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GENOME ANALYSIS OF TRADITIONAL RICE VARIETIES OF KERALA USING ISSR AND RAPD MARKERS

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DECLARATION

I hereby declare that this thesis entitled "**Genome analysis of traditional rice varieties of Kerala using ISSR and RAPD markers**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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DEDICATED
TO
THE FOND
MEMORIES OF
MY BROTHER

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Reshmi Manohar

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LIST OF ABBREVIATIONS

%	Per cent
°	Degree
µl	Microlitre
µM	Micromolar
AFLP	Amplified Fragment Length Polymorphism
BC	Before Christ
bp	Base pair(s)
C	Celsius
CMS	Cytoplasmic Male Sterility
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
et al.	And others
Fig.	Figure
g	Gram(s)
hrs	Hours
HCl	Hydrogen Chloride
IPR	Intellectual Property Rights
ISSR	Inter Simple Sequence Repeat
KAU	Kerala Agricultural University
kb	Kilo base
M	Molar
m	Metre
mg	Microgram
$MgCl_2$	Magnesium Chloride
min	Minute
ml	Millilitre
mM	Millimolar
mm	Millimetre
NATP	National Agricultural Technology Project
NCBI	National Center for Biotechnology Information
ng	Nanogram

nm	Nanometer
NTSYS	Numerical Taxonomy System
OPA	Operon Primer A series
OPB	Operon Primer B series
OPC	Operon Primer C series
OPD	Operon Primer D series
OPF	Operon Primer F series
OPG	Operon Primer G series
OPH	Operon Primer H series
OPK	Operon Primer K series
OPL	Operon Primer L series
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
pmoles	Pico moles
rpm	Revolutions per minute
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RM	Repeat Motif
SCAR	Sequence Characterized Amplified Region
SDS	Sodium Dodecyl Sulphate
sec	Seconds
SHAN	Sequential Agglomerative Hierarchical and Nested
SIMQUAL	Similarity for Qualitative Data
SSR	Simple Sequence Repeat
STMS	Sequence Tagged Microsatellite Site
STS	Sequence Tagged Site
TAE	Tris acetate EDTA buffer
Taq	*Thermus aquaticus*
TE	Tris EDTA buffer
Tris	Tris-hydroxymethyl amino methane
U	Units of enzyme
UPGMA	Unweighted Pair Group Method with Arithmetic average
V	Volts
viz.	Namely

INTRODUCTION

1. INTRODUCTION

Rice, *Oryza sativa* L., member of Poaceae family is one of the world's most important staple food crops, feeding more than half of the world's population. More than ninety per cent of rice is produced and consumed in Asia. It is the most diversified crop species due to its adaptation to a wide range of geographical, ecological and climatic regimes. Rice is grown in over 100 countries spread over all continents, except Australia, extending from 50° North Latitude to 40° South Latitude and from below sea level to an altitude of 3000m. Because of the ancient origin and wide spread distribution of rice, enormous numbers of landraces have been accumulated. These landraces are generally considered to be a rich source of genetic variation for cultivar development. The large variability for complex quantitative traits in these accessions remains unexploited. The genetic potential of these still untapped resources could be unique and different from that of the improved varieties which have a narrow genetic base. Rich genetic diversity in cultivated rice and their wild relatives needs to be collected, characterized, catalogued and conserved for future use.

Kerala is considered as one of the centers of diversity of rice and the antiquity of rice cultivation here dates back to 3000 BC (Manilal, 1990). Rice covers a vast array of ecological niches in Kerala and a vast diversity of germplasm of both cultivated and wild rice exist here (Kumary and Francies, 2002). These varieties possess several unique characteristics. The world renowned 'Pattambi' rice varieties that have contributed sources of resistance to many IRRI varieties are all pureline selection of landraces hailing from the erstwhile Malabar province (Dev *et al.*, 1983). Diversity at ecosystem level, interspecific levels in addition to ethnic diversity is the highest in this stretch of land. These traditional varieties are gene banks for many favourable genes, especially genes for resistance to biotic and abiotic

stresses. Conservation and characterization of these varieties is essential for future genetic improvement of rice.

Characterization based only on morphological and physiological parameters with clear cut features of distinctness are not always possible. Though a range of plant characters are currently available for distinguishing closely related individuals, they can be strongly influenced by environment. Therefore characterization with molecular markers is more reliable and is a necessity with regard to IPR issues. DNA based molecular markers are in abundance and clearly allow the comparison of genetic material avoiding any environmental influence on gene expression.

Under National Agriculture Technology Project on Sustainable Management of Plant Biodiversity at KAU, many landraces of Kerala were collected and documented. From this collection thirty landraces were randomly chosen for the present study on molecular characterization using two DNA markers *viz.* RAPD and ISSR. The major objectives proposed for this study are:-

- To characterize indigenous collection on the basis of RAPD markers
- To standardize the technique for using ISSR markers for assessing the genetic diversity of these accessions
- To compare the ability of RAPD and ISSR markers in assessing the genetic divergence in these rice genotypes

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice, which has been documented as a source of food as far back as 2500 BC in history books, has fed more people over a longer period of time than any other crop in the world (Rost, 1997). It provides the main source of food for approximately half of the world's population and hence, may be the most important plant on this earth (Shimamoto, 1995; Goff, 1999). Rice is one of the three cereals produced annually at worldwide levels of approximately half a billion tons (Goff, 1999). However, unlike other major cereals, more than 90 per cent of the rice is milled almost exclusively as food for human consumption (Goff, 1999) and forms three-fourths of the total diet for millions of people. This member of the grass family (Poaceae) is abundant in carbohydrates and is a major source of protein for the masses of Asia (Chang, 1984). Rice is the only cereal that can withstand flooding and produces more calories to sustain a larger number of persons per unit of land than any other cereal in monsoonal areas (Chang, 1984).

India has the largest area among the rice growing countries, enjoys the second rank in rice production and is considered as one of the centers of origin of rice. A large number of landraces are reported in India which reflects the broad genetic base of this crop in the country. These landraces provide enormous genetic variability for the present day rice improvement program (Zhenshan *et al.* 1996). Though undomesticated, unadapted germplasm and landraces are phenotypically less desirable, breeders have long recognized their intrinsic value for the improvement of simply inherited traits including disease, pest resistance and other useful traits (Shivapriya and Hittalmani, 2006). Thus characterization and conservation of landraces are essential for future use. Knowledge on the molecular biology of these landraces will be useful for realizing their DNA level genetic architecture and nature of genetic base of the breeding stock (Vanaja

and Randhawa, 2004). With the advent of molecular markers a new generation of markers has been introduced which has revolutionized the entire scenario of biological sciences.

2.1 MOLECULAR MARKERS

During the early period of research, classical strategies including comparative anatomy, physiology and embryology were employed in genetic analysis to determine inter and intra species variability. In the past decade, however, molecular markers have very rapidly complemented the classical strategies. Molecular markers include biochemical constituents (e.g. secondary metabolites in plants) and macromolecules, *viz.* proteins and deoxyribonucleic acids (DNA). Amongst the molecular markers used, DNA markers are more suitable and ubiquitous to most of the living organisms and ideally neutral to environmental effects or management practices (Joshi *et al.,* 1999). Over the last few decades plant genomics has been studied extensively bringing about a revolution in this area. Molecular markers, useful for plant genome analysis, have now become an important tool in this revolution. During the last few decades, a broad range of molecular markers have become available, which are being used in a variety of ways not only to supplement and expedite the conventional methods for improvement, but also for characterization and maintenance of plant genetic resources that are so vital for crop improvement programmes (Gupta *et al.*, 2002).

Molecular markers have proved to be prominent tools in augmenting conventional breeding methods for crop improvement in various cultivated species (Mohan *et al.*, 1997). Molecular markers have become fundamental tools for fingerprinting varieties, establishing phylogenetics, tagging desirable genes, characterization of transformants, study of genome organization etc. (Rafalski *et al.*, 1996). The genetic markers are used for clonal identification, linkage mapping, population diversity, taxonomy, germplasm conservation etc. (Brelting and

Widrlechner, 1995). Although each marker system is associated with some advantages and disadvantages, the choice of marker system is dictated to a large measure by the intended application, convenience and cost involved (Gupta *et al.*, 2002).

2.2 DNA MARKERS

The term DNA fingerprinting was introduced by Jeffrey *et al.* (1985). Presently the term DNA fingerprinting or profiling is used to describe combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. Because of its plasticity, ubiquity and stability DNA is the ideal molecule for such analysis (Caetano-Anolles *et al.*, 1991). A major advantage of DNA based marker is the abundance of polymorphic loci, which enables estimation of genetic distance among the germplasm with high precision. Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization based markers and polymerase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

2.2.1 Hybridization Based DNA Markers

The hybridization based DNA markers technology utilize labelled nucleic acid molecule as hybridization probes (Chawla, 2002). Probe molecule range from synthetic oligonucleotides to cloned DNA. In this, DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA, to a labelled probe, which is a DNA fragment of known origin or sequence. Some of the important hybridization based markers are RFLP, Hypervariable sequences etc.

2.2.1.1 *Restriction fragment Length Polymorphism (RFLP)*

RFLP analysis involves digesting the genome with restriction enzyme, separating the fragment electrophoretically and then preferentially visualizing the fragment containing particular homologous sequences by hybridizing them with a specific labelled DNA probe (Deverna and Alpert, 1990).

Lu *et al.* (2002) used RFLP technique to study genetic diversity and relationships of wild relatives of rice with 58 accessions of *Oryza* species and 30 RFLP probes. All the probes detected polymorphism among the *Oryza* accessions with an average of 3.8 polymorphic fragments per probe and considerable genetic diversity was scored among the *Oryza* accessions.

Jena and Kochert (1991) used RFLPs (Restriction fragment length polymorphisms) to study fourteen accessions of CCDD genome allotetraploid wild rice species (*Oryza latifolia*, *O. alta* and *O. grandiglumis*). Fourteen nuclear RFLP markers previously mapped in AA genome cultivated rice were used as probes. By comparing RFLPs from the allotetraploids with those from a CC genome diploid wild species (*O. officinalis*), it was possible to detect RFLPs specific for both the CC and DD genomes of the allotetraploid.

2.2.2 PCR Based DNA Markers

PCR based DNA markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography. PCR is a versatile technique invented during the mid-1980s. Ever since thermo stable DNA polymerase was introduced in 1988, the use of PCR in research and clinical laboratories has increased

tremendously. The primer sequences are chosen to allow base-specific binding to the template in reverse orientation. PCR is extremely sensitive and operates at a very high speed. Its application for diverse purposes has opened up a multitude of new possibilities in the field of molecular biology (Joshi *et al.*, 1999). Among PCR based marker techniques, the important ones are RAPD, ALFP, Minisatellite, Microsatellite, ISSR and SCAR.

2.2.2.1 *RAPD*

The use of polymerase chain reaction in conjunction with random primers for fingerprinting genomes was reported by Welsh and McClelland (1990). RAPD was used for population studies by Astley (1992) and for identification of genome specific markers and other uses by Williams *et al.* (1990) and Erlich *et al.* (1991). Several researchers have applied RAPD technique to investigate genetic variability and found the technique very efficient and reliable (Brown *et al.*, 1993, Munthali *et al.*, 1996). The RAPD procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. On an average each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals. The advantage of RAPD compared to RFLP is that RAPD is less labour intensive, require smaller quantities of genomic DNA, and is less costly and quicker than RFLP. It can be used to detect even single gene mutations (Williams *et al.*, 1990).

RAPD markers are well suited for genetic mapping, for plant and animal breeding application and for DNA fingerprinting with particular utility for studies of population genetics. RAPD marker can also provide an efficient assay for polymorphisms, which should allow rapid identification and isolation of chromosome specific DNA fragments.

Monna *et al.* (1994) mapped 102 RAPD markers on all twelve chromosomes of rice using DNAs of cultivars Nipponbare (*japonica*) and Kasalath (*indica*) and of F_2 population generated by a single cross of these parents and they were able to detect polymorphisms appearing in the range from less than 100 bp to 2000 bp. Genetic variation of nine upland and four lowland rice cultivars was investigated at the DNA level using the randomly amplified polymorphic DNA method *via* the polymerase chain reaction by Yu and Nguyen (1994). Forty two random primers were used to amplify DNA segments and 260 PCR products were obtained. The results of agarose gel electrophoretic analysis of these PCR products indicated that 208 (80 per cent) were polymorphic.

A set of 63 RAPD markers were used to score 47 accessions of rice to determine whether associations between presence or absence of individual markers and performance for two quantitative characters namely culm number and flowering time could be detected, by Virk *et al.* (1996) and they found that such association is possible. Ishii *et al.* (1996) used RAPD analysis to reveal the phylogenetic relationships in A-genome species of rice using 14 decamer primers and 29 accessions. The study indicated that *Oryza sativa* and *Oryza glaberrima* have probably originated from the Asian form of *Oryza perennis* and *Oryza breviligulata,* respectively.

Parsons *et al.* (1997) studied genetic variation between samples of *Oryza sativa* from 19 localities in Bangladesh and Bhutan using 14 decamer primers. They obtained 94 reproducible bands of which fifty per cent were polymorphic. A study using isozyme and RAPD markers was conducted by Buso *et al.* (1998) to estimate the level of genetic diversity of four South American wild rice populations (*Oryza glumaepatula*) collected from Amazon forest and Western Brazilian rivers. Analysis showed that genetic diversity was due to differences among populations and the distribution

pattern of genetic variation of *O. glumaepatula* populations is in agreement with the expectation for an autogamous species.

The genetic relationship between seven *japonica*, two *indica* and one tropical *japonica* rice varieties were analyzed by using PCR with RAPD method by Nadarajan *et al.* (1999) and the results showed sufficient polymorphism which allows identification of individual varieties. Xie *et al.* (2000) evaluated six *Oryza rufipogon* populations from *in situ* conservation sites in China during 1997-98 using RAPD primers. Analysis of the results showed considerable variations within each population and found that major portion of the total genetic variation was within rather than between populations.

Raghunathachari *et al.* (2000) carried out RAPD analysis of genetic variability in Indian scented rice germplasm and found that with the selected primers sufficient polymorphism could be detected to allow identification of individual accessions. Visual examination of banding patterns confirmed that many of the scented rice varieties under cultivation with similar names are genetically quite different. RAPD analysis was used for revealing genetic diversity and discriminating between intra specific groups of *Oryza sativa* germplasm by Virk *et al.* (2000). Genetic diversity among 42 Indian elite rice varieties, which is important for selection of parents for conventional breeding and hybrid programme, was evaluated using RAPD markers by Davierwala *et al.* (2000).

Gaunghua *et al.* (2000) used RAPD analysis for analysis of heterozygosity in rice cultivar Shangyou 63 and found that the heterozygosity of Shangyou 63 is 34.57 per cent. The genetic diversity among 28 rice cultivars that are different in biometric traits, biological cycle and suitability to water limitation was investigated using random amplified polymorphic DNA (RAPD) markers by Porreca *et al.* (2001). Cluster analysis was carried out using 182 polymorphic fragments obtained from 13

decamer primers. High level of polymorphism was found between the *japonica* and *indica* varieties of *Oryza sativa*, whereas the *japonica* long-grain cultivars (tropical) were genetically different from the short-grain cultivars (temperate).

The RAPD analysis of genetic diversity in a set of landraces in comparison to a representative sample of improved varieties by Neeraja *et al.* (2002) indicated that the landraces have wide genetic diversity and are very distinct and distant from improved varieties. Sandhu *et al.* (2002) used eighty operon RAPD primers for analysis of herbicide resistant rice lines that were produced through mutagenesis. The resistant and susceptible lines produced variation in the RAPD patterns and certain bands were found only in certain lines.

Wu *et al.* (2002) identified fifteen dominant and eight co-dominant RAPD markers, which are closely linked with two morphological genes in rice namely brittle culm (bc-1) and lazy (la). Mei *et al.* (2002) analyzed 15 semi-late *indica* hybrid rice parental lines by RAPD with 20 random primers to determine their genetic diversity. Zhongming *et al.* (2003) used RAPD analysis with hundred operon primers in order to evaluate the purity of hybrid seeds of rice cultivar Liangyou Peijiu which was purposely adulterated with seeds of Pei'ai 64s. Four primers amplified stable polymorphic bands. The results indicated that the primer OPN-9 could accurately distinguish the adulterated seeds from the hybrid seeds.

The genetic diversity in 53 rice cultivars from Yunnan, China with resistance to bacterial blight (*Xanthomonas oryzae* pv *oryzae*) was evaluated using 20 random RAPD primers by Guanghai *et al.* (2003) and the results showed that the cultivars exhibited extensive genetic variation and abundant genetic diversity. Sharma *et al.* (2003) used RAPD analysis and field evaluation of double haploid lines of rice for resistance to *Magnaporthe grisea*. A band of 2000 bp present in resistant parent was amplified in all

double haploid lines. Out of 46 primers, the primers OPX-7, OPX-8, OPX-2 and OPX-17, gave polymorphic banding pattern between the resistant and susceptible parents.

In order to estimate genetic relationships of the AA genomes in *Oryza* species RAPD analysis was performed with 45 genotypes which included 13 cultivated varieties and 32 wild accessions by Ren *et al.* (2003). A total of 181 clear and repeatable bands were amplified from 27 selected RAPD primers. Ichii *et al.* (2003) used RAPD marker analysis to characterize Cytoplasmic male sterile (CMS) and maintainer lines in *indica* rice and they found that a 1600 bp fragment derived from CMS mitochondrial DNA could be used as a RAPD marker to distinguish A and B plants at seedling stage.

Ravi *et al.* (2003) carried out molecular marker based genetic diversity analysis using RAPD markers to assess the genetic diversity among forty cultivated varieties and five wild relatives of rice. A total of 499 RAPD markers were produced with 36 decamer primers. The genetic diversity, genotype clustering and classification of 56 Iranian rice genotypes were determined using RAPD markers, UPGMA, and Jaccard's similarity coefficient by Gholaki *et al.* (2003). Some 66 primers were identified, of which 12 random primers produced suitable genetic polymorphisms. Among the 129 RAPD markers, 104 (80.62 per cent) were polymorphic while 25 (19.38 per cent) were monomorphic. The size of generated bands ranged from 0.45 to 3.0 kb. The average band number for each polymorphic primer ranged from 7.00 to 10.75. The genotypes were divided into seven groups and genetic diversity among the genotypes varied from 44 to 91 per cent.

The genetic diversity analysis of traditional Sali rice germplasm of Assam, through RAPD markers was carried out by Barooah and Sarma (2004) using 51 Sali rice accessions and 72 RAPD primers. The results

indicated high level of diversity and emphasized the potentiality of using molecular markers in rice germplasm management of Assam rice collection. Jeung *et al.* (2005) had employed RAPD markers for fingerprinting temperate *japonica* and tropical *indica* rice genotypes. Significant polymorphism was detected among the genotypes of *japonica* and *indica* through analysis of molecular variants while relatively low polymorphism was detected within the genotypes of *japonica* rice cultivars. Saker *et al.* (2005) established the genetic variability and relationships among seven Egyptian rice genotypes by using eight RAPD primers and could reveal 72.2 per cent polymorphism. They could also reveal 17 unique markers using RAPD analysis.

2.2.2.2 *Amplified Fragment Length Polymorphism (AFLP)*

This technique is also called Selective Restriction Fragment Amplification. It is a combination of RFLP and PCR used for obtaining highly informative fingerprints. The technique involves restriction of the DNA and ligation of oligonucleotide adapters, relative amplification of sets of restriction fragments and gel analysis of amplified fragments. It is based on the PCR amplification of genomic restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases (Vos *et al.*, 1995). This approach allows the simultaneous screening of a large number of anonymous markers randomly distributed throughout the genome. AFLP technique uses stringent reaction conditions for primer annealing and combines the reliability of RFLP technique with the power of PCR technique.

Aggarwal *et al.* (1999) used AFLP technique to reveal the phylogenetic relationship among 77 accessions of 23 *Oryza* species, five related genera and three out-group taxa. A total of 1191 polymorphic markers were obtained using five AFLP primer combinations. The genetic diversity and patterns of relationships among 18 rice genotypes

representative of the traditional Basmati, cross bred Basmati and non-Basmati rice varieties were evaluated using five AFLP primer combinations by Saini *et al.* (2004). A total of 171 bands were detected of which 110 were polymorphic.

Jeung *et al.* (2005) used AFLP technique for fingerprinting 14 rice genotypes using eight AFLP primers and generated an average of 92.5 loci and could effectively differentiate all tested rice lines. Genetic variability and relationships among seven Egyptian rice genotypes were evaluated using eight AFLP primer combinations by Saker *et al.* (2005). AFLP analysis could reveal 65 unique markers. Bao *et al.* (2006) utilized AFLP technique using five primer combinations to examine genetic diversity and relationships of 56 waxy rice accessions. A total of 358 AFLP fragments were amplified showing a high level of polymorphism (78.3 per cent).

2.2.2.3 *Minisatellites*

Jeffrey *et al.* (1985) was the first to report on the presence of minisatellite hypervariable sequence in human genome that could be used for DNA fingerprinting. Minisatellites are repeat sequences that are usually 0.2 to 2.0 kb long having repeat units ranging from 11 bp to 60 bp in length. The repeat sequence comprises upto greater than 80 per cent of total DNA in certain plant genomes. The conserved sequence flanking minisatellite can be amplified using a suitable primer to reveal the polymorphism. The polymorphisms are attributed to variation in length of minisatellite.

2.2.2.4 *Microsatellites*

The term microsatellite was coined by Litt and Luty (1989). Microsatellites or Simple Sequence Repeats (SSRs) are two to five base pairs in length and are widely dispersed throughout the nuclear genome of eukaryotes (Tautz and Renz, 1984). They are considered to be the most informative molecular genetic markers (Tautz *et al*, 1986; Tautz, 1989).

These are tandemly arranged repeats of mono, di, tri, tetra and penta nucleotides with different lengths of repeat motifs. These repeats are widely distributed throughout the plant and animal genomes and show comparatively low degree of repetition. The microsatellites can be amplified using a suitable primer to reveal polymorphism. The microsatellites are highly polymorphic and thus highly informative. Being shorter in length they are easy to be cloned, sequenced and amplified through PCR. These simple sequences have been shown to be repetitive and interspersed in many eukaryotic genomes (Tautz and Renz, 1984).

Yang *et al.* (1994) examined the genetic polymorphism of ten microsatellite DNA loci among 238 accessions of landraces and cultivars which included both *indica* and *japonica* groups of cultivated rice. In all, 93 alleles were identified with these ten markers and found that the number of alleles detected at a locus was significantly correlated with the number of simple sequence repeats in the targeted microsatellite DNA.

Akagi *et al.* (1996) found 369 complete microsatellite of which $(CGG/GCC)_n$ was the most frequent in 11798 rice sequences in the database. They also found that of these microsatellites 35 out of 45 could be successfully converted into microsatellite DNA markers using sequence information in their flanking regions. Polymorphisms of 20 microsatellite loci were determined using 59 *japonica* cultivars by Akagi *et al.* (1997). They found that microsatellites consisting of AT repeats are highly polymorphic in rice genomes and can be used to distinguish between even closely related *japonica* cultivars in Japan.

Davierwala *et al.* (2001) examined the potential of $(GATA)_n$ microsatellites from rice for inter and intra specific variability studies. Three polymorphic $(GATA)_n$ harboring loci containing seven to thirteen repeat motifs were identified from a genomic library of *Oryza sativa* var Basmati 370 using oligonucleotide probe $(GATA)_4$. The genetic structure of five

natural populations of common wild rice *Oryza rufipogon* from China was investigated with 21 microsatellite loci by Gao *et al.* (2002) and the microsatellite loci gave much higher levels of genetic diversity.

To estimate the genetic diversity of the residual northern populations of *Oryza rufipogon*, a total of 232 individuals from six populations were analyzed using microsatellites by Song *et al.* (2003). The 23 rice SSR primer pairs selected detected a total of 115 alleles. Ravi *et al.* (2003) carried out molecular marker based genetic diversity analysis in rice using SSR markers among 40 cultivated and five wild varieties. SSR analysis resulted in more definitive separation of clusters of genotypes indicating high level of efficiency of SSR markers.

In order to estimate genetic relationships of the AA genome *Oryza* species, SSR analysis was performed with 45 accessions and 29 SSR primer pairs by Ren *et al.* (2003) and a total of 101 alleles were detected from 29 SSR primer pairs. They found that SSR analysis effectively reveals diminutive variation among accessions or individuals within the same species. Allelic diversity among Basmati and Non Basmati long grain *indica* rice varieties were analyzed using microsatellite markers by Siwach *et al.* (2004). The study showed that grouping of cultivars based on simple sequence repeat polymorphism data corresponds well to their known pedigree data.

Zeng *et al.* (2004) characterized the genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers. A total of 123 alleles were generated at 25 microsatellite loci among 33 genotypes. Genetic relationships among Indian aromatic and quality rice germplasm were assessed using 30 fluorescently labelled rice microsatellite markers and 69 rice genotypes by Jain *et al.* (2004). The results indicated that Indian aromatic and quality germplasm

was genetically distinct from other groups within *Oryza sativa* and is the product of a long independent pattern of evolution.

Saini *et al.* (2004) evaluated the genetic diversity and pattern of relationships among 18 rice genotypes representative of traditional Basmati, cross bred Basmati and non-Basmati rice varieties using SSR markers. A total of 240 bands were detected using 30 SSR markers. DNA fingerprinting of 79 released rice varieties and 14 landraces of Kerala was carried out using microsatellite molecular markers by Vanaja and Randhawa (2004). The study indicated $RM154_{200}$ as putative microsatellite marker for aroma and $RM165_{220}$ as putative microsatellite marker for salinity resistance in rice. Saker *et al.* (2005) established the genetic variability and relationships among seven Egyptian rice genotypes using six SSR primer pairs.

2.2.2.5 *Inter Simple Sequence Repeats (ISSR)*

Among the PCR based marker techniques, ISSR is one of the recent techniques which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. Polymorphism with ISSR primers occurs whenever one genome is missing, the sequence repeated or has a deletion or insertion that modifies the distance between the repeats. For 5' anchored primers polymorphism also occurs due to differences in the length of microsatellite. The utility of microsatellite directed DNA fingerprinting by polymerase chain reaction amplification of the inter-repeat region was first demonstrated by Zietkiewicz *et al.* (1994).

Studies of ISSR locus heritability have demonstrated exceedingly close approximation to classic Mendelian ratios. Nagaoka and Ogihara (1997) reported that ISSR markers produced extremely high variability and high mapping density as compared with RFLP and RAPD data making this new dominant microsatellite based molecular marker ideal for producing

genetic maps of individual species. According to Fang and Roose (1997), these features combined with greater robustness in repeatability experiments and less prone to changing band patterns with change in constituent DNA template concentration make ISSR markers superior to other readily available marker systems in investigations of genetic variation among very closely related individuals and in crop cultivar classification. These ISSR markers are advantageous since no prior genomic information is required for their use (Godwin *et al.*, 1997).

Inter Simple Sequence Repeats amplification was used to analyze microsatellite motif frequency in the rice genome and to evaluate genetic diversity among rice cultivars by Blair *et al.* (1999). A total of 32 primers containing different SSR motifs were tested for amplification on a panel of 59 varieties. The ISSR results suggested that within the dinucleotide class the poly (GA) motif was more common than poly (GT) motif. They found that the ISSR fingerprint could be used to differentiate the genotypes belonging to either *japonica* or *indica* subspecies of cultivated rice and to dissect inner levels of diversity within each subspecies.

Joshi *et al.* (2000) used ISSR polymorphism to determine genetic diversity and phylogenetic relationships in *Oryza*. Eleven ISSR primers used could cluster the 42 genotypes according to their respective genomes. The ISSR analysis suggests that the genus *Oryza* may have evolved following a polyphyletic pathway, also ISSR revealed 87 putative genome/species specific molecular markers for eight of the nine genomes of *Oryza*. Virk *et al.* (2000) used ISSR for revealing genetic diversity and discriminating between intra specific groups of *Oryza sativa* germplasm. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence based ISSR-PCR markers was done by Nagaraju *et al.* (2002). The study indicated that the lowest genetic diversity was observed among the traditional Basmati varieties where as the evolved Basmati varieties showed the highest genetic diversity. A number of

markers, which could unambiguously distinguish the traditional Basmati varieties from, evolved and non-Basmati rice varieties were identified.

Kaushik *et al.* (2003) used ISSR markers for genetic analysis of a CSR 10 (*indica*) x Taraori Basmati F_3 population segregating for salt tolerance. A total of 149 bands (four to eleven bands per primer) ranging from 200-3530 bp was scored using 26 ISSR primers. Genetic diversity among 24 accessions of *Oryza nivara* from 11 states of India and four *Oryza sativa* varieties were analyzed using ISSR-PCR by Sarla *et al.* (2003). The primers based on AG and GA repeats were informative and could together enable grouping accessions on a geographical basis. Saini *et al.* (2004) used ISSR markers to evaluate the genetic diversity and patterns of relationships among 18 rice genotypes. A total of 240 bands were detected using 25 ISSR primers.

Zhou *et al.* (2005) made a study on early stability in rice by ISSR markers. In the F_2 population, crossed from two early stability rice with four cultivars, eight uniform strains were recorded. Four types of marker bands were obtained after the F_2 uniform strains were marked by 26 ISSR primers. The study indicated that the uniform strains and normal strains in rice might be grouped into two classes on the basis of 2000 bp marker band amplified by ISSR marker primer No: 900. Molecular characterization of 32 Indian rice varieties of different agroclimatic zones was done using ISSR by Khandelwal *et al.* (2005). Four 3'anchored primers, two containing $(GA)_n$ repeats and the other two $(GT)_n$ repeats were used for ISSR amplification which resulted in 34 amplicons of which 33 were polymorphic with an average of 8.5 polymorphic bands per primer.

Prasad *et al.* (2005) used ISSR markers for studying polymorphism in three landraces and two elite varieties of rice. Maximum PIC values were observed with trinucleotide ISSR markers (0.67) followed by dinucleotide ISSR primers. ISSR approach was used to detect the genetic diversity of

allelopathic potential in 57 rice accessions by Lin *et al.* (2005). Seven pairs of ISSR primers were used for the study which generated 34 polymorphic bands giving 53 per cent polymorphism. Sarla *et al.* (2005) evaluated the potential of ISSR-PCR for diversity analysis in 86 accessions of Indian rice using 14 anchored primers based on AG and GA repeats. In all, 220 band positions and 5514 bands were generated and all loci were polymorphic. The study indicated that $(AG)_n$ and $(GA)_n$ based primers are highly informative for cost effective assessment of genetic diversity in rice germplasm.

2.2.2.6 *Comparison between ISSR and RAPD*

Genetic variation between samples of *Oryza sativa* from 19 localities in Bangladesh and Bhutan was assessed using two PCR based molecular marker systems, RAPD and ISSR by Parsons *et al.* (1997). Employing RAPD, a set of 14 primers directed the amplification of 94 reproducible marker bands of which 50 per cent were polymorphic and a set of nine ISSR primers directed the amplification of 71 PCR products of which 56 per cent were polymorphic.

Qian *et al.* (2001) investigated the genetic variation within and between five populations of *Oryza granulata* from two regions of China using RAPD and ISSR markers. 20 RAPD primers used in the study amplified reproducible bands with 30-65 per cent polymorphism and 12 ISSR primers amplified 113 bands with 46.02 per cent polymorphism. The results indicated that the percentage of polymorphic bands detected by ISSR is higher than that detected by RAPD. It seems that ISSR is superior to RAPD in terms of polymorphism detected and the amplification reproducibility.

RAPD and ISSR methods were used to detect the genetic diversity of 57 allelopathic rice accessions by He *et al.* (2004). A total of twelve

RAPD primers and seven ISSR primers were identified with polymorphism among the entries. For RAPD markers, 85 polymorphic bands were produced (69.4 per cent) and for ISSR marker, 34 polymorphic bands were generated (53 per cent). The estimates of correlation co-efficient of RAPD and ISSR based on the genetic similarity matrices were significantly correlated.

Jeung *et al.* (2005) used RAPD and ISSR marker systems for fingerprinting 14 rice genotypes consisting of seven temperate *japonica* rice cultivars, three *indica* near isogenic lines, three *indica* introgression lines and one breeding line of *japonica* type adapted to high altitude areas of the tropics with cold tolerance genes. They used 14 RAPD and 21 ISSR markers for fingerprinting. The study detected significant polymorphism among the genotypes of *japonica* and *indica*, while low polymorphism was detected within the genotypes of *japonica* rice cultivars.

2.2.2.6.1 *Comparison in Other Crops*

Four populations of the rare highly clonal grass *Calamagrostis porteri* ssp. *insperata* were examined using allozymes and two PCR based markers RAPD and ISSR by Esselman *et al.* (1999). ISSR markers detected more diversity than RAPD markers in three of the four populations examined. In one population no RAPD diversity was found where as eight different genotypes were found among the ten plants with ISSR markers. The feasibility of identifying ISSR markers associated with seed size in wheat was tested using 113 recombinant inbred lines developed by single seed descent method by Ammiraju *et al.* (2001). The study clearly demonstrated that ISSRs are highly useful for finding markers associated with major and minor genes controlling agronomically important traits in wheat.

The phylogenetic relationships of 16 barley cultivars from different countries all having a known pedigree were analyzed using 125 RAPD and 228 ISSR markers by Fernandez *et al.* (2002). The band profiles generated were reproducible inspite of different DNA extractions, PCR techniques, electrophoretic methods and gel scorings used. The dendrograms obtained using these two molecular markers are in agreement with their known origin showing clusters that separate very well the spring/winter and six rows/two rows cultivars. Tanyolac (2003) used ISSR and RAPD markers to analyze genetic distance among *Hordeum vulgare* subsp. *spontaneum* populations from West Turkey. Fifty five RAPD and ten ISSR primers were used to detect variation among samples. A total of 55 polymorphic loci were found using 65 primers.

Osipova *et al.* (2003) used RAPD and ISSR markers to analyze genetic divergence between regenerated plants derived from callus cultures and the original maize line A188. Analysis of polymorphism by using thirty eight RAPD and ten ISSR oligonucleotide primers showed that the differences between eight examined somaclones and the original line ranged from 6.5 to 23 per cent.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study envisaged to characterize the different landraces of traditional rice of Kerala using ISSR and RAPD markers was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2004-2006.

The experimental material consisted of thirty traditional rice accessions of Kerala whose morphological characterization has been completed under NATP on Sustainable Management of Biodiversity, at KAU (Table 1). The seed material was procured from Rice Research Station, Moncompu, Alappuzha. The facilities available at the Department of Plant Biotechnology, College of Agriculture, Vellayani were utilized for the molecular studies.

3.1 MOLECULAR MARKER ANALYSIS

3.1.1 Isolation of Genomic DNA

Isolation of genomic DNA was done from all the 30 samples following the procedure of Regowsky *et al.* (1991) with required modifications. The procedure is as follows:

- Approximately 1 g of tender leaf sample was taken in a clean autoclaved mortar and crushed by freezing in liquid nitrogen.
- The powder was transferred to 2 ml eppendorf tube and 1 ml of extraction buffer [1.00g SDS (1%), 1.576g Tris HCl (100mM), 0.584 g Sodium chloride (100mM), 0.37224 g EDTA (10mM), volume made up to 100ml with distilled water] was added.
- The tubes were then placed in a water bath maintained at 60° C for 30 min after homogenization.
- The mixture was then centrifuged at 10000 rpm at 4° C for 10 min.
- The aqueous phase was collected and 400µl of phenol chloroform (25: 24) was added and again centrifuged at 10000 rpm at 4° C for 10 min.

Table 1. Morphological characters of the selected thirty rice accessions*

Sl No	Name	Source	LEAF			Days to heading	PANICLE		
			Length (cm)	Width (cm)	Blade colour		Length (cm)	Type	Secondary branching
1	Njavara (yellow)	Allepey	49	0.7	Green	58	18.5	Compact	Light
2	Njavara (black)	Palakkad	61.6	1	Pale green	60	26	Intermediate	Light
3	Cheeravithu	Palakkad	51.5	1.1	Purple blotch	91	24.5	Intermediate	Light
4	Jeerakasala	Wayanad	35	1.1	Pale green	92	36.5	Compact	Heavy
5	Chempan	Trivandrum	48.5	0.9	Pale green	104	29.5	Intermediate	Light
6	Kannikayama	Kannur	52.5	0.8	Green	96	27.23	Compact	Light
7	Thowan	Kannur	42.2	1	Green	102	23.3	Compact	Light
8	Kozhivalan	Kannur	41.7	1.3	Pale green	121	23.4	Compact	Heavy
9	Kururayima	Kannur	64.51	1.2	Green	107	27.2	Compact	Light
10	Karuthacheera	Perumbavur	53.5	0.9	Green	89	26.7	Compact	Light
11	Kalladi Aryan	Kannur	35.5	0.7	Green	101	22.5	Compact	Light
12	Veluthittaryan	Palakkad	59	1.5	Green	126	22.5	Intermediate	Light
13	Athikiramundakan	Allepey	45.5	1.2	Green	116	20.6	Intermediate	Light
14	Anakodan	Palakkad	39.1	1.2	Green	125	22.3	Compact	Light
15	Veluthakattamodan	Kannur	63.7	1.1	Green	90	28.8	Intermediate	Light
16	Kattamodan	Palakkad	79.4	1.1	Pale green	100	27.3	Compact	Light
17	Allikannan	Kannur	40.5	1	Green	104	25.2	Compact	Light
18	Parambuvattan	Palakkad	32.5	0.6	Dark green	94	23.3	Compact	Light
19	Vellakkoli	Thrissur	45.2	0.7	Green	89	23.5	Intermediate	Light
20	Vellamundakan	Allepey	35.9	0.8	Dark green	76	17.4	Compact	Light
21	Cheruvirippu	Ernakulam	58.5	1.3	Green	103	26	Open	Light
22	Choottupokkali	Ernakulam	65	1.4	Green	104	27.5	Intermediate	Light
23	Chenthadi	Wayanad	31.5	0.6	Green	87	23.1	Intermediate	Light
24	Kazhama	Kannur	54.3	1.1	Pale green	96	29.8	Intermediate	Light
25	Mundon	Malapuram	57.5	0.8	Dark green	88	27.2	Intermediate	Light
26	Chettivirippu	Ernakulam	43	1	Dark green	100	26.5	Intermediate	Clustering
27	Pokkali 3	Ernakulam	48	0.9	Dark green	102	23.5	Intermediate	Light
28	Ponkuruka	Ernakulam	49.5	1.2	Dark green	95	25.2	Compact	Light
29	Pandivella	Trivandrum	52	1.1	Green	99	28	Compact	Heavy
30	Thavalakannan	Palakkad	43.2	1.1	Green	105	24	Compact	Light

Table 1. continued

Sl. No	Name	Grain							Maturity days
			Lemma and palea		100 grain wt	Seed coat colour	Endosperm		
		Awning	Colour	Pubescence			Type	Scent	
1	Njavara (yellow)	Absent	Brown furrows on straw	Hairs on upper portion	2.01	Brown	Non waxy	Non scented	80
2	Njavara (black)	Absent	Black	Hairs on upper portion	2.18	Brown	Non waxy	Non scented	98
3	Cheeravithu	Absent	Brown furrows on straw	Hairs on upper portion	2.65	Brown	Non waxy	Non scented	109
4	Jeerakasala	Absent	Straw	Hairs on upper portion	2.27	White	Non waxy	Lightly scented	123
5	Chempan	Absent	Straw	Hairs on upper portion	2.52	Brown	Non waxy	Non scented	121
6	Kannikayama	Absent	Brown furrows on straw	Hairs on upper portion	3.5	Brown	Non waxy	Non scented	122
7	Thowan	Absent	Straw	Short hairs	3.33	Red	Non waxy	Non scented	132
8	Kozhivalan	Absent	Straw	Hairs on upper portion	2.36	Brown	Non waxy	Non scented	148
9	Kururayima	Absent	Gold and/or gold furrows on straw background	Short hairs	2.7	Brown	Non waxy	Non scented	133
10	Karuthacheera	Absent	Straw	Short hairs	2.65	Brown	Non waxy	Non scented	109
11	Kalladi Aryan	Absent	Brown furrows on straw	Hairs on upper portion	2.51	Brown	Non waxy	Non scented	109
12	Veluthittaryan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	2.86	Red	Non waxy	Non scented	150
13	Athikiramundakan	Absent	Brown	Hairs on lemma keel	2.24	Brown	Non waxy	Non scented	147
14	Anakodan	Absent	Straw	Hairs on lemma keel	2.41	Red	Non waxy	Non scented	147
15	Veluthakattamodan	Long and fully awned	Gold and/or gold furrows on straw background	Hairs on upper portion	3.25	Brown	Non waxy	Non scented	132
16	Kattamodan	Absent	Brown furrows on straw	Short hairs	2.14	Light brown	Non waxy	Non scented	130
17	Allikannan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	3.03	White	Non waxy	Non scented	135
18	Parambuvattan	Long and partly awned	Black	Hairs on upper portion	2.84	Light brown	Non waxy	Non scented	120
19	Vellakkoli	Absent	Brown furrows on straw	Short hairs	2.45	Brown	Non waxy	Non scented	130
20	Vellamundakan	Absent	Brown furrows on straw	Hairs on upper portion	2.62	Red	Non waxy	Non scented	117
21	Cheruvirippu	Short and partly awned	Brown furrows on straw	Hairs on upper portion	3.36	Brown	Non waxy	Non scented	135
22	Choottupokkali	Absent	Straw	Hairs on upper portion	2.72	Brown	Non waxy	Non scented	135
23	Chenthadi	Absent	Straw	Hairs on upper portion	3.32	Brown	Non waxy	Non scented	127
24	Kazhama	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.52	Brown	Non waxy	Non scented	135
25	Mundon	Absent	Straw	Hairs on upper portion	2.33	Brown	Non waxy	Non scented	129
26	Chettivirippu	Absent	Straw	Hairs on upper portion	1.8	Red	Non waxy	Non scented	122
27	Pokkali 3	Absent	Straw	Short hairs	2.63	Brown	Non waxy	Non scented	136
28	Ponkuruka	Short and partly awned	Brown	Hairs on upper portion	2.38	Brown	Non waxy	Non scented	121
29	Pandivella	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.91	Red	Non waxy	Non scented	122
30	Thavalakannan	Absent	Gold and/or gold furrows on straw background	Hairs on lemma keel	2.91	Light brown	Non waxy	Non scented	134

* Data obtained from NATP on Sustainable Management of Biodiversity [Principal Investigator, Dr. S. Leenakumary]

- The supernatant was collected to which 200µl of chloroform iso-amylalcohol (24: 1) was added and centrifuged at 10000 rpm at 4°C for 10 min.
- The aqueous phase was collected and 200µl of chloroform iso-amylalcohol (24: 1) was added again and centrifuged at 10000 rpm at 4°C for 5 min.
- The supernatant was collected and 60µl of 3M sodium acetate and 600µl of ice cold iso-propanol were added and kept overnight at -20°C for precipitation.
- The solution was centrifuged after about 16 hrs at 12000 rpm for 10 min and the supernatant was discarded without dislodging the pellet.
- The precipitate was then washed twice using 70% ethanol and dried.
- After drying, the precipitate was dissolved in 100µl 0.1x TE buffer [Tris buffer 0.12g (10mM), EDTA 0.037g (1mM)] and stored at -20°c.

3.1.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit. Required amount of agarose was weighed and melted in 1x TAE buffer. For genomic DNA 0.8 per cent gel was used. After cooling the solution to 42-45° C, ethidium bromide was added at the rate of 12µl per 100ml. The solution was then poured on to a preset, sealed gel casting tray with a comb fixed in position, to a height of 3mm - 5mm. The gel was allowed to solidify for 15- 20 min. The comb and sealing tapes were then removed and the tray was submerged in the electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to a height of 1mm. Required volume of DNA sample and loading dye [glycerol 30 % + bromophenol blue] were mixed in the ratio 5:1 and loaded into the slots of gel using a micropipette near the negative terminal. The cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 60V was used for the run. The power supply was turned off when the loading dye move about $3/4^{th}$ of the gel. The gel was then documented using gel documentation system (BIORAD).

3.1.3 Quantification of DNA

After ensuring the quality of DNA in samples by electrophoresis the quality and quantity was measured using spectrophotometer. 5µl of DNA dissolved in 0.1x TE was added to 3ml of distilled water and absorbance at 260nm and 280nm was read against distilled water as blank, using UV spectrophotometer (Spectronic Genesys 5). The concentration of DNA in sample was calculated using the formula

$$\text{Amount of DNA (µg/ml)} = \frac{A_{260} \times 50 \times \text{Dilution factor}}{1000}$$

where A_{260} = absorbance at 260nm

The quality of DNA was judged from ratio of absorbance values at 260nm and 280nm. A ratio of 1.8 - 2.0 indicates best quality of DNA.

3.1.4 Random Amplified Polymorphic DNA (RAPD)

The procedure of Williams *et al.* (1990) was used for the amplification of DNA. The amplification was done using 20 reported arbitrarily designed primers, which included primers from different Operon primer series *viz* OPA-5, OPB-05, OPB-08, OPB-10, OPC-07, OPC-15, OPD-03, OPD-18, OPF-01, OPF-03, OPF-04, OPF-05, OPF-06, OPF-13, OPG-18, OPG-19, OPH-19, OPK-14, OPK-19 and OPL-17 [Barooah and Sarma (2004); Raghunathachari *et al.* (2000); Saker *et al.* (2005)].

The reaction was carried out in 25µl reaction mixture containing 20ng template DNA, 2.5µl of 1x PCR buffer, 2µl of 2.5mM $MgCl_2$, 1µl of 10mM dNTP mix, 1unit of Taq DNA polymerase and 1µl of 10pmoles primer. Amplification was done in a programmable thermocycler (MJ Research Inc., USA) that was programmed as follows:

An initial denaturation at 94° C for 5 min followed by 40 cycles of denaturation at 94° C for 1 min, annealing at 36° C for 1 min and extension

at 72° C for 90 sec. The synthesis step of final cycle was extended further by 7 min. Finally the products of amplification were cooled to 4° C. Amplified products were separated by agarose gel electrophoresis using 1.4 per cent gel as described earlier and photographed using gel documentation system.

3.1.5 Inter Simple Sequence Repeats (ISSR)

The DNA isolated by the above method was also utilized for ISSR analysis. The amplification was done following the method of Joshi *et al.* (2000) with certain modifications.

The reaction was carried out in 25 µl reaction mixture containing 20ng template DNA, 2.5µl of 1x PCR buffer, 2µl of 2.5mM $MgCl_2$, 2 µl of 10mM dNTP mix, 1unit of Taq DNA polymerase, 1µl of 100pmoles primer and 2% formamide. Amplification was done in a programmable thermocycler that was programmed as follows:

An initial denaturation at 94° C for 5 min followed by 30 cycles of denaturation at 94° C for 1 min, annealing at specific temperature for 45 sec and extension at 72° C for 2 min. The synthesis step of final cycle was extended further by 5 min. Finally the products of amplification were cooled to 4° C. Amplified products were separated by agarose gel electrophoresis using 2 per cent gel as described earlier and photographed using gel documentation system.

3.1.6 Data Analysis

The reproducible bands were scored for their presence (1) or absence (0) for all the genotypes studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908).

$$Sj = \frac{a}{(a+b+c)}$$

where a = number of bands present in both genotypes in a pair

b = number of bands present in first genotype but not in other

c = number of bands present in second genotype but not in other

Based on the similarity coefficient, the distance between the accessions was computed with the help of software package 'NTSYS' (Rholf, 1998). Similarity indices were computed as Jaccard's coefficient through SIMQUAL routine and clustering was done using SAHN routine of the package. Using the values of the distance between accessions, a dendrogram was constructed by UPGMA. Association between the accessions was found out from the dendrogram.

RESULTS

4. RESULTS

In the present investigation thirty traditional rice accessions were characterized and the genetic diversity was analyzed using RAPD and ISSR molecular markers. The results are elaborated in this chapter.

The genomic DNA from the thirty accessions was isolated following the procedure of Regowsky *et al.* (1991) with required modifications. The procedure yielded good DNA whose quality and quantity was checked by gel electrophoresis using 0.8 per cent agarose gel (Plate 1) and by spectrophotometer, respectively. The quantity of DNA obtained ranged from 60 ng/µl to 690 ng/µl (Table 2).

4.1 RANDOM AMPLIFIED POLYMORPHIC DNA

The RAPD reaction was carried out with twenty oligonucleotide primers randomly selected from the previous reports. The procedure of Williams *et al.* (1990) was followed with certain modifications for amplification.

The primers produced multiple band profiles with a number of amplified fragments ranging from 2.0 kb to less than 0.5 kb with mean number of 11.10 amplicons per primer (Fig. 1). The primer OPF-04 gave the maximum number of amplicons (15) while the lowest number (7) was amplified by the primer OPG-18 (Table 3). The average number of polymorphic bands per primer was found to be 9.10. A total of 222 amplicons were produced which showed a polymorphism of 81.98 per cent. The polymorphism produced by the different primers ranged from 100 per cent to 54.54 per cent (Fig. 2).

4.1.1 OPA-05

Nine amplicons were produced by this primer (Plate 2) of which six were polymorphic giving 66.67 per cent polymorphism. The amplicons are numbered from A1 to A9. This primer produced five intense and four faint

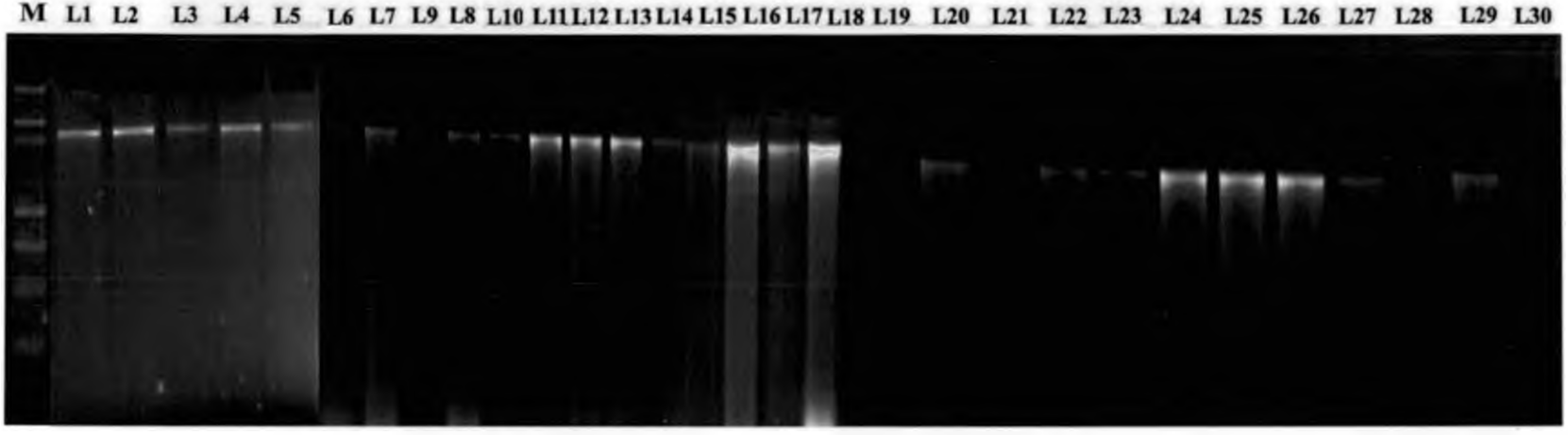

Plate 1. Genomic DNA of the 30 rice accessions

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Table 2. Quality and Quantity of DNA of the 30 rice accessions used in the study

Sl No:	Accession name	A 260 nm	A 280 nm	A260/A280	DNA Yield ng/µl
1	Njavara yellow	0.012	0.007	1.71	360
2	Njavara black	0.012	0.007	1.71	360
3	Cheeravithu	0.003	0.003	1.00	90
4	Jeerakasala	0.013	0.009	1.44	390
5	Chempan	0.013	0.008	1.62	390
6	Kannikayama	0.023	0.014	1.64	690
7	Thowan	0.008	0.007	1.14	240
8	Kozhivalan	0.013	0.008	1.62	390
9	Kururayima	0.016	0.013	1.23	480
10	Karuthacheera	0.007	0.004	1.75	210
11	Kalladi Aryan	0.023	0.013	1.76	690
12	Veluthittaryan	0.002	0.001	2.00	60
13	Athikiramundakan	0.012	0.007	1.71	360
14	Anakodan	0.008	0.005	1.60	240
15	Veluthakattamodan	0.015	0.009	1.60	240
16	Kattamodan	0.027	0.015	1.80	810
17	Allikannan	0.002	0.001	2.00	60
18	Parambuvattan	0.012	0.008	1.50	360
19	Vellakkoli	0.016	0.008	2.00	480
20	Vellamundakan	0.035	0.019	1.84	1050
21	Cheruvirippu	0.006	0.004	1.50	180
22	Choottupokkali	0.009	0.004	2.25	270
23	Chenthadi	0.003	0.002	1.50	90
24	Kazhama	0.002	0.001	2.00	60
25	Mundon	0.014	0.009	1.50	420
26	Chettivirippu	0.003	0.002	1.50	90
27	Pokkali 3	0.010	0.009	1.60	450
28	Ponkuruka	0.015	0.010	1.50	450
29	Pandivella	0.016	0.009	1.77	480
30	Thavalakannan	0.023	0.012	1.91	690

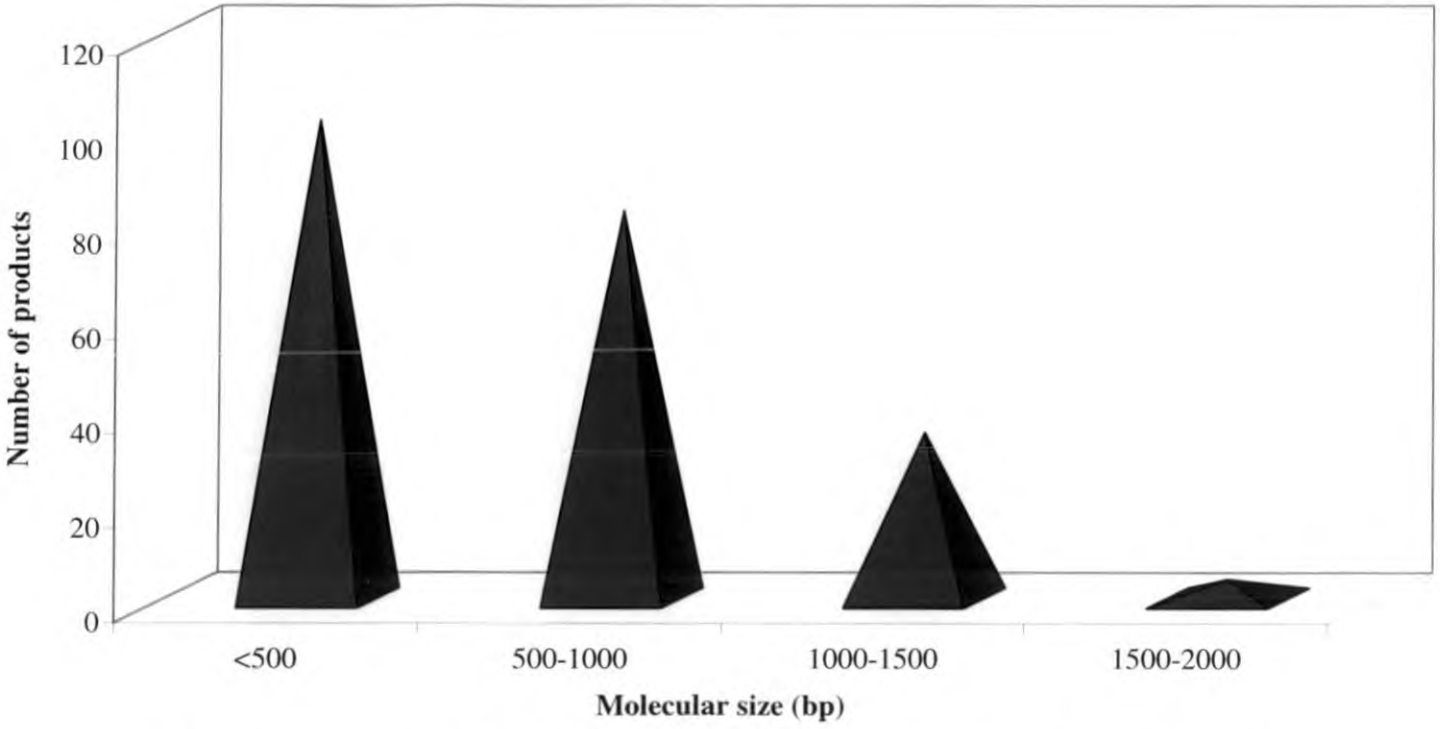

Fig. 1. Representation of molecular size of the products amplified by RAPD primers

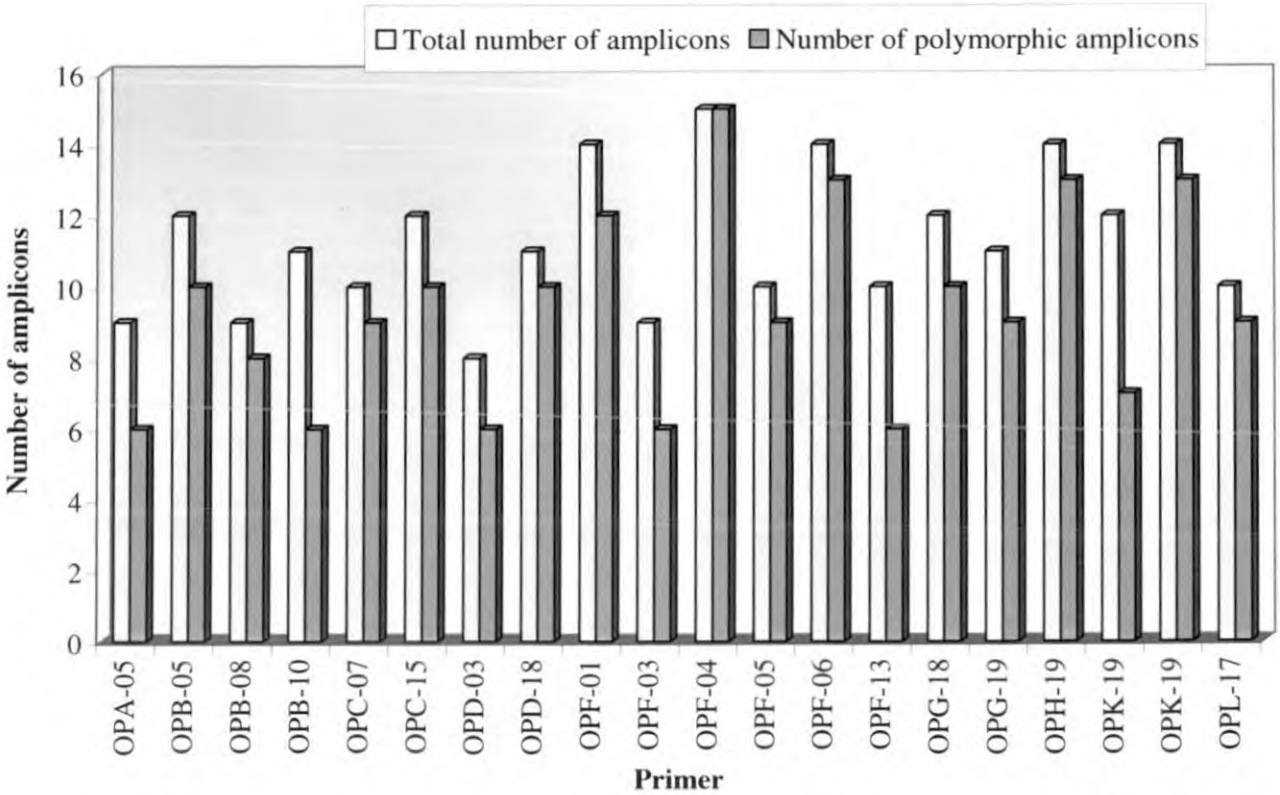

Fig. 2. Total number of amplicons and polymorphic amplicons produced by RAPD primers

Plate 2. Amplification profile of the DNA of thirty rice accessions using the primer OPA-05

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

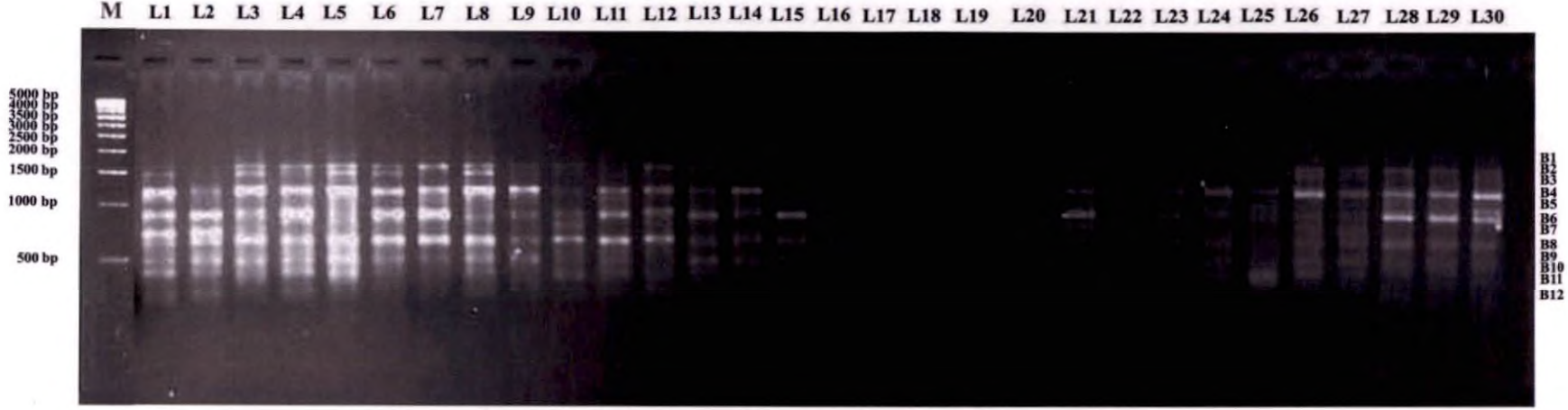

Plate 3. Amplification profile of the DNA of thirty rice accessions using the primer OPB-05

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 3. Representation of the amplification profile of the rice accessions using the primer OPA-05

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
+	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+
-	-	+	+	+	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+	-	-	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
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+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	+	-	-

Fig 4. Representation of the amplification profile of the rice accessions using the primer OPB-05

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-
-	-	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+	+	+	+
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-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
+	+	+	+	+	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

bands (Fig. 3). The size of the amplification products was found to be below 1500 bp. The products A4 (faint), A5 (intense) and A8 (faint) were monomorphic.

The amplicon A1 (faint) was absent in the accessions Njavara black, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Veluthakattamodan, Kattamodan, Parambuvattan, Vellakkoli, Choottupokkali, Mundon and Pandivella. The amplicon A2 of size 100 bp was absent in five accessions namely Athikiramundakan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. The product A3 having a size between 500-1000 bp was totally absent in eleven accessions *viz.* Njavara yellow, Njavara black, Karuthacheera, Athikiramundakan, Kattamodan, Vellakkoli, Choottupokkali, Chettivirippu, Pokkali 3 and Pandivella. The product A6 at less than 500 bp was prominent only in two accessions *viz.* Chempan and Pandivella. The product A7 at less than 500 bp was totally absent in the six accessions *viz.* Cheeravithu, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan and Allikannan. The final product A9 (faint) was present in the accessions Njavara yellow, Njavara black, Cheeravithu, Jeerakasala, Chempan, Thowan, Karuthacheera, Vellamundakan, Cheruvirippu, Choottupokkali, Kazhama, Chettivirippu, Pokkali 3 and Ponkuruka.

4.1.2 OPB-05

The primer OPB-05 produced twelve amplification products whose size was below 2000 bp (Plate 3). They were numbered from B1 to B12. Of the 12 products only two products, B4 and B9, were monomorphic giving a polymorphism of 83.33 per cent. Nine intense and three faint products were produced by this primer (Fig. 4).

The first product B1 were present in the nine accessions Cheeravithu, Jeerakasala, Chempan, Kozhivalan, Veluthittaryan, Athikiramundakan, Vellamundakan, Cheruvirippu and Chettivirippu. The amplicon B2 was absent in the accessions Njavara yellow, Njavara black,

Kururayima, Anakodan, Veluthakattamodan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Choottupokkali, Chenthadi and Mundon. The product B3 (faint) was present in the accessions Njavara yellow, Cheeravithu, Jeerakasala, Chempan, Kannikayama, Kozhivalan, Karuthacheera, Kalladi Aryan, Veluthittaryan, Kattamodan, Vellamundakan, Cheruvirippu, Chenthadi, Chettivirippu and Thavalakannan. The product B5 (faint) was present in the accessions Kannikayama, Thowan, Kururayima, Kalladi Aryan, Anakodan, Veluthakattamodan, Kattamodan, Allikannan, Vellamundakan, Cheruvirippu, Mundon and Thavalakannan. The amplicons B6 was absent in Chempan, Choottupokkali and Pokkali 3. The product B7 was present in Karuthacheera, Veluthittaryan, Kattamodan, Parambuvattan, Cheruvirippu and Pokkali 3. The amplicon B8 was present in the accessions Njavara yellow and Njavara black only with a size of less than 1000 bp. The product B10 was absent in the accessions Karuthacheera, Kalladi Aryan, Veluthittaryan and Mundon. The product B11 was present in Njavara yellow, Njavara black, Cheeravithu, Jeerakasala, Chempan, Kozhivalan, Karuthacheera, Veluthakattamodan, Parambuvattan and Ponkuruka. The final product B12 (faint) was present in the accessions Njavara yellow, Njavara black, Jeerakasala, Chempan and Ponkuruka.

4.1.3 OPB-08

The primer OPB-08 produced nine amplification products and the size of the products was less than 2000 bp (Plate 4). The products were serially numbered from C1 to C9. This primer produced good polymorphism in the accessions studied (88.88 per cent). The primer produced five intense and four faint bands (Fig. 5). The product C8 was monomorphic.

The first product C1 (faint) was present in eleven accessions *viz.* Cheeravithu, Kannikayama, Thowan, Kozhivalan, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu, Pokkali 3 and

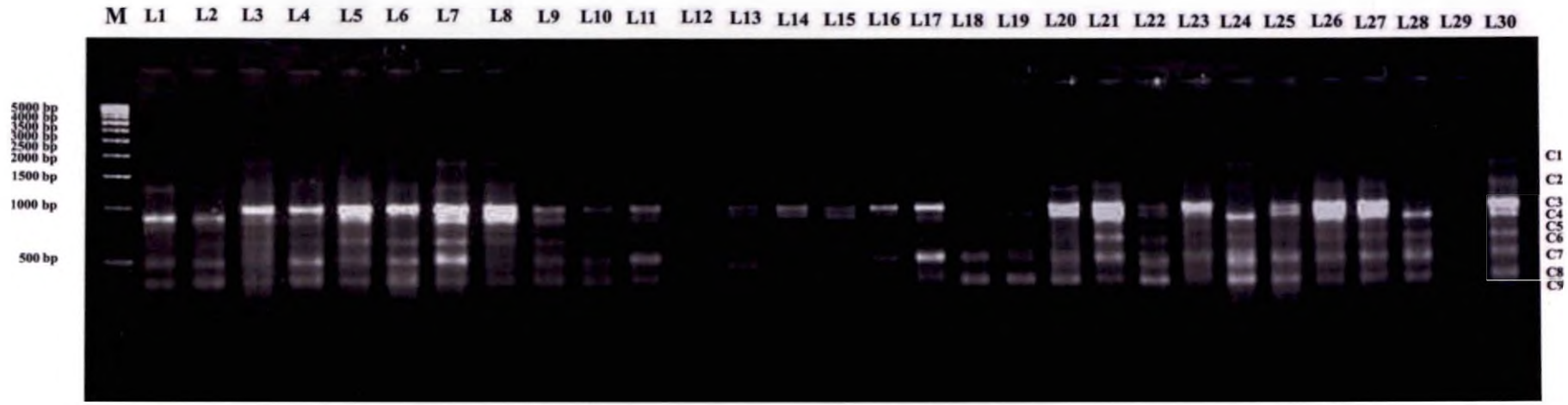

Plate 4. Amplification profile of the DNA of thirty rice accessions using the primer OPB-08

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

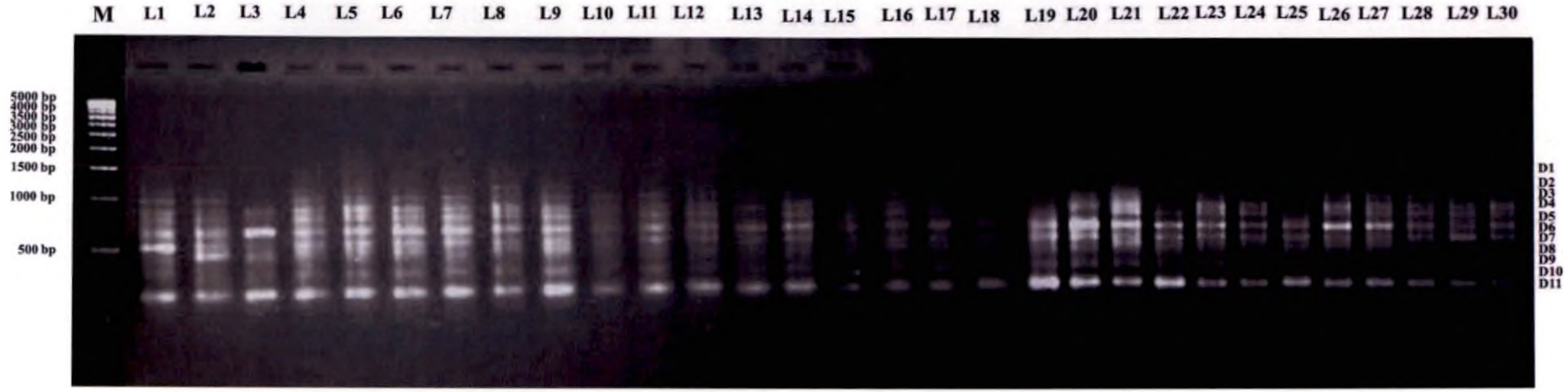

Plate 5. Amplification profile of the DNA of thirty rice accessions using the primer OPB-10

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 5. Representation of the amplification profile of the rice accessions using the primer OPB-08

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	+
+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	+	-	+	+	-	+	+	+	+	+
-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+
+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+	-	+	-	+	+	+	+	+
+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
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-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-

Fig 6. Representation of the amplification profile of the rice accessions using the primer OPB-10

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
+	+	+	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	-	-	+	+	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ → presence of bright products + → presence of faint products - → absence of amplification products

Thavalakannan. The amplicon C2 (faint) was absent in twelve accessions *viz.* Jeerakasala, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Allikannan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. The amplicon C3 (less than 1000 bp) was absent in the accessions Njavara yellow, Njavara black, Veluthittaryan, Parambuvattan, Kazhama and Ponkuruka. The product C4 was absent in only three accessions Kannikayama, Karuthacheera and Kattamodan. The amplicon C5 (faint) was absent in nine accessions *viz.* Njavara yellow, Karuthacheera, Veluthittaryan, Anakodan, Parambuvattan, Vellakkoli, Vellamundakan, Chenthadi and Mundon. The product C6 was also absent in three accessions Cheeravithu, Anakodan and Choottupokkali. The amplicon C7 seen at less than 500 bp and very close to amplicon C6 was prominent only in the three accessions *viz.* Vellamundakan, Choottupokkali and Kazhama. The product C9 (faint) was present in Njavara black, Cheeravithu, Veluthittaryan, Anakodan, Choottupokkali and Kazhama.

4.1.4 OPB-10

Eleven products were formed by the primer OPB-10 of which six were polymorphic giving a polymorphism of 54.54 per cent (Plate 5). The amplification products were numbered from D1 to D11 and were having a size of below 1500 bp. The primer produced five intense and six faint products (Fig. 6). The monomorphic products were D4, D6, D7, D10 (faint) and D11.

The product D1 (faint) was found in seven accessions *viz.* Njavara yellow, Njavara black, Cheeravithu, Kozhivalan, Kururayima, Anakodan and Kattamodan. The amplicon D2 (faint) was absent in Cheeravithu, Choottupokkali and Mundon. The amplification product D3 (faint) was absent in Cheeravithu, Allikannan, Parambuvattan, Vellakkoli, Vellamundakan, Cheruvirippu, Choottupokkali, Kazhama and Mundon. The product D5 was absent in Cheeravithu, Karuthacheera, Athikiramundakan, Parambuvattan, Vellakkoli and Vellamundakan. The amplicon D8 (faint)

was absent in the accessions Karuthacheera, Vellakkoli, Vellamundakan, Chenthadi and Kazhama. The product D9 (faint) was present in the accessions Anakodan, Vellakkoli, Vellamundakan, Choottupokkali, Mundon, Chettivirippu, Ponkuruka, Pandivella and Thavalakannan.

4.1.5 OPC-07

Altogether ten products were produced by the primer OPC-07 of which nine products were polymorphic giving a polymorphism of 90 per cent (Plate 6). The products were numbered from E1 to E10 and had a size of less than 1500 bp. There were five intense and five faint bands. The monomorphic product was E5 (Fig. 7).

The product E1 (faint) was present in the accessions Cheeravithu, Jeerakasala, Kannikayama, Thowan, and Pandivella. The amplicon E2 was absent in the accessions Kururayima, Athikiramundakan, Allikannan, Parambuvattan, Vellakkoli, Vellamundakan, Choottupokkali and Mundon. The product E3 was absent only in the accessions Njavara yellow and Choottupokkali, with a size of nearly 1000 bp. The amplicon E4 was absent in the accessions Karuthacheera, Anakodan, Veluthakattamodan, Kattamodan and Vellamundakan. The product E6 (faint) was present in the accessions Njavara yellow, Njavara black, Kozhivalan and Pandivella. The amplicon E7 (faint) was present in the accessions Njavara yellow, Njavara black, Cheeravithu, Jeerakasala, Chempan, Kannikayama, Thowan, Kururayima, Karuthacheera, Kalladi Aryan and Pandivella. The product E8 (faint) was present in Njavara black, Cheeravithu, Karuthacheera, Veluthittaryan, Choottupokkali and Pandivella. The product E9 was absent in Athikiramundakan, Anakodan, Veluthakattamodan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Vellamundakan, Cheruvirippu, Choottupokkali and Chenthadi. The final amplicon E10 (faint) was present in the accessions Njavara yellow, Njavara black, Kozhivalan and Kalladi Aryan.

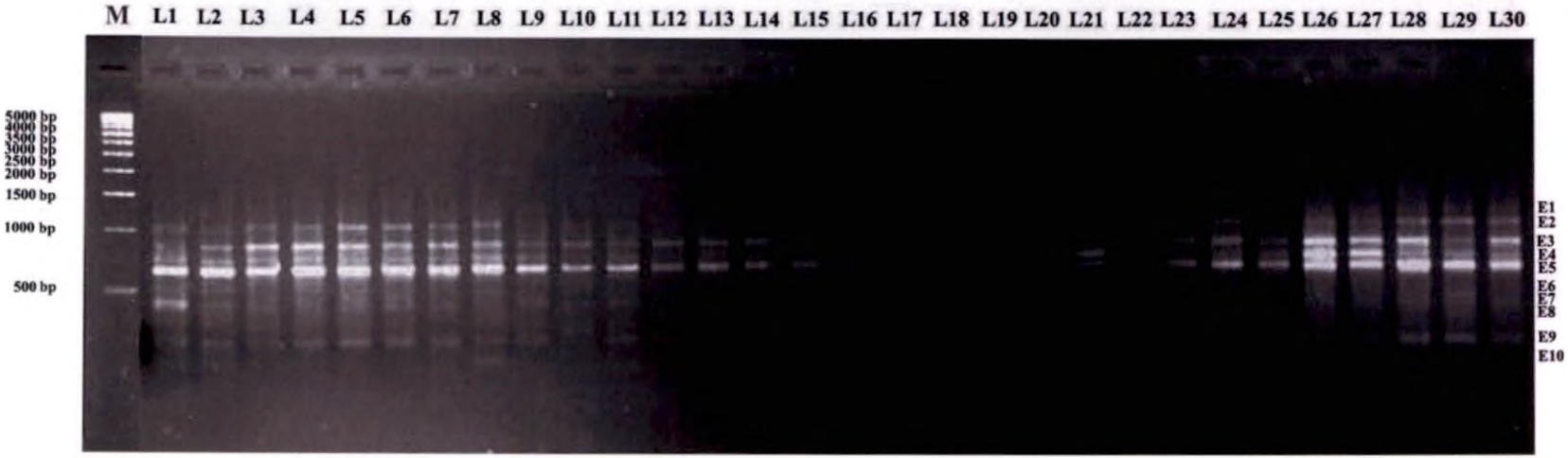

Plate 6. Amplification profile of the DNA of thirty rice accessions using the primer OPC-07

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan
M - λ DNA Hind III digest marker

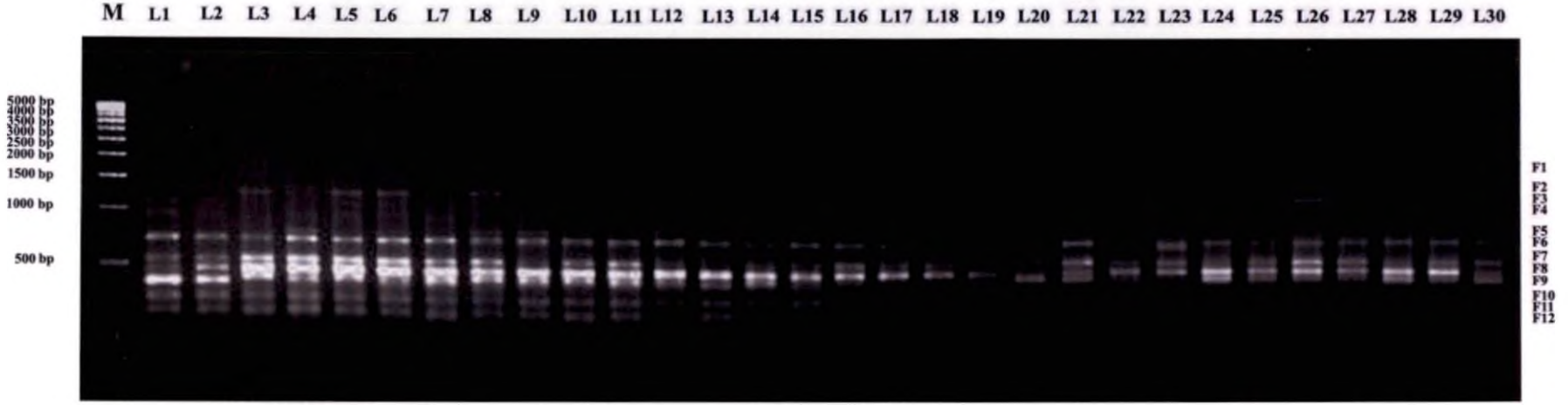

Plate 7. Amplification profile of the DNA of thirty rice accessions using the primer OPC-15

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 7. Representation of the amplification profile of the rice accessions using the primer OPC-07

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+
-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
-	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig 8. Representation of the amplification profile of the rice accessions using the primer OPC-15

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	+
-	-	+	+	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	-	+	+	-	-	+
+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-
-	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ → presence of bright products + → presence of faint products - → absence of amplification products

4.1.6 OPC-15

The primer OPC-15 produced a total of twelve amplicons of which ten were polymorphic giving a polymorphism per cent of 83.33 (Plate 7). The products had a size of less than 1500 bp and were numbered from F1 to F12. The amplicons F7 and F12 (faint) were monomorphic. The primer produced six intense and six faint bands (Fig. 8).

The product F1 (faint) was present in the accessions Cheeravithu, Kannikayama and Kozhivalan. The product F2 was unique since it was present in the accession Cheruvirippu alone and had a size of nearly 1000 bp but the expression of this product was faint. The amplicon F3 was present in the accessions Njavara yellow, Chempan, Kannikayama, Anakodan, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu, Pokkali 3 and Thavalakannan. The product F4 (faint) was present in Cheeravithu, Jeerakasala, Chempan, Kannikayama, Kozhivalan, Anakodan, Veluthakattamodan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu, Pokkali 3 and Thavalakannan. The amplicon F5 (faint) was present in the accessions Njavara yellow, Njavara black, Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Kururayima, Karuthacheera, Kalladi Aryan, Allikannan, Ponkuruka and Pandivella. The product F6 (faint) was present in the accessions Jeerakasala, Kozhivalan, Kururayima, Kalladi Aryan, Veluthittaryan, Cheruvirippu, Mundon, Chettivirippu, Ponkuruka, Pandivella and Thavalakannan. The product F8 was absent in the accessions Kururayima, Veluthittaryan, Athikiramundakan, Anakodan, Vellakkoli, Kazhama and Ponkuruka. The amplicon F9 was absent in the accessions Vellamundakan and Thavalakannan. The product F10 was absent in the accessions Kattamodan, Allikannan, Parambuvattan and Vellakkoli. The amplicon F11 was absent in the accessions Allikannan and Thavalakannan alone.

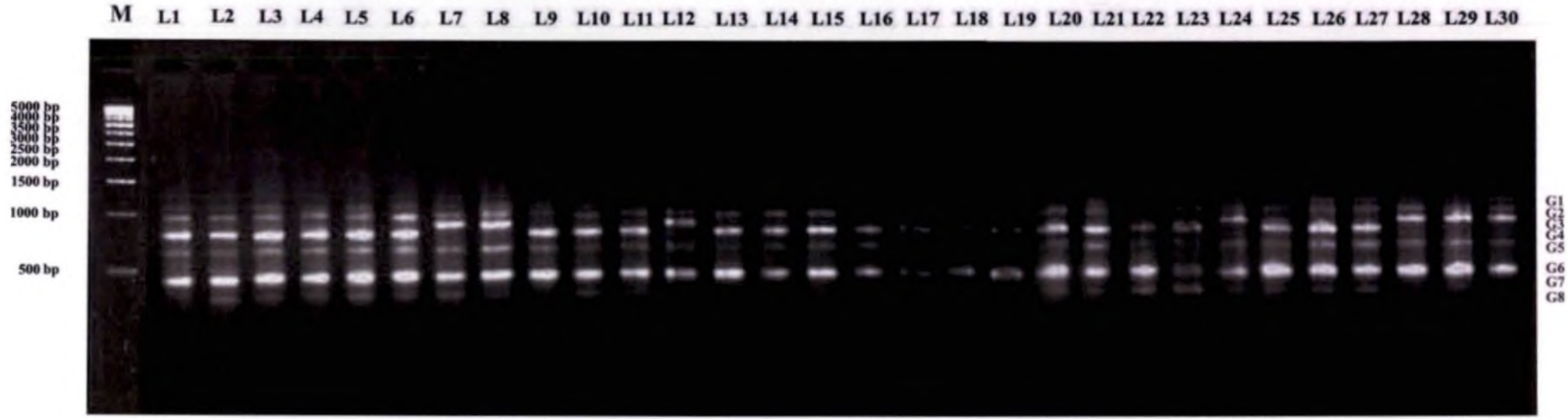

Plate 8. Amplification profile of the DNA of thirty rice accessions using the primer OPD-03

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan
M - λ DNA Hind III digest marker

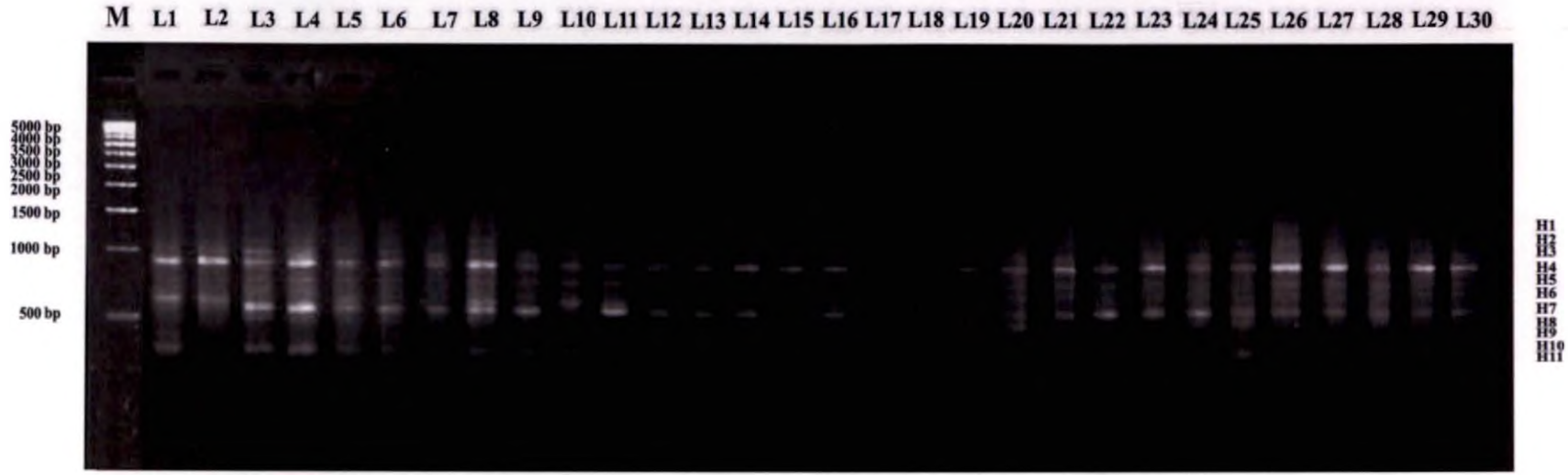

Plate 9. Amplification profile of the DNA of thirty rice accessions using the primer OPD-18

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 9. Representation of the amplification profile of the rice accessions using the primer OPD-03

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+	-	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+
+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig 10. Representation of the amplification profile of the rice accessions using the primer OPD-18

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-
-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	+	+	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	+	+	-	-	-	+	-	-	-	+	+	+	-	-	-	-	+	+	+	+	-	+	-	+	+	-	+	+
+	+	-	-	+	-	-	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	-	-	+	+	+	-	-	+
-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
-	-	+	+	+	-	-	+	-	+	-	+	+	+	+	-	-	-	+	+	-	+	-	+	+	-	-	-	-	+
+	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	+	+	-	+	-	+	-	-	-	-	-	-
+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

4.1.7 OPD-03

Eight products were formed by the primer OPD-03 (Plate 8) of which six were polymorphic (75 per cent). The amplicons were numbered from G1 to G8. The amplification products had a size of nearly 1000 bp and below. The monomorphic products were G5 and G6. The products were all bright and easily visible (Fig. 9).

The product G1 was absent in the accessions Athikiramundakan, Vellakkoli, Choottupokkali, Chenthadi, Mundon, Ponkuruka and Pandivella. The amplification product G2 was absent only in the accession Choottupokkali and had a size of nearly 1000 bp. The product G3 was present in the accessions Thowan, Kozhivalan, Veluthittaryan, Kazhama, Ponkuruka, Pandivella and Thavalakannan. Amplicon G4 was absent in the accessions Thowan, Kozhivalan, Veluthittaryan, Kazhama, Ponkuruka, Pandivella and Thavalakannan. The product G7 was absent in Njavara yellow, Kururayima, Veluthittaryan, Athikiramundakan, Anakodan and Vellakkoli. The product G8 was unique and present only in Njavara black with a size of less than 500 bp.

4.1.8 OPD-18

The primer OPD-18 produced eleven amplicons and is serially numbered from H1 to H11 (Plate 9). Only the amplicon H4 was monomorphic giving a polymorphism of 90.9 per cent. The products had a size of less than 1500 bp. There were four intense and seven faint bands (Fig. 10).

The product H1 (faint) was present in the accessions Kozhivalan, Cheruvirippu, Mundon, Chettivirippu and Pokkali 3. The amplicon H2 (faint) was present in Kozhivalan, Cheruvirippu, Mundon and Chettivirippu. The product H3 was absent in the accessions Veluthakattamodan, Kattamodan, Allikannan, Vellakkoli, Choottupokkali and Pandivella. The

product H5 (faint) was absent in the accessions Njavara black and Kattamodan. The amplification product H6 (faint) was absent in the accessions Njavara yellow, Njavara black, Chempan, Kannikayama, Thowan, Kururayima, Karuthacheera, Kalladi Aryan, Veluthakattamodan, Kattamodan, Allikannan, Parambuvattan, Chenthadi, Mundon and Ponkuruka. The product H7 (faint) was absent in the accessions Cheeravithu, Jeerakasala, Kannikayama, Thowan, Kururayima, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Cheruvirippu, Chenthadi, Kazhama, Ponkuruka and Pandivella. Amplicon H8 was absent in the accessions Njavara yellow, Njavara black, Karuthacheera, Veluthakattamodan, Parambuvattan and Vellakkoli. The product H9 (faint) was absent in Njavara yellow, Njavara black, Kannikayama, Thowan, Kururayima, Kalladi Aryan, Kattamodan, Allikannan, Parambuvattan, Cheruvirippu, Chenthadi, Chettivirippu, Pokkali 3, Ponkuruka and Pandivella. The product H10 (faint) was present in the accessions Njavara yellow, Chempan, Kannikayama, Thowan, Kozhivalan, Athikiramundakan, Veluthakattamodan, Parambuvattan, Vellakkoli, Vellamundakan, Choottupokkali and Kazhama. The final product H11 was absent in Njavara black, Anakodan, Kattamodan, Vellakkoli, Vellamundakan, Cheruvirippu, Choottupokkali, Pokkali 3 and Thavalakannan.

4.1.9 OPF-01

The primer OPF-01 produced fourteen products (Plate 10). The products are numbered from I 1 to I 14. The primer showed 85.71 per cent polymorphism. All the products had a size of less than 1500 bp and most of them clustered below 1000 bp. The monomorphic products were I 5 and I 13. There were five intense and nine faint bands (Fig. 11).

The product I 1 (faint) was present in the accessions Chempan, Kannikayama, Thowan, Allikannan, Chenthadi, Kazhama and Chettivirippu. The amplicon I 2 (faint) was absent in the accessions Njavara

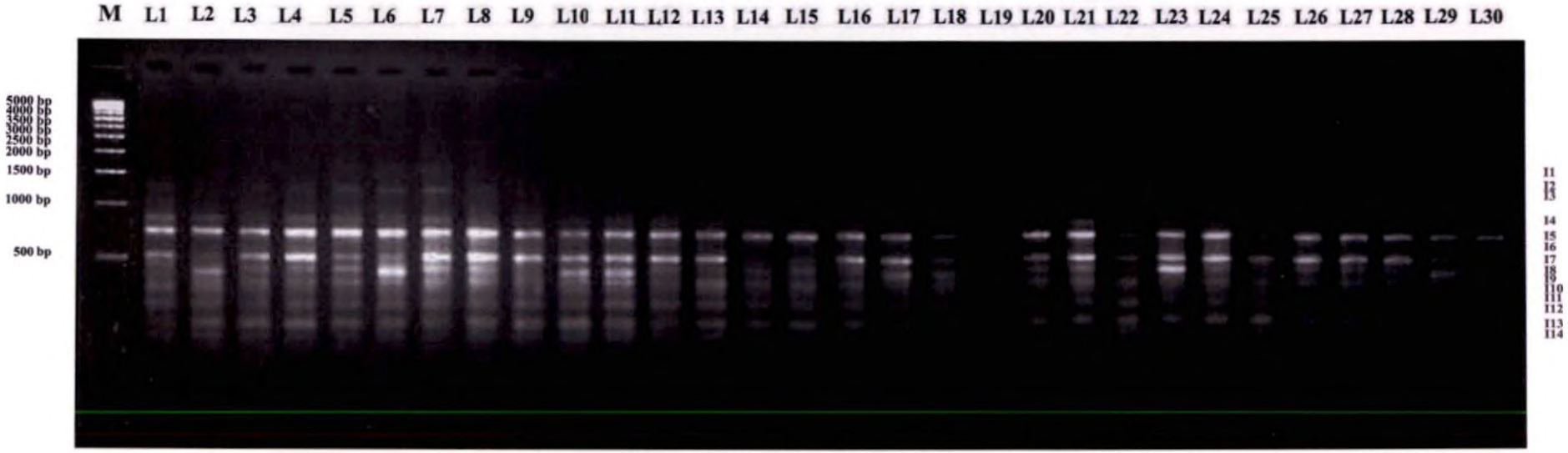

Plate 10. Amplification profile of the DNA of thirty rice accessions using the primer OPF-01

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan
M - λ DNA Hind III digest marker

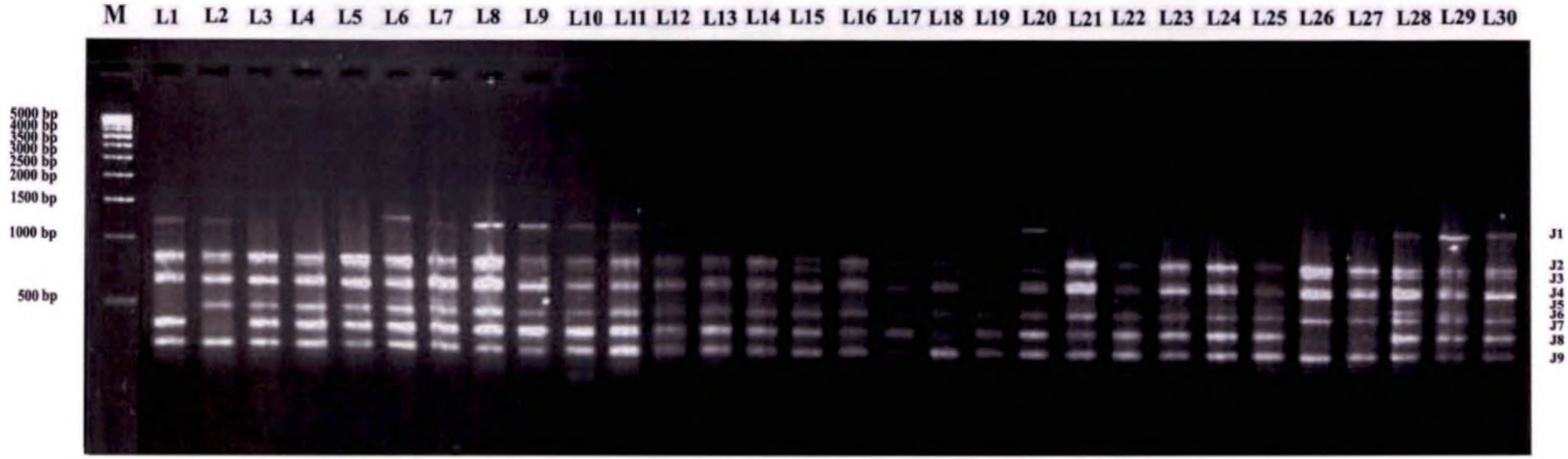

Plate 11. Amplification profile of the DNA of thirty rice accessions using the primer OPF-03

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 11. Representation of the amplification profile of the rice accessions using the primer OPF-01

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-
+	-	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	-	+
+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	-	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	-	-
-	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-

Fig 12. Representation of the amplification profile of the rice accessions using the primer OPF-03

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+
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-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	+	+	-
-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ → presence of bright products + → presence of faint products - → absence of amplification products

black, Kururayima, Karuthacheera, Kalladi Aryan, Athikiramundakan, Allikannan, Parambuvattan, Vellakkoli, Mundon and Pandivella. The product I 3 (faint) was present in the accessions Njavara yellow, Cheeravithu, Kururayima, Cheruvirippu and Kazhama. The amplicon I 4 (faint) was absent in Cheeravithu, Kururayima, Karuthacheera, Parambuvattan, Vellakkoli, Choottupokkali, Mundon, Pokkali 3 and Pandivella. The product I 6 (faint) was present in the accessions Jeerakasala, Chempan, Kannikayama, Kozhivalan, Karuthacheera, Kalladi Aryan, Veluthittaryan, Choottupokkali and Chettivirippu. The amplicon I 7 was absent in three accessions Njavara black, Anakodan and Vellakkoli. The product I 8 (faint) was absent in the accessions Njavara black, Kannikayama, Kururayima, Karuthacheera, Kalladi Aryan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Mundon, Pandivella and Thavalakannan. The amplicon I 9 (faint) was present in Njavara black, Kannikayama, Karuthacheera, Kalladi Aryan, Allikannan, Parambuvattan, Pandivella and Thavalakannan. The product I 10 (faint) was absent in Njavara yellow, Njavara black, Kururayima, Veluthittaryan, Chenthadi, Pandivella and Thavalakannan. The product I 11 was found only in the accessions Njavara yellow and Njavara black and had a size of below 500 bp. Amplicon I 12 was absent in the accessions Chenthadi, Mundon and Thavalakannan. The amplicon I 14 (faint) was present in seven accessions *viz.* Athikiramundakan, Allikannan, Parambuvattan, Vellakkoli, Vellamundakan, Choottupokkali and Pandivella.

4.1.10 OPF-03

The primer OPF-03 produced nine products (Plate 11). The amplification products had a size of less than 1500 bp and were serially numbered from J1 to J9. The primer produced seven intense and two faint products (Fig. 12). Of the nine amplicons, three were monomorphic (J2, J4 and J9) giving a polymorphism of 66.66 per cent.

The product J1 was absent in the accessions Cheeravithu, Jeerakasala, Chempan, Athikiramundakan, Anakodan, Veluthakattamodan, Vellakkoli, Cheruvirippu, Choottupokkali, Chenthadi, Kazhama, Mundon, Chettivirippu and Pokkali 3. The amplicon J3 (faint) was absent in the accessions Njavara yellow, Cheeravithu, Veluthittaryan, Anakodan, Allikannan, Vellakkoli, Choottupokkali and Mundon. The product J5 was present in the accession Allikannan alone and had a size of nearly 500 bp. The product J6 (faint) was present in Cheeravithu, Thowan, Kozhivalan, Veluthakattamodan, Vellamundakan, Kazhama, Mundon, Ponkuruka and Pandivella. The amplicon J7 was absent from the accessions Njavara yellow and Allikannan only. The product J8 was absent in the three accessions *viz.* Njavara black, Chettivirippu and Pokkali 3.

4.1.11 OPF-04

Amplification of the thirty accessions using the primer OPF-04 gave fifteen products (Plate 12). The products had a size below 1500 bp and were serially numbered from K1 to K15. This primer gave the highest percentage of polymorphism (100 per cent). The primer produced eight intense and seven faint amplicons (Fig. 13).

The first product K1 was present in the accessions Cheeravithu, Kannikayama, Thowan, Veluthittaryan, Kazhama and Thavalakannan. The amplicon K2 was present in the accessions Cheeravithu and Thowan. The amplicon K3 was unique and present in the accession Cheruvirippu alone with a size between 1000 and 1500 bp. The product K4 (faint) was present in the accessions Cheeravithu, Kannikayama, Thowan, Kozhivalan, Karuthacheera, Athikiramundakan, Anakodan, Kattamodan, Allikannan, Vellamundakan, Cheruvirippu, Mundon, Chettivirippu and Pokkali 3. The product K5 was absent in the accessions Kalladi Aryan and Vellakkoli. The product K6 (faint) was present in Karuthacheera, Anakodan, Vellamundakan, Choottupokkali, Mundon and Thavalakannan. The product K7 (faint) was absent in Njavara yellow, Karuthacheera, Allikannan,

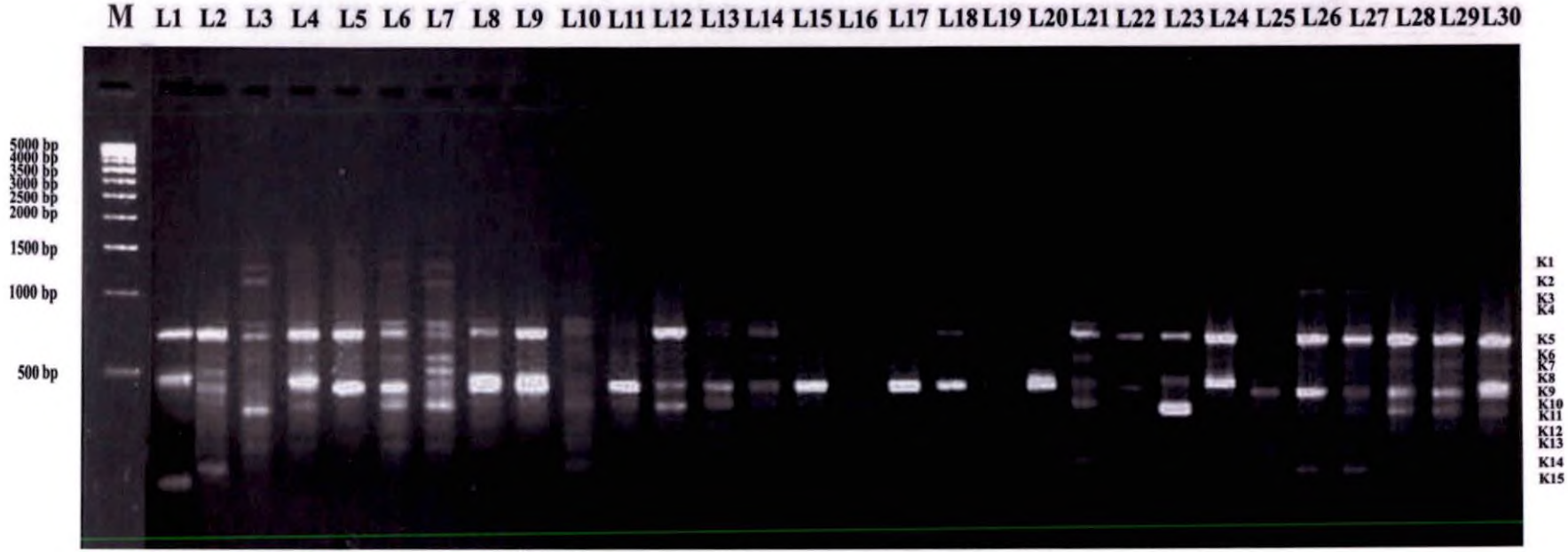

Plate 12. Amplification profile of the DNA of thirty rice accessions using the primer OPF-04

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

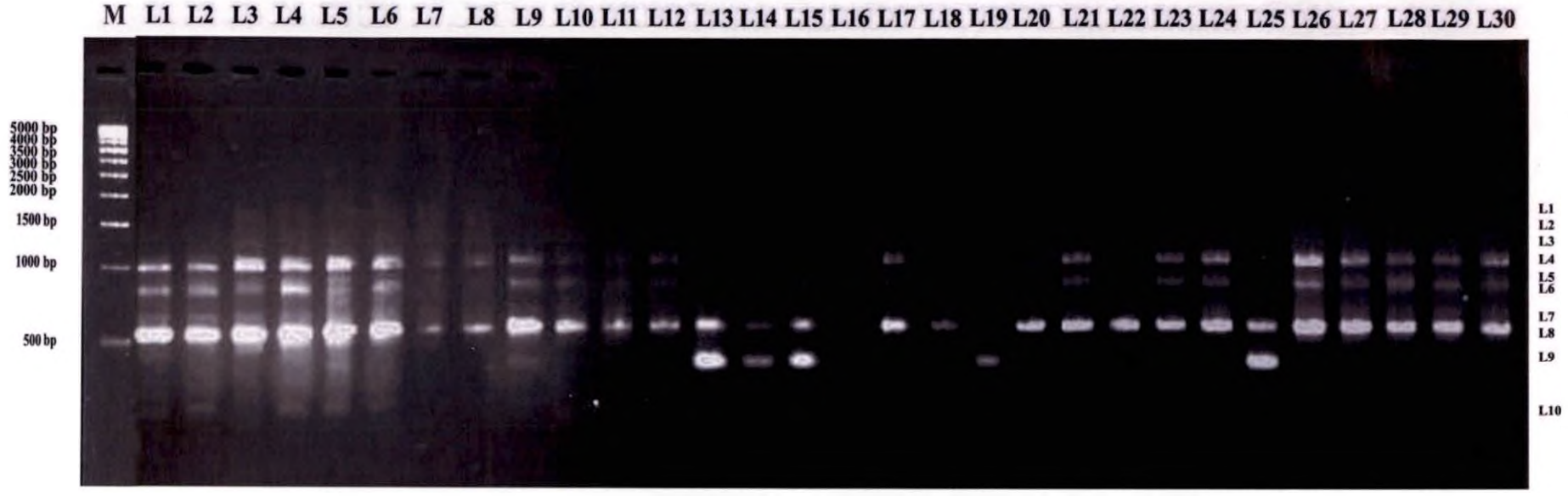

Plate 13. Amplification profile of the DNA of thirty rice accessions using the primer OPF-05

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan
M - λ DNA Hind III digest marker

Parambuvattan, Vellakkoli, Choottupokkali and Chenthadi. The amplicon K8 (faint) was present in the accessions Njavara black, Thowan, Veluthittaryan, Athikiramundakan, Allikannan, Vellamundakan, Kazhama, Mundon, Ponkuruka and Pandivella. The product K9 (faint) was absent in the accessions Cheeravithu and Karuthacheera only. The amplicon K10 was present in the accessions Njavara black, Jeerakasala, Kannikayama, Thowan, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Kattamodan, Cheruvirippu, Choottupokkali, Chenthadi, Chettivirippu, Pokkali 3, Ponkuruka, Pandivella and Thavalakannan. The product K11 was present in two accessions *viz.* Cheeravithu and Chenthadi only. The amplicon K12 (faint) was absent in Njavara yellow, Njavara black, Kozhivalan, Kururayima, Kalladi Aryan, Veluthittaryan, Parambuvattan, Vellakkoli, Vellamundakan, Choottupokkali, Chenthadi, Kazhama and Chettivirippu. The product K13 (faint) was absent in Njavara yellow, Chempan, Kururayima, Veluthittaryan, Allikannan, Parambuvattan, Chenthadi and Pandivella. The product K14 was present in the accessions Njavara black, Cheeravithu, Karuthacheera, Kalladi Aryan, Kattamodan, Parambuvattan, Cheruvirippu, Choottupokkali, Mundon and Chettivirippu. The final product K15 was present in the accession Njavara yellow alone with a size of less than 500 bp.

4.1.12 OPF-05

The amplification of the thirty accessions using the primer OPF-05 gave ten products (Plate 13). The products are serially numbered from L1 to L10. The products had size below 2000 bp with most of the amplicons below 1000 bp. The amplicon L8 alone was monomorphic thus showing a polymorphism of ninety per cent. The primer produced five intense and five faint products (Fig. 15).

The first product L1 (faint) was present in the accessions Cheeravithu and Chenthadi. The product L2 (faint) was present in the accessions Cheeravithu, Jeerakasala, Allikannan, Cheruvirippu, Pokkali 3

Fig 13. Representation of the amplification profile of the rice accessions using the primer OPF-04

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
-	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	-	-	+	+	-	-	-	+	+	+	-	-	-
+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+
-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+
-	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	-	-	+	-	-	-	+	+	-	-	+	+	-
+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	+	-	+	+	-	-	+	+	+	+	+	-	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+
-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+
-	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+
-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig 17. Representation of the amplification profile of the rice accessions using the primer OPG-18

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	+	-	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	+	+	-	-	-	+	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	-	+	-	-	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

Fig 14. Representation of the amplification profile of the rice accessions using the primer OPF-05

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+
-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+
-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+
+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	+	+	-	-	-	+	-	-	-	+	+	+	-	-	-	+	-	+	+	-	-	+	+	+	-	-	-
+	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	+	-	+

Fig 15. Representation of the amplification profile of the rice accessions using the primer OPF-06

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+
-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	+
-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-
+	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+
+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+
-	+	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	+	-	+
+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	+	-	-	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+
+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

Plate 14. Amplification profile of the DNA of thirty rice accessions using the primer OPF-06

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

and Thavalakannan. The amplicon L3 was a unique product present in the accession Cheeravithu only which had a size of nearly 1000 bp. The product L4 was absent in five accessions *viz.* Kattamodan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. The product L5 was also unique present in the accession Chempan only having a size of less than 1000 bp. The amplicon L6 was absent in Thowan, Anakodan, Kattamodan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. The amplicon L7 (faint) which overlays the product L8 was absent in seven accessions Kannikayama, Thowan, Kozhivalan, Anakodan, Allikannan, Parambuvattan and Vellakkoli. The product L9 (faint) was present in the accessions Jeerakasala, Chempan, Kururayima, Athikiramundakan, Anakodan, Veluthakattamodan, Vellakkoli, Cheruvirippu, Choottupokkali, Mundon, Chettivirippu and Pokkali 3. The amplicon L10 (faint) was present in Njavara yellow, Njavara black, Jeerakasala, Chempan, Kannikayama, Kururayima, Allikannan, Choottupokkali, Chettivirippu, Pokkali 3, Ponkuruka and Thavalakannan.

4.1.13 OPF-06

The primer OPF-06 produced fourteen products having a size of less than 2000 bp (Plate 14). They are numbered from M1 to M14. Of the total 14 products only one product, M8 was monomorphic giving 92.85 per cent polymorphism. There were six intense and eight faint products (Fig. 16).

The first product M1 (faint) was present in the accessions Cheeravithu, Chempan, Kannikayama, Thowan, Kozhivalan, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu and Thavalakannan. Amplicon M2 (faint) was present in Cheeravithu, Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Chettivirippu and Thavalakannan. The amplicon M3 was absent in Karuthacheera, Athikiramundakan, Anakodan, Veluthakattamodan, Parambuvattan, Vellakkoli, Choottupokkali, Mundon, Ponkuruka and Pandivella. The

product M4 was present in Cheeravithu, Kozhivalan, Allikannan, Cheruvirippu and Chettivirippu. The amplicon M5 (faint) was present in the accessions Njavara yellow, Chempan, Kannikayama, Veluthittaryan, Cheruvirippu and Thavalakannan. The product M6 (faint) was absent in Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Kattamodan, Parambuvattan, Vellakkoli, Choottupokkali, Mundon and Ponkuruka. The product M7 (faint) was absent in Njavara yellow, Chempan, Veluthittaryan, Athikiramundakan, Veluthakattamodan, Cheruvirippu, Choottupokkali, Chenthadi, Kazhama, Mundon and Ponkuruka. The amplification product M9 (faint) was present in the accessions Njavara yellow, Njavara black, Chempan, Kannikayama, Thowan, Kozhivalan, Parambuvattan, Vellakkoli, Vellamundakan, Chenthadi, Kazhama, Pokkali 3, Ponkuruka and Thavalakannan. Amplicon M10 was absent in Cheeravithu and Veluthittaryan only. The product M11 (faint) was present in the accessions Njavara black, Jeerakasala, Thowan, Karuthacheera, Kalladi Aryan, Anakodan, Veluthakattamodan, Parambuvattan, Vellakkoli and Thavalakannan. The product M12 was present in Njavara yellow, Njavara black, Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Kururayima, Karuthacheera, Kalladi Aryan, Athikiramundakan and Parambuvattan. The product M13 was absent in the accessions Chenthadi and Thavalakannan only. The final product M14 (faint) was present in accessions Cheeravithu, Karuthacheera, Choottupokkali, Mundon, Pokkali 3 and Ponkuruka.

4.1.14 OPF-13

Amplification of the thirty accessions using the primer OPF-13 gave ten products (Plate 15). The products are serially numbered from N1 to N10. The amplicons had size below 1500 bp. Of the ten products six were polymorphic giving 60 per cent polymorphism. The four monomorphic products were N4, N6, N8 and N9. There were six intense and four faint amplicons (Fig. 17).

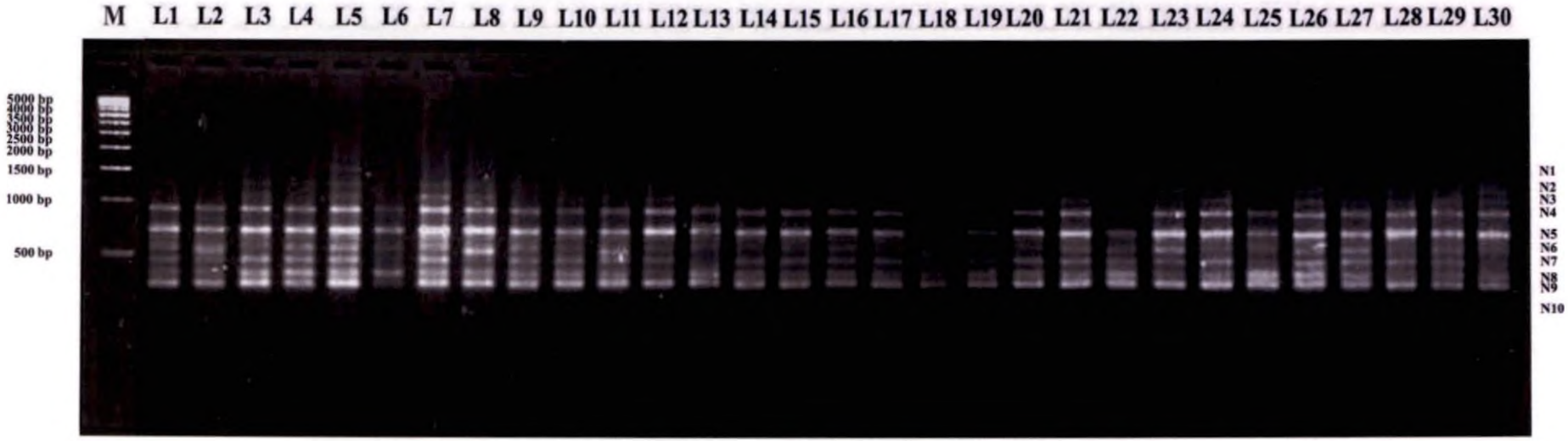

Plate 15. Amplification profile of the DNA of thirty rice accessions using the primer OPF-13

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

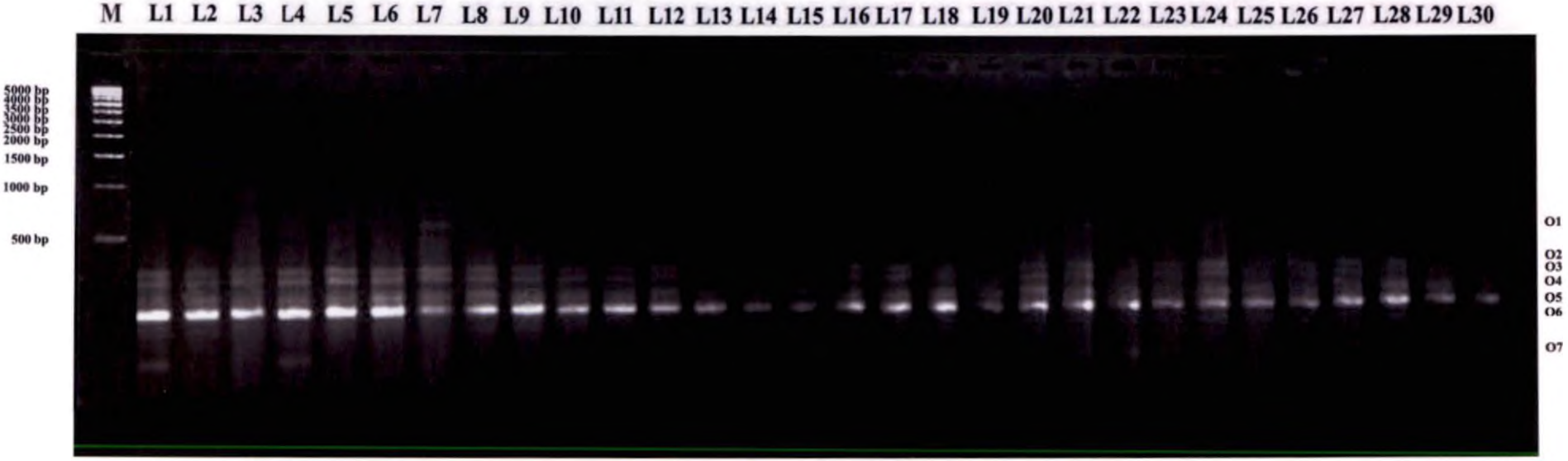

Plate 16. Amplification profile of the DNA of thirty rice accessions using the primer OPG-18

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

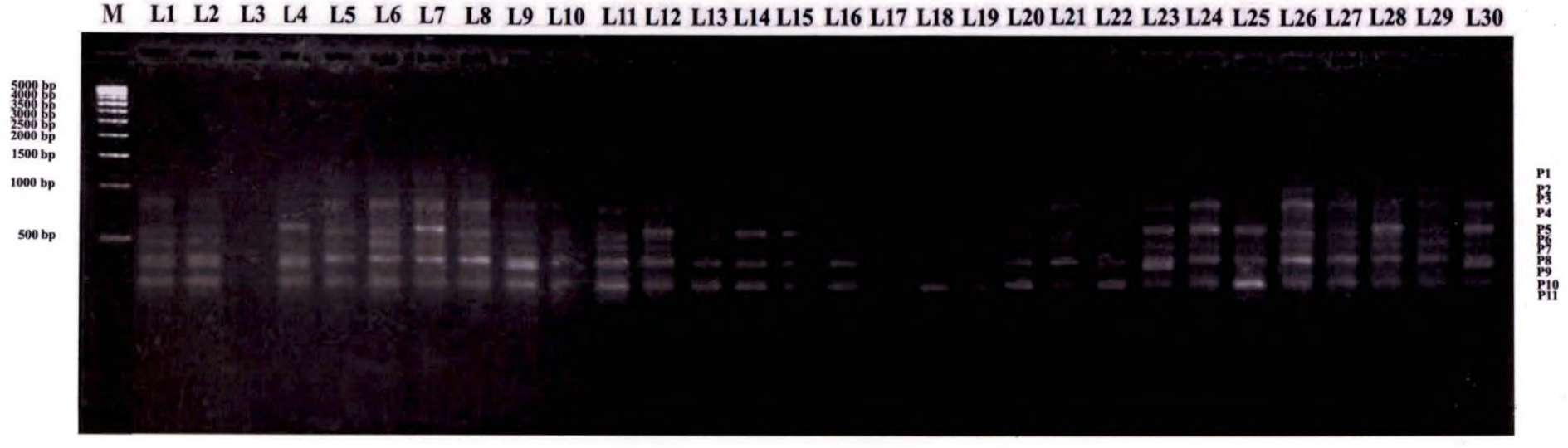

Plate 17. Amplification profile of the DNA of thirty rice accessions using the primer OPG-19

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 16. Representation of the amplification profile of the rice accessions using the primer OPF-13

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
-	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	+	+	-	-	+	+	+	+	+
+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
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+	+	+	+	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-
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Fig 18. Representation of the amplification profile of the rice accessions using the primer OPG-19

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+	+
+	+	-	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+
-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+
+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
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-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

The first product N1 (faint) was present in the accessions Chempan, Veluthittaryan and Chenthadi. The product N2 (faint) was absent in Njavara yellow, Njavara black, Kalladi Aryan, Athikiramundakan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Cheruvirippu, Kazhama and Mundon. The product N3 was absent in Njavara black, Kannikayama, Parambuvattan and Choottupokkali. The amplicon N5 (faint) was present in the accessions Njavara yellow, Njavara black, Cheeravithu, Jeerakasala, Karuthacheera, Kalladi Aryan, Anakodan, Veluthakattamodan, Kattamodan, Allikannan, Cheruvirippu and Chenthadi. The product N7 was absent in three accessions Kannikayama, Kozhivalan and Athikiramundakan. The final product N10 (faint) was absent in Jeerakasala, Veluthittaryan, Chenthadi, Ponkuruka, Pandivella and Thavalakannan.

4.1.15 OPG-18

The primer OPG-18 gave seven amplicons (Plate 16). The products had a size of less than 1000 bp and were serially numbered from O1 to O7. The monomorphic products were O5 and O6, thus giving 71.42 per cent polymorphism. There were five intense and two faint bands (Fig. 14).

The product O1 was unique present in the accession Thowan alone having a size of between 500 and 1000 bp. The product O2 (faint) was present in the accessions Njavara yellow, Chempan, Thowan, Kozhivalan, Veluthittaryan, Kattamodan, Allikannan, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama and Ponkuruka. The product O3 and O4 were absent from the accessions Veluthakattamodan, Vellakkoli and Choottupokkali. The final product O7 (faint) was present in the accessions Njavara black, Jeerakasala, Kattamodan, Allikannan, Parambuvattan, Cheruvirippu, Choottupokkali, Chenthadi, Kazhama, Mundon and Pokkali 3.

4.1.16 OPG-19

Amplification of the thirty accessions using the primer OPG-19 gave eleven amplicons (Plate 17). The products had a size of less than 1500 bp

and were numbered from P1 to P11. Of the eleven products two were monomorphic thus showing a polymorphism of 81.81 per cent. The monomorphic products are P8 and P10. There were six intense and five faint products (Fig. 18).

The product P1 (faint) was present in Kannikayama, Thowan, Kozhivalan, Kalladi Aryan, Vellamundakan and Chettivirippu. The amplicon P2 (faint) was present in Kannikayama, Thowan, Kozhivalan, Kururayima, Veluthittaryan, Vellamundakan, Cheruvirippu, Chettivirippu, Pokkali 3, Ponkuruka and Pandivella. The product P3 was absent in Allikannan, Choottupokkali and Mundon. The product P4 (faint) was present in Njavara yellow, Njavara black, Chempan, Kannikayama, Thowan, Kozhivalan, Veluthittaryan, Veluthakattamodan, Vellakkoli, Kazhama and Thavalakannan. The amplicon P5 was present in the accessions Jeerakasala, Thowan, Veluthittaryan, Chenthadi, Kazhama, Mundon, Ponkuruka and Thavalakannan. The product P6 (faint) was absent in Jeerakasala, Thowan, Veluthittaryan, Chenthadi, Kazhama, Mundon and Thavalakannan. The product P7 was absent only in Vellakkoli and had a size of less than 500 bp. P9, the amplicon very close to P8 was present in Njavara yellow, Njavara black, Jeerakasala, Kururayima, Chenthadi and Thavalakannan. The final product P11 (faint) was present in the accessions Kozhivalan, Kururayima, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Vellamundakan, Choottupokkali, Mundon and Chettivirippu.

4.1.17 OPH-19

The primer OPH-19 formed fourteen products with size less than 2000 bp (Plate 18). The products were serially numbered from Q1 to Q14 and of these only Q7 is monomorphic thus showing 92.85 per cent polymorphism. There were nine intense and five faint amplicons (Fig. 19).

The product Q1 (faint) was present in the accessions Cheruvirippu, Chenthadi, Kazhama, Chettivirippu, Pokkali 3 and Thavalakannan. This product was bright in Thavalakannan. The product Q2 was absent in the

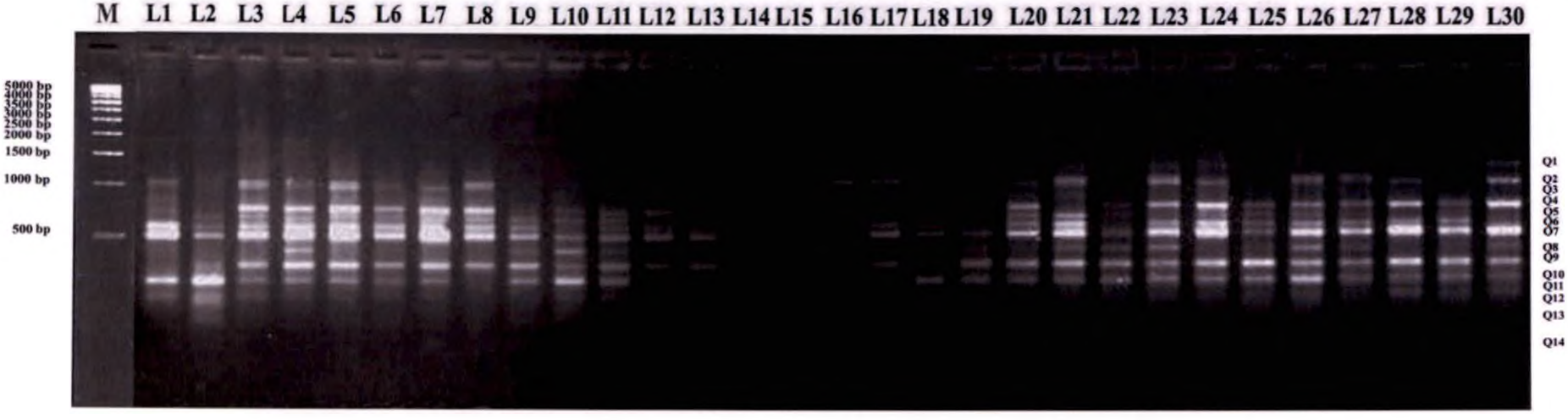

Plate 18. Amplification profile of the DNA of thirty rice accessions using the primer OPH-19

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Plate 19. Amplification profile of the DNA of thirty rice accessions using the primer OPK-14

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig 19. Representation of the amplification profile of the rice accessions using the primer OPH-19

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	+
+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	+	-	+
+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
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-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-

Fig 20. Representation of the amplification profile of the rice accessions using the primer OPK-14

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
+	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	+	+	-	+	+	+	+	+
-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+
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+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

accessions Njavara black, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Parambuvattan, Vellakkoli, Choottupokkali, Mundon and Pandivella. The product Q3 (faint) was present in Njavara yellow, Thowan, Kozhivalan, Veluthittaryan, Vellamundakan and Cheruvirippu. The amplicon Q4 was absent in the accessions Njavara black, Kururayima, Athikiramundakan, Veluthakattamodan, Kattamodan, Allikannan and Cheruvirippu. Amplicon Q5 was absent in Njavara yellow, Veluthittaryan, Athikiramundakan, Veluthakattamodan, Chenthadi, Kazhama, Ponkuruka and Thavalakannan. The product Q6 was absent in Karuthacheera, Anakodan, Veluthakattamodan, Vellakkoli, Choottupokkali and Thavalakannan. The product Q8 was present in Jeerakasala, Kannikayama, Kururayima, Karuthacheera, Kalladi Aryan, Vellakkoli, Cheruvirippu, Choottupokkali, Chenthadi, Mundon, Chettivirippu, Pokkali 3, Pandivella and Thavalakannan. The amplicon Q9 was absent in the accessions Njavara yellow, Njavara black and Parambuvattan. The product Q10 was absent only in the accession Kozhivalan and had a size of less than 500 bp. The product Q11 was absent in the accessions Anakodan, Veluthakattamodan, Kattamodan and Allikannan. The amplicon Q12 (faint) was present in Njavara black, Parambuvattan, Vellakkoli, Choottupokkali, Chettivirippu and Pandivella. The product Q13 (faint) was present in Njavara black, Vellakkoli, Vellamundakan, Choottupokkali, Kazhama, Mundon, Ponkuruka and Pandivella. The final product Q14 (faint) was present in Vellakkoli, Vellamundakan, Kazhama and Mundon.

4.1.18 OPK-14

Amplification of the thirty accessions using the primer OPK-14 gave twelve amplicons (Plate 19). The products were numbered fromR1 to R12 and had a size less than 2000 bp. Of the 12 products seven were polymorphic thus giving 58.33 per cent polymorphism. There were six

intense and six faint products (Fig. 20). The monomorphic products were R7, R9 (faint), R10 (faint), R11 and R12 (faint).

The product R1 (faint) was absent in the accessions Thowan, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. This product was bright only in Kozhivalan. The amplicon R2 was present in Kozhivalan, Anakodan, Kattamodan, Cheruvirippu, Chettivirippu and Thavalakannan. The product R3 (faint) was present in Chempan, Kozhivalan, Kururayima, Karuthacheera, Kalladi Aryan, Athikiramundakan, Veluthakattamodan, Cheruvirippu, Chettivirippu, Pokkali 3, Ponkuruka and Thavalakannan. The product R4 was absent in Chempan, Kannikayama, Kozhivalan, Kururayima, Karuthacheera, Athikiramundakan, Veluthakattamodan, Choottupokkali, Kazhama, Pandivella and Thavalakannan. The amplicon R5 was absent in the accessions Njavara yellow, Njavara black, Cheeravithu, Jeerakasala, Thowan, Athikiramundakan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Choottupokkali and Mundon. The product R6 was absent in the accessions Njavara yellow, Jeerakasala, Kannikayama, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Allikannan, Vellakkoli, Cheruvirippu, Choottupokkali, Mundon and Ponkuruka. The product R8 (faint) was present in the accessions Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Kattamodan, Vellamundakan, Cheruvirippu, Kazhama, Mundon and Chettivirippu.

4.1.19 OPK-19

Fourteen amplification products were formed by the primer OPK-19 of which only one was monomorphic giving 92.85 per cent polymorphism (Plate 20). The products were serially numbered from S1 to S14 and had a size of less than 1000 bp. There were nine intense and five faint products (Fig. 21). The monomorphic product was S9.

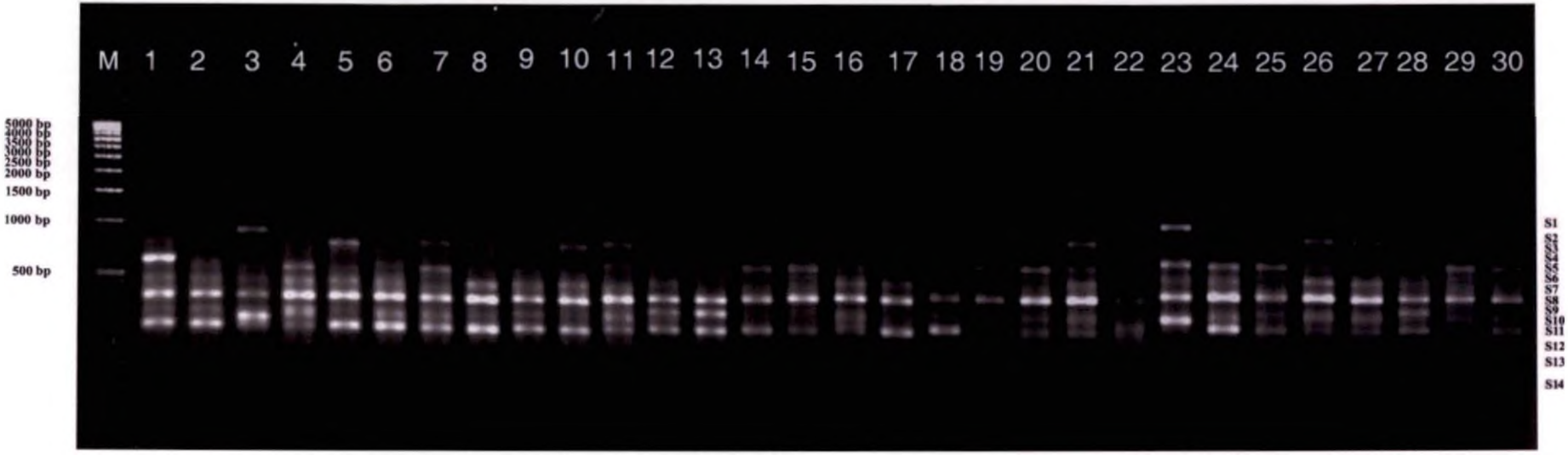

Plate 20. Amplification profile of the DNA of thirty rice accessions using the primer OPK-19

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

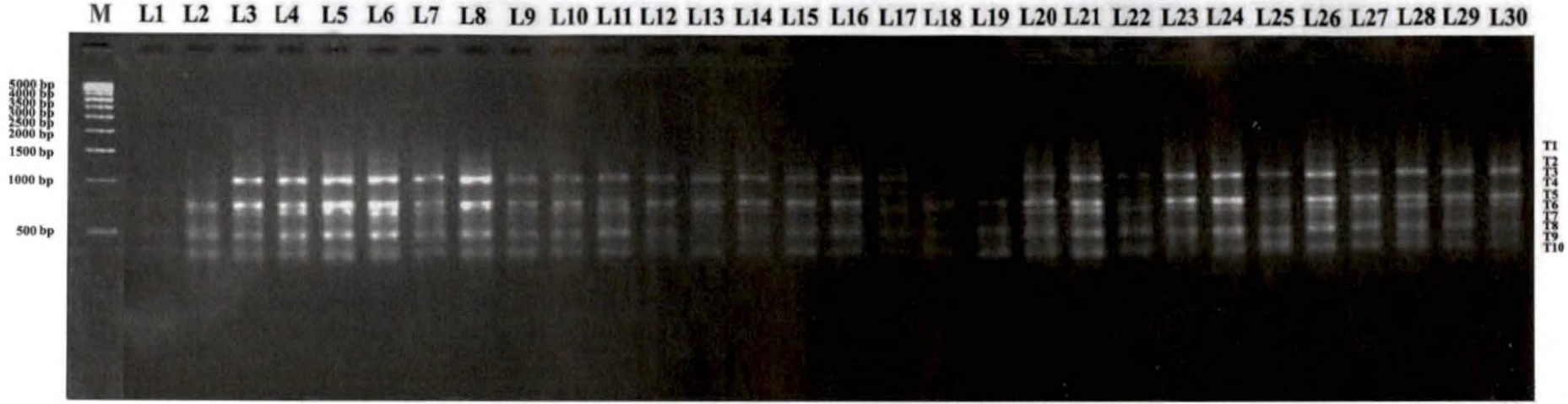

Plate 21. Amplification profile of the DNA of thirty rice accessions using the primer OPL-17

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

marker

Fig 21. Representation of the amplification profile of the rice accessions using the primer OPK-19

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+	-	+	-
-	-	-	+	+	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	+	+
-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	+	-
+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-
-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+	-
+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+

Fig 22. Representation of the amplification profile of the rice accessions using the primer OPL-17

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-
-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	+	-	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
-	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ → presence of bright products + → presence of faint products - → absence of amplification products

The amplicon S1 was present in the accessions Cheeravithu and Chenthadi. The product S2 was absent in the accessions Njavara black, Cheeravithu, Kannikayama, Kururayima, Veluthittaryan, Athikiramundakan, Vellakkoli, Choottupokkali, Chenthadi, Mundon, Ponkuruka and Thavalakannan. The product S3 (faint) was present in the accessions Jeerakasala, Chempan, Kannikayama, Kozhivalan, Karuthacheera, Kattamodan, Kazhama, Pokkali 3, Pandivella and Thavalakannan. The amplicon S4 (faint) was present in Kannikayama, Veluthakattamodan, Vellamundakan, Cheruvirippu, Chettivirippu and Pandivella. The product S5 was unique, present in the accession Njavara yellow alone and had a size between 500 and 1000 bp. The product S6, very close to S5 was present in Njavara black and Chenthadi only. Amplicon S7 (faint) was absent in Njavara yellow, Choottupokkali, Chenthadi, Mundon and Pokkali 3. The product S8 was absent in Athikiramundakan, Anakodan, Veluthakattamodan, Vellakkoli, Choottupokkali and Thavalakannan. Amplicon S10 was absent in Anakodan, Veluthakattamodan, Allikannan and Choottupokkali. The product S11 was absent only in Chenthadi at less than 500 bp. The amplicon S12 (faint) was absent in Njavara yellow, Njavara black, Cheeravithu, Veluthittaryan, Kattamodan, Allikannan, Parambuvattan, Vellakkoli, Chettivirippu, Pokkali 3, Ponkuruka, Pandivella and Thavalakannan. The product S13 (faint) was absent in Njavara yellow, Cheeravithu, Chempan, Kannikayama, Cheruvirippu, Choottupokkali, Chenthadi, Chettivirippu, Pokkali 3, Ponkuruka and Thavalakannan. The final product S14 was absent in the accessions Cheeravithu, Choottupokkali and Pandivella.

4.1.20 OPL-17

Amplification of the thirty accessions using the primer OPL-17 gave ten amplicons (Plate 21). The products are numbered from T1 to T10 and were having a size of less than 1000 bp. The primer shows a polymorphism

of ninety per cent with a single monomorphic band, T10. There were five intense and five faint products (Fig. 22).

The first product T1 (faint) was present in the accessions Cheeravithu, Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Kururayima, Karuthacheera, Cheruvirippu, Chenthadi, Kazhama, Mundon and Chettivirippu. The amplicon T2 was absent in Njavara yellow, Njavara black, Thowan, Karuthacheera, Parambuvattan and Vellakkoli. The product T3 (faint) was absent in Njavara yellow, Njavara black and Parambuvattan. The product T4 (faint) was present in Njavara black, Kururayima, Karuthacheera, Athikiramundakan, Vellakkoli, Vellamundakan, Chenthadi, Mundon, Chettivirippu, Pokkali 3, Ponkuruka, Pandivella and Thavalakannan. The amplicon T5 was absent only in the accession Allikannan, having a size of less than 1000 bp. The product T6 was also absent in a single accession Vellakkoli having a size of less than 1000 bp. The amplicon T7 (faint) was absent in Njavara yellow, Njavara black, Thowan, Kururayima, Veluthittaryan, Allikannan, Ponkuruka, Pandivella and Thavalakannan. The amplicon T8 was absent in the accession Athikiramundakan alone at less than 500 bp. Amplicon T9 (faint) was present in Vellakkoli, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama and Ponkuruka.

4.1.21 RAPD Analysis

Genetic similarity computed from the RAPD profiles (Table 4.) ranged from 0.451 to 0.825 for the different genotypes. The maximum similarity was between the accessions Chettivirippu and Pokkali 3.

Jaccard's similarity coefficient values were used for the nested clustering of the genotypes to develop dendrogram (Fig. 23). At a similarity coefficient of 59 per cent the accessions clustered into two main clusters. The first cluster (I) consisted of three accessions *viz.* Mundon, Choottupokkali and Vellakkoli while all the other twenty seven accessions formed the second cluster (II). The number of clusters and sub clusters

Table 4. Similarity indices for the DNA amplicons obtained by RAPD analysis in rice accessions

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
A1	1.000																													
A2	0.707	1.000																												
A3	0.563	0.557	1.000																											
A4	0.653	0.645	0.715	1.000																										
A5	0.684	0.620	0.668	0.801	1.000																									
A6	0.611	0.604	0.660	0.735	0.798	1.000																								
A7	0.614	0.616	0.653	0.716	0.747	0.763	1.000																							
A8	0.627	0.594	0.689	0.729	0.779	0.775	0.745	1.000																						
A9	0.621	0.664	0.593	0.703	0.694	0.689	0.651	0.674	1.000																					
A10	0.574	0.635	0.614	0.704	0.686	0.680	0.643	0.657	0.686	1.000																				
A11	0.622	0.674	0.593	0.753	0.703	0.728	0.680	0.722	0.761	0.774	1.000																			
A12	0.601	0.565	0.593	0.662	0.654	0.610	0.651	0.655	0.662	0.602	0.672	1.000																		
A13	0.515	0.546	0.548	0.636	0.648	0.602	0.596	0.619	0.678	0.635	0.655	0.633	1.000																	
A14	0.538	0.550	0.601	0.668	0.631	0.615	0.600	0.613	0.636	0.617	0.668	0.625	0.673	1.000																
A15	0.557	0.550	0.572	0.658	0.690	0.615	0.609	0.641	0.625	0.628	0.637	0.575	0.697	0.722	1.000															
A16	0.579	0.641	0.590	0.660	0.643	0.646	0.658	0.634	0.628	0.651	0.692	0.597	0.620	0.689	0.655	1.000														
A17	0.600	0.592	0.587	0.662	0.635	0.619	0.641	0.598	0.662	0.581	0.673	0.578	0.600	0.625	0.603	0.729	1.000													
A18	0.611	0.657	0.524	0.615	0.608	0.591	0.595	0.581	0.632	0.692	0.677	0.558	0.601	0.552	0.573	0.664	0.654	1.000												
A19	0.474	0.535	0.451	0.524	0.508	0.491	0.497	0.494	0.561	0.586	0.565	0.490	0.619	0.600	0.566	0.558	0.517	0.656	1.000											
A20	0.580	0.565	0.636	0.654	0.711	0.677	0.697	0.729	0.624	0.607	0.653	0.624	0.646	0.668	0.648	0.660	0.633	0.606	0.591	1.000										
A21	0.583	0.536	0.659	0.719	0.721	0.688	0.680	0.693	0.627	0.593	0.655	0.608	0.610	0.651	0.641	0.692	0.635	0.545	0.485	0.729	1.000									
A22	0.446	0.490	0.519	0.562	0.552	0.518	0.479	0.500	0.534	0.558	0.560	0.493	0.585	0.602	0.557	0.545	0.513	0.559	0.609	0.563	0.524	1.000								
A23	0.615	0.534	0.620	0.676	0.698	0.643	0.627	0.622	0.614	0.568	0.588	0.614	0.556	0.590	0.590	0.612	0.574	0.554	0.516	0.676	0.678	0.493	1.000							
A24	0.616	0.564	0.649	0.702	0.754	0.689	0.720	0.723	0.625	0.580	0.617	0.664	0.617	0.610	0.650	0.613	0.595	0.586	0.540	0.752	0.703	0.518	0.720	1.000						
A25	0.494	0.523	0.578	0.629	0.567	0.568	0.590	0.586	0.647	0.617	0.668	0.605	0.639	0.631	0.559	0.623	0.603	0.594	0.600	0.619	0.595	0.649	0.570	0.601	1.000					
A26	0.593	0.604	0.687	0.741	0.752	0.738	0.673	0.761	0.686	0.649	0.715	0.628	0.611	0.652	0.614	0.654	0.600	0.573	0.529	0.722	0.780	0.575	0.680	0.687	0.614	1.000				
A27	0.612	0.614	0.674	0.738	0.750	0.725	0.677	0.700	0.670	0.672	0.670	0.593	0.623	0.646	0.616	0.679	0.630	0.602	0.537	0.698	0.768	0.589	0.684	0.691	0.616	0.825	1.000			
A28	0.656	0.628	0.605	0.715	0.717	0.662	0.703	0.687	0.677	0.638	0.676	0.709	0.628	0.611	0.631	0.623	0.635	0.585	0.529	0.676	0.650	0.526	0.660	0.719	0.611	0.699	0.715	1.000		
A29	0.572	0.651	0.597	0.676	0.652	0.666	0.652	0.656	0.722	0.670	0.698	0.673	0.620	0.584	0.585	0.601	0.612	0.613	0.551	0.664	0.601	0.541	0.612	0.646	0.598	0.678	0.683	0.748	1.000	
A30	0.573	0.576	0.600	0.706	0.718	0.723	0.675	0.679	0.638	0.602	0.649	0.638	0.544	0.614	0.595	0.597	0.589	0.523	0.496	0.650	0.652	0.460	0.682	0.700	0.540	0.720	0.715	0.692	0.670	1.000

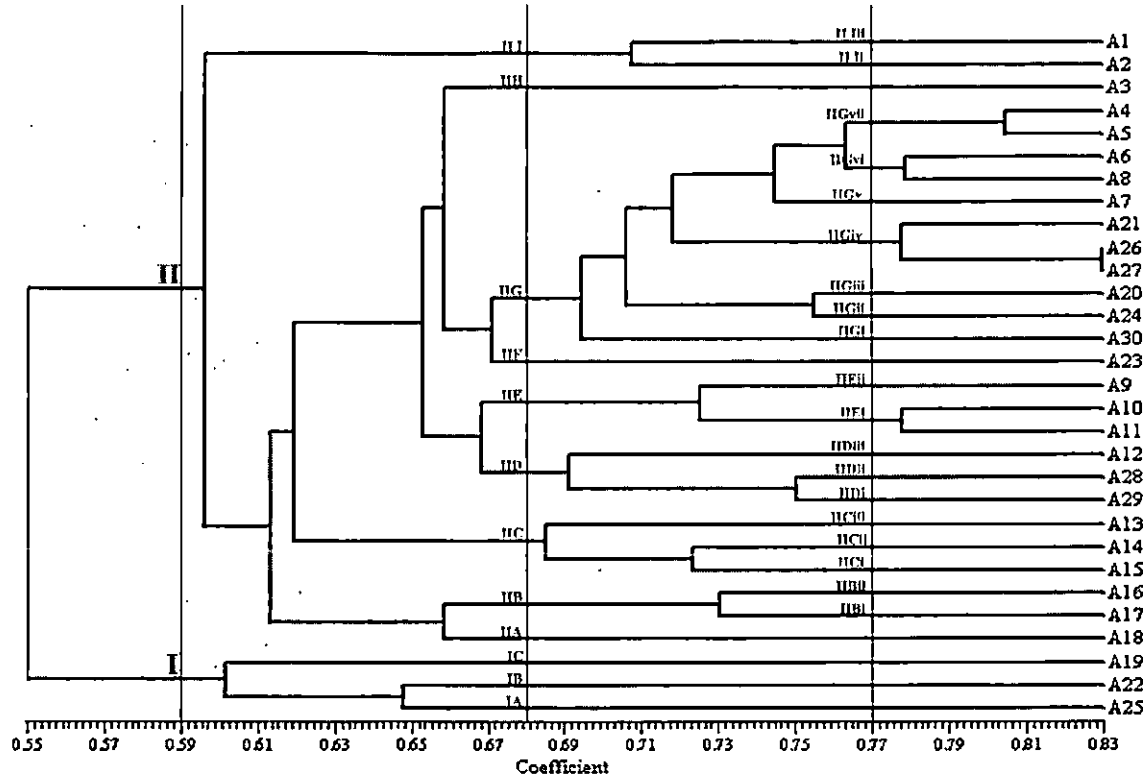

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Fig. 23. Clusters/ Sub clusters at different phenon levels in the dendrogram constructed based on similarity indices for DNA amplification products of rice accessions

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Table 5. Clusters/ Sub clusters at different phenon levels in the dendrogram constructed based on UPGMA clustering

No : of clusters/ subclusters at different phenon levels		
59%	68%	77%
I (3)	I A (1)	I A (1)
	I B (1)	I B (1)
	I C (1)	I C (1)
II (27)	II A (1)	II A (1)
	II B (2)	II B i(1)
		II B ii (1)
	II C (3)	II C i (1)
		II C ii (1)
		II C iii (1)
	II D (3)	II D i (1)
		II D ii (1)
		II D iii (1)
	II E (3)	II E i (2)
		II E ii (1)
	II F (1)	II F (1)
	II G (11)	II G i (1)
		II G ii (1)
		II G iii (1)
		II G iv (3)
		II G v (1)
		II G vi (2)
		II G vii (2)
	II H (1)	II H (1)
	II I (2)	II I i (1)
		II I ii (1)

Table 6. List of accessions coming under the subclusters at different phenon levels in the dendrogram

Cluster/Sub cluster	Name of the accession/s
I A (1)	Mundon
I B (1)	Choottupokkali
I C (1)	Vellakkoli
II A (1)	Parambuvattan
II B i(1)	Allikannan
II B ii (1)	Kattamodan
II C i (1)	Veluthakattamodan
II C ii (1)	Anakodan
II C iii (1)	Athikiramundakan
II D i (1)	Pandivella
II D ii (1)	Ponkuruka
II D iii (1)	Veluthittaryan
II E i (2)	Karuthacheera, Kalladi Aryan
II E ii (1)	Kururayima
II F (1)	Chenthadi
II G i (1)	Thavalakannan
II G ii (1)	Kazhama
II G iii (1)	Vellamundakan
II G iv (3)	Pokkali 3, Chettivirippu, Cheruvirippu
II G v (1)	Thowan
II G vi (2)	Kozhivalan, Kannikayama
II G vii (2)	Jeerakasala, Chempan
II H (1)	Cheeravithu
II I i (1)	Njavara black
II I ii (1)	Njavara yellow

formed at different phenon levels were selected as suggested by Mahapatra *et al.*, 1995 (Table 5 and 6)

The main clusters were separated into sub clusters at 68 per cent similarity values. The first main cluster of three accessions got separated into three independent clusters IA, IB and IC. The second main cluster was separated into nine sub clusters. Among this the first sub cluster IIA consisted of a single accession Parambuvattan. The second sub cluster, IIB had two accessions Allikannan and Kattamodan which clustered at 73 per cent similarity. The third sub cluster II C included three accessions *viz.* Veluthakattamodan, Anakodan and Athikiramundakan. Of these the accessions Veluthakattamodan and Anakodan formed a cluster at 72 percentage similarity. The accessions Pandivella, Ponkuruka and Veluthittaryan clustered together to form the fourth sub cluster II D. Ponkuruka and Pandivella clustered at 75 per cent similarity. The fifth sub cluster II E also showed clustering of three accessions Kalladi Aryan, Karuthacheera and Kururayima. IIF, the sixth sub cluster had a single accession Chenthadi. The seventh sub cluster (IG) was the largest with maximum number of accessions (eleven) namely Thavalakannan, Kazhama, Vellamundakan, Pokkali 3, Chettivirippu, Cheruvirippu, Thowan, Kozhivalan, Kannikayama, Chempan, and Jeerakasala. Kazhama and Vellamundakan clustered at 76 per cent similarity. Thowan, Kozhivalan, Kannikayama, Chempan and Jeerakasala formed a single cluster at the value of 0.75 similarity. The accession Cheeravithu formed the eighth sub cluster II H. The nineth sub cluster II I consisted of two accessions *viz.* Njavara yellow and Njavara black which clustered at 71 per cent similarity.

At similarity coefficient of 0.77 the sub clusters got separated into sub sub clusters. The sub clusters of the first main cluster did not separate into clusters again and they remained as such. The first sub cluster of the second main cluster (IIA) also remains as such. The sub cluster IIB separated into two sub sub clusters each (IIBi, IIBii) with single accessions Kattamodan and Allikannan, respectively. The third sub cluster IIC also got

separated into three independent clusters (IICi, IICii, and IICiii) with single accession *viz.* Veluthakattamodan, Anakodan and Athikiramundakan. The cluster IID got separated into three clusters IIDi with the accession Pandivella, IIDii with Ponkuruka and IIDiii with Veluthittaryan. The cluster IIE got separated into two sub sub clusters. The sub sub cluster IIEi included two accessions Kalladi Aryan and Karuthacheera, which clustered at 78 per cent similarity, while IIEii had a single accession Kururayima. The cluster IIF and IIH did not separate into further clusters. IIG with eleven accessions got clustered into seven sub sub clusters. The first, IIGi consisted of the accessions Thavalakannan alone. IIGii included a single accession Kazhama while IIGiii had the accession Vellamundakan only. The cluster IIGiv grouped three accessions *viz.* Pokkali 3, Chettivirippu and Cheruvirippu together at a similarity of 75 per cent. IIGv again had a single accession, Thowan. IIGvi consisted of two accessions Kozhivalan and Kannikayama [76 per cent similarity] while IIGvii grouped the accessions Chempan and Jeerakasala [80 per cent similarity]. These four accessions clustered into a single cluster at 76 per cent similarity. The cluster II I got separated into two clusters with single accession each. II Ii included Njavara black while II Iii included Njavara yellow. The maximum similarity value was 83 per cent and was between the accessions Chettivirippu and Pokkali3.

4.2 INTER SIMPLE SEQUENCE REPEATS

4.2.1 Standardization of ISSR

The ISSR protocol of Joshi *et al.* (1999) was modified for getting good and clear amplification products. For the rice accessions under study the DNA content in the cocktail was tested with 40, 30, 20 and 10 ng. From this 20 ng that gave comparatively good amplification was adopted. 10 ng of template DNA did not give amplification at all. The primer concentrations of 75, 100, 200 and 400 pM were tried. Of these 100pM gave better results. The dNTP concentration was tested with 100 and

200µM and of this 100µM gave good result. Annealing temperature was tested for 50° C, 45° C and 42° C. The best was 42° C. Both primers, which gave good amplification, had a melting temperature below 50° C. The number of cycles of amplification was standardized at 30 after checking 45 and 35 cycles. Finally the ISSR protocol for rice accessions in this study was standardized as follows.

The reaction was carried out in 25 µl reaction mixture containing 20ng template DNA, 2.5µl of 1x PCR buffer, 2µl of 2.5mM $MgCl_2$, 2 µl of 10mM dNTP mix, 1unit of Taq DNA polymerase, 1µl of 100pmoles primer and 2 per cent formamide. Amplification was done in a programmable thermocycler that was programmed as follows:

An initial denaturation at 94° C for 5 min followed by 30 cycles of denaturation at 94° C for 1 min, annealing at specific temperature for 45 sec and extension at 72° C for 2 min. The synthesis step of final cycle was extended further by 5 min. Finally the products of amplification were cooled to 4° C. Amplified products were separated by agarose gel electrophoresis using two per cent gel and photographed using gel documentation system.

Selection of Primers

Ten ISSR primers were screened for the study. Of these two were reported (Sarla *et al.*, 2003). The rest eight primers were designed based on the rice genome sequence information available at NCBI. A software named Tandem Repeat Finder was used for locating the repeats in the selected sequence. Based on that information the eight primers were designed. But none of these primers could produce amplification using the above protocol. The protocol need to be modified based on the annealing temperatures of the primers.

4.2.2 Inter Simple Sequence Repeats Amplification

The Inter Simple Sequence Repeats reaction was carried out with two 3' anchored primers *viz.* $(AG)_8T$ and $(GA)_8T$. The primers produced

Table 7. Base sequence of ISSR primers, number of amplicons and percentage of polymorphism in rice genomic DNA

Primer name	Sequence	Number of amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Percentage of polymorphism
V-1	AGAGAGAGAGAGAGAGT [$(AG)_8$ T]	10	9	1	90.00
V-2	GAGAGAGAGAGAGAGAT [$(GA)_8$ T]	9	7	2	77.77

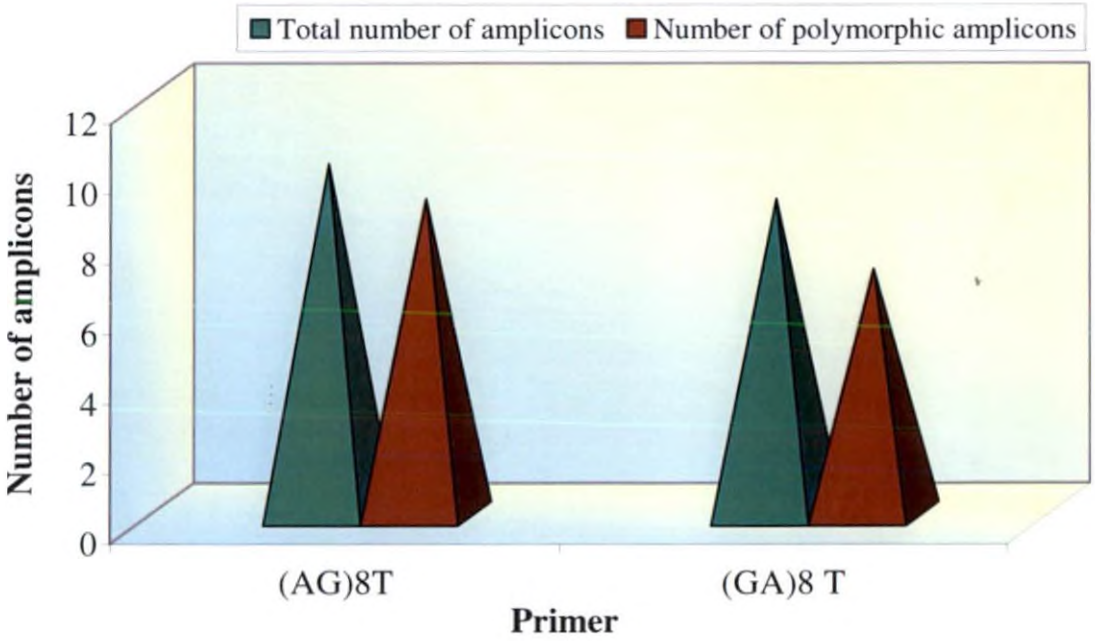


Fig. 24. Total number of amplicons and polymorphic amplicons produced by the ISSR primers

multiple band profiles with a number of amplified DNA fragments ranging from 200 bp to more than 1000 bp with a mean of 9.50 products per primer (Table 7). The average number of polymorphic products per primer was found to be 8.00. A total of 19 amplification products were produced by the two primers which showed a polymorphism of 84.21 per cent (Fig. 24).

4.2.2.1 *ISSR Primer (AG)* $_8$*T*

Amplification of the thirty rice accessions using the 3' anchored ISSR primer (AG) $_8$T produced ten amplification products of which nine were polymorphic thus giving a polymorphism of ninety per cent (Plate 22).The amplicons were serially numbered from U1 to U10 and the products had size ranging between 200 bp and 1000 bp. The monomorphic amplicon was U8 (Fig. 25).

The first product U1 (faint) was present in the accessions Thowan, Kozhivalan and Karuthacheera. The amplicon U2 was present in the accessions Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Karuthacheera, Vellamundakan, Kazhama, Mundon, Chettivirippu and Pokkali 3. The product U3 was absent in the accessions Njavara black, Kururayima, Kalladi Aryan, Athikiramundakan, Veluthakattamodan, Choottupokkali and Pandivella. The amplicon U4 (faint) was absent in the accessions Njavara black, Kozhivalan, Kururayima, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Parambuvattan, Vellakkoli, Choottupokkali, Mundon, Ponkuruka and Pandivella. The product U5 was present in the accessions Njavara yellow, Cheeravithu, Jeerakasala, Chempan, Kannikayama, Thowan, Karuthacheera, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu and Thavalakannan. The amplicon U6 was absent in the four accessions *viz.* Kalladi Aryan, Veluthittaryan, Choottupokkali and Pandivella. The product U7 was absent in Njavara black, Cheeravithu, Chempan, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Athikiramundakan, Anakodan, Veluthakattamodan, Kattamodan,

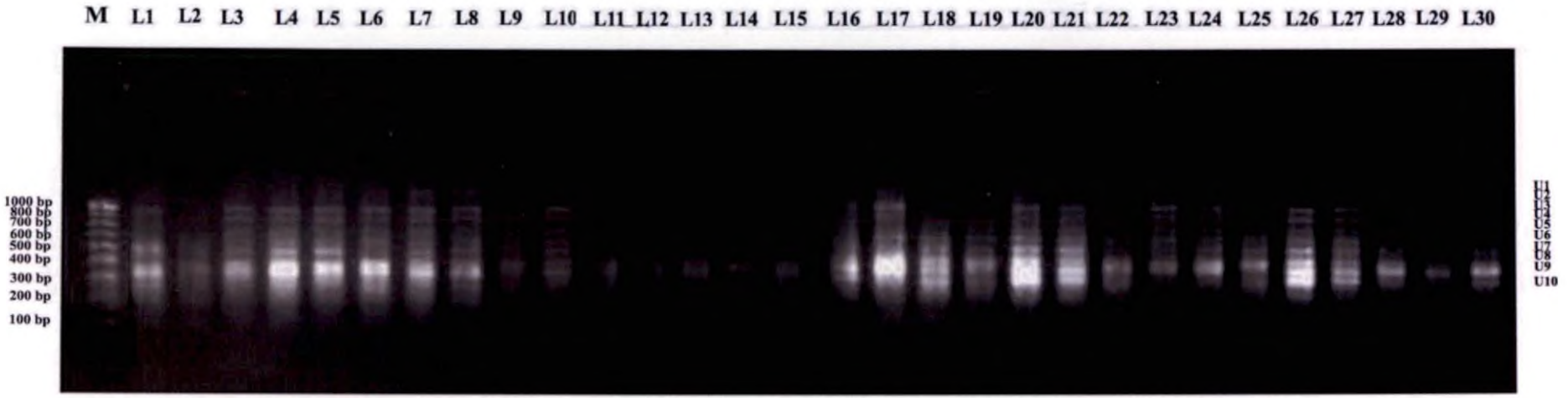


Plate 22. Amplification profile of the DNA of thirty rice accessions using the primer $(AG)_8T$

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

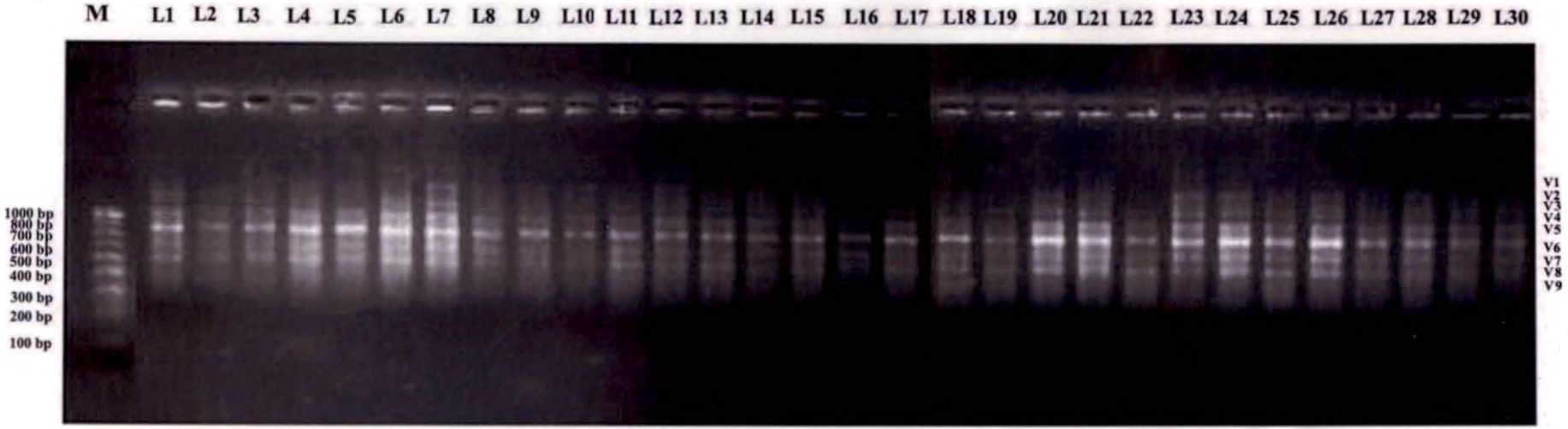


Plate 23. Amplification profile of the DNA of thirty rice accessions using the primer $(GA)_8T$

L 1 = Njavara yellow, L 2 = Njavara black, L 3 = Cheeravithu, L 4 = Jeerakasala, L 5 = Chempan, L 6 = Kannikayama, L 7 = Thowan, L 8 = Kozhivalan, L 9 = Kururayima, L 10 = Karuthacheera, L 11 = Kalladi Aryan, L 12 = Veluthittaryan, L 13 = Athikiramundakan, L 14 = Anakodan, L 15 = Veluthakattamodan, L 16 = Kattamodan, L 17 = Allikannan, L 18 = Parambuvattan, L 19 = Vellakkoli, L 20 = Vellamundakan, L 21 = Cheruvirippu, L 22 = Choottupokkali, L 23 = Chenthadi, L 24 = Kazhama, L 25 = Mundon, L 26 = Chettivirippu, L 27 = Pokkali 3, L 28 = Ponkuruka, L 29 = Pandivella, L 30 = Thavalakannan

M - λ DNA Hind III digest marker

Fig. 25. Representation of the amplification profile of thirty rice accessions using the primer $(AG)_8$ T

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-
+	-	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
+	-	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	+
+	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+
+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+
+	-	-	+	-	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-

Fig. 26. Representation of the amplification profile of thirty rice accessions using the primer $(GA)_8$ T

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
-	-	-	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	+	-	-
+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	-	-
+	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-

+ → presence of bright products + → presence of faint products - → absence of amplification products

Chenthadi, Kazhama and Pandivella. The amplicon U9 was in Njavara black, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Anakodan, Vellakkoli, Choottupokkali, Chenthadi, Kazhama and Mundon. The final product U10 (faint) was absent in the accessions Njavara black, Cheeravithu, Chempan, Kururayima, Karuthacheera, Kalladi Aryan, Veluthittaryan, Kattamodan, Allikannan, Chenthadi, Kazhama, Ponkuruka, Pandivella and Thavalakannan.

4.2.2.2 *ISSR Primer (GA)* $_8$*T*

Amplification of the thirty rice accessions using the 3' anchored primer (GA) $_8$T gave nine amplicons (Plate 23). The products are serially numbered from V1 to V9 and had size ranging from 200 bp to more than 1000 bp. Of the nine products, V5 and V6 were monomorphic giving a polymorphism of 77.77 per cent. Seven intense and two faint products were amplified with this primer (Fig. 26).

The first product V1 (faint) was present in three accessions only *viz.* Kannikayama, Thowan and Chenthadi. The amplicon V2 was present in Jeerakasala, Chempan, Kannikayama, Thowan, Kozhivalan, Kururayima, Karuthacheera, Veluthittaryan, Vellamundakan, Cheruvirippu, Chenthadi, Kazhama, Chettivirippu and Ponkuruka. The product V3 was present in the accessions Njavara yellow, Cheeravithu, Jeerakasala, Vellamundakan, Chenthadi, Kazhama, Mundon, Chettivirippu and Pokkali 3. The amplicon V4 was absent in the accessions Njavara black, Kozhivalan, Kururayima, Karuthacheera, Kalladi Aryan, Pokkali 3, Pandivella and Thavalakannan. The amplicon V7 was absent in two accessions only *viz.* Njavara black and Karuthacheera. The product V8 was unique since it was absent only in the accession Karuthacheera and had a size of nearly 600 bp. The final product V9 (faint) was present in the accessions Parambuvattan, Vellamundakan, Cheruvirippu, Choottupokkali, Kazhama, Mundon and Chettivirippu.

Table 8. Similarity indices for the DNA amplicons obtained by ISSR analysis in rice accessions

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
A1	1.000																													
A2	0.357	1.000																												
A3	0.857	0.416	1.000																											
A4	0.875	0.312	0.750	1.000																										
A5	0.687	0.384	0.785	0.812	1.000																									
A6	0.764	0.312	0.647	0.882	0.812	1.000																								
A7	0.722	0.294	0.611	0.833	0.764	0.941	1.000																							
A8	0.647	0.357	0.529	0.764	0.687	0.764	0.823	1.000																						
A9	0.400	0.714	0.461	0.437	0.538	0.437	0.411	0.500	1.000																					
A10	0.411	0.363	0.466	0.529	0.642	0.529	0.588	0.600	0.416	1.000																				
A11	0.357	0.666	0.416	0.312	0.384	0.312	0.294	0.357	0.714	0.250	1.000																			
A12	0.500	0.500	0.583	0.437	0.538	0.437	0.411	0.400	0.555	0.307	0.714	1.000																		
A13	0.642	0.555	0.615	0.562	0.571	0.562	0.529	0.533	0.600	0.266	0.555	0.600	1.000																	
A14	0.642	0.555	0.615	0.562	0.571	0.562	0.529	0.533	0.600	0.357	0.555	0.777	0.800	1.000																
A15	0.642	0.555	0.615	0.562	0.571	0.562	0.529	0.533	0.600	0.266	0.555	0.600	1.000	0.800	1.000															
A16	0.714	0.500	0.833	0.625	0.769	0.625	0.588	0.600	0.545	0.428	0.500	0.700	0.727	0.727	0.727	1.000														
A17	0.785	0.454	0.769	0.687	0.714	0.687	0.647	0.666	0.500	0.400	0.454	0.636	0.666	0.666	0.666	0.909	1.000													
A18	0.733	0.416	0.600	0.647	0.562	0.647	0.611	0.625	0.461	0.294	0.416	0.583	0.750	0.750	0.750	0.692	0.769	1.000												
A19	0.714	0.500	0.571	0.625	0.533	0.625	0.588	0.600	0.545	0.333	0.500	0.700	0.727	0.900	0.727	0.666	0.750	0.833	1.000											
A20	0.823	0.294	0.705	0.941	0.764	0.833	0.789	0.722	0.411	0.500	0.294	0.411	0.529	0.529	0.529	0.588	0.647	0.705	0.588	1.000										
A21	0.812	0.333	0.687	0.823	0.750	0.823	0.777	0.705	0.466	0.470	0.333	0.466	0.600	0.600	0.600	0.666	0.733	0.800	0.666	0.882	1.000									
A22	0.533	0.400	0.400	0.470	0.375	0.470	0.444	0.437	0.454	0.187	0.555	0.600	0.636	0.636	0.636	0.461	0.538	0.750	0.727	0.529	0.600	1.000								
A23	0.687	0.384	0.785	0.705	0.733	0.705	0.666	0.500	0.538	0.533	0.384	0.538	0.466	0.571	0.466	0.642	0.600	0.470	0.533	0.666	0.647	0.375	1.000							
A24	0.647	0.357	0.733	0.764	0.800	0.666	0.631	0.555	0.500	0.600	0.357	0.500	0.437	0.533	0.437	0.600	0.562	0.529	0.500	0.823	0.705	0.437	0.800	1.000						
A25	0.687	0.384	0.562	0.705	0.529	0.611	0.578	0.588	0.428	0.352	0.384	0.538	0.571	0.692	0.571	0.533	0.600	0.785	0.769	0.764	0.647	0.692	0.529	0.687	1.000					
A26	0.823	0.294	0.705	0.941	0.764	0.833	0.789	0.722	0.411	0.500	0.294	0.411	0.529	0.529	0.529	0.588	0.647	0.705	0.588	1.000	0.882	0.529	0.666	0.823	0.764	1.000				
A27	0.800	0.384	0.666	0.812	0.625	0.705	0.666	0.800	0.428	0.437	0.384	0.428	0.571	0.571	0.571	0.642	0.714	0.666	0.642	0.764	0.647	0.466	0.529	0.588	0.733	0.764	1.000			
A28	0.666	0.454	0.642	0.687	0.714	0.687	0.647	0.666	0.636	0.400	0.454	0.636	0.666	0.666	0.666	0.750	0.833	0.769	0.750	0.647	0.733	0.538	0.600	0.562	0.600	0.647	0.600	1.000		
A29	0.428	0.571	0.500	0.375	0.461	0.375	0.352	0.428	0.625	0.230	0.833	0.625	0.666	0.500	0.666	0.600	0.545	0.500	0.454	0.352	0.400	0.500	0.357	0.333	0.357	0.352	0.461	0.545	1.000	
A30	0.785	0.454	0.769	0.687	0.714	0.687	0.647	0.666	0.500	0.500	0.454	0.500	0.538	0.538	0.538	0.750	0.833	0.642	0.615	0.647	0.733	0.428	0.600	0.562	0.500	0.647	0.714	0.692	0.545	1.000

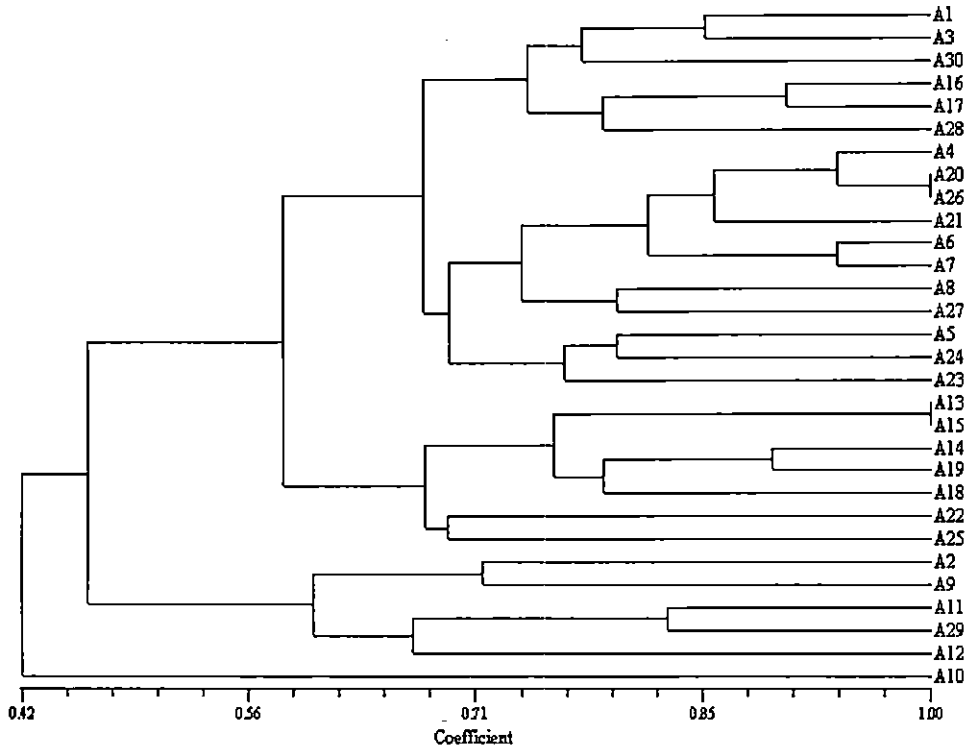


Fig. 27. Dendrogram constructed on the basis of similarity indices for DNA amplification products of rice accessions using ISSR scores

A 1 = Njavara yellow, **A 2** = Njavara black, **A 3** = Cheeravithu,

A 4 = Jeerakasala, **A 5** = Chempan, **A 6** = Kannikayama, **A 7** = Thowan,

A 8 = Kozhivalan, **A 9** = Kururayima, **A 10** = Karuthacheera,

A 11 = Kalladi Aryan, **A 12** = Veluthittaryan, **A 13** = Athikiramundakan,

A 14 = Anakodan, **A 15** = Veluthakattamodan, **A 16** = Kattamodan,

A 17 = Allikannan, **A 18** = Parambuvattan, **A 19** = Vellakkoli,

A 20 = Vellamundakan, **A 21** = Cheruvirippu, **A 22** = Choottupokkali,

A 23 = Chenthadi, **A 24** = Kazhama, **A 25** = Mundon, **A 26** = Chettivirippu,

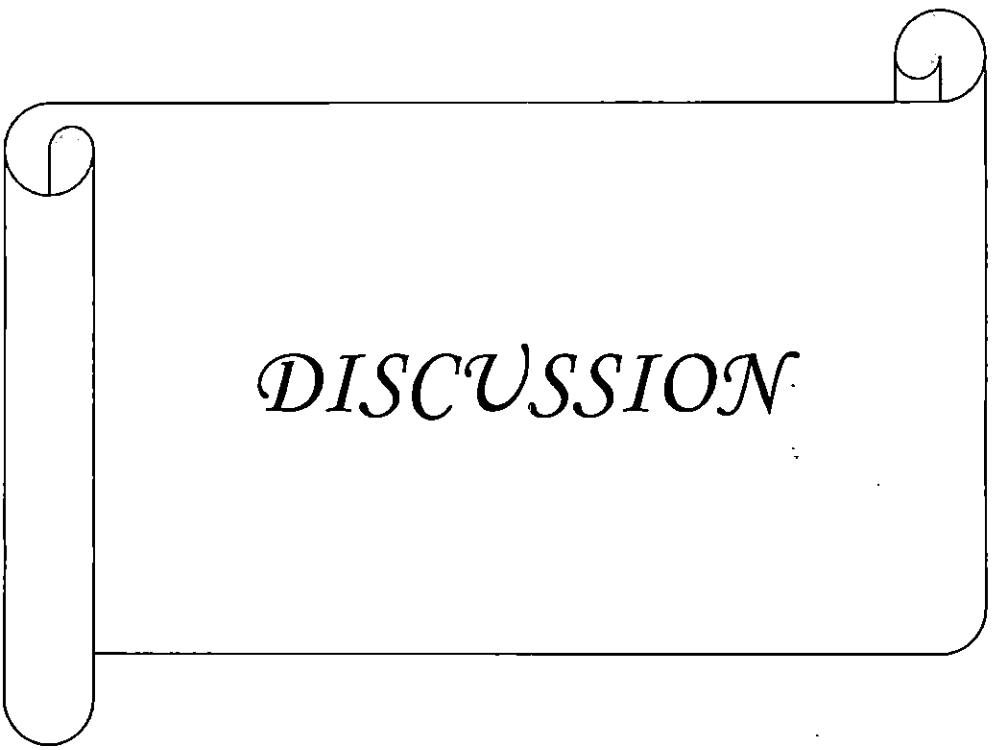
A 27 = Pokkali 3, **A 28** = Ponkuruka, **A 29** = Pandivella,

A 30 = Thavalakannan

4.2.3 ISSR Analysis

Genetic similarity computed from the ISSR profile (Table 8) ranged from 0.230 to 1.000 for the different accessions. The maximum similarity was between the accessions Vellamundakan and Chettivirippu and between Athikiramundakan and Veluthakattamodan.

Jaccard's similarity coefficient values were used for the clustering of the genotypes to develop dendrogram (Fig. 27). As per the dendrogram accessions Athikiramundakan and Veluthakattamodan had hundred per cent similarity and also the accessions Vellamundakan and Chettivirippu had hundred per cent similarity. Karuthacheera accession was unique and clustered with the rest only at forty two per cent similarity.


DISCUSSION

5. DISCUSSION

Rice (*Oryza sativa* L.) is one of the few crop plants with rich genetic diversity. The land races and wild/weedy species together constitute 80 per cent of the rice germplasm. Traditional accessions are rich source of genetic diversity. They are also reservoirs of unique genes, which confer resistance to biotic and abiotic stresses. So characterization and conservation of land races are of utmost importance for future breeding programmes. Even though portions of land races have been collected and maintained in form of gene banks in several germplasm programmes, there have been very few reported studies designed to assess the relative richness of genetic variability in the land races. Molecular markers are more reliable for characterization since it is not affected by changes in environment.

Under the NATP on Sustainable management of biodiversity conducted during 1998-2003, a very exhaustive survey and collection of landraces of rice was made which include a total of 189 accessions. Eventhough these accessions had been characterized based on morphological traits no attempt to characterize them based on molecular markers had been done. The present study was thus designed to characterize thirty traditional accessions of Kerala randomly chosen from the above collection using two molecular markers, RAPD and ISSR. The results are discussed elaborately in this chapter.

5.1 MOLECULAR MARKER STUDIES

Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting varieties, establishing the fidelity of progenies etc. The advent of automated PCR technology made a new set of markers available to scientists interested in comparing organisms at

molecular level. Williams *et al.* (1990) were the first to use RAPD markers obtained by PCR amplification of DNA segments with single arbitrary primers. The RAPD reaction performed on genomic DNA with arbitrary oligonucleotides results in the amplification of several discrete DNA products. They are usually separated in agarose gels and visualized by ethidium bromide staining. The polymorphism between individuals results from change in sequence in one or both of the primer binding sites and is visible as presence or absence of a particular product. Such polymorphism, in general, behaves as dominant genetic markers. The banding pattern differences existing between two species or varieties can be used for species or varietal identification. Also it can be used to study the pattern of introgression in hybrids.

The RAPD amplification generated can be classified into two types: constant (monomorphic) and variable (polymorphic). These differences can be used to examine and establish systematic relationship (Hardys *et al.*, 1992).

In the present study, twenty random primers were used to amplify the genomic DNA of thirty rice accessions. These primers were selected based on the reports on RAPD analysis in rice (Raghunathachari *et al.*, 2000; Barooah and Sarma, 2004; Saker *et al.*, 2005). Of the twenty primers studied, 13 gave a polymorphism of above 80 per cent. This shows the effectiveness of the selected primers in genetic analysis of the different accessions. Primer OPF-04 gave 100 per cent polymorphism in the studied accessions. Barooah and Sarma (2004) had reported 100 per cent polymorphism for the primer OPK-19. In the present study, this primer produced 92.85 per cent polymorphism. Barooah and Sarma (2004) had reported that the primer OPK-14 gave the least percentage of polymorphism (33.33) in the genetic diversity analysis of traditional Sali rice. In the present study this primer produced 58.33 per cent polymorphism. Saker *et al.* (2005)

had reported a unique positive marker with the primer OPB-05 in the study of genetic diversity in Egyptian rice genotypes.

The twenty primers used in this study produced a total of 222 amplicons of which 182 were polymorphic giving a polymorphism of 81.98 per cent, with mean number of 11.10 bands per primer. Raghunathachari *et al.* (2000) reported an average of 11.4 bands per primer in the study of genetic variability in Indian scented rice germplasm using RAPD analysis. Forty cultivated varieties of rice were evaluated for polymorphism after amplification with thirty six decamer RAPD primers by Ravi *et al.* (2003). A total of 499 RAPD markers were produced with a polymorphism percentage of ninety. He *et al.* (2004) had reported 69.4 per cent polymorphism while using twelve RAPD primers in assessment of genetic diversity of allelopathic rice germplasm. Saker *et al.* (2005) had reported on an average 10.90 bands per primer in the study of RAPD analysis in Egyptian rice genotypes.

In this RAPD analysis of rice accessions the amplicons produced had a molecular weight ranging from 2.0 kb to less than 0.5 kb. Raghunathachari *et al.* (2000) had reported amplicons of size ranging from 4.0 kb to 0.5 kb. Wu *et al.* (2002) had reported amplicons of size 2.5 kb to 0.5 kb. Neeraja *et al.* (2002) reported amplicons of size 3.9 kb to 0.25 kb whereas Monna *et al.* (1994) had reported amplicons of size 2.0 kb to less than 0.1 kb.

The primers chosen for study reveal the advantage of GC rich primers in bringing about amplification. Williams *et al.* (1990) tested a set of primers, with GC content ranging from 0–100 per cent in the amplification of soybean genomic DNA to find that GC content of 40 per cent or more generated detectable levels of amplification products. Fukuoka *et al.* (1992) found that in rice increasing GC content in the range of 40 per

Table 9. Unique positive and negative markers identified in the RAPD analysis of the thirty accessions of rice

Unique positive markers				Unique negative markers			
Accession	Size of marker (bp)	Primer	Total no: of markers/variety	Size of marker (bp)	Primer	Total no: of markers/variety	Grand total
Njavara yellow	< 500	OPF-04	1	-	-	-	2
	500 - 1000	OPK-19	1	-	-	-	
Njavara black	< 500	OPD-03	1	-	-	-	1
Cheeravithu	~ 1000	OPF-05	1	-	-	-	1
Chempan	< 1000	OPF-05	1	-	-	-	1
Thowan	500 - 1000	OPG-18	1	-	-	-	1
Kozhivalan	-	-	-	< 500	OPH-19	1	1
Athikiramundakan	-	-	-	~ 500	OPL-17	1	1
Allikannan	~ 500	OPF-03	1	< 1000	OPL-17	1	2
Vellakkoli	-	-	-	< 500	OPG-19	1	2
	-	-	-	< 1000	OPL-17	1	
Cheruvirippu	1000-1500	OPF-04	1	-	-	-	2
	~ 1500	OPC-15	1	-	-	-	
Choottupokkali	-	-	-	~ 1000	OPD-03	1	1
Chenthadi	-	-	-	< 500	OPK-19	1	1

cent to 60 per cent tend to increase the number of amplification products. In the present study, all primers, which gave good amplification, had GC content between 60 and 70 per cent.

In the present study, 9 unique positive amplicons were obtained by the primers OPC-15, OPD-03, OPF-03, OPF-04, OPF-05, OPG-18 and OPK-19 (Table 9). These bands could distinguish Njavara yellow, Njavara black, Cheeravithu, Chempan, Thowan, Cheruvirippu and Allikannan. These markers can be used as fingerprints for these accessions. Primers OPB-05 and OPF-01 produced amplicons for the Njavara group alone at less than 1000 bp. Njavara group includes Njavara yellow and Njavara black, two ecotypes of the medicinal rice Njavara. This may be helpful in the isolation of genes related to medicinal properties of Njavara rice. The primers OPD-03, OPH-19, OPG-19, OPK-19 and OPL-17 produced 7 unique negative amplicons (Table 9). These amplicons could distinguish the accessions Athikiramundakan, Vellakkoli, Allikannan, Kozhivalan, Chenthadi and Choottupokkali.

In the similarity indices, worked out based on Jaccard's coefficient analysis, accessions Chettivirippu and Pokkali 3 had the highest similarity index of 0.825. These accessions formed a cluster with maximum similarity in UPGMA clustering. These two accessions are the landraces of Ernakulam district suited especially for saline sodic soils of Kerala (Pokkali). The morphological data (Table 1) also supports the similarity. The least similarity coefficient (0.451) was between Njavara yellow and Choottupokkali. Njavara yellow is the medicinal rice and Choottupokkali is an accession suited for saline soil. Njavara yellow and Njavara black, the two ecotypes of the medicinal rice Njavara, had a similarity coefficient of 0.707.

In the cluster analysis, based on dendrogram, Jeerakasala and Chempan formed a cluster with 80 per cent similarity. But these two accessions were collected from two ecological zones of Kerala state, Wayanad and Trivandrum. Even though other morphological details show some similarity Jeerakasala is famous for its scented endosperm. The two accessions Kannikayama and Kozhivalan from Kannur district clustered at 78 per cent similarity and these accessions also had similar morphological characters. Cheruvirippu and Chettivirippu, both from Ernakulam district suited to saline soils also clustered at 78 per cent. Even though Karuthacheera and Kalladi Aryan clustered at 78 per cent these are from two different ecological zones *viz.* Perumbavur (Central Kerala) and Kannur (North Kerala). These accessions had similar morphological characters. Ponkurukka and Pandivella which clustered at 75 per cent are from nearby locations, Ernakulam and Trivandrum. At 73 per cent similarity Kururayima from Kannur district clustered with Karuthacheera from Perumbavur and Kalladi Aryan from Kannur. But the other accession from Kannur district did not show much similarity with these. Accessions Kattamodan and Allikannan, which clustered at 73 per cent, were also from two different ecological zones, Palakkad and Kannur. Anakodan and Veluthakattamodan clustered at 72 per cent. These accessions are also from Palakkad and Kannur, respectively.

Vellamundakan from Allepey district and Kazhama from Kannur district clustered at 76 per cent. But Athikiramundakan the other accession from Allepey apart from Njavara Yellow, the medicinal rice from Allepey, was distant from Vellamundakan. This shows that the accessions from a single location were distinct from each other and the collections were representative of the locality.

10.a Accessions from Alleppey

A 1 --- Njavara yellow
A 13 --- Athikiramundakan
A 20 --- Vellamundakan

10.b Accessions from Ernakulam

A 21 --- Cheruvirippu
A 22 --- Choottupokkali
A 26 --- Chettivirippu
A 27 --- Pokkali 3
A 28 --- Ponkuruka

10.c Accessions from Palakkad

A 2 --- Njavara black
A 3 --- Cheeravithu
A 12 --- Veluthittaryan
A 14 --- Anakodan
A 16 --- Kattamodan
A 18 --- Parambuvattan
A 30 --- Thavalakannan

10.d Accessions from Kannur

A 6 --- Kannikayama
A 7 --- Thowan
A 8 --- Kozhivalan
A 9 --- Kururayima
A 11 --- Kalladi Aryan
A 15 --- Veluthakattamodan
A 17 --- Allikannan
A 24 --- Kazhama

Table 10. Similarity indices for the accessions in different locations

10.a Accessions from Alleppey

	A1	A13	A20
A1	1.000		
A13	0.515	1.000	
A20	0.580	0.646	1.000

10.b Accessions from Ernakulam

	A21	A22	A26	A27	A28
A21	1.000				
A22	0.524	1.000			
A26	0.780	0.575	1.000		
A27	0.768	0.589	0.825	1.000	
A28	0.650	0.526	0.699	0.715	1.000

10.c Accessions from Palakkad

	A2	A3	A12	A14	A16	A18	A30
A2	1.000						
A3	0.557	1.000					
A12	0.565	0.593	1.000				
A14	0.550	0.601	0.625	1.000			
A16	0.641	0.590	0.597	0.689	1.000		
A18	0.657	0.524	0.558	0.552	0.664	1.000	
A30	0.576	0.600	0.638	0.614	0.597	0.523	1.000

10.d Accessions from Kannur

	A6	A7	A8	A9	A11	A15	A17	A24
A6	1.000							
A7	0.763	1.000						
A8	0.775	0.745	1.000					
A9	0.689	0.651	0.674	1.000				
A11	0.728	0.680	0.722	0.761	1.000			
A15	0.615	0.609	0.641	0.625	0.637	1.000		
A17	0.619	0.641	0.598	0.662	0.673	0.603	1.000	
A24	0.689	0.720	0.723	0.625	0.617	0.650	0.595	1.000

5.2 LOCATION SPECIFIC RAPDS

In order to access the location specific similarity of the accessions the similarity indices of the 30 accessions were grouped. The 30 accessions were collected from nine locations in Kerala State. Of this Thrissur, Perumbavur and Malappuram had only one accession each. Wayanad and Trivandrum had two accessions each. Similarity indices within each location with more than two accessions were grouped for analysis (Table 10). The two accessions from Wayanad, Jeerakasala and Chenthadi, showed a similarity of 67.6 per cent. The accession from Trivandrum, Pandivella and Chempan, showed a similarity of 65.2 per cent. Within the accessions from Ernakulam district only Pokkali 3 and Chettivirippu had above 80 per cent similarity. Within the accessions of Kannur district Kannikayama and Thowan, Kannikayama and Kozhivalan, Thowan and Kozhivalan, Thowan and Kazhama, and Kozhivalan and Kazhama had above 70 per cent similarity. Among the accessions within Palakkad and Allepey district, none had similarity above 70 per cent. In this study no marker was obtained for grouping the accessions within the location.

5.3 RAPDS FOR KERNEL COLOUR

Vanaja and Randhawa (2004) reported that with primer OPK-14 a bright thick amplicon of size 1000 bp was detected in fifty per cent of white kernelled varieties, which were totally absent in red kernelled varieties. In the present study with the primer OPK-14 an amplicon at 1000 bp was detected. Two of the three white kernelled varieties had the amplicon but faintly. Among the red kernelled varieties eleven had the amplicon, four brightly and the rest faintly. The total absence of this amplicon in red kernelled rice as reported by Vanaja and Randhawa (2004) was not observed in this study.

5.4 INTER SIMPLE SEQUENCE REPEATS

The marker system called ISSR (Inter Simple Sequence Repeats), recently developed, is a PCR based method that assesses variation in the numerous microsatellite regions dispersed throughout the various genomes. In this technique reported by Zietkiewicz *et al.* (1994) primers based on microsatellites are utilized to amplify inter SSR DNA sequences. When the primer successfully locates two microsatellite regions within an amplifiable distance away on the DNA strands of the template DNA, the PCR reaction will generate a band of a particular molecular weight for that locus representing the intervening stretch of DNA between the microsatellites. In general primers with AG, GA, TC, AC, CA repeats show higher polymorphism than primers with other di, tri or tetra nucleotide repeats. Though it is reported that plant genome contains more (AT) rich repeats (Akagi *et al.*, 1997), primers with (AT) rich repeats fail to generate any amplification due to self annealing of the primers. Blair *et al.* (1999) reported that the more common dinucleotide motifs were more amenable to ISSR analysis than the more infrequent tri, tetra and penta nucleotide motifs. Also within the dinucleotide class, the poly (GA) motif was more common than the poly (GT) motif.

ISSR markers are dominant like RAPD and are more stable and reproducible. Because of these properties these markers have recently been used extensively for fingerprinting, phylogenetic analysis, population structure analysis, varietal/line identification, genetic mapping, seed testing, determination of colonization history of plant communities, determining frequency of SSR motif in the genome etc. As ISSR markers are one of the cheapest and easiest marker systems with high efficiency in generating polymorphism among closely related varieties, they would play a major role in plant genome analysis in the future.

In the present study the DNA of thirty traditional rice accessions were amplified using two 3' anchored ISSR primers *viz.* (AG) $_8$T and (GA) $_8$T. The primers produced a total of 19 amplicons of which 16 were polymorphic giving a polymorphism of 84.21 per cent and an average of 9.50 products per primer. Parsons *et al.* (1997) obtained polymorphism of 56 per cent with nine ISSR primers in the study of genetic diversity relationships in rice using different marker systems. They obtained 7.80 products per primer during the study. Qian *et al.* (2001) had reported an average of 9.42 products per primer in the study of genetic variation in *Oryza granulata*. Saini *et al.* (2004) has reported 99.37 per cent polymorphism while assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using ISSR markers. Bao *et al.* (2006) has reported a total of 190 ISSR bands generated during analysis of genetic diversity in waxy rice showing a high level of polymorphism (92.2 per cent).

The primer (AG) $_8$T gave ninety per cent polymorphism in the present study. Sarla *et al.* (2003) had obtained hundred per cent polymorphism (15 polymorphic amplicons) while using this primer in the study of genetic diversity in *Oryza* accessions from India. Lin *et al.* (2005) had reported a polymorphism of 42.90 per cent with the primer (AG) $_8$T in the study of genetic diversity in rice and barley allelopathy. The primer (GA) $_8$T gave a polymorphism of 77.77 per cent during the present study. Joshi *et al.* (2000) had reported that dinucleotide repeats (AG) $_8$ and (GA) $_8$ with a number of anchors gave the best polymorphic and informative patterns during the study of genetic diversity in genus *Oryza*.

During the present study the primers produced amplicons having size of 200 bp to more than 1000 bp. Blair *et al.* (1999) reported that the amplicons amplified by dinucleotide based primers ranged from 100 bp to 1500 bp with a peak concentration around 700 bp. Qian *et al.* (2001)

reported amplicons of size ranging from 230 bp to 1500 bp while studying genetic diversity in rice. Amplicons of size ranging from 300 bp to 2500 bp with many amplicons around 1000 bp were reported by Hariprasad *et al.* (2005). Kaushik *et al.* (2003) reported amplicons of size ranging from 200 bp to 3530 bp in Basmati rice. But Joshi *et al.* (2000) reported a wider size range of 80 bp to 4.5 kb.

In the cluster analysis Athikiramundakan and Veluthakattamodan which showed hundred per cent similarity are from two different ecological zones, Allepey and Kannur, respectively. But these accessions did not show much similarity in morphological traits (Table 1). Chettivirippu from Ernakulam district and Vellamundakan from Allepey district also showed hundred per cent similarity. These accessions also did not show much similarity in the morphological traits. In this study only two primers were used for screening the accessions. Analysis using more number of ISSR primers would have given more congruent results.

5.4.1 Comparison of ISSR and RAPD Markers

In this study RAPD analysis using twenty primers gave 81.98 per cent polymorphism and ISSR analysis using two primers gave 84.21 per cent polymorphism. Even though ISSR analysis gave higher percentage of polymorphism only one unique product (negative) was produced whereas RAPD analysis produced nine unique positive and seven unique negative markers. Previous investigators have demonstrated that ISSR analysis usually detects a higher level of polymorphism than that detected with RFLP or RAPD analysis. It was also reported by Qian *et al.* (2001) while studying genetic variation in rice using RAPD and ISSR markers. The average number of amplification products produced by RAPD reaction was 11.10 whereas that by ISSR reaction was only 9.50. The present study used two per cent agarose gel to separate amplification products. The use of

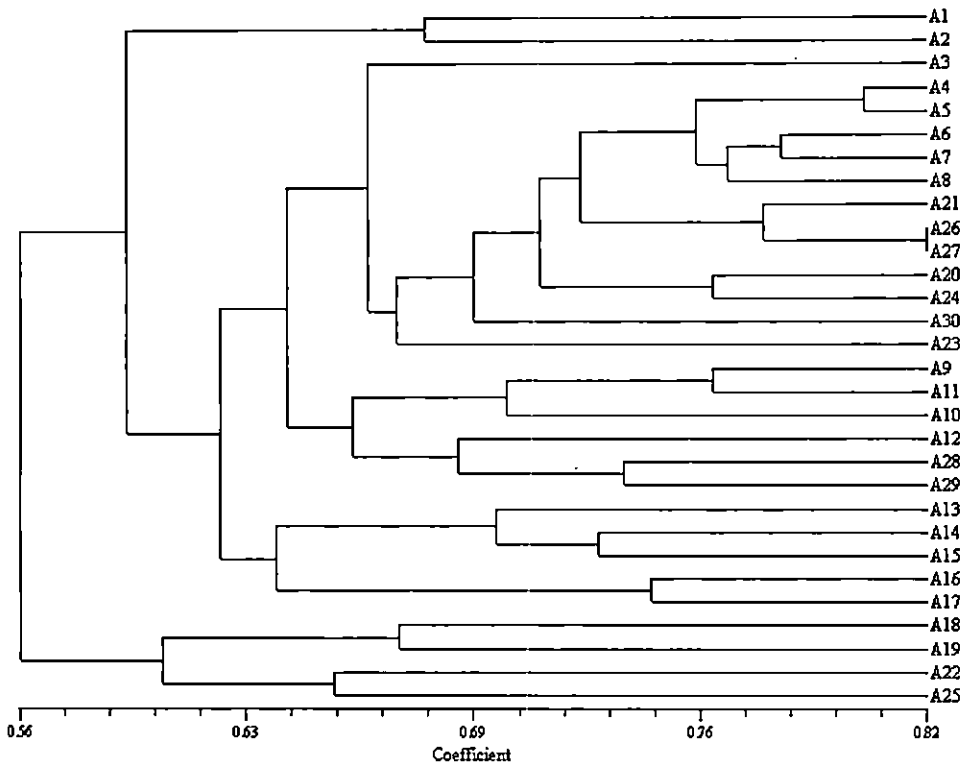


Fig. 28. Dendrogram obtained based on the combined scores of RAPD and ISSR analysis

A 1 = Njavara yellow, **A 2** = Njavara black, **A 3** = Cheeravithu,

A 4 = Jeerakasala, **A 5** = Chempan, **A 6** = Kannikayama, **A 7** = Thowan,

A 8 = Kozhivalan, **A 9** = Kururayima, **A 10** = Karuthacheera,

A 11 = Kalladi Aryan, **A 12** = Veluthittaryan, **A 13** = Athikiramundakan,

A 14 = Anakodan, **A 15** = Veluthakattamodan, **A 16** = Kattamodan,

A 17 = Allikannan, **A 18** = Parambuvattan, **A 19** = Vellakkoli,

A 20 = Vellamundakan, **A 21** = Cheruvirippu, **A 22** = Choottupokkali,

A 23 = Chenthadi, **A 24** = Kazhama, **A 25** = Mundon, **A 26** = Chettivirippu,

A 27 = Pokkali 3, **A 28** = Ponkuruka, **A 29** = Pandivella,

A 30 = Thavalakannan

polyacrylamide non-denaturing gel for better resolution was suggested by Qian *et al.* (2001).

The clustering pattern for the individual marker systems did not show any congruence with each other. The non-congruency was also reported by Khandelwal *et al.* (2005) while studying RAPD, ISSR and STMS markers in rice. In this study only two ISSR primers were used for analysis and this may be the reason for non-congruency. However the cluster analysis of combination of two marker systems (Fig. 28) produced a better picture of the genetic relationship. The dendrogram produced by the combination of markers were almost similar to the dendrogram produced by RAPD except at two regions. The accessions Kannikayama and Kozhivalan formed a cluster at a similarity of 78 per cent and the accession Thowan joined this cluster at a similarity of 74 per cent in the RAPD analysis whereas in the combined analysis Kannikayama and Thowan formed a cluster at around 78 per cent and the accession Kozhivalan joined them at more than 76 per cent similarity. Similarly the accessions Karuthacheera and Kalladi Aryan formed a cluster at 78 per cent similarity value and the accession Kururayima joined them at a value of 72 per cent in RAPD analysis whereas in the combined analysis accessions Kururayima and Kalladi Aryan formed a cluster at around 76 per cent and the accession Karuthacheera joined them at a similarity of around 70 per cent. The combined cluster analysis showed highest similarity between the accessions Chettiviripppu and Pokkali 3 (82 per cent). From the overall view of the dendrograms it can be concluded that the two ISSR markers could not bring any significant change in the clustering of the genotypes.

In conclusion, as two dominant DNA markers, RAPDs and ISSRs are effective and promising for detecting genetic variation. Furthermore, ISSR is superior to RAPD in terms of polymorphism detected.

SUMMARY

6. SUMMARY

The present study "Genome analysis of traditional rice varieties of Kerala using ISSR and RAPD markers" was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the year 2004-2006. The molecular studies were done at Department of Plant Biotechnology, College of Agriculture, Vellayani. The morphological data and the seed material were obtained from the Principle Investigator of the NATP on Sustainable Management of Biodiversity, carried out at KAU during 1998-2003.

The study was intended to characterize the thirty traditional rice accessions of Kerala using the two molecular markers *viz.*, RAPD and ISSR and also to assess the genetic diversity using molecular marker technique.

Molecular studies were done using RAPD and ISSR markers. The RAPD analysis produced 222 amplification products of which 182 were polymorphic. Twenty primers were used for the RAPD analysis. GC rich primers were found to give more number of polymorphic products. The size of the amplification products ranged from 2.0 kb to less than 0.5 kb, with maximum number of amplicons having a size of less than 0.5 kb. The analysis revealed nine unique positive products and seven unique negative products. The primer OPC-15, OPD-03, OPF-03, OPG-18 and OPK-19 gave single unique positive products each in accessions Cheruvirippu, Njavara black, Allikannan, Thowan and Njavara yellow, respectively. The primer OPF-04 gave the highest number of total amplicons and also the highest number of polymorphic amplicons. This primer produced two unique positive products. The first in the accession Cheruvirippu which had a size of between 1.0 kb and 1.5 kb and the second in the accession Njavara yellow which had a size of less than 0.5 kb. The primer OPF-05 also produced two unique positive products, first in the accession Cheeravithu (nearly 1.0 kb) and second in the accession Chempan (less than 1.0 kb).

The primers OPB-5 and OPF-1 could distinguish the Njavara group of accessions *viz.* Njavara black and Njavara yellow from the other accessions. OPB-05 produced unique products in the Njavara accessions alone at a size of less than 1.0 kb. OPF-01 produced similar products at a size of less than 0.5 kb. The unique negative products were produced by the primers OPD-03 in Choottupokkali, OPH-19 in Kozhivalan, OPG-19 in Vellakkoli, OPK-19 in Chenthadi and OPL-17 in three accessions *viz.* Allikannan, Vellakkoli and Athikiramundakan.

The UPGMA clustering based on Jaccard's similarity coefficient grouped the thirty accessions into two main clusters at 59 per cent. The highest similarity was shown by the accessions Chettivirippu and Pokkali 3 (83 per cent). The Njavara group of accessions clustered at a similarity of 71 per cent. The location wise grouping of accessions showed that, of the five accessions coming within Ernakulam district, three showed a similarity of more than 80 per cent while out of the eight accessions of Kannur district, five showed a similarity of more than 70 per cent.

The ISSR analysis of the thirty accessions of rice using two primers gave 84.21 per cent polymorphism. Only one unique negative product in accession Karuthacheera was detected by ISSR primers. Amplification using the primers produced amplicons within the range of 0.2 kb to more than 1.0 kb. The poly (AG) primer gave more polymorphism than the poly (GA) primer. The cluster diagram obtained by UPGMA clustering of the ISSR scores did not show any congruence with that produced using RAPD scores. However the cluster analysis of the combination of the two marker systems produced a better picture of genetic relationship.

The present study using the two dominant DNA markers, RAPD and ISSR, showed that both the DNA markers are effective and promising for detecting genetic variation. It has been observed that ISSR is superior to RAPD in terms of polymorphism detected.

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GENOME ANALYSIS OF TRADITIONAL RICE VARIETIES OF KERALA USING ISSR AND RAPD MARKERS

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Abstract of the
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ABSTRACT

The research project "Genome analysis of traditional rice varieties of Kerala using ISSR and RAPD markers" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2004-2006. The major objectives of the study were to characterize indigenous rice collection of Kerala on the basis of two molecular markers *viz.* ISSR and RAPD and to assess the genetic diversity using molecular marker technique.

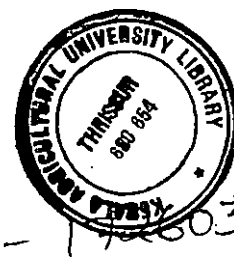
The study using RAPD markers produced 222 amplicons of which 182 were polymorphic thus giving a polymorphism of 81.98 per cent. Twenty primers were used for the study. Of these the primer OPF-04 gave maximum number of polymorphic products and also produced two unique positive products in the accession Cheruvirippu (size between 1.0 kb and 1.5 kb) and in the accession Njavara yellow (size of less than 0.5 kb). The amplification products had size ranging from 2.0 kb to less than 0.5 kb. The analysis produced nine unique positive products and seven unique negative products.

Clustering based on Jaccard's similarity coefficient revealed the highest similarity between the accessions Chettivirippu and Pokkali 3 (0.825). The least similarity index of 0.451 was between the accessions Cheeravithu and Vellakkoli. The Njavara group of accessions, Njavara yellow and Njavara black, clustered at a similarity value of 0.707. The primer OPB-05 and OPF-01 could distinguish the Njavara accessions from others. OPB-05 produced unique product with a size of less than 1.0 kb and OPF-01 produced product at size of less than 0.5 kb.

ISSR analysis was carried out using two primers. The amplification using the two primers produced 19 amplicons of which 16 were

polymorphic giving 84.21 per cent polymorphism. The amplification products had size ranging from 0.2 kb to more than 1.0 kb. A unique negative marker was amplified by the primer $(GA)_8T$ in the accession Karuthacheera with a size of nearly 0.6 kb. The UPGMA clustering was done using Jaccard's similarity coefficient values. The highest similarity of 1.00 was shown by the accessions, Athikiramundakan and Veluthakattamodan and by Vellamundakan and Chettivirippu. The least similarity was between the accessions Karuthacheera and Pandivella (0.230). The accession Karuthacheera was unique and formed a single cluster at similarity value of 0.420.

The pattern of clustering for the individual marker systems did not show any congruence with each other. However the cluster analysis of combination of the two marker systems produced a better picture of the genetic relationship. The present study using the two dominant DNA markers, RAPD and ISSR, showed that both the DNA markers are effective and promising for detecting genetic variation. It has been observed that ISSR is superior to RAPD in terms of polymorphism detected.



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