistant/susceptible lines into different groups based on the resistance genes present in them.

Chen *et al.* (2002) observed sequence divergence of rice microsatellites in *Oryza* and other plant species. They concluded that allelic diversity within a species was predominantly due to changes in the number of repeats in the microsatellite region, but the frequency of insertions/deletions (indels) and base substitutions increased as the genetic distance between samples increased. This study suggests that electromorph size polymorphism is an adequate measure of genetic difference in studies involving closely-related individuals, but that when phylogenetic or evolutionary inferences are being made over longer time scales, evaluation of SSR variation at the sequence level is essential.

Ni *et al.* (2002) evaluate the genetic diversity within a diverse collection of rice (*Oryza sativa* L.) accessions, and determine differences in the patterns of diversity within the two rice subspecies *indica* and *japonica*. Thirty-eight rice cultivars and two wild species accessions (*O. rufipogon* and *O. nivara*) were evaluated by means of 111 microsatellite markers distributed over the whole rice genome. All rice cultivars and lines could be uniquely distinguished, and the resulting groups corresponded exactly to the *indica* and *japonica* subspecies, with *japonica* divided into temperate and tropical types. The results suggested that a relatively small number of microsatellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars.

Ren *et al.* (2003) studied genetic relationships among the AA-genome *Oryza* species using SSR markers 13 cultivated varieties (eight *Oryza sativa* and five *Oryza glaberrima*) and 32 wild accessions (*Oryza rufipogon*, *Oryza nivara*, *Oryza glumaepatula*, *Oryza longistaminata, Oryza barthii*, and *Oryza meridionalis*). The SSR analysis effectively reveals diminutive variation among accessions or individuals within the same species, given approximately the same number of primers or primer-pairs used in the studies.

Yu *et al.* (2003) studied molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program and were evaluated using 101 well-distributed simple sequence repeat (SSR) markers. SSR markers for this assay were chosen because of their obvious advantages such as abundance in the genome, high level of polymorphism, co-dominance and cost-effectiveness (Yang *et al.*, 1994; McCouch *et al.*, 1997; Ni *et al.*, 2002), which were clearly demonstrated in their study.

Jain *et al.* (2004) reported fingerprinting of 69 diverse rice accessions including Basmati and other aromatic/quality rice germplasm collections using four fluorescent-labeled panels of 7-8 SSR markers. Bligh (2000) also reported fluorescent detection of SSLPs between known Basmati rice cultivars and likely long grain adulterants in order to detect the presence of adulterant and crude estimation of amount of adulteration present was also discussed. Saini *et al. (*2004) evaluated the genetic diversity and patterns of relationships among the 18 rice genotypes representative of the traditional Basmati, cross-bred Basmati and non-Basmati (*indica* and *japonica*) rice varieties using AFLP, ISSR and SSR markers. AFLP, ISSR and SSR marker data-sets showed moderate levels of positive correlation. The study emphasized the need for using a combination of different marker systems for a comprehensive genetic analysis of Basmati rice germplasm.

Pal *et al.* (2004) assessed the microsatellite diversity among the thirteen rice cultivars (4 commercial traditional Basmati, 6 cross-bred Basmati and 3 non-Basmati varieties) using DNA derived from milled rice samples. Similarly, Siwach *et al.* (2004) prepared a SSR DNA fingerprint database of 24 rice genotypes including three premium traditional Basmati, 9 cross-bred Basmati, a local scented selection, eight *indica* and three *japonica* rice varieties. Nagaraju *et al.* (2002) reveal genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. All these studies have led to the identification of a number of useful markers that can be used to differentiate between premium traditional Basmati from cheaper cross-bred Basmati/ long-grain non-Basmati rice varieties.

Population structure and breeding pattern of rice genotypes was analysed using 169 SSR by Lu *et al.* (2005) and identified three groups as temperate *japonica*, tropical *japonica* and *indica* based on SSR database.

### 2.7.3 Genetic diversity/ phylogenetic analysis

Population genetic diversity and phylogenetic relationships may not only illustrate the evolutionary process and biological conservation but also provide information useful for selection of parents for conventional breeding and hybrid programs. Nagaraju *et al.*

(2002) reported that lowest genetic diversity was observed among Basmati varieties compared to non-Basmati varieties and both types were delineated by using either ISSR or SSR marker systems. Ni *et al.* (2002) reported that a relatively small number of microsatellite marker could be used for the estimation of genetic diversity and identification of rice cultivars. The well-characterized, Basmati rice-specific molecular markers could serve as marker tags for any of the distinct traits of Basmati and could be used in marker-assisted selection programs. Davierwala *et al.* (2000) used RAPD, ISSR and STMS markers to estimate genetic diversity among 42 elite rice cultivars. Ashikawa *et al.* (1999) reported application of AFLP technique using non-radioactive fluorescent primers for studying genetic diversity in Japanese rice cultivars and they were able to differentiate more than 75% rice cultivars using six primer pairs.

Phylogenetic relationships among *Oryza* species were also studied using AFLP markers and the findings suggest common ancestry to genus *Oryza* and multiple lineages occurred due to independent divergence during evolution (Aggarwal *et al.*, 1999). Microsatellites have proved especially useful in evaluating diversity in narrowly defined gene pools in which other kinds of molecular markers such as AFLP, RFLP or RAPD are unable to detect polymorphism (Powell *et al.*, 1996) such as rice from west Africa (Lorieux *et al.*, 2000; Talag *et al.*, 2000; Semon *et al.*, 2001), Basmati rice from northern India (Jain *et al.*, 2004) and *japonica* rice from California (Ni *et al.*, 2002) and Korea (Ji *et al.*, 1998; Kwon *et al.*, 2000). When evolutionary divergent accessions of *Oryza* and other grasses were compared, chloroplast SSRs proved to be a reliable tool for comparative phylogenetic analysis (Ishii *et al.*, 2001). Effectiveness of different marker systems has been studied for revealing genetic diversity and inter- and intra-specific relationships of *Oryza sativa* germplasm (Virk *et al.*, 2000; Saini *et al.*, 2004).

Joshi *et al.* (2000) used a total of 30 ISSR primers representing di, tri-, tetra- and penta- nucleotide repeats to determine genetic diversity and phylogenetic relationships in *Oryza*. Of all the amplified profiles, the 11 best and highly polymorphic patterns were selected for further analysis. Genetic diversity between 19 rice cultivars was determined using RAPD and ISSR markers and it provided the evidence that pattern of variation corresponds with Glaszmann classification using isozyme analysis (Parsons *et al.*, 1997). Based on SSR analysis of aromatic/quality rice germplasm, Jain *et al.* (2004) concluded that Basmati rice types are distinct from other rice types in genus *Oryza* and may have a long, independent history of evolution.

### 2.7.4 Genes/QTLs analysis in wild genotypes

A comparative study of genetic relationships among the AA-genome *Oryza* species using SSR markers was studied by Fugang *et al.* (2003) by using 45 accessions, including 13 cultivated varieties (*Oryza sativa* and *Oryza glaberrima*) and 32 wild accessions (*Oryza rufipogon*, seven *Oryza nivara*, *Oryza glumaepatula*, *Oryza longistaminata*, *Oryza barthii*, and *Oryza meridionalis*). SSR analysis is a powerful method for detecting polymorphisms among the different AA-genome *Oryza* accessions. The SSR analysis effectively reveals diminutive variation among accessions or individuals within the same species.

Lorieux *et al.* (2000) developed a genetic linkage map based on PCR markers of a cross between *O. glaberrima* × *Oryza sativa*, essentially by microsatellites and STSs. Segregation of markers was examined in a backcross (*O. sativa*/*O. glaberrima*/*O. sativa*) population and will constitute a valuable tool for the utilisation of *O. glaberrima* in the breeding of rice varieties adapted to the African environment.

The African rice *Oryza glaberrima* is a potential source of genes to enhance the eating, cooking, and milling properties of the rice grain. Aluko *et al.* (2003) analysed QTL for grain quality traits from the interspecific cross *Oryza sativa*× *O. glaberrima.* The objective of this research was to identify and characterize quantitative trait loci (QTLs) among 312 doubled haploid lines derived from the $BC_3F_1$ of an interspecific cross of *O. sativa* × *O. glaberrima.* Seven QTLs for improved percentage rice bran, percentage milled rice, alkali-spreading score, percentage protein, and grain length/width ratio were derived from the *O. glaberrima* accession. Three new QTLs for percentage rice bran are reported here for the first time. Results suggested that the African rice might be a valuable new source for introgression and improvement of several traits that affect quality traits demanded by the different rice export markets. They concluded that hybridization of *O. sativa* and *O. glaberrima* can be successfully exploited to improve the milling, cooking, and eating properties of rice for international export markets in Africa, Asia, South America, and other rice-growing regions. Finally, the new QTLs detected in this study for milling quality that are derived from *O. glaberrima* could serve as candidates for future fine mapping and positional cloning projects.

### 2.7.5 Advantages of SSR over other markers

High level of SSR polymorphism detected among the *japonica* accessions, however, was surprising since *japonica* accessions tended to show a low level of polymorphism for other types of markers such as isozyme and RFLP loci (Glaszmann, 1987; Zhang *et al.*, 1992; Li and Rutger, 2000). This could be attributed primarily to the diverse origins of the *japonica* accessions sampled in our materials. Our results revealed several unique features of the SSR diversity in *O. sativa.* The first unique feature of the SSRs in rice is its high level of diversity within both *japonica* and *indica* groups. This was different from the result from isozymes of the same set of materials, which revealed a much higher diversity in the *indica* accessions than the *japonica* ones.

Second, the SSRs clearly show the well-documented *indica-japonica* differentiation that was reflected as subspecies-specific alleles at many loci, but this subspecific differentiation accounted for only

6.5% of the total SSR diversity in the samples and was much-less pronounced than the isozyme variation. While this is expected, based on the genetic neutrality of most SSR loci and easier generation of SSR diversity by mutation, unequal crossover and other mechanisms (McCouch *et al.*, 1997), it does suggest the independent evolution of *japonica* from *indica*.

Third, the *japonica* group exhibited larger geographic differentiation at both single-locus and multi-locus levels than the *indica* group, and the *japonica* group was clearly differentiated into two subgroups, providing evidence supporting the presence of tropical and temperate *japonica* ecotypes first proposed by Chang (1976).

The fourth feature of SSRs in rice was the prevalence of the non-random associations between or among unlinked SSR loci resulting from the subspecific and geographic differentiation within *O. sativa*. The multilocus combinations tended to show a greater level of differentiation than alleles at single SSR loci within various subdivisions. This result is much the same as that observed for the isozyme loci in *O. sativa* (Li and Rutger, 2000). Evidence supporting this interpretation came also from the observed genomic region-specific diversity of the SSRs, which suggests that genes involved in sub-specific and geographic differentiations were not randomly distributed in the rice genome.

### 2.7.6 SNPs (Single Nucleotide Polymorphism)

A good progress has also been made towards the identification of closely-linked molecular markers (SSR, SNP, etc) for important traits such as Basmati aroma, kernel elongation, semi-dwarfing gene (sd-1), waxy locus, yield and tolerance/resistance against biotic and abiotic stresses in rice. It will be interesting to investigate the genetic diversity at these loci and go for the mining of new alleles in Basmati rice germplasm. Such markers can be used to exploit the diversity of complex phenotypes within the rice primary gene pool by transferring useful genetic diversity from the primary gene pool into the elite rice cultivars. A genome-wide map with abundant highly efficient molecular markers shall greatly facilitate the allele mining and map-based cloning in rice.

Public accessibility to the genome sequences of several organisms has enabled the study of sequence variations between individuals, cultivars, and subspecies. These studies revealed that single-nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) are highly abundant and distributed throughout the genome in various species including plants (Garg *et al.*, 1999; Drenkard *et al.*, 2000; Nasu *et al.*, 2002; Batley *et al.*, 2003a). By comparing sequences from a *japonica* rice cultivar to those from an *indica* cultivar, Yu *et al.* (2002) identified, on average, one SNP every 170 bp and one InDel every 540 bp in rice. Nasu *et al.* (2002) reported almost the same frequency of rice SNPs. The abundance of these polymorphisms in plant genomes makes the SNP marker system an attractive tool for marker-assisted breeding and map-based cloning (Gupta *et al.*, 2001; Rafalski, 2002; Batley *et al.*, 2003b).

Several SNP genotyping methods have already been developed (Chen *et al.*, 1998; Livak, 1999; Ahmadian *et al.*, 2000; Alderborn *et al.*, 2000). Hayashi *et al.* (2004) demonstrate the potential of SNPs as the next generation of genetic markers for rapid and effective genotyping for genetic mapping, marker-assisted breeding, and map-based cloning.

### 2.7.7 EST-SSR: marker of the future

Locus-specific primers flanking EST or genic SSRs can be designed to amplify the microsatellite loci present in the genes. Thus, the generation of SSR markers is relatively easy and inexpensive because they are a byproduct of the sequence data from genes or ESTs that are publicly available. Genic SSRs have some intrinsic advantages over genomic SSRs as they are quickly obtained and present in expressed regions of the genome (Varshney *et al.*, 2005). The usefulness of these genic SSRs also lies in their expected transferability because the primers are designed from the more conserved coding regions of the genome. These EST-SSRs are useful as molecular markers because their development is inexpensive, they represent transcribed genes and a putative function can often be deduced by a homology search.

These studies suggested that EST-SSR markers could be used in related plant species for which little information is available on SSRs or ESTs. In addition, the genic SSRs are good candidates for the development of conserved orthologous markers for genetic analysis and breeding of different species. In the longer term, development of allele-specific markers for the genes controlling agronomic traits will be important for advancing the science of plant breeding. The choice of the most appropriate marker system needs to be decided upon on a case by case basis and will depend on many issues, including the availability of technology platforms, costs for marker development, species transferability, information content and ease of documentation.

### 2.7.8 Role of transposon in phylogeny, evolution and gene tagging

The endogenous retrotransposon *Tos17* has been shown to be an efficient insertional mutagen (Hirohico, 2001). Considering the ease of mutagenesis with *Tos17* and its multiple-copy nature, saturation mutagenesis with this retrotransposon should be feasible in rice. It is now clear that the *Tos17* system can significantly contribute to the functional genomics of rice. In addition to this system, several other systems are being developed and several problems inherent to the systems have been noted. However, some of these problems are expected to be alleviated in the near future. One of the important goals for functional genomics is to construct saturation mutant libraries for rice. To achieve this goal, it will be necessary to construct insertion lines using several insertion elements, because, to some extent, each element seems to have different target-site specificity.

Panaud *et al.* (2002) suggested that the genomic differentiation that is observed in the genus *Oryza* may be the result of the recent amplification of several retroelements. If this is confirmed in further experiments, it would show that active TEs are an important source of structural differentiation in small grass genomes.

Jiang *et al.* (2004) reported over 3,000 Pack-MULEs in rice containing fragments derived from more than 1,000 cellular genes. Pack-MULEs frequently contain fragments from multiple chromosomal loci that are fused to form new open reading frames, some of which are expressed as chimaeric transcripts. Comparison of the cellular genes and Pack-MULE counterparts indicates that fragments of genomic DNA have been captured, rearranged and amplified over millions of years. Given the abundance of Pack-MULEs in rice and the widespread occurrence of MULEs in all characterized plant genomes, gene fragment acquisition by Pack-MULEs might represent an important new mechanism for the evolution of genes in higher plants. Because some elements contain two or more fragments, we estimate that more than 1,000 different gene fragments have been captured by MULEs. Although the mechanism of sequence capture is not yet understood, it is likely to involve the acquisition of genomic DNA rather than the cDNA copies of cellular transcripts as reported previously for human L1 retrotransposons. Furthermore, about one-fifth of the identified Pack-MULEs contain fragments acquired from multiple genomic loci, thus demonstrating their potential to create novel genes through the duplication, rearrangement and fusion of diverse genomic sequences.

Nakazaki *et al.* (2003) identified an active rice trasnsposon named miniature ping (mPing) which has inserted in the slender glume (*slg*) mutant allele but not in wild type alleles. Excision of the mPing fron *slg* plant results in the reversion to a wild type phenotype. The mobility of the transposon mPing in the intact plants represents a useful alternative tool for the functional analysis of the rice genome.

Santiago *et al.* (2005) identified a novel family of mobile elements in plants with similarity to both MITEs and the hAT elements. The lettuce element, LS00-7405a, is only one nucleotide larger than dTph2, the smallest class II mobile element described to date that is capable of transposition (MITEs excluded) (Kunze and Weil, 2002), but it is within the normal size range of MITE elements. The potential of LS00-7405a to form a highly stable hairpin-like secondary structure suggests that LS00-7405a could be related to MITEs.

Kenji *et al.* (2005) identified an active transposon in intact rice plant, belong to the hAT superfamily of class II transposons. The 607-bp element itself, termed non-autonomous DNA based active rice transposon (nDart), has no coding capacity. Because the transposons are active in intact rice plants, they should be useful tools for tagging genes in studies of functional genomics.

Kazuo *et al.* (2006) found an active DNA transposon nDart causing leaf variegation and identified a novel mutable dwarf allele thl-m caused by an insertion of nDart1 in rice.

# CHAPTER -III

# Material and Methods

## 3.1 MATERIALS

### 3.1.1 Plant material

A total of 50 rice genotypes representing the commercially important premium traditional, cross-bred Basmati, *indica*, *japonica* and wild rice (*O. glaberrima, O. meridionalis, O. barthii, O. rufipogon, O. nivara, O. glumalpatula*) groups, were selected for the molecular polymorphism studies: the characteristics and origin of these genotypes are given in Table 3.1. Nurseries of all these genotypes were raised in a net house at CCS Haryana Agricultural University, Hisar, India.

### 3.1.2 Chemicals

Chemicals for DNA extraction and PCR amplification were obtained from Sigma-Aldrich (3050 Spruce Station, St. Louis, MO 63103, USA) Chemicals Co. USA, Life Technologies (India) Pvt. Ltd., Imperial Bio Medics (Chandigarh, India), PerkinElmer Inc. (45 William Street, Wellesley, MA 02481-4078, USA), Applied Biosystems (850 Lincoln Center Drive, Foster city, CA-94404) and Bangalore Genei (No. 6, 6th Main, Near SRS Road, Peenya, Bangalore-560058, India). Silver staining kit used for SSR analysis was obtained from Promega Corporation (2800 Woods Hollow Road Madison, WI 53711 USA). All other chemicals used in this study were of molecular biology or analytical grade and procured them from Sigma-Aldrich, Promega Corporation and E. Merck (India) Ltd. (Worli, Mumbai-400018, India). Chemical Composition of some solutions and general precautions for using some chemicals for DNA isolation/gel electrophoresis have been mentioned in ANNEXURE I and II, respectively.

### 3.1.3 Molecular Markers

#### 3.1.3.1 Microsatellite markers

Table 3.2 gives the general information about the microsatellite DNA markers used in this study. The rice microsatellite primer pairs were obtained from Research Genetics, Inc. (Huntsville, AL, USA). The original source, repeat motifs, primer sequences and chromosomal position of these markers can also be found in Temnykh *et al.* (2000) and in the Rice Genes database. (http://www.gramene. Org/micrsat/RMprimers.htm1).

#### 3.1.3.2 TE based markers

Table 3.3 gives the general information about the TE based markers used in this study. The sequence information was provided by Dr. Susan McCouch, Department of Plant Breeding, 252 Emerson Hall, Cornell University, Ithaca, NY, USA. The primers were synthesized by Genetix Biotech Asia (P) Ltd., 71/1, Najafgarh Road, New Delhi-110015, India.

## 3.2 METHODS

### 3.2.1 Preparation of DNA fingerprint database using SSR and TE based markers

#### 3.2.1.1 Genomic DNA isolation:

Genomic DNA of all the genotypes/accessions was isolated from bulked leaf samples (20 mg each) from 5 plants using modified CTAB method of Saghai–Maroof *et al.* (1984; Annexure III).

#### 3.2.1.2 Quality and quantity of DNA

After DNA isolation, its quality and concentration was checked by running DNA samples on 0.8% agarose gels electrophoresis using a standard containing 100 ng/μl genomic λ DNA. By comparing the flourescence intensity of the bands with the standard, DNA concentration was estimated.

### 3.2.1.3 RNase treatment

RNA contamination was removed by adding 5 µl of 10 mg/ml RNase (RNase-A, Sigma chemical Co. USA, No. R- 5503) to the DNA samples. The samples were mixed gently and incubated at 37°C for 20-30 minutes. DNA was extracted by adding equal volume of chloroform: isoamyl alcohol (24:1) mixture. The samples were mixed gently and centrifuged at 10,000 rpm for 10 minutes. Supernatant was collected and transferred to new Eppendorf-tubes. DNA was precipitate by adding 1/10th volume of 3M sodium acetate (pH 6.8) or 5M NaCl and 2 volume of chilled absolute alcohol. The tubes were centrifuged to pellet down the DNA and washed with 70% alcohol. The tubes were kept open overnight for drying the DNA pellet at room temperature. DNA was dissolved in appropriate volume of TE buffer and the samples were stored at -20°C till further use.

### 3.2.1.4 Microsatellite marker analysis

Fifty primer pairs covering all the twelve chromosomes were used for preparation of DNA fingerprint database in 50 rice genotypes/accessions. Short sequence repeat amplifications were performed using PTC-100™ 96V thermocycler (MJ Research, Inc., Watertown, MA, USA) and Taq DNA polymerase (Perkin Elmer, Inc., Madison, WI, USA). The PCR reaction was conducted in a reaction volume of 20 µl containing 1 X PCR buffer, 100 µM dNTPs, 0.4 µM of each primer, 1.2 mM $MgCl_2$, 1 unit Taq DNA polymerase and 40-60 ng template DNA. PCR amplification was performed with initial denaturation at $94^0$C for 5 min followed by 35 cycles of $94^0$C for 1 min, $55^0$C for 1 min, $72^0$C for 2 min and final extension at $72^0$C for 7 min before cooling at $4^0$C. Amplification products were stored at $-20^0$C till further use.

#### 3.2.1.4.1 Electrophoretic separation of amplification products

Polyacryamide gel was prepared (ANNEXURE IV, Plate 1) and amplified products were resolved by 4% polyacrylamide gel electrophoresis using Aluminium Based Sequencing System Model # 535 (Owl Scientific, Inc., Voburn, USA) as described by Chen *et al.*

(1997). Gels were pre-run until an adequate temperature (50-60°C) was reached. DNA bands were then visualized using the Silver staining (ANNEXURE V) DNA Sequencing System (Promega Technical Manual, Part # TM023).

### 3.2.1.5 Transposon Based Element Marker analysis

TE amplifications were performed using PTC-100™ 96V thermocycler as described above for SSR analysis. The PCR reaction mix contained 1 X PCR buffer, 200 μM dNTPs, 0.4 μM of primer, 2 mM $MgCl_2$, 1 unit Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed with initial denaturation at $94^0$C for 4 min followed by 35 cycles of $94^0$C for 1 min, $50^0$C for 1 min, $72^0$C for 2 min and final extension at $72^0$C for 15 min before cooling at $4^0$C. Amplification products were run on 1.5% (w/v) agarose gel electrophoresis (ANNEXURE VI) in 0.5X TBE and were stained with ethidium bromide.

### 3.2.1.6 Allele scoring

For microsatellite markers a cluster of two to five discrete bands (stutter) or blurred stutters was obtained (Chen *et al.*, 1997; Panaud *et al.*, 1996; Temnykh *et al.*, 2000). An allele that was observed in only one of the rice genotype was taken as rare or unique, allele. A genotype was assigned a null allele for a SSR locus whenever amplification product(s) were not detected for a particular genotype and marker combination.

Bands for TE analysis were scored based on the presence or absence of bands for each rice variety. Only clear and apparently unambiguous bands were scored. Nipponbare for which allele size information was available were used as reference for molecular size determination.

The size (in nucleotide base pairs) of the most intensely amplified band was determined based on its migration relative to molecular weight size markers (wide range DNA ladder 50 bp to 1000 bp from Sigma chemicals Co. USA and Lambda DNA/*EcoRI* + *HindIII* double digested from Bangalore, Genei Pvt. Ltd.) for TE markers and 10 bp DNA ladder from Gibco BRL Md. for SSR markers.

The polymorphism information content was determined for microsatellite markers according to Anderson *et al.* (1993) based on allele pattern of all the varieties analyzed.

$$PIC = 1 - \sum_{J=1}^{n} P^{ij}$$

Where $P^{ij}$ is the frequency of the $j^{th}$ allele for $i^{th}$ marker and summation extends over n alleles.

### 3.2.1.7 Data analysis

The frequency of polymorphism between selected varieties of rice for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of common bands (Ghosh *et al.*, 1997). The two different sets of data gathered (based on SSR and TE markers) were subjected to cluster analysis. Sequential Agglomerative Hierarchial Nonoverlapping (SAHN) clustering was performed on squared euclidean distance matrix and similarity matrix using Jaccard coefficient for quantitative and binary data respectively utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. Data analysis was done using NTSYS-PC version 2.02 (Rohlf, 1994). The resultant distance matrix was employed to construct dendrograms by the unweighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC. Allele molecular weight data were also used to determine the genetic distance for phylogeny reconstruction based on the neighbor-joining method (Saitou and Nei, 1987) implemented in PowerMarker, a comprehensive set of statistical methods for genetic marker data analysis, designed especially for SSR/SNP data analysis. The Mantel test of significance (Mantel, 1967) was also used to compare each pair of similarity matrices.

# CHAPTER -IV

# *Results*

The present investigation was undertaken to prepare a DNA fingerprint database of Indian Basmati and other rice types using SSR and transposon element based markers. The database prepared was used: (i) to identify markers that differentiate within/among Basmati and non-Basmati rice varieties and (ii) to determine the genetic relationships among the various rice genotypes.

## 4.1 Preparation of DNA fingerprint database using microsatellite DNA (SSR)

A microsatellite (SSR) DNA fingerprint database was generated for 50 rice genotypes using 50 SSR markers (Table 4.1). Silver stained gels displaying allelic polymorphism among 50 rice genotypes for some of the SSR markers have been shown in Plate 2-11. Salient features of microsatellite marker analysis are as follows:

A total of 341 alleles were detected in 50 rice varieties (Table 4.2). The number of alleles ranged from two (RM321 to sixteen (RM252), with an average of 6.8 alleles per locus. SSR markers with di-nucleotide repeat detected 7.1 alleles per locus followed by those with tri-nucleotide (6.3 alleles per locus) and tetra-nucleotide (4.0 alleles per locus) repeats. The overall size of amplified products ranged from 74 bp (RM248) to 389 bp (RM422). The size difference between the smallest and largest allele at a given SSR locus varied from 3 (RM323) to 150 (RM582).

A total of 40 unique alleles (11.7%) were observed at 24 of the 50 SSR loci. Maximum number of unique alleles was observed at RM229 and RM255 loci (4 alleles each) followed by 3 alleles each at RM547, RM259 and RM580. Null alleles were observed at 31/50 SSR loci in 35 genotypes. Highest frequency of null allele was observed at RM252 (eight) followed by RM424 (five) and RM527 (five) loci. *O. meridionalis* and *O. rufipogon* showed the largest proportion of null alleles of 7 and 5, respectively.

Multiple alleles (when two clusters of bands were observed for a particular marker) were observed in one or more genotypes at 31/50 SSR loci with an average of 0.62 alleles per locus. At RM488 and RM562, multiple alleles were detected at 11 and 10 rice genotypes, respectively. Among the 50 rice genotypes, CSR 30 and Sharbati had multiple alleles at as many as five SSR loci.

The frequency of most common allele ranged from 13% (RM252) to 60% (RM255). On average 43.6% of the rice genotypes shared a common allele at any given locus.

PIC values, which are indicative of genetic diversity, ranged from 0.375 to 0.922, with an overall average of 0.698 per locus. PIC values showed a positive correlation of 54% (Significant at 0.01 level) with number of alleles at a SSR locus.

### 4.1.1 Genetic similarity among various rice genotypes/ groups

Jaccard similarity coefficient data based on the proportion of shared alleles using 'simqual' sub-program of NTSYS-PC software was used to calculate the coefficient values among the various rice genotypes. Highest similarity coefficient of 0.6349 was observed between Nipponbare/Taipei 309 (ANNEXURE VII). Among the traditional Basmati rice varieties Basmati 370/HBC 19 and Basmati 370/Type III were quite close with similarity coefficient values of 0.5385 and 0.5000. A Similarity coefficient of 0.5147 between the two salt tolerant *indica* rice genotypes, CSR 1/CSR 10, was observed. Cross-bred Basmati rice variety CSR 30 showed a similarity coefficient of 0.6000 with HBC 19. Lowest similarity coefficient values of 0.0209 and 0.0303 were observed for Taipei 309/Sabarmati and Nipponbare/Pokkali, respectively.

Group wise mean similarity coefficient values were also calculated for the various rice groups including Traditional Basmati, *indica*, *japonica*, *O. glaberrima*, *O. rufipogon*, *O. nivara*, *O glumalpatula*, *O. barthii*, and *O. meridionalis* (Table 4.3). Traditional Basmati/traditional Basmati, *japonica*/*japonica*, and *indica*/*indica* showed mean similarity of 0.320, 0.300, and 0.225, respectively. Traditional Basmati group had greater similarity with *indica* (0.165) compared to *japonica* (0.112) rice groups. *Japonica*/*indica* group had a mean similarity of 0.074. Among the six wild rice groups the traditional Basmati rice group had maximum similarity with *O. glumalpatula* (0.122) followed by *O. glaberrima* (0.115) and *O. barthii* (0.114); these similarity values were much higher compared to corresponding values involving *indica* or *japonica* rice group instead of traditional Basmati group.

### 4.1.2 Genetic diversity analysis:

The SSR profiles/diversity data were used to determine genetic relationship among the various rice varieties using NTSYS-PC, PCA and PowerMarker software.

#### 4.1.2.1 NTSYS-PC

The NTSYS-PC UPGMA cluster tree analysis led to the grouping of 50 varieties in two major groups at a similarity coefficient of 0.09 (Figure 4.1). Major group I is further divided into two major-sub groups. Major-sub group I comprised of all the *japonica* rice varieties, 9311 (*japonica–indica*) and *O. nivara* genotypes, with a similarity coefficient of 12%. Major- sub group I is further divided into two sub groups, I and II. Sub group I consists of all the six *japonica* varieties, 9311 and *O. nivara*. Sub group I is further divided into two sub-sub groups. The sub-sub group I comprised tropical *japonica* genotypes (Azucena, NPTII and NPTIII) and sub-sub group II consisted of temperate *japonicas* (Nipponbare, TNG 67 and Taipei 309) and 9311 (*japonica- indica*). A similarity coefficient of 64% was observed between Taipei 309 and Nipponbare. Sub group II had only one genotype, *O. nivara*. Major sub group II comprised most of the wild genotypes (*O. rufipogon, O meridionalis, O. nivara and O. glumalpatula*) except *O. glaberrima* and *O. barthii*.

Major group II is divided into two major sub groups at a similarity coefficient of 10%. Major sub group I includes all the *indica*, traditional Basmati, cross-bred Basmati, Aus genotypes and *O. glaberrima*. Major-sub group I is further divided into two sub groups. Sub group I includes most of the cross-bred Basmati and *indica* genotypes and the sub group II consisted of traditional Basmati, few of the cross-bred Basmati, Aus genotypes and *O. glaberrima*. Sub group I is further divided into two sub-sub groups at a similarity coefficient of 18%. Sub-sub group I consists of all the *indica*, some of the cross-bred (Pusa Sugandha 2, Pusa Sugandha 3, Pusa Sugandha 4, HKR 228, Mahisugandha, Sharbati, Sabarmati, Improved Sabarmati) and one of the traditional Basmati, Basmati 217. CSR 1 and CSR 10 showed a similarity level of 52%. Sub-sub group II comprised mainly of the traditional Basmati and some of the cross-bred Basmati genotypes with a similarity coefficient of 31%. Basmati 370 and Type III showed a similarity coefficient of 51% whereas a similarity coefficient of 61% was observed between HBC 19 and CSR 30 (cross-bred Basmati). Major sub group II includes only one genotype *O. barthii*.

#### 4.1.1.2 PCA analysis

Genetic relationships as determined by NTSYS two-dimensional and three-dimensional PCA scaling of various rice genotypes are shown in Figures 4.2 and 4.3. The clustering of different rice varieties was essentially similar to that obtained by NTSYS-PC (UPGMA) tree clustering. Most of the rice genotypes were placed in four major clusters of Basmati, *japonica*, *indica* and the wild rice genotypes. The traditional Basmati varieties except Basmati 217 and some cross-bred Basmati rice varieties were clustered together in one group.

*Japonica* rice varieties formed another group and *indica* varieties with some of the cross-bred Basmati varieties formed a separate cluster. Cross–bred Basmati (CSR 30, Pusa Basmati 1, HKR 228) were closer to the traditional Basmati group than the *indica* rice group. All the wild rice genotypes formed a separate group placed between *indica* and *japonica* rice groups. The PCA analysis also showed a close relationship between two Aus genotypes, KR 1-24 and BR 4-10, which were closer to the wild rice group.

#### 4.1.1.3 PowerMarker analysis

The SSR fingerprint database was used to generate radial and phylogenetic trees based on PowerMarker analysis (Figure 4.4 and 4.5). The groups formed were essentially the same as observed by NTSYS-PC UPGMA and PCA analysis. Most of the Basmati rice varieties including traditional Basmati (except Basmati 217) and some cross-bred Basmati rice varieties (Pusa Sugandha 2, Pusa Sugandha 3, Pusa Sugandha 4 and HKR 228) formed one group. Most of the *indica* rice varieties, some of the cross-bred Basmati and Basmati 217 were clustered together. The wild rice genotypes showed variable clustering with *O. nivara* 2, *O. nivara* 3 and *O. rufipogon* 1 in one group and *O. glabberima*, *O. rufipogon* 2 and *O. nivara* 3 in another group. All of the *japonica* rice genotypes formed a separate cluster which merged with the two clusters of wild rice genotypes to form a big cluster. This study shows a close relationship between BR 4-10/KR 1-24, CSR 1/CSR 10, Nipponbare/TNG 67, Nipponbare/Taipei 309, NPTII/NPTIII, HBC 19/CSR 30 and Basmati 370/TypeIII. BR 4-10 and KR 1-24 formed a distinct group closer to the *japonica*-wild rice group.

### 4.1.3 Identification of markers for varietal identification

Table 4.4 shows a list of SSR markers which can be used to differentiate between the closely related traditional Basmati rice genotypes and traditional Basmati from cross-bred Basmati, *indica* and *japonica* rice genotypes. These markers can be used in combination for varietal identification.

Table 4.5 shows the number of microsatellite alleles amplified by various rice groups/types. In seven traditional Basmati varieties, RM224 amplified five alleles followed by amplification of four alleles at RM594, RM551, RM321, RM541, RM 562, RM426, RM252, RM488 and RM547 loci. These SSR markers can be effectively used to differentiate between closely related seven traditional Basmati rice varieties. RM252, RM547, RM222, RM541 and RM562, amplified 6-8 alleles in 12 cross-bred Basmati rice genotypes and are considered best for differentiating among them. The *japonica* rice genotypes can be distinguished from each other by allelic polymorphism at RM400 and RM248, which amplified four alleles in six *japonica* genotypes. RM252 and RM547 amplified eight and seven alleles, respectively, in 15 indica rice genotypes and can be used to distinguish between *indica* genotypes.

## 4.2 TE marker based analysis

### General features

A total of 30 TE based markers were used; the list of primers for TE markers is given in Table 3.3. Out of 30 TE based primers, 16 Dasheng elements specific, nine Nipponbare specific mPing elements and five cv. 9311 specific mPing elements were used.

Figure 4.6 shows the designing of primers for mPing and Dasheng markers. For mPing, two primers (1 forward and 1 reverse) were used. The amplification of a bigger fragment (700bp, Figure 4.6) will show the presence of mPing element. If there is no element then a DNA fragment of 300 bp, Figure 4.6 shall be amplified. Amplification of no DNA product signifies the alteration in the sequence of primer binding site(s).

In case of Dasheng elements, three primers were used (1 forward, 1 reverse and 1 internal) as shown in Figure 4.6. In this case, amplification of a specific small size product shall show the presence Dasheng element.

A DNA fingerprinting database of 50 rice genotypes was prepared using these 30 TE based markers. Examples of TE based marker profiles obtained among 50 rice varieties are shown in Plate 12-16. Status of different rice genotypes with respect to the presence of Dasheng elements, Nippon mPing and cv. 9311 mPing elements is shown in Table 4.6.

Dasheng elements were present in high frequencies in cv. 9311 (75%) followed by *japonica* rice group (54.2%), cross-bred Basmati (24%) and traditional Basmati (21%) rice groups (Tables 4.7, 4.8 and 4.9). In wild rice genotypes, frequencies of Dasheng elements were minimum (13.9%). In these genotypes null alleles were detected at higher frequencies (44.4%).

Nipponbare mPing elements were present in highest frequency in cv. 9311 (88%) followed by wild rice (44.4%) and *japonica* rice (37.0%) group. In traditional Basmati and *indica* rice groups, they were present at very low frequencies ranging from 1.9% to 3.0%. The frequencies of 9311 mPing elements were maximum in *indica* rice (44%) followed by cross-bred Basmati (21.6%), traditional Basmati (12.7%) and cv. 9311 (6.6%). These elements were detected at a relatively low frequency in *japonica* (6.6%) and wild (6.6%) rice groups.

High level of variation was also obtained among the traditional Basmati rice varieties for the presence of the TE elements. It is interesting to note that Dasheng C7 was present in all the traditional Basmati rice varieties while Dasheng E3, Dasheng F1 and Dasheng G3 were present in 5 of the seven traditional Basmati and absent in Karnal Local and Basmati 217 (Table 4.10, 4.11 and 4.12). The mPing elements were entirely missing in three (HBC 19, Basmati 370 and Type III) of the seven traditional Basmati rice varieties, while other traditional Basmati showed the presence of these mPing elements at 1, 2, and 3 of the 14 loci.

Among the *japonica* rice varieties, 9311 mPing elements were detected only in Azucena (tropical *japonica*). The Dasheng elements were observed at variable frequencies (31.25% to 75%) in all the *japonica* rice varieties (Tables 4.13, 4.14 and 4.15). Nippon mPing elements were present in higher frequencies in temperate *japonica* and at a low frequency (11.11%) in Azucena (tropical *japonica*) and absent in other two tropical *japonica* (NPTII and NPTIII) rice varieties.

PIC values, which is indicative of genetic diversity varied, from one TE locus to another and ranged from 0.110 to 0.374, with an overall average of 0.258 per locus (Table 4.16). Example of TE based marker profiles obtained among 50 rice genotypes are shown in Plate 26-40.

### 4.2.1 Similarity between various rice genotypes

Jaccard similarity coefficient data based on the proportion of shared alleles using 'simqual' sub-program of NTSYS-PC software was used to calculate the similarity coefficient values among the various rice genotypes (ANNEXURE VIII). Highest similarity coefficient of 0.9655 was observed between traditional Basmati rice genotypes (Basmati 385 and Basmati 370). Among the *japonica* and *indica* rice genotypes, similarity indices of 0.7059 and 0.8571 were observed between Taipei 309/Nipponbare and MI 48/Pokkali, respectively. Azucena rice variety showed a similarity coefficient of 0.8125 and 0.7143 with Basmati 370 and Super Basmati, respectively. Cross-bred Basmati rice genotypes Kasturi/Pusa Basmati 1 and HKR 93-401/Mahisugandha showed a similarity coefficient of 0.7857. Lowest similarity coefficient of 0.1026 was observed between TNG 67/Improved Sabarmati, followed by Nipponbare/ Karnal Local (0.1346).

Group wise mean similarity values were calculated for the various rice groups (Traditional Basmati, *indica*, *japonica*, *O. glaberrima*, *O. rufipogon*, *O. nivara*, *O glumapatula*, *O. barthii*, and *O. meridionalis*) (Table 4.17) using the TE similarity indices. Similarity values of 0.669, 0.420 and 0.545 were observed for traditional Basmati/traditional Basmati, *japonica*/*japonica* and *indica*/*indica*, respectively. Traditional Basmati had grater mean similarity with *indica* (0.514) compared to *japonica* (0.400) rice groups. Among the wild rice types, traditional Basmati had grater similarity with *O. glumapatula*

(0.348) followed by *O. meridionalis* (0.327) and *O. nivara* (0.038); these values were higher compared to corresponding mean similarity values involving *indica* or *japonica* rice groups in place of traditional Basmati group.

### 4.2.2 Genetic diversity and phylogenetic analysis

Transposon Element based markers diversity data were used to determine genetic similarity among the various rice genotypes using NTSYS-PC and PowerMarker software.

### 4.2.2.1 NTSYS-PC

The UPGMA cluster tree analysis led to the grouping of 50 varieties comprising traditional Basmati, *indica*, *japonica*, and wild rice genotypes in two major groups at a similarity coefficient of 0.18 (Figure 4.7). Major group I comprised of three temperate *japonica* rice varieties (Nipponbare, Taipei 309, TNG 67 and *japonica-indica* cv. 9311. The major group II consisted of two major sub groups. Major sub group I had all the wild rice genotypes. Major sub group II had all the *indica*, traditional Basmati, cross-bred Basmati and tropical *japonica* rice genotypes. Most of the traditional Basmati rice varieties (except Basmati 217 and Karnal Local), cross-bred Basmati (CSR 30, Super Basmati, Kasturi, Pusa Basmati 1, HKR 228, Sabarmati) and Azucena (scented aromatic *japonica*) rice genotype formed a separate group.

### 4.2.2.2 PCA analysis

Genetic relationship as determined by NTSYS-PC two-dimensional and three-dimensional PCA scaling of various rice genotypes is shown in Figure 4.8 and 4.9. The clustering of different rice varieties was essentially similar to that obtained by NTSYS-PC (UPGMA) tree clustering. The three temperate *japonica* rice and 9311 (*japonica-indica*) varieties formed a distinct cluster at left upper corner of the PCA. All the wild rice genotypes formed a distinct cluster at the left bottom corner and all the other rice genotypes were scattered in the right half. Traditional Basmati except Basmati 217 and Karnal Local and cross-bred Basmati were clustered in the right upper region. The status of three tropical *japonica* rice varieties and the five traditional

Basmati varieties except Basmati 217 and Karnal Local and *indica* rice varieties remains the same as identified by NTSYS-PC, UPGMA. The two Aus rice genotypes BR 4-10 and KR 1-24 are not that close as identified by SSR analysis.

#### 4.2.2.3 PowerMarker analysis

The TE fingerprint database was used to generate radial and phylogenetic tree based on PowerMarker analysis (Figures 4.10 and 4.11). The groups formed were essentially the same as observed by NTSYS-PC UPGMA and PCA analysis. The analysis showed a clear distinct group comprising three temperate *japonica* rice Nipponbare, TNG 67, Taipei 309 and *japonica-indica* (9311) varieties. Most of the wild rice genotypes including KR 1-24 formed a separate cluster. The traditional Basmati rice varieties (except Basmati 217 and Karnal Local) and some of the cross-bred Basmati rice varieties formed a small cluster. Most of the *indica* rice varieties, some of the cross-bred Basmati and the remaining two traditional Basmati (Basmati 217 and Karnal Local) formed a separate cluster. The tropical *japonica* rice varieties, NPT II and NPT III, were quite divergent from the temperate *japonica* group. The third tropical aromatic *japonica* rice variety, Azucena, grouped with traditional Basmati group.

## 4.3 Correlation between the similarity values measured using two marker system

Mantel test of significance (Mantel, 1967) was used to compare each pair of similarity matrices produced using the two marker systems and level of correlation between the two. The values of the Mantel test correlation showed a positive correlation between the two marker types (Figure 4.12, ANNEXURE IX). The correlation coefficient (r) was 0.655 between SSR and TE based markers.

**Table 3.1: A brief description of rice varieties used for genetic diversity and phylogenetic analysis.**

| Sr. No. | Genotype | Source | Abbre. | Remarks |
|---|---|---|---|---|
| 1 | Azucena | CCSHAU,1014/141(A) | Azu | ***Tropical japonica rice variety*** |
| 2 | NPTII | **IRRI,IR68552-100-1-2-2** | NPTII | Tropical japonica rice variety |
| 3 | NPTIII | IARI,N Delhi | ***NPTIII*** | Tropical japonica rice variety |
| 4 | Taipei309 | IARI,N Delhi | T309 | Temperate japonica rice variety |
| 5 | TNG67 | IARI,N Delhi | TNG67 | Temperate japonica rice variety |
| 6 | Nipponbare | IARI,N Delhi | Nippon | Temperate japonica rice variety |
| 7 | Basmati 217 | DRR,1051/16 | Bas217 | Traditional Basmati rice variety |
| 8 | Basmati 370 | CCSHAU,1176/838 | B370 | Traditional Basmati rice variety |
| 9 | HBC19 | CCSHAU, Hisar | HBC19 | Pure line selection from local Basmati Traditional Basmati rice variety |
| 10 | Karnal Local | CSSRI, karnal | KLocal | Traditional Basmati rice variety |
| 11 | Ranbir Basmati | DRR,1340/335 | RanBas | Pure line selection from Basmati-370-90-95 Traditional Basmati rice variety |
| 12 | TypeIII | DRR,1320/138 | TypeIII | A selection from Basmati of Dehradun Traditional Basmati rice variety |
| 13 | Basmati 385 | HAU –Kaul | Bas385 | Obtained from crosses and backcrosses of T (N)1/Bas370 Traditional Basmati rice variety |
| 14 | CSR30 | CSSRI, Karnal | CSR30 | Salt tolerant cross bred Basmati rice variety |

| | | | | |
|---|---|---|---|---|
| | | | | obtained by crosses and backcrosses of salt tolerant indica rice variety from Maharastra, BR4-10/Pakistan Basmati |
| 15 | Improved Sabarmati | IARI, N. Delhi | ImpSabar | Released in 1970 by IARI, New Delhi, obtained from crosses & backcrosses of T (N)-161/Basmati370 |
| 16 | HKR 228 | CCSHAU, Hisar | HKR228 | Re17leased in 1991, obtained from crosses and back18crosses of Sona/Bas370 |
| 17 | Kasturi | GBPU, Pant Nagar | Kasturi | Basma19ti-370 x CRR-88-17-1-5 Released in 1989 by DRR, obtained from crosses and backcrosses of Basmati 370/CR-88-17-1-5 |
| 18 | Mahisugandha | IARI, N. Delhi | Mahi | BK-79 x Basmati-370 |
| 19 | Pusa Basmati 1 | CCSHAU, Hisar | PB1 | Released in 1989 by IARI, New Delhi, obtained from crosses and backcrosses of Pusa 150/karnal local |
| 20 | Pusa Sugandha 2 | IARI,N Delhi | PS2 | Cross-bred Basmati |
| 21 | Pusa Sugandha 3 | IARI,N Delhi | PS3 | Cross-bred Basmati |
| 22 | Pusa Sugandha4 | IARI,N Delhi | PS4 | Cross-bred Basmati |
| 23 | Sabarmati | GBPU, Pant Nagar | Sabar | Released in 1970 by IARI, obtained from crosses and backcrosses of T (N) 1/Bas370 |
| 24 | Super Basmati | CCSHAU, Hisar | Super | Released in 1996 by RRI, obtained from crosses and backcrosses of IR-661/Bas370 |
| 25 | HKR 93-401 | HAU –Kaul | 93-401 | Obtained from crosses and backcrosses of Basmati/indica varieties |
| 26 | Punjab Rice 118 | PAU, Ludhiana | PR118 | Indica rice variety |

| | | | | |
|---|---|---|---|---|
| 27 | Punjab Rice 106 | PAU, Ludhiana | PR106 | (IR-8 x Peta 5) x Bella Patna |
| 28 | IR 36 | IARI, N. Delhi | IR36 | IR-8 x Tadukan x TKM-62 x T(N)1 x IR-243 x Oryza Nivara-4 x IR-8 x PTB-21 & PTB-18<br>Indica rice variety |
| 29 | BR4-10 | IARI, N. Delhi | BR4-10 | Aus |
| 30 | CSR 1 | CSSRI, Karnal | CSR1 | Indica rice variety |
| 31 | CSR 10 | CSSRI, Karnal | CSR10 | M-40-431-24-114 x Jaya Salt tolerant cross bred indica rice variety developed from crosses & backcrosses by CSRI/Jaya |
| 32 | CSR 21 | CSSRI, Karnal | CSR21 | Indica rice variety |
| 33 | HKR 120 | CCSHAU,1870/975 | HKR120 | PTB-33/4 x IR-3403-267-1<br>High yielding indica variety |
| 34 | IR 24 | IARI, N. Delhi | IR24 | Indica Rice variety |
| 35 | IR 72 | IRRI, Phillipines | IR72 | Indica Rice variety |
| 36 | Kala Ratta 1-24 | CSSRI, Karnal | KR1-24 | Aus |
| 37 | MI 48 | CSSRI, Karnal | MI48 | Indica rice variety |
| 38 | Pokkali | CSSRI, Karnal | Pokkali | Salt tolerant indica from South-India (Kerela) |
| 39 | Sharbati | GBPU, Pant Nagar | Sharbati | A local selection from Uttar Pradesh |
| 40 | IR 64 | IRRI, Phillipines | IR64 | IR-5857-33-2-1 x IR-2061-465-1-5-5<br>Indica rice variety |
| 41 | 9311 | IARI, N. Delhi | 9311 | Indica/Japonica |

| | | | | |
|---|---|---|---|---|
| 42 | *O. glaberrima* - 102206 | PAU, Ludhiana | Oglab | Liberia, Africa |
| 43 | *O. barthii*- 100117 | PAU, Ludhiana | Obart | Guinea |
| 44 | *O. rufipogon*- 81885 | PAU, Ludhiana | Orufi1 | India |
| 45 | *O. rufipogon* - 103404 | PAU, Ludhiana | Orufi2 | Bangladesh |
| 46 | *O. nivara* -81847 | PAU, Ludhiana | Oniv1 | India |
| 47 | *O. nivara*- 81859 | PAU, Ludhiana | Oniv2 | India |
| 48 | *O. nivara* - 104688 | PAU, Ludhiana | Oniv3 | India |
| 49 | *O. glumalpatula* - 104387 | PAU, Ludhiana | Oglumal | Brazil, South America |
| 50 | *O. meridionalis* - 101146 | PAU, Ludhiana | Omeridio | Australia |

**Table 3.2: A brief description of microsatellite markers used in this study.**

| Sr. No. | Marker Name | Chr. Position | Repeat Motif | Forward Primer | Reverse Primer | Size | Var |
|---|---|---|---|---|---|---|---|
| 1 | RM213 | 2 | (CT)17 | ATCTGTTTGCAGGGGACAAG | AGGTCTAGACGATGTCGTGA | 139 | IR36 |
| 2 | RM215 | 9 | (CT)16 | CAAAATGGAGCAGCAAGAGC | TGAGCACCTCCTTCTCTGTAG | 148 | IR36 |
| 3 | RM220 | 1 | (CT)17 | GGAAGGTAACTGTTTCCAAC | GAAATGCTTCCCACATGTCT | 127 | IR36 |
| 4 | RM222 | 10 | (CT)18 | CTTAAATGGGCCACATGCG | CAAAGCTTCCGGCCAAAAG | 213 | IR36 |
| 5 | RM224 | 11 | (AAG)8(AG)13 | ATCGATCGATCTTCACGAGG | TGCTATAAAAGGCATTCGGG | 157 | IR36 |
| 6 | RM229 | 11 | (TC)11(CT)5 C3(CT)5 | CACTCACACGAACGACTGAC | CGCAGGTTCTTGTGAAATGT | 116 | IR36 |
| 7 | RM234 | 7 | (CT)25 | ACAGTATCCAAGGCCCTGG | CACGTGAGACAAAGACGGAG | 156 | IR36 |
| 8 | RM242 | 9 | (CT)26 | GGCCAACGTGTGTATGTCTC | TATATGCCAAGACGGATGGG | 225 | IR36 |
| 9 | RM248 | 7 | (CT)25 | TCCTTGTGAAATCTGGTCCC | GTAGCCTAGCATGGTGCATG | 102 | IR36 |
| 10 | RM252 | 4 | (CT)19 | TTCGCTGACGTGATAGGTTG | ATGACTTGATCCCGAGAACG | 216 | IR36 |
| 11 | RM255 | 4 | (AGG)5(AG)2-(GA)16 | TGTTGCGTGTGGAGATGTG | CGAAACCGCTCAGTTCAAC | 144 | IR36 |
| 12 | RM259 | 1 | (CT)17 | TGGAGTTTGAGAGGAGGG | CTTGTTGCATGGTGCCATGT | 162 | IR36 |
| 13 | RM282 | 3 | (GA)15 | CTGTGTCGAAAGGCTGCAC | CAGTCCTGTGTTGCAGCAAG | 136 | IR36 |
| 14 | RM286 | 11 | (GA)16 | GGCTTCATCTTTGGCGAC | CCGGATTCACGAGATAAACTC | 110 | IR36 |
| 15 | RM316 | 9 | T)8-(TG)9 (TTTG)4(TG)4 | CTAGTTGGGCATACGATGGC | ACGCTTATATGTTACGTCAAC | 192 | IR36 |
| 16 | RM321 | 9 | (CAT)5 | CCAACACTGCCACTCTGTTC | GAGGATGGACACCTTGATCG | 200 | IR36 |
| 17 | RM323 | 1 | (CAT)5 | CAACGAGCAAATCAGGTCAG | GTTTTGATCCTAAGGCTGCTG | 244 | NIP |
| 18 | RM400 | - | (ATA)63 | ACACCAGGCTACCCAAACTC | CGGAGAGATCTGACATGTGG | 321 | NIP |
| 19 | RM410 | 9 | (TA)13 | GCTCAACGTTTCGTTCCTG | GAAGATGCGTAAAGTGAACGG | 183 | NIP |
| 20 | RM422 | 3 | (AG)30 | TTCAACCTGCATCCGCTC | CCATCCAAATCAGCAACAGC | 385 | NIP |
| 21 | RM423 | 2 | (TTC)9 | AGCACCCATGCCTTATGTTG | CCTTTTTCAGTAGCCCTCCC | 273 | NIP |
| 22 | RM424 | 2 | (CAT)9 | TTTGTGGCTCACCAGTTGAG | TGGCGCATTCATGTCATC | 239 | NIP |
| 23 | RM426 | 3 | (CA)10 | ATGAGATGAGTTCAAGGCCC | AACTCTGTACCTCCATCGCC | 150 | NIP |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | RM432 | 7 | (CATC)9 | TTCTGTCTCACGCTGGATTG | AGCTGCGTACGTGATGAATG | 187 | NIP |
| 25 | RM439 | 6 | (AAT)13 | TCATAACAGTCCACTCCCCC | TGGTACTCCATCATCCCATG | 269 | NIP |
| 26 | RM440 | 5 | (CTT)22 | CATGCAACAACGTCACCTTC | ATGGTTGGTAGGCACCAAAG | 169 | NIP |
| 27 | RM444 | 9 | (AT)12 | GCTCCACCTGCTTAAGCATC | TGAAGACCATGTTCTGCAGG | 162 | NIP |
| 28 | RM450 | 2 | (AG)17 | AAACCACAGTAGTACGCCGG | TCCATCCACATCTCCCTCTC | 143 | NIP |
| 29 | RM472 | 1 | (GA)21 | CCATGGCCTGAGAGAGAGAG | AGCTAAATGGCCATACGGTG | 296 | NIP |
| 30 | RM475 | 2 | (TATC)8 | CCTCACGATTTTCCTCCAAC | ACGGTGGGATTAGACTGTGC | 235 | NIP |
| 31 | RM488 | 1 | (GA)17 | CAGCTAGGGTTTTGAGGCTG | TAGCAACAACCAGCGTATGC | 177 | NIP |
| 32 | RM490 | 1 | (CT)13 | ATCTGCACACTGCAAACACC | AGCAAGCAGTGCTTTCAGAG | 101 | NIP |
| 33 | RM500 | 7 | (AAG)9 | GAGCTTGCCAGAGTGGAAAG | GTTACACCGAGAGCCAGCTC | 259 | NIP |
| 34 | RM511 | 12 | (GAC)7 | CTTCGATCCGGTGACGAC | AACGAAAGCGAAGCTGTCTC | 130 | NIP |
| 35 | RM518 | 4 | (TC)15 | CTCTTCACTCACTCACCATGG | ATCCATCTGGAGCAAGCAAC | 171 | NIP |
| 36 | RM526 | 2 | (TAAT)5 | CCCAAGCAATACGTCCCTAG | ACCTGGTCATGACAAGGAGG | 240 | NIP |
| 37 | RM525 | 2 | (AAG)12 | GGCCCGTCCAAGAAATATTG | CGGTGAGACAGAATCCTTACG | 131 | NIP |
| 38 | RM527 | - | (GA)17 | GGCTCGATCTAGAAAATCCG | TTGCACAGGTTGCGATAGAG | 233 | NIP |
| 39 | RM528 | 6 | (AGAT)9 | GGCATCCAATTTTACCCCTC | AAATGGAGCATGGAGGTCAC | 232 | NIP |
| 40 | RM536 | 11 | (CT)16 | TCTCTCCTCTTGTTTGGCTC | ACACACCAACACGACCACAC | 243 | NIP |
| 41 | RM541 | 6 | (TC)16 | TATAACCGACCTCAGTGCCC | CCTTACTCCCATGCCATGAG | 158 | NIP |
| 42 | RM547 | - | (AAT)20 | TAGGTTGGCAGACCTTTTCG | GTCAAGATCATCCTCGTAGCG | 235 | NIP |
| 43 | RM551 | 4 | (AG)18 | AGCCCAGACTAGCATGATTG | GAAGGCGAGAAGGATCACAG | 192 | IR36 |
| 44 | RM562 | - | (AAG)13 | CACAACCCACAAACAGCAAG | CTTCCCCCAAAGTTTTAGCC | 243 | NIP |
| 45 | RM567 | 4 | (GA)21 | ATCAGGGAAATCCTGAAGGG | GGAAGGAGCAATCACCACTG | 261 | NIP |
| 46 | RM575 | 1 | (AG)24 | CAATTTCCATAGGCTGCATG | GCTTGGGTTAGCGACGAC | 201 | NIP |
| 47 | RM580 | 1 | (CTT)19 | GATGAACTCGAATTTGCATCC | CACTCCCATGTTTGGCTCC | 221 | NIP |
| 48 | RM582 | 1 | (TC)20 | TCTGTTGCCGATTTGTTCG | AAATGGCTTACCTGCTGTCTC | 231 | NIP |
| 49 | RM585 | 6 | (TC)45 | CAGTCTTGCTCCGTTTGTTG | CTGTGACTGACTTGGTCATAGG | 233 | NIP |
| 50 | RM594 | 1 | (GA)N | GCCACCAGTAAAAGCAATAC | TTGATCTGCTAGTGAGACCC | 300 | NIP |

**Table 3.3: A brief description of Transposon element based markers used in this study.**

| Sr. No. | PRIMER NUMBER | SEQUENCE(F) | SEQUENCE(R) |
|---|---|---|---|
| 1 | DashengD9 | ACgTACTCTCgCTTggATgg | CgATCCATgCTCATCATCAC |
| 2 | DashengD7 | ggTggTgTCgTgTgTTACTgA | ggCTATggTCCACATCgACT |
| 3 | DashengD11 | ggggTCCTCACTCgTCAATA | TAgCCCTTTCATgCAACCAT |
| 4 | Dasheng E3 | ATTgCAATTCgCACACAAAA | CgAggAACATCCCgTCTAAT |
| 5 | Dasheng C11 | ACACACACCCCCAAATTgTT | TgTgAgTATgAgAAggggTgA |
| 6 | Dasheng A3 | ATATTTgAggggCgCTTTTT | TgCTACgATCACCAgTCCAA |
| 7 | Dasheng G5 | AAgTTggACCggCAAAgAAT | TgAAAgAtgATgggCATgTg |
| 8 | Dasheng A7 | AAATTgCCTgTgCgTTACAA | TCAACATAggCTggACCAAA |
| 9 | Dasheng C7 | TggACgCTgTCAAgTTCAgA | AgATTgTAgCAgCgCCCTAA |
| 10 | Dasheng C9 | TgCgggATgTgTACAgTgAC | AggCTTCCgTggTgACTTC |
| 11 | Dasheng E11 | ACACAAATgCCACACACAgAg | ACTCCgTCCCAAAAAgAATC |
| 12 | Dasheng G7 | gTTggCTTTggTCAgAgCTT | gTCCggCCTTgTgTTgTAgT |
| 13 | Dasheng F1 | TCgAgCATTgAgTgTTgAgC | gTgTgAgCATTgTggCAgAg |
| 14 | Dasheng C5 | ggTggTgTCgTgTgTTACTgA | ggCTATggTCCACATCgACT |
| 15 | Dasheng G11 | AACAAAAggAgAgCCCACAA | gTACgTACgTCgCATgTggT |
| 16 | Dasheng F3 | ACCTTACCCCTCCACAAAgg | AAgCACCTAgAgggTgACCA |
| 17 | mPing8-2 | AAAAAgTgTCggAAgCTCTg | ATCgCTgTACTCCACTCTgC |
| 18 | mPing7-1 | CCTCgATACTgTTgCCTgTT | TAgAgCTggTAgTgggCTgT |
| 19 | mPing6-1 | ACAATCTATggCggAAACAg | gCCCTCCCATAggATTAgAA |
| 20 | mPing12-1 | ggCAAgTggAAgATCATACg | CCTCAAgAACAgTgCCAACT |
| 21 | mPing3-3 | CCACgAgTcAAgCTAAAggT | CgTCTCTCggTgACACAgTA |
| 22 | mPing3-4 | gCTgAAgTTTggACACAACC | CCTTCgCTCTTTggACATAA |
| 23 | mPing3-2 | AAAgTCAgAAgCgAATgTgg | CATTTTgCCTTTCTgCTgAT |
| 24 | mPing3-1 | CTTCAgCAgCCTATgTTAgTCg | gCAggCAgATgTTgATggTA |
| 25 | mPingU1 | CAggAgTgTTgggTTATTgg | gAATTATTTggggACgACCT |
| 26 | mPing9311-1 | TATACCCACTTTATCCCATTgC | ACgATTTCAgTgACCTCATCA |
| 27 | mPing9311-4 | gCCTTgAAACATgTCCACAC | AgAggCAAgAgCTACTCCAAAC |
| 28 | mPing9311-3 | CgAgAAgTCCgACACAAATg | ggTATCCCTgTAgCTAgATgTgC |
| 29 | mPing9311-6 | AgTACTTCATCTCCAgggACAg | gCgTCAgCAAgAAggTTAAg |
| 30 | mPing9311-5 | ATTCTCgCCTCTTggTTCTC | TTACTTCAgCTgTACCCgTAgC |

## Table4.1: SSR fingerprint database of 50 rice genotypes.

| Marker | Azu | NPT II | NPT III | T309 | TNG67 | Nippon | Bas217 | B370 | HBC19 |
|---|---|---|---|---|---|---|---|---|---|
| **RM213** | 135 | 163 | 135 | 127 | 127 | 127 | 139 | 139 | 139 |
| **RM215** | 152 | 150 | 150 | 150 | 152 | 150 | 148 | 152 | 152 |
| **RM220** | 108 | 104 | 104 | 108,124 | 124 | 124 | 124 | 108 | 108 |
| **RM222** | 205 | 215 | 205 | 205 | 205 | 199 | 215 | 205 | 209 |
| **RM224** | 159 | 124 | 124 | 124 | 124 | 124 | 145 | 157 | 133 |
| **RM229** | 106 | 138 | 106 | 130 | 130 | 130 | 124 | 124 | 124 |
| **RM234** | 140 | 134 | 0 | 134 | 134 | 134 | 0 | 134 | 134 |
| **RM242** | 193 | 197 | 197 | 193 | 197 | 197 | 225 | 193 | 193 |
| **RM248** | 78 | 82 | 82 | 82 | 88 | 80 | 74 | 74 | 74 |
| **RM252** | 194 | 194 | 198 | 198 | 198 | 198 | 210 | 236 | 242 |
| **RM255** | 145 | 145 | 145 | 145 | 145 | 145 | 145 | 145 | 145 |
| **RM259** | 174 | 172 | 172 | 172 | 172 | 172 | 162 | 176 | 176 |
| **RM282** | 127 | 127 | 127 | 127 | 127 | 127 | 135 | 127 | 127 |
| **RM286** | 118 | 130 | 130 | 122 | 122 | 122 | 112 | 112 | 112 |
| **RM316** | 212 | 196 | 192 | 196 | 196 | 196 | 192 | 196 | 192 |
| **RM321** | 206 | 200 | 200 | 206 | 206 | 206 | 200 | 206 | 206 |
| **RM323** | 241 | 241 | 241 | 241 | 244 | 241 | 244 | 244 | 244 |
| **RM400** | 312 | 190 | 240 | 0 | 240 | 321 | 217 | 255 | 255 |
| **RM410** | 185 | 185 | 169 | 183 | 183 | 183 | 183 | 185 | 185 |
| **RM422** | 389 | 389 | 389 | 385 | 385 | 385 | 385 | 385 | 385 |
| **RM423** | 291 | 273 | 273 | 273 | 273 | 273 | 291 | 291 | 273 |
| **RM424** | 239 | 239 | 239 | 239 | 239 | 239 | 272 | 263 | 263 |
| **RM426** | 152 | 152 | 152 | 148 | 150 | 150 | 0 | 160 | 168 |
| **RM432** | 171 | 187 | 187 | 187 | 187 | 187 | 187 | 175 | 175 |
| **RM439** | 278 | 278 | 242 | 269 | 269 | 269 | 248 | 248 | 248 |
| **RM440** | 220 | 205 | 0 | 169 | 169 | 169 | 205 | 151 | 151 |
| **RM444** | 162 | 212 | 162 | 162 | 162 | 162 | 226 | 226 | 226 |
| **RM450** | 141 | 139 | 143 | 143 | 143 | 143 | 135 | 139 | 139 |
| **RM472** | 260 | 260 | 262 | 262 | 260 | 262 | 206 | 262 | 262 |
| **RM475** | 200 | 200 | 235,200 | 200 | 210 | 235 | 188 | 194 | 194 |
| **RM488** | 195 | 165,195 | 165 | 177,195 | 175 | 177 | 171 | 195 | 195 |
| **RM490** | 103 | 103 | 103 | 101 | 101 | 101 | 97 | 107 | 103 |
| **RM500** | 259 | 259 | 256 | 259 | 256 | 259 | 253 | 253 | 259 |
| **RM511** | 133 | 133 | 133 | 130 | 130 | 130 | 133 | 133 | 127 |
| **RM518** | 163 | 171 | 171 | 171 | 171 | 171 | 161 | 159 | 163 |
| **RM525** | 137 | 140 | 131 | 131 | 131 | 131 | 137 | 107 | 140 |
| **RM526** | 240 | 240 | 232 | 240 | 232 | 240 | 240,264 | 264 | 240 |
| **RM527** | 239 | 0 | 239 | 233 | 233 | 233 | 221 | 239 | 221 |
| **RM528** | 248 | 248 | 0 | 232 | 232 | 232 | 256 | 278 | 278 |
| **RM536** | 231 | 231 | 231 | 243 | 0 | 243 | 219 | 231 | 231 |
| **RM541** | 156 | 156 | 158 | 158 | 158 | 158 | 170 | 180 | 180 |
| **RM547** | 211 | 235 | 235 | 274,235 | 229 | 235 | 274,229 | 241 | 256 |
| **RM551** | 196 | 192 | 192 | 192 | 192 | 192 | 220 | 186 | 186 |
| **RM562** | 219 | 219 | 219 | 243 | 243 | 243 | 249 | 231 | 231 |
| **RM567** | 253 | 261 | 249 | 261 | 249 | 261 | 249 | 239 | 239 |
| **RM575** | 203 | 193 | 193 | 201 | 201 | 201 | 199 | 201 | 201 |
| **RM580** | 230 | 230 | 230 | 218 | 221 | 221 | 206 | 206 | 206 |
| **RM582** | 227 | 231 | 231 | 227 | 227 | 231 | 223 | 227 | 227 |
| **RM585** | 230 | 230 | 230 | 250 | 250 | 270 | 170 | 190 | 190 |
| **RM594** | 308 | 308 | 0 | 292,304 | 304 | 304 | 292 | 292 | 304 |

| Marker | KLocal | RanBas | TypeIII | Bas385 | CSR30 | ImpShar | HKR228 | Kasturi | Mahi |
|---|---|---|---|---|---|---|---|---|---|

Contd…

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **RM213** | 139 | 157 | 143 | 139 | 139 | 139 | 143 | 139 | 139 |
| **RM215** | 148 | 152 | 152 | 152 | 152 | 148 | 152 | 152 | 158 |
| **RM220** | 108 | 108 | 108 | 108 | 0 | 108 | 118 | 108 | 120 |
| **RM222** | 209 | 199 | 205 | 205 | 209 | 213 | 209 | 215 | 213 |
| **RM224** | 159 | 157 | 133 | 154 | 133 | 133,172 | 133 | 133 | 154 |
| **RM229** | 124 | 124 | 124 | 124 | 124 | 0 | 120 | 116 | 116 |
| **RM234** | 156 | 134 | 134,164 | 156 | 134 | 156 | 156 | 156 | 156 |
| **RM242** | 193 | 193 | 193 | 209 | 193 | 219 | 0 | 219 | 225 |
| **RM248** | 74,82 | 74 | 102,74 | 74 | 74 | 102 | 102,150 | 102 | 102,150 |
| **RM252** | 242 | 0 | 234 | 234 | 242 | 242 | 222 | 234 | 0 |
| **RM255** | 145 | 145 | 145 | 145 | 145 | 145 | 151 | 151 | 151 |
| **RM259** | 176 | 176 | 176 | 154 | 174 | 174 | 158 | 158 | 174 |
| **RM282** | 127 | 127 | 127 | 135 | 127 | 131 | 127 | 131 | 131 |
| **RM286** | 112 | 122 | 112 | 112 | 112 | 116 | 108 | 112 | 112 |
| **RM316** | 192 | 192 | 192 | 192 | 192 | 192 | 196 | 192 | 192 |
| **RM321** | 206 | 206 | 206 | 200 | 200 | 200 | 206 | 206 | 200 |
| **RM323** | 241 | 241 | 244 | 241 | 241 | 241 | 241 | 241 | 241 |
| **RM400** | 255 | 217,255 | 255 | 255 | 255 | 201 | 255 | 255 | 201 |
| **RM410** | 183 | 185 | 185 | 185 | 185 | 183 | 185 | 185 | 183 |
| **RM422** | 385 | 385 | 385 | 385 | 385 | 389 | 389 | 385 | 385 |
| **RM423** | 250 | 291 | 291 | 291 | 291 | 270 | 273 | 270 | 291 |
| **RM424** | 263 | 263 | 263 | 263 | 263 | 267 | 263 | 263 | 281 |
| **RM426** | 168 | 0 | 158 | 162 | 168,162 | 168 | 168 | 168 | 150 |
| **RM432** | 175 | 175 | 175 | 175 | 175 | 167 | 167 | 175 | 175 |
| **RM439** | 248 | 248 | 248 | 248 | 248 | 248 | 0 | 248 | 269 |
| **RM440** | 151 | 151 | 149 | 151 | 151 | 205 | 169 | 151 | 205 |
| **RM444** | 226 | 174,226 | 226 | 226 | 226 | 226 | 226 | 226 | 226 |
| **RM450** | 135 | 139 | 139 | 139 | 139 | 135 | 139 | 141 | 137 |
| **RM472** | 270 | 266 | 266 | 266 | 262 | 262 | 270 | 262 | 270 |
| **RM475** | 186 | 194 | 194 | 194 | 194 | 190 | 194 | 190 | 178 |
| **RM488** | 165,195 | 0 | 177 | 195 | 177,195 | 171 | 171 | 177,195 | 171 |
| **RM490** | 107 | 107 | 97 | 107 | 93,103 | 97 | 93,107 | 97 | 103 |
| **RM500** | 259 | 256 | 253 | 259 | 259 | 253 | 256 | 256 | 256 |
| **RM511** | 133 | 133 | 133 | 133 | 130 | 133 | 133 | 130 | 133 |
| **RM518** | 161 | 161 | 159 | 161 | 165 | 163 | 163 | 165 | 159 |
| **RM525** | 140 | 107 | 140,107 | 140 | 140 | 107 | 140 | 140 | 110 |
| **RM526** | 248 | 264 | 240 | 240 | 240 | 240 | 248 | 240 | 248 |
| **RM527** | 221 | 0 | 233 | 233 | 239 | 221 | 221 | 221 | 0 |
| **RM528** | 248 | 256 | 278 | 278 | 278 | 248 | 248,278 | 232,278 | 248 |
| **RM536** | 225 | 231,219 | 231 | 225 | 231 | 225 | 225 | 231 | 225 |
| **RM541** | 170 | 160 | 160 | 158 | 170 | 170,188 | 170 | 170 | 180,188 |
| **RM547** | 274 | 235 | 274 | 274 | 241,256 | 274 | 274 | 199 | 241 |
| **RM551** | 186 | 192 | 186 | 188 | 186 | 192 | 0 | 186 | 186 |
| **RM562** | 219,231 | 219,231 | 231 | 231 | 231 | 219,253 | 249 | 231 | 249 |
| **RM567** | 249 | 253 | 239 | 249 | 239 | 249,261 | 249 | 239,249 | 239 |
| **RM575** | 201 | 201 | 201 | 20093 | 201 | 201 | 201,203 | 201 | 193 |
| **RM580** | 209 | 209 | 203 | 206 | 203 | 206 | 203 | 203 | 203 |
| **RM582** | 223 | 223 | 0 | 331 | 227 | 223 | 223 | 223 | 223 |
| **RM585** | 170 | 170 | 190 | 174 | 190 | 170 | 190 | 190 | 170 |
| **RM594** | 308 | 292 | 308 | 292,308 | 298,308 | 298 | 308 | 298 | 288 |

Contd…

| **Marker** | **PB1** | **PS2** | **PS3** | **PS4** | **Sabarm** | **Super** | **PR118** | **PR106** | **IR36** |
|---|---|---|---|---|---|---|---|---|---|
| **RM213** | 139 | 139 | 139 | 139 | 139 | 139 | 139 | 139 | 139 |
| **RM215** | 152 | 158 | 148 | 158 | 148 | 150 | 148 | 148 | 148,152 |
| **RM220** | 108 | 108 | 116 | 120 | 116 | 120 | 108 | 120 | 120 |
| **RM222** | 203 | 205 | 205 | 209 | 213 | 205 | 215 | 209 | 213 |
| **RM224** | 133 | 133,154 | 133,154 | 133,172 | 159 | 172 | 133 | 157 | 145 |
| **RM229** | 116 | 124 | 124 | 124 | 124 | 124 | 116 | 124 | 116,124 |
| **RM234** | 134 | 156 | 134,164 | 134 | 156 | 134 | 156 | 156 | 156 |
| **RM242** | 193 | 225 | 225 | 193 | 225 | 193 | 225 | 225 | 225 |
| **RM248** | 102,74 | 102 | 102 | 102,150 | 102,135 | 74 | 74 | 102 | 102 |
| **RM252** | 246 | 0 | 200,222 | 200,222 | 236 | 244 | 222 | 0 | 220 |
| **RM255** | 151 | 151 | 151 | 151 | 151 | 151 | 151 | 151 | 145 |
| **RM259** | 154 | 158 | 160 | 158 | 160 | 158 | 174 | 158 | 162 |
| **RM282** | 127 | 135 | 135 | 135 | 131 | 127 | 135 | 135 | 135 |
| **RM286** | 112 | 104 | 108 | 108 | 108 | 112 | 112 | 112 | 112 |
| **RM316** | 0 | 192 | 194 | 192 | 194 | 192 | 194 | 194 | 192 |
| **RM321** | 206 | 200 | 200 | 200 | 200 | 206 | 200 | 206 | 200 |
| **RM323** | 244 | 241 | 241 | 241 | 244 | 241 | 244 | 241 | 244 |
| **RM400** | 201 | 201,255 | 201,255 | 201,255 | 217 | 255 | 255 | 201 | 255 |
| **RM410** | 185 | 183 | 183 | 183,185 | 183 | 185 | 183 | 183 | 185 |
| **RM422** | 383 | 383 | 383 | 385 | 385 | 383 | 385 | 385 | 383 |
| **RM423** | 273 | 273 | 270 | 273 | 270 | 291 | 270 | 273 | 291 |
| **RM424** | 263 | 263 | 263 | 263 | 281 | 239 | 263 | 263 | 281 |
| **RM426** | 168 | 168 | 150 | 160 | 168 | 152 | 168 | 168 | 168 |
| **RM432** | 167 | 171 | 171 | 171 | 175 | 175 | 175 | 187 | 167 |
| **RM439** | 269 | 266 | 248 | 248 | 248 | 248 | 269 | 248 | 248 |
| **RM440** | 205 | 157 | 0 | 205 | 205 | 151 | 205 | 202 | 211 |
| **RM444** | 226 | 170 | 170 | 170,226 | 226 | 226,170 | 226 | 170 | 170 |
| **RM450** | 139 | 137 | 139 | 139 | 137 | 139 | 135 | 135 | 137 |
| **RM472** | 262 | 270 | 270 | 262 | 270 | 262 | 262 | 270 | 270 |
| **RM475** | 194 | 186 | 186 | 194 | 186 | 194 | 190 | 186 | 194 |
| **RM488** | 171,195 | 181 | 181 | 171 | 181 | 177,195 | 195 | 181 | 171 |
| **RM490** | 93 | 103 | 107 | 103 | 97,107 | 103 | 103 | 107 | 107,97 |
| **RM500** | 256 | 256 | 256 | 256 | 256 | 259 | 256 | 259 | 256 |
| **RM511** | 130 | 130 | 133 | 130 | 133 | 130 | 130 | 118 | 118 |
| **RM518** | 163 | 163 | 163 | 163 | 163 | 159 | 163 | 163 | 173 |
| **RM525** | 140,107 | 140 | 140 | 140 | 107 | 110,140 | 107 | 107 | 107 |
| **RM526** | 240 | 248 | 240 | 232 | 232 | 240 | 264 | 264 | 240 |
| **RM527** | 0 | 221 | 221 | 221 | 221 | 221 | 221 | 233 | 221 |
| **RM528** | 278 | 248,278 | 278 | 278 | 248 | 278 | 248 | 248,278 | 248 |
| **RM536** | 225 | 231 | 231 | 225 | 225 | 235 | 225 | 225 | 225 |
| **RM541** | 208 | 170 | 158 | 18008 | 156 | 170 | 170 | 170 | 180 |
| **RM547** | 274 | 199 | 211 | 235 | 271 | 235 | 241 | 199,277 | 235 |
| **RM551** | 188 | 188 | 188 | 188 | 186 | 186 | 188 | 192 | 188 |
| **RM562** | 231,253 | 253 | 253 | 249,253 | 229 | 231 | 249 | 249 | 219 |
| **RM567** | 239 | 249 | 249 | 249 | 249 | 239 | 249 | 249 | 249 |
| **RM575** | 201 | 201 | 201 | 199 | 201 | 201 | 201 | 201 | 199 |
| **RM580** | 206 | 206 | 206 | 203 | 206 | 203 | 206 | 206 | 206 |
| **RM582** | 223 | 223 | 223 | 223 | 223 | 231 | 223 | 223 | 223 |
| **RM585** | 190 | 170 | 170 | 190 | 170 | 190 | 190 | 174 | 174 |
| **RM594** | 298 | 298 | 0 | 288 | 288 | 288 | 288 | 288 | 304 |

Contd…

| Marker | BR4-10 | CSR1 | CSR10 | CSR21 | HKR120 | 93-401 | IR24 | IR72 | KR1-24 |
|---|---|---|---|---|---|---|---|---|---|
| **RM213** | 135 | 139 | 139 | 139 | 139 | 139 | 143 | 143 | 143 |
| **RM215** | 154 | 150 | 150 | 148 | 152 | 156 | 148 | 148 | 148 |
| **RM220** | 120 | 116 | 122 | 120 | 118 | 120 | 116 | 120 | 116 |
| **RM222** | 199 | 217 | 217 | 215,221 | 219 | 221 | 213 | 213 | 199 |
| **RM224** | 157 | 145 | 167 | 172 | 167 | 157 | 133 | 145 | 154,172 |
| **RM229** | 116 | 116 | 116 | 120 | 116 | 124 | 120 | 124 | 124 |
| **RM234** | 150 | 156 | 156 | 164 | 156 | 134 | 156 | 156 | 134 |
| **RM242** | 193 | 225 | 219 | 225 | 219 | 193 | 225 | 225 | 193 |
| **RM248** | 78 | 96 | 94 | 94 | 96 | 102 | 102 | 102 | 78 |
| **RM252** | 212 | 210 | 210 | 236 | 224 | 246 | 224 | 224 | 0 |
| **RM255** | 145 | 145 | 145 | 145 | 145 | 145 | 145 | 145 | 145 |
| **RM259** | 154 | 158 | 158 | 154 | 154 | 158 | 154 | 158 | 160 |
| **RM282** | 135 | 131 | 131 | 135 | 135 | 135 | 127 | 135 | 135 |
| **RM286** | 112 | 112 | 112 | 112 | 112 | 112 | 112 | 104 | 104 |
| **RM316** | 196 | 192 | 194 | 192 | 196 | 192 | 192 | 192 | 196 |
| **RM321** | 206 | 200 | 200 | 200 | 200 | 206 | 206 | 200 | 206 |
| **RM323** | 244 | 241 | 241 | 241 | 241 | 244 | 241 | 241 | 241 |
| **RM400** | 312 | 312 | 312 | 0 | 217 | 180,321 | 201 | 180,201 | 255 |
| **RM410** | 169 | 185 | 185 | 183 | 169 | 183 | 185 | 169 | 183 |
| **RM422** | 373 | 389 | 389 | 389 | 383 | 383 | 383 | 363,385 | 383 |
| **RM423** | 270 | 291 | 291 | 0 | 270,273 | 291 | 270 | 270 | 0 |
| **RM424** | 281 | 281 | 0 | 263 | 281 | 272 | 263 | 263 | 281 |
| **RM426** | 168 | 178,184 | 178,184 | 172 | 172,174 | 178 | 172,174 | 172 | 172 |
| **RM432** | 171 | 175 | 175 | 175 | 175 | 175 | 175 | 175 | 175 |
| **RM439** | 248 | 248 | 248 | 248 | 248 | 230 | 248 | 266 | 269 |
| **RM440** | 169 | 205 | 205 | 211 | 205 | 211 | 205 | 220 | 157 |
| **RM444** | 174 | 226 | 162,226 | 226 | 218 | 226 | 170 | 170 | 174 |
| **RM450** | 135,139 | 141 | 135 | 135 | 135 | 139 | 135 | 139 | 143 |
| **RM472** | 270 | 260 | 270 | 270 | 270 | 270 | 270 | 0 | 266 |
| **RM475** | 178 | 194 | 194 | 194 | 194 | 186 | 186 | 178 | 186 |
| **RM488** | 0 | 171,195 | 181 | 173 | 181 | 173 | 181 | 173 | 179 |
| **RM490** | 97 | 97 | 97 | 97 | 93,103 | 97 | 103 | 103 | 107 |
| **RM500** | 253 | 256 | 256 | 253 | 256 | 259 | 256 | 256 | 253 |
| **RM511** | 133 | 130 | 127 | 118 | 127 | 130 | 133 | 118 | 133 |
| **RM518** | 165 | 163 | 163 | 163 | 171 | 171 | 163 | 163 | 163 |
| **RM525** | 110 | 140 | 107 | 107,110 | 140 | 107 | 107 | 107 | 110 |
| **RM526** | 240 | 240 | 264 | 264 | 264 | 240 | 264 | 240 | 240 |
| **RM527** | 221 | 233 | 233 | 233 | 221 | 221 | 221 | 221 | 221 |
| **RM528** | 248 | 256 | 256 | 248 | 248 | 248 | 248 | 248,278 | 248 |
| **RM536** | 219 | 219 | 225 | 225 | 225 | 225 | 225 | 225 | 219 |
| **RM541** | 180 | 208 | 170 | 210 | 170 | 0 | 170 | 188 | 174 |
| **RM547** | 232 | 226 | 226 | 241 | 226 | 244 | 235 | 235 | 241 |
| **RM551** | 220 | 220 | 220 | 220 | 188 | 188 | 188 | 188 | 188 |
| **RM562** | 249 | 253 | 253 | 243,267 | 219 | 267 | 219,249 | 249 | 249 |
| **RM567** | 253 | 24061 | 249 | 0 | 249 | 249 | 239,249 | 239,249 | 253 |
| **RM575** | 199 | 187 | 187 | 207 | 193 | 201 | 193 | 193 | 187 |
| **RM580** | 230 | 206 | 206 | 203 | 206 | 203 | 206 | 206 | 230 |
| **RM582** | 223 | 0 | 223 | 213 | 331 | 331 | 223 | 223 | 223 |
| **RM585** | 182 | 182 | 182 | 174 | 174 | 182 | 190 | 250 | 174 |
| **RM594** | 298 | 298 | 304 | 298 | 312 | 298 | 298 | 288 | 298 |

Contd…

| Marker | MI48 | Pokkali | Sharbati | 9311 | IR64 | Oglab | Obart | Orufi1 | Orufi2 |
|---|---|---|---|---|---|---|---|---|---|
| **RM213** | 139 | 139 | 139 | 131 | 139 | 143 | 131 | 131 | 139 |
| **RM215** | 148 | 152 | 146 | 154 | 150 | 146 | 136 | 142 | 146 |
| **RM220** | 120 | 118 | 120 | 120 | 120 | 116 | 124 | 124 | 104 |
| **RM222** | 213 | 213 | 215 | 199 | 213 | 221 | 203 | 205 | 209 |
| **RM224** | 159 | 145 | 133 | 124 | 157 | 124 | 157 | 133 | 145 |
| **RM229** | 116 | 116 | 124 | 126 | 116 | 124 | 116 | 130 | 114 |
| **RM234** | 156 | 156 | 156 | 134,164 | 164 | 134 | 138 | 138 | 138 |
| **RM242** | 225 | 219 | 225 | 197 | 225 | 193 | 193 | 193 | 193 |
| **RM248** | 94 | 96 | 102 | 82 | 96 | 96 | 88 | 96,82 | 82 |
| **RM252** | 232 | 222 | 244 | 198 | 220 | 204,232 | 228 | 200 | 200 |
| **RM255** | 145 | 145 | 145 | 145 | 145 | 127 | 135 | 139 | 135 |
| **RM259** | 160 | 158 | 160 | 168 | 152 | 0 | 148 | 0 | 168 |
| **RM282** | 135 | 135 | 135 | 131 | 127 | 135 | 127 | 0 | 0 |
| **RM286** | 122 | 104 | 122 | 118 | 112 | 108 | 104 | 110 | 108 |
| **RM316** | 194 | 0 | 192 | 192 | 194 | 196 | 202 | 198 | 196 |
| **RM321** | 200 | 200 | 200 | 206 | 200 | 206 | 200 | 206 | 206 |
| **RM323** | 244 | 244 | 244 | 241 | 241 | 241 | 244 | 244 | 241 |
| **RM400** | 201 | 240 | 217 | 270 | 201 | 312 | 312,321 | 201 | 255 |
| **RM410** | 185 | 169 | 169 | 185 | 183 | 185 | 169 | 185 | 183 |
| **RM422** | 383 | 383 | 383 | 385 | 383 | 0 | 0 | 373 | 373 |
| **RM423** | 291 | 297 | 297 | 273 | 270 | 250 | 0 | 250 | 250 |
| **RM424** | 263 | 272 | 281 | 239 | 263 | 281 | 0 | 267 | 267 |
| **RM426** | 168 | 178 | 168 | 150 | 168 | 160 | 152 | 150 | 160 |
| **RM432** | 175 | 171 | 171 | 167 | 175 | 175 | 175 | 175 | 175 |
| **RM439** | 269 | 248 | 269 | 266 | 248 | 242 | 248 | 0 | 0 |
| **RM440** | 202 | 202 | 205 | 169 | 211 | 169 | 151 | 211 | 169 |
| **RM444** | 226 | 226 | 226 | 162 | 226 | 174 | 170,218 | 212 | 226 |
| **RM450** | 135 | 139 | 137 | 143 | 135 | 127 | 137 | 130 | 137 |
| **RM472** | 270 | 260 | 266 | 266 | 270 | 266 | 266 | 260 | 260 |
| **RM475** | 178 | 178 | 178 | 178,200 | 194 | 186 | 194 | 190 | 186 |
| **RM488** | 179,195 | 173 | 173 | 165,195 | 175 | 175 | 181 | 173 | 171 |
| **RM490** | 93,107 | 97 | 93,103 | 101 | 93 | 103 | 107 | 97 | 97 |
| **RM500** | 253 | 253,256 | 256,259 | 0 | 256 | 253 | 253 | 256 | 256 |
| **RM511** | 118 | 127 | 127 | 130 | 127 | 118 | 133 | 127 | 118 |
| **RM518** | 163 | 163 | 163 | 159 | 171 | 153 | 153 | 151 | 159 |
| **RM525** | 107 | 107 | 107 | 131 | 107 | 107 | 119 | 110 | 110,119 |
| **RM526** | 264 | 240 | 264 | 240,264 | 264 | 0 | 232 | 248 | 248 |
| **RM527** | 233 | 233 | 221 | 214 | 233 | 239 | 233 | 0 | 233 |
| **RM528** | 248 | 256 | 248 | 248 | 248 | 0 | 270 | 232 | 248 |
| **RM536** | 225 | 225 | 225 | 243 | 225 | 231 | 231 | 0 | 0 |
| **RM541** | 174 | 174 | 170 | 158 | 180 | 180 | 170 | 146 | 146 |
| **RM547** | 277 | 226 | 226 | 235 | 235 | 205 | 211 | 229 | 235 |
| **RM551** | 186 | 188 | 192 | 192 | 188 | 188 | 220 | 192 | 188 |
| **RM562** | 219,249 | 249 | 249,267 | 243 | 267 | 229 | 267 | 249 | 253 |
| **RM567** | 249 | 249 | 249,261 | 261 | 249 | 239 | 261 | 253 | 261 |
| **RM575** | 199 | 187 | 201 | 201 | 206 | 203 | 206 | 206 | 193 |
| **RM580** | 203 | 206 | 206 | 221 | 206 | 190 | 209 | 179 | 199 |
| **RM582** | 223 | 223 | 223 | 231 | 223 | 213 | 181 | 201 | 193 |
| **RM585** | 182 | 250 | 170 | 174 | 250 | 190 | 182 | 270 | 190 |
| **RM594** | 288 | 308 | 288 | 312 | 288 | 292 | 298 | 308 | 288 |

Contd…

| Marker | Oniv1 | Oniv2 | Oniv3 | Oglumal | Omerdio |
|---|---|---|---|---|---|
| **RM213** | 127 | 135 | 135 | 127 | 139 |
| **RM215** | 136 | 136 | 148 | 142 | 142 |
| **RM220** | 122 | 108 | 120,124 | 124 | 124 |
| **RM222** | 199 | 203 | 215 | 199 | 213 |
| **RM224** | 154 | 133 | 159 | 145 | 0 |
| **RM229** | 130 | 130 | 126 | 128 | 0 |
| **RM234** | 150 | 126 | 126 | 130 | 138 |
| **RM242** | 197 | 197 | 193 | 0 | 0 |
| **RM248** | 80 | 80 | 74 | 74 | 80 |
| **RM252** | 228 | 212 | 0 | 0 | 0 |
| **RM255** | 127 | 133 | 141 | 137 | 135 |
| **RM259** | 154 | 152 | 158 | 172 | 0 |
| **RM282** | 0 | 131 | 131 | 127 | 135 |
| **RM286** | 130 | 110 | 108 | 108 | 108 |
| **RM316** | 198 | 198 | 194 | 196 | 194 |
| **RM321** | 206 | 206 | 206 | 200 | 200 |
| **RM323** | 244 | 241 | 241 | 241 | 241 |
| **RM400** | 255 | 217 | 312 | 312 | 201 |
| **RM410** | 169 | 185 | 169 | 169 | 183 |
| **RM422** | 363 | 373 | 383 | 385 | 363 |
| **RM423** | 270,250 | 250 | 250 | 250 | 250 |
| **RM424** | 239 | 239 | 267 | 0 | 0 |
| **RM426** | 152 | 150 | 172 | 148 | 148 |
| **RM432** | 175 | 187 | 171 | 171 | 167 |
| **RM439** | 266 | 269 | 230 | 248 | 269 |
| **RM440** | 202 | 149 | 220 | 202 | 157 |
| **RM444** | 226 | 212 | 226 | 170 | 162 |
| **RM450** | 141 | 139 | 141 | 130 | 139 |
| **RM472** | 260 | 262 | 266 | 266 | 262 |
| **RM475** | 178 | 178 | 186 | 178 | 178 |
| **RM488** | 0 | 177 | 179 | 195 | 175 |
| **RM490** | 101 | 101 | 107 | 93 | 93 |
| **RM500** | 256 | 259 | 256 | 253 | 259 |
| **RM511** | 130 | 130 | 0 | 133 | 127 |
| **RM518** | 159 | 159 | 161 | 151 | 151 |
| **RM525** | 137 | 107 | 107 | 116 | 110 |
| **RM526** | 232 | 220 | 220,232 | 240 | 220 |
| **RM527** | 233 | 221 | 214 | 221 | 214 |
| **RM528** | 232 | 256 | 232 | 270 | 278 |
| **RM536** | 219 | 215 | 219 | 219 | 215 |
| **RM541** | 146 | 180 | 146 | 156 | 156 |
| **RM547** | 241 | 0 | 235 | 277 | 0 |
| **RM551** | 192 | 188 | 188 | 186 | 188 |
| **RM562** | 243 | 249 | 231 | 219 | 219 |
| **RM567** | 253,261 | 249 | 261 | 239 | 239 |
| **RM575** | 193 | 187 | 203 | 199 | 203 |
| **RM580** | 185 | 185 | 190 | 181 | 181 |
| **RM582** | 193 | 183 | 201 | 193 | 193 |
| **RM585** | 174 | 250 | 174 | 190 | 270 |
| **RM594** | 308 | 312 | 304 | 312 | 308 |

**Table 4.2:** **Data on number of alleles, numbers of unique alleles, number of genotypes with multiple alleles, allele size range, highest frequency allele and polymorphic information content (PIC) obtained using 50 SSR markers in 50 rice genotypes.**

| Sr. No. | Marker Name | Number of Alleles | Genotype with | | Size Range (bp) | Difference (bp) | Highest Alleles | % Highest frequency Allele | PIC |
|---|---|---|---|---|---|---|---|---|---|
| | | | Unique Alleles | Multiple Alleles | | | | | |
| 1 | RM 213 | 7 | 2 | 0 | 127-163 | 36 | 29 | 58 | 0.5976 |
| 2 | RM 215 | 9 | 1 | 1 | 136-158 | 22 | 15 | 29 | 0.7883 |
| 3 | RM 220 | 7 | 0 | 2 | 104-124 | 20 | 14 | 27 | 0.7688 |
| 4 | RM 222 | 9 | 1 | 1 | 199-221 | 22 | 22 | 43 | 0.8291 |
| 5 | RM 224 | 8 | 0 | 5 | 124-172 | 48 | 15 | 27 | 0.8245 |
| 6 | RM 229 | 11 | 4 | 1 | 106-138 | 32 | 20 | 40 | 0.7152 |
| 7 | RM 234 | 8 | 2 | 3 | 126-164 | 38 | 19 | 37 | 0.6809 |
| 8 | RM 242 | 5 | 1 | 0 | 193-225 | 32 | 19 | 40 | 0.6489 |
| 9 | RM 248 | 10 | 1 | 8 | 74-150 | 76 | 16 | 27 | 0.818 |
| 10 | RM 252 | 16 | 1 | 4 | 194-244 | 50 | 6 | 13 | 0.9224 |
| 11 | RM 255 | 8 | 4 | 0 | 127-151 | 24 | 30 | 60 | 0.5444 |
| 12 | RM 259 | 10 | 3 | 0 | 148-176 | 28 | 13 | 27 | 0.8413 |
| 13 | RM 282 | 3 | 0 | 1 | 127-135 | 8 | 20 | 42 | 0.5589 |
| 14 | RM 286 | 8 | 1 | 0 | 104-130 | 26 | 22 | 44 | 0.7149 |
| 15 | RM 316 | 6 | 2 | 0 | 192-212 | 20 | 22 | 45 | 0.6391 |
| 16 | RM 321 | 2 | 0 | 0 | 200-206 | 6 | 25 | 50 | 0.375 |
| 17 | RM 323 | 2 | 0 | 0 | 241-244 | 3 | 33 | 66 | 0.3481 |
| 18 | RM 400 | 9 | 1 | 6 | 180-321 | 141 | 18 | 33 | 0.7645 |
| 19 | RM 410 | 3 | 0 | 1 | 169-185 | 16 | 22 | 43.1 | 0.5618 |
| 20 | RM 422 | 5 | 0 | 1 | 363-389 | 26 | 20 | 40.8 | 0.6651 |
| 21 | RM 423 | 5 | 0 | 3 | 250-291 | 41 | 14 | 28 | 0.7195 |
| 22 | RM 424 | 5 | 0 | 0 | 239-281 | 42 | 20 | 43 | 0.6711 |
| 23 | RM | 11 | 1 | 5 | 148- | 36 | 16 | 30.1 | 0.8141 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 426 | | | | 184 | | | | |
| 24 | RM 432 | 4 | 0 | 0 | 163-187 | 24 | 26 | 52 | 0.5904 |
| 25 | RM 439 | 6 | 0 | 0 | 230-278 | 48 | 26 | 55.3 | 0.5838 |
| 26 | RM 440 | 8 | 0 | 0 | 149-220 | 71 | 13 | 27.1 | 0.8121 |
| 27 | RM 444 | 6 | 0 | 5 | 162-226 | 64 | 28 | 50.9 | 0.6336 |
| 28 | RM 450 | 7 | 1 | 3 | 127-143 | 16 | 18 | 34 | 0.749 |
| 29 | RM 472 | 4 | 0 | 0 | 260-270 | 10 | 16 | 32 | 0.6975 |
| 30 | RM 475 | 6 | 1 | 2 | 172-230 | 58 | 16 | 30.7 | 0.7456 |
| 31 | RM 488 | 8 | 0 | 11 | 165-195 | 30 | 17 | 29.3 | 0.8284 |
| 32 | RM 490 | 5 | 0 | 7 | 93-107 | 14 | 15 | 26.7 | 0.744 |
| 33 | RM 500 | 3 | 0 | 2 | 253-259 | 6 | 25 | 49 | 0.5579 |
| 34 | RM 511 | 4 | 0 | 0 | 118-133 | 15 | 19 | 38 | 0.6566 |
| 35 | RM 518 | 8 | 1 | 0 | 151-173 | 22 | 20 | 30 | 0.7436 |
| 36 | RM 525 | 7 | 1 | 5 | 107-140 | 33 | 21 | 38.1 | 0.7061 |
| 37 | RM 526 | 5 | 0 | 3 | 220-264 | 44 | 22 | 42 | 0.66 |
| 38 | RM 527 | 4 | 0 | 0 | 214-239 | 25 | 22 | 48 | 0.5681 |
| 39 | RM 528 | 5 | 0 | 7 | 232-278 | 46 | 25 | 45 | 0.6599 |
| 40 | RM 536 | 6 | 1 | 1 | 215-243 | 28 | 21 | 43 | 0.652 |
| 41 | RM 541 | 10 | 1 | 3 | 146-210 | 64 | 15 | 28 | 0.821 |
| 42 | RM 547 | 13 | 3 | 5 | 199-277 | 78 | 13 | 24 | 0.8421 |
| 43 | RM 551 | 5 | 1 | 0 | 186-220 | 34 | 19 | 38 | 0.6766 |
| 44 | RM 562 | 7 | 0 | 10 | 219-276 | 48 | 15 | 25.8 | 0.7996 |
| 45 | RM 567 | 4 | 0 | 7 | 239-261 | 22 | 26 | 46 | 0.6388 |
| 46 | RM 575 | 6 | 0 | 2 | 187-207 | 20 | 23 | 44 | 0.709 |
| 47 | RM 582 | 9 | 2 | 0 | 181-331 | 150 | 24 | 50 | 0.6862 |
| 48 | RM580 | 11 | 3 | 0 | 179-230 | 51 | 19 | 38 | 0.7607 |
| 49 | RM 585 | 7 | 0 | 1 | 174-270 | 96 | 24 | 47 | 0.7974 |
| 50 | RM 594 | 6 | 0 | 4 | 288-312 | 24 | 13 | 25 | 0.7795 |

**Table 4.3: Mean similarity between various rice types based on SSR analysis (NYSYS-PC, UPGMA).**

| Sr. No. | GROUPS | SSR-J |
|---|---|---|
| 1 | Traditional Basmati / Traditional Basmati | 0.320 |
| 2 | *Japonica* / *Japonica* | 0.300 |
| 3 | *Indica* / *Indica* | 0.225 |
| 4 | *Japonica* / Traditional Basmati | 0.112 |
| 5 | *Japonica / Indica* | 0.074 |
| 6 | *Indica* / Traditional Basmati | 0.165 |
| 7 | *O. glaberrima* / Traditional Basmati | 0.115 |
| 8 | *O. barthii* / Traditional Basmati | 0.114 |
| 9 | *O. rufipogon* / Traditional Basmati | 0.089 |
| 10 | *O. nivara* / Traditional Basmati | 0.084 |
| 11 | *O. glumalpatula* / Traditional Basmati | 0.122 |
| 12 | *O. meridonalis* / Traditional Basmati | 0.066 |
| 13 | *O. glaberrima* / *Japonica* | 0.078 |
| 14 | *O. barthii* / *Japonica* | 0.064 |
| 15 | *O. rufipogon* / *Japonica* | 0.082 |
| 16 | *O. nivara* / *Japonica* | 0.100 |
| 17 | *O. glumalpatula* / *Japonica* | 0.094 |
| 18 | *O. meridonalis* / *Japonica* | 0.083 |
| 19 | *O. glaberrima* / *Indica* | 0.096 |
| 20 | *O. barthii* / *Indica* | 0.098 |
| 21 | *O. rufipogon* / *Indica* | 0.085 |
| 22 | *O. nivara* / *Indica* | 0.085 |
| 23 | *O. glumalpatula* / *Indica* | 0.087 |
| 24 | *O. meridonalis* / *Indica* | 0.087 |

**Table 4.4: SSR markers which can be used for differentiating between particular combinations of Traditional Basmati, cross-bred Basmati, *Indica* and *Japonica* rice varieties.**

| Sr. No. | Varietal combinations | SSR markers for differentiation |
|---|---|---|
| 1 | Traditional Basmati and Cross-bred Basmati varieties | RM222, RM224, RM229, RM248, RM252, RM259, RM286, RM400, RM426, RM440, RM518, RM528, RM536, RM541, RM551, RM567. |
| 2 | Traditional Basmati and *Indica* rice varieties | RM222, RM224, RM229, RM248, RM252, RM259, RM286, RM400, RM424, RM426, RM439, RM440, RM444, RM525, RM547, RM541, RM551, RM575. |
| 3 | Traditional Basmati and *Japonica* rice varieties | RM213, RM215, RM220, RM224, RM229, RM248, RM252, RM259, RM286, RM424, RM426, RM432, RM439, RM440, RM444, RM450, RM475, RM518, RM526, RM528, RM536, RM541, RM547, RM551, RM580, RM585. |
| 4 | *Indica* and *Japonica* rice varieties | RM213, RM220, RM222, RM224, RM229, RM234, RM242, RM248, RM252, RM259, RM286, RM580, RM439, RM282, RM400, RM424, RM426, RM432, RM440, RM444, RM475, RM525, RM528, RM541, RM547, RM551, RM585. |
| 5 | Between closely related Traditional Basmati rice varieties | RM222, RM224, RM252, RM423, RM426, RM440, RM475, RM518, RM536, RM541, RM547, RM551, RM580, RM582, RM594. |
| 6 | Between *Japonica* rice varieties | RM213, RM215, RM220, RM229, RM248, RM282, RM316, RM400, RM410, RM426, RM440, RM450, RM488, RM567, RM575, RM580, RM585, RM594. |
| 7 | Between *Indica* rice varieties | RM215, RM222, RM224, RM229, RM248, RM252, RM400, RM426, RM440, RM450, RM475, RM488, RM 490, RM541, RM547, RM562, RM575, RM585, RM594, |

**Table 4.5: SSR marker analysis: number of alleles amplified in traditional Basmati, cross-bred Basmati, *japonica*, *indica* and wild rice genotypes.**

| **Sr. No.** | **Marker** | **Number Of Alleles** | **Traditional Basmati (7)** | **Cross-bred (12)** | ***Japonica* (6)** | ***Indica* (15)** | ***Oryza glaberrima* (1)** | ***Oryza barthii* (1)** | ***Oryza rufipogon* (2)** | ***Oryza nivara* (3)** | ***Oryza glumalpatula* (1)** | ***Oryza merdionalis* (1)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RM 213 | 7 | 3 | 2 | 3 | 3 | 1 | 1 | 2 | 2 | 1 | 1 |
| 2 | RM 215 | 9 | 2 | 5 | 3 | 5 | 1 | 1 | 2 | 2 | 1 | 1 |
| 3 | RM 220 | 7 | 2 | 4 | 3 | 4 | 0 | 1 | 2 | 4 | 1 | 1 |
| 4 | RM 222 | 9 | 4 | 6 | 3 | 6 | 1 | 1 | 2 | 3 | 1 | 1 |
| 5 | RM 224 | 8 | 5 | 5 | 2 | 7 | 1 | 1 | 2 | 3 | 1 | 0 |
| 6 | RM 229 | 11 | 2 | 3 | 3 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |
| 7 | RM 234 | 8 | 3 | 3 | 2 | 4 | 1 | 1 | 1 | 2 | 1 | 1 |
| 8 | RM 242 | 5 | 3 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 0 | 0 |
| 9 | RM 248 | 10 | 3 | 4 | 4 | 5 | 1 | 0 | 2 | 2 | 1 | 1 |
| 10 | RM 252 | 16 | 4 | 8 | 2 | 8 | 1 | 1 | 2 | 2 | 0 | 0 |
| 11 | RM 255 | 8 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 3 | 1 | 1 |
| 12 | RM 259 | 10 | 2 | 3 | 2 | 3 | 1 | 1 | 1 | 3 | 1 | 0 |
| 13 | RM 282 | 3 | 2 | 3 | 2 | 3 | 1 | 1 | 0 | 1 | 1 | 1 |
| 14 | RM 286 | 8 | 2 | 4 | 3 | 4 | 1 | 1 | 2 | 3 | 1 | 1 |
| 15 | RM 316 | 6 | 2 | 3 | 3 | 3 | 1 | 1 | 2 | 2 | 1 | 1 |
| 16 | RM 321 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 17 | RM 323 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 |
| 18 | RM 400 | 9 | 2 | 5 | 4 | 6 | 1 | 1 | 2 | 3 | 1 | 1 |
| 19 | RM 410 | 3 | 2 | 2 | 3 | 3 | 1 | 1 | 2 | 2 | 1 | 1 |
| 20 | RM 422 | 5 | 1 | 3 | 2 | 4 | 0 | 0 | 1 | 3 | 1 | 1 |
| 21 | RM 423 | 5 | 2 | 3 | 2 | 4 | 1 | 0 | 1 | 1 | 1 | 1 |
| 22 | RM 424 | 5 | 2 | 5 | 1 | 4 | 1 | 0 | 1 | 2 | 0 | 0 |
| 23 | RM 426 | 11 | 4 | 5 | 3 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | RM 432 | 4 | 2 | 3 | 2 | 4 | 1 | 1 | 1 | 3 | 1 | 1 |
| 25 | RM 439 | 6 | 1 | 4 | 3 | 3 | 1 | 1 | 0 | 3 | 1 | 1 |
| 26 | RM 440 | 8 | 3 | 5 | 3 | 6 | 1 | 1 | 2 | 3 | 1 | 1 |
| 27 | RM 444 | 6 | 2 | 2 | 2 | 5 | 1 | 2 | 2 | 2 | 1 | 1 |
| 28 | RM 450 | 7 | 2 | 3 | 3 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |
| 29 | RM 472 | 4 | 3 | 2 | 2 | 4 | 1 | 1 | 1 | 3 | 1 | 1 |
| 30 | RM 475 | 6 | 3 | 4 | 2 | 5 | 1 | 1 | 2 | 2 | 1 | 1 |
| 31 | RM 488 | 8 | 4 | 5 | 3 | 6 | 1 | 1 | 2 | 2 | 1 | 1 |
| 32 | RM 490 | 5 | 3 | 4 | 2 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |
| 33 | RM 500 | 3 | 3 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 1 | 1 |
| 34 | RM 511 | 4 | 2 | 3 | 2 | 4 | 1 | 1 | 2 | 2 | 1 | 1 |
| 35 | RM 518 | 8 | 3 | 4 | 2 | 2 | 1 | 1 | 2 | 3 | 1 | 1 |
| 36 | RM 525 | 7 | 3 | 3 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 1 |
| 37 | RM 526 | 5 | 3 | 3 | 2 | 2 | 0 | 1 | 1 | 2 | 1 | 1 |
| 38 | RM 527 | 4 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 1 | 1 |
| 39 | RM 528 | 5 | 3 | 3 | 2 | 3 | 0 | 1 | 2 | 2 | 1 | 1 |
| 40 | RM 536 | 6 | 3 | 3 | 2 | 3 | 1 | 1 | 0 | 2 | 2 | 2 |
| 41 | RM 541 | 10 | 4 | 6 | 2 | 6 | 1 | 1 | 1 | 2 | 1 | 1 |
| 42 | RM 547 | 13 | 4 | 8 | 3 | 7 | 1 | 1 | 2 | 2 | 1 | 0 |
| 43 | RM 551 | 5 | 4 | 3 | 2 | 4 | 0 | 1 | 2 | 2 | 1 | 1 |
| 44 | RM 562 | 7 | 4 | 6 | 2 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |
| 45 | RM 567 | 4 | 3 | 3 | 3 | 4 | 1 | 1 | 2 | 3 | 1 | 1 |
| 46 | RM 575 | 6 | 3 | 3 | 3 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |
| 47 | RM580 | 11 | 3 | 2 | 3 | 4 | 1 | 1 | 2 | 2 | 1 | 1 |
| 48 | RM 582 | 9 | 3 | 4 | 2 | 3 | 2 | 1 | 2 | 3 | 1 | 1 |
| 49 | RM 585 | 7 | 3 | 3 | 3 | 5 | 1 | 1 | 2 | 2 | 1 | 1 |
| 50 | RM 594 | 6 | 4 | 3 | 3 | 5 | 1 | 1 | 2 | 3 | 1 | 1 |

**Table 4.6: TE fingerprint database of 50 rice genotypes.**

| Marker | Nippon | T309 | Azu | TNG67 | NPT2 | NPT3 | 9311 | TypeIII | B370 | RanBas | Bas217 | Bas385 | HBC19 | KLocal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DAS A 3** | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 0 | 220 | 220 | 220 | 220 | 220 | 220 |
| **DAS A 7** | 210 | 210 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 410 | 0 | 410 |
| **DAS C5** | 430 | 230 | 430 | 0 | 230 | 230 | 230 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |
| **DAS C7** | 230 | 230 | 430 | 230 | 430 | 430 | 230 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |
| **DAS C9** | 240 | 0 | 440 | 0 | 240 | 0 | 240 | 440 | 440 | 0 | 0 | 440 | 0 | 440 |
| **Das C11** | 210 | 210 | 410 | 0 | 210 | 0 | 410 | 0 | 410 | 0 | 410 | 410 | 0 | 410 |
| **DAS D7** | 200 | 200 | 400 | 200 | 200 | 200 | 200 | 0 | 400 | 400 | 400 | 400 | 400 | 400 |
| **DAS D9** | 210 | 410 | 410 | 0 | 410 | 0 | 0 | 410 | 410 | 410 | 410 | 410 | 410 | 410 |
| **DAS D11** | 250 | 250 | 450 | 0 | 450 | 0 | 250 | 450 | 450 | 450 | 450 | 0 | 0 | 0 |
| **DAS E3** | 210 | 210 | 210 | 0 | 210 | 0 | 210 | 210 | 210 | 210 | 410 | 210 | 210 | 410 |
| **DasE11** | 220 | 220 | 420 | 220 | 0 | 420 | 220 | 420 | 420 | 220 | 420 | 420 | 420 | 420 |
| **DAS F1** | 240 | 240 | 240 | 440 | 440 | 240 | 240 | 240 | 240 | 240 | 440 | 240 | 240 | 440 |
| **DAS F3** | 210 | 210 | 410 | 210 | 210 | 210 | 210 | 410 | 410 | 210 | 0 | 410 | 410 | 410 |
| **DAS G5** | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 430 | 230 | 230 | 430 |
| **DAS G 7** | 430 | 430 | 430 | 430 | 0 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 230 |
| **DAS G11** | 240 | 240 | 440 | 240 | 440 | 440 | 240 | 440 | 440 | 440 | 440 | 440 | 440 | 440 |
| **9311-1** | 240 | 240 | 530 | 240 | 240 | 240 | 530 | 0 | 240 | 530 | 240 | 240 | 240 | 530 |
| **9311-3** | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 |
| **9311-4** | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 600 | 200 | 200 | 200 |
| **9311-5** | 230 | 230 | 640 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 640 |
| **9311-6** | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 0 | 230 | 230 | 630 |
| **mPing-3-1** | 650 | 650 | 250 | 650 | 250 | 0 | 650 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| **mPing3-2** | 490 | 490 | 210 | 490 | 210 | 210 | 490 | 210 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-3** | 510 | 510 | 210 | 510 | 210 | 0 | 510 | 210 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-4** | 650 | 250 | 250 | 250 | 250 | 250 | 650 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **mPing6-1** | 400 | 400 | 200 | 400 | 200 | 200 | 400 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| **mPing7-1** | 425 | 425 | 225 | 425 | 225 | 225 | 425 | 225 | 225 | 225 | 225 | 225 | 225 | 225 |
| **mPing8-2** | 220 | 0 | 220 | 510 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 |
| **mPing12-1** | 260 | 660 | 260 | 660 | 260 | 260 | 660 | 260 | 260 | 260 | 260 | 260 | 260 | 260 |
| **mPingU-1** | 250 | 250 | 450 | 250 | 250 | 250 | 450 | 0 | 450 | 250 | 250 | 450 | 250 | 250 |

Contd…

| Marker | CSR30 | Kasturi | Super | PB1 | PS2 | PS3 | PS4 | HKR228 | ImpShar | Sabarm | Mahi | 93-401 | CSR1 | CSR10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DAS A 3** | 220 | 220 | 220 | 220 | 220 | 220 | 420 | 0 | 0 | 220 | 0 | 220 | 220 | 220 |
| **DAS A 7** | 410 | 0 | 410 | 0 | 410 | 0 | 0 | 0 | 410 | 210 | 410 | 0 | 0 | 0 |
| **DAS C5** | 430 | 430 | 430 | 430 | 430 | 0 | 430 | 0 | 0 | 0 | 0 | 0 | 230 | 430 |
| **DAS C7** | 430 | 430 | 430 | 430 | 0 | 430 | 430 | 0 | 430 | 430 | 430 | 430 | 430 | 430 |
| **DAS C9** | 440 | 0 | 440 | 440 | 0 | 440 | 440 | 0 | 0 | 440 | 0 | 0 | 240 | 0 |
| **Das C11** | 210 | 0 | 410 | 0 | 0 | 410 | 0 | 210 | 0 | 410 | 410 | 410 | 0 | 0 |
| **DAS D7** | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 0 | 200 | 400 |
| **DAS D9** | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 0 | 0 | 410 | 410 | 410 | 410 | 410 |
| **DAS D11** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DAS E3** | 210 | 410 | 410 | 210 | 210 | 210 | 410 | 210 | 210 | 210 | 210 | 210 | 210 | 410 |
| **DasE11** | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 |
| **DAS F1** | 240 | 240 | 240 | 240 | 0 | 440 | 240 | 240 | 240 | 440 | 240 | 240 | 240 | 240 |
| **DAS F3** | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 210 | 210 |
| **DAS G5** | 230 | 230 | 230 | 230 | 430 | 430 | 430 | 230 | 430 | 230 | 430 | 430 | 230 | 430 |
| **DAS G 7** | 0 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 0 | 430 | 0 | 430 | 430 | 0 |
| **DAS G11** | 440 | 440 | 440 | 440 | 440 | 440 | 440 | 440 | 440 | 440 | 440 | 240 | 440 | 440 |
| **9311-1** | 240 | 240 | 240 | 240 | 240 | 240 | 240 | 240 | 240 | 0 | 240 | 240 | 240 | 240 |
| **9311-3** | 220 | 220 | 220 | 620 | 0 | 620 | 220 | 220 | 0 | 620 | 620 | 620 | 620 | 620 |
| **9311-4** | 200 | 200 | 200 | 200 | 600 | 600 | 600 | 200 | 200 | 200 | 600 | 600 | 600 | 600 |
| **9311-5** | 230 | 230 | 230 | 230 | 0 | 0 | 230 | 230 | 0 | 0 | 640 | 640 | 640 | 640 |
| **9311-6** | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 630 | 230 | 230 | 630 | 630 |
| **mPing-3-1** | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 0 | 250 | 250 | 250 | 250 | 250 | 250 |
| **mPing3-2** | 210 | 210 | 210 | 210 | 0 | 210 | 210 | 0 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-3** | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **mPing6-1** | 200 | 0 | 400 | 0 | 200 | 200 | 200 | 200 | 0 | 400 | 200 | 200 | 200 | 400 |
| **mPing7-1** | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 |
| **mPing8-2** | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 |
| **mPing12-1** | 260 | 260 | 260 | 260 | 0 | 260 | 0 | 260 | 260 | 260 | 260 | 260 | 260 | 260 |
| **mPingU-1** | 450 | 250 | 450 | 250 | 0 | 250 | 250 | 0 | 250 | 250 | 250 | 250 | 250 | 250 |

Contd…

| Marker | CSR21 | HKR120 | IR24 | IR36 | IR64 | IR72 | Pokkali | PR106 | PR118 | Sharbati | MI48 | KR1-24 | BR4-10 | Obart |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DAS A 3** | 0 | 220 | 220 | 220 | 220 | 0 | 220 | 220 | 220 | 0 | 220 | 420 | 220 | 420 |
| **DAS A 7** | 0 | 0 | 0 | 410 | 410 | 0 | 0 | 410 | 410 | 0 | 0 | 0 | 410 | 0 |
| **DAS C5** | 0 | 0 | 430 | 430 | 430 | 0 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |
| **DAS C7** | 0 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 0 |
| **DAS C9** | 440 | 0 | 440 | 440 | 440 | 0 | 440 | 440 | 440 | 0 | 440 | 440 | 0 | 440 |
| **Das C11** | 0 | 0 | 0 | 0 | 0 | 410 | 0 | 410 | 410 | 0 | 410 | 410 | 0 | 0 |
| **DAS D7** | 0 | 0 | 0 | 400 | 400 | 0 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 200 |
| **DAS D9** | 410 | 410 | 410 | 0 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 410 | 410 | 210 |
| **DAS D11** | 0 | 0 | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 0 |
| **DAS E3** | 0 | 410 | 410 | 410 | 410 | 0 | 410 | 410 | 410 | 410 | 410 | 410 | 0 | 410 |
| **DasE11** | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 420 | 0 | 420 | 420 | 420 | 420 | 0 |
| **DAS F1** | 440 | 0 | 440 | 0 | 440 | 440 | 440 | 240 | 440 | 240 | 440 | 440 | 0 | 440 |
| **DAS F3** | 410 | 0 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 410 | 0 | 0 | 0 |
| **DAS G5** | 430 | 430 | 430 | 430 | 430 | 430 | 0 | 430 | 430 | 430 | 430 | 430 | 230 | 430 |
| **DAS G 7** | 430 | 430 | 0 | 430 | 430 | 430 | 430 | 230 | 430 | 230 | 0 | 0 | 0 | 430 |
| **DAS G11** | 240 | 240 | 440 | 440 | 240 | 440 | 0 | 440 | 440 | 440 | 440 | 440 | 0 | 240 |
| **9311-1** | 240 | 240 | 530 | 240 | 0 | 240 | 240 | 0 | 240 | 240 | 240 | 0 | 240 | 240 |
| **9311-3** | 0 | 220 | 220 | 220 | 220 | 620 | 620 | 620 | 620 | 620 | 620 | 220 | 220 | 0 |
| **9311-4** | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 200 | 0 | 200 | 0 | 200 | 200 | 200 |
| **9311-5** | 0 | 640 | 640 | 230 | 640 | 230 | 640 | 640 | 640 | 640 | 640 | 0 | 640 | 0 |
| **9311-6** | 0 | 630 | 630 | 630 | 630 | 630 | 630 | 630 | 630 | 630 | 630 | 230 | 230 | 230 |
| **mPing-3-1** | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 0 |
| **mPing3-2** | 0 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-3** | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 650 |
| **mPing6-1** | 200 | 400 | 200 | 200 | 400 | 200 | 200 | 200 | 400 | 200 | 200 | 200 | 200 | 0 |
| **mPing7-1** | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 225 | 0 |
| **mPing8-2** | 220 | 220 | 220 | 220 | 510 | 220 | 220 | 220 | 510 | 220 | 220 | 220 | 0 | 220 |
| **mPing12-1** | 0 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 |
| **mPingU-1** | 0 | 250 | 250 | 250 | 250 | 250 | 250 | 450 | 450 | 250 | 250 | 250 | 250 | 0 |

Contd…

| Marker | Oglab | Oglumal | Omerdio | Oniv1 | Oniv2 | Oniv3 | Orufi1 | Orufi2 |
|---|---|---|---|---|---|---|---|---|
| **DAS A 3** | 420 | 0 | 420 | 420 | 420 | 420 | 0 | 420 |
| **DAS A 7** | 0 | 0 | 410 | 0 | 0 | 410 | 410 | 410 |
| **DAS C5** | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |
| **DAS C7** | 0 | 0 | 0 | 0 | 230 | 0 | 230 | 0 |
| **DAS C9** | 440 | 0 | 440 | 440 | 0 | 240 | 0 | 0 |
| **Das C11** | 0 | 410 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DAS D7** | 200 | 200 | 0 | 200 | 0 | 0 | 0 | 0 |
| **DAS D9** | 210 | 210 | 0 | 210 | 210 | 0 | 210 | 0 |
| **DAS D11** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DAS E3** | 410 | 410 | 410 | 410 | 410 | 0 | 0 | 0 |
| **DasE11** | 420 | 420 | 420 | 420 | 420 | 0 | 0 | 0 |
| **DAS F1** | 440 | 440 | 0 | 440 | 0 | 0 | 0 | 0 |
| **DAS F3** | 410 | 410 | 410 | 0 | 210 | 410 | 210 | 410 |
| **DAS G5** | 430 | 430 | 430 | 430 | 430 | 230 | 430 | 430 |
| **DAS G 7** | 0 | 0 | 0 | 0 | 430 | 230 | 0 | 0 |
| **DAS G11** | 240 | 0 | 440 | 0 | 240 | 240 | 240 | 0 |
| **9311-1** | 530 | 240 | 530 | 240 | 240 | 240 | 240 | 240 |
| **9311-3** | 0 | 220 | 0 | 220 | 220 | 220 | 0 | 220 |
| **9311-4** | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| **9311-5** | 0 | 230 | 230 | 230 | 640 | 230 | 0 | 230 |
| **9311-6** | 230 | 230 | 230 | 230 | 230 | 230 | 230 | 230 |
| **mPing-3-1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **mPing3-2** | 0 | 210 | 0 | 210 | 210 | 210 | 210 | 210 |
| **mPing3-3** | 210 | 210 | 210 | 210 | 210 | 0 | 210 | 210 |
| **mPing3-4** | 650 | 650 | 650 | 650 | 650 | 650 | 650 | 650 |
| **mPing6-1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **mPing7-1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **mPing8-2** | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 |
| **mPing12-1** | 0 | 260 | 260 | 260 | 260 | 260 | 260 | 260 |
| **mPingU-1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4.7: Data on percentage of TE inserts, no insert and null alleles in *japonica*, traditional Basmati, *indica*, cross-bred Basmati and wild rice genotypes.**

| **Sr. No.** | **Rice group** | **Dasheng elements** | | | **Nippon mPing elements** | | | **9311 mPing elements** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Insert (%)** | **No insert (%)** | **Null allele (%)** | **Insert (%)** | **No insert (%)** | **Null allele (%)** | **Insert (%)** | **No insert (%)** | **Null allele (%)** |
| 1 | *Japonica* rice group | 54.16 | 28.14 | 17.70 | 37.03 | 57.42 | 5.55 | 6.66 | 94.33 | - |
| 2 | Traditional Basmati rice group | 21.42 | 66.98 | 11.60 | 3.17 | 84.14 | 12.69 | 14.29 | 80 | 5.71 |
| 3 | *Indica* rice group | 17.91 | 60.18 | 21.91 | 2.96 | 82.97 | 14.07 | 44 | 40.66 | 9.33 |
| 4 | Cross-bred Basmati rice group | 23.95 | 57.3 | 18.75 | 1.85 | 76.86 | 21.29 | 21.66 | 66.66 | 11.66 |
| 5 | cv. 9311 (*indica-japonica*) | 75 | 12.50 | 12.50 | 88 | 12 | - | 20 | 80 | - |
| 6 | Wild rice group | 13.88 | 41.68 | 44.44 | 44.38 | 44.51 | 11.11 | 6.66 | 77.79 | 15.55 |

**Table 4.8: TE insert Dasheng primers in *japonica*, traditional Basmati, *indica*, cross-bred Basmati, 9311 and wild rice genotypes.**

| Sr. No. | Marker | # of *japonica* rice genotypes with | | | # of Traditional Basmati rice genotypes with | | | # *Indica* rice genotypes with | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TE insert | No insert | Null allele | TE insert | No insert | Null allele | TE insert | No insert | Null allele |
| 1 | DashengA3 | 6 | 0 | 0 | 6 | 0 | 1 | 11 | 1 | 3 |
| 2 | DashengA 7 | 3 | 0 | 3 | 0 | 2 | 5 | 0 | 5 | 10 |
| 3 | DashengC5 | 3 | 2 | 1 | 0 | 7 | 0 | 1 | 10 | 4 |
| 4 | DashengC7 | 3 | 3 | 0 | 7 | 0 | 0 | 14 | 0 | 1 |
| 5 | DashengC9 | 2 | 1 | 3 | 0 | 4 | 3 | 1 | 9 | 5 |
| 6 | DashengC11 | 3 | 1 | 2 | 0 | 4 | 3 | 0 | 5 | 10 |
| 7 | Dasheng D7 | 5 | 1 | 0 | 0 | 6 | 1 | 1 | 10 | 4 |
| 8 | Dasheng D9 | 1 | 3 | 2 | 0 | 7 | 0 | 1 | 14 | 0 |
| 9 | DashengD11 | 2 | 2 | 2 | 0 | 7 | 0 | 0 | 14 | 1 |
| 10 | Dasheng E3 | 4 | 0 | 2 | 5 | 2 | 0 | 1 | 11 | 3 |
| 11 | Dasheng 11 | 3 | 2 | 1 | 1 | 6 | 0 | 0 | 15 | 0 |
| 12 | Dasheng F1 | 4 | 2 | 0 | 5 | 2 | 0 | 4 | 8 | 3 |
| 13 | Dasheng F3 | 5 | 1 | 0 | 1 | 5 | 1 | 2 | 10 | 3 |
| 14 | Dasheng G5 | 6 | 0 | 0 | 5 | 2 | 0 | 2 | 12 | 1 |
| 15 | Dasheng G7 | 0 | 5 | 1 | 1 | 6 | 0 | 2 | 8 | 5 |
| 16 | DashengG11 | 3 | 3 | 0 | 0 | 7 | 0 | 3 | 10 | 2 |
| | Mean | 3.31 | 1.62 | 1.06 | 1.93 | 4.18 | 0.87 | 2.68 | 8.87 | 3.43 |

| Sr. No. | Marker | # of Cross-bred Basmati rice genotypes with | | | 9311 rice genotype | | | # of Wild rice genotypes with | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TE insert | No insert | Null allele | TE insert | No insert | Null allele | TE insert | No insert | Null allele |
| 1 | DashengA3 | 8 | 1 | 3 | 1 | 0 | 0 | 0 | 7 | 2 |
| 2 | DashengA 7 | 1 | 5 | 6 | 0 | 0 | 1 | 0 | 4 | 5 |
| 3 | DashengC5 | 0 | 6 | 6 | 1 | 0 | 0 | 0 | 9 | 0 |
| 4 | DashengC7 | 9 | 1 | 2 | 1 | 0 | 0 | 0 | 4 | 5 |
| 5 | DashengC9 | 0 | 6 | 6 | 1 | 0 | 0 | 1 | 4 | 4 |
| 6 | DashengC11 | 2 | 5 | 5 | 0 | 1 | 0 | 0 | 1 | 8 |
| 7 | Dasheng D7 | 0 | 11 | 1 | 1 | 0 | 0 | 4 | 0 | 5 |
| 8 | Dasheng D9 | 0 | 10 | 2 | 0 | 0 | 1 | 6 | 0 | 3 |
| 9 | DashengD11 | 0 | 11 | 1 | 1 | 0 | 0 | 0 | 0 | 9 |
| 10 | Dasheng E3 | 9 | 3 | 0 | 1 | 0 | 0 | 0 | 6 | 3 |
| 11 | Dasheng 11 | 0 | 12 | 0 | 1 | 0 | 0 | 0 | 5 | 4 |
| 12 | Dasheng F1 | 9 | 2 | 1 | 1 | 0 | 0 | 0 | 4 | 5 |
| 13 | Dasheng F3 | 0 | 12 | 0 | 1 | 0 | 0 | 1 | 2 | 6 |
| 14 | Dasheng G5 | 6 | 6 | 0 | 1 | 0 | 0 | 1 | 8 | 0 |
| 15 | Dasheng G7 | 0 | 9 | 3 | 0 | 1 | 0 | 1 | 2 | 6 |
| 16 | DashengG11 | 1 | 11 | 0 | 1 | 0 | 0 | 5 | 1 | 3 |
| | Mean | 2.81 | 6.93 | 2.25 | 0.75 | 0.12 | 0.12 | 1.18 | 3.56 | 4.25 |

**Table 4.9: TE insert mPing and 9311 primers in *japonica*, traditional Basmati, *indica*, cross-bred Basmati, 9311 and wild rice genotypes.**

| Sr. No. | Marker | # of *japonica* rice genotypes with | | | # of Traditional Basmati rice genotypes with | | | # *Indica* rice genotypes with | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TE insert | No insert | Null allele | TE insert | No insert | Null allele | TE insert | No insert | Null allele |
| 1 | 9311 mPing-1 | 1 | 5 | 0 | 2 | 4 | 1 | 1 | 11 | 3 |
| 2 | 9311 mPing-3 | 0 | 6 | 0 | 0 | 7 | 0 | 8 | 6 | 1 |
| 3 | 9311 mPing-4 | 0 | 6 | 0 | 1 | 6 | 0 | 10 | 5 | 0 |
| 4 | 9311 mPing-5 | 1 | 5 | 0 | 1 | 6 | 0 | 11 | 2 | 2 |
| 5 | 9311 mPing-6 | 0 | 6 | 0 | 1 | 5 | 1 | 12 | 2 | 1 |
| 6 | mPing3-1 | 3 | 2 | 1 | 0 | 7 | 0 | 0 | 15 | 0 |
| 7 | mPing3-2 | 3 | 3 | 0 | 0 | 7 | 0 | 0 | 14 | 1 |
| 8 | mPing3-3 | 3 | 2 | 1 | 0 | 7 | 0 | 0 | 15 | 0 |
| 9 | mPing3-4 | 1 | 5 | 0 | 0 | 0 | 7 | 0 | 0 | 15 |
| 10 | mPing6-1 | 3 | 3 | 0 | 0 | 7 | 0 | 4 | 11 | 0 |
| 11 | mPing7-1 | 3 | 3 | 0 | 0 | 7 | 0 | 0 | 15 | 0 |
| 12 | mPing8-2 | 1 | 5 | 1 | 0 | 7 | 0 | 2 | 13 | 1 |
| 13 | mPing12-1 | 2 | 4 | 0 | 0 | 7 | 0 | 0 | 14 | 1 |
| 14 | mPingU-1 | 1 | 5 | 0 | 2 | 4 | 1 | 2 | 12 | 1 |
| | Mean | 1.57 | 4.28 | 0.21 | 0.50 | 5.78 | 0.71 | 3.57 | 9.64 | 1.85 |

| Sr. No. | Marker | # of Cross-bred Basmati rice genotypes with | | | 9311 rice genotype with | | | # of Wild rice genotypes with | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TE insert | No insert | Null allele | TE insert | No insert | Null allele | TE insert | No insert | Null allele |
| 1 | 9311 mPing-1 | 0 | 11 | 1 | 1 | 0 | 0 | 2 | 7 | 0 |
| 2 | 9311 mPing-3 | 5 | 5 | 2 | 0 | 1 | 0 | 0 | 5 | 4 |
| 3 | 9311 mPing-4 | 5 | 7 | 0 | 0 | 1 | 0 | 0 | 9 | 0 |
| 4 | 9311 mPing-5 | 2 | 6 | 4 | 0 | 1 | 0 | 1 | 5 | 3 |
| 5 | 9311 mPing-6 | 1 | 11 | 0 | 0 | 1 | 0 | 0 | 9 | 0 |
| 6 | mPing3-1 | 0 | 11 | 1 | 1 | 0 | 0 | 0 | 0 | 9 |
| 7 | mPing3-2 | 0 | 10 | 2 | 1 | 0 | 0 | 0 | 7 | 2 |
| 8 | mPing3-3 | 0 | 12 | 0 | 1 | 0 | 0 | 0 | 8 | 1 |
| 9 | mPing3-4 | 0 | 0 | 12 | 1 | 0 | 0 | 9 | 0 | 0 |
| 10 | mPing6-1 | 2 | 7 | 3 | 1 | 0 | 0 | 0 | 0 | 9 |
| 11 | mPing7-1 | 0 | 12 | 0 | 1 | 0 | 0 | 0 | 0 | 9 |
| 12 | mPing8-2 | 0 | 11 | 1 | 0 | 1 | 0 | 0 | 9 | 0 |
| 13 | mPing12-1 | 0 | 10 | 2 | 1 | 0 | 0 | 0 | 8 | 1 |
| 14 | mPingU-1 | 2 | 8 | 2 | 1 | 0 | 0 | 0 | 0 | 9 |
| | Mean | 1.21 | 8.64 | 2.14 | 0.64 | 0.35 | 0 | 0.85 | 4.78 | 3.35 |

**Table 4.10: Data on percentage of TE inserts, no insert and null alleles in traditional Basmati rice genotypes.**

| Sr. No. | Marker type | Type III | | | Ranbir Basmati | | | Karnal Local | | | Basmati 370 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) |
| 1 | Dasheng elements | 25 | 50 | 25 | 43.75 | 37.50 | 18.75 | 18.75 | 81.25 | 0 | 31.25 | 62.50 | 6.25 |
| 2 | Nippon mPing elements | 0 | 77.77 | 22.22 | 0 | 88.88 | 11.11 | 0 | 88.88 | 11.11 | 11.11 | 77.77 | 11.11 |
| 3 | 9311 mPing elements | 0 | 80 | 20 | 20 | 80 | 0 | 60 | 40 | 0 | 0 | 100 | 0 |

| Sr. No. | Marker type | Basmati 217 | | | Basmati385 | | | HBC 19 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) |
| 1 | **Dasheng elements** | 12.50 | 68.75 | 18.75 | 31.25 | 68.75 | 0 | 31.25 | 50 | 18.75 |
| 2 | **Nippon mPing elements** | 0 | 88.88 | 11.11 | 77.77 | 11.11 | 0 | 0 | 88.88 | 11.11 |
| 3 | **9311 mPing elements** | 20 | 60 | 20 | 0 | 100 | 0 | 0 | 100 | 0 |

**Table 4.11: Status of Dasheng Elements in traditional Basmati rice genotypes.**

| **Sr. No.** | **Marker** | **Type III** | | | **Ranbir Basmati** | | | **Karnal Local** | | | **Basmati 370** | | | **Basmati 217** | | | **Basmati 385** | | | **HBC 19** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** |
| 1 | Dasheng A 3 | | | + | + | | | + | | | + | | | + | | | + | | | + | | |
| 2 | Dasheng A 7 | | | + | | | + | | + | | | | + | | | + | | + | | | | + |
| 3 | Dasheng C5 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 4 | Dasheng C7 | + | | | + | | | + | | | + | | | + | | | + | | | + | | |
| 5 | Dasheng C9 | | + | | | | + | | + | | | + | | | | + | | + | | | | + |
| 6 | Dasheng C11 | | | + | | | + | | + | | | + | | | + | | | + | | | | + |
| 7 | Dasheng D7 | | | + | | + | | | + | | | + | | | + | | | + | | | + | |
| 8 | Dasheng D9 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 9 | Dasheng D11 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 10 | Dasheng E3 | + | | | + | | | | + | | + | | | | + | | + | | | + | | |
| 11 | Dasheng E11 | | | + | + | | | | + | | | + | | | + | | | + | | | + | |
| 12 | Dasheng F1 | + | | | + | | | | + | | + | | | | + | | + | | | + | | |
| 13 | Dasheng F3 | | + | | + | | | | + | | | + | | | | + | | + | | | + | |
| 14 | Dasheng G5 | + | | | + | | | | + | | + | | | | + | | + | | | + | | |
| 15 | Dasheng G 7 | | + | | | + | | + | | | | + | | | + | | | + | | | + | |
| 16 | Dasheng G11 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |

**A- Insert**
**B-No insert**
**C-Null allele**

**Table 4.12: Status of Nippon mPing and 9311 mPing Elements in traditional Basmati rice genotypes.**

| Sr. No. | Marker | Type III | | | Ranbir Basmati | | | Karnal Local | | | Basmati 370 | | | Basmati 217 | | | Basmati 385 | | | HBC 19 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** |
| 1 | 9311 mPing-1 | | | + | + | | | + | | | | + | | | + | | | + | | | + | |
| 2 | 9311 mPing-3 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 3 | 9311 mPing-4 | | + | | | + | | | + | | | + | | + | | | | + | | | + | |
| 4 | 9311 mPing-5 | | + | | | + | | + | | | | + | | | + | | | + | | | + | |
| 5 | 9311 mPing-6 | | + | | | + | | + | | | | + | | | | + | | + | | | + | |
| 6 | mPing3-1 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 7 | mPing3-2 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 8 | mPing3-3 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 9 | mPing3-4 | | | + | | | + | | | + | | | + | | | + | | | + | | | + |
| 10 | mPing6-1 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 11 | mPing7-1 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 12 | mPing8-2 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 13 | mPing12-1 | | + | | | + | | | + | | | + | | | + | | | + | | | + | |
| 14 | mPingU-1 | | | + | | | + | | + | | | + | | | + | | + | | | | + | |

**A- Insert**
**B-No insert**
**C-Null allele**

**Table 4.13: Data on percentage of TE inserts, no insert and null alleles in *Japonica* rice genotypes.**

| Sr. No. | Marker type | Taipei 309 | | | TNG 67 | | | Nipponbare | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) |
| 1 | Dasheng elements | 75 | 18.75 | 6.25 | 37.5 | 18.75 | 43.75 | 81.25 | 18.75 | 0 |
| 2 | Nippon mPing elements | 66.66 | 22.22 | 11.11 | 88.88 | 11.11 | 0 | 66.66 | 33.33 | 0 |
| 3 | 9311 mPing elements | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 |

| Sr. No. | Marker type | NPT II | | | NPT III | | | Azucena | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) | Insert (%) | No insert (%) | Null allele (%) |
| 1 | Dasheng elements | 56.25 | 31.25 | 12.5 | 43.75 | 12.5 | 43.75 | 31.25 | 62.5 | 6.25 |
| 2 | Nippon mPing elements | 0 | 100 | 0 | 0 | 100 | 0 | 11.11 | 88.88 | 0 |
| 3 | 9311 mPing elements | 0 | 100 | 0 | 0 | 100 | 0 | 40 | 60 | 0 |

**Table 4.14: Status of Dasheng Elements in *Japonica* rice genotypes.**

| Sr. No. | Marker | Taipei 309 | | | TNG 67 | | | Nipponbare | | | NPT II | | | NPT III | | | Azucena | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** |
| 1 | Dasheng A 3 | + | | | + | | | + | | | + | | | + | | | + | | |
| 2 | Dasheng A 7 | + | | | | | + | + | | | | | + | | | + | + | | |
| 3 | Dasheng C5 | + | | | | | + | | + | | + | | | + | | | | + | |
| 4 | Dasheng C7 | | + | | | + | | | + | | + | | | + | | | + | | |
| 5 | Dasheng C9 | | | + | | | + | + | | | + | | | | | + | | + | |
| 6 | Dasheng C11 | + | | | | | + | + | | | + | | | | | + | | + | |
| 7 | Dasheng D7 | + | | | + | | | + | | | + | | | + | | | | + | |
| 8 | Dasheng D9 | | + | | | | + | + | | | | + | | | | + | | + | |
| 9 | Dasheng D11 | + | | | | | + | + | | | | + | | | | + | | + | |
| 10 | Dasheng E3 | + | | | | | + | + | | | + | | | | | + | + | | |
| 11 | Dasheng E11 | + | | | + | | | + | | | | | + | | + | | | + | |
| 12 | Dasheng F1 | + | | | | + | | + | | | | + | | + | | | | | + |
| 13 | Dasheng F3 | + | | | + | | | + | | | + | | | + | | | | + | |
| 14 | Dasheng G5 | + | | | + | | | + | | | + | | | + | | | + | | |
| 15 | Dasheng G 7 | | + | | | + | | | + | | | + | | | | + | | + | |
| 16 | Dasheng G11 | + | | | + | | | + | | | | + | | | + | | | + | |

**A- Insert**
**B-No insert**
**C-Null allele**

**Table 4.15: Status of Nippon mPing and 9311 mPing Elements in *Japonica* rice genotypes.**

| Sr. No. | Marker | Taipei 309 | | | TNG 67 | | | Nipponbare | | | NPT II | | | NPT III | | | Azucena | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C |
| 1 | 9311 mPing-1 | | + | | | + | | | + | | | + | | | + | | + | | |
| 2 | 9311 mPing-3 | | + | | | + | | | + | | | + | | | + | | | + | |
| 3 | 9311 mPing-4 | | + | | | + | | | + | | | + | | | + | | | + | |
| 4 | 9311 mPing-5 | | + | | | + | | | + | | | + | | | + | | + | | |
| 5 | 9311 mPing-6 | | + | | | + | | | + | | | + | | | + | | | + | |
| 6 | mPing3-1 | + | | | + | | | + | | | | + | | | | + | | + | |
| 7 | mPing3-2 | + | | | + | | | + | | | | + | | | + | | | + | |
| 8 | mPing3-3 | + | | | + | | | + | | | | + | | | | + | | + | |
| 9 | mPing3-4 | | + | | | + | | + | | | | + | | | + | | | + | |
| 10 | mPing6-1 | + | | | + | | | + | | | | + | | | + | | | + | |
| 11 | mPing7-1 | + | | | + | | | + | | | | + | | | + | | | + | |
| 12 | mPing8-2 | | | + | + | | | | + | | | + | | | + | | | + | |
| 13 | mPing12-1 | + | | | + | | | | + | | | + | | | + | | | + | |
| 14 | mPingU-1 | | + | | + | | | | + | | | + | | | + | | + | | |

**A- Insert**
**B-No insert**
**C-Null allele**

**Table 4.16: PIC values for Dasheng elements, Nippon mPing elements and 9311 mPing elements.**

| Sr. No. | Marker | PIC |
|---|---|---|
| 1 | Dasheng A3 | 0.2839 |
| 2 | Dasheng A7 | 0.2688 |
| 3 | Dasheng C5 | 0.1986 |
| 4 | Dasheng C9 | 0.2447 |
| 5 | Dasheng C7 | 0.2608 |
| 6 | Dasheng C11 | 0.2896 |
| 7 | Dasheng D7 | 0.3230 |
| 8 | Dasheng D9 | 0.2608 |
| 9 | Dasheng D11 | 0.1379 |
| 10 | Dasheng E3 | 0.3744 |
| 11 | Dasheng E11 | 0.1780 |
| 12 | Dasheng F1 | 0.3713 |
| 13 | Dasheng F3 | 0.3047 |
| 14 | Dasheng G5 | 0.3698 |
| 15 | Dasheng G 7 | 0.1820 |
| 16 | Dasheng G11 | 0.3265 |
| 17 | 9311 mPing -3 | 0.3329 |
| 18 | 9311 mPing -1 | 0.2282 |
| 19 | 9311 mPing -4 | 0.3405 |
| 20 | 9311 mPing -5 | 0.3627 |
| 21 | 9311 mPing -6 | 0.3278 |
| 22 | mPing 3-1 | 0.1671 |
| 23 | mPing 3-4 | 0.3374 |
| 24 | mPing 6-1 | 0.3126 |
| 25 | mPing 7-1 | 0.1606 |
| 26 | mPing 8-2 | 0.1103 |
| 27 | mPing 12-1 | 0.1145 |
| 28 | mPing U-1 | 0.2815 |
| 29 | mPing 3-2 | 0.1489 |
| 30 | mPing 3-3 | 0.1411 |

**Table 4.17: Mean similarity between various rice types based on TE analysis (NYSYS-PC, UPGMA).**

| Sr. No. | GROUPS | TE-J |
|---|---|---|
| 1 | Traditional Basmati / Traditional Basmati | 0.669 |
| 2 | *Japonica* / *Japonica* | 0.420 |
| 3 | *Indica* / *Indica* | 0.545 |
| 4 | *Japonica* / Traditional Basmati | 0.400 |
| 5 | *Japonica / Indica* | 0.269 |
| 6 | *Indica* / Traditional Basmati | 0.514 |
| 7 | *O. glaberrima* / Traditional Basmati | 0.215 |
| 8 | *O. barthii* / Traditional Basmati | 0.214 |
| 9 | *O. rufipogon* / Traditional Basmati | 0.284 |
| 10 | *O. nivara* / Traditional Basmati | 0.308 |
| 11 | *O. glumalpatula* / Traditional Basmati | 0.348 |
| 12 | *O. meridonalis* / Traditional Basmati | 0.327 |
| 13 | *O. glaberrima* / *Japonica* | 0.175 |
| 14 | *O. barthii* / *Japonica* | 0.230 |
| 15 | *O. rufipogon* / *Japonica* | 0.221 |
| 16 | *O. nivara* / *Japonica* | 0.261 |
| 17 | *O. glumalpatula* / *Japonica* | 0.269 |
| 18 | *O. meridonalis* / *Japonica* | 0.173 |
| 19 | *O. glaberrima* / *Indica* | 0.227 |
| 20 | *O. barthii* / *Indica* | 0.274 |
| 21 | *O. rufipogon* / *Indica* | 0.235 |
| 22 | *O. nivara* / *Indica* | 0.264 |
| 23 | *O. glumalpatula* / *Indica* | 0.304 |
| 24 | *O. meridonalis* / *Indica* | 0.277 |

**Figure 4.1: Dendrogram (Jaccard, UPGMA, NYSYS-PC) showing genetic relationships among 50 rice genotypes based on genetic distance matrix data obtained using 50 SSR primers.**

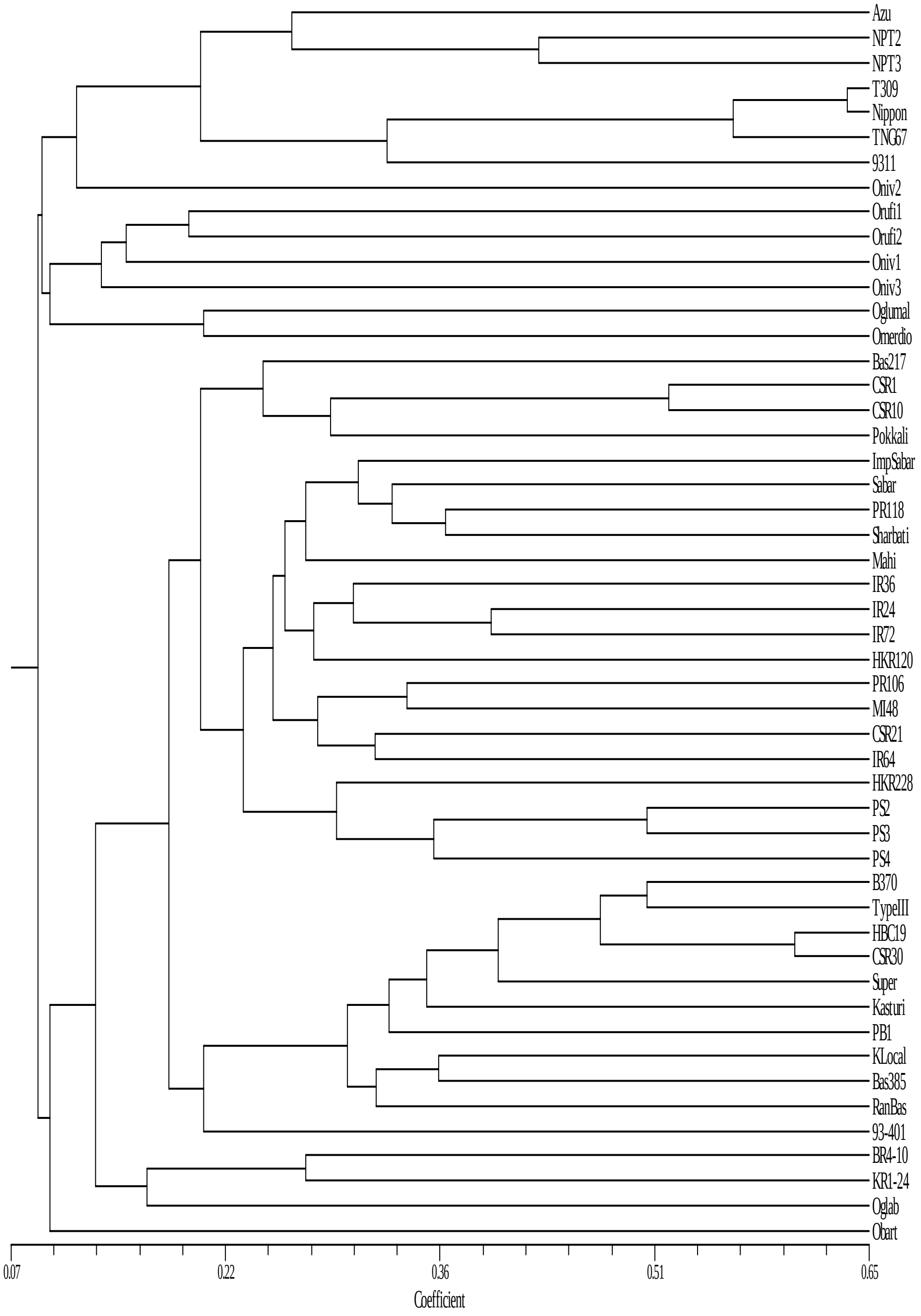


**Figure 4.2: Two-dimensional scaling of 50 rice genotypes by principal component analysis (PCA, NTSYS-PC) using SSR genetic distance matrix data.**

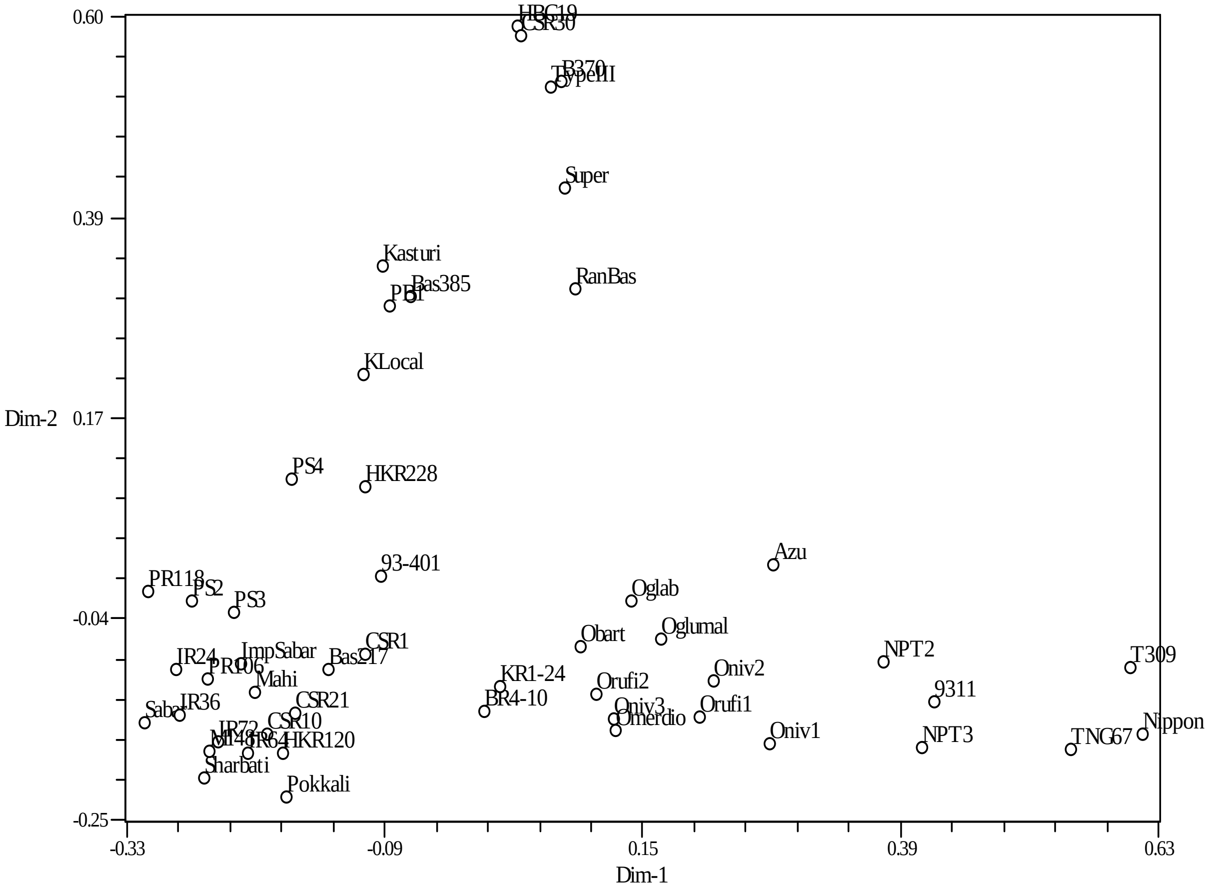


**Figure 4.3:** **Three-dimensional scaling of 50 rice genotypes by principal component analysis (PCA, NTSYS-PC) using SSR genetic distance matrix data.**

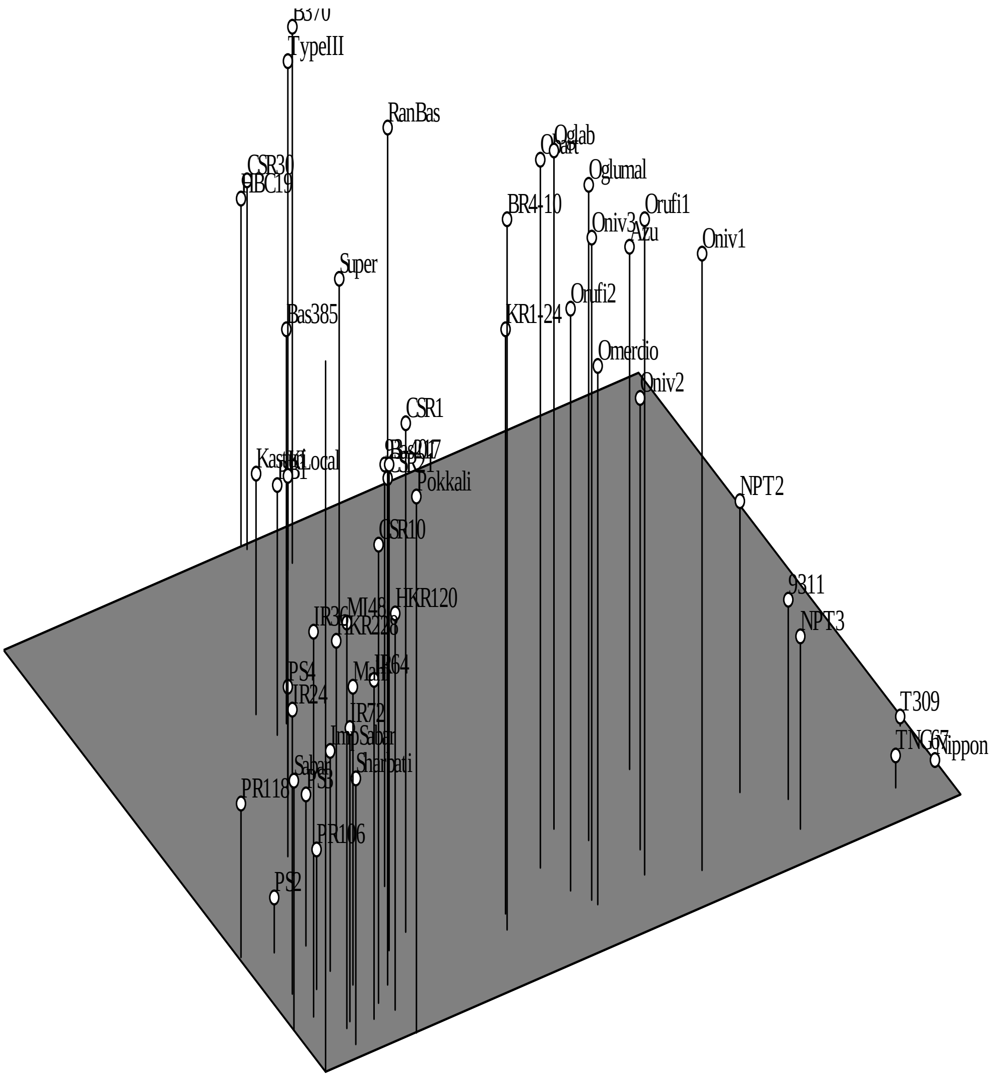


**Figure 4.4: Radial tree analysis (PowerMarker) showing genetic relationship among the 50 rice genotypes using the SSR diversity data.**

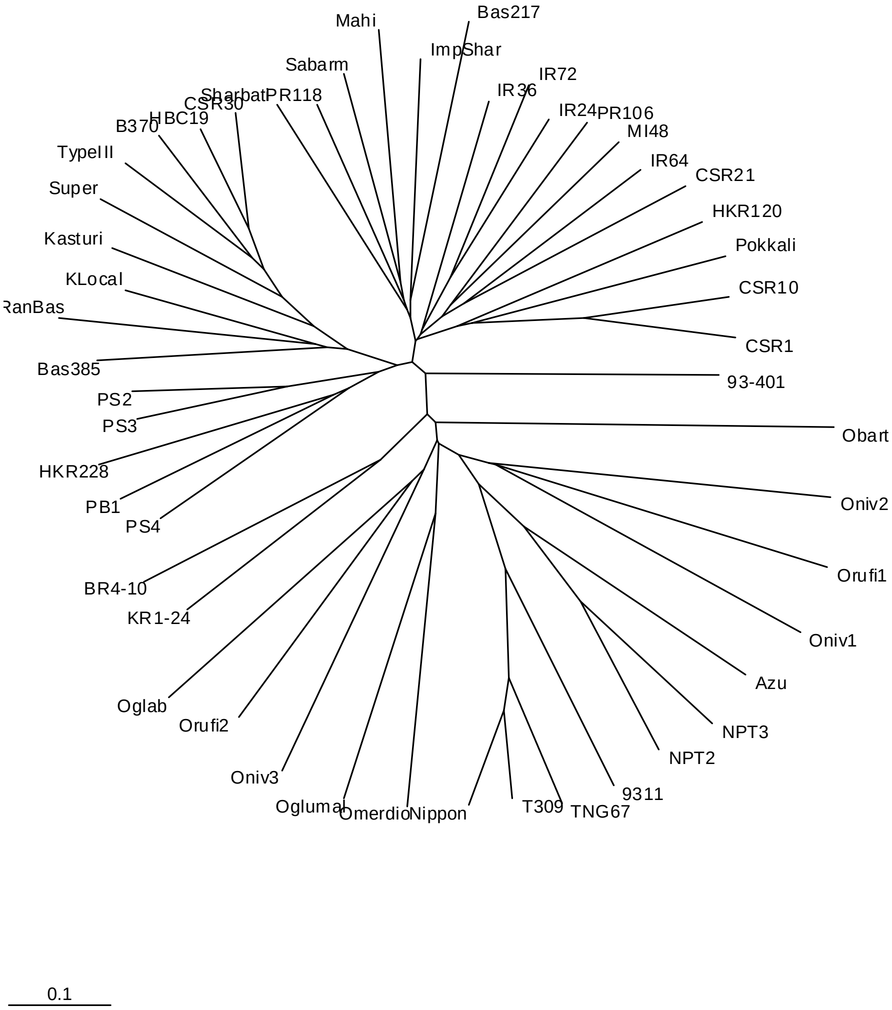


**Figure 4.5:** **Phylogenetic tree analysis (PowerMarker) showing genetic relationship among the 50 rice genotypes using the SSR diversity data.**

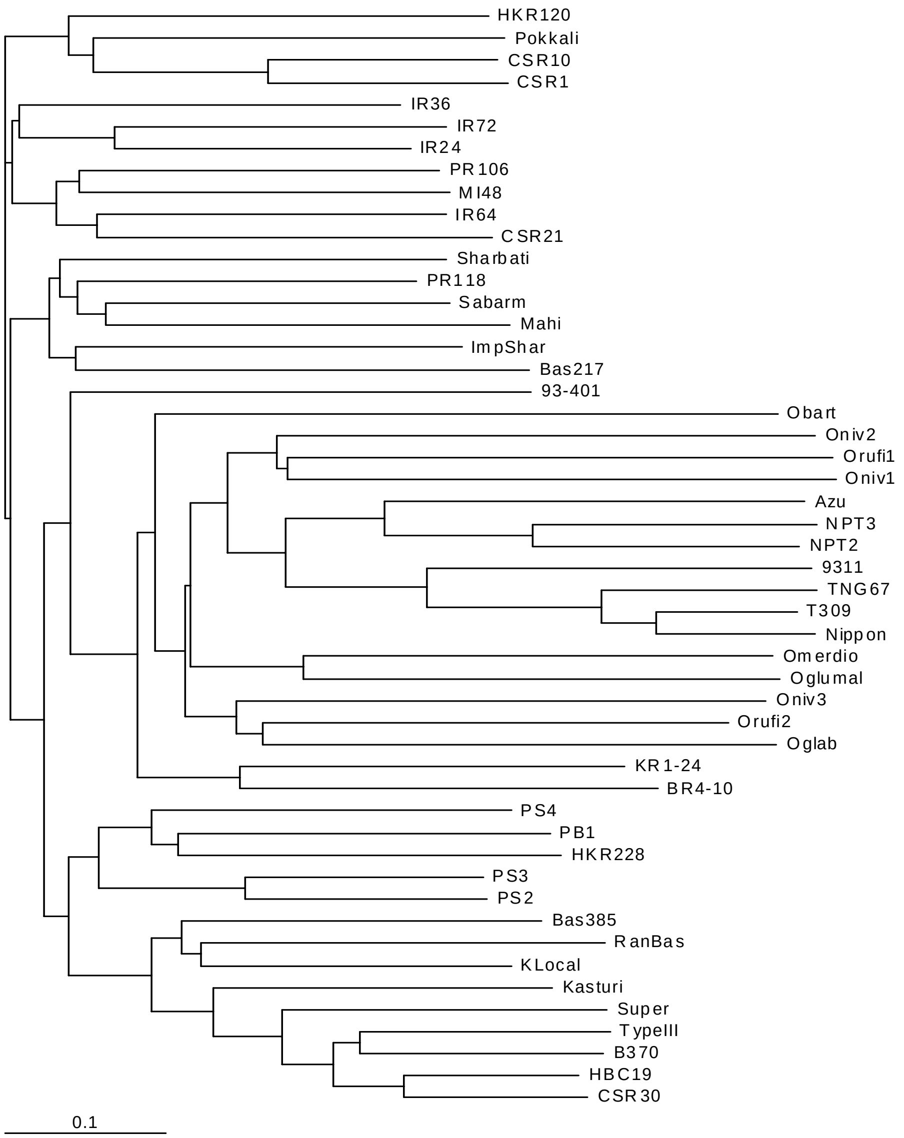


**Figure 4.7: Dendrogram (Jaccard, UPGMA, NTSYS-PC) showing genetic relationships among 50 rice genotypes based on genetic distance matrix data obtained using 30 TE primers.**

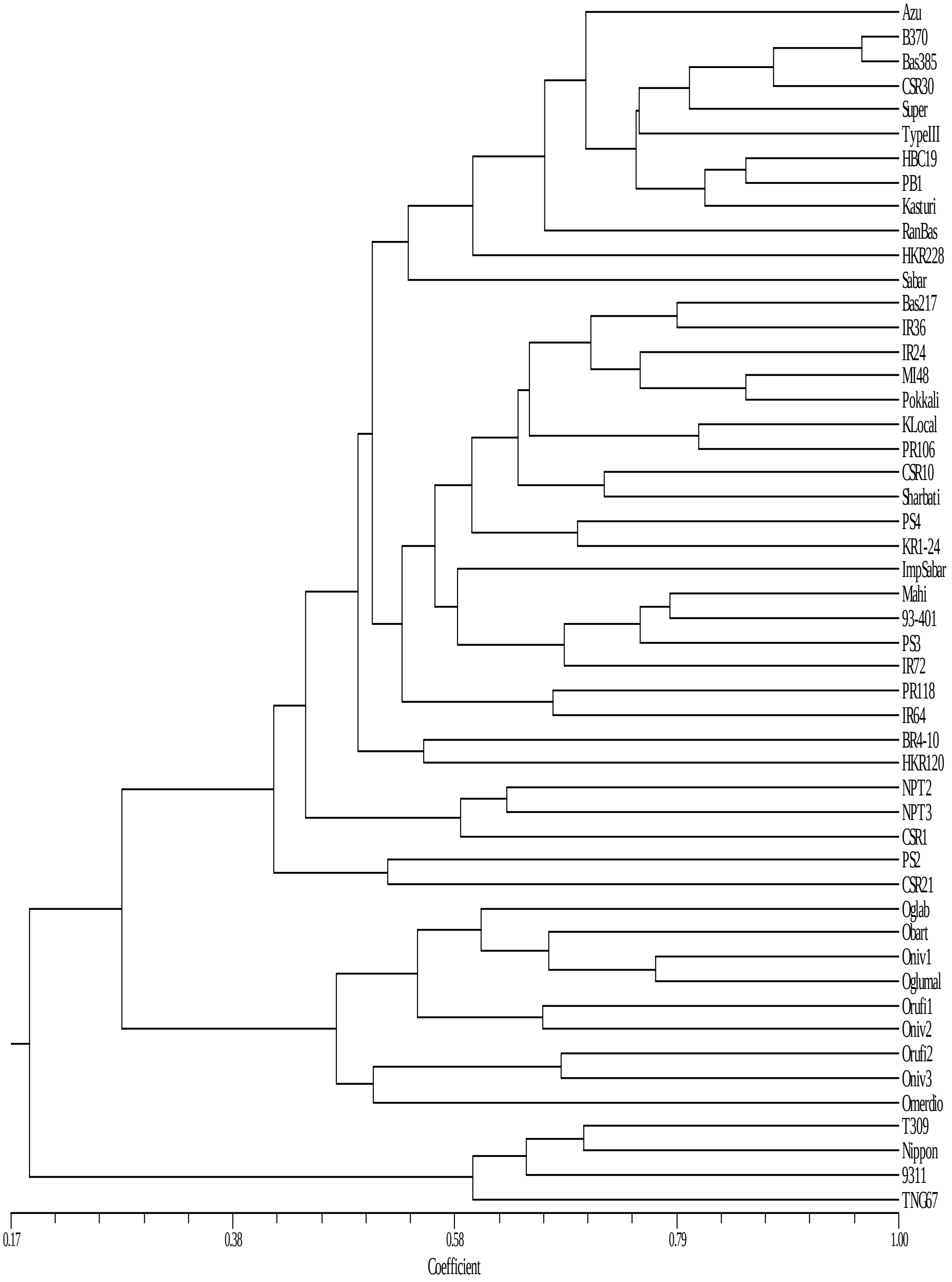


**Figure 4.8: Two-dimensional scaling of 50 rice genotypes by principal component analysis (PCA, NTSYS-PC) using the TE genetic distance matrix data.**

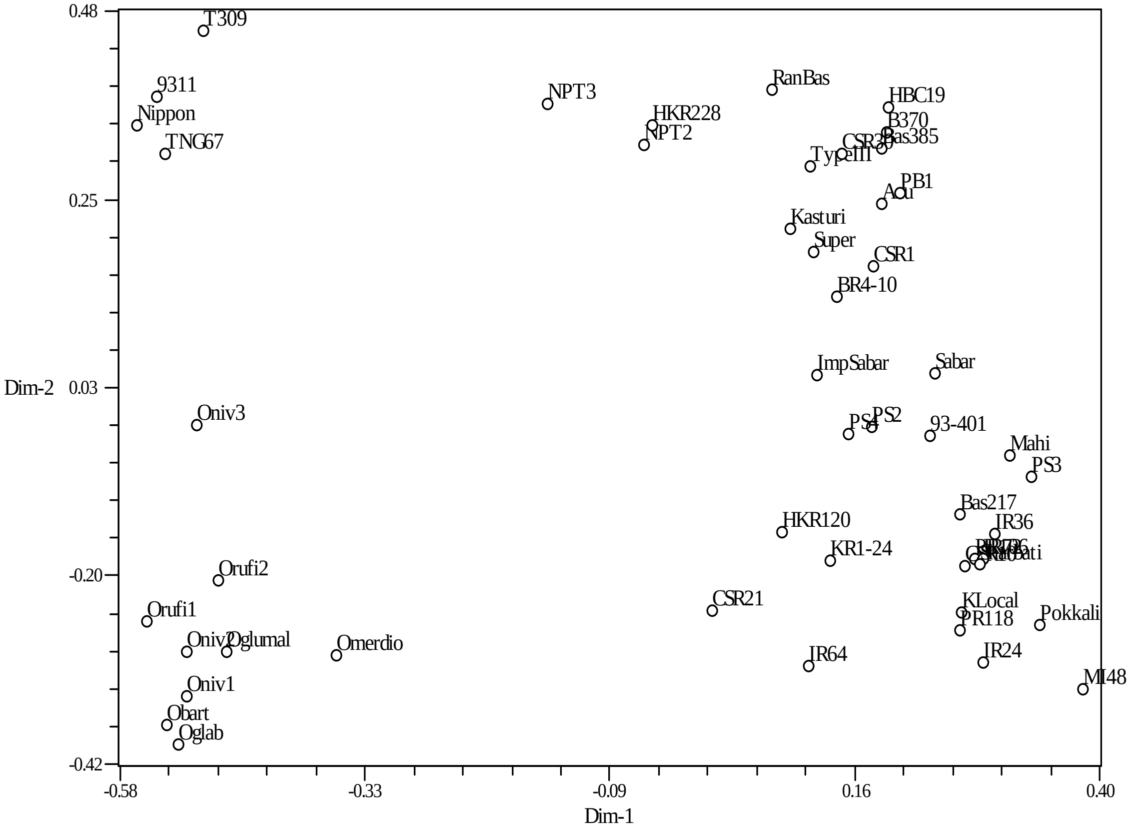


**Figure 4.9: Three-dimensional scaling of 50 rice genotypes by principal component analysis (PCA, NTSYS-PC) using the TE genetic distance matrix data.**

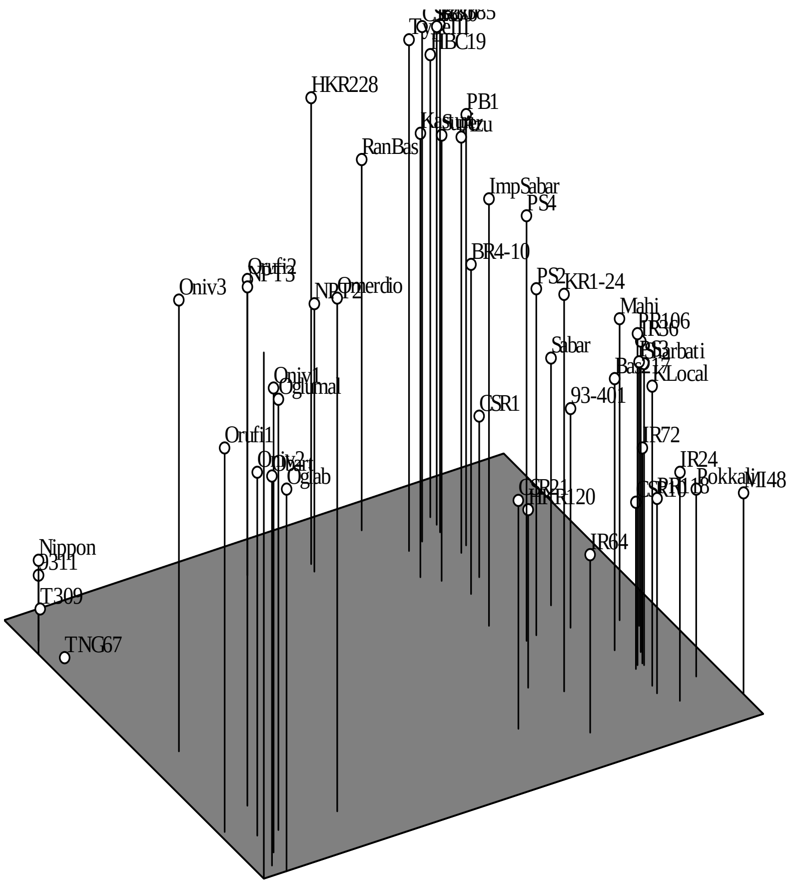


**Figure 4.10: Radial tree analysis (PowerMarker) showing genetic relationship among the 50 rice genotypes using the TE diversity data.**

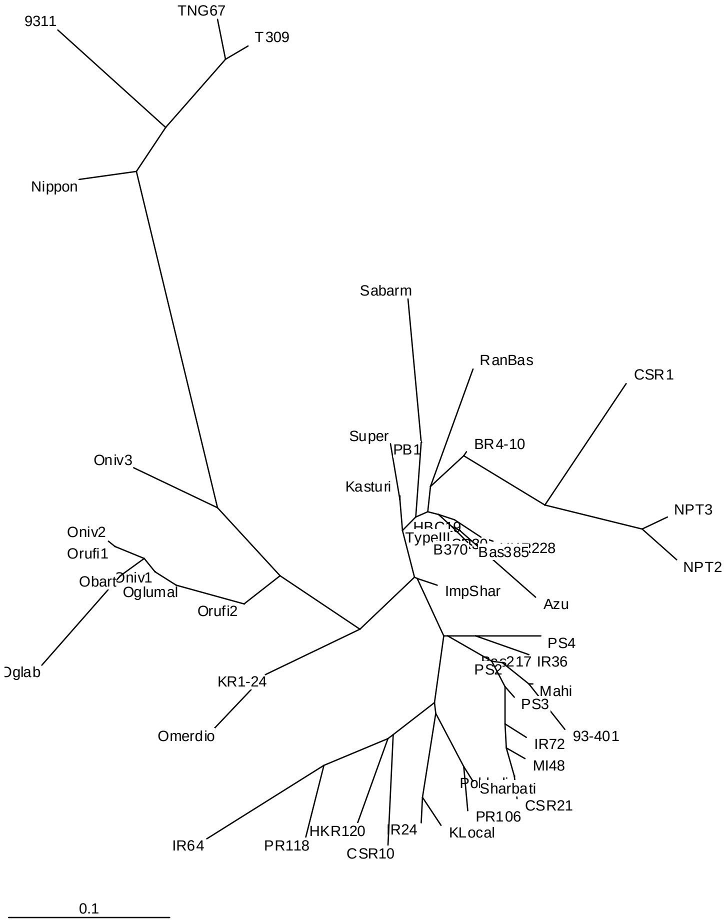


**Figure 4.10:** **Radial tree analysis (PowerMarker) showing genetic relationship among the 50 rice genotypes using the TE diversity data.**

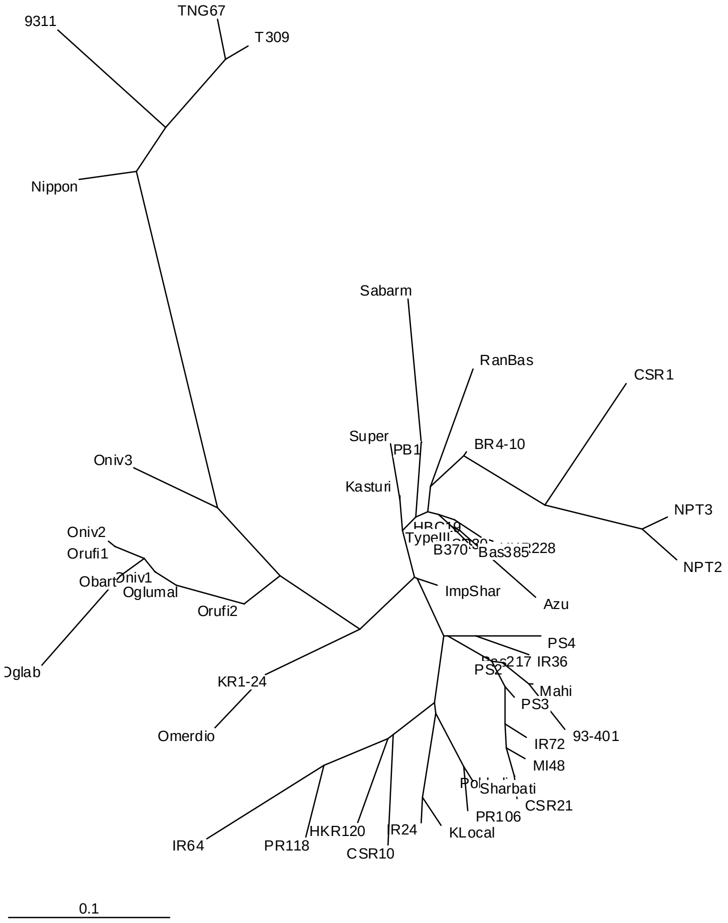


**Figure 4.11: Phylogenetic tree analysis (PowerMarker) showing genetic relationship among the 50 rice genotypes using the TE diversity data.**

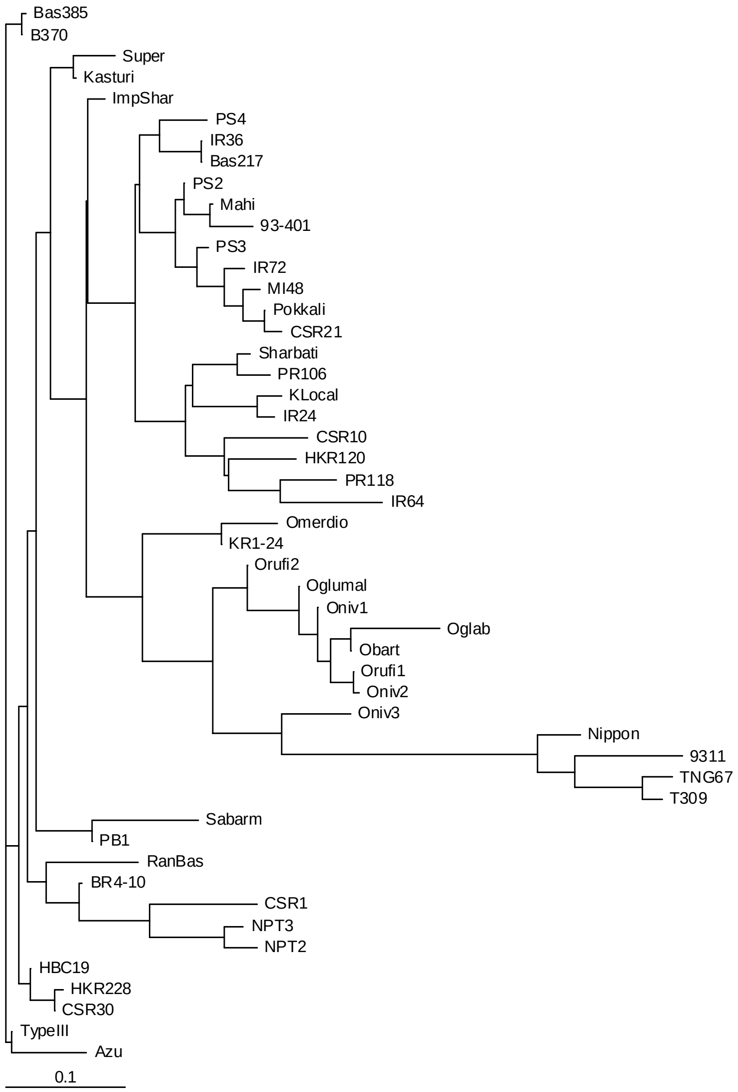
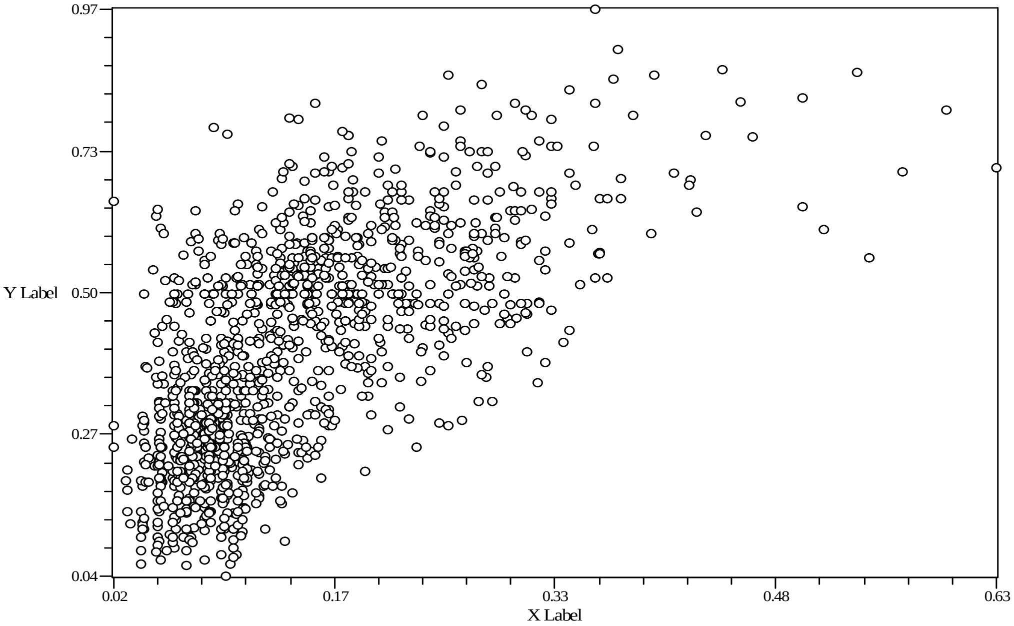


**Figure 4.12: Matrix correlation between SSR and TE markers based diversity data.**



N = 1225

Mean X = 0.1481 SSx = 9.0903; Mean Y = 0.4083 SSy = 38.5210

Tests for association:

Matrix correlation: r = 0.65569

(= normalized Mantel statistic Z)

Approximate Mantel t-test: t = 10.4284

Prob. random Z < obs. Z: p = 1.0000

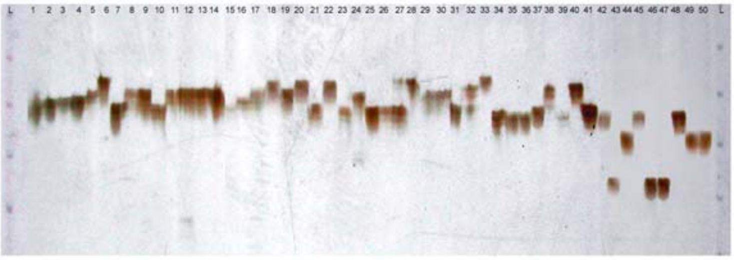


Plate. 2a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-215 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

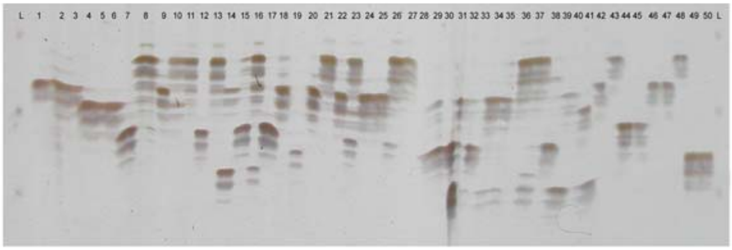


Plate. 2b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-490 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

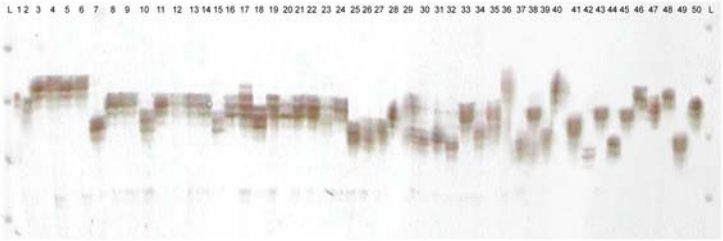


Plate. 3a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-450 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

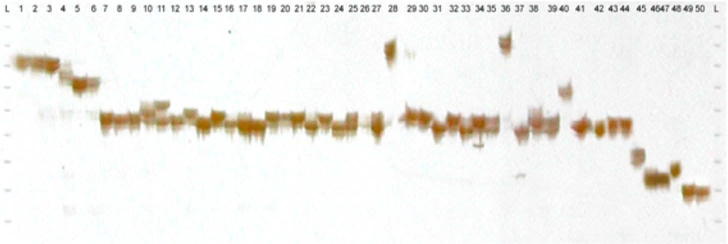


Plate. 3b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-580 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

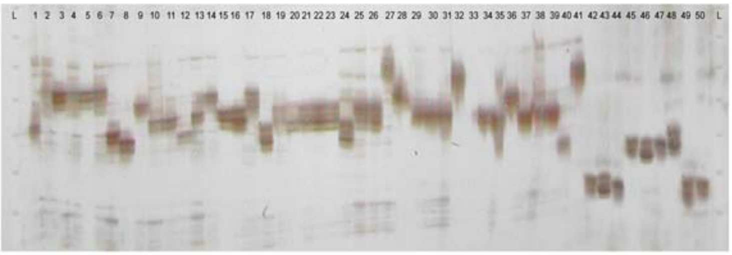


Plate. 4a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-518 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

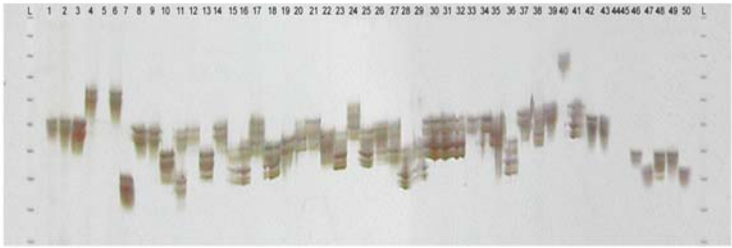


Plate. 4b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-536 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

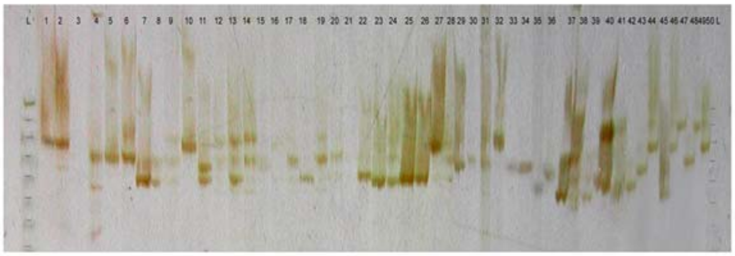


Plate. 5a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-594 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

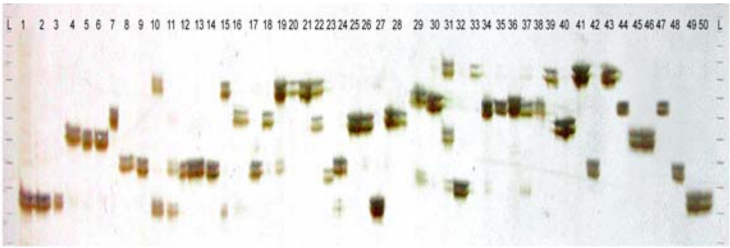


Plate. 5b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-562 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

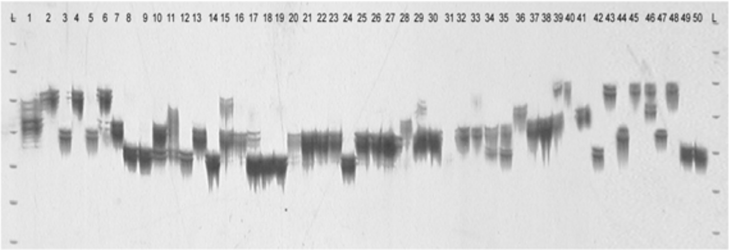


Plate. 6a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-567 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

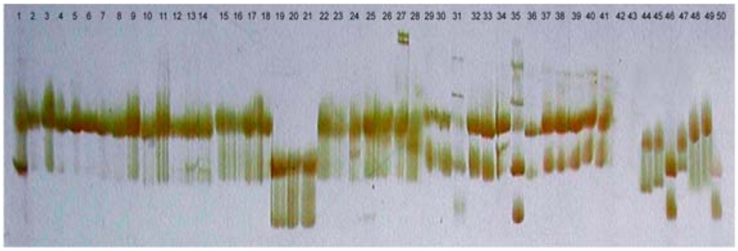


Plate. 6b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-422 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50)

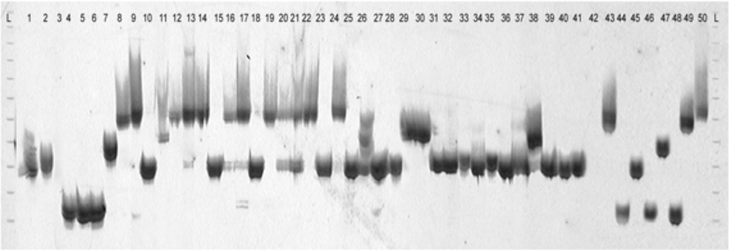


Plate. 7a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-528 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

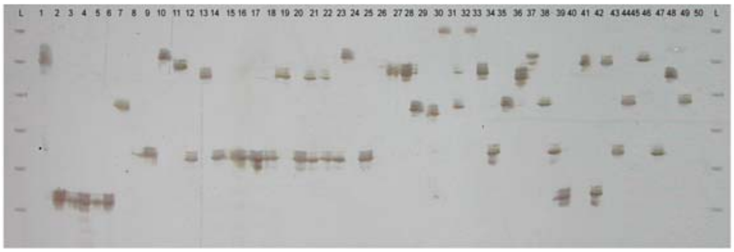


Plate. 7b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-224 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

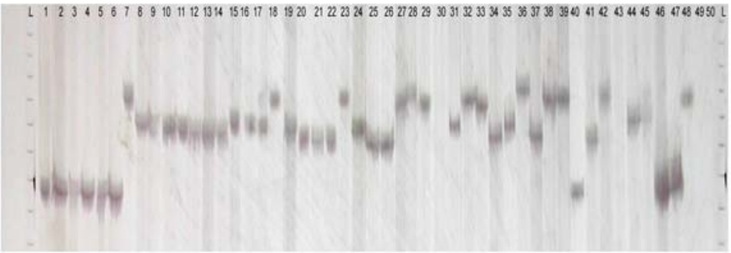


Plate. 8a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-424 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

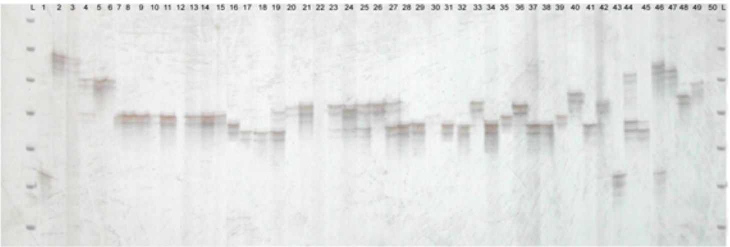


Plate. 8b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-229 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

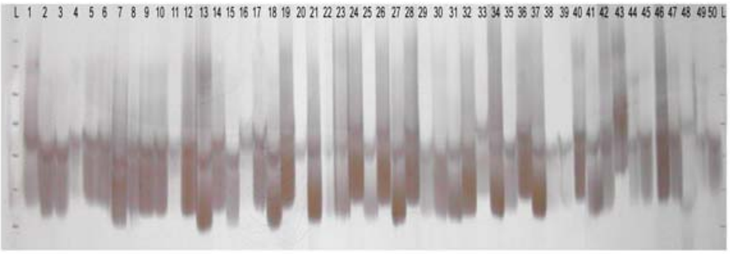


Plate. 9a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-321 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

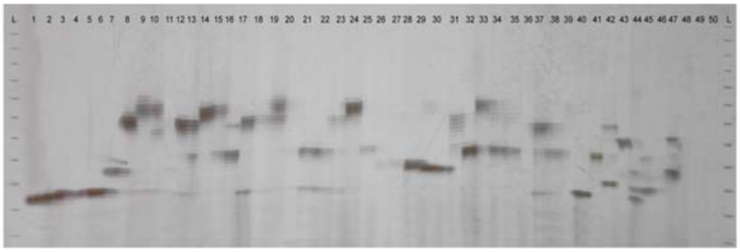


Plate. 9b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-252 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

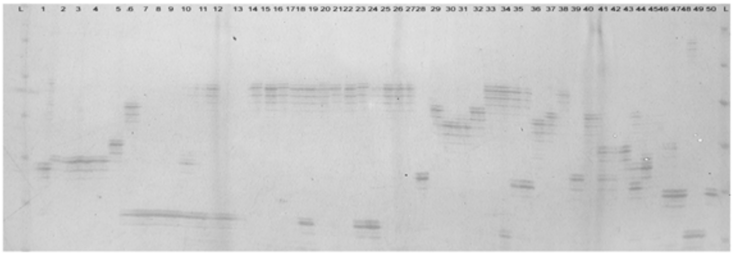


Plate. 10a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-248 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

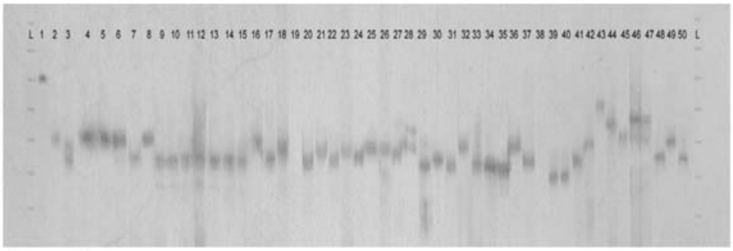


Plate. 10b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-316 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

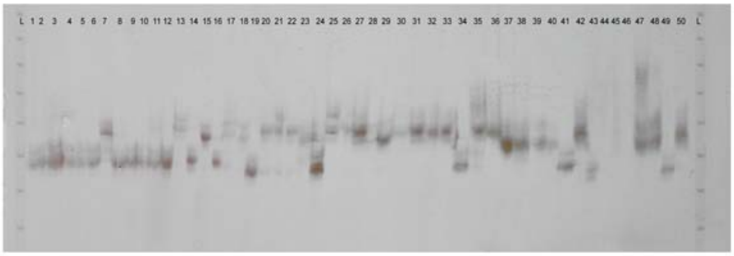


Plate. 11a

A Silver stained gel showing polymorphism among 50 rice varieties at RM-282 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

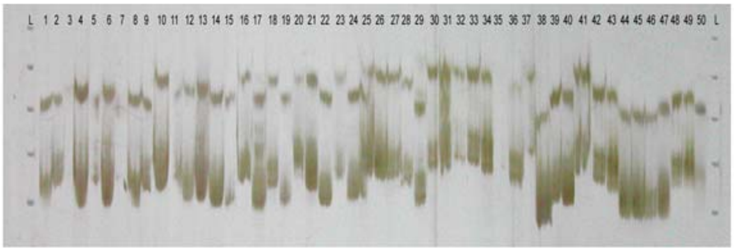


Plate. 11b

A Silver stained gel showing polymorphism among 50 rice varieties at RM-472 locus. Lane 1-50 Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48) O. glumalpatula (49) O. meridionali (50) Ladder (L)

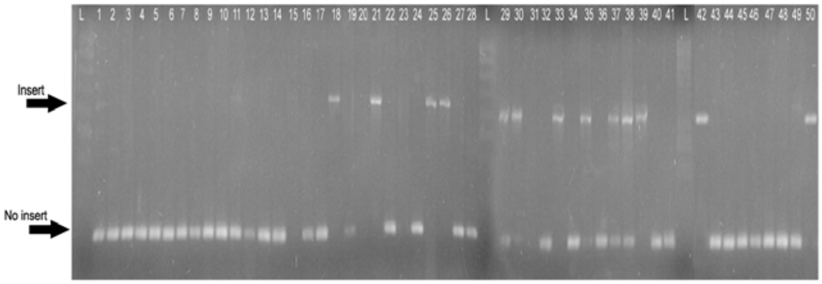


Plate. 12a

Transposon element based marker banding profile of 50 rice varieties using mPing-9311-1 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

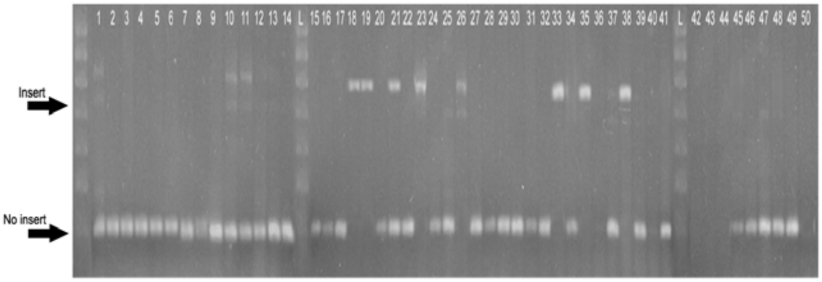


Plate. 12b

Transposon element based marker banding profile of 50 rice varieties using mPing-9311-3 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

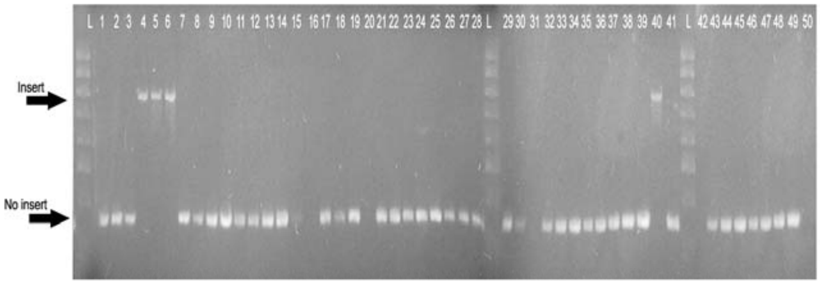


Plate. 13a

Transposon element based marker banding profile of 50 rice varieties using mPing-3-2 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

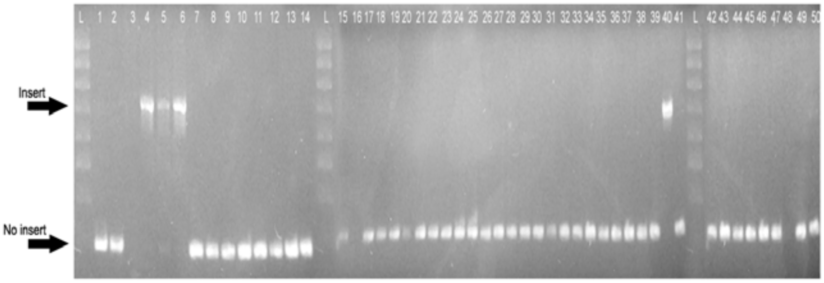


Plate. 13b

Transposon element based marker banding profile of 50 rice varieties using mPing-3-3 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

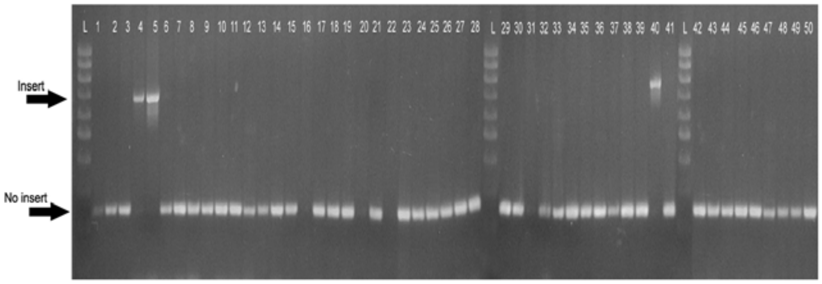


Plate. 14a

Transposon element based marker banding profile of 50 rice varieties using mPing-12-1 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

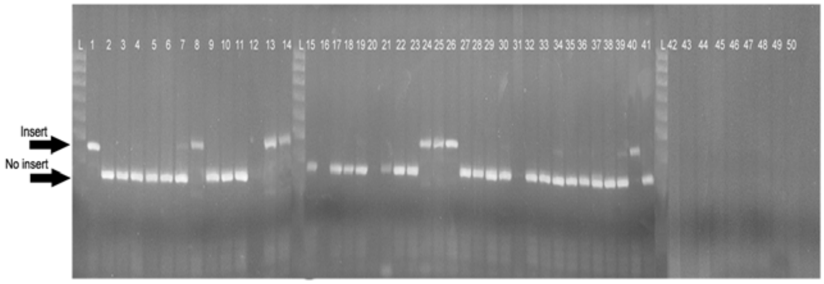


Plate. 14b

Transposon element based marker banding profile of 50 rice varieties using mPing-U-1 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

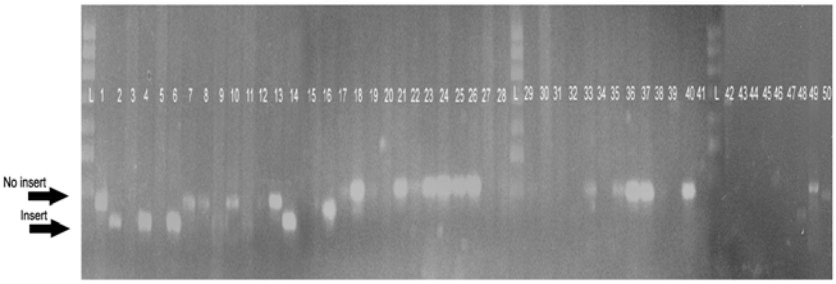


Plate. 15a

Transposon element based marker banding profile of 50 rice varieties using Dasheng-C-11 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

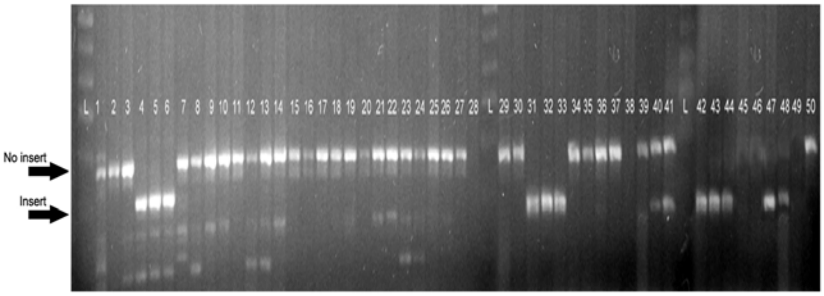


Plate. 15b

Transposon element based marker banding profile of 50 rice varieties using Dasheng-G-11 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

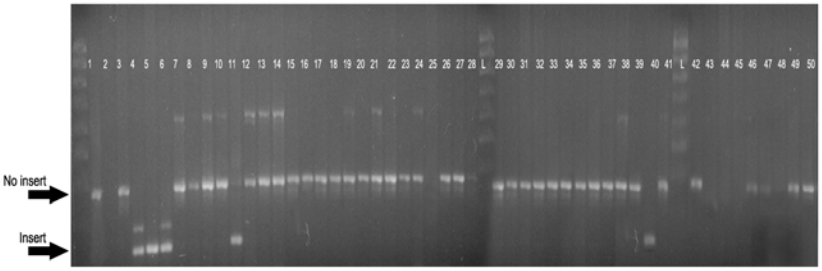


Plate. 16a

Transposon element based marker banding profile of 50 rice varieties using Dasheng-E-11 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

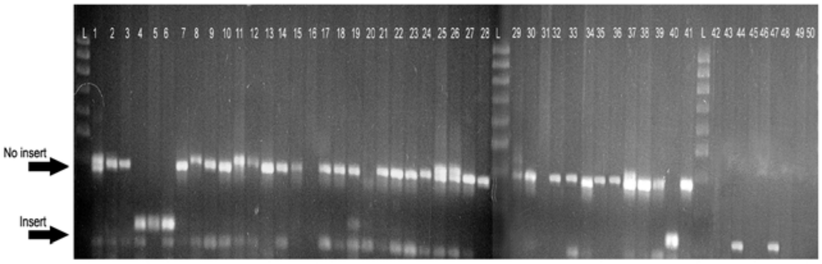


Plate. 16b

Transposon element based marker banding profile of 50 rice varieties using Dasheng-C-7 resolved on 1.5% agarose gel. Lane 1-50 represent Azucena (1) NPTII (2) NPTIII (3) Taipei309 (4) TNG67 (5) Nipponbare (6) Basmati217 (7) Basmati 370 (8) HBC19 (9) Karnal Local (10) Ranbir Basmati (11) TypeIII (12) Basmati 385 (13) CSR30 (14) Improved Sabarmati (15) HKR 228 (16) Kasturi (17) Mahisugandha (18) Pusa Basmati 1 (19) Pusa Sugandha 2 (20) Pusa Sugandha 3 (21) Pusa Sugandha4 (22) Sabarmati (23) Super Basmati (24) Punjab Rice 118 (25) Punjab Rice 106 (26) IR 36 (27) BR4-10 (28) CSR 1 (29) CSR 10 (30) CSR 21 (31) HKR 120 (32) HKR 93-401 (33) IR 24 (34) IR 72 (35) Kala Ratta 1-24 (36) MI 48 (37) Pokkali (38) Sharbati (39) 9311 (40) IR 64 (41) O. glaberrima (42) O. barthii(43) O. rufipogon1(44) O. rufipogon2(45) O. nivara1(46) O. nivara2(47) O. nivara3(48), O. glumalpatula (49) O. meridionali (50) Ladder (L)

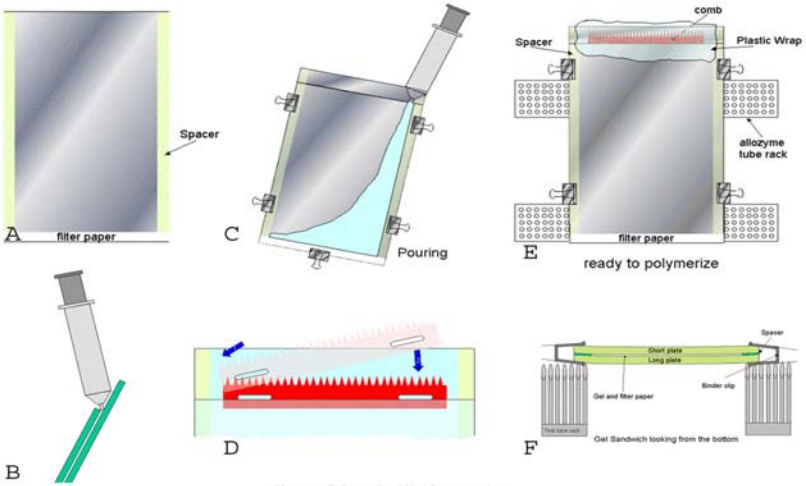


Plate. 1 : Polyacrylamide gel preparation

# CHAPTER -V

# Discussion

Rice (*Oryza sativa*) belongs to the genus *Oryza* that includes more than 25 wild species either perennial or annual, which are either diploid or tetraploid. Rice is composed of two major subspecies, *indica* and *japonica*, and several ecotypes, which are adapted to diverse environmental conditions ranging from upland to lowland and from tropical to temperate zones. With such a wide range of geographic distribution, rice is well-known for its rich genetic resources maintained worldwide with more than 250,000 germplasm collections of more than 20 cultivated and wild species (Evernson *et al.*, 1998).

Rice is predominantly autogamous and, hence, gene flow is restricted. As a result, geographically or ecologically distinct groups of rice are expected to show greater genetic differentiation than would be the case in an out-crossing species. The exotic collection of diverse cultivated and wild rice germplasm has made great contributions to rice breeding. Due to the continuous selection of domesticated wild rice for the development of high yielding and well adapted varieties, which occupied more than half of world's rice growing area, has led to genetic erosion which in-turn narrowed variability in rice.

Basmati rice varieties cultivars are one of the premium quality rices of the world. They fetch a premium price in the world market. Most of the European Union and South-East Asian countries have even exempted the high quality, sweet-scented Basmati rice from export duty. As a result, premium traditional Basmati rice supplies are mixed with the cheaper, low quality cross-bred Basmati and long-grain *indica* rice varieties to achieve good earnings. Earlier no appropriate methods were there to perceive the level of adulteration, but now the consumer and the regulatory authorities have become aware of such malpractices and advocated the need of most sensitive, reliable DNA markers based methods for varietal identification and determine the levels of adulteration. A good progress has already been made in this direction and several microsatellite DNA markers have been identified for differentiating between different grades of Basmati rice (Pal *et al.*, 2004).

Basmati rice varieties of Indian subcontinent have been conventionally classified as aromatic *indica*. But, both isozymatic (Glaszmann 1987, 1988) and DNA marker (Bligh *et al.*, 1999; Aggarwal *et al.*, 2002; Nagaraju *et al.*, 2002; Jain *et al.*, 2004; Saini *et al.*, 2004) analyses have placed most of the Basmati rice genotypes in a separate group/cluster distinct from that of *indica* and *japonica* rice genotypes. The actual phylogenetic status and origin of Basmati rice is still not clear and needs further investigation.

In the present study, experiments were conducted to: (1) develop a DNA fingerprint database of 50 rice genotypes including commercially important Basmati rice varieties which can be used for varietal identification and (2) clarify the phylogenetic status of Basmati rice vis-à-vis *indica*, *japonica* and wild type rice genotypes having AA genome.

## 5.1 Microsatellite marker analysis

Molecular markers would be of great value to detect alien introgression and to develop DNA fingerprints for the characterization of genetic resources of rice. The use of SSRs has already been demonstrated for DNA fingerprinting and varietal identification (Olufowote *et al.* 1997; Bligh *et al.* 1999; Nagaraju *et al.*, 2002; Jain *et al.*, 2004), assessment of genetic diversity and phylogenetic relationships in *Oryza* species (Akagi *et al.* 1997; Cho *et al.*, 2000; Chen *et al.*, 1997; Temnykh *et al.*, 2001; Saini *et al.*, 2004; Jain *et al.*, 2004;

Ren *et al.*, 2003; Rave *et al.*,2003; Ni *et al.*, 2002), population structure and breeding pattern (Lu *et al.*, 2005; Gao *et al.*, 2005), detection of cases of adulteration (Bligh, 2000) and identification of hybrids (Yun *et al.*, 2005).

In the present study, a DNA fingerprint database of 50 rice genotypes including traditional Basmati, *japonica*, *indica*, cross-bred Basmati and wild rice genotypes have been developed using 50 SSR markers. SSR markers showed a very high level of polymorphism. A total of 341 alleles were detected with an average of 6.82 alleles (range 2-16 alleles) per locus. These results were comparable to 7.4 alleles per locus as reported by Olufowote *et al.* (1997) in 71 cultivars of rice but were on higher side in comparison to Cho *et al.* (2000), who reported an average of 2.0-5.5 alleles per locus in a different set of rice germplasm. Panaud *et al.* (1996) reported 2 to 9 alleles for microsatellite markers in 22 *japonica* and *indica* cultivars. The accessions assessed by Olufowote *et al.* (1997) and Panaud *et al.* (1996) consisted of a wide range of *indica* and *japonica* cultivars and landraces and therefore are more likely to possess higher number of alleles. Akagi *et al.* (1997) reported 2-10 alleles among 59 closely related *japonica* cultivars. Nagaraju *et al.* (2002) reported 2-8 alleles in 24 rice varieties including Basmati rice varieties, with an average of 3.8 alleles per locus. Garland *et al.* (1999) detected 5-23 alleles in 43 Australian breeding lines of rice using 10 SSR markers with an average of 11.5 alleles per locus. Greater number of alleles per locus is likely to depend up on the constitution of plant material being subjected to SSR analysis. More the number of genetically diverse genotypes or wild species involved in the study more shall be the number of alleles per locus.

As many as 40 unique alleles were observed in 50 rice genotypes including 17 unique alleles from the nine wild rice genotypes. Four and six unique alleles were present in traditional Basmati and cross-bred Basmati rice varieties, respectively. High number of unique alleles may be attributed to the collection of genotypes targeted in this study, which belongs to the genetically diverse groups and wild species of rice. Nagaraju *et al.* (2002) showed preponderance of SSR alleles in Basmati varieties. Of 70 alleles, 9 were unique to traditional Basmati and some of the evolved-Basmati and were absent in all seven non-Basmati varieties. Further analysis of seven more non-Basmati varieties confirmed that these nine alleles are confined only to the traditional Basmati varieties analyzed in the study. While occurrence of such rare or unique alleles in rice varieties remains to be explained, it may have resulted from unequal crossing-over, translocation or other types of mutations.

Null alleles were observed at 31/50 loci in 35/50 rice genotypes. While the length variation at microsatellite loci generally results from a change in the number of repeats, null alleles are the consequence of polymorphism in the primer binding site(s). Cho *et al.* (2000) reported null alleles at 17% of the total microsatellites (323) mapped; 1-6 varieties out of a panel of 13 cultivars showed the presence of null alleles. Most often null allele was due to an accumulation of sequence differences coupled with insertion/deletion events in the flanking regions outside the SSR domains. The prevalence of high number of null alleles in *O. meridionalis* and *O. rufipogon* strongly suggests that sequence divergence in flanking regions may play a significant role in interspecific SSR variation. Null alleles have also been reported earlier to be most frequent in distant wild *Oryza* species perhaps due to greater variability in the primer annealing sequences (Panaud *et al.*, 1996; Temnykh *et al.*, 2000). Large number of null alleles was observed in this study, may be due to inclusion of several wild rice varieties/species and wide range of rice germplasm.

Multiple alleles were observed at 31 of the 50 SSR loci with an average frequency of 0.62 alleles per locus. At RM488 locus, 11 of the 50 genotypes showed multiple alleles. Cross-bred rice varieties, CSR-30 and Sharbati, both were having multiple alleles at 5 SSR loci. Some other rice varieties, Pusa Sugandha 3, Sabarmati, Improved Sabarmati, also had multiple alleles at four of the 50 SSR loci. Pokkali, Kasturi HKR228, Pusa Basmati 1, Pusa Sugandha 2, Basmati 385, Ranbir Basmati showed multiple alleles at three of the 50 SSR loci. Higher number of multiple alleles was observed in cross-bred Basmati (3.08 multiple alleles per genotype) in comparison to *indica* (2.33 multiple alleles per genotype), *japonica* (0.71 multiple alleles per genotype), traditional Basmati (1.28 multiple alleles per genotype) and wild rice genotypes (1.0 multiple alleles per genotype). The detection of multiple alleles is probably due to heterogeneity rather than the genetic heterozygosity. Rice is predominantly self-fertilized crop and most variants appeared to be homozygous, however heterozygous individuals may occur due to out-crossing or residual heterozygosity in case of cross-bred varieties. Garland *et al.* (1999) also observed heterogeneity in Della cultivar, which may be due to out crossing. Intracultivar polymorphism was detected by the identification of multiple products for a specific primer. The multiple alleles may occur due to enhanced intensity of amplification for some alleles in allelic mixtures and the presence of overlapping stutters of separating alleles in bulked DNA preparations from five plants; however screening of numerous individuals for each cultivar is necessary to adequately assess intracultivar polymorphism.

SSR primers are designed based on the frequency and average length of specific SSR motif. The most frequently observed microsatellites in rice genome are poly $(GA)_n$ and poly $(GT)_n$ (Panaud *et al.*, 1995). These SSR repeats have been found interspersed in various combinations of two or more SSRs (compound SSRs) as well as with other single and multicopy sequences. Chen *et al.* (1997) reported that simple sequence repeats (SSR) were pre-dominantly poly (GA) motifs, because of their abundance in the rice genome. Among the 94 SSLP loci, 86 poly (GA), 6 poly (TCT), 1 poly (ATT) motifs were observed from two (an enzyme digested and a sheared library) libraries. GA containing microsatellites were found to be most polymorphic among all the di-nucleotide repeats and the highest degree of genetic diversity was reported from GA-containing microsatellites of genomic library origin (Cho *et al.*, 2000). Twenty nine of the fifty SSR markers used in this study had di-nucleotide repeat motifs including GA (7), CT (11), AG (4), and TC (4) motifs. On an average, number of alleles amplified using the markers with CT and TC di-nucleotide repeat motif was 8.36 and 8.5, respectively, whereas GA, AG, and AT repeat based marker amplified 5.28, 5.75 and 6.0 alleles, respectively. A total of 13 tri-nucleotide repeat motifs were used and CTT and AAT repeat motifs amplified 9.5 alleles per loci. Four complex repeat primers (RM229, RM316, RM224 and RM255) detected on an average 8.25 alleles per loci. When di, tri, tetra and complex repeat primers were compared, it was observed that complex primer generated highest number of alleles (8.25) followed by di-nucleotide (7.1) and tri-nucleotide (6.3) repeat based primers. Tetra-nucleotide repeats primer detected lowest number of alleles (5) per locus. PIC values were analyzed, CT and TC repeat showed highest values of 0.753 and 0.762 whereas complex repeat primers amplified maximum average alleles but the PIC values was 0.680, which is less than the CT and TC repeat primer. The average PIC values of di-nucleotide repeat primers were 0.721 in comparison to complex repeat primer (0.680). This shows that di-nucleotide repeat primers are more polymorphic than any other repeat primers. Also, the polymorphism obtained using the markers with TC and CT was maximum in comparison to other di-nucleotide repeat (AT, GA, AG, CA and GT) repeat primer.

Genetic relationships and diversity data generated using SSR marker systems was in agreement with the Glaszmann (1987) classification of Asian rice germplasm. The study shows that Group V traditional Basmati rice landraces/varieties (Basmati 370, HBC 19, Basmati 385, Karnal Local, Ranbir Basmati) are genetically distinct from Group 1 *indica* (HKR 120, CSR 10, IR24, IR36, IR64) and Group VI *japonica* (Nipponbare, Azucena, NPTII, NPTIII, TNG 67 and Taipei 309) rice varieties. Higher levels of genetic diversity between Basmati and non-Basmati (*indica*, *japonica* and wild genotypes) suggest that former may have a long history of independent evolution and diverged from non-Basmati rices a long time ago through conscious selection and patronage for this cluster of germplasm (Nagaraju *et al.*, 2002; Saini *et al.*, 2004; Jain *et al.*, 2004). However, the traditional Basmati variety, Basmati 217, had an allelic profile, which was quite different from the other traditional Basmati rice varieties. Basmati 217 had ten of 16 unique alleles observed in a total of seven traditional Basmati rice varieties. Nagaraju *et al.* (2002) also reported low level of genetic similarity between Basmati 217 and other traditional Basmati rice varieties using both SSR and ISSR markers. Basmati 217 may have resulted due to crossing (natural out-crossing or manual) between Basmati and an *indica* rice variety and probably represents a separate lineage. This point is further supported by the fact that it shares several alleles with *indica* varieties and was clustered with *indica* sub-group. Another point that needs to be investigated relates to the diversity that exists in Basmati rice gene pool. This is an interesting observation and needs further research.

Among the twelve cross-bred (*indica* × Basmati) Basmati rice varieties, there were two categories: first (CSR30, Pusa Basmati 1, Kasturi, and Super) that invariably clustered with traditional Basmati group and the second (Improved Sabarmati, Mahisugandha, Pusa Sugandha 2, Pusa Sugandha 3, Pusa Sugandha 4 and Sabarmati) closer to *indica* rice genotypes. The cross-bred varieties may have different levels of genetic content from their *indica* and Basmati parents. The variable degree of diversity between traditional Basmati and crossbred Basmati rice varieties may be due to the complex parentage of cross-bred varieties involving several recombination events and is the indicative of different levels of genomic fractions from their respective *indica* rice parent(s) (Singh *et al.*, 2000b; Nagaraju *et al.*, 2002; Saini *et al.*, 2004).

The six *japonica* and one *japonica-indica* (9311) rice varieties used in this study were always clustered together, which indicates that the *japonica* group is quite distinct from all the other three other groups (traditional Basmati, *indica* and wild genotypes). This observation was in agreement with Glazmann (1987) classification of Asian rice germplasm, where most of the *japonica* rice varieties were placed in a distinct Varietal group VI and reports of Jain et al. (2004), Gao et al. (2005) and Saini et al. (2004) on the basis of SSR analyses showed the clustering of most of the japonica rice varieties in one group/cluster.

The Aus rices were traditionally grown in a short summer season in Bangladesh under rainfed conditions (Parsons *et al.*, 1999). Adaptation to flowering under long days required evolution of day length neutrality, fostering temporal reproductive isolation and divergence. Although the Aus types have a historically smaller geographical distribution and receive less attention than *indica* and *japonica* rices in breeding programs, their drought tolerance and early maturity are adaptive traits could be usefully targeted in breeding applications. Aus rice genotypes (BR4-10 and KR1-24) used in this study always form a separate group were in agreement with studies conducted by Glaszmann (1987). Garris *et al.* (2005) reported the genotyping of 234 rice accessions at 169 nuclear SSRs and two chloroplast loci. The data analysis showed the grouping of the genotypes into five groups, which corresponded to *indica*, Aus, aromatic, temperate *japonica* and tropical *japonica*.

The availability of markers that distinguish traditional Basmati rice cultivars from other rice types is also of great interest to exporters/ consumers to ensure the varietal purity. In this study several microsatellite markers have been identified which can be used to differentiate between Basmati/non-Basmati rice types (RM213, RM215, RM220, RM222, RM224, RM229, RM248, RM252, RM259, RM286, RM400, RM424,RM426, RM440, RM518, RM528, RM536, RM541, RM551, RM567), within traditional Basmati rice varieties (RM222, RM224, RM252, RM423, RM426, RM440, RM475, RM518, RM536, RM541, RM547, RM551, RM580, RM582, RM594) and traditional Basmati/cross-bred rice genotypes (RM222, RM224, RM229, RM248, RM252, RM259, RM286, RM400, RM426, RM440, RM518, RM528, RM536, RM541, RM551, RM567). A number of new SSR markers have been reported here, which may broaden the list of SSR markers already recommended for varietal identification of Basmati rice supplies by Food Standards Agency (www.food.gov.uk). Thus with minimal upfront investment, public available, low-cost, and highly efficient SSR fingerprinting technology could be used to help the safeguard the interests of both the consumers and producers for traditional Basmati.

### 5.2 Transposon element based marker analysis

Transposable elements constitute a major portion of plant and animal genomes (Kidwell and Lisch, 2000). Over millions of years of evolution, mobile elements have achieved a balance between detrimental effects on the individual and long-term beneficial effects on a species through genome modification. Indeed, we may soon learn that the shaping of the genome by mobile elements has played an important role in events leading to speciation. Some may have had an important function long ago, but have lost that role today. Others may never have had a function, yet the cluttering of our genomes with nonfunctional DNA was a small price to pay for the genome malleability they provided.

The rice genome is populated by representatives from all known transposon super-families, including elements that cannot be easily classified into either class I or II (Turcotte *et al.*, 2001). Previous estimates of the transposon content in the rice genome range from 10 to 25% (Turcotte *et al.*, 2001; Mao *et al.*, 2000). However, the increased availability of transposon query sequences indicates that the transposon content of the *O. sativa* ssp. *japonica* genome is at least 35% (IRGSP, 2005). Availability of several well-characterized wild relatives provides the material necessary to analyze the impact of TEs on genome evolution and speciation. Recently, a new class (mPing and Dasheng) of markers based on transposable elements (TEs) have been developed, which provides a ubiquitous source of polymorphism and genome size variation in eukaryotes (Zhang and Wessler, 2004; Kazazian, 2004). Since, transposable elements are known to play a vital role in speciation/evolution, markers based on such elements might give a better account of genetic relationships between different varieties, sub-species and/or species in rice. Mini-inverted transposable elements (mPing; Jiang *et al.*, 2003; Kikuchi *et al.*, 2003) and non-autonomous (Dasheng, Edwards *et al.,* 2004) have been used for rice genome analysis and provided important clues for rice evolution. “Master” or limited amplification model given by Deininger et al. (1992) concluded that most TE copies (of retro types) in the genome arouse from a few active master copies and that different sub-families were active at different evolutionary periods. Therefore, TE sub-families that are active after the radiation of two sub-species (or two phylogenetic groups) should generate new copies at specific loci not shared between the species.

Evolutionary studies suggest that some of the MITEs (miniature inverted-repeat transposable elements; Bureau and Wessler, 1992; Zhang *et al.*, 2001b) in plants and animal genomes have spread recently (Feschotte *et al*, 2002). A new class of transposon in MITE family, called miniature Ping (mPing) belongs to class II transposable elements, transposes by cut and paste mechanism, was observed in genomes of *Oryza sativa*. The sequences of mPing are highly conserved: the nucleotide sequences of 34 members (type A1) of this family are completely identical, and the other seven members have only one base change from type A1 members (Kikuchi *et al*, 2003). The copy number of mPing (60-80) even in *japonica* rice is much lower than those of other MITE’s (>1000’s) suggests that the amplification of mPing elements may be currently at an early stage of MITE accumulation, because *japonica* rice has evolved from *O. rufipogon* in a relatively short period of time (10,000 years ago).

A sequence classified as a Tourist-like MITE of 430 base pairs, called miniature Ping (mPing) has been reported to be present in Nipponbare (~70 copies) and in 93-11 (14 copies) (Jiang *et al.*, 2003). Since the domestication of rice mPing MITEs have been amplified preferentially in cultivars adapted to environmental extremes—a situation that is reminiscent of the genomic shock theory for transposon activation. The difference in the estimated copy number of mPing elements in a *japonica* (Nipponbare) and an *indica-japonica* (93-11) genome (70 versus 14 copies) suggested the recent amplification of this MITE family, perhaps since domestication. To assess the timing of amplification, Jiang *et al.* (2001) carried out transposon display with a series of *O. sativa* DNAs to determine the approximate copy number of mPing and Pong elements. Temperate *japonica*s contained the largest number of different mPing-anchored amplicons, whereas the tropical *japonica*s had the least. This marked difference in mPing copy number between the two sub-groups of *japonica* is significant because the temperate and tropical cultivars are thought to have diverged some 5,000–7,000 years ago and are more closely related to each other than either is to *indica*. Our results were in agreement with that of Jiang *et al.* (2002) as only temperate *japonicas* (Nipponbare, TNG67 and Taipei 309) and cv. 9311 (*japonica-indica*) rice genotypes showed the presence of most of the mPing elements. These elements were largely missing in *indica* and Basmati rice genotypes but were present in wild rice genotypes (*O. glaberrima*, *O. nivara*, *O. barthii*, *O. rufipogon*, *O. glumaepatula* and *O. meridionalis*) at variable frequencies.

The observation that mPing elements seem to have undergone amplification more extensively in temperate than in tropical *japonica* cultivars can be explained by an alternative hypothesis. As mentioned above, temperate and tropical *japonicas* are thought to have diverged from a common ancestor since domestication. These two varietal groups are adapted to radically different temperature and water regimes. Tropical *japonica* cultivars (previously known as *javanica*) are broadly adapted to tropical and subtropical environments, whereas temperate *japonica*s represent an evolutionary extreme, having been selected for productivity in cool, temperate zones with very short growing seasons. Thus, in a situation reminiscent of the genome shock theory, stress activation of mPing elements during the domestication of temperate *japonica*s, followed by their preferential insertion into genic regions, might have diversified these cultivars and hastened their domestication by creating new allelic combinations that might be favoured by human selection. Notably, it was speculated that an apparent insertion in an intron of the rice homologue of the CONSTANS gene (Hd1) was responsible for a quantitative change in flowering time (Jiang *et al.*, 2003). On close inspection, this insertion is identifiable as an mPing element.

Nakajaki *et al.* (2003) showed that mPing has a lower copy number in *indica* subspecies than in *japonica* subspecies. Therefore, mPing is a newly originated MITE family in the rice genome whose amplification began after differentiation into the *japonica* and *indica* subspecies. Kikuchi *et al.* (2003) studied the mobilization of mPing and found that *japonica* has pattern with many bands, *indica* and *O. rufipogon* have a pattern consistent with fewer mPing elements. These results indicate that mPing was amplified in the *japonica* lineage after divergence from *O. rufipogon*. None of the *indica* and *O. rufipogon* cultivars had a ping sequence. This might account for the lower number of mPing elements in these species than in *japonica* rice. Copy number of mPing (60-80) even in *japonica* rice is much lower than those of the other MITEs (1000s) suggests that the amplification of the mPing may be currently at an early stage of MITEs accumulation, because *japonica* rice has evolved from *O. rufipogon* in a relatively short time period. It was observed that mPing seems to be inserted in the genic region like other MITEs, it should be an useful molecular tool for gene isolation and gene knockouts in crop species.

Presence of mPing elements in some of wild rice genotypes though at low frequency indicates that these elements may have evolved long back but had greater amplification during speciation of japonica lineages, temperate and tropical. However, more research is required to confirm this hypothesis.

Dasheng is among the rice transposable elements of highest copy number (800-1300) and most recent amplification. Dasheng elements are concentrated in the gene-poor pericentromeric regions of the chromosomes, which might be the reason for Dasheng's success in the small genome of rice.

Dasheng primers detected high level of polymorphism within *indica* and *japonica* subspecies indicating that Dasheng elements can serve as a valuable marker system. Dasheng elements were present at high frequencies in cv. 9311 (75.0%) and *japonica* rice varieties (54.2%). These elements were also present albeit at low frequencies in *indica* (17.9%), traditional Basmati (21.4%), cross-bred Basmati (24.0%) and wild rice genotypes (13.9%). Frequency of Dasheng elements in temperate *japonica* rice genotypes (64.6%) was higher in comparison to tropical *japonica* rice varieties (43.75%). Data shows that Dasheng elements is wide spread in *Oryza* genus which supports the hypothesis that this family originated earlier than or around the time of speciation, approximately 5 to 10 million years ago (Kellogg, 2001; Jiang *et al.*, 2002). However their abundance in *japonica* rice genotypes is indicative of its recent activity and amplification after speciation of sub-species *japonica*.

While TE based marker analysis was able to differentiate between the tropical and temperate *japonica* rice varieties, SSR analysis failed to do so. This clearly demonstrates the importance of TE based markers, mPing and Dasheng elements in case, for studies on rice speciation/evolution.

The values of Mantel test correlation showed a positive correlation (r = 0.655) between the two marker type which are known to target different sequences in the genomic fractions. SSR markers more or less equally distributed on the entire genome amplify short repeat sequences whereas TE based markers amplifies the sequences inserted in the genome during the course of evolution. Marker-based differences in the genetic relationships between rice genotypes do emphasize the need of using a combination of different marker systems for a comprehensive genetic analysis.

## CHAPTER -VI

# Summary and Conclusion

The present investigation was undertaken to prepare a DNA fingerprint database of 50 rice genotypes including traditional Basmati, *indica*, *japonica*, cross-bred Basmati and wild rice genotypes using SSR and transposon element (mPing and Dasheng) based markers. The database was used to identify markers that can differentiate within/among Basmati and non-Basmati rice varieties and to determine the genetic relationships among the various rice genotypes/groups.

Microsatellite (SSR) marker analysis of 50 rice genotypes using 50 primer pairs showed the amplification of a total of 341 alleles. The number of alleles ranged from two (RM321 to sixteen (RM252), with an average of 6.8 alleles per locus. SSR markers with di-nucleotide repeat detected 7.1 alleles per locus followed by those with tri-nucleotide (6.3 alleles per locus) and tetra-nucleotide (4.0 alleles per locus) repeats. The overall size of amplified products ranged from 74 bp (RM248) to 389 bp (RM422). The size difference between the smallest and largest allele at a given SSR locus varied from 3 (RM323) to 150 (RM582). A total of 40 unique alleles (11.7%) were observed at 24 of the 50 SSR loci. Maximum number of unique alleles was observed at RM229 and RM255 loci (4 alleles each) followed by 3 alleles each at RM547, RM259 and RM580. Null alleles were observed at 31/50 SSR loci in 35 genotypes. Highest frequency of null allele was observed at RM252 (eight) followed by RM424 (five) and RM527 (five) loci. *O. meridionalis* and *O. rufipogon* showed the largest proportion of null alleles of 7 and 5, respectively. Multiple alleles (when two clusters of bands were observed for a particular marker) were observed in one or more genotypes at 31/50 SSR loci with an average of 0.62 alleles per locus. At RM488 and RM562, multiple alleles were detected at 11 and 10 rice genotypes, respectively. CSR 30 and Sharbati had multiple alleles at as many as five SSR loci. The frequency of most common allele ranged from 13% (RM252) to 60% (RM255). On average 43.6% of the rice genotypes shared a common allele at any given locus. PIC values ranged from 0.375 to 0.922, with an overall average of 0.698 per locus and showed a positive correlation of 54% (Significant at 0.01 level) with number of alleles at a SSR locus.

Jaccard similarity coefficient data based on 'simqual' sub-program of NTSYS-PC software was used to calculate the coefficient values among the various rice genotypes. Highest similarity coefficient of 0.6349 was observed between Nipponbare/Taipei 309. Among the traditional Basmati rice varieties Basmati 370/HBC 19 and Basmati 370/Type III were quite close with similarity coefficient values of 0.5385 and 0.5000. A Similarity coefficient of 0.5147 between the two salt tolerant *indica* rice genotypes, CSR 1/CSR 10, was observed. Cross-bred Basmati rice variety CSR 30 showed a similarity coefficient of 0.6000 with HBC 19. Traditional Basmati/traditional Basmati, *japonica/japonica*, and *indica/indica* showed mean similarity of 0.320, 0.300, and 0.225, respectively. Traditional Basmati group had greater similarity with *indica* (0.165) compared to *japonica* (0.112) rice groups. *Japonica/indica* group had a mean similarity of 0.074. Among the six wild rice groups the traditional Basmati rice group had maximum similarity with *O. glumalpatula* (0.122) followed by *O. glaberrima* (0.115) and *O. barthii* (0.114); these similarity values were much higher compared to corresponding values involving *indica* or *japonica* rice group instead of traditional Basmati group.

The SSR profiles/diversity data were used to determine genetic relationship among the various rice varieties using NTSYS-PC, PCA and PowerMarker software. The NTSYS-PC UPGMA cluster tree analysis led to the grouping of 50 varieties in two major groups at a similarity coefficient of 0.09. Major group I is further divided into two major-sub groups. Major-sub group I comprised of all the *japonica* rice varieties, 9311 (*japonica–indica*) and *O. nivara* genotypes, with a similarity coefficient of 12%. Major sub group II comprised most of the wild genotypes (*O. rufipogon, O meridionalis, O. nivara and O. glumalpatula*) except *O. glaberrima* and *O. barthii.* Major group II is divided into two major sub groups at a similarity coefficie nt of 10%. Major sub group I was further divided into two subgroups with sub-sub group I consisting of all the *indica*, some of the cross-bred Basmati (Pusa Sugandha2, Pusa Sugandha 3, Pusa Sugandha 4, HKR228, Mahisugandha, Sharbati, Sabarmati and Improved Sabarmati) and traditional Basmati cv. Basmati 217. Sub-sub group II comprised mainly of the traditional Basmati and some of the cross-bred Basmati genotypes. Major sub group II had only one genotype *O. barthii.* The clustering of rice varieties obtained by two-dimensional and three-dimensional PCA scaling were essentially similar to that obtained by NTSYS-PC (UPGMA) tree clustering. Most of the rice genotypes were placed in four major clusters of Basmati, *japonica*, *indica* and the wild rice genotypes. The traditional Basmati varieties except Basmati 217 and some cross-bred Basmati rice varieties were clustered together in one group. *Japonica* rice varieties formed another group and *indica* varieties with some of the cross-bred Basmati varieties formed a separate cluster. Cross–bred Basmati (CSR 30, Pusa Basmati 1, HKR 228) were closer to the traditional Basmati group than the *indica* rice group. All the wild rice genotypes formed a separate group placed between *indica* and *japonica* rice groups. The PCA analysis also showed a close relationship between two Aus genotypes, KR 1-24 and BR 4-10, which were closer to the wild rice group. The groups formed by

PowerMarker analysis were essentially the same as observed by NTSYS-PC UPGMA and PCA analysis.

Several SSR markers have been identified which can be used to differentiate between the closely related traditional Basmati rice genotypes and traditional Basmati genotypes from cross-bred Basmati, *indica* and *japonica* rice genotypes. In seven traditional Basmati varieties, RM224 amplified five alleles followed by amplification of four alleles at RM594, RM551, RM321, RM541, RM 562, RM426, RM252, RM488 and RM547 loci. These SSR markers can be effectively used to differentiate between closely related seven traditional Basmati rice varieties. RM252, RM547, RM222, RM541 and RM562, amplified 6-8 alleles in 12 cross-bred Basmati rice genotypes and are considered best for differentiating among them. The *japonica* rice genotypes can be distinguished from each other by allelic polymorphism at RM400 and RM248, which amplified four alleles in six *japonica* genotypes. RM252 and RM547 amplified eight and seven alleles, respectively, in 15 indica rice genotypes and can be used to distinguish between *indica* genotypes. These markers can be used in combination for varietal identification.

A total of 30 TE based markers including 16 Dasheng elements specific, nine Nipponbare specific mPing elements and five cv. 9311 specific mPing elements, were used in 50 rice genotypes. In this case, PIC values ranged from 0.110 to 0.374, with an overall average of 0.258 per locus. Dasheng elements were present in high frequencies in cv. 9311 (75%) followed by *japonica* rice group (54.2%), cross-bred Basmati (24%) and traditional Basmati (21%) rice groups. Frequencies of Dasheng elements were lowest in the wild rice genotypes (13.9%).

Nipponbare mPing elements were present in highest frequency in cv. 9311 (88%) followed by wild rice (44.4%) and *japonica* rice (37.0%) genotypes. Nippon mPing elements were detected at relatively higher frequencies in temperate *japonica* (74.06%) compared to that (11.11%) in Azucena (tropical *japonica*) and were absent in other two bred tropical *japonica* (NPTII and NPTIII) rice varieties. In traditional Basmati and *indica* rice groups, they were present at very low frequencies ranging from 1.9% to 3.0%. The frequencies of 9311 mPing elements were maximum in *indica* rice (44%) followed by cross-bred Basmati (21.6%), traditional Basmati (12.7%) and cv. 9311 (6.6%). These elements were detected at a relatively low frequency in wild (6.6%) rice genotypes. Among *japonicas*, 9311 mPing elements were detected only in Azucena (tropical).

High level of variation was also obtained among the traditional Basmati rice varieties for the presence of the TE elements. It is interesting to note that Dasheng C7 was present in all the traditional Basmati rice varieties while Dasheng E3, Dasheng F1 and Dasheng G3 were present in 5 of the seven traditional Basmati and absent in Karnal Local and Basmati 217. The mPing elements were entirely missing in three (HBC 19, Basmati 370 and TypeIII) of the seven traditional Basmati rice varieties, while other traditional Basmati showed the presence of these mPing elements at 1, 2, and 3 of the 14 loci.

Jaccard similarity coefficient data based on 'simqual' sub-program of NTSYS-PC software was used to calculate the similarity coefficient values among the various rice genotypes.

Group wise mean similarity values of 0.669, 0.420 and 0.545 were observed for traditional Basmati/traditional Basmati, *japonica*/*japonica* and *indica*/*indica*, respectively. Traditional Basmati had grater mean similarity with *indica* (0.514) compared to *japonica* (0.400) rice groups. Among the wild rice types, traditional Basmati had grater similarity with *O. glumalpatula* (0.348) followed by *O. meridionalis* (0.327) and *O. nivara* (0.038); these values were higher compared to corresponding mean similarity values involving *indica* or *japonica* rice groups in place of traditional Basmati group.

The UPGMA cluster tree analysis led to the grouping of 50 varieties comprising traditional Basmati, *indica*, *japonica*, and wild rice genotypes in two major groups at a similarity coefficient of 0.18. Major group I comprised of three temperate *japonica* rice varieties (Nipponbare, Taipei 309, TNG 67 and *japonica-indica* cv. 9311. The major group II includes all the wild rice genotypes *indica*, traditional Basmati, cross-bred Basmati and tropical *japonica* rice genotypes. Most of the traditional Basmati rice varieties (except Basmati 217 and Karnal Local), cross-bred Basmati (CSR 30, Super Basmati, Kasturi, Pusa Basmati 1, HKR 228, Sabarmati) and Azucena (scented aromatic *japonica*) rice genotype formed a separate group. Genetic relationship as determined by NTSYS-PC two-dimensional and three-dimensional PCA scaling of various rice genotypes was essentially similar to that obtained by NTSYS-PC (UPGMA) tree clustering. The three temperate *japonica* rice and 9311 (*japonica-indica*) varieties formed a distinct cluster at left upper corner of the PCA. All the wild rice genotypes formed a distinct cluster at the left bottom corner and all the other rice genotypes were scattered in the right half. Traditional Basmati except Basmati 217 and Karnal Local and cross-bred Basmati were clustered in the right upper region. The status of three tropical *japonica* rice varieties and the five traditional Basmati varieties except Basmati 217 and Karnal Local and *indica* rice varieties remains the same as identified by NTSYS-PC, UPGMA. The groups formed by PowerMarker analysis were essentially the same as observed by NTSYS-PC UPGMA and PCA analysis.

Mantel test of significance (Mantel, 1967) showed a positive correlation (correlation coefficient r = 0.655) between SSR and TE based markers.

# Bibiliography

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999). Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theoretical and Applied Genetics* **98**: 1320-1328.

Aggarwal RK, Shenoy VV, Ramadevi J, Rajkumar R, Singh L (2002). Molecular characterization of some Indian Basmati and other elite rice genotypes using fluorescent- AFLP. *Theoretical and Applied Genetics* **105**: 680-690.

Ahmadian A, Gharizadeh B, Gustafsson AC, Sterky F, Nyren P, Uhlen M and Lundeberg J (2000) Single-nucleotide polymorphism analysis by pyrosequencing. *Anal Biochem* **280**: 103–110.

Ahn SN, Bollich CN, McClung AMand Tanksley SD (1993) RFLP analysis of genomic regions associated with cooked-kernel elongation in rice. *Theoretical and Applied Genetics* **87**: 27-32.

Ahuja SC, Panwar DVS, Ahuja U, Gupta KR (1995) *Basmati Rice-The Scented Pearl*. CCS Haryana Agricultural University, Hissar (India). p 63.

Akagi H, Yokozeki Y, Inagaki A, Fujimura T (1996) Microsatellite DNA markers for rice chromosomes. *Theoretical and Applied Genetics* **93**: 1071-1077.

Akagi H, Yokozeki Y, Inagaki A, Fujimura T (1997) Highly polymorphic microsatellites of rice consist of AT repeats and a classification of closely related cultivars with microsatellite loci. *Theoretical and Applied Genetics* **94**: 61-67.

Alderborn A, Kristofferson A and Hammerling U (2000) Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. *Genome Research* **10**: 1249–1258.

Aluko G, Martinez C, Tohme J, Castano C, Bergman C and Oard JH (2004) QTL mapping of grain quality traits from the interspecific cross *Oryza sativa*× *O. glaberrima*. *Theoretical and Applied Genetics* **109**: 630–639.

Anderson JA, Churchill GA, Autique JE, Tanksley SD and Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. *Genome* **36**: 181-186.

Arcot SSZ, Wang JL, Weber PL, Deininger and Batzer MA (1995) Alu repeats: a source for the genesis of primate microsatellites. *Genomics* **29**: 136-144.

Ashikawa I, Fukuta Y, Tamura K, Yagi T (1999). Application of AFLP technique that uses non-radioactive fluorescent primers to the detection of genetic diversity in Japanese rice cultivars and cloning of DNA sequences derived from an *indica* genome. *Breeding Science* **49**(**4**): 225-231.

Bao JS, Zheng XW, Xia YW, He P, Shu QY, Lu X, Chen Y, and Zhu LH (2000)QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **100**: 280–284.

Bao Z and Eddy SR (2002) Automated de novo identification of repeat sequence families in sequenced genomes. *Genome Research* **12**: 1269–1276.

Batley J, Barker G, O'Sullivan H, Edwards KJ and Edwards D (2003a) Mining for single nucleotide polymorphisms and insertions/

deletions in maize expressed sequence tag data. *Plant Physiology* **132**: 84–91.

Batley J, Mogg R, Edwards D, O'Sullivan H and Edwards KJ (2003b) A high-throughput SNuPE assay for genotyping SNPs in the flanking regions of *Zea mays* sequence tagged simple sequence repeats. *Molecular Breeding* **11**: 111–120.

Bessey CE (1906) Crop improvement by tilizing wild species. American Breeders' Association **II**: 112–118.

Bhasin VK (2000) India and the emerging global rice trade. In: Singh RK, Singh US, Khush GS (eds) Aromatic rices. Oxford & IBH, New Delhi, pp. 252-276.

Blair MW, Hedetale V and McCouch SR (2002) Fluorescentlabeled microsatellite panels useful for detecting allelic diversity in cultivated rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **105**: 449–457.

Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **98**: 780-792.

Bligh HFJ (2000) Detection of adulteration of Basmati rice with non-premium long-grain rice. *International Journal of Food Science and Technology* **35**: 257-265.

Bligh HFJ, Blackhall NW, Edwards KJ and McClung AM (1999) Using Amplified Fragment length polymorphisms and Simple Sequence Length Polymorphisms to identify cultivars of Brown and White Milled Rice. *Crop Science* **39**: 1715-1721.

Braquart C and Buhin VR (1999) DEC: a new miniature inverted-repeat transposable element from the genome of the beetle Tenebrio molitor. *Insect Molecular Biology* **8**: 571–574.

Bres-Patry, C, Loreux, M, Clement, G, Bangratz, M, and Ghesquiere, A (2001) Heredity and genetic mapping of domestication related traits in a temperate *japonica* weedy rice. *Theoretical and Applied Genetics* **102**: 118–126.

Burbank L (1914) How plants are trained to work for man, Volume 1. New York: P. F. Collier and Son. 302 p.

Bureau TE and Wessler SR (1992). *Tourist*: A large family of small inverted repeat elements frequently associated with maize genes. *Plant cell* **4**: 1283-1294.

Bureau TE, Ronald PC and Wessler SR (1996) A computer based systematic survey reveals the predominance of small inverted-repeat elements in wild-type rice genes. *Proceedings of National Academy of Sciences* USA, **93**: 8524-8529.

Buttery RG, Ling LC, Juliano BO, Turnbough JG (1983) Cooked rice aroma and 2-acetyl, 1- Pyrrolline. *Journal of Agricultural Food Chemistry* **31**: 823-826.

Casa, A. M., C. Brouwer, A. Nagel, L. Wang, Q. Zhang et al., 2000 The MITE family heartbreaker (Hbr): molecular markers in maize. *Proceedings of National Academy of Sciences* USA **97**: 10083–10089.

Causse M, Fulton TM, Cho YG, Ahn SN, Wu K, Xiao J, Chunwongse J, Yu Z, Ronald PC, Harrington SE, Second GA, McCouch SR and Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**: 1251-1274.

Chang TT (1975) Exploration And Survey In Rice. Crop Genetic Resources For Today And Tomorrow. International Biological Programme, Vol 2. Cambridge, UK: Cambridge University Press, 159 -165.

Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. *Euphytica* **25**: 425-441.

Chang TT (1984) Conservation of rice genetic resources: luxury or necessity? *Science* **224**: 251–256.

Charlesworth B, Sniegowski P and Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**: 215-220.

Chen M, Presting G, Barbazuk WB, Goicoechea JL, Blackmon B, Fang G, Kim H, Frisch D, Yu Y, Sun S, Higingbottom S, Phimphilai J, Phimphilai D, Thurmond S, Gaudette B, Li P, Liu J, Hatfield J, Main D, Farrar K, Henderson C, Barnett L, Costa R, Williams B, Walser S, Atkins M, Hall C, Budiman MA, Tomkins JP, Luo M,Bancroft I, Salse J, Regad F, Mohapatra T, Singh NK, Tyagi AK, Soderlund C, Dean RA and Wing RA (2002) An integrated physical and genetic map of the rice genome. *Plant Cell* **14**: 537–545.

Chen M, SanMiguel P, de Oliveira AC, Woo SS, Zhang H, Wing RA and Bennetzen JL (1998) Microcolinearity in sh2-homologous regions of the maize, rice and sorghum genomes. *Proceedings of National Academy of Sciences* USA **94**: 3431-3435.

Chen X, Cho YG and McCouch SR (2002) Sequence divergence of rice microsatellites in Oryza and other plant species. *Molecular Genetics and Genomics* **268**: 331–343.

Chen X, Temnykh S, Xu Y, Cho YG and McCouch SR (1997) Development of microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **95**: 553-567.

Cheng, KS (1985) A statistical evaluation of the classification of rice cultivars into hsien and keng subspecies. *Rice Genetics Newsletter* **2**: 46–48.

Cho YG, Blair MW, Panaud O, McCouch SR (1996). Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphisms (AFLP) from silver stained polyacrylamide gels. *Genome* **39**: 373-378.

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N and Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and gene bank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **100**: 713-722.

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM and Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa.* L.). *Theoretical and Applied Genetics* **97**: 370-380.

Clayton WD and Renvoize SA (1986) Genera graminum. Grasses of the world. Key Bulletin Series XIII. London, Her Majesty's Stationary Office.

Coburn JR, Temnykh SV, Paul EM and McCouch SR (2002). Design and application of microsatellite marker panels for semiautomated genotyping of rice (*Oryza sativa* L.). *Crop Science* **42**: 2092-2099.

Colbert T, Till BJ, Tompa R, Reynolds S, Steine MN, Yeung AT, McCallum CM, Comai L and Henikoff S (2001) High-throughput screening for induced point mutations. *Plant Physiology* **126**: 480–484.

Davierwala AP, Chowdari KV, Kumar S, Reddy APK, Ranjekar PK, Gupta VS (2000) Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica-Dordrecht* **108**(**3**): 269-284

Davierwala AP, Reddy APK, Lagu MD, Ranjekar PK and Gupta VS (2001) Marker assisted selection of bacterial blight resistance genes in rice. *Biochem. Genet.* **39**: 261-278.

Deininger PL (1989) SINES: short interspersed repeated DNA elements in higher eukaryotes. In Mobile DNA (Berg, D. and Howe, M., eds), pp. 619–636, American Society of Microbiology.

Deininger, PL (1989) SINES: short interspersed repeated DNA elements in higher eukaryotes. In Mobile DNA (Berg, D. and Howe, M., eds), pp. 619– 36, American Society of Microbiology.

Deininger, PL, Batzer, MA, Ill, Hutchinson, CA and EdgeR, MH (1992) *Trends in Genetics* **8***,* 307-311.

Delseny M, Sales J, Richard C, Sallaud C, Regad F, Lagoda P (2001) Rice genomics: present and future. *Plant Physiology and Biochemistry* **39**: 323–334.

Drenkard E, Richter BG, Rozen S, Stutius LM, Angell NA, Mindrinos M, Cho RJ, Oefner PJ, Davis RW and Ausubel FM (2000) A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. *Plant Physiology* **124**: 1483–1492.

Duwick DN (1984) Genetic diversity in major farm crops on the farm and reserve. *Econ. Bot.* **32**: 161–178.

Edwards JD, Lee VM and McCouch SR (2004) Sources and predictors of resolvable indel polymorphism assessed using rice as a model. *Molecular Genetics Genomics* **271**: 298-307.

Evenson RE (1998) The economic value of genetic improvement in rice. In *Sustainability of Rice in the Global Food System* (Dowling, N.G. *et al.*, eds), pp. 303–320, Pacific Basin Center, San Francisco, CA, USA, and International Rice Research Institute, Philippines.

Feschotte C and Mouche`s C (2000a) Evidence that a family of miniature inverted-repeat transposable elements (MITEs) from the *Arabidopsis thaliana* genome has arisen from a pogo-like DNA transposon. *Molecular Biology and Evolution* **17**: 730-737.

Feschotte C and Wessler SR (2002b) Mariner-like transposases are widespread and diverse in flowering plants. *Proceedings of National Academy of Sciences* USA **99**: 280–285.

Feschotte C, Jiang N. and Wessler SR (2002a) Plant transposable elements: where genetics meets genomics. *Nature Review Genetics* **3**: 329–341.

Feschotte C, Swamy L and Wessler SR (2003) Genome-wide analysis of mariner-like transposable elements in rice reveals complex relationships with Stowaway MITEs. *Genetics* **163**: 747-758.

Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN and Willimitzer L (2000) Metabolite profiling for plant functional genomics. *Nature Biotechnology* **18**: 1157–1161.

Fu PY, Pai C (1979). Genetic studies on isozymes in rice plant. 2. Classification and geographical distribution of cultivated rice through isozyme studies (in Chinese with English summary). *Journal of Agricultural Association of China* **107**: 1-16.

Gao Li-Zhi and Zhang Chi-Hong (2005) Comparisons of Microsatellite variability and population genetic structure of two endangered wild rice species, *Oryza rufipogon* and *O. officinalis*, and their conservation implications. *Biodiversity and Conservation* **14**: 1663–1679.

Gao LZ, Ge S and Hong DY (2000) A preliminary study on ecological differentiation within common wild rice *Oryza rufipogon* Griff. (In Chinese with English abstract). *Acta Agronomica Sinica* **26**: 210–216.

Gao LZ, Ge S and Hong DY (2000a) Allozymic diversity and genetic structure of common wild rice *Oryza rufipogon* Griff., China. *Theoretical and Applied Genetics* **101**: 494–502.

Gao LZ, Ge S and Hong DY (2002a) Low levels of allozyme variability and conservation genetics of common wild rice *Oryza rufipogon* Griff. from Yunnan, China. *Euphytica* **124**: 273–281.

Gao LZ, Schaal BA, Zhang CH, Jia JZ and Dong YS (2002b) Assessment of population genetic structure of common wild rice *Oryza rufipogon* Griff. detected by microsatellite DNA and allozyme loci. *Theoretical and Applied Genetics* **106**: 173–180.

Gao LZ, Zhang CH, Chang LP, Jia JZ, Qui ZE and Dong YS (2005) Microsatellite diversity within *Oryza sativa* with emphasis on *indica-japonica* divergence. *Genetics Research, Cambridge* **85**: 1-14.

Garg K, Green P, Nickerson DA (1999) Identification of candidate coding region single nucleotide polymorphisms in 165 human genes using assembled expressed sequence tags. *Genome Research* **9**: 1087–1092.

Garland S, Lewin L, Blakeney A, Reinke R and Henry R (2000) PCR-based molecular markers for the fragrance gene in rice (*Oryza sativa.* L.). *Theoretical and Applied Genetics* **101**: 364-371.

Garland SH, Lewin L, Abedinia M, Henry R and Blakeney A (1999) The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryzas sativa* L.). *Euphytica* **108**: 53-63.

Garris AJ, McCouch SR and Kresovich S (2003) Population Structure and Its Effect on Haplotype Diversity and Linkage Disequilibrium Surrounding the *xa5* Locus of Rice (*Oryza sativa* L.) *Genetics*

Ge S, Sang T, Lu BR and Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetroaploid species. *Proceedings of National Academy of Sciences* USA **96**: 14400–14405.

Ghosh S, Karanjawala ZE and Hauser ER (1997) Methods for precise sizing, automated binning of alleles, and reduction of error rates in large-scale genotyping using fluorescently labeled dinucleotide markers. *Genome Research* **7**: 165-178.

Glaszmann JC (1987). Isozyme and Classification of Asian rice varieties. *Theoretical and Applied Genetics* **74**: 21-30.

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, T Migue, Paszkowski U, Zhang S, Colbert M, Sun W, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A and Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp *japonica*). *Science* **296**: 92–100.

Gowda M, Venu RC, Roopalakshmi K, Sreerekha MV and Kulkarni RS (2003) Advances in rice breeding, genetics and genomics. *Molecular Breeding* **11**: 337–352.

Grosch W, Schieberle P (1997) Flavor of cereal products-a review. *Cereal Chemistry* **74**: 91-97.

Gupta PK, Roy JK and Prasad M (2001) Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current Science* **80**(**4**): 524-535.

Harlan JR (1972a) Breeding success brings a peril. *Crops Soils* **72**: 5–6.

Harrington, S (2000) A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers. MS thesis, Cornell University, Ithaca, NY.

Harushima Y, Jano M, and Shomura A (1998) A high density rice genetic linkage map with 2275 markers using a single $F_2$ population, *Genetics* **148**: 479-494.

Hayashi K, Hashimoto N, Daigen M, Ashikawa I (2004) Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus O. *Theoretical and Applied Genetics* **108**:1212–1220.

Henikoff S, Greene EA, Pietrokovski S, Bork P, Attwood TK and Hood L (1997) Gene families: the taxonomy of protein paralogs and chimeras. *Science* **278**: 609–614.

Hess KR, Zhang W, Baggerly KA, Stivers DN and Coombes KR (2001) Microarrays: handling the deluge of data and extracting reliable information. *Trends in Biotechnology* **11**: 463–468.

Hirohiko Hirochika (2001) Contribution of the *Tos17* retrotransposon to rice functional genomics. *Current Opinion in Plant Biology* **4**: 118–122.

International Human Genome Sequencing Consortium(2001) *Nature* **409**: 860.

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. *Nature* **436**: 793-800.

Ishii T, Xu Y, McCouch SR (2001). Nuclear and chloroplast microsatellite variation in A-genome species of rice (*Oryza spp.*). Genome (In press).

Jack Harlan:*The Living Fields: Our Agricultural Heritage* (Cambridge Univ. Press, New York, 1995), pp.30–31.

Jackson MT (1994) Preservation of rice strains. *Nature (London)* **371**: 470.

Jackson MT (1997) Conservation of rice genetic resources: the role of the International Rice Genebank at IRRI. *Plant Molecular Biology* **35**: 61–67.

Jackson MT and Juggan R (1993) Sharing the diversity of rice to feed the world. *Diversity* **9**: 22-25.

Jain N (2003) Assessment of molecular polymorphism among commercially important Basmati rice varieties using PCR based markers. Ph.D thesis, CBSH, CCS Haryana Agriculture University, Hisar.

Jain S, Jain RK and McCouch S (2004) Genetic analysis of aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theoretical and Applied Genetics* **109**: 965–977.

Jarvik T and Lark KG (1998) Characterization of Soymar1, a Mariner element in soybean. *Genetics*, **149**: 1569-1574.

Ji HS, Koh HJ, Park SU, McCouch SR (1998) Varietal identification in japonica rice using microsatellite DNA markers. *Korean Journal of Breeding* **30**: 350-360.

Jiang N and Wessler SR (2001) Insertion preference of maize and rice miniature inverted repeat transposable elements as revealed by the analysis of nested elements. *Plant Cell* **13**: 2553–2564.

Jiang N, Bao Z, Temnykh S, Cheng Z, Jiang J (2002) Dasheng: a recently amplified nonautonomous long terminal repeat element that is a major component of pericentromeric regions in rice. *Genetics* **161**: 1293–1305.

Jiang N, Bao Z, Zhang X, Eddy SR and Wessler SR (2004) Pack-MULE transposable elements mediate gene evolution in plants. *Nature* **431**: 567-573.

Jiang N, Bao Z, Zhang X, Hirochika H, Eddy SR, McCouch SR, Wessler SR (2003) An active DNA transposon family in rice. *Nature* **21**: 163–167.

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000). Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics* **100**: 1311-1320.

Juliano and Duff (1991) Setting priorities for rice grain quality research. Pages 201-211 in Grain Postharvest Research and Development: Priorities for the Nineties. Proceedings of the 12th ASEAN Seminar on Grain Postharvest Technology, Surabaya, Indonesia, 29-31 Aug. 1989. J. 0. Naewbanij, ed. ASEAN Grain Postharvest Programme: Bangkok, Thailand.

Juliano BO (1971) A simplified assay for milled-rice amylose. *Cereal Sci Today* **16**: 334–360.

Juliano, BO (1998) Varietal impact on rice quality. *Cereal Foods World.* **43**: 207-222.

Jurka J and Pethiyagoda C (1995) Sequences from primates: Compilation and analysis. *Journal of Molecular Evolution* **40**: 120-126.

Kato S, Kosaka H and Hara S (1928) On the affinity of rice varieties as shown by the fertility of rice plants. Central Agricultural Institute Kyushu Imp University **2**: 241-276.

Kazazian HH (2004) Mobile Elements: Drivers of Genome Evolution. *Science* **303**: 1626-1632.

Kazazian, HH (2004) Mobile Elements: Drivers of Genome Evolution. *Science* **303**: 1626-1632.

Kazuo Tsugane, Masahiko Maekawa, Kyoko Takagi, Hiroyuki Takahara, Qian Qian, Chang-Ho Eun and ShigeruIida (2006)An active DNA transposon nDart causing leaf variegation and mutable dwarfism and its related elements in rice. *Plant Journal* **45**: 46–57.

Kellogg EA (2000) The grasses: a case study in macroevolution. *Annual Review of Ecology and Systematics* **31**: 217-238.

Kellogg EA (2001) Evolutionary history of the grasses. *Plant Physiology* **125**: 1198–1205.

Kenji Fujino, Hiroshi Sekiguchi, Tadahiko Kiguchi (2005) Identification of an active transposon in intact rice plants. *Moleular Genetics and Genomics* **273**: 150–157.

Khush GS (1997) Origin, dispersal, cultivation and variation of Wright, S., 1969 *Evolution and the Genetics of Populations*, Vol. 2. rice. *Plant Mol. Biol.* **35**: 25–34.

Khush GS (2000) Taxonomy and Origin of Rice. In: Aromatic Rices (eds Singh RK, Singh US, Khush GS), Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, p 47-69.

Khush GS and Brar DS (2001). Rice genetics from Mendal to functional genomics. In: Rice Genetics IV. Proceedings of Fourth International Rice Genetics Symposium, IRRI, Los, Banos, Philippines, edited by GS Khush, DS Brar, B Hardy, IRRI, Science Publishers, Inc., New Delhi, Los Banos p 3-25.

Khush GS and dela Cruz N (1998) Developing Basmati rices with high yield potential. International Symposium or rice Quality – Nottingham, U.K., November 24-27.

Khush GS and dela Cruz N (2002) Developing Basmati rices with high yield potential. In: R. Duffy (Ed.), Speciality Rices of the World: Breeding, Production and Marketing, pp. 15–18. Science Publisher, Enfield, USA.

Khush GS and Juliano BO (1985) Breeding for high-yielding rices of excellent cooking and eating qualities. Pages 6l-69 in Proceedings, Rice Grain Quality and Marketing, 1985. Intern. Rice Res. Inst., Los Baños, Laguna.

Khush GS and Juliano BO (1991) Research priorities for improving rice grain quality. In: Rice Grain Marketing and Quality Issues, pp. 65–66, IRRI, Manila, Philippines.

Kidwell MG and Lisch DR (2000) Transposable elements and host genome evolution. *Trends Ecology and Evolution* **15**: 95-99.

Kikuchi K, Terauchi K, Wada M, Hirano H (2003) The plant MITE mPing is mobilized in anther culture. *Nature* **421**: 167–170.

King TL, Kalinowski ST, Schill WB, Spidle AP and Lubinski BA (2001) Population structure of Atlantic salmon (*Salmo salar* L.): a rangewide perspective from microsatellite DNA variation. *Molecular Ecology*, **10**: 807–821.

Ko HL, Cowan DC, Henry RJ, Graham GC, Blakeney AB, Lewin LG (1994) Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. *Euphytica* **80**: 179-189.

Kraic J, Zakova M and Gregova (1998) Comparison of differentiation capability of RAPD and SSR markers in commercial barley (*Hordeum vulgare* L.) cultivars. Cereal Research Communication **20**(**4**): 375.

Kunze R and Weil CF (2002) The *hAT* and CACTA superfamilies of plant transposons. In *Mobile DNA II*, Craig, N, Craigie, R., M Gellert, M.

and Lambowitz, A. eds., ASM Press, Washington, DC, pp. 565-610.

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin Sy, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhang HS, Tamura Y, Wang ZX, Momma Y, Umehara Y, Yano M, Sasaki T and Minobe Y (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature Genetics* **8**: 365-375.

Kwon SJ, Ahn SN, Yang CI, Hong HC, Kim YK, Suh JP, Hwang HG, Moon HP, Choi HC (2000). Genetic diversity of korean *japonica* rice cultivars. In: Abstracts. International Rice Genetics Symposium, International Rice Research Institute, Los Banos, Philippines, Oct. 2000. p 41.

Lander ES (2001) Initial sequencing and analysis of the human genome. *Nature* **409**: 860–921.

Lapointe FJ and Legendre P (1992 A statistical framework to test the consensus among additive trees (cladograms). *System Biology* **41**: 158-171.

Le QH, Wright S, Yu Z and Bureau T (2000) Transposon diversity in Arabidopsis thaliana. *Proceedings of National Academy of Sciences USA*, **97**: 7376-7381.

Li Z (2001) QTL mapping in rice: a few critical considerations. In: Khush GS, Brar DS, Hardy B, eds. Rice genetics IV. Los Baños (Philippines): International Rice Research Institute. pp. 153-171.

Li Z and Rutger JN (2000) Geographic distribution and multi-locus structure of isozyme variation in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **101**: 379-387.

Liu XC and Wu JL (1998) SSR heterogenic patterns of parents for making and predicting heterosis in rice breeding. *Molecular Breeding* **4**: 263-268.

Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 50 nuclease assay. *Genet Anal* **14**: 143–149.

Lockhart DJ and Winzeler EJ (2000) Genomics, *gene expression and DNA arrays*. *Nature* **405**: 827-836.

Lorieux M, Ndjiondjop M-N, Ghesquiere A (2000). A first interspecific *Oryza sativa* X *oryza glaberrima* microsatellite based genetic map. *Theoretical and Applied Genetics* **100**: 591-601.

Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquiere A (1996). Aroma in rice: genetic analysis of a quantitative trait. *Theoretical and Applied Genetics* **93**: 1145-1151.

Lu BR, Zheng KL, Qian HR and Zhuang JY (2002) Genetic differentiation of wild relatives of rice as referred by the RFLP analysis. *Theoretical and Applied Genetics* **106**: 101–106.

Lu H, Redus M, Coburn J, Rugter N, McCouch S and Tai T (2005) Population structure and breeding patterns of 145 U.S. rice cultivars based on SSR markers analysis. *Crop Science* **45**: 66-76.

MacBeath G and Schreiber SL (2000) Printing Proteins as Microarrays for High-Throughput Function Determination *Science* Vol. 289. no. 5485, pp. 1760 – 1763.

Mackill DJ (1995) Classifying *japonic*a rice cultivars with RAPD markers. *Crop Science* **35**: 889–894.

Mackill DJ and Li J (2001) Molecular mapping and markerassisted selection for major-gene traits in rice. In: Khush GS, Brar DS, Hardy B, eds. Rice genetics IV. Los Baños (Philippines): International Rice Research Institute. pp. 137-151.

Mackill DJ, Zhang Z, Redona ED and Colowit PM (1996) Level of polymorphism and genetic mapping of AFLP marker in rice. *Genome* **39**: 969-977.

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC and Huang N (1997). Polymorphism, distribution and segregation of AFLP markers in a double haploid rice population. *Theoretical and Applied Genetics* **94**: 39-45.

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209-220.

Mao L, Wood TC and Yu Y (2000) Rice transposable elements: a survey of 73,000 sequence-tagged connectors. *Genome Research* **10**: 982-990.

Marri PR, Sarla N, Reddy LV and Siddiq EA (2005) Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. *BMC Genetics* **6**: 33.

Matsuo T (1952) Genecological studies on cultivated rice Bull National Institute of Agricultural Sciences, Japan **D3**: 1-111.

McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T and Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology* **35**: 88-99.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR and Tanksley SD (1988) Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* **76**: 815-829.

McCouch SR, Temnykh S, Lukashova A, Coburn J, DeClerck G, Cartinhour S, Harrington S, Thomson M, Septiningsih E, Semon M, Moncada P and Li J (2001) Microsatellite markers in rice: abundance, diversity, and applications. In: G.S. Khush, D.S. Brar & B. Hardy (Eds.), Rice Genetics IV, IRRI, Los Ba˜nos, Philippines, pp. 117–135, Science Publishers, New Delhi, India.

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Zing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D and Stein L

(2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research* **9**: 199-207.

Melchinger, AE, Messmer M, Lee M, Woodman WL &. Lamkey KR (1991) Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. *Crop Science* **31**: 669–678.

Meyers BC, Tingey SV and Morgante M (2001) Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. *Genome Research* **11**: 1660–1676.

Miflin B (2000) Crop improvement in the 21st century. *Journal of Experimental Botany* **51**: 1-8.

Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H, Guimaraes E, Tohme J and McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC2F2 population evaluated in an upland environment. *Theoretical and Applied Genetics* **102**: 41-52.

Moore G, Devos KM, Wang Z and Gale MD (1995) Cereal genome evolution: Grasses, line up and form a circle. *Current Biology* **5***:* 737–-739.

Morinaga T (1954) Classification of rice varieties on the basis of affinity. *In*. International Rice Commission, Working party on Rice breeding, Reports of the 15th meeting: 1-14.

Morishima H and Oka HI (1981) Phylogenetic differentiation of cultivated rice, 22. Numerical evaluation of the *Indica-Japonica* differentiation. *Japanese Journal of Breeding* **31**: 402-413.

Mouse Genome Sequencing Consortium, *Nature* **420**: 520 (2002).

Nagaraju J, Kathirvel M, Ramesh Kumar R, Siddiq EA and Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence based ISSR-PCR and SSR markers. *Proceedings of National Academy of Sciences* USA **99**(**9**): 5836-5841.

Nakagahra, M (1978) The differentiation, classification and center of genetic diversity of cultivated rice (*Oryza sativa* L.) by isozyme analysis. *Tropical Agriculture Research Service* **11**: 77–82.

Nakazaki T, Okumoto Y, Horibata A, Yamahira S, Teraishi M, Nishida H, Inoue H and Tanisaka T (2003) Mobilization of a transposon in the rice genome. *Nature* **421**: 170–172.

Nasu S, Suzuki J, Ohta R, Hasegawa K, Yui R, Kitazawa N, Monna L and Minobe Y (2002) Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (*Oryza sativa*, *Oryza rufigogon*) and establishment of SNP markers. *DNA Research* **9**: 163–171.

Nene YL (1998) Basmati rice: a distinct variety (cultivar) of the Indian subcontinent. *Asian-Agri-History* **2**(**3**): 175-188.

Nevo E (1998) Genetic diversity in wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. *Genetic Resources and Crop Evolution*, **45**: 355–370.

Nevo E (2001) Evolution of genome–phenome diversity under environmental stress. PNAS **98**: 6233–6240.

Ni J, Colowit PM, Oster JJ, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using SSLP markers. *Crop Science* **42**: 601-607.

Oka HI (1958) Intervarietal variation and classification of cultivated rice. *Indian Journal of Genetics and Plant Breeding* **18**: 79-89.

Oka HI (1964) Considerations on the genetic basis of intervarietal sterility in *Oryza sativa*. In: *Rice Genetics and Cytogenetics*, 159–174. Symposium on Rice Genetics and Cytogenetics, Manila, The Philippines.

Oka HI (1988) Origin of cultivated rice. Japanese Scientific Societies Press, Tokyo.

Olsen KM and Schaal BA (2001) Microsatellite variation in cassava (*Manihot esculenta*, Euphorbiaceae) and its wild relatives: further evidence for a southern Amazonian origin of domestication. *American Journal of Botany*, **88** (**1**): 131–142.

Olufowote JO, Xu Y, Chen X, Park WO, Beachell HM, Dilday RH, Goto M and McCouch SR (1997) Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genome* **40**: 370-378.

Osterlund MT and Paterson AH (2002). Applied plant genomics: the secret is integration. *Cur. Opinion in Plant Bio.* **5**: 141–145. *Outlook. Progress in Irrigated Rice Research.* International Rice Research Institute, Manila, Philippines

Pai C, Endo T, Oka HI (1975). Genic analysis for acid phosphatase isozymes in *Oryza perennis* and *O.sativa. Canadian Journal of Genetics and Cytology* **17**: 637-650.

Pal S, Jain S, Saini N and Jain RK (2001). DNA isolation from milled rice samples for PCR-based molecular marker analysis. *Rice Genetics Newsletter* **18**: 94-97.

Pal S, Jain S, Saini N, Aarti, Jain RK (2004) Identification of microsatellite markers for differentiating some Basmati and non-Basmati rice varieties. *Indian Journal of Biotechnology* **3**: 519-526.

Panaud O, Chen X and McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.) *Molecular and General Genetics* **252**: 597-607.

Panaud O, Vitte C, Hivert J, Muzlak S, Talag J, Brar D, Sarr A (2002)Characterization of transposable elements in the genome of rice (*Oryza sativa* L.) using representational difference analysis (RDA). *Molecular Genetics and Genomics* **268**: 113-121.

Parsons BJ, Newbury HJ, Jackson MT and Ford-Lloyd BV (1999) The genetic structure and conservation of aus, aman and boro rices from Bangladesh. *Genet. Res. Crop Evol.* **46**: 587–598.

Parsons JB, Newbusy HT, Jackson MT, Ford-Lloyd BV (1997). Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Molecular *Breeding* **3:** 115-125.

Philips RL and Vasil IK eds (2001) DNA-Based Markers in Plants, Kluwer Academic Publishers.

Piperno, DR and Flannery KV (2001) The earliest archaeological maize (*Zea mays* L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. *Proceedings of National Academy of Sciences* USA **98**: 101–2103.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). Comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**: 225-238.

Provan JG, Corbett JW, McNiol and Powell W (1997) Chloroplast DNA variability in wild and cultivated rice (*Oryza* spp.) revealed by polymorphic chloroplast simple sequence repeats. *Genome* **40**: 104–110.

Qian W, Ge S, Hong D-Y (2001). Genetic variation within and among populations of a wild rice *Oryza granulata* from china detected by RAPD and ISSR markers. *Theoretical and Applied Genetics* **102**: 440-449

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology* **5**: 94-100.

Rafalski JA (2002) Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science* **162**: 329-333.

Ramsay L, Macaulay M, Cardle L, Morgante M, degli Ivanissevich S, Maestri E, Powell W and Waugh R (1999) Intimate association of microsatellite repeats with retrotransposons and other dispersed repetitive elements in barley. *Plant Journal* **17**: 415–425.

Ren F, Lu BR, Li S, Huang J and Zhu Y (2003) A comparative study of genetic relationships among the AA-genome Oryza species using

RAPD and SSR markers. *Theoretical and Applied Genetics* **108**: 113–120.

Rohlf FJ (1994) NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.80, Exeter Software, New York.

Saghai- Maroof MA, Soliman KM, Jorgensen RA, Allerd RW (1984). Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of National Academy of Sciences* USA **81**: 8014-8019.

Saini N, Jain N, Jain S and Jain RK (2004) Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica* **140**: 133–146.

Saito A, Yano M, Kishimoto N, Nakagahara M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata N and Sugiura M (1991) Linkage map of restriction fragment length polymorphism loci in rice. *Japanese Journal of Breeding* **41**: 665-670.

SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL (1998) The paleontology of intergene retrotransposons of maize. *Nature Genetics* **20**: 43–45.

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, and Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the maize genome. *Science* **274**: 765–768.

Santiago Moreno-Va´ zquez, Jianchang Ning and Blake C Meyers (2005) hATpin, a family of MITE-like hAT mobile elements conserved in diverse plant species that forms highly stable secondary structures .*Plant Molecular Biology* **58**: 869–886.

Second G (1982) Origin of the genic diversity of cultivated rice (*Oryza* aromatic and quality rice (*Oryza sativa* L.) germplasm using panels spp.): study of the polymorphism scored at 40 isozyme loci. *Japanese Journal of Genetics* **57**: 25–57.

Semon M, Coburn J, Cadalen T, Jones MP, McCouch SR (2001) Microsatellite variation in *O. glaberrima* Stend accessions from West Africa. (Abstracts: Poter P 3b-33). Proceedings Plant and Animal Genome IX conference, San Diego, California, 13-17 Jan, 2001.

Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. *Theoretical and Applied Genetics* **107**: 1433-1411.

Sharma A, Sharma R and Machii H (2000) Assessment of genetic diversity in a *Morus* germplasm collection using fluorescence-based AFLP markers. *Theoretical and Applied Genetics* **101**: 1049-1055.

Sharma GR and Manda D (1980) *Excavations at Mahagara 1977– 1978: A Neolithic Settlement in Belan Valley* (Archaeology of Vindhyas and Ganga Valley 6). Department of Ancient History, Culture and Archeology, Allahabad, India.

Sharma SD (1983) Conservation of wild species of *Oryza*. In: Rice germplasm conservation workshop. The International Rice Research Institute and the International Board for Plant Genetic Resources, pp 21–25.

Shoba, Rani N. 1992 'Research efforts to develop scented quality rices for increased productivity and export purposes' paper presented at the Special Seminar for the Awardees. International Rice Research Conference 21-25 April, 1992, IRRI, P.O. Box 933, Manila, Philippines.

Singh RK, Khush GS, Singh US, Singh AK and Singh S (2000b) Breeding aromatic rice for high yield, improved aroma and grain quality. In: R.K. Singh, U.S. Singh & G.S. Khush (Eds.), Aromatic Rices, pp. 71–105, Oxford & IBH Pub. Co., New Delhi, India.

Singh RK, Singh US, Khush GS, Rohilla R (2000). Genetics and biotechnology quality traits in aromatic rices. In: Aromatic Rices (eds Singh RK, Singh US, Khush GS), Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, p 47-69.

Singh VP, Siddiq EA, Zaman FU, Sadananda AR (1988). Improved Basmati donors. *International Rice Research Newsletter* **13**(**6**): 22-25.

Sitch LA, Dalmacio RD and Romero GO (1989) Crossability of wild Oryza species and their potential use for improvement of cultivated rice. *Rice Genetics Newsletter* **6**: 58–60.

Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK (2004) Allelic diversity among Basmati and non Basmati long grain *indica* rice varieties using microsatellite markers. *Journal of plant Biochemistry and Biotechnology* **13**: 25-32.

Smit AF and Riggs AD (1996) Tiggers and DNA Transposon fossils in the human genome. *Proceedings of National Academy of Sciences.* USA **93**: 1443–1448.

Solheim WG (1972) An earlier agricultural revolution. *Scientific America* **266**: 34-41.

Song ZP, Xu X, Wang B, Chen JK and Lu BR (2003) Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theoretical and Applied Genetics* **107**: 1492-1499.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science* **270**: 1804-1806.

Song ZP, Lu BR, Zhu YG and Chen JK (2003) Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytologist* **157**: 657–665.

Squirrell J, Hollingsworth PM, Woodhead M, Russell J, Lowe AJ, Gibby M and Powell W 2003. How much effort is required to isolate nuclear microsatellites from plants? *Molecular Ecology* **12**: 1339-1348.

Suh HS, Sato YI and Morishima H (1997) Genetic characterization of weedy rice (*Oryza sativa* L.) based on morpho-physiology, isoenzyme and RAPD markers. *Theoretical and Applied Genetics* **94**: 316-321.

Sun CQ, Wang XK and Yoshimura A (2002) Genetic differentiation for nuclear, mitochondrial and chloroplast genomes in common wild rice (*Oryza rufipogon* Gri.) and cultivated rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **104**: 1335–1345.

Sun CQ, Wang XK, Li ZX, Yoshimura A and Iwata N (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (O. sativa L) using RFLP markers. *Theoretical and Applied Genetics* **102**: 157-162.

Talag JD, Brar DS, Li ZK, Jones M, Khush GS (2000). Molecular characterization of introgression from *O. glaberrima* into *O.sativa* using microsatellite markers. In: Abstracts, International Rice Genetics Symposium, International Rice Research Institute, Los Banos, Philippines, Oct. 2000 p. 68.

Tanksley SD and McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* **77**: 1063-1066.

Tanksley SD and Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics* **92**: 191-203.

Tarchini R, Biddle P, Wineland R, Tingey S and Rafalski A (2000) The complete sequence of 340 Kb of DNA around the rice Adh1-Adh2 region reveals interrupted colinearity with maize chromosome 4. *Plant Cell* **12**: 381-391.

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S and McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, Transposon associations, and genetic marker potential. *Genome Research* **11**: 1441-1452.

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T and McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.) *Theoretical and Applied Genetics* **100**: 697-712.

Terao H and Mizushima U (1942) Some consideration on the classification of Oryza sativa L. into two subspecies, so-called *Japonica* and *Indica*. *Japanese Journal of Botany* **10**: 213-258.

Thomson MJ, Tai TH, McClung AM, Hinga ME, Lobos KB, Xu Y, Martinez C, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components, and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theoretical and Applied Genetics* **107**: 479– 493

Tu Z (1997) Three novel families of miniature inverted-repeat transposable elements are associated with genes of the yellow fever mosquito, *Aedes aegypti*. *Proceedings of National Academy of Sciences* USA **94**: 7475–7480.

Tu Z (2001) Eight novel families of miniature inverted repeat transposable elements in the African malaria mosquito, *Anopheles gambiae*. *Proceedings of National Academy of Sciences* USA **98**: 1699–1704.

Turcotte K, Srinivasan S and Bureau T (2001) Survey of transposable elements from rice genomic sequences. *Plant Journal* **25**: 169–179

Uozo S, Ikehashi H, Ohmido N, Ohtsubo H and Ohtsubo E (1997) Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus *Oryza*. *Genome Research*. **10**: 982–990.

Van den Broeck D (1998) Transposon display identifies individual transposable elements in high copy number lines. *Plant Journal* **13**: 121–129.

Varshney RK, Grane A and Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* **23**: 48-55.

Vaughan DA (1989) The Genus *Oryza* L. Current Status Of Taxonomy. IRPS 138. IRRI, Manila, Philippines.

Vaughan DH (1994) The relationship between the genus *Oryza* and other grasses. In: The Wild Relatives of Rice, pp 3-6. International Rice Research Institute. Philippines.

Virk PS, Zhu J, Newbury HJ, Bryan GJ, Jackson MT, Ford-Lloyd BV (2000) Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*oryza sativa*) germplasm. *Euphytica* **112**: 275-284.

Wang Z, Weber JL, Zhong G and Tanksley SD (1994): Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics*, **88**: 1-6.

Wang ZY and Tanksley SD (1989) Restriction fragment length polymorphism in *Oryza sativa* L. *Genome* **32**: 1113–1118.

Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT and Powell W (1997) Genetic distribution of Bare-1-like retro-transposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Molecular Genomics and Genetics* **253**: 687–694.

Weising K, Kaemmer D, Epplen JT, Weigand F, Saxena M, Kahl G (1991) DNA fingerprinting of *Ascochyta rabiei* with synthetic oligonucleotides. *Current Genetics* **19** (**6**): 483-489.

Wessler SR, Bureau TE and White SE (1995) LTR retrotransposons and MITEs: important players in the evolution of plant genomes. *Current Opinion in Genetics Development* **5**: 814-821.

Wu J, Maehara T, Shimokawa T, Yamamoto S, Harada C, Takazaki Y, Ono N, Mukai Y, Koike K, Yazaki J (2002) A comprehensive rice transcript map containing 6591 expressed sequence tag sites. *Plant Cell* **14**: 525–535.

Wu KS and Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular Genetics and Geneomics* **241**: 225-235.

Xiao J, Li J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Yuan L (1996). A wild species contains genes that may significantly increase the yield of rice. *Nature* **384**: 223-224.

Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, Tanksley SD and McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon. Genetics* **150**: 899-909.

Xu Y (2002) Global view of QTL: rice as a model. In: Kang MS, ed. Quantitative genetics, genomics and plant breeding. Wallingford (UK): CAB International. p 109-134.

Yang GP, Saghai-Maroof MA, Zu CG, Zhang Q, Biyashev RM (1994). Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular Genetics and Genomics* **245**: 187-194.

Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS and Li ZK (2003) Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. *Theoretical and Applied Genetics* **108**: 131–140.

Yu, J *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp *indica*). *Science* **296**: 79–92.

Yuan LP, Virmani SS, Mao CX (1989) Hybrid rice: achievements and further outlook. In: Progress in irrigated rice research. International Rice Research Institute, Manila, The Philippines, pp 219–223.

Yun XIN Ye, Zhan Z, Yi-ping X and Long-ping Y (2005) Identification and Purity Test of Super Hybrid Rice with SSR Molecular Markers. *Rice Science* **12**(1): 7–12.

Zhang L, Dawson A and Finnegan D J (2001a) DNA-binding activity and subunit interaction of the *mariner* transposase. *Nucleic Acids Research*. **29**: 3566–3575.

Zhang Q, Saghai Maroof MA, Liu TY, Shen BZ (1992) Genetic diversity and differentiation of *indica* and *japonica* rice detected by RFLP analysis. *Theoretical and Applied Genetics* **83**: 495–499.

Zhang X and Wessler SR (2004) Genome wide comparative analysis of the transposable elements in the related species *Arabidopsis thaliana* and *Brassica oleracea*. *Proceedings of National Academy of Sciences* **101**: 5589-5594.

Zhang X, Feschotte C, Zhang Q, Jiang N and Eggleston WB (2001b) *P Instability Factor*: an active maize transposon system associated with the amplification of *Tourist*-like MITEs and a new superfamily of transposases. *Proceedings of National Academy of Sciences* USA **98**: 12572– 12577.

Zhang XY and Wessler SR (2004) Genome-wide comparative analysis of the transposable elements in the related species *Arabidopsis thaliana* and *Brassica oleracea. Proceedings of National Academy of Sciences* USA **101**: 5589–5594.

Zhao MF, Li XH, Yang JB, Xu CG, Hu RY, Liu DJ and Zhang Q(1999) Relationship between molecular marker hetero zygosity and hybrid performance in intra and inter subspecific crosses of rice. *Plant Breeding* **118**: 139–144.

Zhao Q, Zhang Y, Cheng Z, Chen M, Wang S, Feng Q, Huang Y, Li Y, Tang Y, Zhou B, Chen Z, Yu S, Zhu J, Hu X, Mu J, Ying K, Hao

P, Zhang L, Lu Y, Zhang LS, Liu Y, Yu Z, Fan D, Weng Q, Chen L, Lu T, Liu X, Jia P, Sun T, Wu Y, Zhang Y, Lu Y, Li C, Wang R, Lei H, Li T, Hu H, Wu M, Zhang R, Guan J, Zhu J, Fu G, Gu M, Hong G, Xue Y, Wing R, Jiang J, Han B (2002) A fine physical map of the rice chromosome-4. *Genome Research* **12**: 817–823.

Zhao W, Wang J, He X, Jiao Y (2004) BIG-RIS: An integrated information resource and comparative analysis workbench for rice genomics. *Nucleic Acids Research* **32**: D377-D382.

Zhao X and Kochert G (1992) Characterization and genetic mapping of a short, highly repeated, interspersed DNA sequence from rice (Oryza sativa L.). *Molecular Genetics and Genomics* **231**: 353-359.

Zhao XP and Kochert G (1993) Phylogenetic distribution and genetic mapping of a $(GGC)_n$ microsatellite from rice (*Oryza sativa* L.). *Plant Molecular Biology* **21**: 607-614.

Zhu J, Gale MD, Quarrie S, Jackson M.T and Bryan GJ (1998) AFLP markers for study of rice biodiversity. *Theoretical and Applied Genetics* **96**: 602-611.

Zohary D and Hopf M (2000) *Domestication of Plants in the Old World.* Oxford University Press, Oxford.

Zou, JH, Pan, XB, Chen, ZX, Xu, JY, Lu, JF, Zhai, WX, and Zhu, LH (2000) Mapping quantitative trait loci controlling sheath blight resistance into rice cultivars (*Oryza sativa* L.) *Theoretical and Applied Genetics* **101**: 569–573.

# ANNEXURE I

## Composition of solutions used for DNA extraction and gel electrophoresis

1. **CTAB extraction buffer (100ml)**

| | |
|---|---|
| 1 M Tris-HCl (pH 8.0) | 20 ml |
| 0.5 M EDTA (pH 8.0) | 4.33 ml |
| 5M NaCl | 28 ml |
| Distilled $H_2O$ | 45.8 ml |
| CTAB | 2 g |
| β-mercaptoethanol | 2 g |

2. **TE buffer (100 ml)**

| | |
|---|---|
| 1M Tris (pH 8.0) | 1 ml |
| 0.5M EDTA (pH 8.0) | 0.2 ml |
| Distilled $H_2O$ | 98.8 ml |

3. **4% acrylamide solution (500ml)**

| | |
|---|---|
| Urea | 210 g |
| Distilled $H_2O$ | 225 ml |
| 5x TBE | 50 ml |
| Acrylamide | 23.7 g |
| Bis-acrylamide | 1.25g |

4. **Binding Silane Solution**

0.5% acetic acid in 95% ethanol.

Add 1ml of glacial acetic acid to 95% ethanol.

Take 1ml of this and add 3µl of binding silane.

5. **10% APS (ammonium persulfate)**

| | |
|---|---|
| Ammonium Persulfate | 1 g |
| Distilled $H_2O$ | 10 ml |

6. **Developing Solution**

| | |
|---|---|
| Sodium Carbonate | 60 g |
| Formaldehyde (37%) | 3 ml |
| Sodium Thiosulfate(10mg/ml) | 400 µl |
| Distilled $H_2O$ | 2000 ml |

Prepare fresh solution and chill to 4-10°C before use.

**7. Staining Solution**

| | |
|---|---|
| Silver Nitrate | 2 g |
| Formaldehyde (37%) | 3 ml |
| Distilled $H_2O$ | 2000ml |

**8. Fix/Stop Solution**

| | |
|---|---|
| Glacial acetic acid | 200 ml |
| Distilled $H_2O$ | 1800 ml |

**9. 10x TBE**

| | |
|---|---|
| Tris base | 54 g |
| Boric Acid | 27.6 g |
| EDTA | 4.6 g |

Add distilled water to final volume of 500 ml.

**10. 3x STR Loading Solution**

| | |
|---|---|
| 5M NaOH | 0.2 ml |
| Formamide (95%) | 95 ml |
| Bromophenol Blue | 50 mg |
| Xylene Cyanol FF | 50 mg |

Add distilled water to final volume of 100 ml.

**11. 10 bp Ladder**

| | |
|---|---|
| Sterile Distilled $H_2O$ | 66µl |
| 3 x STR | 33µl |
| 10bp ladder (stock 50 µg, 1 µg/µl, Gibco BRL) | 1µl |

Load 6-8 µl of the above per lane of the ladder.

## ANNEXURE II

### General precautions for using some chemicals for DNA isolation and gel electrophoresis, etc.

**Ethidium Bromide:** Ethidium bromide is a powerful mutagen and is toxic. Consult the local institutional safety officer for specific handling and disposal procedures. Avoid breathing the dust. Wear appropriate gloves when working with solutions that contain this dye.

**NaOH:** The preparation of 10 N NaOH involves a highly exothermic reaction, which can cause breakage of glass containers. Prepare this solution with extreme care in plastic beakers. To 800 ml of $H_2O$, slowly add 400g of NaOH pellets, stirring continuously. As an added precaution, place the beaker on ice. When the pellets have dissolved completely, adjust the volume to 1 liter with $H_2O$. Store the solution in a plastic container at room temperature. Sterilization is not necessary.

**TBE:** TBE is usually made and stored as a 5x or 10x stock solution. The pH of the concentrated stock buffer should be approx. 8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10x as opposed to 5x). However, 5x stock solution is more stable because the solutes do not precipitate during storage. Passing the 5x or 10x buffer stocks through a 0.22-μm filter can prevent or delay formation of precipitates.

**Tris-Cl;** If the 1M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature-dependent and decreases approx. 0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.

**Chloroform:** Chloroform $CHCl_3$ is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is also volatile. Avoid breathing the vapors. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood.

**Ammonium Persulfate:** Ammonium persulfate decays slowly in solution, so replace the stock solution every 2-3 weeks. Ammonium persulfate is used as a catalyst for the copolymerization of acrylamide and bisacrylamide gels. The polymerization reaction is driven by free radicals generated by an oxido-reduction reaction in which a diamine (e.g., TEMED) is used as the adjunct catalyst. Ammonium persulfate $(NH_4)_2S_2O_8$ is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Wear appropriate gloves, safety glasses, and protective clothing. Always use in a chemical fume hood. Wash thoroughly after handling.

**Acrylamide:** Acrylamide (unpolymerized) is a potent neurotoxin and is absorbed through the skin (effects are cumulative). Avoid breathing the dust. Wear appropriate gloves and a face mask when weighing powdered acrylamide and methylene-bisacrylamide. Use in a chemical fume hood. Polyacrylamide is considered to be nontoxic, but it should be handled with care because it might contain small quantities of unpolymerized acrylamide.

**TEMED:** TEMED *N,N,N',N'*-Tetramethylethylenediamine is extremely destructive to tissues of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Prolonged contact can cause severe irritation or burns. Wear appropriate gloves, safety glasses, and other protective clothing. Use only in a chemical fume hood. Wash thoroughly after handling. Flammable: Vapor may travel a considerable distance to source of ignition and flash back. Keep away from heat, sparks, and open flame.

**Glacial acetic acid:** Acetic acid (concentrated) must be handled with great care. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and goggles. Use in a chemical fume hood.

## ANNEXURE III

**CTAB method for genomic DNA isolation** (Saghai-Maroof *et al.*, 1984)

1. Grind thirty-day-old fresh leaf tissues from 3-5 plants in liquid nitrogen with the help of pestle and mortar. Transfer around 0.5 g of leaf powder to a 50 ml polypropylene tube.
2. Add fifteen ml of preheated ($65^{0}$C) CTAB-DNA extraction buffer (100 mM Tris HCl (pH 8.0), 600nM NaCl, 10mM EDTA, 2% β-mercaptoethanol, 0.1% CTAB) and mix well.
3. Incubate for 2 hours at $65^{0}$C with gentle shaking after every 10 minutes by inverting the tubes.
4. Remove tubes from the water bath, cool for 5 minutes and add 15 ml of Chloroform: isoamyl alcohol (24:1v/v). Mix gently and centrifuge at 10,000 rpm for 10 minutes.
5. Pour off the top aqueous layer to new 50 ml tubes, add equal vol. of chloroform: isoamyl alcohol (24:1v/v). Centrifuge at 10000 rpm for 10 minutes.
6. Transfer the supernatant to a fresh tube and add equal volume of ice-cold isopropanol. Keep the tubes undisturbed for 15 minutes.
7. Pellet down the DNA by centrifugation at 12,000 rpm for 5 minutes. Wash with 70% ethanol for 1-2 minutes.
8. Keep for air-drying overnight. Dissolve in minimum amount of TE buffer.
9. Add DNAse free RNAse (30 μg /100 μl DNA) to the dissolved DNA. Incubate for 30 min at $37^{0}$C.
10. Again extract the DNA with equal volume of chloroform: isoamyl alcohol (24:1v/v). Centrifuge at 10,000 rpm for 10

minutes. Add 1/10th volume of 3M Sodium Acetate (pH 6.8) and 2 volumes of 95% ethanol.

11. Pellet out the precipitated DNA and dissolve in TE buffer (10mM Tris HCL pH8.0), 1mM EDTA (pH8.0).

**12.** When DNA is completely dissolved in TE, quality and concentration can be checked. To check this, run an aliquot of DNA sample on 0.8% agarose gel in 1X TBE buffer for 45 min along with molecular weight standard of lambda phage cut with *Hind III* and *Eco RI*.

**ANNEXURE IV**

## Procedure for denaturing Polyacrylamide Gel Preparation and Electrophoresis

Long glass plate was marked on one side in one corner to distinguish the treated side. The short and the long glass plates were thoroughly cleaned twice with 95% ethanol (Plate 1). Binding silane (y-methacryloxypropyltrimethoxy silane, Promega, Inc., USA) solution was prepared in a 1.5 ml microcentrifuge tube. Short glass plate was wiped using tissue paper saturated with the freshly prepared binding solution. Be certain to wipe the entire plate surface with the saturated tissue. Wait for 5 minutes for binding solution to dry. Apply 3 ml of Sigmacote® (SIGMA) on one the side of the long plate. With a dry tissue paper, spread the sigmacote over the entire surface of the plate. Wait for 5 minutes.

(**Note**: To prevent the binding solution from contaminating the long plate, the long plate needs to be prepared first. If short plate is prepared first then gloves need to be changed before the preparation of long plate.)

Assemble the plates with 0.4 mm spacers sandwiched at three corners and tighten it with clamps. Pour 4% acrylamide solution (75 ml) into a squeeze bottle. For each 75 ml solution, add 75µl of TEMED (N,N,N, N'-Tetraethyl-methylenediamine) and 625µl of 10% APS (Ammonium persulfate) to the acrylamide solution and mix gently. Carefully pour the acrylamide solution into the space between the plates, avoiding the formation of bubbles and maintaining a constant flow of the solution.

Insert inverted multi-well sharktooth comb, straight side into the gel, between the glass plates (approximately 6-8 mm of the comb should be between the two glass plates). Allow polymerization to proceed for at least two hours*.

*The gel may be stored overnight at room temperature if a paper towel saturated with dH2O and plastic wrap are placed around the end of the gel to prevent the gel from drying out. As an alternative, the gel can be stored overnight by setting in the electrophoresis apparatus with 0.5 x TBE buffer in the bottom and top chamber of the apparatus.

**Denaturing Polyacrylamide Gel Electrophoresis**

Excess of polyacrylamide was removed from the comb. Pull out the comb was pulled out and excess of polyacrylamide was shaved from the top of the glass plates. Electrophoretic unit was assembled, 400 ml of 0.5X TBE buffer was added in the top and bottom chamber. Air bubbles and unpolymerised acrylamide were removed on the top of the gel using a 20ml syringe or plastic Pasteur pipette filled with buffer. Be certain the well area is free from of air bubbles and small pieces of Polyacrylamide. Using a syringe with a bend needle, air bubbles between the glass plates on the bottom of the gel were removed. Gel was pre-run at constant power of 55 W to achieve the gel surface temperature of approximately 45-50°C. Samples for loading were prepared by adding 10µl of 3X STR loading solution to each 0.2 ml tube containing 20µl of the PCR product. 10 bp was prepared ladder as molecular weight marker for each marker lane. The samples and markers were denatured by heating at 95°C for and immediately transferred to chill on ice.

After the pre-run, the sharktooth comb was carefully inserted into the gel approximately 1-2 mm. 8µl of each sample and marker was loaded into respective wells. To prevent the gel from cooling, gel loading should not exceed 20 minutes. Run the gel using the same condition as pre- run step).

**Note:** In a 4% gel, bromophenol blue migrates at approximately 40 bases, and xylene cyanol migrates at approximately 170 bases.

## ANNEXURE V

### Silver Staining protocol for detection of SSRs

After electrophoresis, the sequencing unit was disassembled. The comb was removed. A plastic wedge or flat end spatula was used to carefully separate the two glass plates. The gel should be strongly affixed to the short glass plate. The gel (attached to the short plate) was placed in a shallow plastic tray.

For silver staining, steps listed below were followed:

1. The gel was in fix/stop solution for 30 minutes or until the dye gets invisible.
2. The gel was washed three times (2 minutes each) with distilled water.
3. The gel was stained in staining solution for 30 minutes.
4. Rinsed the gel for no more than 10 seconds.
5. Put the gel in the prechilled Developer solution (4-10°C) for 2-5 minutes or until the bands were visible.
6. Fix the gel in Fix/stop solution using the same solution from step (a) for 3-6 minutes.
7. Rinse the gel with distilled water for 2-3 minutes.

*All steps should be done on a shaker.

The short plate was positioned with gel upright and allows it to dry overnight. The gel plate was photographed using the fluorescent transilluminator of Chemilmager™ (Alpha Innotech Corporation) gel documentation unit or by digital camera (Nikon Coolpix-850).

# ANNEXURE VI

## Agarose gel electrophoresis procedure

PCR amplified DNA fragments for TE based markers were resolved by submerged horizontal electrophoresis in 1.5% (w/v) agarose gels. Gel casting plate was washed, air-dried and its ends were sealed with tape. Agarose was melted in 0.5 X TBE buffer and ethidium bromide (5 μg/ml) was added. Gel solution was then poured into the gel casting plate inserted with an appropriate comb to get a 0.5 cm thick gel. After setting of gel, sealing tapes were removed from both the ends. Gel plate was placed in the electrophoresis chamber and submerged using 0.5 X TBE buffer and comb was removed gently. Samples were prepared by adding 6 X loading dye (sucrose 4 gm, Bromophenol Blue 0.025 g, Xylene cyanol 0.025g, final volume 10 ml) and pulse centrifuged for proper mixing. Samples were loaded in the wells and electrophoresis was carried out at constant voltage (3 v/cm of gel) until the dye migrated to the other end of the gel. PCR amplified products were viewed under UV light fluorescence using photo dyne UV transilluminator.

# ANNEXURE-VII

## Similarity matrix data of 50 rice genotypes obtained using the allelic diversity data at 50 SSR loci.

| | **Azu** | **NPT2** | **NPT3** | **T309** | **TNG67** | **Nippon** | **Bas217** | **B370** | **HBC19** | **KLocal** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | 1 | | | | | | | | | |
| **NPT2** | 0.2949 | 1 | | | | | | | | |
| **NPT3** | 0.225 | 0.4265 | 1 | | | | | | | |
| **T309** | 0.1556 | 0.241 | 0.2658 | 1 | | | | | | |
| **TNG67** | 0.1111 | 0.1647 | 0.28 | 0.5692 | 1 | | | | | |
| **Nippon** | 0.086 | 0.2346 | 0.2763 | 0.6349 | 0.5469 | 1 | | | | |
| **Bas217** | 0.0632 | 0.087 | 0.0659 | 0.0842 | 0.1 | 0.0638 | 1 | | | |
| **B370** | 0.1609 | 0.0989 | 0.0778 | 0.1573 | 0.125 | 0.087 | 0.1765 | 1 | | |
| **HBC19** | 0.1609 | 0.1364 | 0.0778 | 0.1705 | 0.125 | 0.1236 | 0.1494 | 0.5385 | 1 | |
| **KLocal** | 0.1429 | 0.1319 | 0.0989 | 0.1398 | 0.0737 | 0.0842 | 0.241 | 0.2716 | 0.3553 | 1 |
| **RanBas** | 0.1461 | 0.1222 | 0.1136 | 0.1429 | 0.1111 | 0.1222 | 0.2024 | 0.4225 | 0.3117 | 0.3165 |
| **TypeIII** | 0.1444 | 0.1087 | 0.0645 | 0.129 | 0.1099 | 0.0968 | 0.1724 | 0.5 | 0.4571 | 0.2651 |
| **Bas385** | 0.1429 | 0.1444 | 0.0989 | 0.1398 | 0.0851 | 0.0842 | 0.2262 | 0.3553 | 0.3377 | 0.359 |
| **CSR30** | 0.1798 | 0.1556 | 0.0978 | 0.1383 | 0.0842 | 0.1183 | 0.1429 | 0.4444 | 0.6 | 0.321 |
| **ImpSabar** | 0.1183 | 0.1196 | 0.1111 | 0.1042 | 0.0515 | 0.0842 | 0.2716 | 0.1196 | 0.1705 | 0.3086 |
| **HKR228** | 0.1209 | 0.1348 | 0.0889 | 0.0947 | 0.087 | 0.086 | 0.0978 | 0.1609 | 0.2169 | 0.284 |
| **Kasturi** | 0.0938 | 0.0722 | 0.0745 | 0.1146 | 0.0957 | 0.1064 | 0.1429 | 0.2683 | 0.3684 | 0.2738 |
| **Mahi** | 0.0632 | 0.0753 | 0.0778 | 0.0404 | 0.0532 | 0.0526 | 0.1905 | 0.1364 | 0.1111 | 0.1977 |
| **PB1** | 0.1075 | 0.0968 | 0.0421 | 0.1413 | 0.1099 | 0.0968 | 0.1209 | 0.3077 | 0.3973 | 0.1932 |
| **PS2** | 0.0729 | 0.0737 | 0.1 | 0.0714 | 0.0745 | 0.0515 | 0.1461 | 0.1087 | 0.2 | 0.2651 |
| **PS3** | 0.0833 | 0.0957 | 0.087 | 0.0707 | 0.0851 | 0.0729 | 0.1705 | 0.1573 | 0.1839 | 0.2326 |
| **PS4** | 0.0481 | 0.102 | 0.1053 | 0.0882 | 0.0808 | 0.08 | 0.1739 | 0.1868 | 0.2857 | 0.1935 |
| **Sabar** | 0.0505 | 0.051 | 0.0526 | 0.0291 | 0.0737 | 0.03 | 0.2262 | 0.1573 | 0.1444 | 0.2771 |
| **Super** | 0.1429 | 0.1705 | 0.1236 | 0.1648 | 0.0737 | 0.1573 | 0.1196 | 0.3553 | 0.411 | 0.2471 |
| **PR118** | 0.0632 | 0.0638 | 0.0543 | 0.0842 | 0.0879 | 0.0638 | 0.2658 | 0.2195 | 0.25 | 0.2716 |
| **PR106** | 0.0515 | 0.0632 | 0.0538 | 0.1064 | 0.0989 | 0.1099 | 0.1882 | 0.1477 | 0.1609 | 0.3 |
| **IR36** | 0.0833 | 0.0729 | 0.0753 | 0.0392 | 0.0625 | 0.0404 | 0.2875 | 0.1573 | 0.1839 | 0.2045 |
| **BR4-10** | 0.1222 | 0.0753 | 0.0543 | 0.0619 | 0.0532 | 0.0638 | 0.2048 | 0.1364 | 0.1236 | 0.1319 |
| **CSR1** | 0.1196 | 0.1333 | 0.0879 | 0.0825 | 0.0632 | 0.0737 | 0.2593 | 0.1209 | 0.1591 | 0.1413 |
| **CSR10** | 0.0851 | 0.0745 | 0.0889 | 0.0612 | 0.0638 | 0.0632 | 0.2169 | 0.1348 | 0.1348 | 0.1685 |
| **CSR21** | 0.0632 | 0.0638 | 0.0543 | 0.051 | 0.0421 | 0.0526 | 0.2048 | 0.1494 | 0.1111 | 0.1705 |
| **HKR120** | 0.0612 | 0.1319 | 0.1236 | 0.0495 | 0.0737 | 0.051 | 0.1573 | 0.1075 | 0.1444 | 0.1778 |
| **93-401** | 0.086 | 0.0753 | 0.043 | 0.1075 | 0.1 | 0.1111 | 0.1765 | 0.1765 | 0.1905 | 0.2118 |
| **IR24** | 0.1064 | 0.1075 | 0.1111 | 0.0392 | 0.0515 | 0.0404 | 0.1839 | 0.1573 | 0.1839 | 0.2619 |
| **IR72** | 0.0722 | 0.0957 | 0.1111 | 0.06 | 0.0515 | 0.051 | 0.1839 | 0.1075 | 0.1573 | 0.1522 |
| **KR1-24** | 0.1236 | 0.0879 | 0.0549 | 0.1087 | 0.0769 | 0.1124 | 0.1379 | 0.1379 | 0.1124 | 0.1724 |
| **MI48** | 0.0842 | 0.0625 | 0.0421 | 0.05 | 0.0632 | 0.0408 | 0.186 | 0.1591 | 0.1209 | 0.2069 |
| **Pokkali** | 0.0619 | 0.0625 | 0.0645 | 0.0396 | 0.0978 | 0.0303 | 0.2439 | 0.1087 | 0.1209 | 0.1053 |
| **Sharbati** | 0.06 | 0.0938 | 0.0851 | 0.0693 | 0.0833 | 0.0714 | 0.2651 | 0.0938 | 0.1667 | 0.1868 |
| **9311** | 0.1304 | 0.2118 | 0.2195 | 0.325 | 0.275 | 0.3733 | 0.0619 | 0.0957 | 0.1075 | 0.1042 |
| **IR64** | 0.0521 | 0.087 | 0.1023 | 0.1075 | 0.1 | 0.087 | 0.1494 | 0.1628 | 0.1494 | 0.1977 |
| **Oglab** | 0.1 | 0.0769 | 0.0674 | 0.0978 | 0.0659 | 0.0652 | 0.0426 | 0.1951 | 0.1264 | 0.0745 |
| **Obart** | 0.0753 | 0.0645 | 0.0787 | 0.0515 | 0.0652 | 0.0532 | 0.1 | 0.1379 | 0.0879 | 0.0968 |
| **Orufi1** | 0.0778 | 0.0667 | 0.0449 | 0.0879 | 0.131 | 0.0787 | 0.0667 | 0.0667 | 0.0787 | 0.0761 |
| **Orufi2** | 0.0532 | 0.1011 | 0.0674 | 0.1099 | 0.0778 | 0.0889 | 0.0769 | 0.1136 | 0.0889 | 0.1744 |
| **Oniv1** | 0.086 | 0.0989 | 0.1023 | 0.1075 | 0.1647 | 0.1494 | 0.0638 | 0.0753 | 0.0526 | 0.0619 |
| **Oniv2** | 0.0753 | 0.0879 | 0.0787 | 0.1333 | 0.1264 | 0.1647 | 0.0761 | 0.0879 | 0.1 | 0.0737 |
| **Oniv3** | 0.0978 | 0.0417 | 0.0659 | 0.0842 | 0.0645 | 0.0753 | 0.0753 | 0.0753 | 0.0638 | 0.1196 |
| **Oglumal** | 0.0889 | 0.1149 | 0.0805 | 0.1111 | 0.0667 | 0.1023 | 0.1548 | 0.1279 | 0.1149 | 0.1236 |
| **Omerdio** | 0.093 | 0.0814 | 0.0588 | 0.1034 | 0.0575 | 0.1071 | 0.069 | 0.0568 | 0.0814 | 0.0787 |

Contd…

| | **RanBas** | **TypeIII** | **Bas385** | **CSR30** | **ImpSabar** | **HKR228** | **Kasturi** | **Mahi** | **PB1** | **PS2** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | 1 | | | | | | | | | |
| **TypeIII** | 0.3733 | 1 | | | | | | | | |
| **Bas385** | 0.3165 | 0.3816 | 1 | | | | | | | |
| **CSR30** | 0.2963 | 0.4324 | 0.3718 | 1 | | | | | | |
| **ImpSabar** | 0.1556 | 0.1538 | 0.191 | 0.1758 | 1 | | | | | |
| **HKR228** | 0.1591 | 0.2262 | 0.2093 | 0.2209 | 0.2235 | 1 | | | | |
| **Kasturi** | 0.2209 | 0.3418 | 0.2738 | 0.4211 | 0.2738 | 0.2651 | 1 | | | |
| **Mahi** | 0.1099 | 0.1333 | 0.1705 | 0.1685 | 0.241 | 0.1882 | 0.1954 | 1 | | |
| **PB1** | 0.2118 | 0.3165 | 0.25 | 0.325 | 0.25 | 0.3038 | 0.325 | 0.1591 | 1 | |
| **PS2** | 0.1319 | 0.1304 | 0.2209 | 0.191 | 0.2651 | 0.2716 | 0.2927 | 0.2439 | 0.253 | 1 |
| **PS3** | 0.1556 | 0.1798 | 0.2619 | 0.163 | 0.2471 | 0.253 | 0.2022 | 0.1839 | 0.2209 | 0.5 |
| **PS4** | 0.172 | 0.1957 | 0.2065 | 0.2727 | 0.2198 | 0.3457 | 0.2584 | 0.2706 | 0.3253 | 0.358 |
| **Sabar** | 0.1429 | 0.129 | 0.1648 | 0.1031 | 0.3086 | 0.1954 | 0.2159 | 0.3038 | 0.1538 | 0.2805 |
| **Super** | 0.2381 | 0.3636 | 0.2771 | 0.4658 | 0.1158 | 0.2093 | 0.3544 | 0.1839 | 0.2805 | 0.1932 |
| **PR118** | 0.1348 | 0.1461 | 0.2262 | 0.2381 | 0.3205 | 0.2317 | 0.3333 | 0.2821 | 0.3247 | 0.3077 |
| **PR106** | 0.1461 | 0.1319 | 0.2093 | 0.1538 | 0.2235 | 0.275 | 0.2209 | 0.2169 | 0.1705 | 0.3377 |
| **IR36** | 0.1556 | 0.1538 | 0.2471 | 0.1383 | 0.2619 | 0.1685 | 0.1889 | 0.2561 | 0.2069 | 0.25 |
| **BR4-10** | 0.1477 | 0.1209 | 0.0957 | 0.0947 | 0.1444 | 0.1222 | 0.1304 | 0.1364 | 0.1209 | 0.0851 |
| **CSR1** | 0.1444 | 0.1429 | 0.2209 | 0.191 | 0.2353 | 0.1444 | 0.2471 | 0.186 | 0.2093 | 0.1685 |
| **CSR10** | 0.1461 | 0.1196 | 0.1818 | 0.129 | 0.253 | 0.1724 | 0.1932 | 0.1744 | 0.1573 | 0.1573 |
| **CSR21** | 0.1222 | 0.1591 | 0.1977 | 0.1556 | 0.2262 | 0.1222 | 0.1304 | 0.1905 | 0.1087 | 0.1333 |
| **HKR120** | 0.1064 | 0.0714 | 0.2471 | 0.1383 | 0.191 | 0.1685 | 0.163 | 0.1705 | 0.1667 | 0.2069 |
| **93-401** | 0.1609 | 0.2143 | 0.2118 | 0.1954 | 0.1705 | 0.1348 | 0.1818 | 0.1494 | 0.2143 | 0.2 |
| **IR24** | 0.1818 | 0.1798 | 0.191 | 0.163 | 0.3086 | 0.253 | 0.2299 | 0.241 | 0.25 | 0.2963 |
| **IR72** | 0.1304 | 0.1413 | 0.2184 | 0.1383 | 0.2326 | 0.1818 | 0.1758 | 0.2716 | 0.1538 | 0.2963 |
| **KR1-24** | 0.2048 | 0.1348 | 0.1591 | 0.1075 | 0.1461 | 0.1364 | 0.0842 | 0.1379 | 0.1099 | 0.1882 |
| **MI48** | 0.1319 | 0.1304 | 0.1798 | 0.1398 | 0.1932 | 0.1705 | 0.1398 | 0.2593 | 0.1818 | 0.1556 |
| **Pokkali** | 0.0957 | 0.1304 | 0.1932 | 0.0928 | 0.2069 | 0.1444 | 0.1398 | 0.1209 | 0.1685 | 0.1556 |
| **Sharbati** | 0.1522 | 0.1031 | 0.1613 | 0.1354 | 0.2857 | 0.1778 | 0.1474 | 0.25 | 0.1889 | 0.2892 |
| **9311** | 0.1429 | 0.117 | 0.1277 | 0.1031 | 0.1042 | 0.0833 | 0.1146 | 0.0957 | 0.0938 | 0.0714 |
| **IR64** | 0.1609 | 0.1209 | 0.1839 | 0.1429 | 0.2118 | 0.1609 | 0.1429 | 0.2346 | 0.2 | 0.186 |
| **Oglab** | 0.1379 | 0.1494 | 0.086 | 0.1209 | 0.0412 | 0.1 | 0.0851 | 0.0652 | 0.087 | 0.0753 |
| **Obart** | 0.1494 | 0.1222 | 0.1087 | 0.1075 | 0.0737 | 0.0526 | 0.0729 | 0.0532 | 0.0745 | 0.0978 |
| **Orufi1** | 0.0778 | 0.1011 | 0.0421 | 0.0526 | 0.0645 | 0.0778 | 0.087 | 0.0787 | 0.0769 | 0.0538 |
| **Orufi2** | 0.0879 | 0.0989 | 0.0978 | 0.0851 | 0.1099 | 0.1512 | 0.0968 | 0.1667 | 0.0989 | 0.1236 |
| **Oniv1** | 0.0978 | 0.0851 | 0.0957 | 0.0612 | 0.0404 | 0.0521 | 0.0947 | 0.087 | 0.0625 | 0.0515 |
| **Oniv2** | 0.087 | 0.0978 | 0.0737 | 0.0842 | 0.0851 | 0.087 | 0.1196 | 0.0879 | 0.1222 | 0.0745 |
| **Oniv3** | 0.1477 | 0.0737 | 0.0957 | 0.0505 | 0.0729 | 0.086 | 0.1064 | 0.0526 | 0.0968 | 0.0737 |
| **Oglumal** | 0.1136 | 0.125 | 0.0989 | 0.1348 | 0.087 | 0.0889 | 0.0978 | 0.0778 | 0.0761 | 0.0532 |
| **Omerdio** | 0.033 | 0.044 | 0.1034 | 0.1149 | 0.1034 | 0.093 | 0.0543 | 0.1205 | 0.1176 | 0.1047 |

Contd…

| | **PS3** | **PS4** | **Sabar** | **Super** | **PR118** | **PR106** | **IR36** | **BR4-10** | **CSR1** | **CSR10** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | 1 | | | | | | | | | |
| **PS4** | 0.3537 | 1 | | | | | | | | |
| **Sabar** | 0.359 | 0.2065 | 1 | | | | | | | |
| **Super** | 0.1778 | 0.3214 | 0.1042 | 1 | | | | | | |
| **PR118** | 0.2716 | 0.3012 | 0.3377 | 0.1839 | 1 | | | | | |
| **PR106** | 0.3 | 0.2824 | 0.3165 | 0.1818 | 0.3117 | 1 | | | | |
| **IR36** | 0.2471 | 0.2333 | 0.2927 | 0.1522 | 0.241 | 0.253 | 1 | | | |
| **BR4-10** | 0.1196 | 0.1134 | 0.1196 | 0.0957 | 0.1236 | 0.1348 | 0.2118 | 1 | | |
| **CSR1** | 0.1413 | 0.1957 | 0.1798 | 0.1798 | 0.1724 | 0.1075 | 0.2209 | 0.1333 | 1 | |
| **CSR10** | 0.1429 | 0.1596 | 0.2235 | 0.1304 | 0.2317 | 0.2143 | 0.2093 | 0.1222 | 0.5147 | 1 |
| **CSR21** | 0.1444 | 0.1613 | 0.1705 | 0.1319 | 0.2195 | 0.2625 | 0.2118 | 0.1628 | 0.2 | 0.2469 |
| **HKR120** | 0.1778 | 0.1563 | 0.191 | 0.1158 | 0.241 | 0.1818 | 0.2471 | 0.1573 | 0.2069 | 0.3 |
| **93-401** | 0.1839 | 0.2 | 0.1839 | 0.241 | 0.1765 | 0.2317 | 0.2561 | 0.1905 | 0.1724 | 0.1609 |
| **IR24** | 0.2927 | 0.2198 | 0.2771 | 0.1648 | 0.3205 | 0.3 | 0.2771 | 0.1573 | 0.1798 | 0.2381 |
| **IR72** | 0.2619 | 0.2907 | 0.2326 | 0.1648 | 0.2716 | 0.284 | 0.325 | 0.1319 | 0.1538 | 0.1429 |
| **KR1-24** | 0.2439 | 0.1383 | 0.1591 | 0.1333 | 0.1512 | 0.1494 | 0.1591 | 0.2692 | 0.1099 | 0.0638 |
| **MI48** | 0.1798 | 0.1702 | 0.2805 | 0.117 | 0.3077 | 0.3377 | 0.3291 | 0.1591 | 0.1685 | 0.241 |
| **Pokkali** | 0.1667 | 0.1702 | 0.1667 | 0.0714 | 0.2143 | 0.1705 | 0.2805 | 0.1724 | 0.2683 | 0.3038 |
| **Sharbati** | 0.2135 | 0.2151 | 0.3171 | 0.1368 | 0.3636 | 0.2619 | 0.2857 | 0.1538 | 0.163 | 0.2045 |
| **9311** | 0.0816 | 0.0882 | 0.0392 | 0.1522 | 0.0619 | 0.1064 | 0.1042 | 0.0957 | 0.0938 | 0.0612 |
| **IR64** | 0.2262 | 0.2 | 0.241 | 0.1444 | 0.2987 | 0.2785 | 0.3038 | 0.1494 | 0.2 | 0.2949 |
| **Oglab** | 0.1099 | 0.1277 | 0.0978 | 0.1099 | 0.0769 | 0.0761 | 0.086 | 0.1264 | 0.0753 | 0.0532 |
| **Obart** | 0.0968 | 0.07 | 0.0968 | 0.0968 | 0.0645 | 0.087 | 0.0851 | 0.1379 | 0.1099 | 0.1111 |
| **Orufi1** | 0.0645 | 0.0722 | 0.0421 | 0.0645 | 0.0667 | 0.043 | 0.0532 | 0.0909 | 0.0652 | 0.0543 |
| **Orufi2** | 0.1222 | 0.191 | 0.1222 | 0.1348 | 0.1136 | 0.1124 | 0.1222 | 0.0889 | 0.1364 | 0.0879 |
| **Oniv1** | 0.0404 | 0.0485 | 0.0619 | 0.0842 | 0.087 | 0.0521 | 0.051 | 0.1111 | 0.0968 | 0.0521 |
| **Oniv2** | 0.0737 | 0.1146 | 0.0515 | 0.1209 | 0.1124 | 0.0753 | 0.0737 | 0.1 | 0.0745 | 0.0753 |
| **Oniv3** | 0.1075 | 0.1134 | 0.1196 | 0.1075 | 0.087 | 0.1099 | 0.1075 | 0.087 | 0.0968 | 0.0978 |
| **Oglumal** | 0.0989 | 0.1053 | 0.087 | 0.1236 | 0.0659 | 0.0652 | 0.0989 | 0.1548 | 0.0879 | 0.0426 |
| **Omerdio** | 0.1294 | 0.1222 | 0.0787 | 0.0909 | 0.0941 | 0.093 | 0.0909 | 0.0449 | 0.0326 | 0.0682 |

Contd…

| | **CSR21** | **HKR120** | **93-401** | **IR24** | **IR72** | **KR1-24** | **MI48** | **Pokkali** | **Sharbati** | **9311** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | | | | | | | | | | |
| **PS4** | | | | | | | | | | |
| **Sabar** | | | | | | | | | | |
| **Super** | | | | | | | | | | |
| **PR118** | | | | | | | | | | |
| **PR106** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | 1 | | | | | | | | | |
| **HKR120** | 0.1977 | 1 | | | | | | | | |
| **93-401** | 0.25 | 0.1573 | 1 | | | | | | | |
| **IR24** | 0.2262 | 0.359 | 0.1839 | 1 | | | | | | |
| **IR72** | 0.1977 | 0.2184 | 0.2118 | 0.3947 | 1 | | | | | |
| **KR1-24** | 0.1786 | 0.1333 | 0.1786 | 0.2143 | 0.186 | 1 | | | | |
| **MI48** | 0.2593 | 0.1667 | 0.186 | 0.2353 | 0.25 | 0.1744 | 1 | | | |
| **Pokkali** | 0.1724 | 0.2651 | 0.2 | 0.1667 | 0.2805 | 0.1477 | 0.253 | 1 | | |
| **Sharbati** | 0.1932 | 0.2706 | 0.2209 | 0.2558 | 0.2857 | 0.1429 | 0.3049 | 0.2892 | 1 | |
| **9311** | 0.0957 | 0.0707 | 0.0957 | 0.0707 | 0.1042 | 0.1087 | 0.0714 | 0.0396 | 0.102 | 1 |
| **IR64** | 0.3158 | 0.3038 | 0.2195 | 0.2875 | 0.2561 | 0.0879 | 0.3077 | 0.2439 | 0.25 | 0.0729 |
| **Oglab** | 0.0889 | 0.0978 | 0.1136 | 0.1609 | 0.1222 | 0.1975 | 0.0753 | 0.0526 | 0.0729 | 0.0745 |
| **Obart** | 0.125 | 0.1087 | 0.1 | 0.0851 | 0.0625 | 0.1011 | 0.0978 | 0.0978 | 0.0947 | 0.0303 |
| **Orufi1** | 0.0667 | 0.0421 | 0.0787 | 0.0761 | 0.0532 | 0.0674 | 0.0538 | 0.1011 | 0.0745 | 0.0645 |
| **Orufi2** | 0.1136 | 0.086 | 0.1136 | 0.0978 | 0.1099 | 0.1279 | 0.0753 | 0.087 | 0.0842 | 0.0978 |
| **Oniv1** | 0.0753 | 0.0729 | 0.0526 | 0.0619 | 0.0842 | 0.1 | 0.0625 | 0.1087 | 0.0714 | 0.1444 |
| **Oniv2** | 0.0206 | 0.0625 | 0.0879 | 0.0968 | 0.0968 | 0.0769 | 0.0632 | 0.0978 | 0.0947 | 0.1333 |
| **Oniv3** | 0.087 | 0.0729 | 0.1111 | 0.0957 | 0.1196 | 0.1512 | 0.0968 | 0.0968 | 0.1053 | 0.1075 |
| **Oglumal** | 0.043 | 0.0989 | 0.0211 | 0.0989 | 0.1111 | 0.1034 | 0.1124 | 0.1 | 0.0737 | 0.087 |
| **Omerdio** | 0.069 | 0.0909 | 0.069 | 0.0667 | 0.1294 | 0.0824 | 0.1176 | 0.1047 | 0.0889 | 0.0549 |

Contd…

| | IR64 | Oglab | Obart | Orufi1 | Orufi2 | Oniv1 | Oniv2 | Oniv3 | Oglumal | Omerdio |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | | | | | | | | | | |
| **PS4** | | | | | | | | | | |
| **Sabar** | | | | | | | | | | |
| **Super** | | | | | | | | | | |
| **PR118** | | | | | | | | | | |
| **PR106** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | | | | | | | | | | |
| **HKR120** | | | | | | | | | | |
| **93-401** | | | | | | | | | | |
| **IR24** | | | | | | | | | | |
| **IR72** | | | | | | | | | | |
| **KR1-24** | | | | | | | | | | |
| **MI48** | | | | | | | | | | |
| **Pokkali** | | | | | | | | | | |
| **Sharbati** | | | | | | | | | | |
| **9311** | | | | | | | | | | |
| **IR64** | 1 | | | | | | | | | |
| **Oglab** | 0.0889 | 1 | | | | | | | | |
| **Obart** | 0.1124 | 0.0778 | 1 | | | | | | | |
| **Orufi1** | 0.0787 | 0.0682 | 0.0795 | 1 | | | | | | |
| **Orufi2** | 0.1264 | 0.1707 | 0.0899 | 0.1899 | 1 | | | | | |
| **Oniv1** | 0.0526 | 0.0538 | 0.1 | 0.1429 | 0.1529 | 1 | | | | |
| **Oniv2** | 0.0761 | 0.0778 | 0.0208 | 0.131 | 0.0659 | 0.1379 | 1 | | | |
| **Oniv3** | 0.0989 | 0.1395 | 0.0761 | 0.1034 | 0.1529 | 0.1364 | 0.1 | 1 | | |
| **Oglumal** | 0.0543 | 0.1047 | 0.1163 | 0.0568 | 0.0795 | 0.0899 | 0.0549 | 0.1149 | 1 | |
| **Omerdio** | 0.1341 | 0.0964 | 0.0575 | 0.1266 | 0.1235 | 0.069 | 0.1358 | 0.1071 | 0.2 | 1 |

## Similarity matrix data of 50 rice genotypes obtained using the allelic diversity data at 50 TE based marker loci.

| | Azu | NPT2 | NPT3 | T309 | TNG67 | Nippon | Bas217 | B370 | HBC19 | KLocal |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | 1 | | | | | | | | | |
| **NPT2** | 0.4615 | 1 | | | | | | | | |
| **NPT3** | 0.4444 | 0.6333 | 1 | | | | | | | |
| **T309** | 0.234 | 0.375 | 0.3889 | 1 | | | | | | |
| **TNG67** | 0.1522 | 0.3158 | 0.3636 | 0.7 | 1 | | | | | |
| **Nippon** | 0.25 | 0.3571 | 0.3684 | 0.7059 | 0.5588 | 1 | | | | |
| **Bas217** | 0.4865 | 0.4857 | 0.4242 | 0.1522 | 0.1707 | 0.1957 | 1 | | | |
| **B370** | 0.8125 | 0.5278 | 0.5152 | 0.2444 | 0.186 | 0.2889 | 0.6061 | 1 | | |
| **HBC19** | 0.697 | 0.6061 | 0.6 | 0.2857 | 0.225 | 0.3333 | 0.6452 | 0.8621 | 1 | |
| **KLocal** | 0.5946 | 0.4 | 0.3077 | 0.0962 | 0.1064 | 0.1346 | 0.6364 | 0.5405 | 0.5278 | 1 |
| **RanBas** | 0.6471 | 0.6061 | 0.5484 | 0.3171 | 0.2564 | 0.3659 | 0.5455 | 0.6875 | 0.7931 | 0.4865 |
| **TypeIII** | 0.7097 | 0.5152 | 0.5 | 0.2143 | 0.15 | 0.2619 | 0.5 | 0.8214 | 0.8148 | 0.4857 |
| **Bas385** | 0.7879 | 0.5135 | 0.5 | 0.2391 | 0.1818 | 0.2826 | 0.5882 | 0.9655 | 0.8333 | 0.5676 |
| **CSR30** | 0.7059 | 0.5714 | 0.4706 | 0.2444 | 0.1591 | 0.2889 | 0.5143 | 0.8667 | 0.8 | 0.5405 |
| **ImpSabar** | 0.4706 | 0.4242 | 0.4 | 0.1429 | 0.1026 | 0.1905 | 0.4516 | 0.5484 | 0.6429 | 0.4848 |
| **HKR228** | 0.5152 | 0.4688 | 0.5 | 0.2632 | 0.1944 | 0.3158 | 0.4063 | 0.6552 | 0.7037 | 0.3243 |
| **Kasturi** | 0.5882 | 0.5 | 0.5862 | 0.2683 | 0.2368 | 0.3171 | 0.6333 | 0.7333 | 0.8519 | 0.5143 |
| **Mahi** | 0.5278 | 0.4054 | 0.3429 | 0.1277 | 0.0667 | 0.1458 | 0.5625 | 0.5588 | 0.5938 | 0.5429 |
| **PB1** | 0.6471 | 0.5143 | 0.5 | 0.2558 | 0.1951 | 0.3023 | 0.5455 | 0.8 | 0.8571 | 0.4865 |
| **PS2** | 0.4706 | 0.3429 | 0.2727 | 0.1429 | 0.1026 | 0.1628 | 0.5517 | 0.5484 | 0.5862 | 0.4412 |
| **PS3** | 0.5556 | 0.4722 | 0.3714 | 0.1489 | 0.1395 | 0.1667 | 0.7 | 0.6364 | 0.625 | 0.5714 |
| **PS4** | 0.5405 | 0.4211 | 0.4412 | 0.1957 | 0.1628 | 0.2128 | 0.6774 | 0.6667 | 0.7097 | 0.5556 |
| **Sabar** | 0.6286 | 0.4211 | 0.3243 | 0.1957 | 0.1628 | 0.2128 | 0.4857 | 0.5714 | 0.5588 | 0.6 |
| **Super** | 0.7143 | 0.425 | 0.4444 | 0.2609 | 0.2045 | 0.3043 | 0.5714 | 0.8125 | 0.697 | 0.5526 |
| **PR118** | 0.5 | 0.2667 | 0.2093 | 0.1154 | 0.1277 | 0.1321 | 0.5714 | 0.4872 | 0.4 | 0.5946 |
| **PR106** | 0.6389 | 0.3023 | 0.275 | 0.0755 | 0.04 | 0.1132 | 0.5 | 0.5833 | 0.4865 | 0.8125 |
| **IR36** | 0.5 | 0.4211 | 0.4 | 0.1458 | 0.1364 | 0.1875 | 0.7931 | 0.6176 | 0.6563 | 0.697 |
| **BR4-10** | 0.5152 | 0.5161 | 0.4483 | 0.2 | 0.1944 | 0.1905 | 0.5 | 0.5484 | 0.6429 | 0.5313 |
| **CSR1** | 0.4872 | 0.6176 | 0.5625 | 0.2727 | 0.186 | 0.2889 | 0.5143 | 0.5135 | 0.5882 | 0.425 |
| **CSR10** | 0.4103 | 0.3684 | 0.3429 | 0.1522 | 0.1163 | 0.1957 | 0.6129 | 0.4324 | 0.5 | 0.5429 |
| **CSR21** | 0.2857 | 0.2353 | 0.1935 | 0.1026 | 0.1176 | 0.0976 | 0.4286 | 0.3438 | 0.3226 | 0.3333 |
| **HKR120** | 0.4054 | 0.4 | 0.375 | 0.2195 | 0.2162 | 0.2381 | 0.5667 | 0.4286 | 0.5 | 0.5455 |
| **93-401** | 0.5278 | 0.4054 | 0.3824 | 0.2045 | 0.1429 | 0.2222 | 0.5625 | 0.5588 | 0.5938 | 0.4595 |
| **IR24** | 0.5278 | 0.4054 | 0.3056 | 0.0816 | 0.0909 | 0.1224 | 0.6667 | 0.4722 | 0.5 | 0.8 |
| **IR72** | 0.3947 | 0.4286 | 0.3636 | 0.1087 | 0.122 | 0.1277 | 0.7143 | 0.5 | 0.5313 | 0.5294 |
| **KR1-24** | 0.5429 | 0.4571 | 0.3529 | 0.1064 | 0.119 | 0.1489 | 0.6897 | 0.5758 | 0.5625 | 0.7097 |
| **MI48** | 0.5 | 0.3846 | 0.2895 | 0.0784 | 0.087 | 0.1176 | 0.7333 | 0.5278 | 0.5143 | 0.75 |
| **Pokkali** | 0.4865 | 0.3684 | 0.3056 | 0.1042 | 0.1163 | 0.1458 | 0.6667 | 0.5143 | 0.5455 | 0.6364 |
| **Sharbati** | 0.4865 | 0.3684 | 0.3429 | 0.1042 | 0.0667 | 0.1458 | 0.5625 | 0.5143 | 0.5938 | 0.6875 |
| **9311** | 0.2609 | 0.2791 | 0.3158 | 0.6471 | 0.5455 | 0.6571 | 0.1277 | 0.2727 | 0.2273 | 0.1176 |
| **IR64** | 0.3902 | 0.2558 | 0.225 | 0.1224 | 0.1905 | 0.1875 | 0.5294 | 0.375 | 0.3947 | 0.6 |
| **Oglab** | 0.2308 | 0.1538 | 0.1429 | 0.122 | 0.1714 | 0.2308 | 0.1944 | 0.2105 | 0.1892 | 0.3056 |
| **Obart** | 0.2308 | 0.25 | 0.25 | 0.15 | 0.2059 | 0.2973 | 0.303 | 0.2778 | 0.2571 | 0.2703 |
| **Orufi1** | 0.1842 | 0.2353 | 0.2333 | 0.1622 | 0.1875 | 0.3235 | 0.2121 | 0.2286 | 0.2424 | 0.2222 |
| **Orufi2** | 0.25 | 0.2727 | 0.2759 | 0.1316 | 0.1515 | 0.25 | 0.2903 | 0.3438 | 0.3667 | 0.2941 |
| **Oniv1** | 0.2564 | 0.3143 | 0.3226 | 0.1463 | 0.2 | 0.2895 | 0.375 | 0.3429 | 0.3235 | 0.3333 |
| **Oniv2** | 0.2821 | 0.2368 | 0.3125 | 0.2 | 0.2286 | 0.3514 | 0.3235 | 0.2973 | 0.3143 | 0.2895 |
| **Oniv3** | 0.2368 | 0.2941 | 0.3 | 0.1842 | 0.2121 | 0.3429 | 0.2 | 0.3235 | 0.3438 | 0.2432 |
| **Oglumal** | 0.2821 | 0.3056 | 0.3125 | 0.1707 | 0.2286 | 0.3158 | 0.4063 | 0.3714 | 0.3529 | 0.3611 |
| **Omerdio** | 0.3056 | 0.1892 | 0.2188 | 0.0714 | 0.0811 | 0.175 | 0.2727 | 0.3235 | 0.303 | 0.3939 |

Contd…

| | RanBas | TypeIII | Bas385 | CSR30 | ImpSabar | HKR228 | Kasturi | Mahi | PB1 | PS2 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | 1 | | | | | | | | | |
| **TypeIII** | 0.6897 | 1 | | | | | | | | |
| **Bas385** | 0.6667 | 0.7931 | 1 | | | | | | | |
| **CSR30** | 0.6364 | 0.7586 | 0.9 | 1 | | | | | | |
| **ImpSabar** | 0.4839 | 0.5357 | 0.5806 | 0.6 | 1 | | | | | |
| **HKR228** | 0.5333 | 0.6538 | 0.6333 | 0.6552 | 0.5385 | 1 | | | | |
| **Kasturi** | 0.6667 | 0.6786 | 0.7097 | 0.6774 | 0.5714 | 0.5714 | 1 | | | |
| **Mahi** | 0.4571 | 0.5 | 0.5882 | 0.5588 | 0.7308 | 0.4516 | 0.4848 | 1 | | |
| **PB1** | 0.6774 | 0.75 | 0.7742 | 0.7419 | 0.6429 | 0.5862 | 0.7857 | 0.5938 | 1 | |
| **PS2** | 0.4375 | 0.4828 | 0.5806 | 0.5484 | 0.5385 | 0.4815 | 0.4667 | 0.6071 | 0.5333 | 1 |
| **PS3** | 0.4857 | 0.5313 | 0.6176 | 0.5429 | 0.5862 | 0.4375 | 0.5152 | 0.7586 | 0.6774 | 0.6429 |
| **PS4** | 0.5588 | 0.6667 | 0.6471 | 0.6176 | 0.5667 | 0.5161 | 0.7 | 0.5758 | 0.6563 | 0.5667 |
| **Sabar** | 0.5143 | 0.5152 | 0.5556 | 0.4865 | 0.4688 | 0.3824 | 0.5 | 0.4857 | 0.6563 | 0.3824 |
| **Super** | 0.5556 | 0.6563 | 0.8438 | 0.7576 | 0.5152 | 0.5152 | 0.7419 | 0.4865 | 0.697 | 0.4706 |
| **PR118** | 0.3023 | 0.359 | 0.5128 | 0.45 | 0.3889 | 0.25 | 0.4211 | 0.5278 | 0.4737 | 0.4706 |
| **PR106** | 0.4474 | 0.4857 | 0.6111 | 0.5833 | 0.4848 | 0.3243 | 0.4722 | 0.5882 | 0.5278 | 0.4412 |
| **IR36** | 0.5143 | 0.5625 | 0.6471 | 0.6176 | 0.5161 | 0.4242 | 0.6452 | 0.5758 | 0.6061 | 0.6207 |
| **BR4-10** | 0.5333 | 0.4828 | 0.5806 | 0.6 | 0.5385 | 0.4286 | 0.5714 | 0.5517 | 0.5333 | 0.4286 |
| **CSR1** | 0.5429 | 0.5 | 0.5 | 0.4737 | 0.4118 | 0.4118 | 0.4857 | 0.5588 | 0.5429 | 0.4118 |
| **CSR10** | 0.4571 | 0.3714 | 0.4211 | 0.4324 | 0.5 | 0.2857 | 0.5313 | 0.6129 | 0.5455 | 0.4516 |
| **CSR21** | 0.2059 | 0.3571 | 0.3333 | 0.303 | 0.2963 | 0.2963 | 0.3 | 0.3793 | 0.3226 | 0.5217 |
| **HKR120** | 0.4118 | 0.4063 | 0.4167 | 0.3889 | 0.4483 | 0.3125 | 0.5333 | 0.4242 | 0.4545 | 0.3548 |
| **93-401** | 0.4571 | 0.5 | 0.5429 | 0.4722 | 0.5517 | 0.4063 | 0.4848 | 0.7857 | 0.5938 | 0.5517 |
| **IR24** | 0.4571 | 0.5 | 0.4595 | 0.4722 | 0.4063 | 0.2857 | 0.4848 | 0.5152 | 0.4571 | 0.4516 |
| **IR72** | 0.4 | 0.4839 | 0.4857 | 0.4167 | 0.4828 | 0.3871 | 0.4688 | 0.6552 | 0.5313 | 0.4828 |
| **KR1-24** | 0.5152 | 0.5667 | 0.5588 | 0.5294 | 0.5172 | 0.375 | 0.5484 | 0.5313 | 0.5152 | 0.4194 |
| **MI48** | 0.3947 | 0.4286 | 0.5135 | 0.4865 | 0.4688 | 0.3056 | 0.5 | 0.6774 | 0.5588 | 0.5161 |
| **Pokkali** | 0.4167 | 0.4545 | 0.5 | 0.4722 | 0.4063 | 0.3235 | 0.5313 | 0.5625 | 0.5938 | 0.5 |
| **Sharbati** | 0.4571 | 0.5 | 0.5 | 0.5143 | 0.6071 | 0.4063 | 0.5806 | 0.6667 | 0.5938 | 0.4516 |
| **9311** | 0.3171 | 0.2143 | 0.2667 | 0.2174 | 0.1163 | 0.2308 | 0.2093 | 0.1042 | 0.2 | 0.1163 |
| **IR64** | 0.325 | 0.3514 | 0.4 | 0.375 | 0.3824 | 0.2368 | 0.4167 | 0.4054 | 0.3947 | 0.3824 |
| **Oglab** | 0.1579 | 0.2424 | 0.2051 | 0.2105 | 0.2258 | 0.1875 | 0.2353 | 0.1622 | 0.2222 | 0.2258 |
| **Obart** | 0.2222 | 0.2813 | 0.2703 | 0.2432 | 0.2667 | 0.2258 | 0.3125 | 0.1944 | 0.2941 | 0.2258 |
| **Orufi1** | 0.2424 | 0.2258 | 0.2571 | 0.2647 | 0.3462 | 0.2069 | 0.2581 | 0.25 | 0.2424 | 0.25 |
| **Orufi2** | 0.2813 | 0.3571 | 0.375 | 0.3871 | 0.4 | 0.3462 | 0.3929 | 0.2903 | 0.3226 | 0.2963 |
| **Oniv1** | 0.25 | 0.3548 | 0.3333 | 0.3429 | 0.3 | 0.3 | 0.3871 | 0.2222 | 0.3235 | 0.2188 |
| **Oniv2** | 0.2778 | 0.303 | 0.2895 | 0.2632 | 0.2903 | 0.2903 | 0.375 | 0.25 | 0.2778 | 0.25 |
| **Oniv3** | 0.2647 | 0.3333 | 0.3529 | 0.3636 | 0.2759 | 0.3214 | 0.3667 | 0.2 | 0.303 | 0.1935 |
| **Oglumal** | 0.2432 | 0.3438 | 0.3611 | 0.3333 | 0.3333 | 0.3333 | 0.4194 | 0.2857 | 0.3143 | 0.25 |
| **Omerdio** | 0.2647 | 0.3793 | 0.3529 | 0.3636 | 0.3704 | 0.3214 | 0.3667 | 0.2727 | 0.3438 | 0.3214 |

Contd…

| | **PS3** | **PS4** | **Sabar** | **Super** | **PR118** | **PR106** | **IR36** | **BR4-10** | **CSR1** | **CSR10** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | 1 | | | | | | | | | |
| **PS4** | 0.6061 | 1 | | | | | | | | |
| **Sabar** | 0.6563 | 0.4211 | 1 | | | | | | | |
| **Super** | 0.5135 | 0.6286 | 0.5833 | 1 | | | | | | |
| **PR118** | 0.6 | 0.4615 | 0.5833 | 0.6216 | 1 | | | | | |
| **PR106** | 0.5278 | 0.5135 | 0.5556 | 0.5946 | 0.6389 | 1 | | | | |
| **IR36** | 0.6563 | 0.7419 | 0.5 | 0.6286 | 0.6286 | 0.6 | 1 | | | |
| **BR4-10** | 0.4839 | 0.4688 | 0.4242 | 0.5152 | 0.3889 | 0.4412 | 0.5161 | 1 | | |
| **CSR1** | 0.5429 | 0.4474 | 0.4865 | 0.4146 | 0.4146 | 0.425 | 0.5278 | 0.5 | 1 | |
| **CSR10** | 0.5455 | 0.5294 | 0.4857 | 0.4865 | 0.6176 | 0.5882 | 0.625 | 0.4516 | 0.6061 | 1 |
| **CSR21** | 0.5185 | 0.4483 | 0.3125 | 0.2857 | 0.3636 | 0.2941 | 0.4483 | 0.25 | 0.303 | 0.2903 |
| **HKR120** | 0.4545 | 0.4848 | 0.4848 | 0.4857 | 0.4857 | 0.4571 | 0.5806 | 0.5556 | 0.5152 | 0.6207 |
| **93-401** | 0.7586 | 0.5294 | 0.4857 | 0.4474 | 0.4865 | 0.5 | 0.5294 | 0.5 | 0.6061 | 0.5625 |
| **IR24** | 0.5938 | 0.5758 | 0.5294 | 0.4474 | 0.5714 | 0.6364 | 0.7333 | 0.4516 | 0.5143 | 0.6129 |
| **IR72** | 0.75 | 0.5625 | 0.5625 | 0.4324 | 0.5588 | 0.4857 | 0.6667 | 0.3871 | 0.5455 | 0.5484 |
| **KR1-24** | 0.6129 | 0.7 | 0.5 | 0.5429 | 0.4595 | 0.5588 | 0.5938 | 0.5172 | 0.3684 | 0.4848 |
| **MI48** | 0.7667 | 0.5882 | 0.5882 | 0.5 | 0.7273 | 0.697 | 0.7419 | 0.4688 | 0.5278 | 0.7333 |
| **Pokkali** | 0.7 | 0.5758 | 0.5758 | 0.4865 | 0.6667 | 0.5882 | 0.7333 | 0.5 | 0.5588 | 0.6667 |
| **Sharbati** | 0.5455 | 0.625 | 0.4857 | 0.4865 | 0.5278 | 0.7419 | 0.625 | 0.5 | 0.5143 | 0.7241 |
| **9311** | 0.125 | 0.1458 | 0.1957 | 0.2609 | 0.0943 | 0.14 | 0.1 | 0.1163 | 0.2444 | 0.1042 |
| **IR64** | 0.4722 | 0.4595 | 0.4595 | 0.4615 | 0.6765 | 0.4737 | 0.6364 | 0.4242 | 0.375 | 0.5294 |
| **Oglab** | 0.2222 | 0.3235 | 0.2162 | 0.2308 | 0.2 | 0.2703 | 0.2162 | 0.1176 | 0.0952 | 0.1622 |
| **Obart** | 0.2941 | 0.3636 | 0.2162 | 0.2973 | 0.2632 | 0.2368 | 0.2857 | 0.1875 | 0.1795 | 0.2286 |
| **Orufi1** | 0.2059 | 0.2353 | 0.1351 | 0.25 | 0.1842 | 0.2222 | 0.2353 | 0.25 | 0.1622 | 0.25 |
| **Orufi2** | 0.2424 | 0.4 | 0.1667 | 0.3636 | 0.2162 | 0.2571 | 0.3548 | 0.2963 | 0.1622 | 0.2121 |
| **Oniv1** | 0.2857 | 0.4375 | 0.2105 | 0.3611 | 0.2564 | 0.2632 | 0.3529 | 0.2581 | 0.2051 | 0.2571 |
| **Oniv2** | 0.2432 | 0.3824 | 0.175 | 0.3158 | 0.25 | 0.2564 | 0.3056 | 0.2903 | 0.2632 | 0.3235 |
| **Oniv3** | 0.1622 | 0.2941 | 0.1579 | 0.3429 | 0.1463 | 0.2105 | 0.2571 | 0.2759 | 0.1842 | 0.1351 |
| **Oglumal** | 0.3143 | 0.3824 | 0.2368 | 0.3889 | 0.2821 | 0.2895 | 0.3429 | 0.25 | 0.2 | 0.25 |
| **Omerdio** | 0.2647 | 0.4194 | 0.2571 | 0.3824 | 0.2703 | 0.3939 | 0.375 | 0.1935 | 0.125 | 0.2353 |

Contd…

| | CSR21 | HKR120 | 93-401 | IR24 | IR72 | KR1-24 | MI48 | Pokkali | Sharbati | 9311 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | | | | | | | | | | |
| **PS4** | | | | | | | | | | |
| **Sabar** | | | | | | | | | | |
| **Super** | | | | | | | | | | |
| **PR118** | | | | | | | | | | |
| **PR106** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | 1 | | | | | | | | | |
| **HKR120** | 0.3704 | 1 | | | | | | | | |
| **93-401** | 0.4815 | 0.5667 | 1 | | | | | | | |
| **IR24** | 0.4286 | 0.5667 | 0.5152 | 1 | | | | | | |
| **IR72** | 0.52 | 0.5 | 0.6552 | 0.6 | 1 | | | | | |
| **KR1-24** | 0.3448 | 0.4839 | 0.4412 | 0.6333 | 0.5161 | 1 | | | | |
| **MI48** | 0.4483 | 0.5313 | 0.625 | 0.7931 | 0.7241 | 0.6452 | 1 | | | |
| **Pokkali** | 0.4815 | 0.5667 | 0.6129 | 0.7241 | 0.6552 | 0.5313 | 0.8571 | 1 | | |
| **Sharbati** | 0.3333 | 0.5667 | 0.5625 | 0.6129 | 0.6 | 0.5806 | 0.7333 | 0.6667 | 1 | |
| **9311** | 0.075 | 0.1628 | 0.1778 | 0.0816 | 0.0851 | 0.1064 | 0.0577 | 0.06 | 0.06 | 1 |
| **IR64** | 0.3548 | 0.5806 | 0.4444 | 0.625 | 0.4286 | 0.4571 | 0.5882 | 0.625 | 0.4444 | 0.1 |
| **Oglab** | 0.32 | 0.2121 | 0.1944 | 0.303 | 0.1714 | 0.3548 | 0.25 | 0.2286 | 0.2286 | 0.2105 |
| **Obart** | 0.32 | 0.3333 | 0.2647 | 0.2647 | 0.2424 | 0.4 | 0.2857 | 0.303 | 0.2647 | 0.1795 |
| **Orufi1** | 0.2 | 0.2759 | 0.25 | 0.1765 | 0.1875 | 0.2581 | 0.2 | 0.1765 | 0.25 | 0.1944 |
| **Orufi2** | 0.2 | 0.2759 | 0.25 | 0.25 | 0.2667 | 0.3448 | 0.2353 | 0.2121 | 0.2903 | 0.1622 |
| **Oniv1** | 0.2593 | 0.3226 | 0.2222 | 0.3333 | 0.2727 | 0.4828 | 0.3143 | 0.2941 | 0.2941 | 0.175 |
| **Oniv2** | 0.25 | 0.4483 | 0.3235 | 0.2857 | 0.2286 | 0.375 | 0.2703 | 0.2857 | 0.3235 | 0.2308 |
| **Oniv3** | 0.1429 | 0.2188 | 0.2 | 0.1667 | 0.1765 | 0.2424 | 0.1579 | 0.1667 | 0.2353 | 0.25 |
| **Oglumal** | 0.25 | 0.3125 | 0.2857 | 0.3235 | 0.3438 | 0.4194 | 0.3429 | 0.2857 | 0.3235 | 0.2308 |
| **Omerdio** | 0.2308 | 0.2188 | 0.2 | 0.3548 | 0.25 | 0.4138 | 0.2941 | 0.2353 | 0.3125 | 0.1538 |

Contd…

| | **IR64** | **Oglab** | **Obart** | **Orufi1** | **Orufi2** | **Oniv1** | **Oniv2** | **Oniv3** | **Oglumal** | **Omerdio** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Azu** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **T309** | | | | | | | | | | |
| **TNG67** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **RanBas** | | | | | | | | | | |
| **TypeIII** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **ImpSabar** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **PS2** | | | | | | | | | | |
| **PS3** | | | | | | | | | | |
| **PS4** | | | | | | | | | | |
| **Sabar** | | | | | | | | | | |
| **Super** | | | | | | | | | | |
| **PR118** | | | | | | | | | | |
| **PR106** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | | | | | | | | | | |
| **HKR120** | | | | | | | | | | |
| **93-401** | | | | | | | | | | |
| **IR24** | | | | | | | | | | |
| **IR72** | | | | | | | | | | |
| **KR1-24** | | | | | | | | | | |
| **MI48** | | | | | | | | | | |
| **Pokkali** | | | | | | | | | | |
| **Sharbati** | | | | | | | | | | |
| **9311** | | | | | | | | | | |
| **IR64** | 1 | | | | | | | | | |
| **Oglab** | 0.2857 | 1 | | | | | | | | |
| **Obart** | 0.3235 | 0.6364 | 1 | | | | | | | |
| **Orufi1** | 0.2353 | 0.4348 | 0.5714 | 1 | | | | | | |
| **Orufi2** | 0.2353 | 0.375 | 0.5 | 0.5789 | 1 | | | | | |
| **Oniv1** | 0.3143 | 0.6087 | 0.7619 | 0.4783 | 0.619 | 1 | | | | |
| **Oniv2** | 0.3429 | 0.52 | 0.6522 | 0.6667 | 0.5217 | 0.625 | 1 | | | |
| **Oniv3** | 0.1892 | 0.2963 | 0.4 | 0.4545 | 0.6842 | 0.44 | 0.4231 | 1 | | |
| **Oglumal** | 0.3056 | 0.5833 | 0.5833 | 0.5217 | 0.5909 | 0.7727 | 0.6 | 0.4231 | 1 | |
| **Omerdio** | 0.2571 | 0.5909 | 0.4583 | 0.3913 | 0.6 | 0.5652 | 0.4231 | 0.4167 | 0.48 | 1 |

## ANNEXURE IX

### Similarity matrix data of 50 rice genotypes obtained using PowerMarker analysis

| **OTU** | **9311** | **93-401** | **Azu** | **B370** | **Bas217** | **Bas385** | **BR4-10** | **CSR1** | **CSR10** | **CSR21** |
|---|---|---|---|---|---|---|---|---|---|---|
| **9311** | 0.0000 | | | | | | | | | |
| **93-401** | 0.7518 | 0.0000 | | | | | | | | |
| **Azu** | 0.7280 | 0.7717 | 0.0000 | | | | | | | |
| **B370** | 0.7648 | 0.6247 | 0.6482 | 0.0000 | | | | | | |
| **Bas217** | 0.8237 | 0.6233 | 0.8167 | 0.6291 | 0.0000 | | | | | |
| **Bas385** | 0.7280 | 0.5979 | 0.6940 | 0.4336 | 0.5744 | 0.0000 | | | | |
| **BR4-10** | 0.7518 | 0.6104 | 0.6798 | 0.6898 | 0.6146 | 0.7633 | 0.0000 | | | |
| **CSR1** | 0.7651 | 0.6479 | 0.7081 | 0.7081 | 0.5571 | 0.5795 | 0.6752 | 0.0000 | | |
| **CSR10** | 0.8081 | 0.6580 | 0.7633 | 0.6898 | 0.5762 | 0.6163 | 0.7041 | 0.2915 | 0.0000 | |
| **CSR21** | 0.7559 | 0.5407 | 0.8045 | 0.6617 | 0.5819 | 0.5938 | 0.6475 | 0.5676 | 0.5301 | 0.0000 |
| **CSR30** | 0.7463 | 0.5916 | 0.6362 | 0.3322 | 0.6617 | 0.4103 | 0.7620 | 0.6049 | 0.6854 | 0.6475 |
| **HBC19** | 0.7464 | 0.6063 | 0.6482 | 0.2701 | 0.6666 | 0.4419 | 0.7081 | 0.6530 | 0.6898 | 0.7088 |
| **HKR120** | 0.7916 | 0.6431 | 0.8020 | 0.7203 | 0.6479 | 0.5499 | 0.6630 | 0.5880 | 0.4593 | 0.5850 |
| **HKR228** | 0.7649 | 0.6972 | 0.7103 | 0.6441 | 0.7311 | 0.6002 | 0.7062 | 0.6956 | 0.6369 | 0.6863 |
| **ImpShar** | 0.7331 | 0.6291 | 0.7265 | 0.6982 | 0.5204 | 0.6262 | 0.6666 | 0.5775 | 0.5470 | 0.5496 |
| **IR24** | 0.7633 | 0.6163 | 0.7300 | 0.6580 | 0.6307 | 0.6317 | 0.6630 | 0.6247 | 0.5428 | 0.5379 |
| **IR36** | 0.7449 | 0.5344 | 0.7840 | 0.6595 | 0.5283 | 0.5514 | 0.6078 | 0.5810 | 0.5711 | 0.5675 |
| **IR64** | 0.7916 | 0.5696 | 0.8283 | 0.6482 | 0.6666 | 0.6302 | 0.6714 | 0.6063 | 0.4877 | 0.4613 |
| **IR72** | 0.7534 | 0.5963 | 0.7816 | 0.7464 | 0.6441 | 0.6094 | 0.6955 | 0.6565 | 0.6666 | 0.5888 |
| **Kasturi** | 0.7395 | 0.6163 | 0.7480 | 0.5334 | 0.6768 | 0.5252 | 0.6798 | 0.5458 | 0.6078 | 0.6808 |
| **KLocal** | 0.7449 | 0.5696 | 0.6857 | 0.5154 | 0.5455 | 0.4449 | 0.6898 | 0.7112 | 0.6530 | 0.6130 |
| **KR1-24** | 0.7295 | 0.6130 | 0.6940 | 0.6752 | 0.6760 | 0.6666 | 0.4980 | 0.7088 | 0.7854 | 0.6279 |
| **Mahi** | 0.7383 | 0.6617 | 0.7878 | 0.6854 | 0.6067 | 0.6479 | 0.6808 | 0.6042 | 0.6233 | 0.5910 |
| **MI48** | 0.8026 | 0.6002 | 0.7732 | 0.6630 | 0.6337 | 0.6446 | 0.6479 | 0.6510 | 0.5353 | 0.4999 |
| **Nippon** | 0.4057 | 0.7265 | 0.7563 | 0.7563 | 0.7979 | 0.7660 | 0.7901 | 0.7816 | 0.8000 | 0.8149 |
| **NPT2** | 0.6119 | 0.7690 | 0.4877 | 0.7449 | 0.7766 | 0.7097 | 0.7792 | 0.6987 | 0.7690 | 0.7935 |
| **NPT3** | 0.5652 | 0.8203 | 0.5586 | 0.7633 | 0.7803 | 0.7348 | 0.8003 | 0.7603 | 0.7511 | 0.7956 |
| **Obart** | 0.8416 | 0.7486 | 0.7870 | 0.6808 | 0.7311 | 0.7191 | 0.6671 | 0.7062 | 0.7000 | 0.7019 |
| **Oglab** | 0.7711 | 0.7002 | 0.7242 | 0.5872 | 0.8185 | 0.7543 | 0.7002 | 0.7603 | 0.8003 | 0.7555 |
| **Oglumal** | 0.7755 | 0.8612 | 0.7279 | 0.6896 | 0.6711 | 0.7279 | 0.6459 | 0.7543 | 0.8237 | 0.8185 |
| **Omerdio** | 0.8047 | 0.7717 | 0.7538 | 0.7956 | 0.7905 | 0.7232 | 0.8262 | 0.8360 | 0.7860 | 0.7775 |
| **Oniv1** | 0.6720 | 0.8045 | 0.7604 | 0.7690 | 0.8220 | 0.7518 | 0.7331 | 0.7415 | 0.8149 | 0.7711 |
| **Oniv2** | 0.6854 | 0.7503 | 0.7717 | 0.7533 | 0.7854 | 0.7717 | 0.7417 | 0.7878 | 0.7690 | 0.8718 |
| **Oniv3** | 0.7191 | 0.7191 | 0.7128 | 0.7690 | 0.7739 | 0.7315 | 0.7574 | 0.7383 | 0.7383 | 0.7727 |
| **Orufi1** | 0.7886 | 0.7571 | 0.7603 | 0.7803 | 0.8070 | 0.8311 | 0.7366 | 0.7886 | 0.7980 | 0.7833 |
| **Orufi2** | 0.7242 | 0.7046 | 0.8045 | 0.7088 | 0.7803 | 0.7574 | 0.7543 | 0.6866 | 0.7543 | 0.7102 |
| **PB1** | 0.7622 | 0.5762 | 0.7417 | 0.4908 | 0.7274 | 0.5572 | 0.7000 | 0.5954 | 0.6632 | 0.7144 |
| **Pokkali** | 0.8643 | 0.5916 | 0.7717 | 0.7365 | 0.5866 | 0.6262 | 0.6479 | 0.5267 | 0.4908 | 0.6581 |
| **PR106** | 0.7331 | 0.5541 | 0.8184 | 0.6714 | 0.6042 | 0.6078 | 0.6955 | 0.7315 | 0.5627 | 0.5105 |
| **PR118** | 0.8100 | 0.6247 | 0.7923 | 0.5762 | 0.5166 | 0.5859 | 0.7081 | 0.6530 | 0.5612 | 0.5762 |
| **PS2** | 0.7792 | 0.5916 | 0.7633 | 0.7365 | 0.6513 | 0.6094 | 0.7604 | 0.6377 | 0.6377 | 0.6760 |
| **PS3** | 0.7957 | 0.6233 | 0.7503 | 0.6580 | 0.6173 | 0.5369 | 0.7191 | 0.6704 | 0.6513 | 0.6711 |
| **PS4** | 0.7548 | 0.5994 | 0.8020 | 0.6332 | 0.6307 | 0.5874 | 0.7280 | 0.6377 | 0.6576 | 0.6441 |
| **RanBas** | 0.6619 | 0.6419 | 0.6671 | 0.3661 | 0.6143 | 0.4672 | 0.6882 | 0.6727 | 0.6544 | 0.7040 |
| **Sabarm** | 0.8268 | 0.6262 | 0.8103 | 0.6580 | 0.5541 | 0.6677 | 0.7081 | 0.6347 | 0.5711 | 0.6337 |
| **Sharbati** | 0.7464 | 0.5810 | 0.8118 | 0.7383 | 0.5470 | 0.6775 | 0.6660 | 0.6660 | 0.6094 | 0.6193 |
| **Super** | 0.6760 | 0.5428 | 0.6760 | 0.4336 | 0.7143 | 0.5252 | 0.7548 | 0.6392 | 0.6928 | 0.6944 |
| **T309** | 0.4665 | 0.7128 | 0.6630 | 0.6729 | 0.7917 | 0.7142 | 0.7878 | 0.7737 | 0.8081 | 0.8149 |
| **TNG67** | 0.5166 | 0.7315 | 0.7350 | 0.6982 | 0.7574 | 0.7633 | 0.8065 | 0.8065 | 0.7979 | 0.8326 |
| **TypeIII** | 0.7229 | 0.5932 | 0.6615 | 0.3054 | 0.6441 | 0.4073 | 0.7041 | 0.6714 | 0.7143 | 0.6507 |

Contd…

| OTU | CSR30 | HBC19 | HKR120 | HKR228 | ImpShar | IR24 | IR36 | IR64 | IR72 | Kasturi |
|---|---|---|---|---|---|---|---|---|---|---|
| 9311 | | | | | | | | | | |
| 93-401 | | | | | | | | | | |
| Azu | | | | | | | | | | |
| B370 | | | | | | | | | | |
| Bas217 | | | | | | | | | | |
| Bas385 | | | | | | | | | | |
| BR4-10 | | | | | | | | | | |
| CSR1 | | | | | | | | | | |
| CSR10 | | | | | | | | | | |
| CSR21 | | | | | | | | | | |
| CSR30 | 0.0000 | | | | | | | | | |
| HBC19 | 0.2235 | 0.0000 | | | | | | | | |
| HKR120 | 0.6798 | 0.6857 | 0.0000 | | | | | | | |
| HKR228 | 0.5651 | 0.5571 | 0.6281 | 0.0000 | | | | | | |
| ImpShar | 0.6408 | 0.6347 | 0.6277 | 0.5920 | 0.0000 | | | | | |
| IR24 | 0.6545 | 0.6220 | 0.4531 | 0.5204 | 0.4868 | 0.0000 | | | | |
| IR36 | 0.6729 | 0.6137 | 0.5237 | 0.6193 | 0.5359 | 0.4974 | 0.0000 | | | |
| IR64 | 0.6813 | 0.6662 | 0.4877 | 0.6720 | 0.5795 | 0.4779 | 0.4599 | 0.0000 | | |
| IR72 | 0.6971 | 0.6729 | 0.6009 | 0.6279 | 0.5689 | 0.3889 | 0.4807 | 0.5443 | 0.0000 | |
| Kasturi | 0.3705 | 0.4254 | 0.6497 | 0.5044 | 0.5106 | 0.5582 | 0.6332 | 0.6760 | 0.6476 | 0.0000 |
| KLocal | 0.4570 | 0.4254 | 0.6220 | 0.4908 | 0.4922 | 0.5267 | 0.6152 | 0.5942 | 0.6729 | 0.5004 |
| KR1-24 | 0.7295 | 0.7128 | 0.6854 | 0.6819 | 0.6648 | 0.5830 | 0.6682 | 0.7503 | 0.6042 | 0.7503 |
| Mahi | 0.6337 | 0.7229 | 0.6291 | 0.6110 | 0.5411 | 0.5556 | 0.5556 | 0.5541 | 0.5411 | 0.6018 |
| MI48 | 0.6932 | 0.7081 | 0.6476 | 0.6475 | 0.6049 | 0.5612 | 0.4838 | 0.4693 | 0.5470 | 0.6844 |
| Nippon | 0.7081 | 0.7022 | 0.8200 | 0.7766 | 0.7633 | 0.8103 | 0.8283 | 0.7563 | 0.8184 | 0.7577 |
| NPT2 | 0.6713 | 0.6898 | 0.6997 | 0.6956 | 0.7143 | 0.7081 | 0.7717 | 0.7533 | 0.7417 | 0.7847 |
| NPT3 | 0.7311 | 0.7633 | 0.7062 | 0.7538 | 0.7219 | 0.7062 | 0.7633 | 0.7242 | 0.7111 | 0.7935 |
| Obart | 0.7258 | 0.7574 | 0.7295 | 0.8202 | 0.7845 | 0.7678 | 0.7694 | 0.7191 | 0.8041 | 0.7662 |
| Oglab | 0.6911 | 0.6850 | 0.7543 | 0.7232 | 0.8403 | 0.6760 | 0.7543 | 0.7633 | 0.7111 | 0.7739 |
| Oglumal | 0.6866 | 0.7088 | 0.7383 | 0.7511 | 0.7543 | 0.7486 | 0.7471 | 0.8045 | 0.7258 | 0.7486 |
| Omerdio | 0.7092 | 0.7538 | 0.7441 | 0.7603 | 0.7232 | 0.7974 | 0.7538 | 0.6700 | 0.7109 | 0.8183 |
| Oniv1 | 0.8061 | 0.8065 | 0.7823 | 0.8003 | 0.8476 | 0.7979 | 0.8253 | 0.8167 | 0.7678 | 0.7518 |
| Oniv2 | 0.7604 | 0.7350 | 0.7901 | 0.7437 | 0.7706 | 0.7548 | 0.7901 | 0.7717 | 0.7417 | 0.7181 |
| Oniv3 | 0.8045 | 0.7878 | 0.7792 | 0.7619 | 0.7766 | 0.7229 | 0.7432 | 0.7417 | 0.7000 | 0.7229 |
| Orufi1 | 0.8091 | 0.7603 | 0.8311 | 0.7538 | 0.7886 | 0.7711 | 0.8111 | 0.7711 | 0.8091 | 0.7511 |
| Orufi2 | 0.7437 | 0.7471 | 0.7471 | 0.6511 | 0.7258 | 0.7088 | 0.7000 | 0.6896 | 0.7152 | 0.7279 |
| PB1 | 0.4780 | 0.3970 | 0.6494 | 0.4684 | 0.5531 | 0.5369 | 0.5947 | 0.5916 | 0.6840 | 0.4767 |
| Pokkali | 0.7518 | 0.7081 | 0.5344 | 0.6475 | 0.5932 | 0.6645 | 0.5542 | 0.5527 | 0.4994 | 0.6813 |
| PR106 | 0.6580 | 0.6530 | 0.6262 | 0.5211 | 0.5744 | 0.4892 | 0.5542 | 0.5060 | 0.5181 | 0.5841 |
| PR118 | 0.5726 | 0.5402 | 0.5597 | 0.5571 | 0.4708 | 0.4696 | 0.5597 | 0.4862 | 0.5259 | 0.4516 |
| PS2 | 0.6424 | 0.6178 | 0.5994 | 0.5317 | 0.5517 | 0.4991 | 0.5443 | 0.6262 | 0.4923 | 0.5022 |
| PS3 | 0.6441 | 0.6307 | 0.6291 | 0.5760 | 0.5618 | 0.4994 | 0.5658 | 0.5830 | 0.5323 | 0.6033 |
| PS4 | 0.5091 | 0.5169 | 0.6497 | 0.4557 | 0.6139 | 0.5934 | 0.5626 | 0.6152 | 0.5136 | 0.5544 |
| RanBas | 0.4776 | 0.4730 | 0.7258 | 0.6316 | 0.6560 | 0.5920 | 0.6597 | 0.6369 | 0.6711 | 0.5814 |
| Sabarm | 0.7265 | 0.6662 | 0.6040 | 0.6225 | 0.4708 | 0.5057 | 0.5154 | 0.5402 | 0.5726 | 0.6054 |
| Sharbati | 0.6997 | 0.6497 | 0.5334 | 0.6696 | 0.5076 | 0.5589 | 0.5252 | 0.5709 | 0.5121 | 0.6707 |
| Super | 0.3238 | 0.3894 | 0.7217 | 0.6161 | 0.7280 | 0.6317 | 0.6497 | 0.6760 | 0.6545 | 0.4531 |
| T309 | 0.6565 | 0.6545 | 0.8184 | 0.7649 | 0.7432 | 0.8184 | 0.8467 | 0.7449 | 0.8081 | 0.7365 |
| TNG67 | 0.7503 | 0.6982 | 0.7816 | 0.7348 | 0.8167 | 0.8184 | 0.8000 | 0.7350 | 0.8268 | 0.7732 |
| TypeIII | 0.3509 | 0.3422 | 0.7816 | 0.5545 | 0.6682 | 0.6362 | 0.6744 | 0.7181 | 0.7072 | 0.4539 |

Contd…

| **OTU** | **KLocal** | **KR1-24** | **Mahi** | **MI48** | **Nippon** | **NPT2** | **NPT3** | **Obart** | **Oglab** | **Oglumal** |
|---|---|---|---|---|---|---|---|---|---|---|
| **9311** | | | | | | | | | | |
| **93-401** | | | | | | | | | | |
| **Azu** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **HKR120** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **ImpShar** | | | | | | | | | | |
| **IR24** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **IR64** | | | | | | | | | | |
| **IR72** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **KLocal** | 0.0000 | | | | | | | | | |
| **KR1-24** | 0.6190 | 0.0000 | | | | | | | | |
| **Mahi** | 0.5815 | 0.6808 | 0.0000 | | | | | | | |
| **MI48** | 0.6055 | 0.6441 | 0.5084 | 0.0000 | | | | | | |
| **Nippon** | 0.7563 | 0.7128 | 0.8065 | 0.8268 | 0.0000 | | | | | |
| **NPT2** | 0.7181 | 0.7471 | 0.7690 | 0.8112 | 0.5512 | 0.0000 | | | | |
| **NPT3** | 0.7559 | 0.7980 | 0.7571 | 0.8311 | 0.4999 | 0.3418 | 0.0000 | | | |
| **Obart** | 0.7383 | 0.7348 | 0.8003 | 0.7454 | 0.8253 | 0.7935 | 0.7651 | 0.0000 | | |
| **Oglab** | 0.7633 | 0.5729 | 0.7886 | 0.7711 | 0.7829 | 0.7603 | 0.7747 | 0.7588 | 0.0000 | |
| **Oglumal** | 0.7207 | 0.7242 | 0.7633 | 0.7364 | 0.7279 | 0.7152 | 0.7538 | 0.7127 | 0.7119 | 0.0000 |
| **Omerdio** | 0.7651 | 0.7503 | 0.6860 | 0.7092 | 0.7119 | 0.7503 | 0.7849 | 0.8024 | 0.7203 | 0.5863 |
| **Oniv1** | 0.7979 | 0.7454 | 0.7437 | 0.7854 | 0.6666 | 0.7383 | 0.7162 | 0.7311 | 0.8295 | 0.7511 |
| **Oniv2** | 0.7717 | 0.7662 | 0.7574 | 0.7979 | 0.6431 | 0.7503 | 0.7603 | 0.8612 | 0.7603 | 0.8025 |
| **Oniv3** | 0.6955 | 0.6513 | 0.8149 | 0.7590 | 0.7792 | 0.8237 | 0.7886 | 0.7727 | 0.6548 | 0.7152 |
| **Orufi1** | 0.7744 | 0.7747 | 0.7571 | 0.8091 | 0.7603 | 0.7911 | 0.8244 | 0.7503 | 0.7833 | 0.7931 |
| **Orufi2** | 0.6425 | 0.6911 | 0.6310 | 0.7633 | 0.7471 | 0.7242 | 0.7747 | 0.7477 | 0.6072 | 0.7571 |
| **PB1** | 0.6314 | 0.7046 | 0.6560 | 0.6281 | 0.7315 | 0.7549 | 0.8185 | 0.7711 | 0.7477 | 0.7819 |
| **Pokkali** | 0.7265 | 0.6808 | 0.7000 | 0.5267 | 0.8452 | 0.7979 | 0.7911 | 0.7454 | 0.8111 | 0.7348 |
| **PR106** | 0.4693 | 0.6666 | 0.5830 | 0.4532 | 0.7166 | 0.7979 | 0.8003 | 0.7649 | 0.7603 | 0.7957 |
| **PR118** | 0.5237 | 0.6565 | 0.4877 | 0.4792 | 0.7923 | 0.8000 | 0.8025 | 0.7957 | 0.7829 | 0.7854 |
| **PS2** | 0.5344 | 0.6338 | 0.5658 | 0.6580 | 0.8084 | 0.7792 | 0.7203 | 0.7454 | 0.7603 | 0.8045 |
| **PS3** | 0.5728 | 0.5635 | 0.6385 | 0.6337 | 0.7792 | 0.7574 | 0.7437 | 0.7419 | 0.7272 | 0.7203 |
| **PS4** | 0.6234 | 0.7002 | 0.5415 | 0.6492 | 0.7660 | 0.7265 | 0.7046 | 0.7901 | 0.6866 | 0.7191 |
| **RanBas** | 0.4428 | 0.6035 | 0.7152 | 0.6960 | 0.6850 | 0.7062 | 0.7092 | 0.6621 | 0.6779 | 0.7178 |
| **Sabarm** | 0.4959 | 0.6479 | 0.4634 | 0.4907 | 0.8463 | 0.8084 | 0.8025 | 0.7383 | 0.7437 | 0.7471 |
| **Sharbati** | 0.6234 | 0.6666 | 0.5572 | 0.4769 | 0.7938 | 0.7648 | 0.7755 | 0.7694 | 0.7739 | 0.7766 |
| **Super** | 0.5559 | 0.6955 | 0.6205 | 0.7211 | 0.6580 | 0.6476 | 0.6850 | 0.7518 | 0.7046 | 0.7103 |
| **T309** | 0.7043 | 0.7088 | 0.8237 | 0.8198 | 0.1868 | 0.5486 | 0.5018 | 0.8236 | 0.7511 | 0.7258 |
| **TNG67** | 0.7717 | 0.7662 | 0.8045 | 0.7878 | 0.2572 | 0.6565 | 0.5002 | 0.7935 | 0.7803 | 0.7829 |
| **TypeIII** | 0.5106 | 0.6808 | 0.6840 | 0.6854 | 0.7449 | 0.7331 | 0.7803 | 0.6956 | 0.6619 | 0.6956 |

Contd…

| OTU | Omerdio | Oniv1 | Oniv2 | Oniv3 | Orufi1 | Orufi2 | PB1 | Pokkali | PR106 | PR118 |
|---|---|---|---|---|---|---|---|---|---|---|
| **9311** | | | | | | | | | | |
| **93-401** | | | | | | | | | | |
| **Azu** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **HKR120** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **ImpShar** | | | | | | | | | | |
| **IR24** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **IR64** | | | | | | | | | | |
| **IR72** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **KR1-24** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **MI48** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **Obart** | | | | | | | | | | |
| **Oglab** | | | | | | | | | | |
| **Oglumal** | | | | | | | | | | |
| **Omerdio** | 0.0000 | | | | | | | | | |
| **Oniv1** | 0.7804 | 0.0000 | | | | | | | | |
| **Oniv2** | 0.6700 | 0.6808 | 0.0000 | | | | | | | |
| **Oniv3** | 0.7306 | 0.6972 | 0.7383 | 0.0000 | | | | | | |
| **Orufi1** | 0.6695 | 0.6769 | 0.6752 | 0.7232 | 0.0000 | | | | | |
| **Orufi2** | 0.6874 | 0.6671 | 0.7829 | 0.6402 | 0.6219 | 0.0000 | | | | |
| **PB1** | 0.6807 | 0.7829 | 0.7000 | 0.7559 | 0.7571 | 0.7419 | 0.0000 | | | |
| **Pokkali** | 0.7190 | 0.7383 | 0.7417 | 0.7574 | 0.7383 | 0.7543 | 0.6494 | 0.0000 | | |
| **PR106** | 0.7441 | 0.8045 | 0.7690 | 0.7229 | 0.8185 | 0.7152 | 0.6441 | 0.6479 | 0.0000 | |
| **PR118** | 0.7328 | 0.7604 | 0.7166 | 0.7503 | 0.7803 | 0.7088 | 0.4619 | 0.5895 | 0.4693 | 0.0000 |
| **PS2** | 0.7345 | 0.8253 | 0.7792 | 0.7690 | 0.8202 | 0.7062 | 0.5602 | 0.6479 | 0.4609 | 0.4892 |
| **PS3** | 0.6707 | 0.8538 | 0.7766 | 0.7046 | 0.8087 | 0.7219 | 0.5920 | 0.6441 | 0.4805 | 0.5369 |
| **PS4** | 0.6927 | 0.8268 | 0.7380 | 0.7244 | 0.8144 | 0.6265 | 0.4915 | 0.6561 | 0.5190 | 0.5086 |
| **RanBas** | 0.8450 | 0.7636 | 0.7511 | 0.6727 | 0.7503 | 0.7555 | 0.5976 | 0.7527 | 0.6459 | 0.6866 |
| **Sabarm** | 0.7538 | 0.7979 | 0.8084 | 0.7143 | 0.8311 | 0.7000 | 0.6611 | 0.6446 | 0.4892 | 0.4322 |
| **Sharbati** | 0.7555 | 0.7924 | 0.7648 | 0.7432 | 0.7819 | 0.7782 | 0.6221 | 0.5052 | 0.5458 | 0.4449 |
| **Super** | 0.7441 | 0.7604 | 0.7081 | 0.7331 | 0.7911 | 0.6944 | 0.5259 | 0.7916 | 0.6262 | 0.6317 |
| **T309** | 0.7404 | 0.7191 | 0.6768 | 0.7814 | 0.7588 | 0.7152 | 0.6856 | 0.8253 | 0.7128 | 0.7732 |
| **TNG67** | 0.7931 | 0.6321 | 0.6940 | 0.8061 | 0.6802 | 0.7662 | 0.7088 | 0.7417 | 0.7315 | 0.7533 |
| **TypeIII** | 0.8146 | 0.7471 | 0.7417 | 0.7870 | 0.7162 | 0.7242 | 0.4805 | 0.7057 | 0.7057 | 0.6813 |

Contd…

| **OTU** | **PS2** | **PS3** | **PS4** | **RanBas** | **Sabarm** | **Sharbati** | **Super** | **T309** | **TNG67** | **TypeIII** |
|---|---|---|---|---|---|---|---|---|---|---|
| **9311** | | | | | | | | | | |
| **93-401** | | | | | | | | | | |
| **Azu** | | | | | | | | | | |
| **B370** | | | | | | | | | | |
| **Bas217** | | | | | | | | | | |
| **Bas385** | | | | | | | | | | |
| **BR4-10** | | | | | | | | | | |
| **CSR1** | | | | | | | | | | |
| **CSR10** | | | | | | | | | | |
| **CSR21** | | | | | | | | | | |
| **CSR30** | | | | | | | | | | |
| **HBC19** | | | | | | | | | | |
| **HKR120** | | | | | | | | | | |
| **HKR228** | | | | | | | | | | |
| **ImpShar** | | | | | | | | | | |
| **IR24** | | | | | | | | | | |
| **IR36** | | | | | | | | | | |
| **IR64** | | | | | | | | | | |
| **IR72** | | | | | | | | | | |
| **Kasturi** | | | | | | | | | | |
| **KLocal** | | | | | | | | | | |
| **KR1-24** | | | | | | | | | | |
| **Mahi** | | | | | | | | | | |
| **MI48** | | | | | | | | | | |
| **Nippon** | | | | | | | | | | |
| **NPT2** | | | | | | | | | | |
| **NPT3** | | | | | | | | | | |
| **Obart** | | | | | | | | | | |
| **Oglab** | | | | | | | | | | |
| **Oglumal** | | | | | | | | | | |
| **Omerdio** | | | | | | | | | | |
| **Oniv1** | | | | | | | | | | |
| **Oniv2** | | | | | | | | | | |
| **Oniv3** | | | | | | | | | | |
| **Orufi1** | | | | | | | | | | |
| **Orufi2** | | | | | | | | | | |
| **PB1** | | | | | | | | | | |
| **Pokkali** | | | | | | | | | | |
| **PR106** | | | | | | | | | | |
| **PR118** | | | | | | | | | | |
| **PS2** | 0.0000 | | | | | | | | | |
| **PS3** | 0.2977 | 0.0000 | | | | | | | | |
| **PS4** | 0.4486 | 0.4579 | 0.0000 | | | | | | | |
| **RanBas** | 0.7005 | 0.6505 | 0.6254 | 0.0000 | | | | | | |
| **Sabarm** | 0.5160 | 0.4142 | 0.5904 | 0.6777 | 0.0000 | | | | | |
| **Sharbati** | 0.5190 | 0.5932 | 0.6114 | 0.6581 | 0.4711 | 0.0000 | | | | |
| **Super** | 0.6277 | 0.6408 | 0.4711 | 0.5439 | 0.7300 | 0.6955 | 0.0000 | | | |
| **T309** | 0.7792 | 0.7766 | 0.7548 | 0.6760 | 0.8452 | 0.7916 | 0.6347 | 0.0000 | | |
| **TNG67** | 0.7690 | 0.7574 | 0.7633 | 0.7002 | 0.7717 | 0.7732 | 0.7717 | 0.2641 | 0.0000 | |
| **TypeIII** | 0.7260 | 0.6544 | 0.6422 | 0.4159 | 0.7127 | 0.7365 | 0.4386 | 0.6971 | 0.7229 | 0.0000 |

# ABSTRACT

**Title of Thesis** : DNA fingerprinting and phylogenetic analysis of Indian Basmati and other rice types using SSR and transposon element based markers

**Full Name of Degree Holder** : Amit Kaushik (2001BS62D)

**Title of Degree** : Doctor of Philosophy

**Name and Address of Major Advisor** : Dr. R.K. Jain (Professor)
Dept. of Biotechnology and Molecular Biology College of Basic Sciences & Humanities
CCS Haryana Agricultural University
Hisar-125 004, India

**Degree awarding University/Institute** : CCS Haryana Agricultural University
Hisar-125 004, India

**Year of award of degree** : 2006

**Major Subject** : Biotechnology and Molecular Biology

**Total number of pages In Thesis** : **75+xxx+XXV**

**Number of words in abstract** : Approx. 370 words

Molecular markers provide novel tools for varietal identification, diversity analysis and assessing phylogenetic relationships among various rice groups in genus *Oryza*. A DNA fingerprint database has been developed for 50 rice genotypes representative of the traditional Basmati (TB), cross-bred Basmati, *indica, japonic*a and wild rice groups using fifty SSR and thirty transposable element (TE) based markers. The salient features of SSR and TE based marker data analyzed using various clustering algorithms, principal component analysis and Mantel test are as given below: (i) SSR generated higher levels of polymorphism (mean PIC value = 0.698) than TE based markers (PIC = 0.258), (ii) a total of 341 alleles were generated with an average of 6.8 allele per locus using SSR markers, (iii) 40 of these SSR alleles were rare/unique being present in only one of 50 genotypes with 17 unique alleles in the nine wild rice genotypes, (iv) the traditional Basmati rice varieties except Basmati 217 were genetically distinct from *indica*, *japonica* and wild rice varieties and invariably formed a separate cluster (v) NTSYS-PC, PCA and PowerMarker analysis of SSR database clearly exhibited the formation of four distinct groups of Basmati, *indica*, *japonica* and wild rice, (vi) the twelve Basmati rice varieties developed from *indica* x Basmati crosses/backcrosses were scattered between the traditional Basmati and *indica* rice groups with CSR30, Super, Kasturi and Pusa Basmati 1 being closer to the TB group, (vii) a number of SSR markers have been identified which can be used to differentiate within/among the various rice groups, (viii) genetic relationships assessed using TE based markers (mPing and Dasheng) were essentially the same as obtained using SSRs except that it also differentiated between the temperate and tropical *japonica* rice genotypes into separate clusters, (ix) SSR and TE based marker data-set showed high levels of positive correlation (Mantel test, $r$ = 0.655). The study demonstrate that SSRs are best for varietal identification especially for differentiating between the closely related Basmati, *indica* or *japonica* rice varieties, while TE based markers may provide vital clues about evolution/speciation in rice. The high-level polymorphism generated by SSR assay described in this study shall provide novel markers to differentiate between traditional Basmati rice supplies from cheaper cross-bred Basmati and long-grain non-Basmati varieties at commercial level.

**MAJOR ADVISOR** **SIGNATURE OF STUDENT**

**HEAD OF DEPARTMENT**