GENETIC DIVERSITY ANALYSIS OF INDIGENOUS RICE VARIETIES IN KERALA USING MOLECULAR MARKERS

By

AJITH M. K.

(2013-09-120)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

B. Sc. - M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture
Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA

2019

DECLARATION

I hereby declare that the thesis entitled **"Genetic Diversity analysis of indigenous rice varieties in Kerala using molecular 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.

Place: Vellayani
Date: 12/11/2019

AJITH M. K.
(2013-09-120)

CERTIFICATE

Certified that this thesis entitled **"Genetic diversity analysis of indigenous rice varieties in Kerala using molecular markers"** is a record of research work done by **Mr. Ajith M. K. (2013-09-120)** under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Jayalekshmy. V. G

Place: Vellayani
Date: 11/11/2019

Dr. V. G. Jayalekshmy
(Chairperson, Advisory Committee)
Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Ajith M. K. (2013-09-120), a candidate for the degree of B. Sc. - M. Sc. (Integrated) Biotechnology, agree that the thesis entitled **"Genetic Diversity analysis of indigenous rice varieties in Kerala using molecular markers"** may be submitted by Mr. Ajith M. K. in partial fulfilment of the requirement for the degree.

Dr. V. G. Jayalekshmy
(Chairperson, Advisory Committee)
Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

Dr. Swapna Alex
(Member, Advisory Committee)
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

Dr. K. B. Soni
(Member, Advisory Committee)
Professor
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

Dr. Beena R.
(Member, Advisory Committee)
Associate Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

12/11/19

Dr. Bindu R. Nair
(External Examiner)
Associate Professor, Department of
Botany, University of Kerala,
Kariavattom Thiruvananthapuram-695581

ACKNOWLEDGEMENT

With Boundless love and appreciation, I would like to extend my heartfelt gratitude and appreciation to the people who helped me in bringing the study into reality.

It is with genuine pleasure I would like to express my deep sense of gratitude to my beloved advisor Dr. (Mrs.) V. G. Jayalekshmy, Professor and Head, Department of Seed Science and Technology, College of Agriculture, Vellayani, Thiruvananthapuram for her persistent interest in my work, continuous support and constant encouragement as well as thoughtful and meaningful discussions throughout my study. I am highly indebted to her for her invaluable guidance.

I take immense pleasure to express my deep sense of gratitude to Dr. Swapna Alex, Dr. K. B. Soni and Dr. Beena R., not only for their insightful comments and encouragement, but also for their valuable counseling and constructive suggestions that were much helpful throughout my research progress.

My whole hearted thanks to Dr. K. B. Soni, Professor and Course Director for her valuable help, suggestions and advice during my research work.

I will always remember with gratitude our dearest, retired professors and course directors of plant biotechnology department Dr. K. Rajmohan, Dr. B. R. Reghunath, Dr. Lekha Sreekantan for the valuable suggestions and advice during this M.Sc. programme.

I take immense pleasure to express my thanks to my teachers Dr. Geetha Ramesh, Dr. Deepa S Nair, Dr. Kiran A.G., Dr. Jean Cletus, Dr. Bindi Das, Dhanya Madam, for their valuable suggestions and advice during this M.Sc. programme.

My heartfelt thanks to Soumya chechi, Thejasree deedi, Reshma Gopi chechie, Shahiba chechie and Bhagya, for their valuable support, companionship guidance effort made by them to bring the thesis into its final form.

My acknowledgement would be lacking if I don't mention my gratitude to my beloved friends, Bimal, Vishnu, Sabari, Achuth, Athul, Adithya, Anji,

Aryalekshmi, Aswathy, Bhagya, Dhathy, Pakkaran, Retna, RS, Sahla, Shakku, Sruthy, Alina and Vattu for their invaluable care, constant support, motivation and selfless help.

I wish to express my deep gratitude to all the teachers in college, my seniors and juniors for their timely support.

I acknowledge the favor of numerous persons who, though not been individually mentioned here, who have all directly or indirectly contributed to this work.

I wish to express my deep love and gratitude to my sweetheart Appu for always loving me and inspiring me.

I owe this achievement to my beloved parents, my sister who always stood along my side and I will never forget the timely help, mental support, kindness and affection extended by other family members without them this work would have never seen light.

Finally, I humbly thank the Almighty for showering His blessings and bestowing the wisdom, perseverance and physical ability to accomplish this work.

Ajith M. K.

DEDICATED
TO
MY
BELOVED
PARENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVATIONS

%	Percentage
A_{260}	Absorbance at 260 nm wavelength
A_{280}	Absorbance at 280 nm wavelength
AFLP	Amplified fragment length polymorphism
bp	Base pair
cm	centimetre
CTAB	Cetyl Trimethyl Ammonium Bromide
CuMiSat	*Curcuma* Microsatellite marker
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
et al.	et alia
EtBr	Ethidium bromide
F	Forward primer
g	gram
ISSR	Inter simple sequence repeat
KAU	Kerala Agricultural University
kb	Kilo bases
kg	Kilogram
L	Litre
m	Meter
M	Molar
mg	milligram
$MgCl_2$	Magnesium Chloride
min	Minute
ml	Millilitre
mm	Millimeter

mM	Millimolar
NaCl	Sodium chloride
ng	Nanogram
nm	Nanometer
˚C	Degree Celsius
OD	Optical density
PAGE	Poly Acrylamide Gel Electrophoresis
PCA	Principal Component Analysis
PCC	Pearson Correlation Coefficient
PCR	Polymerase chain reaction
PGR	Plant Genetic resources
R	Reverse primer
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
RT	Reverse transcriptase
RT-PCR	Reverse transcription-polymerase chain reaction
s	second
spp.	Species (plural)
SDW	Sterile distilled water
SSR	Simple sequence repeat
Taq	Thermusaquaticus
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
TM	Trademark

Tm	Melting temperature
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UBC	University of British Columbia
UV	Ultra violet
V	Volt
v/v	volume/volume
W	Watt
w/v	weight/volume
µg	Microgram
µl	Microlitre
µM	Micromolar

INDRODUCTION

1. INRODCUTION

Rice (*Oryza sativa* L.) the second most important cereal crop in the world is a member of the Poaceae family. It feeds more than half of the world's population. Asia is the largest producer and consumer of rice. India ranks second in production of rice next to China and it has the second biggest region under rice crop (around 45 million ha) (Thompson *et al.*, 2015). Among the developed species, *Oryza sativa* is the most regularly cultivated species all through Asia, Australia, America, and Africa. More than 90% of the world's rice is developed and consumed in Asia.

The world's rice production has increased many times in the recent years to a great extent because of the utilization of enhanced innovations such as, creation of improved yielding varieties and better post-harvest conservation. The interest for rice cultivation is increasing year by year. The varieties suitable for modification is diminishing because of ceaseless urbanization and unseeing land utilization. To maintain nourishment independence and to meet future sustenance necessities, India needs to build rice efficiency by three percent every year.

Rice has great importance around the world due to its nutritional value. It has a special protein called glutelin having more balanced amino acid compared to other cereals that have prolamine rich storage proteins. Rice is a good source of thiamin, niacin, riboflavin, phosphorus, magnesium, zinc and copper. Short grain of rice is very starchy, cooks soft and sticky while long-grain rice contains less starch. About 477.50 Mt of rice per year is being consumed by the present world population. Though the global production of rice is increasing, this increase is not proportional to the demand of increasing human population. The rice production should rise at least 70% in the coming years to fulfill the demand of increasing human population by 2050 (Leegood *et al.*, 2010).

Kerala is considered as one of the focuses of the high quality varieties of rice and the relics of rice development here goes back to 3000 BC. Rice covers an

immense range of biological specialties in Kerala and a tremendous decent variety of germplasm of both developed and wild rice exist here. In Kerala, rice is grown under various climatic and soil conditions. Assortments have been produced to suit this shifting circumstance.

These conventional rice assortments are quality banks for the numerous ideal qualities, particularly qualities for resistance from biotic and abiotic stresses. Conservation and characterization of these varieties is fundamental for future hereditary change of rice.

In this study, conventional rice assortments of Kerala in particular from four major agro climatic zones *viz.,* slope zones of Wayanad, rice developing tract of Palakkad, saline soils of Pokkali and Kuttanad soils are surveyed through sub-atomic markers to uncover the diversity of rice population in Kerala. This will recognize hereditary disparity in the selected varieties of rice in these areas and help to distinguish disparate assortment as rearing stock. Under this background, the project was proposed with the objective to analyse the diversity among indigenous collection of rice varieties using RAPD and SSR markers using the molecular data.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature survey conducted to collect the reports regarding the present project entitled "Genetic diversity analysis of indigenous rice varieties in Kerala using molecular markers" are presented in this chapter

2.1. RICE AS A STAPLE FOOD

Rice (*Oryza sativa* L.) is one of the most important food crops in Asian countries. It is a widely consumed food all over the world and it can meet 50 to 80% of our daily calories. Rice is one of the most calorie rich foods to used the ever-increasing world population. Crop improvement in rice is necessitated in terms of yield, grain quality and stress resistance.

In India, Rice occupies an area of 38.35 M ha with 103.61 Mt production and 2.3 t/ha of productivity. India was the largest exporter of rice in 2015-16 followed by Thailand, Vietnam and Pakistan (Calpe, 2017).

2.1.1 Traditional varieties of Kerala

In Kerala, rice covers a vast array of ecological niches and vast diversity of germplasm of both cultivated and wild rice exist here (Kumary and Francies, 2002). The traditional varieties exhibit variations in characters such as crop duration, plant height, tillering, pigmentation of various plant parts, panicle characters, grain characters grain size, kernel color, aroma, puffing, popping, flattening, cooking and eating qualities. Nearly 2000 traditional varieties are under cultivation in Kerala. The traditional varieties include aromatic/scented rice, medicinal rice and varieties possessing resistant genes against various types of stresses (Kumary, 2012). The world renowned 'Pattambi' rice varieties that have contributed source of resistance to many IRRI varieties are all pureline selections of landraces hailing from erstwhile Malabar province.

Traditional varieties exhibit high genetic diversity and are the sources of specific stress resistance and quality improvement genes which have more scope in breeding programmes. The urbanization and other related developmental activities exploited the land-use pattern and the natural habitats. The modern varieties are replacing indigenous cultivars and land races, resulting in reduction of genetic diversity. The large variability for complex quantitative traits in the existing varieties remains unused.

2.1.2. Genetic diversity of Rice in Kerala

The rice system in Kerala is highly heterogenous due to different agro-climatic conditions. In Kerala, the rice genetic resources include land races, wild and weedy relatives, commercial leading varieties, pureline selections of farmer varieties, hybrid derivatives and mutants. Among these wild and weedy relatives the land races are known to be the gene reservoirs evolved through natural selection.

Vaughan and Muralidharan (1989) reported that there are five weedy species of Rice in Kerala namely *Oryza viz., O. nivara, O. rufipogon, O. officinalis,, O. granulata* and *O. spontanea. Oryza spontanea* is found to be the closest wild relative of the cultivated rice of Kerala. The hybrids of *O.sativa*, *O.nivara* and *O. rufipogon* are known by the names as *O. fatua* or *O.sativa* var f*atua. Oryza officinal* found to be the resistant source of yellow stem borer, brown plant hopper, green leaf hopper, white backed hopper and zigzag leaf hopper (Heinrichs *et al*., 1985). *Oryza rufipogon* has been identified as a source for cytoplasmic male sterility which can be used for hybridization programmes. (Virmani and Shinjyo, 1988).

Land races of Kerala include medicinal rice, aromatic rice, and traditional lines possessing biotic and abiotic stress resistant genes. The important aromatic rices of Kerala include Gandhagasala, Jeerakasala, PTB 13, Kothampalarik PTB 13, Kunjik PTB 13, Neycheera, Pookkulathari and RajaPTB 13 (Kumary *et al.,* 2002).

Traditional varieties are more commonly used in ayurvedic medicines and they act as a source for resistant genes. The important varieties which are having

good qualities include Chennellu, Kunjinellu, Chembavu, Kalamappari, Neduvali, Velvali, Narikari, Erumakkari, Varakan, Poovali, and Tanavala . "Chennellu" is considered as the most beneficial (Nair, 2004). Showmy and Yusuf (2016) reported that Chennellu possess bacterial blight resistance compared to other varieties.

Varieties resistant to biotic and abiotic stresses will be a boon for farmers. The varieties such as Arikirai, Aruvakari, Parambuvattan, Urulan PTB 13 is found to possess genes for gall midge resistance. Rice varieties like Chuvannamodan, Cheriyaryan, Modan, Karuthamodan, Kattamodan, Kuttimodan, Parambuvattan, Karanellu are mostly cultivated in Palakkad region. In Onattukara region, Kochuvithu, Vykatharyan, Karavala, Champavu, Parapilarppan are the varieties preferred by the farmers. These varieties possess resistance to drought and diseases like blast. Among the Pokkali varieties is known to be saline resistant it., Pallippuram Pokkali, Kuzhippalli Pokkali and Vettikkal Pokkali used for cultivation in different Pokkali fields. The other saline resistant varieties include Cheruviruppu, Chettiviruppu, Choottupokkali, Kuruka. Many of the varieties are found to posess multiple resistantce and can used for resistance breeding programmes (Kumary, 2012).

Traditional varieties are important reservoirs of valuable traits which can be used for breeding programmes. The conservation of these varieties need special attention. Kerala is a rich source of traditional varieties but some of them extinct now. Some of the indigenous rice germplasms of Kerala are conserved in Regional Agricultural Research Station (RARS) Pattambi, Rice Research Station (RRS) Moncompu, and RRS Vytila. Identification of genetic diversity in traditional sources will pave a new way for further rice improvement programmes.

2.2 MOLECULAR MARKERS

Selection of varieties by using conventional methods is not reliable. With the advent of molecular breeding more reliable techniques using DNA markers are available for selection. They clearly depict the information on the variation that exists

in a particular species grown in different agro climatic situations and also help in collection and utilization of genetic resources.

Molecular markers are now widely used in many areas of research mainly genetic mapping, paternal tests, hereditary diseases, cultivars identification, marker assisted breeding of crops, population history, epidemiology and food safety, and population studies (Hartl and Jones, 2005). There are different types of molecular markers which detect the genetic variation within the individual species (Collard *et al.*, 2005). Schhulmann (2007) used genetic markers to construct linkage maps and genetic diversity.

The introduction of molecular markers revolutionized the crop improvement techniques. In a conventional breeding programmes usually follow hybridization by selection method to develop a variety. It is time consuming and laborious and also the variety may sometimes be prone to environmental stresses, it may not reveal the actual potential. World is moving ahead day by day, technologies are also developing fast, so by using newer molecular markers we can reduce the time for developing a variety.

Morphological and biochemical markers are environment sensitive but the DNA based molecular markers are environment insensitive. The different molecular markers can be grouped into hybridization-based markers and polymerase chain reaction (PCR)-based markers. In hybridization-based markers, DNA profiles are visualized by hybridizing the restriction endonuclease digested DNA fragment, to a labelled probe, which is a DNA fragment of known sequence. Analysis using PCR based markers involve *in vitro* amplification of particular DNA sequences with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermo stable DNA polymerase enzyme. The amplified DNA fragments are separated by electrophoresis and banding patterns are detected by different methods such as staining (using ethidium bromide dye) and autoradiography (Saiki *et al.*, 1985; Saiki *et al.*, 1988).

Another classification is based on their different capabilities for exhibiting homozygosity (dominant marker) or heterozygosity (co-dominant marker) (Hartl, 1988). The commonly used molecular markers are Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Sequence Characterized Amplified Region (SCAR), Expressed Sequence Tags (EST), Single Nucleotide Polymorphism (SNP) etc. Dominant and co-dominant markers can be used to assess the genetic polymorphism and there by the level of genetic variation present in different populations and its phylogeny and breeding structure can be assessed (Hartl and Jones, 2005).

There are different markers available for assessing the genetic diversity and the most commonly used molecular marker is RAPD ((RAPD; Williams *et al.*, 1990). The RAPD markers are small oligo nucleotide primers (usually 10 bp in length) of arbitrary sequence used to generate bands profiles. These primers bind to the complementary sequences along the genome and PCR amplification occurs when the regions between the opposing primer sites are within amplifiable distances. The techniques of RAPD involves (i) isolation of DNA, (ii) addition of single arbitrary primer, (iii) polymerase chain reaction (PCR), (iv) Agarose gel electrophoresis (Huff *et al.,* 1993; Vejl, 1997; Hollingsworth *et al.,* 1998), or fluorescently labeled primers or nucleotides (CorleySmith *et al.,* 1997; Weller and Reddy, 1997) or radioactivity (Pammi *et al.*, 1994) are sometimes used.

2.2.1. RAPD markers for assessing genetic diversity in rice

RAPD and SSR markers were employed to assess the genetic diversity among 45 genotypes of rice by Ravi *et al.* (2003). 36 decamer primers and 38 SSR primer pairs were used in the study. Among the 38 SSR primer pairs used, only one locus was monomorphic. The Polymorphism Information Content value was 0.578 and it ranged from a low of zero (RM 115) to a high of 0.890 (RM202). The comparison of the marker efficiency revealed that SSR analysis resulted in a more definitive

separation of clusters of genotypes indicating a higher level of efficiency of SSR markers than RAPD markers.

A study was conducted to evaluate the genetic polymorphism in six different rice (*Oryza sativa* L.) cultivars viz. Basmati 370, DM 25, IRATOM 24, Binadhan 6, TNDB 100 and Y 1281 using Random Amplified Polymorphic DNA (RAPD) by Rahman *et al.*, 2006. Out of the 26 bands, 14 were polymorphic. Proportion of polymorphic bands and gene diversity estimates indicated the existence of high level of intracultivar genetic variation in Basmati 370 and TNDB, respectively 100%. The UPGMA dendrogram based on Nei's genetic distance grouped the rice genotypes into three clusters.

Genetic diversity analysis using 20 true breeding rice varieties of Kerala were performed using 24 RAPD markers and obtained 204 different reproducible amplicons (Vanaja *et al.*, 2006). Out of the 204 bands, 104 bands (50.2%) were polymorphic and the largest number of polymorphic bands were produced by the primers OPB18 and OPB01 with an average of 5.2. In cluster analysis, maximum genetic distance was seen between varieties such as 'Remya' and 'Annapoorna' (0.26), followed by 'Remya' and 'Kairali', and 'Remya' and 'Aiswarya'.

Rabbani *et al.* (2007) evaluated the genetic polymorphisms and identities of 10 traditional, 28 improved and 2 Japanese cultivars of rice using the random amplified polymorphic DNA technique. Twenty-five decamer-primers produced a total of 208 RAPD fragments, of which 186 were polymorphic. The number of amplification products ranged from 4 to 16 with an average of 8.3 bands per primer. Based on cluster analysis performed on a similarity matrix using UPGMA, 40 cultivars were grouped into 3 main clusters corresponding to aromatic, non-aromatic and japonica group. The cluster analysis showed most of the aromatic cultivars into a close relation showing a high level of genetic similarity. They found that improved and traditional cultivars originating from diverse sources were interspersed,

indicating no association between the RAPD patterns and the geographic origin of the cultivars.

Skaria *et al.* (2011) studied genetic variability and diversity in eight rice varieties of Kerala and southern India using RAPD techniques. He used 10 primers for screening and found that out of 101 total RAPD fragments amplified, 28 (27.72%) were found to be shared by individuals of all eight varieties and the rest of the fragments were found to be polymorphic (72.27%).

Rajani *et al.* (2013) used 30 decamer random primers to study the population structure of ten different cultivars of rice varieties from Kerala. Out of 30, 25 RAPD primers shown polymorphism. The maximum number of polymorphic bands were produced by the primer OPB-17. The RAPD data was analysed to determine the genetic similarity coefficients which ranged from 0.46 to 0.81. Cluster analysis was done by using UPGMA the Jaccard's similarity coefficient and the rice genotypes were grouped into two clusters.

Manohar *et al.* (2011) analyzed molecular diversity of traditional rice varieties of Kerala using 10 reported primers. The primers used are OPF-04, OPH-19, OPK-19, OPF-06, OPD-18, OPL-17, OPF-05, OPC-07, OPB-08, OPF-01 (Saker *et al.* 2005).

Bansal *et al.* (2013) used 20 RAPD primers for the molecular characterization of 20 rice genotypes. A total of 116 alleles were produced among the 20 rice genotypes. The overall size of amplified products showed considerable variation from 100 (OPA-10) to 1200 bp (OPA-11). All the RAPD primers used for analysis of genetic diversity and relationship generated polymorphic bands among the genotypes.

Alam *et al.* (2014) studied genetic diversity analysis of rice (*Oryza sativa* L.) landraces through RAPD markers and found that RAPD markers *viz.,* OPC 03, OPC 04 and OPA 01 gave reproducible and distinct polymorphic amplified products.

2.2.2 Simple Sequence Repeats

Microsatellites are also known as simple sequence repeats (SSR) and represent mono-, di-, tri, tetra-, penta- and hexa-nucleotide repeats, respectively. Trinucleotide repeats are most abundant nucleotide repeats in plants (Beckmann and Weber, 1992; Kantety *et al*., 2002; Chen *et al*., 2017, Idrees and Irshad, 2014). SSRs are being widely used because of the high variability. SSR are used in genome mapping in many organisms (Knapik*et al*. 1998). In rice, SSR is the most commonly used DNA marker. They are codominant, hypervariable, abundant and well distributed throughout the rice genome (Temnykh *et al.*, 2001). A variety of microsatellite markers is now available through the published high-density linkage map (McCouch *et al.*, 2002; IRGSP, 2005; Hossain *et al.*, 2007).

2.2.3 SSR marker for genetic diversity analysis in rice

Hossain *et al.* (2007) used thirty SSR markers to identify the genetic diversity of 21 rice genotypes. The number of alleles per locus ranged from three to nine. RM223 was found to be the best marker among the thirty for the identification rice genotypes.

Molecular characterization and genetic diversity were studied by Chen *et al.,* (2017) and the study revealed that 50 rice types clustered into different subpopulations whereas three genotypes were admixtures. 10 specific SSR markers were obtained to identify the 53 rice genotypes.

Genetic diversity in twenty-one elite and high-yielding rice varieties were studied using thirty-four SSR markers distributed across 12 chromosomes by Rahman *et al*., 2012. The study revealed that 14 rice varieties with unique alleles could be used for identification, molecular characterization, and DNA fingerprinting of these varieties and also identified eight SSR markers such as RM10713, RM279, RM424, RM6266, RM1155, RM289, RM20224, and RM5371 suitable for this purpose.

A study was conducted with 20 SSR primers distributed in chromosomes 7 to 12 to estimate genetic diversity among 20 genotypes of rice. The study revealed that

15 out of 20 SSR primer pairs generated polymorphic bands. The size of amplified products ranged from 100 (RM264) to 250 bp (RM286) and the highest PIC value was produced by the primer RM-222 and RM-206 (0.97). The cluster analysis using dendrogram revealed two distinct clusters with a similarity coefficient level of 0.63. Cluster II was the largest and included 19 genotypes and cluster I comprised only one genotype (Bansal *et al.*, 2013).

Morphological and molecular characterization of twenty-six landraces of rice and four high yielding rice accessions were studied using 27 SSR markers which generated 321polymorphic alleles. The results showed that Polymorphism information content (PIC) values ranged between 0.6806 (RM 11) and 0.9416 (RM 474) with an average of 0.8414. Genetic diversity analysis performed using UPGMA revealed that all 30 accessions belongs to 6 clusters based on SSR markers' data at a cut-off similarity coefficient 0.17% (Nadia *et al.,* 2014).

Population structure analysis with 35 SSR markers revealed considerable genetic diversity in the hill rice germplasm containing 64 rice genotypes and 15 control plants. The results of analysis showed that the hill rice was clustered into two broad groups corresponding to Indica and Japonica (Roy *et al.*, 2016).

A set of 729 Indian rice varieties were genotyped using 36 SSR markers and obtained a total of 112 alleles which amplified with an average of 3.11 alleles per locus. Cluster analysis grouped these varieties into two clusters (Singh *et al.*, 2016).

Sixty-five accessions of rice were evaluated to find out the genetic polymorphism and identification of diverse parents using SSR markers. A total of 52 alleles were obtained using 19 polymorphic markers. The markers such as RM-84 and RM- 481 produced a maximum of 4 alleles. The marker RM231 was found to have highest PIC value (0.588). The cluster analysis grouped the sixty accessions in to nine clusters and the cluster IB-1a had maximum thirty-one genotypes followed by cluster IB-1b and cluster V (Rashmi *et al.*, 2017).

Genetic diversity of five rice genotypes were studied using 20 SSR markers and found out that allele diversity frequency among the varieties, ranged from 0.215 to 0.791, with an average of 0.493. RM 260 was found as the best marker for identification of genotypes as it obtained the highest PIC value. The identified markers were utilized in further studies for comparative mapping and marker assisted selection for drought tolerance (Krupa *et al.*, 2017)

Genetic diversity of aromatic rice germplasm was studied by Aljumaili *et al.* (2018) using SSR markers and revealed that, out of 147 SSR markers screened, 32 markers (21.77%), showed distinct polymorphic bands. A total of 131 alleles were produced. The highest number of alleles obtained per locus with the primer RM462 (7) with an average of 4.09. The expected heterozygosity ranged from 0.01 (RM23) to 1.13 (RM172) with an average of 0.60. Cluster analysis based on UPGMA method grouped the 53 accessions into ten distinct clusters at the coefficient of 1.05. Cluster I with 2 accessions, while clusters II and III had 7 and 3 accessions, respectively. Cluster IV had the highest number of accessions with 29 rice accessions. Clusters V, VI, VII, VIII, IX, X had 1, 3, 3, 2, 2, 1, respectively.

Genetic diversity analysis of rice landraces (*Oryza sativa* L.) for salt tolerance using SSR markers were carried out by Rashid *et al.* (2018) in Bangladesh. Seven landraces along with 3 released varieties were included in the study. A total of 31 reproducible polymorphic alleles were identified from the loci with an average of 5.167 alleles per locus (ranges from 4-7) using six SSR markers. The polymorphism information content (PIC) value ranged from 0.595 (RM8094) to 0.797 (AP3206) with an average of 0.697.

A study was conducted by Hue *et al.* (2018) to find out the genetic polymorphism in 90 Vietnamese local rice accessions using 40 SSR markers. The numbers of polymorphic bands showed significant variation and ranged from 3 to 12 alleles per locus with an average of 7.1 alleles per locus. The similarity coefficients of the ninety rice landraces ranged from 0.76 to 0.93. The rice landraces were divided

into five clusters and identified 11 primers such as RM250, RM302, RM10926, RM208, RM227, RM17231, RM23251, RM5647, RM1376, RM339 and RM228 which gave the unique allele.

In the present study, rice varieties from four major agro climatic zones of Kerala are studied using RAPD and SSR markers to uncover the diversity of rice population.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2018. This section accounts for procedures and experimental materials used in the study.

3.1 PLANT MATERIALS

Rice (*Oryza sativa* L.) accessions collected from the various geographic locations are conserved in the rice germplasm repository of different Rice research stations of Kerala Agricultural University. Five varieties were collected from the each agro climatic zones *viz*., hill areas of Wayanad, rice growing tract of Palakkad, saline soils of Pokkali and Kuttanad soils. Represented in Fig.1. The details of the accessions and their place of collection are given in table 1.

Table 1. Passport data of the Rice varieties accessions used for the study

Sl. No.	Variety name	Location	District
1	ABL 12	Ambalavayal	Wayanad
2	ABL 13		
3	AMB 5		
4	AMB 14		
5	AMB 22		
6	TRV 2108	Moncompu	Alappuzha
7	Chettivirippu		
8	Karavala kochu vithu		
9	Kochu vithu-Shornad		
10	Vellakuttadan		
11	T. Virippu	Vytila	Ernakulam
12	Chettivirippu		
13	Pokkali		
14	VTL-1 (Pure line Selection from Choottupokkali		
15	VTL-2 (Pure line Selection from		

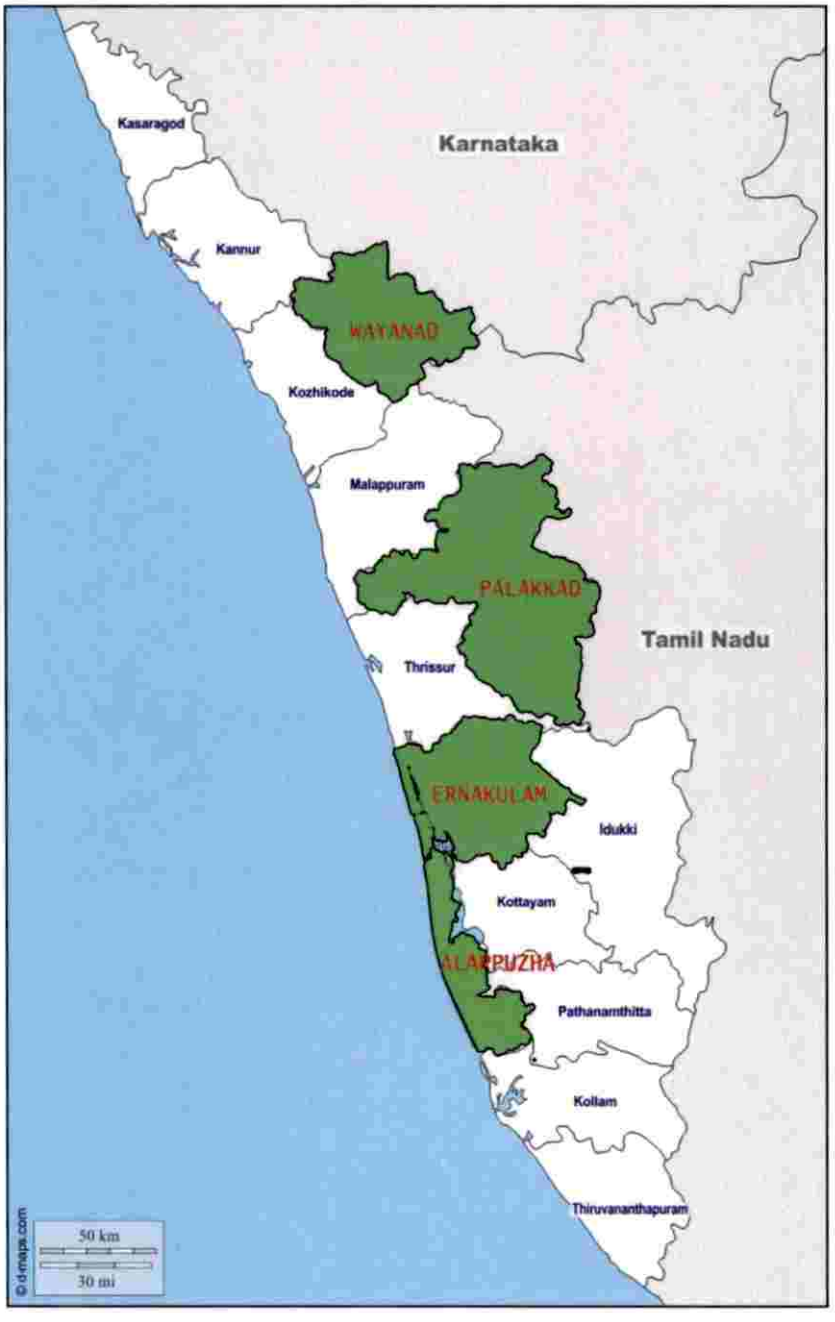

Fig.1. Selected agro climatic zones of Kerala

	Chervirippu)		
16	PTB 2 (Pureline Selection from Ponnaryan)	Pattambi	Palakkad
17	PTB 8 8(Pureline Selection from Chuvnnarithavalakannan)		
18	PTB 12 (Pureline Selection from Thekkanchitteni)		
19	PTB 13 (Pureline Selection from Kayama)		
20	PTB 17 (PurelineSelection from local variety Halliga)		

3.2 MOLECULAR CHARACTERIZATION

3.2.1 Glass wares and other materials

The glass wares and materials required are 1.5 and 2 ml Eppendorf tubes, PCR tubes, mortar and pestle, micropipette tips for 10 µl, 200 µl and 1000 µl were autoclaved and used. 1.5 ml tube stand, PCR tube holders, micropipettes, ice bags, measuring cylinder, bottles, spatula, polythene covers, labels, wipes and weighing pot are the other materials needed for molecular work.

3.2.2 Instruments

The equipments *viz.*, water bath, electronic weighing balance, microwave oven, vortex, spinner, cooling centrifuge (Eppendorf), spectrophotometer, pH meter, deep freezer (-20°C) electrophoresis apparatus (Bio-rad), gel documentation system (Syngene), PCR machine (Bio-rad), hot air oven, autoclave and distilled water unit were used for the study.

3.2.3 Isolation of DNA

HiPurA Plant Genomic DNA Miniprep Purification Kit (MB507) was used to carry out genomic DNA isolation. 100 mg of finely cut leaf material (after removing the mid rib, since it is a source of carbohydrate contamination) were ground with liquid nitrogen to make a fine powder using sterilized mortar and pestle. The finely ground leaf tissue was mixed with 400 µl of lysis buffer, and transferred to a 2.0 ml clean collection tube. After giving a vigorous vortexing, 20µl of RNase (20 mg/ml) was added and incubated at room temperature (15-250C) for 10 minutes. The mixture was then incubated at 65^0C for 10 minutes with intermittent mixing. Then 130µl of precipitation buffer was added to the lysate and incubated for 5 minutes on ice. After that, the entire sample was added to the Hishredder placed in a 2.0 ml collection tube and centrifuged for 5 minutes at 14000 rpm.

The resultant flow-through was transferred to a 2.0ml collection tube without disturbing the cell debris. To the lysate, added 1.5volumes of dil. binding buffer (The binding buffer was prepared by diluting the 14ml Binding Buffer Concentrate with 7ml of 100% ethanol) and from this solution 650µl of this lysate was added to HiElute Miniprep Spin Column, and carried out centrifugation at 8000 rpm for 1minute. The same step was repeated once again and flow-through was discarded. To the same collection tube after discarding the flow-through, 500µl diluted wash solution (15ml wash solution concentrate added to 35 ml 100% ethanol) and centrifuged at 8000 rpm for 1 minute. Discarded the flow-through and reused the collection tube. Again added 500 µl of diluted wash buffer to the column and centrifuged for 2 minutes at 14000 rpm. The tube with column was centrifuged for 2 minutes at 14000 rpm after discarding the flow-through in order to dry the membrane. To a new 2.0 ml collection tube added 100µl of elution buffer, incubated at room temperature (RT) for 5 minutes. For eluting DNA, centrifugation was carried at 10000 rpm for 1 minute. This step was repeated again with another 100µl of elution

buffer for getting high yield of DNA. Finally, the elute was transferred to a fresh capped 2.0 ml collection tube and was stored at -20^{0}C.

3.3 AGAROSE GEL ELECTROPHORESIS

3.3.1Reagents (Stock solutions)

a) 50X TAE Buffer (1000 ml)

Tris base	240.00g
Acetic acid	57.100ml
0.5M EDTA (pH-8.0)	186.120g
Distilled H_2O	942.90ml

b) 6X loading dye	10.00 ml
Sucrose	4.00g
Bromophenol blue	0.025g
Distilled H_2O	10.00ml

After DNA isolation, agarose gel electrophoresis was carried out to resolve the genomic DNA in a horizontal gel electrophoresis Unit of BIO-SYS. For that, 0.8% (w/v) agarose gel was prepared using 1X TAE buffer by boiling it in a microwave oven. After melting, the solution was cooled to 42-45˚C and mixed with 0.3 μg/ml of ethidium bromide. The solution was then poured on to a preset, gel casting tray and allowed to solidify for 15-20 min. After removing the comb, the casting tray was submerged in electrophoresis chamber containing 1X TAE buffer in such a way that the wells are placed closest to the negative terminal of the chamber. The DNA samples were prepared by mixing with loading dye in the ratio 5:1 respectively and

loaded into the corresponding wells of gel. The negative terminal (cathode) and positive terminal (anode) of the electrophoresis unit were attached to the power supply and set a voltage of 60V to run the gel.

The power was turned off when the loading dye reaches about 3/4th of the gel. The gel was visualized under U.V light using SYNGENE gel documentation system in order to check the intactness, shearing of DNA and RNA contamination. Single intact band of high molecular weight showed that DNA was pure.

3.4 QUANTITATIVE AND QUALITATIVE ANALYSIS OF DNA

Quantification of the genomic DNA was estimated by using UV-Visible spectrophotometer by measuring the absorbance (A) at 260 nm. Here, 5µl of DNA was mixed with 0.1X TAE which is then added to 3ml of distilled water. By using distilled water as blank, the absorbance was taken at 260 nm and 280 nm. Since the absorbance, 1.0 O.D. at 260 nm is equivalent to 50ng of DNA per ml, the concentration of DNA was estimated from the following formula:

Concentration of DNA (mg/ml) = A_{260} x 50 x dilution factor / 1000

3.5 ANALYSIS OF RAPD MARKER

The procedure of Manohar *et al.* (2011) was used for the amplification of the DNA. Amplification was done using 10 reported primers for the molecular diversity analysis of traditional rice varieties of Kerala and are OPF-04, OPH-19, OPK-19, OPF-06, OPD-18, OPL-17, OPF-05, OPC-07, OPB-08, OPF-01 (Saker *et al.,* 2005).

Table 2. List of RAPD markers used in the study.

Sl. No.	RAPD Marker	Sequence
1	OPF-04	GGTGATCAGG
2	OPH-19	CTGACCAGCC

3	OPK-19	CACAGGCGGA
4	OPF-06	GGGAATTCGG
5	OPD-18	GAGAGCCAAC
6	OPF-01	ACGGATCCTG
7	OPB-08	GTCCACACGG
8	OPC-07	GTCCCGACGA
9	OPL-17	AGCCTGAGCC1
10	OPF-05	CCGAATTCCC

3.5.1 Polymerase chain reaction of RAPD markers

The reaction executed in 25 µl reaction mixture consisting of 20ng template DNA, 2 µl of dNTP mix, 2.5 µl of PCR buffer, 2.5 µl of $Mgcl_2$ and 0.3 Tag DNA polymerase. Amplification done in an Eppendorf master cycler nexus gradient PCR. Following PCR conditions were used:

An initial denaturation at 94° C for 5 minutes followed by 40 cycles of denaturation at 94 ° C for 1 minute, annealing at 36 ° C (for all primers) for 1 minute and extension at 72 ° for 1.5 minute. The synthesis step of final cycle was extended further by 7 minutes. Finally, the products were separated by agarose gel electrophoresis using 1.4% agarose gel containing 0.3 µg/ml of ethidium bromide in 1X TAE buffer and visualized under SYNGENE G-Box F3 gel documentation unit. Further a 100 bp DNA ladder was used as a standard reference to score the polymorphisms in the DNA profile by comparing the size of the genotypes.

3.6 ANALYSIS OF SSR MARKERS

Amplification was done using 10 reported SSR markers for the molecular diversity analysis of traditional rice varieties of Kerala. SSR primers *viz.*, RM595, RM567, RM210, RM551, RM410, RM260, RM335, RM547, RM592, RM204.

Table 3. List of SSR markers used in the study.

Sl. No.	**Primer name**	**Sequence**	**Annealing temperature**
1.	RM595-F: RM595-R:	CCT TGA CCC TCC TCT TAC TT TCC TAT CAA AAT TTG GCA AC	57.0 °C
2.	RM567-F: RM567R:	ATCAGGGAAATCCTGAAGGG GGAAGGAGCAATCACCACTG	57.8 °C
3.	RM210-F: RM210- R:	TCACATTCGGTGGCATTG CGAGGATGGTTGTTCACTTG	57.0 °C
4.	RM551-F: RM551-R	AGCCCAGACTAGCATGATTG GAAGGCGAGAAGGATCACAG	56.3 °C
5.	RM410-F: RM410-R:	GCTCAACGTTTCGTTCCTG GAAGATGCGTAAAGTGAACGG	56.0°C
6.	RM260-F: RM260-R:	ACTCCACTATGACCCAGAG GAACAATCCCTTCTACGATCG	58.2 °C
7.	RM335-F: RM335-R:	GTACACACCCACATCGAGAAG GCTCTATGCGAGTATCCATGG	56.3 °C

8.	RM547-F: RM547- R	TAGGTTGGCAGACCTTTTCG GTCAAGATCATCCTCGTAGCG	58.4°C
9.	RM592-F: RM592-R	TCTTTGGTATGAGGAACACC AGAGATCCGGTTTGTTGTAA	57.0 °C
10.	RM204-F: RM204-R:	GTGACTGACTTGGTCATAGGG GCTAGCCATGCTCTCGTACC	56.5°C

3.6.1 Polymerase chain reaction of SSR markers

The reaction executed in 25 µl reaction mixture consist of 20ng template DNA, 2 µl of dNTP mix, 2.5 µl of PCR buffer, 2.5 µl of $MgCl_2$ and 0.3 Taq DNA polymerase of both forward and reverse primer. Amplification compassed in an Eppendorf master cycler nexus gradient PCR. Following PCR conditions were used:

An initial denaturation at 94° C for 5 minutes followed by 35 cycles of denaturation at 94 ° C for 30 seconds to 1-minute, annealing temperature is varied for every SSR marker, (normally 55 ° C to 61° C for 30 seconds to 1 minute, extension at 72 ° for 1 minute. for 1 min of primer extension and final extension 5-7 min at 72°C The synthesis step of final cycle was extended further by 7 minutes. Finally, the products were separated by agarose gel electrophoresis using 2.0% agarose gel containing 0.3 µg/ml of ethidium bromide in 1X TAE buffer and visualized under SYNGENE G-Box F3 gel documentation unit. Further a 100 bp DNA ladder was used as a standard reference to score the polymorphisms in the DNA profile by comparing the size of the genotypes.

3.6 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). Distance matrix was subjected to cluster analysis employing UPGMA using SAHN (Sequential Aglomerative Heirarchial and nested Cluster) module of the software NTSYS PC (Rohlf 2002). The value of each of the ten RAPD markers and SSR markers was assessed using two indices PIC which is same as diversity index (DI) and Rp (Prevost and Wilkinson 1999). PIC was estimated as PIC= Σ $(1\text{-}pi^2)$ /n where n is the number of band positions analyzed in the set of accessions, pi is the frequency of the ith pattern. Marker Index was calculated as the product of DI and EMR (Effective multiplex ratio). EMR of a primer is defi ned as the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Milbourne *et al.*, 1997).

RESULTS

4. RESULTS

The study entitled "Genetic diversity analysis of indigenous rice varieties in Kerala using SSR and RAPD markers" was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellyani, Thiruvananthapuram during 2017-2018. The selected 20 varieties of traditional rice were characterized based on both SSR and RAPD markers. The results are depicted in this chapter.

4.1 MOLECULAR DATA ANALYSIS

Isolation of DNA from the 20 varieties of traditional rice was tried by using the HiPurA Plant Genomic DNA Miniprep Purification Kit (MB507) to extract quality DNA with better concentration. This method of DNA isolation yielded good quality DNA (Table 4.) Isolated DNAs were separated on 0.8% agarose gel to check the quality. It is shown in plate 1.

Table 4. Spectrophotometric readings of isolated DNA

Sl. No.	Variety name	Concentration (ng/µl)	A_{260}/A_{280}
1	ABL 12	908.60	1.93
2	ABL 13	603.79	1.85
3	AMB 5	1023.10	1.9
4	AMB 14	1789.00	1.86
5	AMB 22	312.62	1.91
6	TRV 2108	369.00	1.84
7	Chettivirippu	770.70	1.81
8	Karavala kochu vithu	696.10	1.87
9	Kochu vithu-Shornad	974.30	1.9
10	Vellakuttadan	1698.0	1.84
11	T. Virippu	1307.05	1.89
12	Chettivirippu	785.67	1.86
13	Pokkali	1077.13	1.95
14	VTL-1	2236.26	1.82
15	VTL-2	2172.12	1.86

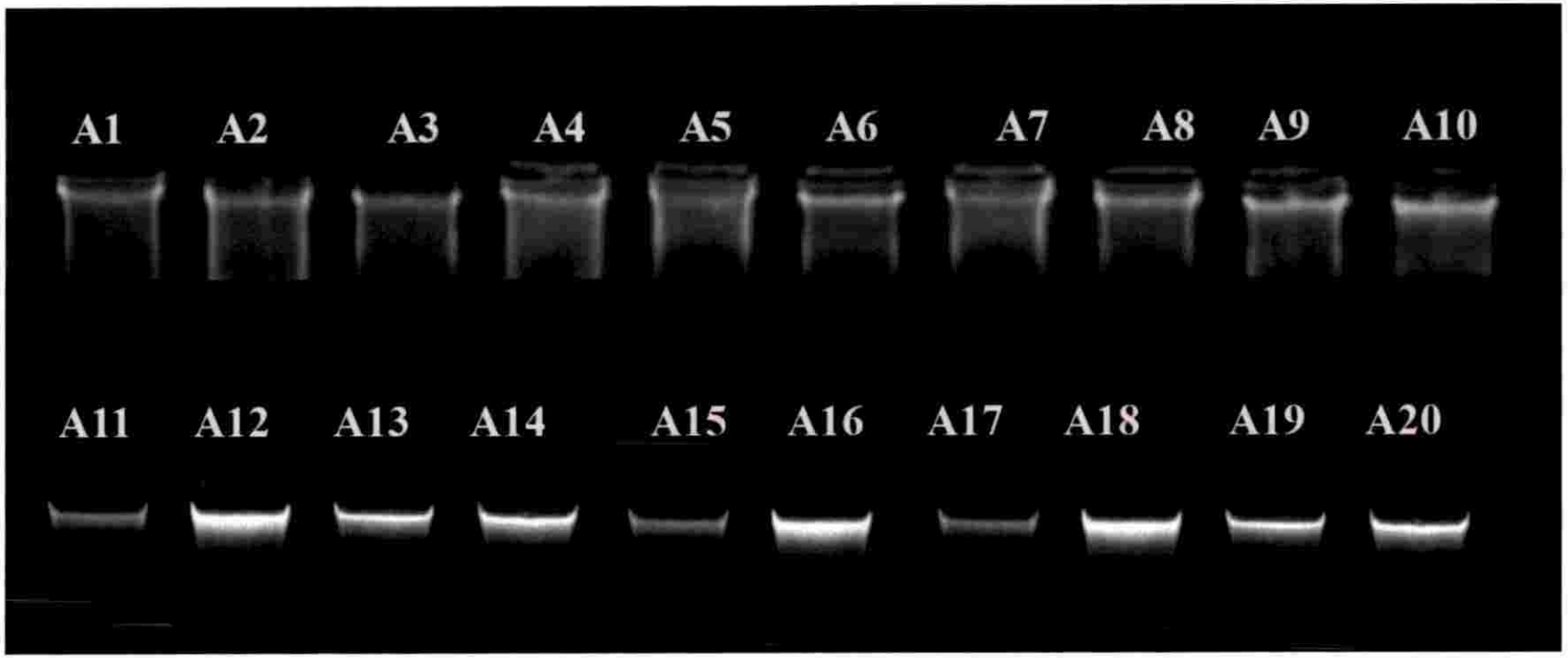

Plate 1. DNA extraction of the twenty rice accessions

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

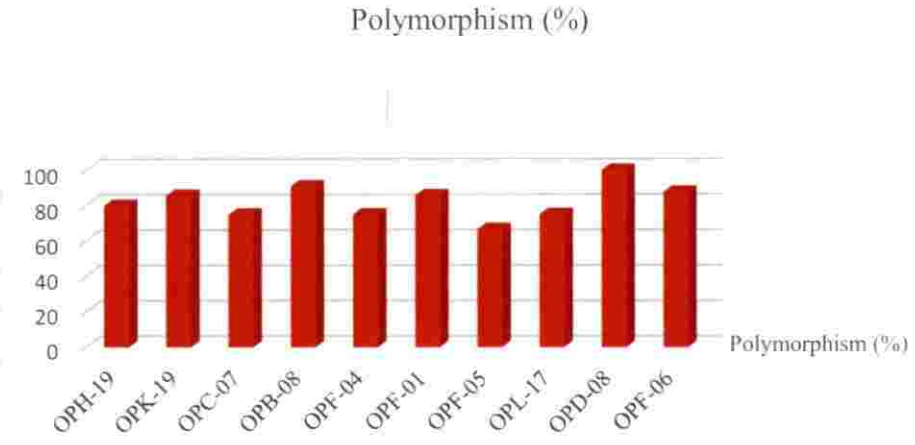

Fig.2. Polymorphism of RAPD

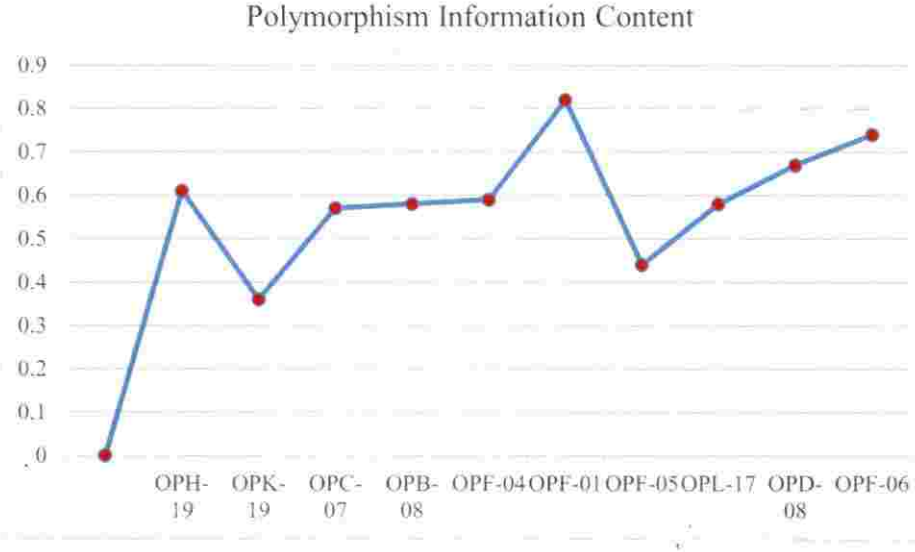

Fig.3. PIC value of RAPD

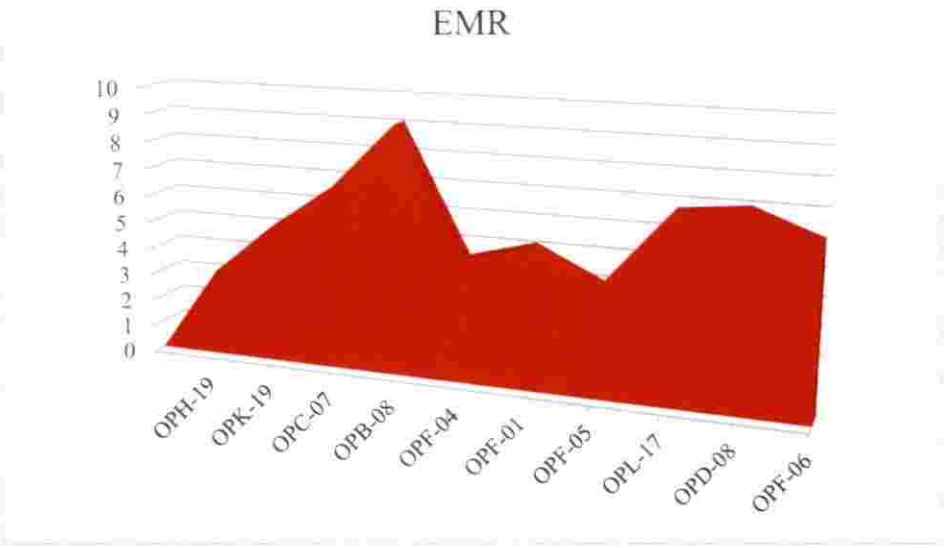

Fig. 4. Effective Multiplex Ratio value of RAPD

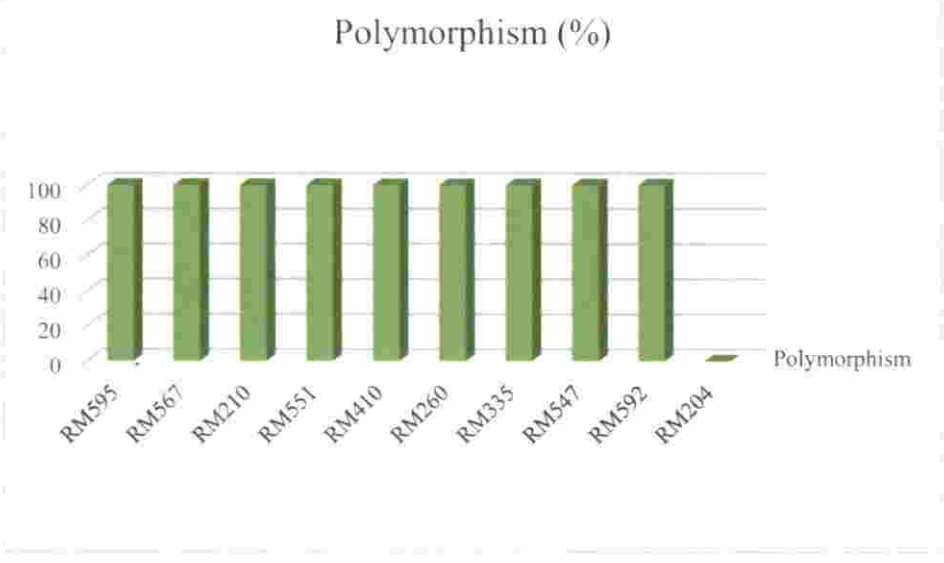

Fig.5. Percentage of Polymorphism of SSR

16	PTB 2	590.0	1.92
17	PTB 8	785.0	1.83
18	PTB 12	696.10	1.91
19	PTB 13	995.80	1.88
20	PTB 17	698.0	1.86

A spectrophotometric value (A260/A280) of 1.81-1.95 and a conspicuous band on 0.8% agarose gel ensured the high quality of isolated DNA.

4.2.1 RAPD analysis

After initial primer screening, 10 Operon primers OPF-04, OPH-19, OPK-19, OPF-06, OPD-18, OPL-17, OPF-05, OPC-07, OPB-08, and OPF-01 which showed polymorphism were chosen for further analysis. PCR was performed using standardized PCR conditions and the annealing temperature is 36° C, obtained from literature was found to be optimum for each primer.

Primer name	Sequence	Number of amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Polymorphism (%)	PIC	Rp	EMR
OPH-19	GGTGATCAGG	05	04	01	80.00	0.61	5.80	3.20
OPK-19	CTGACCAGCC	07	06	01	85.71	0.36	1.90	5.14
OPC-07	CACAGGCGGA	12	09	03	75.00	0.57	15.4	6.75
OPB-08	GGGAATTCGG	11	10	01	90.90	0.58	12.8	9.09
OPF-04	GAGAGCCAAC	08	06	02	75.00	0.59	6.90	4.50
OPF-01	ACGGATCCTG	07	06	01	85.71	0.82	3.90	5.14
OPF-05	GTCCACACGG	09	06	03	66.66	0.44	10.4	4.00
OPL-17	GTCCCGACGA	12	09	03	75.00	0.58	7.50	6.75
OPD-08	AGCCTGAGCC	07	07	00	100.0	0.67	7.30	7.00
OPF-06	CCGAATTCCC	08	07	01	87.50	0.74	7.90	6.13

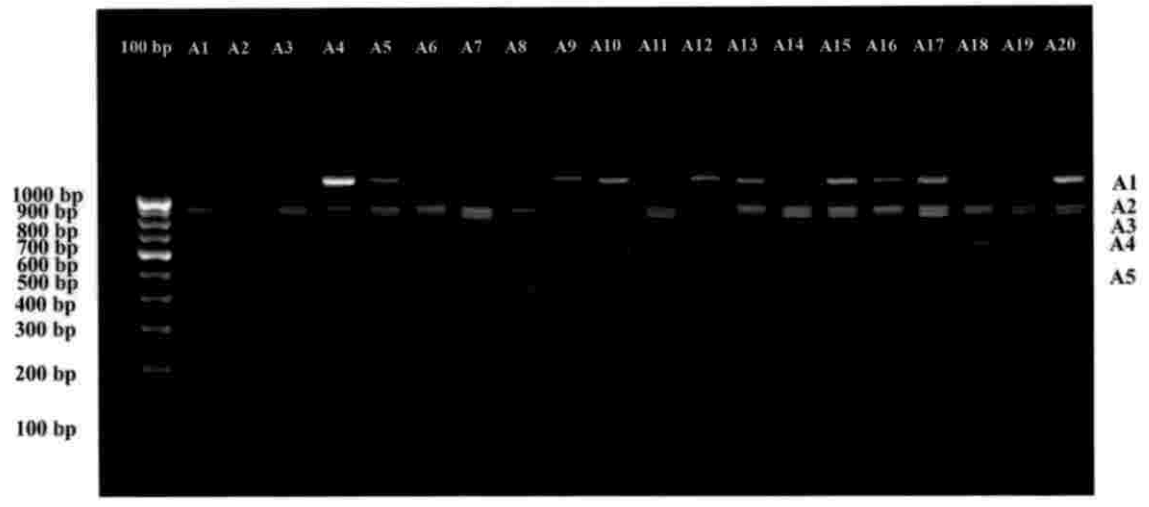

Plate 2. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPH-19

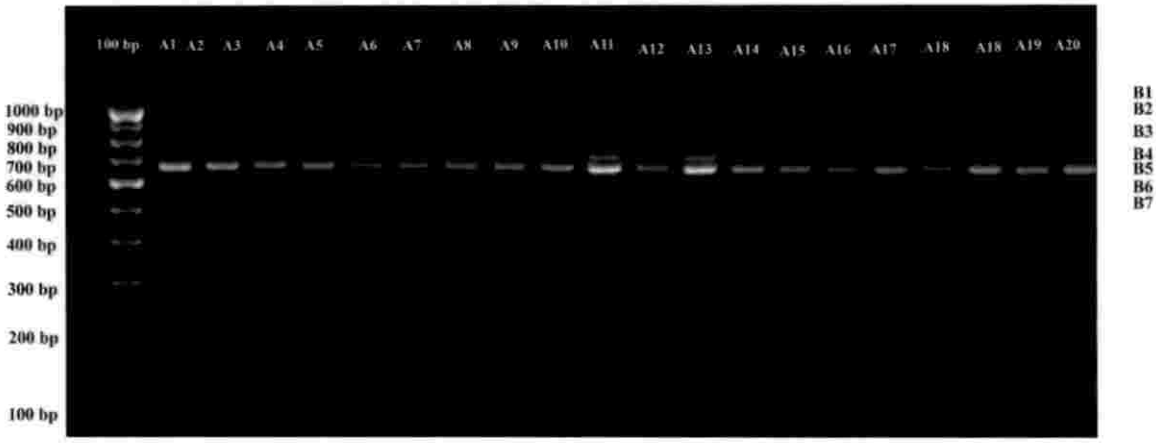

Plate 3. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPK-19

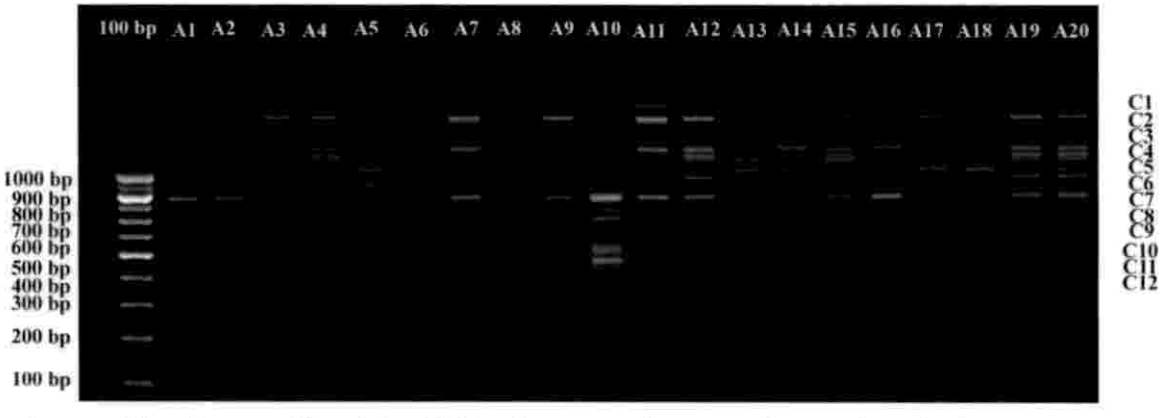

Plate 4. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPC-07

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

After the completion of PCR using selected RAPD primers, the amplicons were resolved in 1.4% agarose gel and amplicons obtained are shown in Figures. A total of 88 scorable bands were produced in the 20 varieties studied, out of which 70 were polymorphic (81.39%). The percentage of polymorphism ranged from a maximum of 100 percentage (shown by OPD-08 primer) and a minimum of 66.66 (shown by OPF-05). The RAPD primers used, the number of bands produced by each primer, number of polymorphic bands and percentage polymorphism are shown in Table 5.

Table 5. Details of the RAPD primers and polymorphism information content

RAPD analysis produced a total of 88 amplicons over 10 operon primers. The percentage of polymorphism produced by the RAPD Markers ranged from 66.66 % to100 %. Highest polymorphism was produced by the marker OPD-08 and lowest polymorphism by OPF-05. Polymorphic information content (PIC) of the RAPD markers ranged from 0.36 to 0.74. Highest PIC value of RAPD marker is 0.82 for OPF-01 and lowest value of PIC is 0.36 for OPK-19. Resolving power (Rp) of RAPD markers ranged from 1.9 to 12.8. Highest value of Rp in RAPD markers is for OPC-07 and lowest for OPK-19.Effective Multiplex ratio (EMR) values ranged from 3.2 to 9.09. Lowest value of EMR is 3.2 for OPH-19 and highest value of EMR 9.08 is for OPB-08.

4.2.1.1 OPH-19

The OPB-19 RAPD marker formed five amplification products whose size were above 300 bp. (Plate2) they were numbered from A1 to A5. Of the 5 products

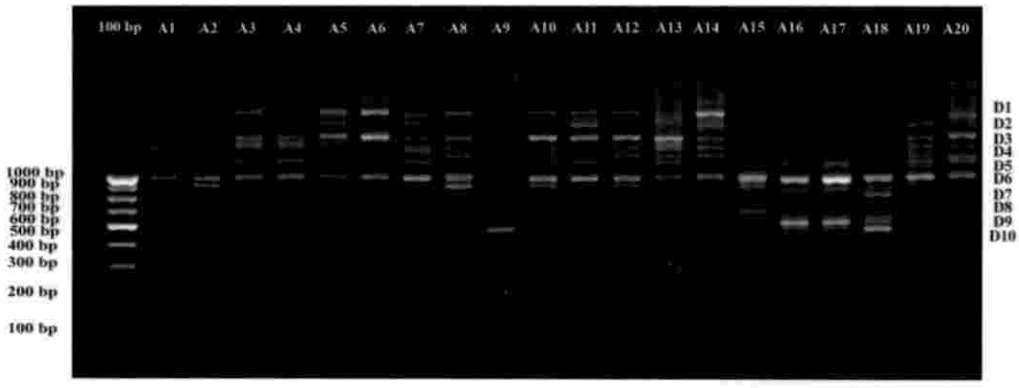

Plate 5. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPB-08

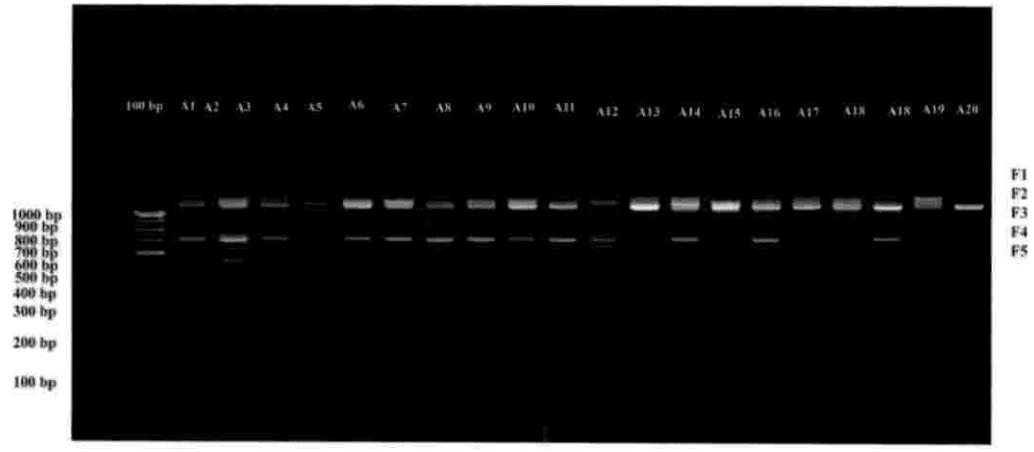

Plate 6. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPF-04

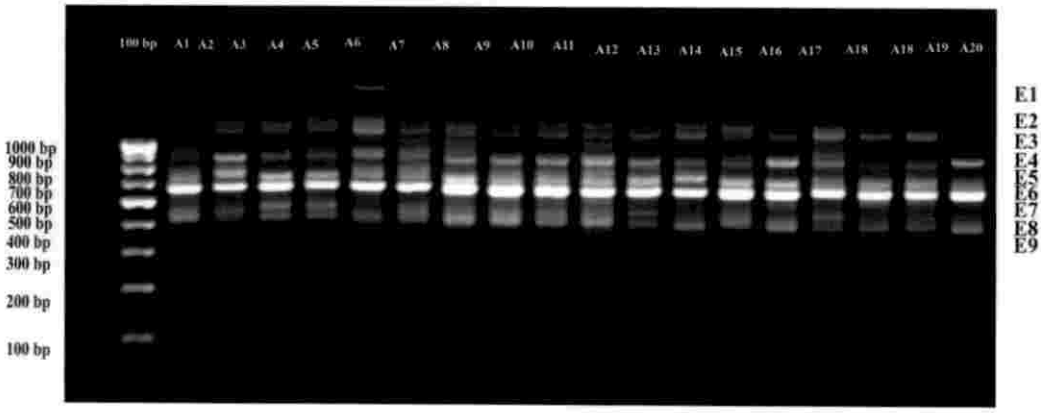

Plate 7. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPF-01

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

A1, A3, A4, and A5 were polymorphic giving a polymorphism of 80.00 percent (plate 2).

The first product A1 was present in the seventeen accessions AMB22, AMB5, TRV2108, Chettivirippu, Karavala kochu vithu, Kochu vithu-shornad, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 17, PTB 13, PTB 2, PTB12, and PTB 8. The product A2 was monomorphic. The product A3 was present in all accessions except two accessions Kochu vithu-shornad and PTB 13. The product A4 was present in the seventeen accessions ABL-12, ABL-13, AMB-14, AMB-22, AMB-5, TRV-2108, Chettivirippu, Karavala kochu vithu, Vellakuttadan, T.virippu, VTL-1, Pokkali, VTL-2, PTB 17, PTB 2, and PTB 12. The final product A5 was present in five accessions ABL-12, ABL-13, AMB-14, Karavala kochu vithu and Vellakuttadan.

4.2.1.2 OPK-19

The OPK-19 RAPD marker formed seven amplification products whose size was above 300 bp. (Plate 3) they were numbered B1 to B7. Six products B1, B2, B3, B4, B6 and B7 were polymorphic giving a polymorphism of 85.71 percent (plate 3)

The first product B1 was present in the twelve accessions ABL-12, ABL-13, AMB-14, AMB-22, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 17, and PTB 2. The product B2 was present sixteen accessions ABL-12, ABL-13, AMB-14, AMB-5, TRV-2108, Chettivirippu, Karavala kochuvithu, Karavala kochu-shornad, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, VTL-2, , PTB 13, and PTB 12, four accessions were absent in the B2. The product of B3 were present in all the accessions except AMB22. The product B4 was seen in seventeen accessions ABL-12, ABL-13, AMB-14, AMB-22, AMB-5, TRV-2108, Karavala kochu-shornad, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 17, , PTB 2, PTB 12, and PTB 8. The product B5 was monomorphic. The product B6 was seen eleven accessions ABL-12, ABL-13, AMB-22, Chettivirippu,

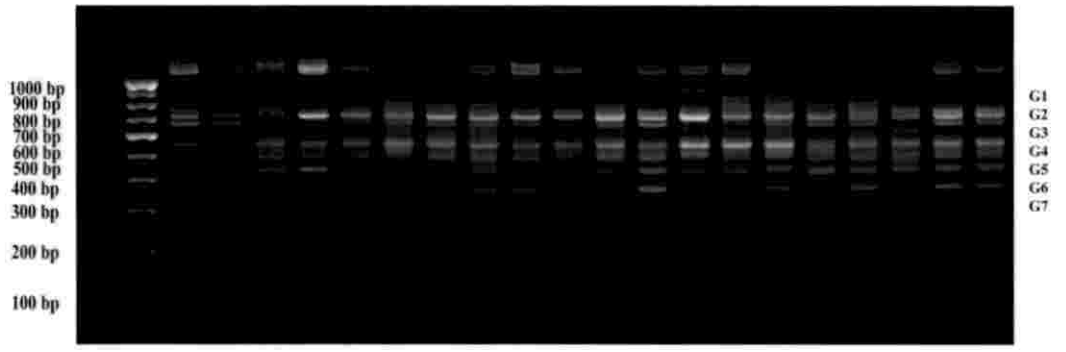

Plate 8. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPF-05

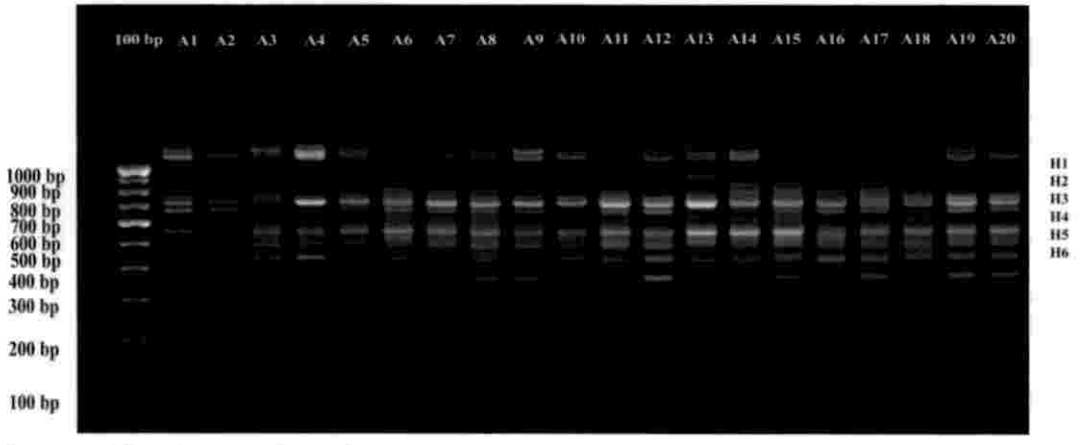

Plate 9. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPL-17

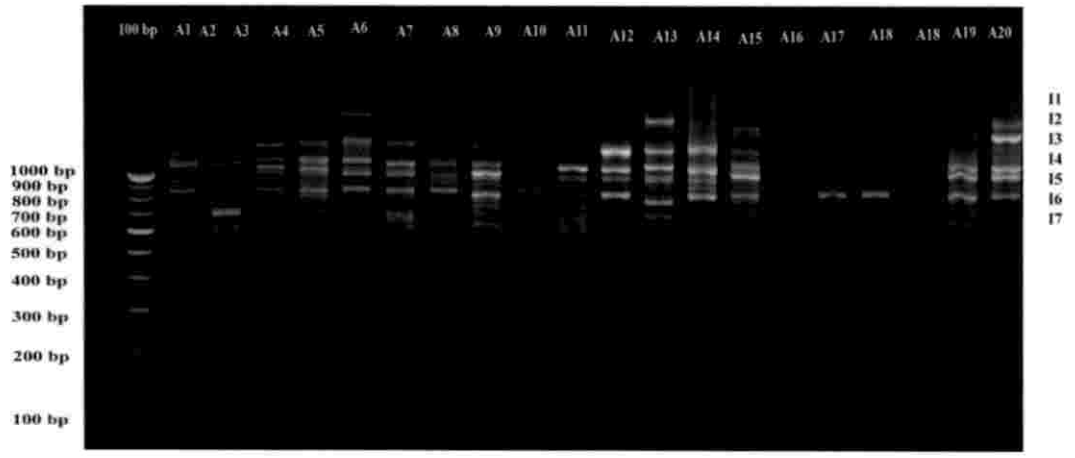

Plate 10. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPD-08

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

Karavala kochuvithu, Karavala kochu-shornad, Vellakuttadan, T.virippu, Chettivirippu, , PTB 17, PTB 13, PTB 12, and PTB 8. The B7 was present in all accessions except two accessions PTB 2 and PTB 8.

4.2.1.3 OPC-07

The OPC-07 RAPD marker formed twelve amplification products whose size was above 400bp. (Plate4) they were numbered C1 to C12. Of the twelve products C1, C2, C3, C4, C5, C6, C10, C11 and C12 were polymorphic giving a polymorphism of 75.00 percent (plate 4).

The product C1 was seen only in accessions AMB-14, T.virippu, PTB 17, and PTB 2. Accessions AMB-5, TRV-2108, Karavala kochu vithu, Vellakuttadan, VTL-1, PTB 17, and PTB 2 showed the presence of C2. The product C3 was seen in fourteen accessions ABL-12, ABL-13, AMB-14, AMB-22, AMB-5, TRV-2108, Karavala kochu-shornad, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 17, PTB 13, and PTB 2. The C4 product, was present in all except Vellakuttadan, C5 and C6. Amplicons C7, C8, and C9 were monomorphic in nature. The amplicon C10 was present in fourteen accessions AMB-14, AMB-22, TRV-2108, Chettivirippu, Karavala kochu vithu, Karavala kochu-shornad, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, PTB 13, PTB 2, PTB 12, and PTB 8. The products C11 and C12 were present only in Vellakuttadan.

4.2.1.4 OPB-08

The OPB-08 RAPD marker formed eleven amplification products whose size was above 400bp. (Plate4) they were numbered D1 to D10. Nine products of ten products D1, D2, D3, D4, D5, D6, D8, and D10 were polymorphic giving a polymorphism of 90.00 percent (plate 5).

The products of D1 was present in ABL-12, ABL-13, AMB-14, AMB-22, AMB-5, TRV-2108, Chettivirippu, Karavala kochuvithu, Vellakuttadan, T.virippu, Chettivirippu, VTL-1, Pokkali, VTL-2, and PTB 8. The product D2was absent in eight accessions ABL-12, ABL-13, AMB-14, AMB-22, AMB-5, TRV-2108, Chettivirippu, Karavala kochu vithu, Vellakuttadan, T.virippu, Chettivirippu, and PTB 8. Amplicon D3 was present in the accessions, TRV-2108, Chettivirippu, T.virippu, VTL-1, Pokkali, PTB 13, PTB 2, and THEKKAN CHITTEN CHUVANNARI. Karavala kochu-shornad did not show the presence of D5. D7 was monomorphic. The product D8 was shown by ABL 12, ABL 13, AMB 14, AMB 22, AMB 5, Karavala kochu vithu Karavala kochu vithu -Shornad , PTB 12 and PTB 8. Monomorphic products were produced in D9. In D10 product was present in ABL 13, TRV 2108, Vellakuttadan, VTL- 2, Pokkali, PTB 12, and PTB 8.

4.2.1.5 OPF-04

The OPF-04 RAPD marker produced eight amplified products (plate 5) they were numbered from E1 to E9. Four products were polymorphic such as E1, E2, E3, and E7. Percentage of polymorphism was 66.7.

The product E1 was absenct in ten accessions ABL12, ABL13, AMB14, AMB22, AMB5, Kochu vithu shornad, Vellakuttadan, T. virippu, VTL-2, PTB17, and PTB2. The amplicon E2 was absent in PTB8 .Product E3 present in all accessions except PTB38.

4.2.1.6 OPF-01

The OPF-01 RAPD Marker produced four amplified products on (plate 6) F1 to F5. Five products were polymorphic such as F1, F3, F4 and F5. Percentage of polymorphism was 80.0%.

The product F1 present in eight accessions AMB5, TRV 2108, Chettivirippu, VTL-1, Pokkali, PTB 17, PTB 13 and PTB 12. The product F2 was monomorphic..

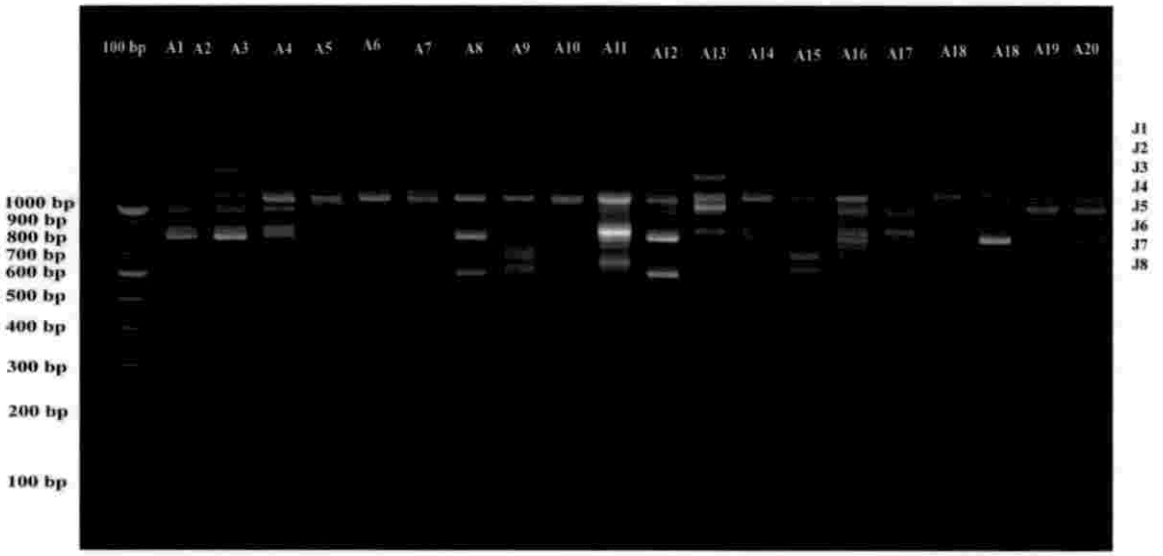

Plate 11. Amplification profile of the DNA of twenty rice accessions using the RAPD marker OPF-06

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12. A20-PTB 8

TRV2108, Chettivirippu, and Karavala kochu vithu-shornad showed the presence of the product F3. AMB5 and PTB 13 alone did not have the product F4. The product F5, was present only in AMB 14, and Karavala kochu vithu-shornad.

4.2.1.7 OPF-05

The OPF 05 RAPD Marker exposed seven amplified products on (plate 7) G1 to G7.Six out of seven bands showed polymorphism and percentage of polymorphism is 85.6 %. The product G1 seen in seventeen accessions and only PTB 17, PTB 13, and PTB 2 did not show the presence of this product. The product G2 seen only in four accessions Kochu vithu-shornad, VTL-1, Pokkali, and VTL-2. Product G3, was absent in AMB 14 and in all others it was present. The product G4 was monomorphic. ABL 12 and ABL 13 did not show the presence of the products G5 and G6. The product G7was absent in eight accessions ABL 12, ABL 13, AMB 14, AMB 5, TRV 2108, Chettivirippu, Vellakuttadan and T.virippu.

4.2.1.8 OPL 17

The OPL 17 RAPD Marker reported six amplified products on (plate 8) H1 to H6. Five out of six products were polymorphic in nature and the percentage of polymorphism was 83.3%.

The product H1 showed in all except PTB 17 and in all others it was present. ABL 12, Kochu vithu-shornad, VTL-1 and VTL-2 had the product H2. H3 was present in all except AMB14. The product H4 was monomorphic .ABL 12 and ABL 13 did not have the products .The product H7 were absent in eight accessions except ABL 12, ABL 13, AMB 14, AMB 5, TRV 2108, Chettivirippu, Vellakuttadan and T.virippu.

4.2.1.9. OPD-08

The OPD-08 RAPD Marker reported seven amplified products on (plate 9) I1 to I7. Seven out of seven were polymorphic in nature and the percentage of polymorphism was 100%.

The product I1 showed that in all accessions except ABL 13, Vellakuttadan, PTB 7, PTB 13 and PTB 2.The product I2 showed the all accessions except Vellakuttadan, PTB 7, PTB 13 and PTB 2. The product I3 showed the all accessions except Vellakuttadan, PTB 7, PTB 13 and PTB 2. The product I4 was monomorphic in nature. The product I5 showed the all accessions except ABL 12, TRV2108, Vellakuttadan, PTB 7, PTB-13 and PTB 2. The product I6 was present in all accessions except Vellakuttadan, PTB-7, PTB -3 and PTB 2. The product I7 present only ABL 13 T. Virippu, Chettivirippu, VTL-1, Pokkali and VTL-2.

4.2.1.10. OPF-06

The OPF-06 RAPD Marker reported seven amplified products on (plate 9) J1 to J8. Seven out of eight products were polymorphic in nature and the percentage of polymorphism was 87.5%.

The product J1present only in ABL 12, ABL 13, VTL-1 and PTB 2. The product J2 present only in ABL 12, ABL 13, AMB 22 and VTL- 1. The product J3 was monomorphic in nature. The product J4 was absent four accessions AMB5, TRV 2108, Chettivirippu, Karavala kochu vithu, Kochu vithu-shornad, Vellakuttadan and T. Virippu. The product J5 was absent three accessions AMB5, TRV 2108 and Chettivirippu. The product J6 was absent only in AMB 22 and Pokkali. The product J7 was absent only in VTL-1 and Pokkali. The product J8 present only Kochu vithu-shornad, VTL-2, PTB 17, PTB 13, PTB 12 and PTB 8.

4.2.2 Clustering of genotypes using RAPD markers

The clustering of genotypes using RAPD markers was done using UPGMA clustering using NTSYS software. The dendrogram constructed is given in Figure 8. The clusters and sub clusters at different phenon levels is given in Table 6.

Table 6. Cluster/ sub clusters at different phenon levels in the dendrogram contsructed based on UPGMA clustering using RAPD scores

<table>
<tr><th colspan="5">No: of Clusters / sub clusters at different phenon levels</th></tr>
<tr><th>40%</th><th>50%</th><th>55%</th><th>60%</th><th>65%</th></tr>
<tr><td>I (1)</td><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="5">II (19)</td><td rowspan="4">IIA (14)</td><td rowspan="3">II AB (11)</td><td rowspan="2">IAB1 (7)</td><td>II AB1A (2)
II AB1 B (2)
II AB1C (3)</td></tr>
<tr><td rowspan="2">IIAB2 (4)</td><td>II AB2A (2)
IIAB2 B (2)</td></tr>
<tr><td>II AC</td><td></td><td></td></tr>
<tr><td>II B (1)</td><td colspan="2"></td><td rowspan="3"></td></tr>
<tr><td rowspan="2">II C(4)</td><td colspan="2">II CA (3)</td></tr>
<tr><td colspan="2">II CB (1)</td></tr>
</table>

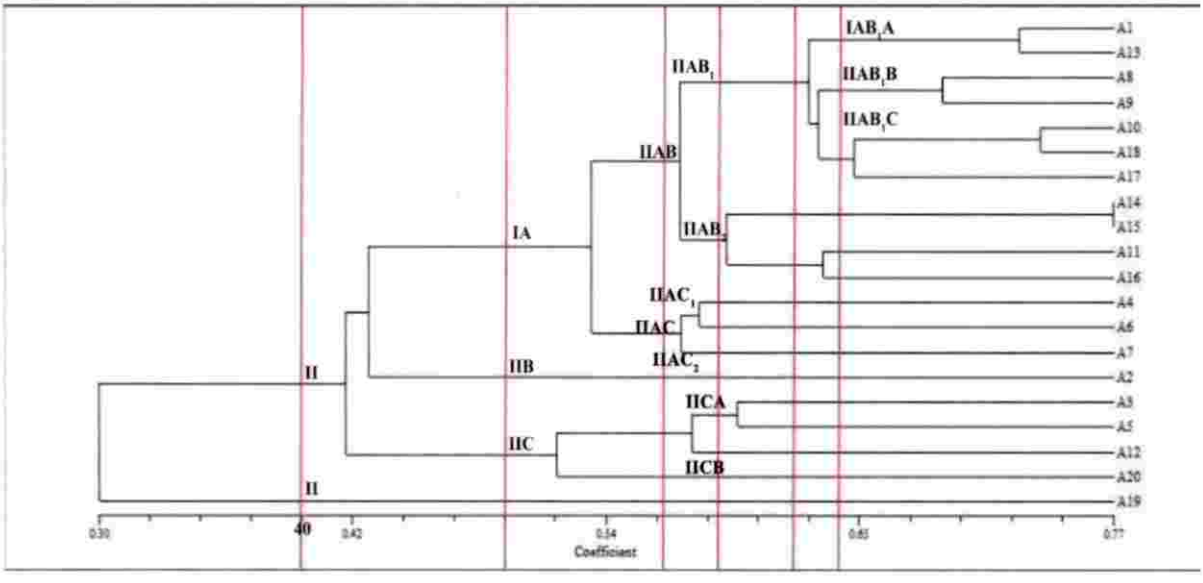

Fig.8. Cluster dendrogram of rice varieties based on RAPD

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

Dendrogram constructed produced two clusters at 40% similarity. Cluster I with only one member (PTB 12).Cluster was larger cluster with 19 members this cluster formed two cluster at 50% similarity 2A with 14 members and 2B with one member A2. Cluster 2A was further sub divided into 2AB with 11 members and 2AC with three members at 55 % similarity at 60 %similarity 2AB1 with 7 members and 2AB2 with 4 members. At 65% similarity 2AB1 formed 3 clusters 2AB1A with 2 members, 2AB1B with 2 members, and 2AB1C with three members. 2AB2 form 2 clusters and 65 % similarity 2AB2A with 2 members, 2AB2B With 2 members.

Cluster 2AC Produced 2 cluster at 60% similarity 2AC1 with 2membsrs and 2AC2 with 1 members. The cluster 2C4 formed 2 clusters at 55% similarity 2CA with three embers 2cb with 1 member. Cluster 2CA formed 2 sub clusters 2CAB with 2 members and 2CAC with one member at 65 % similarity.

4.3 SSR MARKER ANALYSIS

SSR marker analysis was done using 10 RM primers. The details of the primer, Polymorphism percentage, Polymorphism information content, Resolving Power and Effective Multiplex Ratio is given in Table 7.

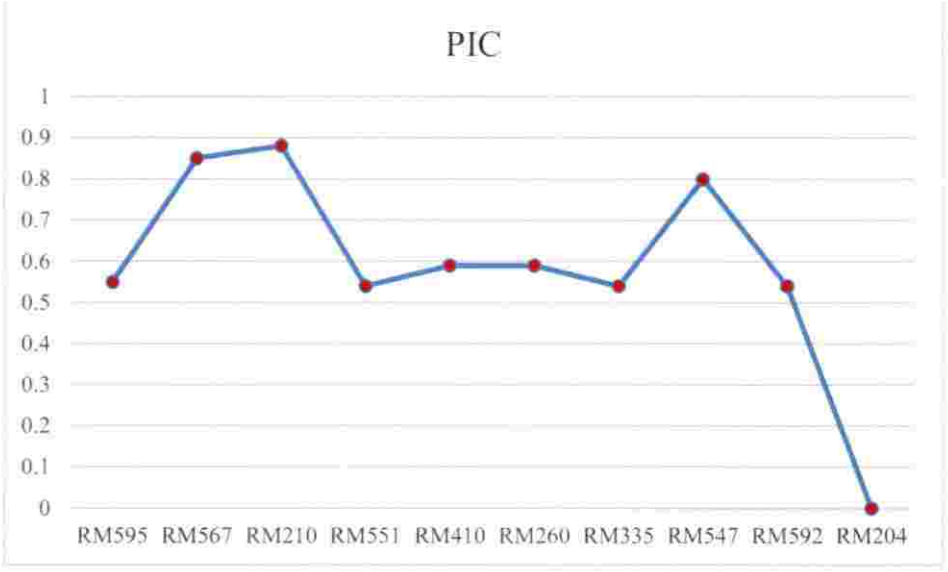

Fig.6. Polymorphism Information Content value of SSR

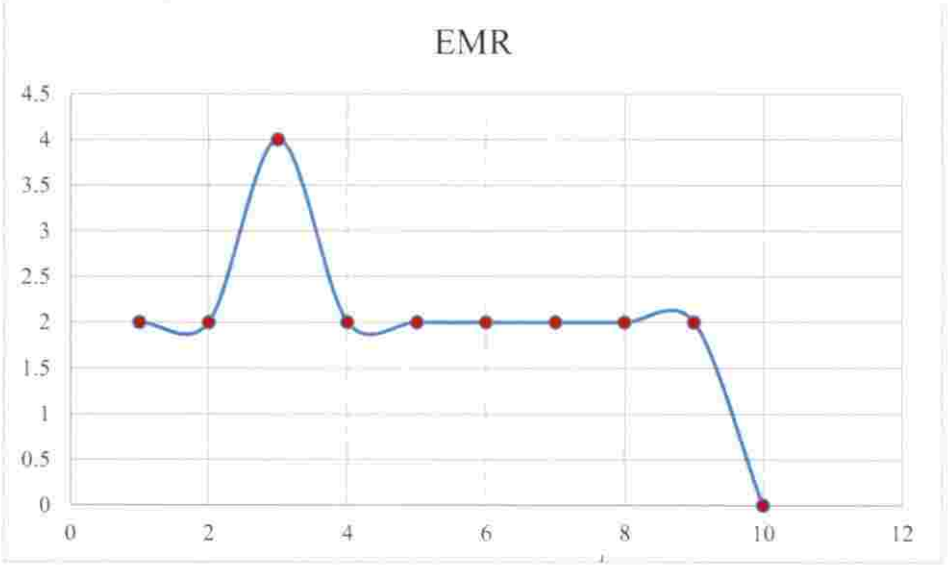

Fig.7. Effective Multiplex Ratio value of SSR

Table 7. Details of SSR markers and their polymorphism information content

Primer name	Sequence	Number of alleles	Number of polymorphic alleles	Polymorphism (%)	PIC	Rp	EMR
RM595-F: RM595-R:	CCT TGA CCC TCC TCT TAC TT TCC TAT CAA AAT TTG GCA AC	2	2	100	0.55	1.00	2.00
RM567-F: RM567R:	ATCAGGGAAATCCTGAAGGG GGAAGGAGCAATCACCACTG	2	2	100	0.85	1.40	2.00
RM210-F: RM210- R:	TCACATTCGGTGGCATTG CGAGGATGGTTGTTCACTTG	4	4	100	0.88	2.05	4.00
RM551-F: RM551-R	AGCCCAGACTAGCATGATTG GAAGGCGAGAAGGATCACAG	2	2	100	0.54	0.90	2.00
RM410-F: RM410-R:	GCTCAACGTTTCGTTCCTG GAAGATGCGTAAAGTGAACGG	2	2	100	0.59	1.05	2.00
RM260-F: RM260-R:	ACTCCACTATGACCCAGAG GAACAATCCCTTCTACGATCG	2	2	100	0.59	1.00	2.00
RM335-F: RM335-R:	GTACACACCCACATCGAGAAG GCTCTATGCGAGTATCCATGG	2	2	100	0.54	0.95	2.00
RM547-F: RM547- R	TAGGTTGGCAGACCTTTTCG GTCAAGATCATCCTCGTAGCG	2	2	100	0.80	1.15	2.00
RM592-F: RM592-R	TCTTTGGTATGAGGAACACC AGAGATCCGGTTTGTTGTAA	2	2	100	0.54	0.90	200
RM204-F: RM204-R:	GTGACTGACTTGGTCATAGGG GCTAGCCATGCTCTCGTACC	1	0	0	0.00	0.00	00.00

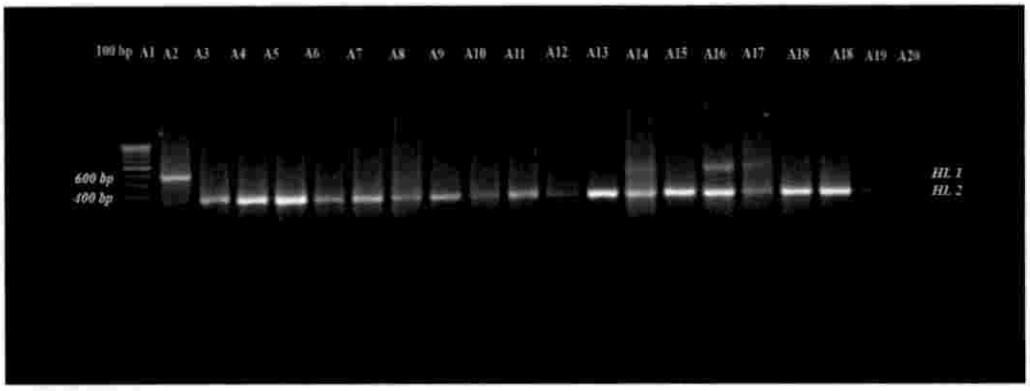

Plate 12. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-595

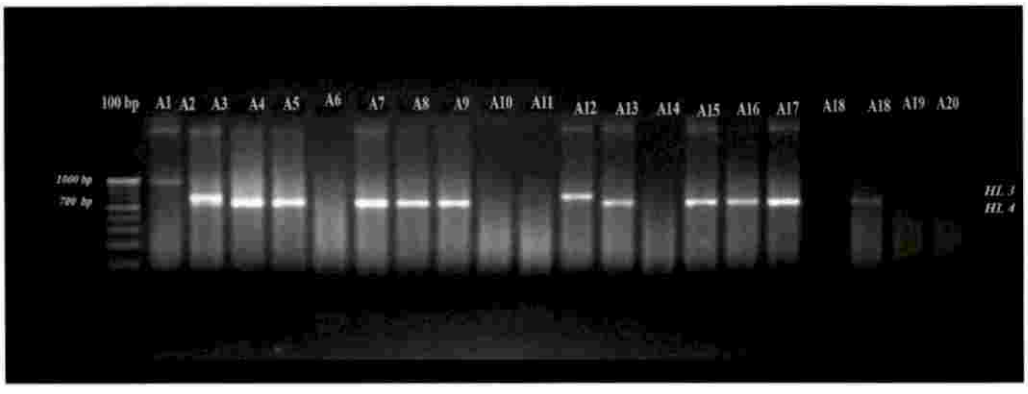

Plate 13. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-567

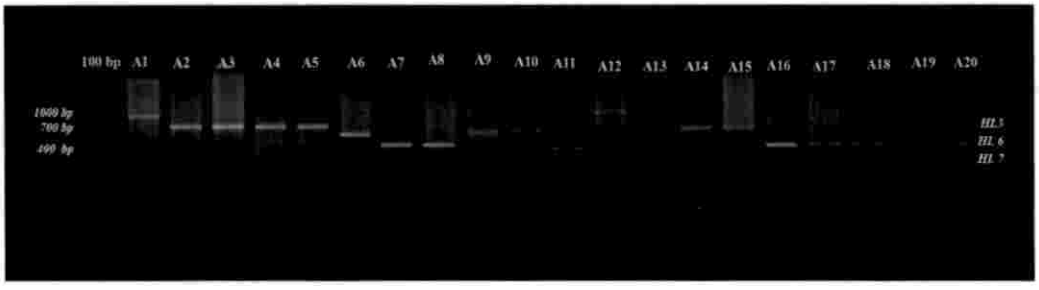

Plate 14. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-210

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12. A20-PTB 8

Percentage of polymorphism in SSR Markers ranged from 0.00 to100 %. Polymorphism information content (PIC) ranged from 0.00 to 0.88. Highest PIC value was for SSR RM-210 marker and lowest value for SSR marker RM-204.

Resolving power (Rp) of SSR markers ranged from 0.00 to 2.05. Highest value of Rp was for SSR marker RM-210 and lowest for RM-204. Effective Multiplex ratio (EMR) values ranged from 3.2 to 9.09. Lowest value of EMR is 0.00in SSR RM-04 and highest value of EMR is 4.00 in RM-210.

4.3.1 RM 595

The amplification profile of the SSR primer RM-595 is given in plate 12.This primer produced two alleles HL1 and HL2 at 600bp and 400bp. The allele HL1 was present only ABL 12, Pokkali and PTB 17. But the allele HL1 was monomorphic in nature.

4.3.2 RM 567

The amplification profile of the SSR primer RM-567 is given in plate 13.The RM 567 primers produced two alleles HL3 and HL4 at 1000bp and 700bp region. Only TRV 2108, Vellakuttadan, T.virippu, PTB 13, PTB 12 and PTB 8 and ABL 12 is present in HL3 and remaining nineteen accessions was absent.

4.3.3 RM210

The amplification profile of SSR Marker RM-210 is given plate 14. RM210 primer produced three alleles HL5, HL6 and HL7 at 1000bp, 700bp and 400bp respectively. The allele HL5 was present only ABL 12 and Chettivirippu. Allele HL6 was present only in TRV2108, Kochuvithu-shornad, Vellakuttadan, VTL-2 and Pokkali. HL7 was present in chettivirippu, Karavalakochivithu-shornad, T.Virippu, PTB 17, PTB 13, PTB 2, PTB 12 and PTB 8.

4.3.4 RM 551

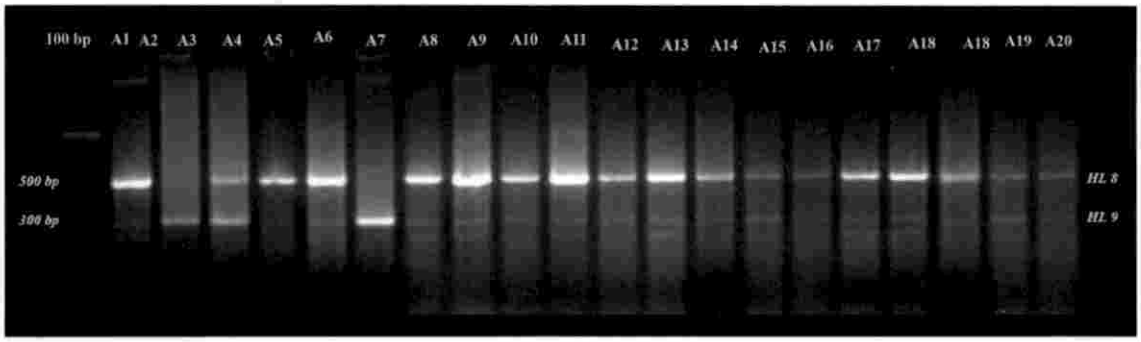

Plate 15. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-551

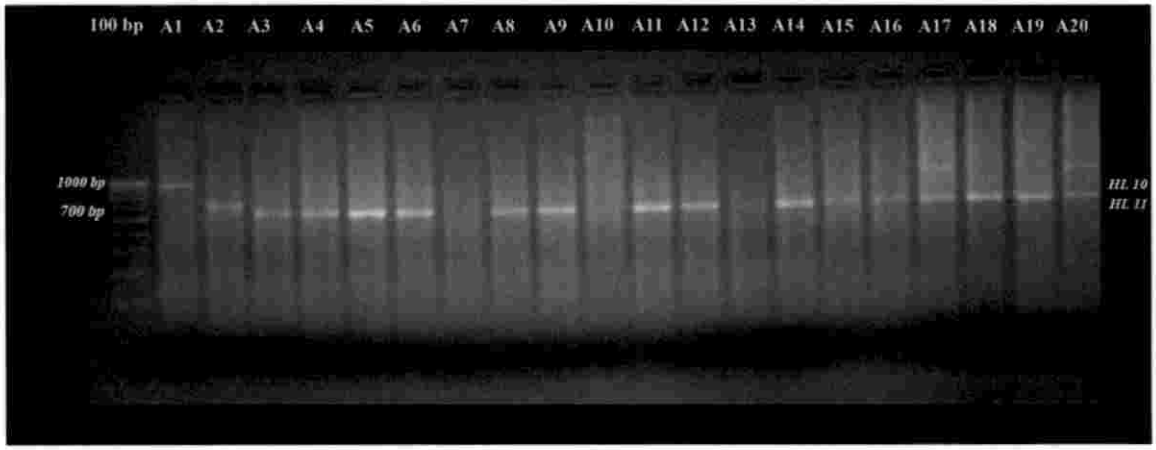

Plate 16. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-410

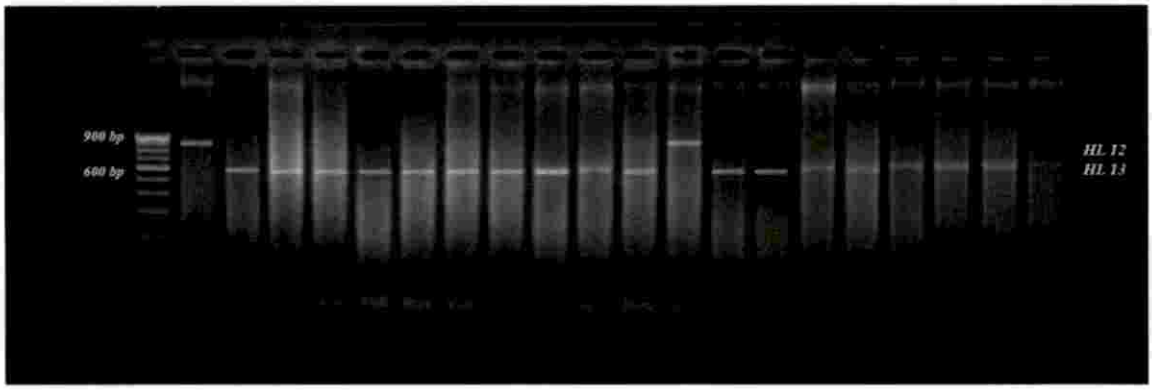

Plate 17. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-260

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12. A20-PTB 8

The amplification profile of SSR Marker RM-551 is given in plate 15. RM551 Primer produced two alleles HL8 and HL9 at 500bp and 300 bp. Allele HL8 was present only in ABL 13 and in all other accessions it was absent. Allele HL9 was present only in ABL13, AMB14 and Chettivirippu.

4.3.5 RM410

The amplification profile of SSR Marker RM-410 is given in plate16.Primer produced two alleles HL10 and HL11 at 1000bp and 700bp. Allele HL10 was present only in ABL 12. Allele HL11 was absent in all accessions except ABL 12.

4.3.6 RM260

The amplification profile of SSR Marker RM-260 is given plate 17. Primer produced two alleles HL12 and HL13 at 900bp and 600bp. Allele HL12 was present in ABL 12 and Chettivirippu. And in all other accessions it was absent. Allele HL13 was present only in ABL 12 and Chettivirippu.

4.3.7 RM335

The amplification profile of SSR Marker RM-335 is given plate 18.primer produced two alleles HL14 and HL15 at 1000bp and 700bp. Allele HL14 was present only in ABL 12, PTB 17 and all other accessions it was absent. Allele HL15 was present in all accessions except ABL12.

4.3.8 RM547

The amplification profile of SSR Marker EM-547 is given plate 19. Primer produced two alleles HL16 and HL17 at 1000bp and 700bp. Primer produced allele HL6 at 1000bp present ABL 12, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 13, and

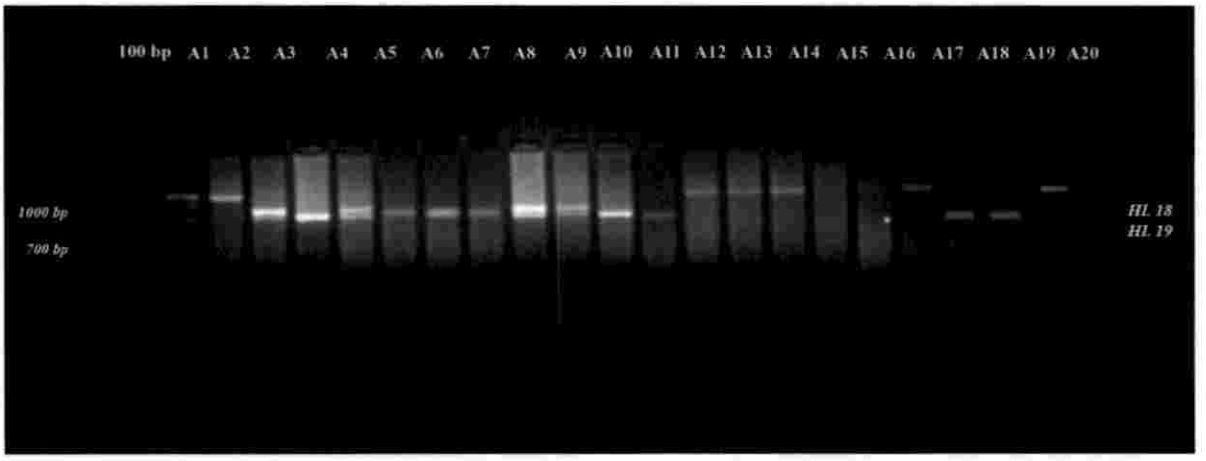

Plate 18. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-335

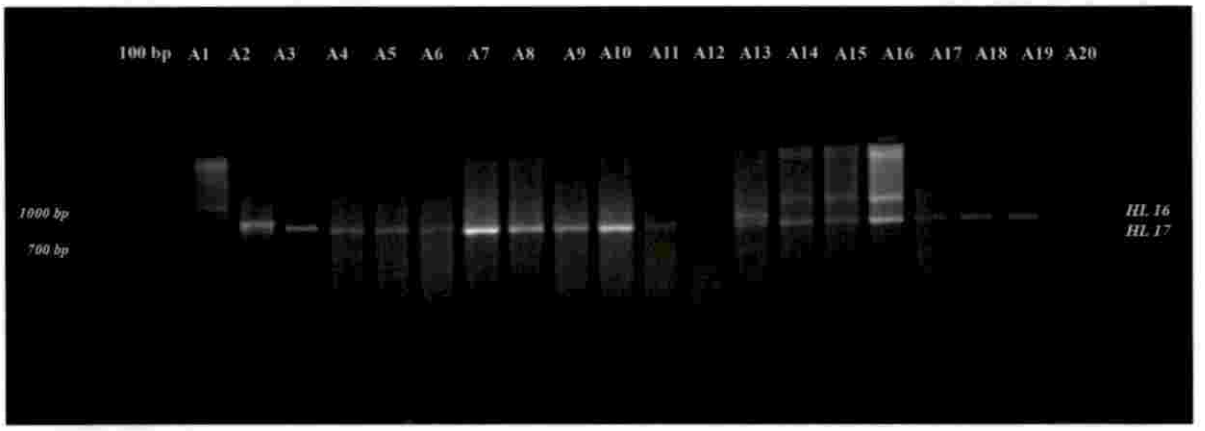

Plate 19. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-547

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12. A20-PTB 8

PTB 8. Primer produced allele HL 7 at 700 bp was absent all accessions except ABL 12, Chettivirippu, VTL-1, Pokkali, VTL-2, PTB 13, and PTB 8.

4.3.9 RM597

The amplification profile of SSR Marker RM-547 is given plate 20. . Primer produced two alleles HL18 and HL19 at 1000bp and 700bp. Allele HL18 was present only PTB 12 and PTB 13 and all other accessions it was absent. In allele HL19 was present all accessions except ABL 12.

4.3.10. RM 204

The amplification profile of SSR Marker RM-204 is given plate 21. Primer produced one allele at 200 bp which is monomorphic.

4.4 CLUSTERING OF GENOTYPES USING SSR MARKERS

The clustering of genotypes using SSR markers was done using UPGMA clustering using NTSYS software. The dendrogram constructed is given in Fig.9. The clusters and sub clusters at different phenon levels is given in Table 8.

Table : 8. Cluster/ sub cluster at different phenon levels in the dendrogram constructed based on UPGMA sub clustering using SSR scores

<table>
<tr><td colspan="4">No: of Clusters / sub clusters at different phenon levels</td></tr>
<tr><td>40%</td><td>50%</td><td>60%</td><td>70%</td></tr>
<tr><td>I</td><td>1</td><td></td><td></td></tr>
<tr><td rowspan="2">II</td><td rowspan="2">IIA</td><td>IIAA
IIAB</td><td>IIAA1(9)
IIAA2(8)</td></tr>
<tr><td rowspan="2">IIB</td><td colspan="2" rowspan="2"></td></tr>
</table>

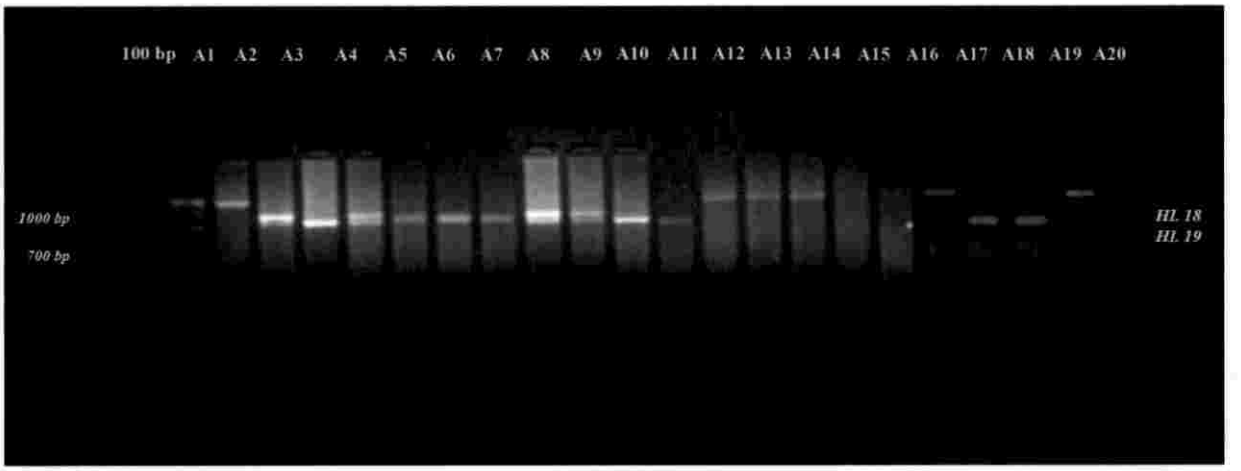

Plate 20. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-592

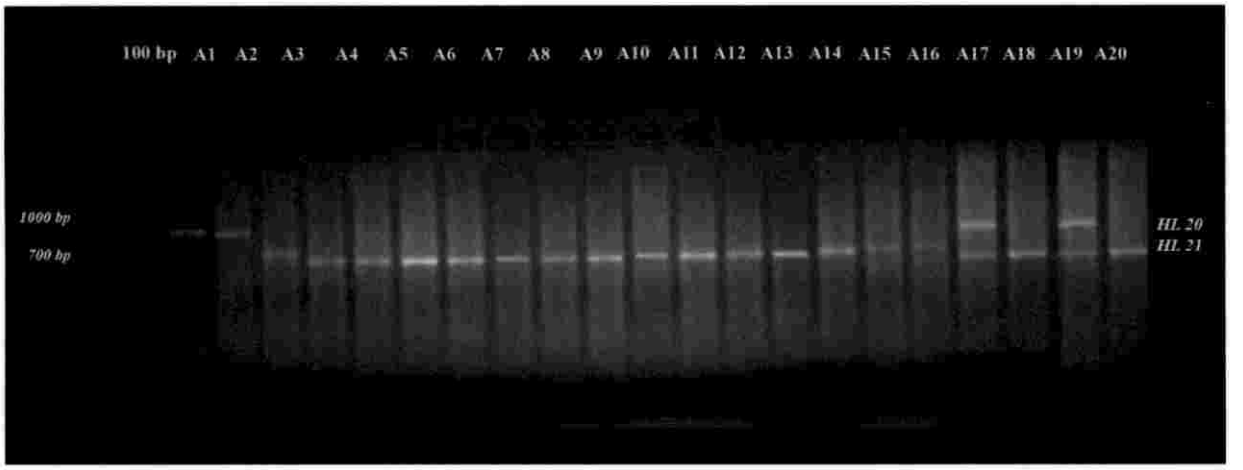

Plate 21. Amplification profile of the DNA of twenty rice accessions using the SSR marker RM-204

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12. A20-PTB 8

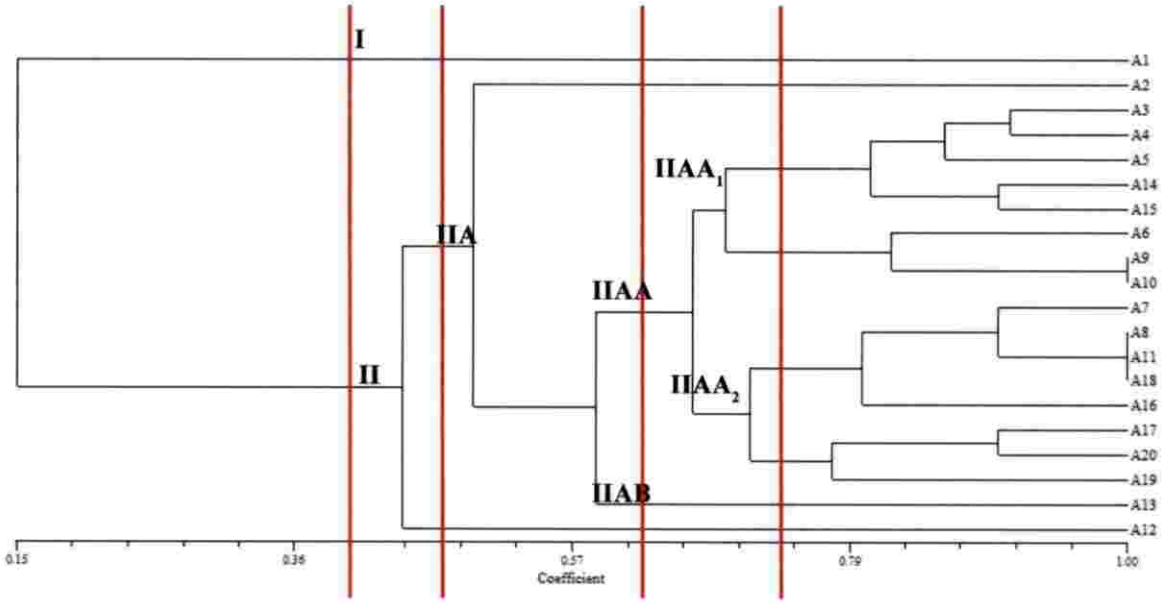

Fig.9. Cluster dendrogram of rice varieties based on SSR markers

A1-ABL12, A2-ABL13, A3-AMB14, A4-AMB22, A5-AMB5, A6-TRV2108, A7-Chettivirippu, A8-Karavala kochuvithu, A9-Kochu vithu – shornad, A10-Vellakuttadan, A11-T. Virippu, A12-Chettivirippu, A13-VTL-1, A14-Pokkali, A15-VTL-2, A16-PTB 17, A17-PTB 13, A18-PTB 2, A19-PTB 12, A20-PTB 8

The Dendrogram constructed based on the scores of SSR marker produced two cluster at 40% similarity. Cluster I had only one member (ABL 12). Cluster II produced two sub clusters at 50% similarity II A with 18 members and II B with single member Chettivirippu. IIA produced two sub cluster at 60% similarity, IIAA with 17 members and IIAB with single member VTL-1. At 70% similarity cluster IIAA produced two sub cluster IIAA1and IIAA2. Accession Kochu vithu - shornad and Vellakuttadan showed 100%similarity. Karavala kochu vithu, T. Virippu and PTB 2 also sowed 100% similarity.

DISCUSSION

5. DISCUSSION

Rice is the staple food for majority of Keralites. Rice (*Oryza sativa*) is cultivated in Kerala traditionally long back. In Kerala rice is cultivated in different agroclimatic zones like the traditional rice tract, Pokkali area, hills of Wayanad and central zone with kole lands. Genetic diversity in the traditional rice varieties of Kerala is also very high (Kumary *et al.*, 2003,). Molecular markers are valuable for diversity analysis is on they are least affected by environment.

Genetic diversity in the present study twenty accessions collected from four agroclimatic zones, Moncompu, Pokkali, Wayanad, and central Kerala is assessed by using two molecular markers, RAPD and SSR. The results obtained are discussed elaborately in this chapter.

5.1 DIVERSITY ANALYSIS WITH RAPD MARKERS

The RAPD markers are small oligo nucleotide primers usually 10 bp in length of arbitrary sequence to generate bands profiles. These primers bind to the complementary sequences along the genome and PCR amplification occurs when the regions between the opposing primer sites are within amplifiable distances

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 (Hardrys *et al.,* 1992).

In the present study ten random primers were used to amplify the genomic DNA and twenty rice accessions. These primers were selected based on the previous reports (Manohar *et al.*, 2011) and one more.

The polymorphism ranged from 66.66 % to 100 %. Skaria *et al.*, 2011 reported 72% polymorphism in his study of ten varieties of Kerala with 30 RAPD primers. OPD-08 gave 100 % polymorphism. The polymorphism information content (PIC) ranged from 0.44 to 0.74. Primer OPF-01 had the highest value of 0.82. Considering

resolving power OPC-07 (15.4) had the highest value but its PIC and EMR were considerably low. Considering all the marker parameters together primer OPF-06 is formed to be the best RAPD primer with considerably high PIC, RP, and EMR. Manohar *et al.*, 2011 hard reported twenty oligonucleotide primer as the best considering these three parameters.

The dendrogram constructed based on the RAPD scoring showed that varieties Pokkali and VTL-2 had maximum similarity. These two were from Pokkali rice, PTB 12 from Pattambi were found to be unique and is clustered using others only at 30% similarity.

The clustering of the genotypes did not show any correlation with the geographic origin. ABL 12 and VTL- 2 showed 70 % similarity but those two were from Wayanad Hills and Pokkali tract. Vellakuttadan from Moncombu clustered with PTB 2 from pattambi at 72% a similarity. Kochuvith and Vellakuttadan from Moncombu clustered at 67 % Manohar *et al.*, 2011 also reported that there was no correlation with the RAPD clustering and Geographic origin.

5.2 DIVERSITY ANALYSIS WITH SSR MARKERS

Simple sequence repeats markers being more stable, reproducible and codominant in inheritance are more reliable on diversity analysis is hence the twenty genotypes were analyzed using ten SSR markers. These markers were selected based on previous reports.

All the SSR markers produced two alleles except RM 210 and RM 204 which produced four alleles and one allele respectively. Hossain et al., 2007 hard reported that number of alleles per locus ranged from three to nine. All the alleles of all the markers wear polymorphic except that of the polymorphism information content RM 204 ranged from 0.44 to 0.88. Ravi *et al.*, 2003 had reported that the PIC ranged from 0 to 0.890 in the case of SSR markers.

Bansal *et al*., 2013 reported that highest PIC value of 0.97 was reported by RM 222 and RM 206 in this study the highest PIC value of 0.88 was reported by RM 210 followed by RM 567 is 0.85. The resolving power and EMR was also highest for RM 210.

The dendrogram constructed based on the SSR markers would give a clustering of genotypes more correlated with the geographic origin. Genotypes Kochuvithu and Vellakuttadan showed 100 % similarity both where from Kuttanad. But Karavalakochuvith, T.virippu, and PTB 2 with and PTB 2 also showed 100% similarity but these three were from monomorphic, Pokkali and Central Kerala respectively. At around 90 % similarity AMB 14, AMB 22, Pokkali, VTL-2, PTB 13 PTB 8, and ABL14 from Wayanad. Pokkali, PTB 13 and PTB 8 from Pattambi Clustered showing more correlation to the Geographic origin.

SSR markers being sequence specific and flanking the repeat sequence which has more role in evolution, are more reliable in predicting the Genetic diversity based on origin.

5.3 COMPARISON OF RAPD MARKERS AND SSR MARKERS

RAPD Markers are randomly hitting the genome and SSR markers hit the genome specifically targeting the repeat sequences. In this study the highest PIC for RAPD marker was 0.82 and for SSR marker was 0.88. Highest resolving power for RAPD was 15.4 and SSR it were only 2.05 this due to the less number of products in SSR. Since both of them could not give a clear-cut clustering based on geographic origin an analysis using both the members together was done.

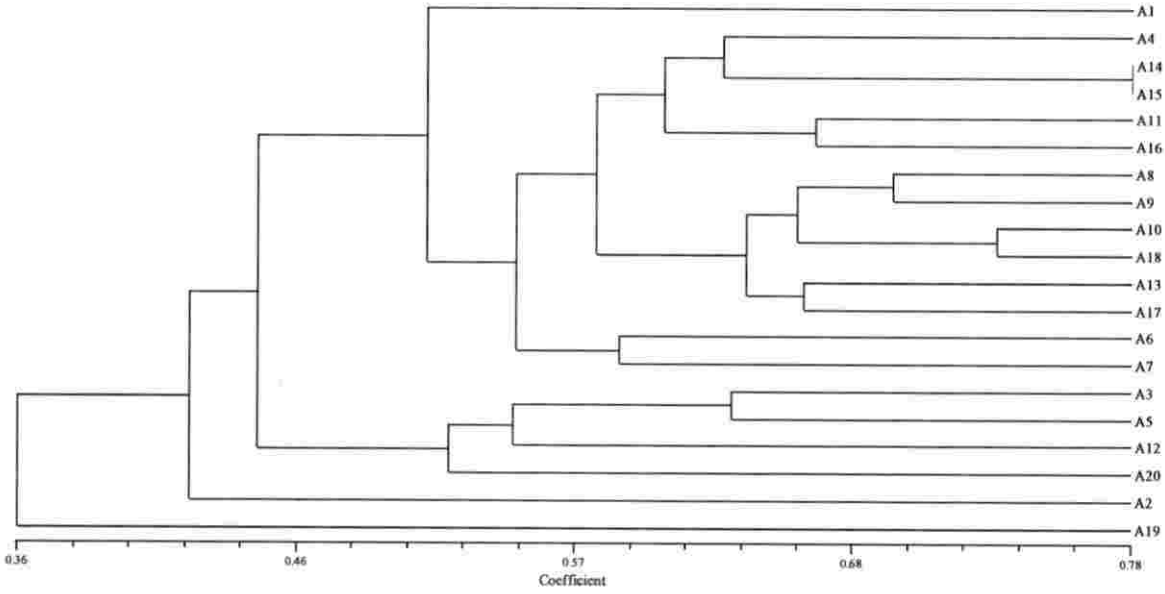

Fig.10 Cluster dendrogram of rice varieties based on RAPD and SSR markers

Dendrogram constructed based on both RAPD and SSR markers gave a better correlation with respect to the geographical origin (Fig.10). But here also the varieties from Wayanad ABL 12 to AAMB 5 were scattered in different clusters. Only AMB 14 and AMB 5 (AMB 14 and AMB 5) clustered at 60 percentage similarity. The accessions from TRV2108 to Vellakuttadan clustered at around 50 % similarity. In the accession from Pokkali tract T.virippu to VTL-2 clustered at 78 % similarity. Accession from central zone zone Pattambi PTB 17 to PTB 8 was scattered into different clusters. PTB 12 and PTB 12 was unique from other accessions.

This molecular diversity analysis of the traditional rice genotypes from four different agroclimatic zones could find that the the maximum similarity was 78% and that too only between two accessions. The diversity among the genotypes was 64% as all the genotypes clustered at 36% similarity. The clustering of the genotypes did not show any correlation with the geographic origin. Exchange of varieties between the farmers and some amount of natural crossing would have led to the mixture of populations of rice genotypes in different agroclimatic zones.

SUMMARY

6. SUMMARY

The present study entitled "Genetic diversity analysis of indigenous rice varieties in Kerala using molecular markers "was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2018.

The study was conducted with the objective to analyse the genetic diversity of traditional rice varieties in four agroclimatic zones of Kerala using RAPD and SSR markers.

Five varieties were collected from the each agro climatic zones *viz*., hill areas of Wayanad, rice growing tract of Palakkad, saline soils of Pokkali and Kuttanad soils. The DNA was isolated and RAPD analysis with 10 Operon primers and SSR analysis was done with ten RM primers.

In the present study ten random primers were used to amplify the genomic DNA and twenty rice accessions. These primers were selected based on the previous reports. The ten operon primers produced 88 amplicons with an average polymorphism 82%. The polymorphism obtained for the different operon primers ranged from 66.66 % to 100 %. OPD-08 gave 100 % polymorphism. The polymorphism information content (PIC) ranged from 0.44 to 0.74. Primer OPF-01 had the highest value of 0.82. Resolving power OPC-07 (15.4) had the highest value but its PIC and Effective Multiplication Ratio (EMR) were considerably low. Considering all the three parameters together primer OPF-06 is found to be the best RAPD primer with considerably high polymorphism information content, resolving power and effective multiplication ratio.

The dendrogram constructed based on the RAPD scoring showed that varieties Pokkali and VTL-2 had maximum similarity. These two were from Pokkali rice tract. PTB 12 from Pattambi was found to be unique and it clustered with others only at 30% similarity.The clustering of the genotypes did not show any correlation

with the geographic origin. ABL 12 and VTL- 2 showed 70 % similarity but those two were from Wayanad Hills and Pokkali tract respectively. Vellakuttadan from Moncombu clustered with PTB 2 from pattambi at 72% a similarity. Kochuvith and Vellakuttadan from Moncombu clustered at 67 %. Manohar *et al.*, 2011 also reported that there is no correlation with the RAPD clustering and Geographic origin.

All the SSR markers produced two alleles except RM 210 and RM 204 which produced four alleles and one allele respectively. All the alleles of all the markers were polymorphic except that of primer RM204. The polymorphism information content of the SSR primers used in the study ranged from 0 to 0.88. In this study the highest PIC value of 0.88 was reported by RM 210 followed by RM 567 (0.85). The resolving power and EMR was also highest for RM 210.

The dendrogram constructed based on the SSR markers could give a clustering of genotypes more correlated with the geographic origin. Genotypes Kochuvithu and Vellakuttadan showed 100 % similarity both where from Kuttanad. But Karavalakochuvith, T.virippu, and PTB 2 also showed 100% similarity but these three were from Moncompu, Pokkali and Palakkad respectively. At around 90 % similarity AMB 14, AMB 22 from Wayanad, Pokkali andVTL-2 from Pokkali, PTB 13 and PTB 8 from Palakkad clustered showing more correlation to the Geographic origin. SSR markers being sequence specific and flanking the repeat sequence which has more role in evolution, are more reliable in predicting the Genetic diversity based on origin.

RAPD Markers are randomly hitting the genome and SSR markers hit the genome specifically targeting the repeat sequences. In this study the highest PIC for RAPD marker was 0.82 and for SSR marker was 0.88. Highest resolving power for RAPD was 15.4 and SSR it was only 2.05 this is due to the less number of products in SSR. Since both of them could not give a clear-cut clustering based on geographic origin an analysis using both the markers together was done. This gave a better

picture of the clustering as it involved more number of variables. But here also the varieties from Wayanad A1 to A5 were scattered in different clusters. Only A 14 and A15 (AMB 14 and AMB 5) clustered at 60 percentage similarity. The accessions from moncompu (A6-A10) clustered at around 50 % similarity. In the accession from Pokkali tract (A11-A15) only T.virippu to VTL-2 clustered at 78 % similarity. Accession from central zone Pattambi (A16-A20) was scattered into different clusters. PTB 12 was unique from other accessions.

This molecular diversity analysis of the traditional rice genotypes from four different agro climatic zones could find that the the maximum similarity was 78% and that too only between two accessions. The diversity among the genotypes was 64% as all the genotypes clustered at 36% similarity. The clustering of the genotypes did not show any correlation with the geographic origin. Exchange of varieties between the farmers and some amount of natural crossing would have lead to the mixture of populations of rice genotypes in different agro climatic zones.

REFERENCES

REFERENCES

Alam, M. S., Begum, S. N., Gupta, R. and Islam, S. N. 2014. Genetic diversity analysis of rice (*Oryza sativa* L.) landraces through RAPD markers. *Int. J. of Agric. Res. Innovation and Technol. 4*(1): 77-87.

Aljumaili, S., Rafii, M. Y., Latif, M. A., Sakimin, S. Z., Arolu, I. W. and Miah, G. 2018. Genetic diversity of aromatic rice germplasm revealed by SSR markers. *BioMed Res. Int.*

Bansal, H., Kumar, R., Vivek, V. and Sanjay, S. 2013. Analysis of diversity in rice (*Oryza sativa* L.) using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers. *African J. of Biotechnol.* 12(35): 5404-5412.

Beckmann, J. S. and Weber, J. L. 1992. Survey of human and rat microsatellites. *Genomics*, 12(4): 627-631.

Calpe, C. 2017. Rice international commodity profile. *Rome: Food and Agricultural Organization of the United Nations.*

Chen, C., He, W., Nassirou, T. Y., Nsabiyumva, A., Dong, X., Adedze, Y. M. N. and Jin, D. 2017. Molecular characterization and genetic diversity of different genotypes of *Oryza sativa* and *Oryza glaberrima. Electr. J. of Biotechnol. 30*, 48-57.

Collard, B. C., Bertrand, C. Y. Jahufer, M. Z., Brouwer, J. B. and Pang, E. C. 2005. "An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts." *Euphytica* 142(2): 169-196.

Corley Smith, G. E., Lim, C. J., Kalmar, G. B., and Brandhorst, B. P. 1997. Efficient detection of DNA polymorphisms by fluorescent RAPD analysis. *BioTechniques* 22: 690-692.

Hadrys, H., Balick, M., and Schienvater, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* 1, 55-63.

Hartl, D. L. and Clark, A. G. 1997. Principles of population genetics. 3rd ed. Sunderland, Massachsetts: Sinauer Associates. 163p.

Hartl, D. L. and Jones, E. W. 2005. DNA Structure and DNA manipulation. *Genetics: analysis of genes and genomes*, 36-85.

Heinrichs, E. A., Medrano, F. G. and Prapusa, H. R. 1985. *Genetic evaluation for insect resistance*. International Rice Research Institute, Manila, Philippines, 365p.

Hollingsworth, W. O., Christie, C. B., Nichols, M. A., and Neilson, H. F. 1998. Detect ion of variation among and within asparagus hybrids using random amplified DNA (RAPD) markers. *New Zealand J. Crop Hortic. Sci.* 26: 1-9.

Hossain, M. Z., Rasul, M. G., Ali, M. S., Iftekharuddaula, K. M., and Mian, M. A. K. 2007. Molecular characterization and genetic diversity in fine grain and aromatic landraces of rice (*Oryza sativa* L.) using microsatellite markers. *Bangladesh J. of Plant Breed. and Genet. 20*(2): 1-10.

Hue, H. T., Nghia, L. T., Minh, H. T., Anh, L. H., Trang, L. T. T., and Khanh, T. D. 2018. Evaluation of Genetic Diversity of Local-Colored Rice Landraces Using SSR Markers. *Int. Lett. of Nat. Sci*. 67, 24-34.

Huff, D. R., Peakall, R., and Smouse, P. E. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) Engelm. *Theor. Appl. Genet.* 86: 927-934.

Idrees, M. and Irshad, M. 2014. Molecular markers in plants for analysis of genetic diversity: a review. *European Acad. Res*. 2(1): 1513-1540.

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

Jaccard, P. 1908. Nouvelles researches sur la la distribution florale. *Bull. Soc. Vandoise des Sci. Naturelles* 44: 223-270.

Kantety, R. V., La Rota, M., Matthews, D. E. and Sorrells, M. E. 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Mol. Biol.* 48(5-6): 501-510.

Knapik, E. W., Clark, M., Lehrach, H., Postlethwait, J. H. and Beier, D. R. 1998. Gene mapping in zebrafish using single-strand conformation polymorphism analysis. *Genomics*, 51(2): 216-222.

Krupa, K. N., Shashidhar, H. E., Ningaraj, D., Reddy, M., Kumara, V. and Swamy, H. V. 2017. Molecular marker based genetic diversity analysis in rice genotypes (*Oryza sativa* L.) using SSR markers. *Int. J. of Pure and Appl. Biosci.* 5(2): 668-674.

Kumary, L. S. and Francies, M. R. 2002. Collection, Management, Conservation and Utilization of genetic diversity of rice in Kerala In: Nair, R. R., Jyothi, M. L.

and Balalchandran, P. V. (eds), Seventy five years of research, -Kerala Agricultural University, RARS, Pattambi, pp.1-19.

Kumary, S. L., Susamma, P., George, D. S., Aipe, K. C. and Rema, B. N. 2003. Aromatic Rices of Kerala. In: Singh, R. K. and Singh, U. S. (eds), A treatise on the Scented Rices of India. Kalyani publishers, New Delhi, pp. 317-326.

Kumary, S. L. 2012. Status Paper on Rice in Kerala. *Rice Knowledge Management Portal (RKMP), 32.*

Leegood, R. C., Evans, J. R. and Furbank, R. T. 2010. Food security requires genetic advances to increase farm yields. *Nature* 464: 831-831.

Manohar, R., Jayalekshmy, V. G. and Leenakumary, S. 2011. Molecular Diversity Analysis of Traditional Rice Varieties of Kerala Using RAPD Markers. *Indian J. Plant Genet. Resour. 24*(3), 309p.

McCouch, S. R., Teytelman, Y. L., Lobos, K., Clare, M., Walton, B., Fu, R., Maghirang, Z., Li, Y., Xing, Q., Zhang, I., Kono, M., Yano, R., Fjellstrom, G., DeClerck, D., Schneider, S., Cartinhour, D. W., and Stein, L. 2002. Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *DNA Res.* 9: 199-207.

Milbourne, D. R., Meyer, J. E. Bradshaw, E., Baird, N., Bonar, J., and Provan, W. 1997. Comparison of PCR based marker systems for the genetic analysis of relationship in cultivated potato. *Mol. Breed.* 3: 127-136.

Nadia, I., Mohiuddin, A. K. M., Sultana, S., and Ferdous, J., 2014. Diversity analysis of indica rice accessions (*Oryza sativa* L.) using morphological and SSR markers. *Annals Biol. Res.* 5(11).

Nair, M. C. 2004. Comparative biochemical characterization of indigenous medicinal rice *Njavara* and selected cultivars of *Oryza sativa* L. Project report submitted to Kerala State Council for Science, Technology and Environment.

Pammi, S., Schertz, K., Xu, G., Hart, G. and Mullet, J. E. 1994. "Random amplified-polymorphic DNA markers in sorghum." *Theor. Appl. Genet.* 89: 80-88.

Prevost, A. and Wilkinson, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98: 107-112.

Rabbani, M. A., Zahida, H. P., and Masood, M. S. 2007. Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electr. J. Biotechnol.* 11(3): 717-3458.

Rahman, M., Azizur, R. M., Hossain, M., and Rasul, G. 2012. Comparative Study on Morphological, Physiological and Molecular Genetic Diversity Analysis in Rice (*Oryza sativa* L.), *Libyan Agric. Res. Cent. J. Int.* 2 (2): 85-93.

Rahman, S. N., Islam, M. S., Alam, M. S., and Nasiruddin, K. M. 2006. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. *Indian J. Biotech.* 6: 224-229.

Rajani, J., Deepu, V., Nair, G. M., and Nair, A. J. 2013. Molecular characterization of selected cultivars of rice, Oryza sativa L. using Random Amplified Polymorphic DNA (RAPD) markers. *Int. Food Res. J.* 20(2): 919-923.

Rashid, M. M., Shahin, I., Islam, M. A., and Lutful, H. 2018. Genetic diversity analysis of rice landraces (*Oryza sativa* L.) for salt tolerance using SSR markers in Bangladesh. *Fundamental and Appl. Agric. 3*(2): 460-466.

Rashmi, D., Bisen, P., Saha, S., Loitongbam, B., Singh, S., and Singh, P. K. 2017. Genetic Diversity Analysis in Rice (*Oryza sativa* L.) Accessions using SSR Markers. *Int. J. of Agric. Environ. and Biotechnol. 10*(4): 457-467.

Ravi, M., Geethanjali, S., Sameeyafarheen, F., and Maheswaran, M. 2003. Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica.*133: 243-252.

Rohlf, R. J. 1998. NTSYS-PC Version 2.0, Exeter Software, Setauket, New York.

Roy, P. S., Rao, G. J. N., Jena, S., Samal, R., Patnaik, A., Patnaik, S. S. C., Jambhulkar, N. N., Sharma, S. and Mohapatra, T. 2016. Nuclear and chloroplast DNA variation provides insights into population structure and multiple origin of native aromatic rices of Odisha, India. *PLoS One*, 11(9): e0162268.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A. 1988. Primerdirected enzymatic amplification of DNA with a thermostable DNA polymerase. *Nat.* 239: 487-497.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N. 1985. Enzymatic amplification of beta-globin genomic

sequences and restriction site analysis for diagnosis of sickle cell anemia. *Sci.* 230: 1350-1354.

Saker, M. M., Youssef, S. S., Abdallah, N. A., Bashandy, H. S., and El-sharkawy, A. M. 2005. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *Afr. J. Biotechnol.* 4 (9): 882-890.

Schulmann, A. H. 2007. Molecular markers to assess genetic diversity. *Euphytica* 158(3): 313-321.

Showmy, K. S. and Yusuf, A. 2016. Chennellu-a resistant variety of traditional rice cultivar in Kerala against bacterial blight. *Int. J. of Curr. Res.* 8(8) 35739-35743.

Singh, N., Choudhury, D. R., Tiwari, G., Singh, A. K., Kumar, S., Srinivasan, K., and Singh, R. 2016. Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC Genet. 17*(1): 127p.

Skaria, R., Sen, S., and Muneer, P. M. A. 2011. Analysis of Genetic Variability in Rice Varieties (*Oryza sativa* L.) of Kerala using RAPD Markers. *Genet. Eng. and Biotechnol. J.* 241: 1-24.

Temnykh, S., Park, W. D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y. G., Ishii, T. and McCouch, S. R. 2001. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 1441-1452.

Thompson, J., Wailes, E. J., Durand-Morat, A., and Leister, A. 2015. Welfare effects of U.S. liberty link rice contamination. *J. Agric. Appl. Econ.*, 47 (2): 243-259.

Vanaja, T., Randhawa, G. J. and Mammootty, K. P. 2006. Pedigree evaluation and molecular diversity of some true breeding rice (*Oryza sativa* L.) genotypes of Kerala . *J. Trop. Agric.* 44 (1-2): 42-47.

Vaughan, D. A. and Muralidharan, V. K. 1989. Collection of wild relatives from Kerala State, India. *Plant Genet. Resour. Newsl.* 42: 2-6.

Vejl, P. 1997. Identification of genotypes in hop (*Humulus lupulus* L.) by RAPD analsysis using program Gel Manager for Windows. *Rostlinna Vyroba* 43: 325-331.

Virmani, S. S. and Shinjyo, C. 1988. Current status of analysis and symbols for male sterile cytoplasms and fertility restoring genes. *Rice Genet. Newsl.* 5: 9-15.

Weller, J. W. and Reddy, A. 1997. Fluorescent detection and analysis of RAPD amplicons using the ABI PRISM DNA sequencers. In Fingerprinting methods based on arbitrarily primed PCR, edited by Micheli MR and Bova R., 81-92. Springer Lab Manual.

Williams, J. G. K., Kubelik, A. R., Livak, K. L., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.

GENETIC DIVERSITY ANALYSIS OF INDIGENOUS RICE VARIETIES IN KERALA USING MOLECULAR MARKERS

By

AJITH M. K.

(2013-09-120)

ABSTRACT

Submitted in partial fulfilment of the

requirement for the degree of

B. Sc. - M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture
Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA

2019

ABSRACT

The present study entitled "Genetic diversity analysis of indigenous rice varieties in Kerala using molecular markers "was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2018.

The study was conducted with the objective to analyze the genetic diversity of traditional rice varieties in four agro climatic zones of Kerala using RAPD and SSR markers.

Five varieties were collected from the each agro climatic zones *viz*., hill areas of Wayanad, rice growing tract of Palakkad, saline soils of Pokkali and Kuttanad soils. The DNA was isolated and RAPD analysis with 10 Operon primers and SSR analysis was done with ten RM primers.

In the RAPD analysis the ten operon primers produced 88 amplicons with an average polymorphism 82%. Resolving power OPC-07 (15.4) had the highest value but its Polymorphism Information Content and Effective Multiplication Ratio (EMR) were considerably low. Considering all the three parameters together primer OPF-06 is found to be the best RAPD primer with considerably high polymorphism information content, resolving power and effective multiplication ratio.

The dendrogram constructed based on the RAPD scoring showed that varieties Pokkali and VTL-2 had maximum similarity. These two were from Pokkali rice tract. PTB 12 from Pattambi was found to be unique and it clustered with others only at 30% similarity.The clustering of the genotypes did not show any correlation with the geographic origin. ABL 12 and VTL- 2 showed 70 % similarity but those two were from Wayanad Hills and Pokkali tract respectively. Vellakuttadan from Moncombu clustered with PTB 2 from pattambi at 72% a similarity. Kochuvith and Vellakuttadan from Moncombu clustered at 67 %.

All the SSR markers produced two alleles except RM 210 and RM 204 which produced four alleles and one allele respectively. All the alleles of all the markers were polymorphic except that of primer RM204. The polymorphism information content of the SSR primers used in the study ranged from 0 to 0.88. In this study the highest PIC value of 0.88 was reported by RM 210 followed by RM 567 (0.85). The resolving power and EMR was also highest for RM 210.

The dendrogram constructed based on the SSR markers could give a clustering of genotypes more correlated with the geographic origin. Genotypes Kochuvithu and Vellakuttadan showed 100 % similarity both where from Kuttanad. But Karavalakochuvith, T.virippu, and PTB 2 also showed 100% similarity but these three were from Moncompu, Pokkali and Palakkad respectively. At around 90 % similarity AMB 14, AMB 22 from Wayanad, Pokkali andVTL-2 from Pokkali, PTB 13 and PTB 8 from Palakkad clustered showing more correlation to the Geographic origin. SSR markers being sequence specific and flanking the repeat sequence which has more role in evolution, are more reliable in predicting the Genetic diversity based on origin.

Since both of them could not give a clear-cut clustering based on geographic origin an analysis using both the markers together was done. This gave a better picture of the clustering as it involved more number of variables. But here also the varieties from Wayanad A1 to A5 were scattered in different clusters. Only A 14 and A15 (AMB 14 and AMB 5) clustered at 60 percentage similarity. The accessions from moncompu (A6-A10) clustered at around 50 % similarity. In the accession from Pokkali tract (A11-A15) only T.virippu to VTL-2 clustered at 78 % similarity. Accession from central zone Pattambi (A16-A20) was scattered into different clusters. PTB 12 was unique from other accessions.

This molecular diversity analysis of the traditional rice genotypes from four different agro climatic zones could find that the maximum similarity was 78% and

that too only between two accessions. The diversity among the genotypes was 64% as all the genotypes clustered at 36% similarity. The clustering of the genotypes did not show any correlation with the geographic origin. Exchange of varieties between the farmers and some amount of natural crossing would have led to the mixture of populations of rice genotypes in different agro climatic zones.