DNA FINGERPRINTING OF PROMISING SELECTIONS OF JACK (*Artocarpus heterophyllus* Lam.) USING MOLECULAR MARKERS

By

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(2015-11-106)

THESIS

Submitted in partial fulfilment of requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY
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KERALA, INDIA
2017

CERTIFICATE

I hereby declare that the thesis entitled **"DNA fingerprinting of promising selections of jack (*Artocarpus heterophyllus* Lam.) using molecular markers"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other university or society.

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Acknowledgement

First and foremost, I humbly bow my head before the **God Almighty** *for the unmerited blessings showered on me to successfully complete the endeavour.*

It is with great respect and devotion, I place on record my deep sense of gratitude and indebtedness to **Dr. Anu G. Krishnan**, *Assistant Professor (Hort.), RARS, Kumarakom and chairperson of my Advisory Committee for her unrelenting and inspiring guidance, untiring help, patience, encouragement, constructive criticism, precious suggestions and gracious approach throughout the course of study and the period of the investigation and preparation of the thesis. I consider myself being fortunate in having the privilege of being guided by her.*

I gratefully express my sincere gratitude to **Dr. M. R. Shylaja**, *Professor and Head, CPBMB, member of my advisory committee for her valuable suggestions, critical assessments, and guidance rendered to me for the completion of the research programme and preparation of the thesis.*

No words can truly express my profound sense of gratitude to **Dr. Minimol J. S.** *Assistant Professor (Plant Breeding and Genetics), Cocoa Research Centre, Vellanikkara and member of my advisory committee for the generous and timely help, valuable suggestions and critical comments always accorded to me during the course of this study.*

I am deeply obliged to **Dr. P. S. Abida**, *Professor (Plant Physiology), RARS, Pattambi and member of my advisory committee for her unfailing support and relevant suggestions throughout the period of the work. I thank her for all the help and cooperation she has extended to me.*

I express my heartiest gratitude to my beloved teachers, **Dr. P. A. Valsala**, *Professor (Plant biotechnology) and* **Dr. Deepu Mathew**, *Assistant Professor (Plant Biotechnology),* **Mrs. Binu** *(Teaching assistant) for their encouragement, valuable help and advice rendered during the course of study.*

I wish to express my sincere gratitude to **Mr. Vinu Prasad** *(Field assistant),* **Mrs. Anu Cyriac** *(SRF), and* **Ms. Reshma John** *(SRF) for the sincere help, timely suggestions and mental support during the research works.*

I wish to express my gratitude to my respected senior **Mr. Sujith. S. and Mrs. Manjusha Rani** *of Dept. of Plant Biotechnology for help and support during the research works.*

I am extremely happy to place on record my sincere appreciation to my beloved friends **Ms. Lekshmi Sekhar, Mrs. Shamla K, Mrs. Nadiya Rehman, Mrs. Aishamol P. B, Ms. Arunima V, Ms. Geethu Gogul, Ms. Deepali Rahi, Ms. Priya sonkamble, Ms. Anandu Chanra Giridari, Mr. Aswin**

Vargeese, Mr. Basil Babu Paul, Mr. Debashis Sahoo, Mr. Kishore Jeghule, Mr. Swapnil Waghmare and Mr. Kabade Pramode for the love, support and affection they rendered towards me.

I thankfully remember the services rendered by all the staff members of CPBMB, Student's Computer Club, Library, Office of COH, and Central Library, KAU.

I am thankful to Kerala Agricultural University for technical and financial assistance for carrying out my study and research work.

*Words cannot really express the love, care and boundless support that I relished from my beloved parents **Mr. Abdulla A. C.** and **Mrs. Beefathummabi P. S,** my brother **Rifayi P. S,** my sister **Nafeesathul Misriya**, my **grandparents** and from my **entire family**. I am affectionately dedicating this thesis to them for their selfless sacrifice, constant encouragement, motivations, warm blessings and unflagging interest towards me throughout these years.*

*Above all, my success would have remained an illusion without the ambitious encouragement, unqualified love, constant support and affection showed on me by **Jabir M. P.** I thank him for all the support he has extended to me*

Marjan P.S.

Dedicated to my beloved parents, sister, brother and teachers

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ABBREVATIONS

%	Percentage
B	Beta
µg	Microgram
µL	Microliter
AFLP	Amplified Fragment Length Polymorphism
Bp	Base pair
Cm	Centimetre
CAPS	Cleaved Amplified Polymorphic Sequence
CPBMB	Centre for Plant Biotechnology and Molecular Biotechnology
CTAB	Cetyl Trimethyl Ammonium Bromide
°C	Degree Celsius
DNA	Deoxyribonucleic acid
DNTPs	Deoxyibo Nucleoside Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EST	Expressed Sequence Tags
G	Gram
IPGRI	International Plant Genetic Resources Institute
IPR	Intellectual Property Rights
ISSR	Inter Simple Sequence Repeat
KAU	Kerala Agricultural University
Kb	Kilo base pairs
L	Litre
M	Molar
MAS	Marker Assisted Selection
Mg	Milligram
MI	Marker Index
Ml	Millilitre
Mm	Milli Molar
ng	Nano gram
OD	Optical Density
PCR	Polymerase Chain Reaction
pH	Hydrogen ion concentration

PIC	Polymorphic Information Content
PVP	Poly Vinyl Pyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
Rpm	Revolution per minute
RNA	Ribonucleic acid
RNase	Ribonuclease
SCAR	Sequence characterized amplified region
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
STS	Sequence Tagged Sites
TAE	Tris Acetate EDTA
TE	Tris EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultra Violet
V	Volts

Introduction

1. INTRODUCTION

The jack (*Artocarpus heterophyllus* Lam.) is a fruit crop which belongs to the family Moraceae. It is originated from Western Ghats of India (Rowe-Dutton, 1985). Malaysia, Bangladesh, Vietnam, Thailand, China, Myanmar, Indonesia, Sri Lanka are the other jack fruit producers. Jackfruit is the National fruit of Bangladesh and it is also known as "Poor man's food" (Rahman *et al.,* 1995). In Western Ghats it is found up to 1500 m and has tremendous diversity (Muralidharan *et al.*, 1997). Jackfruit is the World's largest edible fruit reaching up to 50 kg in weight and 60-90 cm in length and produce higher yield than any other fruit crops (Naik, 1949 and Sturrock, 1959). India is one of the major jackfruit producing country and it is an important component in the homestead gardens of Kerala. In India, Kerala is the largest producer of jackfruit and it is about 28 lakh tonnes from an area of 89702 ha. Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, West Bengal, Assam, Tripura, Bihar and Uttar Pradesh are other jack growing states (APAARI, 2012).

Jack has not yet gained a commercial status. However it is an important component in homesteads of Kerala. It produces about 700 fruits in a year. Jackfruit is a multiple fruit composed of large number of flowers with edible petals. It is also a multipurpose plant, it give food, fuel, timber and medicinal products. Both ripe and unripe fruits are used for different edible purposes. The ripe fruit is fleshy and sweet. It can be used for making jam, jelly, canned products, squash etc. It is a good source of vitamins and minerals and the unripe fruits are used as vegetable. The jackfruit seeds are also edible, it can boil or roast. It also provides flour for baking and flavour for cooked dishes (APAARI, 2012). The aged tree timber has an orange or reddish brown colour with anti- termite properties. So, it is used for the preparation of a dye, which is used to give the orange- red colour to the robes of Buddhist priests (Elevitch and Manner, 2006). The leaves and fruit waste can be used as fodder. Jackfruit is highly nutritious as it contains carbohydrates, proteins, minerals, and vitamins. Jackfruit is used as staple food in some regions during the times of scarcity, because of its higher quantity of carbohydrate (APAARI, 2012). The ripe jackfruit flakes contains 18.9 g carbohydrate, 0.8 g minerals, 30 IU vitamin A and 0.25 mg thiamine per every hundred gram (Samaddar, 1985). It also contains isoflavones, antioxidants, and phytonutrients which help to fight against ulcer, indigestion and cancer. A jack extract called 'Jacaline' is used in treatment of AIDS (Prakash *et al.,* 2009).

Jack is one of the most drought tolerant, hardy fruit crop. The tree is monoecious, cross pollinated and mostly seed propagated. Hence high variability occurs in fruit shape and quality. The knowledge about genetic diversity of jack is important to identify superior genotypes for cultivation. In Kerala, such studies have resulted in the release of a new jack variety 'Sindhur' and selection of some superior genotypes. RARS, Kumarakom also have conducted studies on the variability of jack fruit in Kuttanad region and identified few promising genotypes.

Central Seed Committee established under the Seed Act (1966) insists DNA fingerprint data for the varieties released or proposed to be released. The specific fingerprint data will serve as a mark for identifying the varieties and could be utilized for registration and documentation of varieties, settling IPR issues and to avoid bio piracy. Till date, no one has made any attempt to develop the DNA fingerprints of different jackfruit cultivars in Kerala. The objective of present study was to characterize the released jack variety Sindhur, cultivar Muttom varikka and superior jack selections identified at RARS, Kumarakom.

Review of Literature

2. REVIEW OF LITERATURE

2.1 General background

Jackfruit (*Artocarpus heterophyllus* Lam.) presumably originated in the Western Ghats of India (Rowe-Dutton 1985) and has now been introduced and cultivated in many tropical countries like West Africa (Burkill, 1997), Southeast Asia (Rahman *et al.*, 1999), South Florida (Schnell *et al.,* 2001) and in Northern Australia (Azad *et al.,* 2007). India is one of the leading producers of jackfruit. Kerala accounts for the largest share of jackfruit produced in India, producing about 28 lakhs tonnes from an area of 89702 ha. Jack finds importance as an extensively grown tree in Kerala's homesteads. Other Indian states growing jack include Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, West Bengal, Assam, Tripura, Bihar and Uttar Pradesh (APAARI, 2012). It is the National fruit of Bangladesh and is also known as "Poor man's food" (Rahman *et al.*, 1995).

Jack is an evergreen tree, reaching up to a height of about 8-25m. It produces heavy yield than any other tree species; it is cheap and plentiful during the season (January - February to May -June). Jack bears the largest edible fruit (up to 35 kg) and has several uses. The green fruit and starchy seeds is used as a vegetable and the ripe fruit has sweet flush and eaten as row. The fibrous rind of the fruit and leaves are considered as a fodder (Ying-zhi *et al.*, 2010). Ripe fruits are highly nutritive; every 100 g of ripe flakes contains 287-323 mg potassium, 30.0-73.2 mg calcium and 11-19 g of carbohydrates (Samaddar, 1985). The tree timber has anti-termite property and wood chips yield a dye, which gives orange-red colour to the robes of Buddhist priests (Elevitch and Manner, 2006). Besides this, many other parts of the plant, including the leaves, bark, fruits and roots have medicinal properties (Hakim *et al.*, 2005; Arung *et al.*, 2006). The presence of isoflavones, antioxidants, and phytonutrients in the fruits indicate that jackfruit has cancer fighting properties. It is also known to help cure ulcers and indigestion. It had been reported that jackfruit could be very useful in the treatment of AIDS (Prakash *et al.,* 2009).

Jackfruit is a tetraploid tree crop, its somatic chromosome number is (4n) 56 (Darlington and Wylie, 1956). Being an indigenous, cross pollinated and propagated through seeds, there exist lots of variability in this tree.

Identification of superior types for cultivation is essential for extending the knowledge about genetic diversity. Such studies carried out in Kerala have resulted in the release of a new jack variety 'Sindhur' and selection of some superior genotypes. Studies on the variability of jackfruit in Kuttanad region have been carried out in RARS, Kumarakom and a few promising genotypes have been identified (Krishnan *et al.*, 2015a). Central Seed Committee established under the Seed Act (1966) insists DNA fingerprint data for the varieties released or proposed to be released. The specific fingerprint data will serve as a mark for identifying the varieties and could be utilized for registration and documentation of varieties, settling IPR issues and to avoid bio piracy. The present study would help to characterize the released jack variety Sindhur, cultivar Muttom varikka and the superior jack selections identified at RARS, Kumarakom.

2.2 Usefulness of genetic markers in plants

Variation within or between the populations, generated from genes and/or environmental effects, can be easily evaluated through the use of genetic markers. It has been described before the discovery of nucleic acid and proteins (Park *et al.*, 2009). A genetic marker can be defined in many ways. According to King and Stansfield (1990), it is a gene whose phenotypic expression is usually easily discerned, used to identify an individual or a cell that carries it, or as a probe to mark nucleus, chromosomes, or locus. According to different criteria, genetic markers are broadly classified into three categories: morphological markers (those based on visually assessable traits), biochemical markers (those based on gene product), and molecular markers (those relying on a DNA assay) (Semagn *et al.*, 2006). Applications of genetic markers include: describing mating systems and levels of inbreeding, identifying genetic variation, Inferring taxonomic and phylogenetic relationships among species, Fingerprinting and germplasm identification, Constructing genetic linkage maps and Marker assisted breeding.

2.3 Morphological markers in plant characterization

In earlier times, morphological traits like flower colour, seed colour, pod colour, hilum colour, pubescence colour, rind colour, flesh colour, leaf shape, fruit shape, awn type and length, stem length etc were considered as markers for diversity studies in plants (Ganesan *et al.*, 2014). Morphological markers have a crucial role in

choosing desirable traits for plant breeding programs (Khalid *et al.*, 2010). Monogenic morphological markers are usually easy, fast and cheap to score (Ghafoor, 1999). These markers are usually limited, but procedure of this assay is very simple, and there is no need of sophisticated equipments for performance. Type, number and linkage relationship of markers are depends upon the amount of information provided by the markers (Singh & Singh, 1992). Some molecular markers are appropriate for plant germplasm assessment (Gottlieb, 1984; Hilu, 1984). But morphological traits display continuous deviation between individuals and also show pleiotropism and multifactorial effect, which confuse the characterization of plant populations (Park *et al.*, 2009). Investigations so far have revealed that morphological markers are useful for studying diversity of jack population (Krishnan *et al.*, 2015a; Ashwini, 2015).

2.3.1 Morphological scoring of genotypes

Morphological scoring is simple and easy to conduct. Bioversity international (2006) recommended a list of standard descriptors to characterize jackfruit germplasm. Azad *et al.* (2007) reported that quantitative characters like weight of fruit, length of fruit, diameter of fruit, girth of fruits, number of bulbs per fruit, percentage of pulp, percentage of rachis and percentage of rind were poorly effected by environment, but characters like brix (%), bulb weight and seed weight were highly effected.

Ashwini (2015) conducted a study for morphological characterization of twenty accessions and three varieties of jackfruits. She evaluated five tree characters, five leaf characters, six inflorescence characters and twenty four fruit characters for assessing variability among varieties.

Krishnan *et al.* (2015a) identified ten firm fleshed jack genotypes in Kuttanadu regions. Quantitave characters like fruit weight, bulb mass, total seed weight, flake mass and flake thickness were evaluated in these 10 jack genotypes. Cluster analysis grouped these genotypes into four distinct clusters.

2.4 Biochemical markers in plant characterization

A biochemical marker is evolved from the study of chemical products of gene expression. They are also called as alloenzyme/ isoenzyme markers or protein markers (Karp *et al.*, 1997). Isozyme techniques are quick, simple and low cost and

are used as supplementary markers on molecular genetics maps (Gill *et al.*, 1991; Korzun *et al.*, 2001). Isoenzyme could be useful in breeding as markers of important genes, which is difficult to expose (Stuber, 1989). Allozymes are codominant markers and that have high reproducibility (Dudnikov, 2002).

Protein markers have the drawbacks of tissue and development stage specificity as well as limitation in the number of detectable isozymes (Park *et al.*, 2009). They show low level of polymorphism. Besides, identical electrophoretic mobility proteins may not be homologous for distantly related germplasm and their selective neutrality may be in question (Berry and Kreitman 1993; Hudson *et al.*, 1994; Krieger and Ross, 2002).

Allozymes have been applied in many fields, including fingerprinting (Tao and Sugiura, 1987), diversity studies (Ronning and Schnell, 1994), studies on interspecific relationships (Garvin and Weeden, 1994), to study parents in hybrids (Parani *et al.*, 1997) and mode of genetic inheritance (Warnke *et al.*, 1998).

2.5 Molecular markers in plant characterization

Molecular marker is also called as DNA marker and was developed in 1980. It is a segment of DNA found at specific locations of the genome and is used to 'flag' the inheritance of a particular character or location of a particular gene. It can be detected through exact laboratory techniques (Datta *et al.*, 2011). DNA marker systems have lots of advantages over other marker technologies. Primarily, a limitless amount of DNA markers can be generated; secondly, environmentally unaffected DNA marker profiles can be developed, and thirdly these DNA markers are not inhibited by tissue or developmental stage specificity like isozyme markers (Park *et al.*, 2009).

The finding of molecular markers in recent years has significantly improved the scope for detailed genetic analysis and improved the efficiency of plant breeding programs, which leads to the improvement of plants. DNA markers play two key roles in plant breeding programs; it act as a particular marker linked to phenotypic traits of interest to breeder and as a source of genetic finger prints. These markers are outstanding tools for genetic diversity analysis, to study the genetic relationships,

genome mapping, gene tagging and for using in plant variety rights (PVR) (Gopalsamy *et al.*, 2012).

The Molecular markers can be classified into various groups based on; style of transmission (biparental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance, or paternal organelle inheritance), form of gene action (dominant or codominant markers) and technique used for detection and amplification (hybridization-based or PCR-based markers) (Semagn *et al.*, 2006).

Depending on mode of analysis, DNA marker can be classified into two groups; hybridization-based markers and PCR-based markers. Restriction fragment length polymorphism (RFLP) is a hybridisation based marker where as Random amplified polymorphic DNA (RAPD), Sequence characterized amplified region (SCAR) and Sequence tagged sites (STS), Cleaved amplified polymorphic sequence (CAPS), Amplified fragment length polymorphism (AFLP), Simple sequence repeat (SSR), Inter simple sequence repeat (ISSR) and Single nucleotide polymorphism (SNP) markers are some PCR based markers (Datta *et al.*, 2011).

2.6 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is an enzymatic technique to amplify a specific DNA segment *in vitro* using two site specific primers that hybridise to complimentary DNA strands (Mullis and Floona, 1987). The method produces large amount of specific DNA from a complex DNA template in a single enzymatic reaction within a matter of hours. The invention of PCR was a breakthrough in molecular biology and molecular medicine (Saiki *et al.*, 1985). PCR is a rapid and simple technique for generating infinite copies of any fragment of DNA and it takes analysis of minute quantity of genetic material even injured genetic material to a new level of reliability and accuracy (Joshi and Deshpande, 2011).

The basic PCR principle is simple and is based on a specific enzyme known as DNA polymerases. It helps to the duplication of DNA by continuously adding individual nucleotide. DNA template, nucleotides and primers are three important components of PCR. Primers are short stenches of DNA which are complementary to the targeted genome (Mullis and Floona, 1986).

Three main steps involved in the PCR technique are denaturation, annealing, and extension. First step is denaturation of the DNA and in this step a double-stranded DNA is converted in to single-stranded DNA at high temperature (from 90-97 °C). These mother strands acts as the template for new DNA synthesis. In step two, annealing of primers to the DNA template strands occurs. Extension of the DNA chain by nucleotide addition using DNA polymerase as catalyst in the presence of Mg^{2+} ions is the third step. These newly synthesized DNA strand is a complimentary copy of template DNA. This cycle is repeated several times and generates numerous copies of DNA fragments (Joshi and Deshpande, 2011).

In Mullis's original PCR process, *Escherichia Coli* DNA polymerase enzyme was used for amplification of DNA. Due to thermal instability of this enzyme, it was destroyed at the denaturation temperature. This problem was solved by replacing *E.Coli* DNA polymerase with a thermostable Taq polymerase derived from *Thermus aquqticus* (Saiki *et al.*, 1988).

2.6.1 PCR based molecular marker techniques

PCR based markers are also called as second generation DNA markers. PCR based marker shows more advantages over hybridization based markers. PCR based marker techniques are easy and economical enough to carry out that large scale experiments quickly and requires only a small quantity of DNA. It is more effective in genetic and ecological analyses of population. RAPD, AFLP and SSR are the major PCR based marker systems and other systems are the modifications of these three (Park *et al.*, 2009).

PCR based marker technique are subdivided into two; sequence specific techniques and sequence non specific techniques. RAPD and AFLP are examples of sequence non specific techniques, which do not require prior sequence information to perform. Whereas, SSR and SNP are sequence specific techniques, which need prior sequence information to perform (Agarwal *et al.*, 2008).

2.6.1.1 Inter Simple sequence repeat (ISSR) markers in plants

Inter Simple Sequence Repeat (ISSR) is a reliable and reproducible marker system for the amplification of inter sequence between SSRs (Zietkiewics *et al.*, 1994). The primers used in this technique, usually 16-25 bp long, are complimentary

to the simple sequence repeated region. A single ISSR PCR reaction can amplify the inter SSR sequences of different sizes in multiple locus. The primers usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994). But in some case, it may be unanchored (Meyer *et al.*, 1993; Gupta *et al.*, 1994). Amplification with nonanchored primers are called microsatellite primed PCR (MP PCR) (Meyer *et al.*, 1993). Normally ISSR is a dominant marker (Casasoli *et al.*, 2001), but a longer 5 –anchor marker shows codominance (Fisher *et al.*, 1996).

ISSR is faster and simple like randomly amplified polymorphic DNAs (RAPD), but they show the reproducibility of SSR markers because of the longer primer length. When compare to SSRs, developmental cost is less in the case of ISSR and it does not need prior information about the target sequences like SSRs (Awasthi *et al.*, 2004). ISSR shows a higher level of polymorphism than randomly amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphism. So it is an efficient tool for mapping projects of numerous plant species (Zietkiewicz *et al.*, 1994; Ratnaparkhe *et al.*, 1998; Durán *et al.*, 2004; Doucleff *et al.*, 2004).

This technique is effective in any species that contains adequate number of SSR motifs (Gupta *et al.*, 1994; Goodwin *et al.*, 1997). The primer used in ISSR analysis based on SSR motifs, may be di, tri, tetra or penta-nucleotides (Zietkiewicz *et al.*, 1994). Effectiveness of ISSR analysis depends on the occurrence of pairs of SSRs within a short distance (Zietkiewicz *et al.*, 1994). Polymorphism in ISSRs occurs due to the deletion or insertion in between the SSRs, which modifies the distance between repeats. In the case of 5' anchored primers, variation in the length of the microsatellite is also a reason for the occurrence of polymorphism (Zietkiewicz *et al.*, 1994; Tsumara *et al.*, 1996; Nagaoka *et al.*, 1997). ISSRs usually prefer high annealing temperature (45–60°C), because of its longer length of primer, which leads to higher stringency.

IISR markers have been developed for various important crops in moraceae family including mulberry (Venkateswarlu *et al.*, 2006; Weiguo *et al.*, 2007), fig (Ikegami *et al.*, 2009) and others. But there have been only a few ISSRs markers developed in jack genome till date (Chunhai *et al.*, 2009; Ashwini, 2015).

2.6.1.1.1 ISSR markers for Genetic diversity analysis in plants

Hundred ISSR Primers were used for the analysis of six wheat accessions containing diploid, tetraploid, and hexaploid members (Nagaoka and Ogihara, 1997). Objective of the study was to evaluate the applicability of ISSR in wheat in comparison to RFLP and RAPD markers. Thirty three primers which showed good polymorphism in each of the six accessions were selected for further study. Out of 33, 30 primers gave distinct bands. In total, 224 polymorphic bands were found in Einkorn wheat and 120 bands in common wheat. In conclusion, extent of band polymorphism produced by ISSR makers was similar to that of RFLP markers, greater than that of RAPDs and genetic relationships of wheat accessions estimated by ISSR markers were identical with those inferred by RFLP and RAPD markers.

Qian and Hong (2000) conducted a study to investigate the genetic variation within and between five populations of *Oryza granulata* from two regions of China. Amplification with 20 RAPD primers yields 199 reproducible bands with 61 polymorphic bands. Amplification with 12 ISSR primers yields 113 bands with 52 polymorphic bands. Both markers showed a low level of genetic diversity in wild populations of *O. granulata*. Total genetic diversity between the two regions revealed by RAPD is 78.85% and ISSR is 49. 26 %. Genetic diversity was only 19.45% and 6.70% between populations within regions and within a population, respectively in RAPD makers. Similarly, only 38.07% and 12.66% genetic diversity was present between populations within regions and within a population respectively in ISSR markers. This study shows that percentage polymorphism detected by ISSR (46.02%) is greater than that detected by RAPD markers (30.65%). In terms of the polymorphism detected and the amplification reproducibility, ISSR makers are superior to RAPD markers.

Nineteen RAPD markers and six ISSR markers were used to analyse the fifteen mulberry species (Awasthi *et al.*, 2004). Amplification with 19 RAPD markers yield, 128 discrete bands ranging from 500-3000 bp in size. Among this, 119 were polymorphic (92%). Out of six ISSR, four gave polymorphism, which yields 93 polymorphic bands. Cluster analysis of these two primes resulted into two clusters, one comprising polyploid wild species and the other with domesticated species. This

study recommended that RAPD and ISSR markers are useful for genetic diversity analysis and germplasm characterization in mulberry.

Ten inbred lines of ash gourd [*Benincasa hispida* (Thunb.) Cogn.] were analysed by using 42 RAPD primers and five ISSR primers. RAPD primers produced a total of 282 bands with 130 polymorphic bands. Out of five ISSR primers, four were informative. It yielded 26 ISSR bands with 11 polymorphic bands. ISSR yields higher polymorphic bands (>80%) than RAPD markers (46%). The genetic variation among inbred lines of ash gourd examined in this study acts as reference for further genetic analyses. It helps the selection of potential parents for predicting hybrid performance and heterosis (Verma *et al.*, 2007).

Twenty two ISSR markers and fifteen SSR markers were used to examine genetic diversity of 27 mulberry accessions including 19 cultivated accessions and 8 wild accessions (Weiguo *et al.*, 2007). ISSRs and SSRs were compared in terms of their efficiency and informativeness. SSRs presented a higher level of polymorphism information content (PIC). Cluster analysis of revealed that the wild species are genetically distant from cultivated species. In conclusion, the two marker types successfully analyse the level of polymorphism and diversity in mulberry. The genetic diversity exposed by SSR and ISSR revealed that wild species had higher genetic diversity than cultivated species.

Bajpai *et al.* (2008) analysed genetic diversity of forty six mango cultivars using nine RAPD and eleven ISSR primers. From this study, the RAPD and ISSR primers yielded 87.3% and 79.38% polymorphism, respectively. UPGMA analysis was done on RAPD data, which clustered the cultivar into 3 groups as per geographic separation. But ISSR clustering could not arrange the cultivars as per geographic separation. It was concluded that, there is no clear cut separation among varieties from two regions due to common gene pool origin and similar selection pressure of cultivar.

Behera *et al.* (2008) conducted a study to analyse 38 different Indian bitter gourd (*Momordica charantia* var. *charantia*, and var. *muricata*) accessions using 29 RAPD and 15 ISSR markers. Amplification with RAPD primers yielded 76 polymorphic bands and ISSR primers provided 94 polymorphic bands. The concordance among accession groupings after cluster analysis was high. It indicated

that RAPD and ISSR based diversity analysis in this bitter gourd germplasm were consistent. The genetically distinct domesticated (*M. charantia* var. *charantia*) and wild, free-living (*M. charantia* var. *muricata*) accessions provide the development of new strategies for genetic analyses and crop improvement.

2.6.1.2 Simple sequence repeat (SSR) or microsatellite markers in plants

Microsatellite markers are short tandem repeats of DNA sequences having a length of 2-6 bp. SSR marker system consists of two primers, which are complementary to the flanking region of the repeated sequences and help to amplify the repeated sequence. It produces polymorphic bands among alleles, depending on the number of repeat units. SSRs are ubiquitous, locus-specific, codominant, multiallelic, highly polymorphic, transferable and well distributed throughout the genome (Weber, 1990) and only small amounts of DNA is required for this technique (Rafalski *et al.*, 1996). Total sequence length is determined by the number of repeated units. The heterozygote in diploid genomes can be distinguishable in SSR technique.

SSRs are more variable than RFLP or RAPD (Morgante and Olivieri, 1993; Rongwen *et al.*, 1995; He *et al.*, 2003) and have been extensively used in plant genomic studies like marker-assisted selection, analysis of genetic diversity, population genetic analysis (Budak *et al.*, 2003; Nybom, 2004), genetic mapping and genetic map comparison (Grando *et al.*, 2003; Rosa *et al.*, 2003; Kenis and Keulemans, 2005; Pelgas *et al.*, 2005; Yamanaka *et al.*, 2005), parentage determination (Barreneche *et al.*, 1998; Arnold *et al.*, 2002; Szczys *et al.*, 2005; Pedryc *et al.*, 2009) and fingerprinting in most plant species (Gupta and Varshney, 2000) due to their high level of polymorphism, high information content, high discriminating power, reproducibility, codominant nature, mendelian inheritance and ease of genotyping through polymerase chain reaction (PCR) and electrophoretic methods.

SSR also has some disadvantages, the initial cost required for the development of SSR is very high, generally species specific, and prior sequence information is also required. But once the primers have been developed, the system becomes comparatively inexpensive, and can be working with material from the same species or related ones.

Microsatellite markers have been developed for various important crops in moraceae family including mulberry (Venkateswarlu *et al.*, 2006; Weiguo *et al.*, 2007; Mathithumilan *et al.*, 2013), fig (Giraldo and Lopez-Corrales, 2008; Ikegami *et al.*, 2009), bread fruit (Witherup *et al.*, 2013; De Bellis *et al.*, 2016) and others. But there is only a few SSRs markers developed in jack genome till date (Witherup *et al.*, 2013; De Bellis *et al.*, 2016).

2.6.1.2.1 SSR markers for Genetic diversity analysis in plants

The effectiveness of SSR markers for the genetic diversity analysis in fig was explained by Giraldo and Lopez-Corrales (2008). They used 20 SSR markers, for the characterization of 209 fig accessions conserved in an ex-situ field germplasm collection. Amplification with 20 makers yielded a total of 78 bands with an average of 3.9 alleles per locus and size ranged between 120 and 376 bp. The observed heterozygosity is higher (0.41) than that of mean expected heterozygosity (0.36). This study led to the identification of 98 unique genotypes (46.86%) in fig germplasm.

Ravishankar *et al.* (2011) conducted a study to develop microsatellite markers in mango cultivars (*Mangifera indica*) for their genetic diversity analysis and for testing their amplification in closely related species. Thirty six microsatellite loci were analysed on 30 diverse mango cultivars. Polymorphic information content values varied in the range of 0.185 to 0.920. The study concluded that newly developed SSRs would be transferable to related varieties like *M. odorata, M. anadamanica, M. zeylanica, M. camptosperma* and *M. griffithii.*

El-Assal and Gaber (2012) conducted a study to investigate the discriminating capacity of RAPD, ISSR and SSR markers and of their usefulness in constructing genetic diversity among Egyptian and Saudi wheat cultivars. Five Egyptian wheat and six Saudi wheat landrace cultivars were used for the study. Ten RAPD, nine ISSR and four SSR primers were used for the analysis; among this six RAPD, eight ISSR and two SSR primers which showed clear and repeatable bands were selected for further analysis. In RAPD analysis, a total 141 alleles were detected, among them 74 were polymorphic. In ISSR analysis 36 alleles out of 78 were polymorphic. In SSR reactions, 5 alleles out of 6 alleles were polymorphic. Genotypes were clustered into three major groups based on RAPD, ISSR and SSR analysis with UPGMA. In

conclusion, molecular markers such as RAPD, ISSR and SSR has confirmed a significant genetic diversity and genetic relationship in wheat cultivars

Sheidai *et al.* (2012) have conducted a study to understand the genetic diversity induced in tissue culture plants of cotton cultivars mainly in Mehr, Sindose and their hybrid Mehr X Sindose. Different subcultures of these three cultivars were used for the study. ISSR and SSR primers used in this study showed different levels of molecular polymorphisms. Some of the primers were amplified only in one genotype. Genetic variations among genotypes may be due to the occurrence of unique insertion/deletion in DNA during sub culturing.

Mathithumilan *et al.* (2013) conducted a study for developing a large number of locus specific genic and genomic SSR markers in mulberry species and can be used for molecular characterization. A total of 188 primers were developed, including 137 genomic and 51 genic SSR markers. While, a large proportion of these markers (164) were polymorphic in mulberry species. 149 SSR primers were transferable to other related species like Ficus, Fig and Jackfruit.

Witherup *et al.* (2013) conducted a study with an objective of isolating and characterizing SSR loci from *Artocarpus altilis* (breadfruit) and testing it in four *Artocarpus* species and one hybrid. Twenty one microsatellite loci were analysed on 241 *A. altilis*, 34 *A. amansi*, 15 *A. mariannensis*, and 64 *A. altilis* X *mariannensis* samples. Nine of those loci plus four extra loci were analysed on 426 *A. heterophyllus* (jackfruit) samples. All loci were polymorphic for at least one species. These microsatellite primers facilitate further studies on genetic diversity analysis of *Artocarpus* species.

New microsatellite loci were characterized in *Artocarpus altilis* (Moraceae) and two congeners to increase the number of available markers for genotyping breadfruit cultivars using next-generation sequencing (NGS) technology (De Bellis *et al.* 2016). By sequencing genomic DNA library of breadfruit 47,607 SSR loci were obtained. Among them, 50 SSR markers were used for the analysis of 41 samples which includes 39 *A. altilis*, one *A. camansi*, and one *A. heterophyllus*. All the 50 loci were polymorphic in *A. altilis*, 44 in *A. camansi*, and 21 in *A. heterophyllus*. The number of alleles per locus ranged from two to 19. These newly developed markers

can be useful for examining the identity and genetic diversity of breadfruit cultivars and also helpful to optimize the breadfruit gene bank management.

2.7 Genetic diversity analysis in jackfruit

An experiment was conducted by Pushpakumara and Harris (2007) for identifying RAPD markers to differentiate the two fruit types of jackfruit (*A. Heterophyllus*). Fruit types varikka (hard) and vella (soft-fleshed) was distinguished using RAPD marker OPB-01-1.0.

Shyamalamma *et al.* (2008) evaluated genetic diversity among 50 jackfruit accessions was using 16 AFLP markers. Out of 16 primer pairs used eight were selected for further analysis. Amplification with eight primers yields 5976 bands, 22% of which were polymorphic. Cluster analysis of these 50 accessions form three major clusters. This grouping strongly coincides with geographical localities as well as morphological characters. This information will be useful for further tree breeding programmes in jackfruit.

Chunhai *et al.* (2009) evaluated 76 accessions of jack fruit using 24 ISSR primers. Amplification with 24 ISSR yields 477 bands, out of which 427 bands were polymorphic. Cluster analysis divided 76 accessions in to 4 groups. The result showed that, soft flushed and firm flushed accessions could not be discriminated. The accessions collected from different areas could not form different clusters, but accessions from different sources formed different clusters.

AFLP markers were also employed for analysing genetic diversity of 50 jackfruit accessions from three provinces in China (Ying-zhi *et al.*, 2010). The genetic similarity coefficients varied from 0 to 0.9841, with an average of 0.5000 which shows a moderate genetic diversity among these 50 jackfruit accessions. Dendrogram analysis revealed five major groups. There was no correlation between genetic relationship and geographical origin.

Aswini (2015) conducted genetic diversity analysis in 20 accessions and three varieties of jack fruit using 50 ISSR primers. Out of 50 ISSR primers, 10 primers with reproducible polymorphic bands were selected. Cluster analysis divided jackfruit genotypes into five main clusters. First cluster consisted of firm flushed accessions,

while fifth cluster consists of all soft flushed accessions. The study concluded that, soft flushed and firm flushed accessions could be discriminated using ISSR primers.

Krishnan *et al.* (2015b) conducted a study to realize the genetic relationship among jackfruit selections of Kuttanad region using 30 RAPD primers. Out of the 30 primers, only 10 yielded polymorphic bands. The primer OPA-1 produced the highest number of bands and OPN-05 gave minimum number of bands. A dendrogram was constructed using the binary data of RAPD primers based on UPGMA clustering and showed three major clusters, which followed geographical separation.

De Bellis *el al.* (2016) conducted a study using one *A. heterophyllus* (jackfruit) sample. They selected 50 SSR markers from 47,607 simple sequence repeat loci of breadfruit genomic DNA library. Out of 50 SSR primes, 21 gave bands in *A. heterophyllus* sample. This study concluded that the new markers developed using bread fruit sequence will be useful for assessing the identity and genetic diversity of jackfruit.

2.8 DNA fingerprinting

DNA fingerprinting is the molecular marker based techniques for the identification of cultivars. This technique helps to find the genetic identity. This is mainly based on the polymorphisms occurring at the molecular level (Archak, 2000). The fundamental techniques involved in DNA fingerprinting were developed by Jeffreys and his associates in 1985 for the detection of highly variable DNA fragments by hybridisation of specific multilocus probes. Like barcodes DNA fingerprints are unique to the individual and hence, it can be used in forensics in the same way as in conventional fingerprints (Bhat, 2001).

In plants, DNA profiling is used for the identification of gene diversity and variation, protection of biodiversity, identifying markers for traits *etc* (Archak, 2000). DNA fingerprinting is an important tool for genetic identification in germplasm management and plant breeding (Jondle, 1992; Smith, 1998). The selection of marker system and techniques are very crucial in DNA fingerprinting. PCR based fingerprinting is most commonly used technique. It is rapid, uncomplicated to perform and requires minute quantity of DNA which makes it useful when dealing

with *in vitro* plantlets (McGregor *et al.*, 1999). The most widely used DNA fingerprinting techniques in plants are RFLP, RAPD, ISSR, SSR *etc* (Archak, 2000).

2.8.1 ISSR markers for DNA fingerprinting in plants

Using 15 ISSR and 15 RAPD primers, DNA fingerprinting of 18 mulberry varieties including Indian and Japanese genotypes were conducted for elucidating genetic relationships between genotypes (Vijayan, 2004). The RAPD primers showed 71.78% polymorphism, while ISSR primers provided 81.13 % polymorphism. The ISSR primers UBC-812, UBC-826, UBC827, UBC-881 and RAPD primers OPA-01, OPA-02, OPA-04 and OPH-17 gave higher polymorphic index value. The cluster analysis using UPGMA method clustered the genotypes into two groups. This result agreed with species status of Japanese mulberry genotypes but does not apply to Indian genotypes.

Kalpana *et al.* (2012) conducted a study to estimate the genetic variations and relationships between sixteen mulberry species, including fourteen *Morus alba* and two *Morus lhou* (ser) koidz cultivars. Forty RAPD primers and ten ISSR primers were used for the study. Amplification with 40 RAPD primers yield 66.67% polymorphism, ISSR yields 55.05% and a combination of RAPD + ISSR yields 64.11% polymorphism. Cluster analysis using UPGMA clustering methods clustered the genotypes in to three groups by RAPD primers and four groups by ISSR primers. The ISSR primers UBC-14, UBC-17 and RAPD primers OPA-6, OPA-14, OPA-15 have superior average polymorphic index value. The fingerprints obtained from this study can be used for better plant breeding in mulberry.

DNA fingerprinting of released varieties and selected superior somaclones of ginger were conducted using 10 RAPD and 11 ISSR primers. Clear and distinct bands generated by RAPD and ISSR were utilized for developing fingerprints for each varieties. The Rp value of RAPD and ISSR primers ranged between 6 to 16.25. The PIC value varied between 0.67 to 0.88. The dendrogram analysis placed Maran and Rio-de-Janeiro somaclones into separate clusters. The variety Athira showed maximum variability from source parent cultivar Maran and Somaclone 292R showed maximum variability from source parent cultivar Rio- de- Janeiro (Ghosh, 2013).

DNA fingerprinting of brinjal varieties/ accessions including three *Solanum melongena* vaieties- Surya, Swetha, Haritha and the hybrid Neelima, three accessions – SM 116, SM 396, SM 397 and two wild relatives *S. melongena* var. *insanum* and *S. macrocarpon* were conducted using 10 ISSR and 10 SSR primers (Laxman, 2013). The Rp value of ISSR primers ranged between 9.9 and 28.44. The PIC value varied between 0.83 to 0.96 for ISSR primers and zero for SSR primers. So, SSR primer is not suitable to detect polymorphism. The dendrogram showed that the hybrid Neelima and its maternal parent Surya has more similarity, *i.e.* 84% and *S. macrocarpon* showed maximum variability from other genotypes, *i.e.* 49%.

Sujith (2016) conducted a study for DNA fingerprinting of eight promising cocoa varieties released from KAU (CCRP 1, CCRP 2, CCRP 4, CCRP 5, CCRP 6, CCRP 7, CCRP 8 and CCRP 9). Ten ISSR primers UBC 810, UBC 815, UBC 826, UBC 827, UBC 835, UBC 841, UBC 842, UBC 846, UBC 855 and UBC 866 were used for developing fingerprints of these cocoa varieties. The result showed that some ISSR primers were specific for some varieties. It implied the capability of the selected markers to distinguish the varieties.

2.8.2 SSR markers for DNA fingerprinting in plants

A comparative assessment of DNA fingerprinting in tetraploid potato (*Solanum tuberosum* L.) germplasm using RAPD, ISSR, AFLP and SSR makers was conducted by McGregor *et al.* (2000). The study assessed the DNA fingerprinting of 39 potato cultivars using 20 RAPD primers, 6 ISSR primers, 2 AFLPs primers and 5 SSR primer pairs. Each of the four marker techniques independently identified each cultivar. Out of four techniques, SSR and AFLP techniques were found to be highly reproducible. The reproducibility of SSR, AFLP, ISSR and RAPD methods were found to be 100 %, 99.6 %, 87 % and 84.3 % respectively. This study concluded that AFLP and SSR are very suitable techniques for DNA fingerprinting in potato.

Using 30 SSR primers, genetic diversity and DNA fingerprinting of 15 elite rice genotypes was performed by Chakravarthi and Naravaneni (2006). All SSR primers gave distinct polymorphism among the rice genotypes. It showed the robust nature of SSR in revealing polymorphism. Cluster analysis using UPGMA methods grouped the rice genotypes into 10. The dendrogram showed that, the japonica types DH1 (Azucena) and Moroborekan clustered separately from indica types. This study

concluded that SSR marker yields larger range of similarity values for related cultivars and it provides greater assurance for the evaluation of genetic diversity and relationships.

Ten ISSR, ten RAPD primers and eight SSR primer pairs was used for the DNA fingerprinting of seven black pepper (Panniyur-1 to Panniyur-7)varieties collected from pepper research station, Panniyur (Mogalayi, 2011). Resolving power (Rp) of RAPD and ISSR primers ranged between 7.4 to 9.42 and 5.42 to 12.28 respectively. The PIC value varied between 0.86 to 0.90 for RAPD and 0.80 to 0.89 in ISSR primers. The results showed that Panniyur-1 and Panniyur-3 had maximum similarity *i.e.,* 76% and Panniyur-4 varied the most from other genotypes.

Joshi and Albertse (2013) developed a fingerprinting database of Sugarcane, including all South African Sugarcane Research Institute (SASRI) released varieties. Fingerprints of 84 sugarcane genotypes were developed using four SSR primer pairs. All primer pairs produce variable number of bands with good polymorphism. These bands were resolved on ABI3500 genetic analyser. SoftGenetics GeneMarker software version 2.4.0. was used for the recognition of true microsatellite alleles.

Molecular characterization and DNA fingerprinting of 16 KAU released cashew verities, including three hybrids using 11 ISSR and 11 SSR primers. In ISSR and SSR analysis, 69 ISSR and 12 SSR amplicons were polymorphic. Rp value of ISSR primers ranged from 1.4 to 7.0. The PIC value varied between 0.256 to 0.42 for ISSR primers and 0 to 0.50 for SSR primers. The dendrogram results revealed that the varieties Priyanka and Dhana had more similarity, *i.e.,* 68% and field grown Poornima showed maximum variability (Meena, 2014).

Sujith (2016) conducted a study for DNA fingerprinting of eight promising cocoa varieties released from KAU (CCRP 1, CCRP 2, CCRP 4, CCRP 5, CCRP 6, CCRP 7, CCRP 8 and CCRP 9). Eleven SSR primers mTcCIR 8, mTcCIR 11, mTcCIR 12, mTcCIR 18, mTcCIR 24, mTcCIR 33, mTcCIR 40, mTcCIR 42, mTcCIR 49, mTcCIR 51 and mTcCIR 64 were used for developing fingerprints of these cocoa varieties. The result showed that some SSR primers were specific for some varieties. It implied the capability of the selected markers to distinguish the varieties.

2.8.3 DNA fingerprinting in jackfruit

DNA fingerprinting of five varieties of jackfruit (TCJ4, TCJ2, FH10, FH4, and NC2) was conducted using RAPD primer OPC7-GTCCGACGACGA (Gopalsamy *et al.*, 2012). Cluster analysis gave three main groups, one cluster with two varieties. Genotype in same cluster shows common phylogenetic characteristics. This study helps the selection of superior varieties for further breeding programs and good germplasm management in jackfruit.

Materials and methods

3. MATERIALS AND METHODS

The study on "DNA fingerprinting of promising selections of jack (*Artocarpus heterophyllus* Lam.) using molecular markers" was carried out at Regional Agricultural Research Station, Kumarakom and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during the period 2015-2017. The materials and methods are given below

3.1 Materials

3.1.1 Plant materials

One KAU released jack variety, one cultivar and six superior jackfruit selections identified at RARS, Kumarakom (Krishnan *et al.*, 2015a) were utilized for the study as detailed below (Plate 1).

Released variety: Sindhur

Cultivar: Muttom varikka

Selections from RARS, Kumarakom

Veloor varikka-1

Veloor varikka-2

Pathamuttom varikka-1

Pathamuttom Varikka-2

Kavanar Varikka-1

Chengalam varikka

3.1.2 Laboratory chemicals, glassware's and plastic wares

Laboratory chemicals procured from Merk, HIMEDIA and SIGMA were used for the study. The primers were supplied by IDT, USA. Plastic vessels were obtained from Tarsons Products Pvt. Ltd. and glass wares from Borosil. Master mixture was provided by SIGMA and ladder from Invitrogen.

Veloor varikka-1

Veloor varikka-2

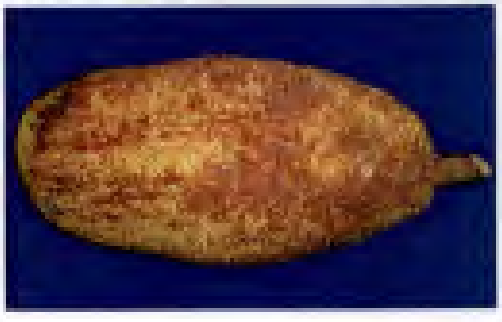
Pathamuttom varikka-1

Pathamuttom varikka-2

Kavanar varikka-1

Chengalam varikka

Muttom varikka



Sindhur

Plate 1. Fruits of genotypes used for the study

3.1.3 Equipments

Work was done by using the equipments available at RARS, Kumarakom. Centrifugation was done in centrifuge 5430R (eppendorf) and SureCycler 8800 PCR (Agilent Technologies) was used for the amplification of DNA. Bio-Rad electrophoresis unit and Nanodrop^{R} ND-1000 Spectrophotometer were used for checking the quality and quantity of DNA respectively. Gel images were taken using Bio-Rad gel documentation system.

3.2 Methods

3.2.1 Fruit characters

The jack variety Sindhur, cultivar Muttom varikka and six superior jack selections (Veloor varikka-1, Veloor varikka-2, Pathamuttom varikka-1, Pathamuttom Varikka-2, Kavanar Varikka-1and Chengalam varikka) maintained at RARS, Kumarakom were used for the study. The recorded information's are;

3.2.1.1 Analysis of qualitative traits

Seven characters *viz,* colour of flake, fruit texture, fruit shape, fruit thickness, flesh aroma, bulb shape and seed shape were recorded in eight genotypes of jack fruit. Cluster analysis was performed using qualitative traits to gather information about divergence among jack genotypes in NTSYS Pc version 2.1 (Rohlf, 1992) and Unweighted Pair Group Method (UPGMA) dendrogram was constructed (Sneath and Sokal, 1973).

3.2.1.2 Analysis of quantitative traits

Five quantitative characters *viz,* fruit weight (kg), single bulb weight (g), single seed weight (g), single flake weight (g) and flake thickness (cm) were recorded. Cluster analysis was performed using quantitative traits to gather information about divergence among jack genotypes in NTSYS Pc version 2.1 (Rohlf, 1992) and Unweighted Pair Group Method (UPGMA) dendrogram was constructed (Sneath and Sokal, 1973).

3.2.2 Biochemical analysis

Six characters viz TSS (°Brix), acidity (%), total sugar (%), reducing sugar (%), non reducing sugar (%) and ascorbic acid (mg/ml) were recorded in eight genotypes of jackfruit. Cluster analysis was performed using biochemical traits to gather information about divergence among jack genotypes in NTSYS Pc version 2.1 (Rohlf, 1992) and Unweighted Pair Group Method (UPGMA) dendrogram was constructed (Sneath and Sokal, 1973).

3.2.3 Molecular analysis

DNA fingerprinting in jack verities were performed using SSR (Simple Sequence Repeat) and ISSR (Inter Simple Sequence Repeat) markers.

3.2.3.1 Genomic DNA extraction

Tender emerging leaves were collected from each genotype early in the morning. After covering with aluminium foil, the leaves were brought to the laboratory in ice box. The leaf surface was washed with sterile water and wiped with 70 percent ethanol. It was then stored at -80°C. CTAB method developed by Doyle and Doyle (1987) was used for the extraction of genomic DNA.

Reagents and equipments

I. CTAB buffer (2X):

- 2 per cent CTAB (w/v)
- 100 mM Tris base (pH8)
- 20 mM EDTA(pH8)
- 1.4 M NaCl
- 1 per cent polyvinyl pyrrolidin (PVP)
- 0.2 percent 2-βmercaptoethanol

II. Chloroform: isoamyl alcohol (24:1 v/v)

III. Chilled isopropanol

IV. Wash buffer

- 76 % ethyl alcohol
- 10 mM ammonium acetate

V. Ethanol 70 percent

VI. Sterile distilled water or

VII. TE buffer:

- 10 mM Tris (pH8)
- 1 mM EDTA

Reagents were autoclaved and stored at room temperature

VIII. Water bath

IX. Centrifuge

X. -20 and -80 Refrigerator

Procedure

CTAB isolation buffer (2X) was preheated in a 50 ml Oakridge centrifuge tube to 60°C in a water bath. Fresh leaf tissue (0.2 – 0.5g) is ground with a pinch of polyvinyl pyrrolidin (PVP), 50 µl of 2-βmercaptoethanol and liquid nitrogen. The powdered sample was transferred to 2 ml eppendorf tube containing 1 ml of preheated CTAB solution. The sample is incubated for 30 minutes at 60°C with optional occasional gentle swirling. Equal volume of chloroform: isoamyl alcohol (24:1) mixture was added to the tube. It was mixed gently by inversion and was centrifuged (Eppendorf) at 12000 rpm for 20 min at room temperature. The content got separated into three distinct phases as detailed below.

Aqueous top layer - DNA with small quantity of RNA

Middle layer - Protein and fine particles

Lower layer - Chloroform, pigments and cell debris

The top aqueous layer was transferred to a sterile eppendorf tube. Equal volume of chloroform: isoamyl alcohol (24:1) was added to this and mixed by inversion. It was centrifuged at 12,000 rpm for 20 minutes at room temperature. After

transferring the aqueous phase into a clean eppendorf tube, 0.6 volume of chilled isopropanol was added. This was mixed by gentle inversion till the DNA was precipitated. These tubes were kept at -20°C for half an hour for complete precipitation. After this, it was centrifuged for 15 minutes at 12,000 rpm at 4°C. The supernatant was gently poured off. The DNA pellet was washed with 10-20 µl of wash buffer with centrifugation at 1000 rpm for 5 min. The supernatant was carefully removed. It was again washed with 70% ethanol. Again the tubes were spun for 5 minutes at 1000 rpm and ethanol was decanted. The remaining pellet was air dried and dissolved in 50µl of TE buffer for storing at -20°C.

3.2.3.2 Purification of DNA

Reagents

I. 10 mg/ml RNAase

II. Phenol: Chloroform: isoamyl alcohol (25:24:1 v/v)

III. Chloroform: Isoamyl alcohol (24:1 v/v)

IV. Chilled isopropanol

V. Wash buffer

- 76 % ethyl alcohol
- 10 mM ammonium acetate

VI. Ethanol 70 percent

VII. Sterile distilled water or

VIII. TE buffer:

- 10 mM Tris (pH8)
- 1 mM EDTA

Reagent are autoclaved and stored at room temperature

Procedure

RNA contaminated DNA was treated with RNaseA. 1µl of 10 mg/l RNaseA was added to 50 µl of sample DNA. Then the sample was incubated in water bath at 37°C for 1 hr. Then equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) mixture was added to the tube. It was mixed gently by inversion and was centrifuged (Eppendorf) at 12000 rpm for 20 min at room temperature. The content was separated into three distinct phases as follows.

Aqueous top layer - DNA with small quantity of RNA

Middle layer - Protein and fine particles

Lower layer - Chloroform, pigments and cell debris

The top aqueous layer was transferred to a sterile eppendorf tube. Equal volume of chloroform: isoamyl alcohol (24:1) was added to this and mixed by inversion. It was centrifuged at 12,000 rpm for 20 minutes at room temperature. After transferring the aqueous phase into a clean eppendorf tube, 0.6 volume of chilled isopropanol was added. This was mixed by gentle inversion till the DNA was precipitated. These tubes were kept at -20°C for half an hour for complete precipitation. After this, it was centrifuged for 15 minutes at 12,000 rpm at 4°C. The supernatant was carefully removed. The DNA pellet was washed with 70% ethanol. Again the tubes were spun for 5 minutes at 1000 rpm and ethanol was decanted. The remaining pellet was air dried and dissolved in 50 µl of TE buffer for storing at -20°C.

3.2.3.3 Assessing the quality of DNA by Agarose gel electrophoresis

The quality of DNA was assessed through Agarose gel electrophoresis (Sambrook *et al.*, 1989).

Reagents and equipments

I. Agarose (0.8 %)

II. 50X TAE buffer (pH8)

- 2M Triss (pH8)
- 1M Acetic acid
- 50mM EDTA (pH8)

III. 1X TAE buffer/ Running buffer
   - 40Mm Triss (pH8)
   - 20Mm Acetic acid
   - 1mM EDTA(pH8)

IV. 6X Loading dye

V. Ethidium bromide(0.5µg/l)

VI. Electrophoresis unit

VII. UV transilluminator

VIII. Gel documentation system

Procedure

The electrophoresis unit, gel casting tray and comb was wiped with 70% ethanol. Then 0.8% agarose was added to the 1X TAE buffer and boiled to dissolve it. Ethidium bromide was added after cooling the solution. The open end of casting tray was sealed and kept it on a horizontal surface and a suitable comb was placed. The agarose solution was poured to the tray and allowed to solidify for 30 minutes. Then the comb was removed and placed the gel in an electrophoresis unit having running buffer with the wells directed towards the cathode. Then the DNA sample and Ladder-100mg/ml (5µl) loaded to the corresponding well after mixing with tracking dye (1µl) and then loaded to the corresponding wells. After closing the electrophoresis unit, anode and cathode were connected to the power pack and set the voltage to 100V. When the tracking dye reached 2/3rd length of the gel, the gel was taken out from the electrophoresis unit, and the DNA band were observed using UV transilluminator and the gel documentation system. Gel images captured using Gel documentation system was saved. The gel image was examined for purity of DNA, presence of RNA and protein, intactness and clarity of DNA band.

3.2.3.4 Assessing the quality and quantity of DNA by NanoDrop method

The quality and quantity of DNA was examined by using Nanodrop^{R} spectrophotometer (ND-1000). The highest absorbance of nucleic acid is found at 260nm and that of protein is at 280nm. Hence OD $_{260}$/OD$_{280}$ ratio was recorded to check the purity of DNA.

If the OD_{260}/OD_{280} value obtained is between 1.8 and 2 the DNA is pure and if it more than 2, then DNA is contaminated with RNA and if < 1.8 it means the DNA is contaminated with proteins.

Procedure

The Nanodrop spectrophotometer was connected to the system having the software ND-1000. Then select the option nucleid acid from software window. Before taking sample reading, a blank reading was set to zero using 1µl autoclaved distilled water as blank solution. For this the sampling arm was opened and transferred the distilled water (blank) to the measurement pedestal of nanodrop apparatus. The sampling arm was closed and the instrument automatically measured the reading. Then the reading was set to zero. After this step 1µl of each sample was transferred to the measurement pedestal and OD value was measured. The OD value and quantity DNA (ng/µl) were recorded. After taking the measurements the apparatus is wiped by using tissue paper.

3.3 DNA fingerprinting

Two types of markers were used for this study, which included SSR (Simple Sequence Repeat) and ISSR (Inter Simple sequence Repeats) markers.

3.3.1 Inter Simple Sequence Repeat (ISSR) analysis.

The genomic DNA of jack was amplified with repeat sequences as primer so as to amplify the inter simple sequence repeat region. The DNA diluted to 40 ng/ µl for the ISSR analysis.

PCR amplification was performed using reaction mixture with following composition.

Genomic DNA (40 ng/ µl)	: 1µl
10X Taq assay buffer A with $MgCl_2$	: 2.5µl
dNTP mix (10mM each)	: 1.25µl
Primer (10pM)	: 2µl
Taq DNA polymerase (5u)	: 0.2 µl

Autoclaved distilled water : 17.75µl

Total volume : 25 µl

Amplification was performed. The following programme was used for PCR amplification.

Initial denaturation : 94°C for 4 min

Denaturation : 94°C for 30 Sec

Annealing : 50°C to 55°C for 1min

Extension : 72°C for 1mint 50 Sec

Final extension : 72°C for 10 min

Storage : 4°C for infinite time

Number of cycles : 35 times

3.3.1.1 Screening and analysis of ISSR primer

Fifty previously reported ISSR markers (Ashwini, 2015) were used for this study. List of ISSR markers used in this studies are provided in Table 1.

Screening was done using bulked DNA from eight samples. Out of fifty primers ten primers were selected for further analysis based on polymorphism. PCR amplified product of these primers showing polymorphism were resolved on 2 % agarose gel along with 1 Kb DNA ladder (Thermo scientific) and scored for making fingerprints.

Table 1. List of primers used for ISSR analysis

Sl. No.	Primer	Nucleotide sequence
1	UBC 807	5'-AGAGAGAGAGAGAGAGT-3'
2	UBC 809	5'-AGAGAGAGAGAGAGAGG-3'
3	UBC 810	5'-GAGAGAGAGAGAGAGAT-3'
4	UBC 811	5'-GAGAGAGAGAGAGAGAC-3'
5	UBC 812	5'-GAGAGAGAGAGAGAGAA-3'
6	UBC 813	5'-CTCTCTCTCTCTCTCTT-3'
7	UBC 814	5'-CTCTCTCTCTCTCTCTA-3'
8	UBC 815	5'-CTCTCTCTCTCTCTCTG-3'
9	UBC 816	5'-CACACACACACACACAT-3'
10	UBC 817	5'-CACACACACACACACAA-3'
11	UBC 818	5'-CACACACACACACACAG-3'
12	UBC 820	5'-GTGTGTGTGTGTGTGTC-3'
13	UBC 825	5'- ACACACACACACACACT-3'
14	UBC 826	5'-ACACACACACACACACC-3'
15	UBC 834a	5'-AGAGAGAGAGAGAGAGCT-3'
16	UBC 834b	5'-AGAGAGAGAGAGAGAGTT-3'
17	UBC 835	5'-AGAGAGAGAGAGAGAGCC-3'
18	UBC 836a	5'-AGAGAGAGAGAGAGAGCA-3'
19	UBC 838	5'-TATATATATATATATAAC-3'
20	UBC 840a	5'-GAGAGAGAGAGAGAGACT-3'
21	UBC 840b	5'-GAGAGAGAGAGAGAGATT-3'
22	UBC 841a	5'-GAGAGAGAGAGAGAGACC-3'
23	UBC 841b	5'-GAGAGAGAGAGAGAGATC-3'
24	UBC 842b	5'-GAGAGAGAGAGAGAGATG-3'
25	UBC 843a	5'-CTCTCTCTCTCTCTCTAA-3'
26	UBC 844a	5'-CTCTCTCTCTCTCTCTAC-3'
27	UBC 845	5'-CTCTCTCTCTCTCTCTRG-3'
28	UBC 848a	5'-CACACACACACACACAAG-3'
29	UBC 850a	5'-GTGTGTGTGTGTGTGTCC-3'
30	UBC 852a	5'-TCTCTCTCTCTCTCTCAA-3'
31	UBC 854	5'-TCTCTCTCTCTCTCTCRG-3'
32	UBC 855	5'-ACACACACACACACACYT-3'
33	UBC 857a	5'-ACACACACACACACACCG-3'
34	UBC 857b	5'-ACACACACACACACACTG-3'
35	UBC 858	5'-TGTGTGTGTGTGTGTGRT-3'
36	UBC 860	5'-TGTGTGTGTGTGTGTGAA-3'
37	UBC 862	5'-AGCAGCAGCAGCAGCAGC-3'
38	UBC 865	5'-CCGCCGCCGCCGCCGCCG-3'
39	UBC 866	5'-CTCCTCCTCCTCCTCCTC-3'

40	UBC 868	5'-GAAGAAGAAGAAGAAGAA-3'
41	UBC 872	5'-GATAGATAGATAGATA-3'
42	UBC 873	5'-GACAGACAGACAGACA-3'
43	UBC 874	5'-CCCTCCCTCCCTCCCT-3'
44	UBC 880	5'-GGAGAGGAGAGGAGA-3'
45	UBC 892	5'-TAGATCTGATATCTGAATTCCC-3'
46	UBC 895	5'-AGAGTTGGTAGCTCTTGATC-3'
47	UBC 899	5'- CATGGTGTTGGTCATTGTTCCA-3'
48	UBC 900	5'-ACTTCCCCACAGGTTAACACA-3'
49	TC 10G	5'-TCTCTCTCTCTCTCTCTCTCG-3'
50	CT 10A	5'-CTCTCTCTCTCTCTCTCTCTA-3'

3.3.2 Simple Sequence Repeat (SSR) analysis.

The genomic DNA of jack was amplified with inter simple sequence repeat as primer so as to amplify the simple sequence repeat region. The DNA diluted to 40ng/ µl for the SSR analysis.

PCR amplification was performed using reaction mixture with following composition.

Genomic DNA (40 ng/ µl)	: 1µl
10X Taq assay buffer A with $MgCl_2$	: 2.5µl
dNTP mix (10mM each)	: 1µl
Forward primer (10pM)	: 2.5 µl
Reverse primer (10pM)	: 2.5 µl
Taq DNA polymerase (5u)	: 0.1µl
Autoclaved distilled water	: 15.4 µl
Total volume	: 25 µl

Amplification was performed. The following programme was used for PCR amplification.

Initial denaturation	: 94°C for 2 min
Denaturation	: 94°C for 30 Sec
Annealing	: 50°C to 55°C for 30 Sec
Extension	: 72°C for 1min
Final extension	: 72°C for 8min
Storage	: 4°C for infinite time
Number of cycles	: 35 times

3.3.2.1 Screening and analysis of SSR primer

Fifty previously reported SSR markers (Mathithumilan *et al.*, 2013; Witherup *et al.*, 2013; Bellis *et al.*, 2016) were used for the study. List of SSR markers used for this study are provided in Table 2.

Screening was done using bulked DNA from eight samples. Out of fifty primers eleven primers were selected for further analysis based on polymorphism. PCR amplified product of these primers showing polymorphism were resolved on 3% high resolution agarose gel along with 100 bp DNA ladder (Thermo scientific) and scored for making fingerprints.

Table 2. List of primers used for SSR analysis

Sl. No.	Primer	Nucleotide sequence
1	MulSSR I	F: 5'-GATCTGAAGTCACCCAGCC-3' R: 5'-GCAGAATCTTTTCAGCTTCCA-3'
2	MulSSR 2	F: 5'-GGTGCCTGAAGATATGTGG-3' R: 5'-CTCTGAGGGAAGCAGAAG-3'
3	MulSSR 23	F: 5'-CGGAAACAGCCCAAAGAAGG-3' R: 5'-AGGAGGGGTTTAGGGG-3'
4	MulSSR 26	F: 5'-CCACTGGTGCCTGAAG-3' R: 5'-CATCTCATACTGGGGC-3'
5	MULSSR 59	F: 5'-GGTTTCATTTTCCCTCTCGA-3' R: 5'-GGCCGATGCGAACAGA-3'
6	MULSSR 69	F: 5'-CAATATTACCACCCTCAC-3' R: 5'-GAAATGGTTTGCATCC-3'
7	MulSSR 82	F: 5'-CAATCACTAACGGGGGAAG-3' R: 5'-GCTCTTTTTGGTGCTCC-3'
8	MULSSR 85	F: 5'-CCGGAGAAATTCCAAAGG-3' R: 5'-CATCCAGGCATCTGATTG-3'
9	M2SSR 1	F: 5'-CTCTCGAGAAAGCCATCA-3' R: 5'-GGTTGTCAAGTAGGACCG-3'
10	M2SSR 5	F: 5'-GCTCAGATTCGGTCATGG-3' R: 5'-CTGCTTCATGGTATCAGAGCAAGG-3'
11	mAaCIR 0019	F: 5'-TGACATTCCCGCAAAA-3' R: 5'-AAGTCTTCTGTTCCTACTGACAA-3'
12	mAaCIR 0033	F: 5'-CGGGTACAGGGTATTGGT-3' R: 5'-AGGAGAGCGTTTGAGGAA-3'
13	mAaCIR 0048	F: 5'-CGAAATCGGAACAGAAAAC-3' R: 5'-GTCCTTGGCTACTATAATCCCT-3'
14	mAaCIR 0049	F: 5'-TACATACAAGCCAACTTCCA-3' R: 5'-CCTTTGTGAGGAAGACCA-3'
15	mAaCIR 0050	F: 5'-TTCCCTGCCTAGTTTTGTG-3' R: 5'-AATAAAGCGCGGACTTACA-3'
16	mAaCIR 0053	F: 5'-GCAACACATTCATCAACA-3' R: 5'-GACTCACCAAGACTTTATTACC-3'
17	mAaCIR 0075	F: 5'-CATTCTTGGGAAGAGTTGA-3' R: 5'-ATAGCGGTGAAAATGGAA-3'
18	mAaCIR 0078	F: 5'-CTTCAACTATTACTACTGCTGCT -3' R: 5'-CTGTTCAGGTTGGTGCT-3'
19	mAaCIR 0081	F: 5'-AATTGGCGGTATTCTATG-3' R: 5'-GGAGGCAGATAAATTAGAAA-3'
20	mAaCIR 0089	F: 5'-CCTGAGTAGGACAAAGACTGAA-3' R: 5'-ATTGCGCTTTTCTTCCC-3'

21	mAaCIR 0090	F: 5'-GGGTGTCCTCGCCTC-3' R: 5'-GGTGGATCATTCAGCAAA-3'
22	mAaCIR 0108	F: 5'-CAATATAGCAGGCACTAATTCA-3' R: 5'-TCTTCTTTCTCTCGTTCGTT-3'
23	mAaCIR 0111	F: 5'-TGCAGGCATCACGAAAC-3' R: 5'-CTTCTGCATGAGCGGTG-3'
24	mAaCIR 0115	F: 5'-ACAGCTTTGCACCGACAC-3' R: 5'-GCCCTCAACCACCCC-3'
25	mAaCIR 0127	F: 5'-TGATTCTCTCTTTACAGGCAC-3' R: 5'-GCTCAGGTGCTTACTTGTTC-3'
26	mAaCIR 0128	F: 5'-CAACCACTGATGGAGATAG-3' R: 5'-ACAACACCGTTTACTGAAG-3'
27	mAaCIR 0134	F: 5'-AGCTGCCAATGATCCC-3' R: 5'-ATGTGAAAAGGTTGGATTTG-3'
28	mAaCIR 0141	F: 5'-TCAAGCCCCTCACTCAA-3' R: 5'-ATGGCATAGCACAACACAA-3'
29	mAaCIR 0146	F: 5'-CTTGCACCATCGTCATTT-3' R: 5'-GTTAATTGGAAGTTGTGTCTCC-3'
30	mAaCIR 0154	F: 5'-TCGAGGCCCTTGTTG-3' R: 5'-GGAAATTCACCTTTCCTTG-3'
31	mAaCIR 0204	F: 5'-TTTAGGGTCCGTTGAAGA-3' R: 5'-GAAGTCTTGTTATTTGTGGAAG-3'
32	MAA 3	F: 5'-TGTTCTAGCTGCACGAATTATG-3' R: 5'-CTTGAATCAAACAGGCCAATTA-3'
33	MAA 9	F: 5'-AACAGGGTTAAAATCCCTTCAC-3' R: 5'-GTTCCCGTTTTGTTCAAAGAG-3'
34	MAA 26	F: 5'-CATGAATGAAACAACATCAGAC-3' R: 5'-ATAGTCATAAAGCCCTGCG-3'
35	MAA 40	F: 5'-AGCATTTCAGGTTGGTGAC-3' R: 5'-GTTGTTCTGTTTGCCTCATC-3'
36	MAA 54a	F: 5'-AACCTCCAAACACTAGGACAAC-3' R: 5'-AGCTACTTCCAAAACGTGACA-3'
37	MAA 54b	F: 5'-AACCTCCAAACACTAGGACAAC-3' R: 5'-AGCTACTTCCAAAACGTGACA-3'
38	MAA 71	F: 5'-TTCCTATTTCTTGCAGATTCTC-3' R: 5'-AGTGGTGGTAAGATTCAAAGTG-3'
39	MAA 85	F: 5'-TCAGGGTGTAGCGAAGACA-3' R: 5'-AGGGCTCCTTTGATGGAA-3'
40	MAA 96	F: 5'-GGACCTCAAGGATGTGATCTC-3' R: 5'-ACACGGTCTTCTTTGGATAGC-3'
41	MAA 105	F: 5'-GTTGGGACACTGTGAACTATTC-3' R: 5'-AAAAGCTAGTGGATTAGATGCA-3'
42	MAA 122	F: 5'-CTGGCCTTCAGTTTTGTCAAC-3'

		R: 5'-CACCAGGCTTCAAGATGAAA-3'
43	MAA 140	F: 5'-CCATCCCCCATCTTTCCT-3' R: 5'-TCCTCGTTTGCCACAGTG-3'
44	MAA 145	F: 5'-CCAACGCATAGCCAAATC-3' R: 5'-AAATCCCAAACCCAACGT-3'
45	MAA 156	F: 5'-CTGGTGCTTCAGCCTAATG-3' R: 5'-TCAGCGTCAAAGATAACTCG-3'
46	MAA 178a	F: 5'-GATGGAGACACTTTGAACTAGC-3' R: 5'-CACCAGGGTTTAAGATGAAAC-3'
47	MAA 178b	F: 5'-GATGGAGACACTTTGAACTAGC-3' R: 5'-CACCAGGGTTTAAGATGAAAC-3'
48	MAA 182	F: 5'-TACTGGGTCTGAAAAGATGTCT-3' R: 5'-CGTTTGCGTTTGGATAAAT-3'
49	MAA 196a	F: 5'-GGAATGTGGTAGATGAAACTCC-3' R: 5'-CGACAAAAAAACAAAGGAAGAC-3'
50	MAA 196b	F: 5'-GAATGTGAGAGATAAATCTCC-3' R:5,-CGACAAAAAAACAAAGGAAGAC-3'

3.4 Scoring of bands and data analysis

Gel picture was taken with image lab software. The bands were scored as 1 for the presence and 0 for absence of the bands respectively. The sizes of bands were detected based on the ladder size. Genetic variability was estimated based on this manually scored bands using NTSYS version 2.1 (Rohlf, 1992) and cluster analysis was done using Unweighted Pair Group Method (UPGMA) (Sneath and Sokal, 1973). Fingerprints were developed using the scored bands of selected primers using Microsoft office excel. Different colour codes were provided to the bands of different genotypes based on their molecular weight, same colours were provided to the amplicones of same size.

Polymorphic information content (PIC) and Marker index (MI) were the parameters used for measuring the performance of markers. Both PIC and MI confirm the suitability of the primer, PIC represents the ability of a marker to detect the polymorphism within a population and MI helps to understand the capacity of primer to detect polymorphic loci among varieties.

Polymorphic information content in dominant marker (Roldan-Ruize *et al.*, 2000),

$$PIC_i = 2f_i (1-f_i)$$

Where, f_i is frequency of amplified allele (band present) and $(1-f_i)$ is frequency of null allele (band absent) of marker i.

Polymorphic information content in co-dominant marker (Milbourne *et al.*, 1997)

$$PIC_i = 1-\Sigma\, p_i^2$$

Where, p_i is the frequency of i^{th} allele

Marker index (Powell *et al.*, 1996) of both markers,

$$MI = PIC \times \text{No. of polymorphic bands}$$

Results

4. RESULTS

The study on "DNA fingerprinting of promising selections of jack (*Artocarpus heterophyllus* Lam.) using molecular markers" was carried out at Regional Agricultural Research Station, Kumarakom and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during the period 2015-2017. The jack variety Sindhur, cultivar Muttom varikka and six superior jack selections (Veloor varikka-1, Veloor varikka-2, Pathamuttom varikka-1, Pathamuttom Varikka-2, Kavanar Varikka-1 and Chengalam varikka) maintained at RARS, Kumarakom were used for the study. The results are given below.

4.1 Fruit characters

Fruit characters of the selected jack genotypes were recorded. Standard descriptors prescribed by Bioversity International (2006) were used as the guideline to describe the qualitative and quantitative characters of jackfruit.

4.1.1 Qualitative characters

Variation in characters like fruit shape, flake texture, pulp colour, flake thickness, pulp flavour, flake shape and seed shape were recorded in eight genotypes of jack fruit (Table 3).

Very low variability was observed between jackfruit genotypes for most of the qualitative characters. The pulp colour of Pathamuttom varikka-1, Pathamuttom varikka-2 and Sindhur was coppery red (Plate 2), which has high fancy value in the market. The pulp colours of all the other genotypes were different shades of yellow. All these genotypes had firm flush. Except Kavanar varikka-1 and Chengalam varikka, fruit of all the other genotypes had strong pulp flavour. All genotypes had thick fruit except Kavanar varikka-1. All the accessions processed elongated seed. Pathamuttom Varikka-2 and Muttom varikka produced fruits which were irregular in shape. Veloor varikka-1 and Pathamuttom vaikka-1 produced oblong fruit. Apart from these four varieties, all other had fruits with ellipsoid shape. Flake shape showed high variability among these genotypes.

4.1.2 Quantitative characters

Table 4 represented the variation in quantitative fruit characters like fruit weight (kg), single bulb weight (g), single seed weight (g), single flake weight (g) and flake thickness (cm) recorded for the eight genotypes of jackfruit studied.

The fruit weight varied between 5.93 to 21.52 kg. Fruit weight was highest for Pathamuttom varikka-1 followed by Veloor varikkka-1. The flake thickness was highest for Muttom varikka (0.63 cm) whereas Veloor varikka-2 recorded lowest value for flake thickness (0.33 cm). The single bulb weight ranged between 15.12 to 39.87 g Pathamuttom varikka-2 recorded highest value for bulb weight. The single seed weight and flake weight varied between 2.38 to12.89 g and 7.7 to 28.36 g respectively.

4.1.3 Biochemical characters

The biochemical characters like TSS (°Brix), acidity (%), total sugar (%), reducing sugar (%), non reducing sugar (%) and ascorbic acid (mg/100g) recorded for the eight genotypes of jackfruit are depicted in Table 5.

The values of total soluble solids (TSS) varied between 18.8 to 32 °Brix. The TSS was maximum for Sindhur (32 °Brix) followed by Chengalam varikka (31.80 °Brix). The total sugar was highest for Chengalam varikka (19.81 %). Chengalam varikka and Muttom varikka (9.39 %) showed highest value for the percentage of reducing sugar. Percentage of non reducing sugar varied from 8.45 to 11.44 %. The ascorbic acid content ranged between 2.40 and 12.00 mg/100g and Pathamuttom varikka-2 recorded maximum value. The acidity was highest for Veloor varikka-2 and Pathamuttom varikka-1 (1.02 %) whereas Kavanar Varikka-1 recorded lowest value for acidity (0.38 %).

Table 3. Qualitative characters of fruits from different jackfruit genotypes

Genotypes	Fruit shape	Flake texture	Pulp (flake flesh) colour	Flake thickness	Pulp flavour	Flake shape	Seed shape
Veloor varikka-1	Oblong	Firm	Deep yellow	Thick	Strong	Irregular	Elongate
Veloor varikka-2	Ellipsoid	Firm	Light yellow	Thick	Strong	Cordate	Elongate
Pathamuttom varikka-1	Oblong	Firm	Coppery red	Thick	Strong	Obovate	Elongate
Pathamuttom Varikka-2	Irregular	Firm	Coppery red	Thick	Strong	Rectangular	Elongate
Kavanar Varikka-1	Ellipsoid	Firm	Orange yellow	Medium	Intermediate	Cordate	Elongate
Chengalam varikka	Ellipsoid	Firm	Orange yellow	Thick	Intermediate	Irregular	Elongate
Muttom varikka	Irregular	Firm	Deep yellow	Thick	Strong	Twisted	Elongate
Sindhur	Ellipsoid	Firm	Coppery red	Thick	Strong	Twisted	Elongate

Table 4.Quantitative characters of fruits from different jackfruit genotypes

Genotypes	Fruit weight (kg)	Single bulb weight (g)	Single seed weight (g)	Flake weight (g)	Flake Thickness (cm)
Veloor varikka-1	13.45	28.46	7.64	20.82	0.51
Veloor varikka-2	5.93	15.12	6.70	8.42	0.33
Pathamuttom varikka-1	21.52	22.87	8.65	14.22	0.50
Pathamuttom varikka-2	10.53	39.87	12.89	26.98	0.60
Kavanar varikka-1	6.16	15.27	7.57	7.70	0.44
Chengalam varikka	9.98	31.30	8.99	22.31	0.39
Muttom varikka	7.85	25.41	8.16	17.25	0.63
Sindhur	12.50	30.74	2.38	28.36	0.55

Table 5. Biochemical characters of fruits from different jackfruit genotypes

Genotypes	TSS (°Brix)	Acidity (%)	Total sugar (%)	Reducing sugar (%)	Non reducing sugar (%)	Ascorbic acid (mg/100g)
Veloor varikka-1	26.03	0.56	16.88	5.44	11.44	3.10
Veloor varikka-2	26.50	1.02	14.96	5.36	9.6	2.40
Pathamuttom varikka-1	30.00	1.02	14.20	4.07	10.13	9.62
Pathamuttom Varikka-2	18.80	0.44	14.17	3.57	10.60	12.00
Kavanar Varikka-1	26.40	0.38	14.65	5.32	9.33	2.40
Chengalam varikka	31.80	0.64	19.81	9.39	10.42	3.22
Muttom varikka	27.00	0.64	17.84	9.39	8.45	10.45
Sindhur	32.00	0.42	12.70	3.27	9.43	7.82

Veloor varikka-1

Veloor varikka-2

Pathamuttom varikka-1

Pathamuttom varikka-2

Kavanar varikka-1

Chengalam varikka

Muttom varikka-1

Sindhur

Plate 2. Flakes of genotypes used for the study

4.2. Molecular characterization

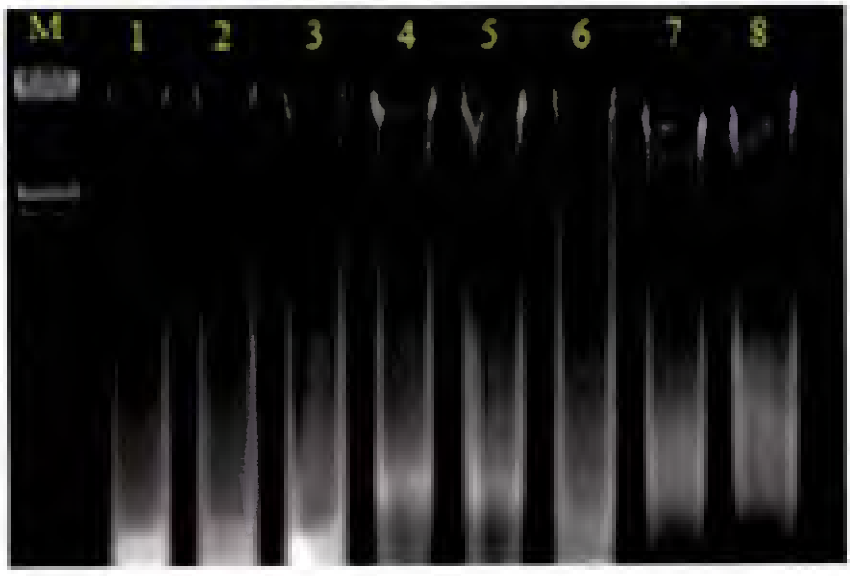
4.2.1 Isolation and quantification of DNA

Tender emerging leaves were collected from each genotype of jackfruit in early morning. DNA isolation was done as per the modified CTAB method described by Doyle and Doyle (1987). Slight modifications were made in the protocol for the isolation of good quality DNA (Plate 3) as reported by Ashwini (2015). In this protocol, sample size was reduced from 0.5-1.0 g to 0.2-0.5 g. Liquid nitrogen was used for powdering of sample, which helped to isolate nick free DNA (Blin and Stafford, 1976). Centrifugation with chloroform-isoamyl alcohol (24:1) was done two times for getting more clear solution. The DNA pellet was washed with wash buffer, followed by 70% ethanol for removing salt and detergents completely. Purification procedure was also performed for getting contamination free DNA (Plate 4).

DNA quantification and quality checking was done using Nanodrop^R spectrophotometer (ND-1000). The highest absorbance of nucleic acid is found at 260 nm and that of protein is at 280 nm. Hence OD_{260}/OD_{280} ratio was recorded to check the purity of DNA. The ratio of DNA sample was found to be in between 1.81 -1.96, which was considered as pure DNA.

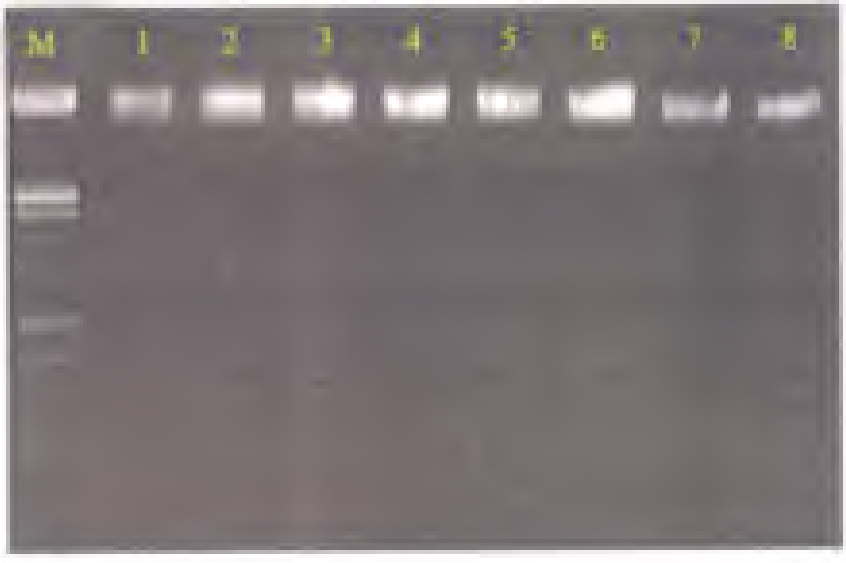
Table 6. Quantification of purified DNA from eight jackfruit genotypes

Genotypes	UV absorbance at 260 nm (A_{260})	UV absorbance at 280 nm (A_{280})	Optical density ($A_{260/280}$)	Quantity (ng/ µl)
Veloor varikka-1	8.48	4.60	1.84	540
Veloor varikka-2	14.02	7.40	1.89	500
Pathamuttom varikka-1	6.5	3.3	1.96	342
Pathamuttom Varikka-2	17.10	9.20	1.86	463
Kavanar Varikka-1	9.57	5.21	1.84	236
Chengalam varikka	10.50	5.80	1.81	371
Muttom varikka	6.02	3.12	1.93	380
Sindhur	7.88	4.30	1.83	411



M- Lambda/Hind III DNA weight marker, 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom Varikka-2, 5- Kavanar Varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 3. Isolated DNA from eight jackfruit genotypes before purification



M-Lambda/Hind III DNA weight marker, 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom Varikka-2, 5- Kavanar Varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 4. Isolated DNA from eight jackfruit genotypes after purification

4.3 Molecular marker analysis

Screening was done using bulked DNA from eight jackfruit genotypes. Fifty SSR and fifty ISSR markers were screened using bulked DNA. From these primers desirable primers were selected and utilized for further amplification of eight jackfruit samples. Reproducibility of selected primers was tested by repeating the PCR reactions. Different dilutions of DNA were tested and 40 ng/µl was found to be optimum for the PCR reactions.

4.3.1 Inter Simple Sequence Repeat (ISSR) analysis

4.3.1.1 Primer screening for ISSR assay

The details of ISSR primers screened were provided in Table 7. Out of the fifty primers, ten showed good polymorphism and reproducibility. These were further utilised in fingerprinting of eight jack genotypes. The details of polymorphic markers are shown in the table (Table 8).

Table 7. DNA amplification pattern obtained with fifty ISSR primers

Sl. No.	Primer	Amplification pattern			
		No. of amplicon	Types of amplicons		Remarks
			Distinct	Faint	
1	UBC 807	17	3	14	Rejected
2	UBC 809	1	0	1	Rejected
3	UBC 810	14	8	6	Selected
4	UBC 811	3	2	1	Rejected
5	UBC 812	3	0	3	Rejected
6	UBC 813	4	4	0	Rejected
7	UBC 814	3	2	1	Rejected
8	UBC 815	15	9	6	Selected
9	UBC 816	8	0	8	Rejected
10	UBC 817	0	0	0	Rejected
11	UBC 818	1	1	0	Rejected
12	UBC 820	5	3	2	Rejected
13	UBC 825	8	3	5	Rejected
14	UBC 826	4	0	4	Rejected
15	UBC 834a	15	8	7	Selected
16	UBC 834b	9	6	3	Selected
17	UBC 835	14	10	4	Rejected
18	UBC 836a	10	5	5	Rejected
19	UBC 838	8	4	4	Rejected
20	UBC 840a	11	6	5	Rejected
21	UBC 840b	10	6	4	Rejected
22	UBC 841a	9	5	4	Selected
23	UBC 841b	17	11	6	Selected
24	UBC 842b	12	7	5	Rejected
25	UBC 843a	11	5	6	Rejected
26	UBC 844a	11	6	5	Selected
27	UBC 845	9	9	0	Rejected
28	UBC 848a	13	8	5	Selected
29	UBC 850a	18	11	7	Rejected
30	UBC 852a	12	7	5	Rejected
31	UBC 854	2	2	0	Rejected
32	UBC 855	19	12	7	Rejected
33	UBC 857a	7	4	3	Selected
34	UBC 857b	9	7	2	Selected
35	UBC 858	4	0	4	Rejected
36	UBC 860	10	4	6	Rejected

37	UBC 862	8	0	8	Rejected
38	UBC 865	0	0	0	Rejected
39	UBC 866	12	6	6	Rejected
40	UBC 868	0	0	0	Rejected
41	UBC 872	0	0	0	Rejected
42	UBC 873	7	5	3	Rejected
43	UBC 874	0	0	0	Rejected
44	UBC 880	0	0	0	Rejected
45	UBC 892	0	0	0	Rejected
46	UBC 895	0	0	0	Rejected
47	UBC 899	8	0	8	Rejected
48	UBC 900	7	3	4	Rejected
49	TC 10G	15	8	7	Rejected
50	CT 10A	14	0	14	Rejected

Table 8. Details of selected ISSR primers

Sl. No.	Primer	Annealing temperature (°C)	Nucleotide sequence
1	UBC 810	52.5	5'-GAGAGAGAGAGAGAGAT-3'
2	UBC 815	52	5'-CTCTCTCTCTCTCTCTG-3'
3	UBC 834a	50	5'-AGAGAGAGAGAGAGAGCT-3'
4	UBC 834b	50	5'-AGAGAGAGAGAGAGAGTT-3'
5	UBC 841a	50	5'-GAGAGAGAGAGAGAGACC-3'
6	UBC 841b	50	5'-GAGAGAGAGAGAGAGATC-3'
7	UBC 844a	52	5'-CTCTCTCTCTCTCTCTAC-3'
8	UBC 848a	52	5'-CACACACACACACACAAG-3'
9	UBC 857a	50	5'-ACACACACACACACACCG-3'
10	UBC 857b	50	5'-ACACACACACACACACTG-3'

4.3.2 Simple Sequence Repeat (SSR) analysis

4.3.2.1 Primer screening for SSR assay

Fifty SSR primer sets were screened (Table 9) for polymorphism in eight jack genotypes studied after bulking the DNA. Eleven SSR primer sets were selected based on their amplification pattern for developing fingerprints of eight jack genotypes. Table 10 describes the details of selected primer sets. Six of the selected primers belonged to mAaCIR series and five belonged to MAA.

Table 9. DNA amplification pattern obtained with fifty SSR primers

Sl. No.	Primer	Amplification pattern			
		No. of amplicon	Types of amplicons		Remarks
			Distinct	Faint	
1	MulSSR I	0	0	0	Rejected
2	MulSSR 2	1	0	1	Rejected
3	MulSSR 23	0	0	0	Rejected
4	MulSSR 26	1	1	0	Rejected
5	MULSSR 59	1	1	0	Rejected
6	MULSSR 69	0	0	0	Rejected
7	MulSSR 82	0	0	0	Rejected
8	MulSSR 85	1	1	0	Rejected
9	M2SSR 1	1	0	1	Rejected
10	M2SSR 5	1	0	1	Rejected
11	mAaCIR 0019	0	0	0	Rejected
12	mAaCIR 0033	0	0	0	Rejected
13	mAaCIR 0048	2	1	1	Rejected
14	mAaCIR 0049	2	1	1	Selected
15	mAaCIR 0050	0	0	0	Rejected
16	mAaCIR 0053	0	0	0	Rejected
17	mAaCIR 0075	1	0	1	Rejected
18	mAaCIR 0078	2	1	1	Selected
19	mAaCIR 0081	1	1	0	Rejected
20	mAaCIR 0089	3	3	0	Rejected
21	mAaCIR 0090	0	0	0	Rejected
22	mAaCIR 0108	0	0	0	Rejected
23	mAaCIR 0111	1	1	0	Rejected
24	mAaCIR 0115	3	1	2	Selected
25	mAaCIR 0127	2	1	1	Selected
26	mAaCIR 0128	1	0	1	Rejected
27	mAaCIR 0134	2	1	1	Selected
28	mAaCIR 0141	2	1	1	Selected

29	mAaCIR 0146	2	0	2	Rejected
30	mAaCIR 0154	0	0	0	Rejected
31	mAaCIR 0204	2	0	2	Rejected
32	MAA 3	0	0	0	Rejected
33	MAA 9	0	0	0	Rejected
34	MAA 26	1	0	1	Rejected
35	MAA 40	0	0	0	Rejected
36	MAA 54a	4	2	2	Selected
37	MAA 54b	4	0	4	Rejected
38	MAA 71	0	0	0	Rejected
39	MAA 85	0	0	0	Rejected
40	MAA 96	0	0	0	Rejected
41	MAA 105	4	3	1	Selected
42	MAA 122	2	1	1	Selected
43	MAA 140	1	0	1	Rejected
44	MAA 145	1	1	0	Selected
45	MAA 156	5	5	0	Rejected
46	MAA 178a	6	0	6	Rejected
47	MAA 178b	6	0	6	Rejected
48	MAA 182	4	4	0	Rejected
49	MAA 196a	4	4	0	Selected
50	MAA 196b	0	0	0	Rejected

Table 10. Details of selected SSR primers

Sl. No.	Primer	Annealing temperature (°C)	Nucleotide sequence
1	mAaCIR 0049	53	F:5'-TACATACAAGCCAACTTCCA-3' R:5'-CCTTTGTGAGGAAGACCA-3'
2	mAaCIR 0078	53	F:5'-CTTCAACTATTACTACTGCTGCT-3' R:5'-CTGTTCAGGTTGGTGCT-3'
3	mAaCIR 0115	54	F: 5'-ACAGCTTTGCACCGACAC-3' R:5'-GCCCTCAACCACCCC-3'
4	mAaCIR 0127	52	F:5'-TGATTCTCTCTTTACAGGCAC-3' R:5'-GCTCAGGTGCTTACTTGTTC-3'
5	mAaCIR 0134	55	F:5'-AGCTGCCAATGATCCC-3' R:5'-ATGTGAAAAGGTTGGATTTG-3'
6	mAaCIR 0141	55	F:5'-TCAAGCCCCTCACTCAA-3' R:5'-ATGGCATAGCACAACACAA-3'
7	MAA 54a	55	F:5'-AACCTCCAAACACTAGGACAAC-3' R:5'-AGCTACTTCCAAAACGTGACA-3'
8	MAA 105	55	F:5'-GTTGGGACACTGTGAACTATTC-3' R:5'-AAAAGCTAGTGGATTAGATGCA-3'
9	MAA 122	55	F:5'-CTGGCCTTCAGTTTTGTCAAC-3' R:5'-CACCAGGCTTCAAGATGAAA-3'
10	MAA 145	55	F:5'-CCAACGCATAGCCAAATC-3' R:5'-AAATCCCAAACCCAACGT-3'
11	MAA 196a	55	F:5'-GGAATGTGGTAGATGAAACTCC-3' R:5'-CGACAAAAAAACAAAGGAAGAC-3'

4.3.3 Polymorphic information content (PIC) of selected ISSR and SSR primes

The total numbers of loci produced by selected 10 ISSR primers among eight jackfruit genotypes were 122 with an average of 12.2 loci per primer (Table 11). An average of 8.8 polymorphic loci was produced by each primer. The percentage of polymorphism varied from 55.55 % (UBC 834b) to 87.5 % (UBC 857a). The PIC values were in the range of 0.23 (UBC 834b) to 0.3 (UBC 857a) with an average 0.27 (Fig. 1a) which indicated the usefulness of these primers.

In the case of SSR analysis, it yielded a total number of 29 alleles with an average of 2.63 alleles per primer (Table 12). An average of 1.81 polymorphic alleles was produced by each primer. The percentage of polymorphism varied from 50 % (mAaCIR 0049, mAaCIR 0078, mAaCIR 0127, mAaCIR 0134, mAaCIR 0141 and MAA 122) to 100 % (mAaCIR 0115 and MAA 145). Calculated SSR PIC value for selected SSR primers were ranged between 0.22 (MAA 54a) to 0.98 (mAaCIR 0078, mAaCIR 0134, mAaCIR 0141 and MAA 122) with an average of 0.75 (Fig. 1b).

4.3.4 Marker index (MI) of selected ISSR and SSR primers

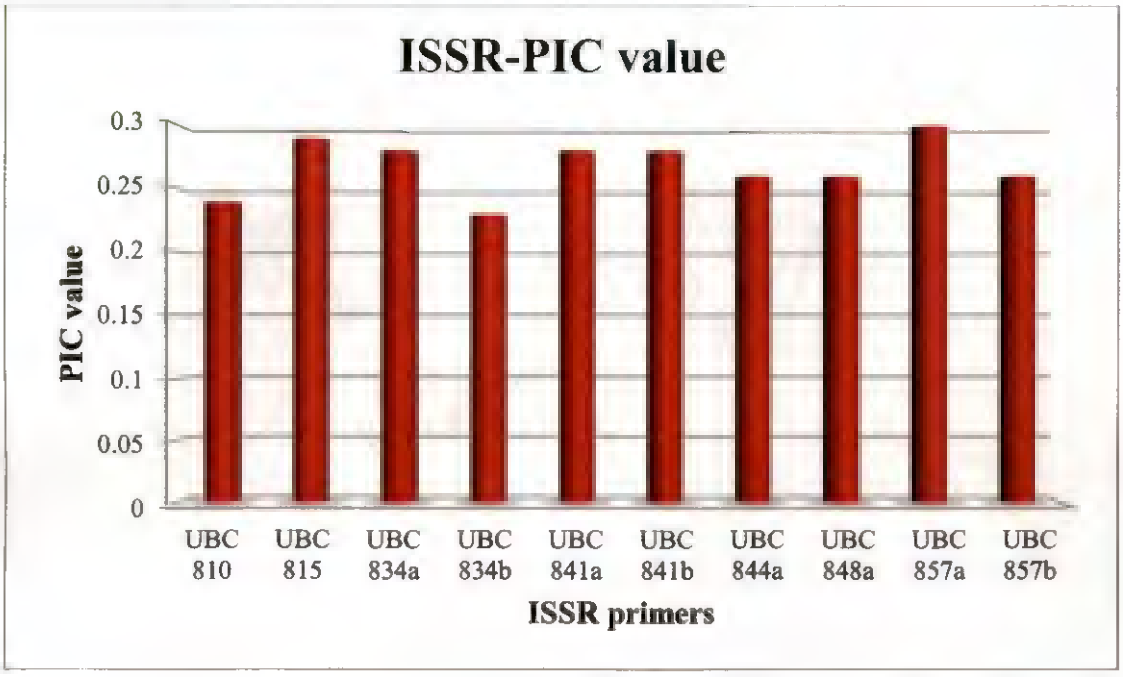
Marker index (MI) of selected ISSR (Table 11, Fig. 2a) and SSR (Table 12, Fig. 2b) primers were calculated. Calculated marker index were in the range of 1.15 (UBC 834b) to 3.77 (UBC 815) for ISSR primers with average being 2.38. Marker index for SSR primers varied between 0.60 (mAaCIR 0115) to 2.7 (MAA 196a) with an average 1.18.

Table 11. Details of DNA amplification with selected 10 ISSR primers

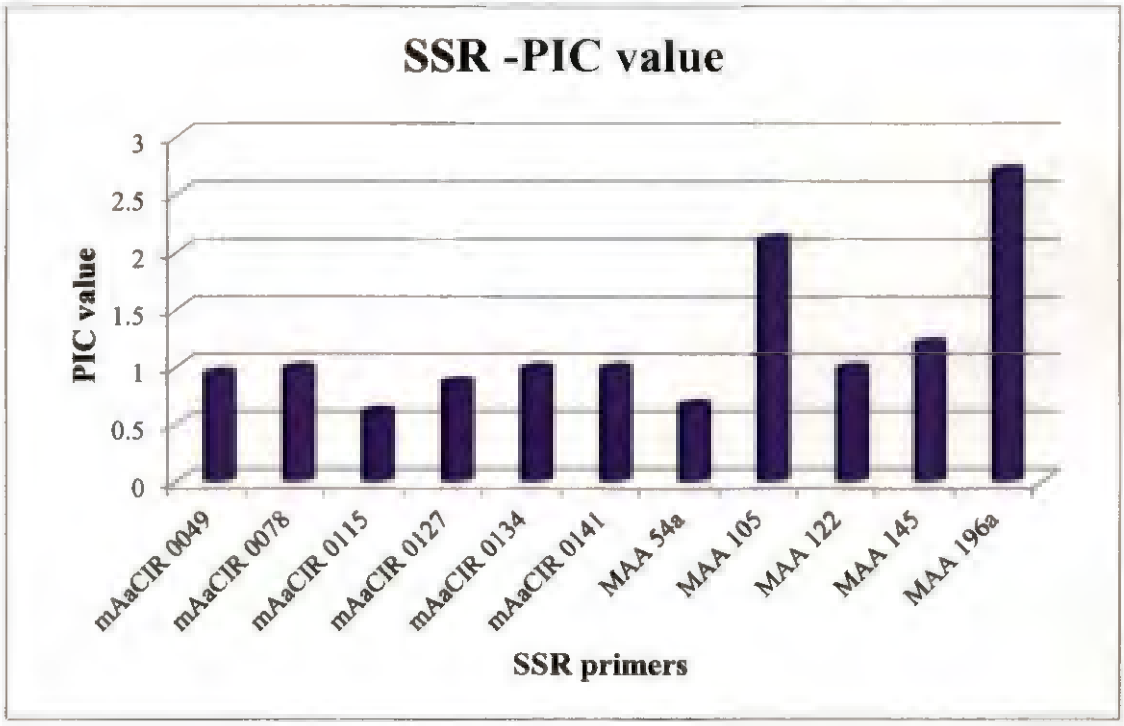
Sl. No.	ISSR Primer	Total no of loci	No of polymorphic loci	Polymorphism (%)	Polymorphic information content (PIC)	Maker Index (MI)
1	UBC 810	16	11	68.75	0.24	2.64
2	UBC 815	16	13	81.25	0.29	3.77
3	UBC 834a	14	11	78.57	0.28	3.08
4	UBC 834b	9	5	55.55	0.23	1.15
5	UBC 841a	9	7	77.77	0.28	1.96
6	UBC 841b	17	11	64.70	0.28	3.08
7	UBC 844a	11	7	63.63	0.26	1.82
8	UBC 848a	13	10	76.92	0.26	2.6
9	UBC 857a	8	7	87.50	0.30	2.1
10	UBC 857b	9	6	66.66	0.26	1.56
Total		**122**	**88**	**721.3**	**2.68**	**23.76**
Average		**12.2**	**8.8**	**72.13**	**0.27**	**2.38**

Table 12. Details of DNA mplification with selected 11 SSR primers

Sl. No.	SSR Primer	Total no of alleles	No of polymorphic alleles	Polymorphism (%)	Polymorphic information content (PIC)	Maker Index (MI)
1	mAaCIR 0049	2	1	50	0.94	0.94
2	mAaCIR 0078	2	1	50	0.98	0.98
3	mAaCIR 0115	2	2	100	0.30	0.60
4	mAaCIR 0127	2	1	50	0.86	0.86
5	mAaCIR 0134	2	1	50	0.98	0.98
6	mAaCIR 0141	2	1	50	0.98	0.98
7	MAA 54a	4	3	75	0.22	0.66
8	MAA 105	4	3	75	0.70	2.1
9	MAA 122	2	1	50	0.98	0.98
10	MAA 145	3	3	100	0.40	1.2
11	MAA 196a	4	3	75	0.90	2.7
Total		**29**	**20**	**725**	**8.24**	**12.98**
Average		**2.63**	**1.81**	**65.90**	**0.75**	**1.18**

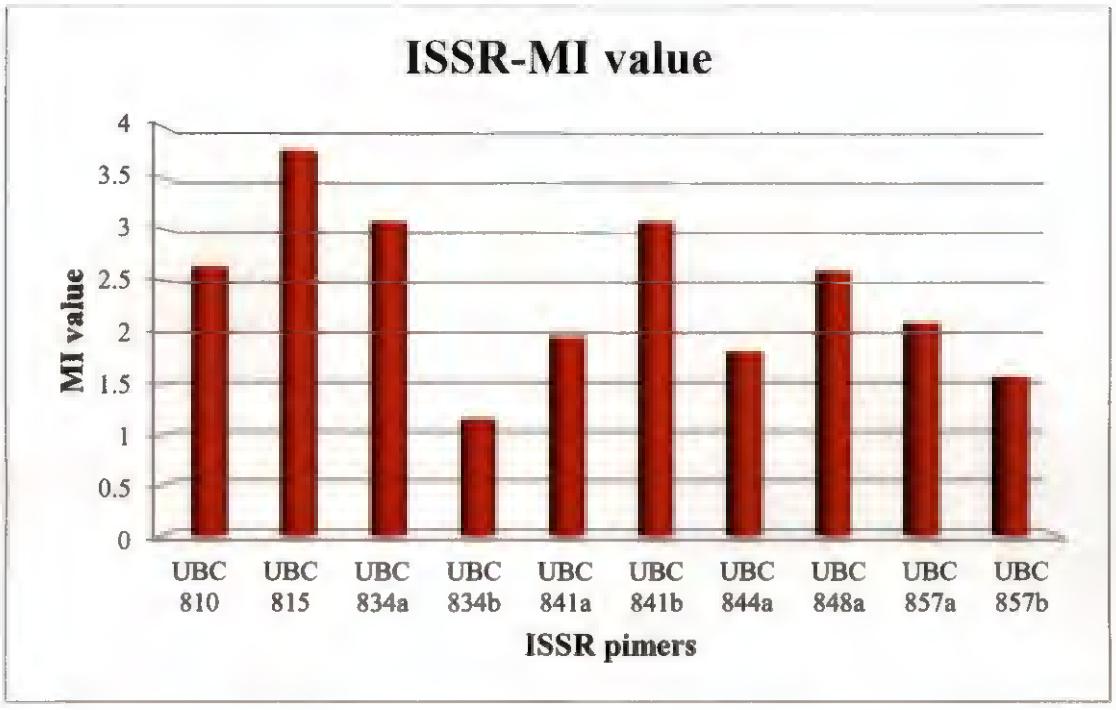


a. PIC value of ISSR primers

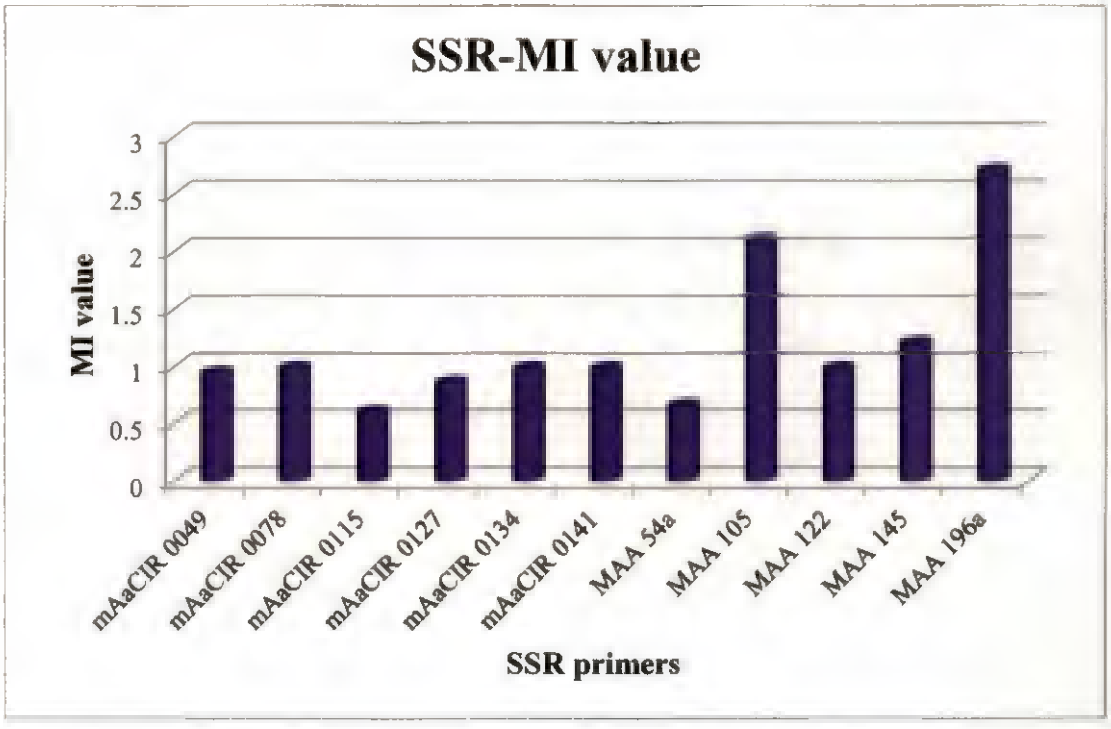


b. PIC value of SSR primers

Fig. 1 Polymorphic information content of selected ISSR and SSR primes



a. MI value of ISSR primers



b. MI value of SSR primers

Fig. 2 Marker index of selected ISSR and SSR primers

4.3.5 DNA amplification pattern of selected ISSR primers

In order to develop fingerprints for the selected jackfruit genotypes, DNA samples were amplified with selected ISSR primers (Table 8). The details of amplification are described below.

4.3.5.1 UBC 810

The DNA samples from eight jackfruit genotypes were amplified using primers UBC 810 (Plate 5; Fig. 3) which generated an average of eleven amplicons in each accession. Molecular size of the amplicons generated ranged between 370 bp to 1600 bp. At 430 bp length, polymorphic bands were present in Veloor varikka-1 and Veloor varikka-2. Another polymorphic band was observed in Kavanar varikka-1 and Muttom varikka at 630 bp. This primer also produced a unique band at 480 bp in Pathamuttom varikka-1 and hence it can be used for identifying this genotype.

4.3.5.2 UBC 815

The primer UBC 815 (Plate 6) produced an average of nine amplicons per accession. Molecular size of the amplicons varied from 410 bp to 8000 bp. The pattern of amplification is depicted in fig. 4. Unique polymorphic bands were found in Muttom varikka at 2500 bp and 3500 bp. Another unique band at 1050 bp was found for Sindhur. At 600 bp length, Pathamuttom varikka-1 and Kavanar varikka-1 shared a polymorphic band. The amplicons detected at 2500 bp and 3500 bp can be a fingerprint of the cultivar Muttom varikka and amplicon at 1050 bp can be a fingerprint for the variety Sindhur.

4.3.5.3 UBC 834a

ISSR analysis of jackfruit accessions using the primer UBC 834a generated an average of 6 amplicons per accession on agarose gel (Plate 7; Fig. 5). Molecular size of the amplicons varied from 370 bp to 2500 bp. On careful observation of gel picture it was observed that the bands formed at 950 bp and 1000 bp are specific for Muttom varikka and bands at 580 bp and 640 bp are distinct for Pathamuttom varikka-1. Hence this primer can be used for identifying Pathamuttom varikka 1 and Muttom varikka. This primer also produced polymorphic bands at 450 bp and 760 bp in Kavanar varikka-1 and Muttom varikka. Another polymorphic band was observed at a length of 370 bp with Chengalam varikka and Sindhur.

4.3.5.4 UBC 834b

On an average seven amplicons were obtained when the DNA samples were amplified with the primer UBC 834b (Plate 8). Molecular size of the amplicons generated varied between 380 bp and 1100 bp. A unique band was found in Chengalam varikka at 760 bp. Thus, the primer UBC 834b can be used for identifying the genotype Chengalam varikka (Fig. 6).

4.3.5.5 UBC 841a

The primer UBC 841a generated an average of five amplicons per accession (Plate 9). Molecular size of the amplicons generated ranged between 240 bp and 520 bp. Unique polymorphic bands were observed in Pathamuttom varikka-1 at 520 bp and Pathamuttom varikka-2 at 370 bp (Fig. 7) on close observation of gel picture. Thus, the primer UBC 841a can be used for identifying Pathamuttom varikka-1 and Pathamuttom varikka-2 of jackfruit.

4.3.5.6 UBC 841b

Amplification pattern observed using the primer UBC 841b consisted an average of eleven amplicons (Plate 10; Fig. 8). Molecular size of the amplicons generated varied from 380 bp to 1400 bp. At 700 bp length, a specific polymorphic band was formed in Pathamuttom varikka-2. Further amplicon scoring, revealed polymorphic bands at 1450 bp in Kavanar varikka-1 and Muttom varikka. Polymorphic bands were also observed at 950 bp in Pathamuttom varikka-1 and Chengalam varikka. Thus, the primer UBC 841b can be used for generating fingerprint for Pathamuttom varikka-2 and also for identifying Pathamuttom varikka 1, Kavanar varikka-1, Chengalam varikka and Muttom varikka from a mixed population.

4.3.5.7 UBC 844a

UBC 844a generated an average of eight amplicons in all genotypes studied. (Plate 11). Molecular size of the amplicons generated ranged between 350 bp and 1200 bp. Polymorphic band was observed in Pathamuttom varikka-2 and Muttom varikka at 1050 bp. Thus, the primer UBC 844a can be used for identifying Pathamuttom varikka-2 and Muttom varikka accessions of jackfruit (Fig. 9).

4.3.5.8 UBC 848a

On an average eight amplicons were obtained when the DNA samples were amplified with the primer UBC 848a (Plate 12). Molecular size of the amplicons developed varied from 300 bp to 1400 bp. A specific polymorphic band was formed at 1340 bp (Fig. 10) in Chengalam varikka. Kavanar varikka-1 and Muttom varikka produced polymorphic band at 600bp. Veloor varikka-1 and Veloor varikka-2 also showed polymorphism at two sites i.e., 1370 bp and 1400 bp. Thus, the primer UBC 841b can be used for identifying Veloor varikka-1, Veloor varikka-2, Kavanar varikka-1, Chengalam varikka and Muttom varikka varieties of jackfruit.

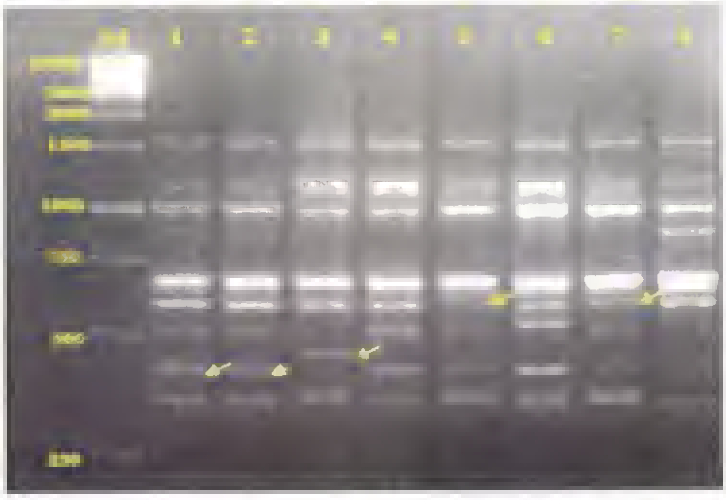
4.3.5.9 UBC 857a

ISSR analysis of DNA samples using the primer UBC 857a produced an average of four amplicons per genotype on agarose gel (Plate 13; Fig. 11). The molecular size of the amplicons generated ranged from 300 bp to 1490 bp. Kavanar varikka-1 and Muttom varikka-1 showed polymorphic band at 800 bp. Specific polymorphic bands were found in Chengalam varikka at two sites *i.e.*, 1250 bp and 1490 bp. Thus, the primer UBC 857a was found ideal for identifying Kavanar varikka-1, Chengalam varikka and Muttom varikka accessions of jackfruits. The unique amplicon scored for Chengalam varikka will serve as a fingerprint for the genotypes.

4.3.5.10 UBC 857b

The primer UBC 857b developed an average of six amplicons in all the eight jackfruit genotypes (Plate 14; Fig. 12). The molecular size of the amplicons generated varied from 360 bp to 950 bp. Chengalam varikka-1 developed a specific polymorphic band at 680 bp and Veloor varikka-1 and Veloor varikka-2 yielded a polymorphic band at 360 bp. Thus, the primer UBC 857b can be utilised for identifying Veloor varikka-1, Veloor varikka-2 and Chengalam varikka-1 genotypes of jackfruit.

In the present study all the selected ten primes developed at least one useful polymorphic band among the eight selected genotypes of jack fruit. These selected primers belong to the series, University of British Colombia (UBC). Out of the ten primers selected, nine of them generated unique bands which can acts as genotype specific DNA fingerprints.



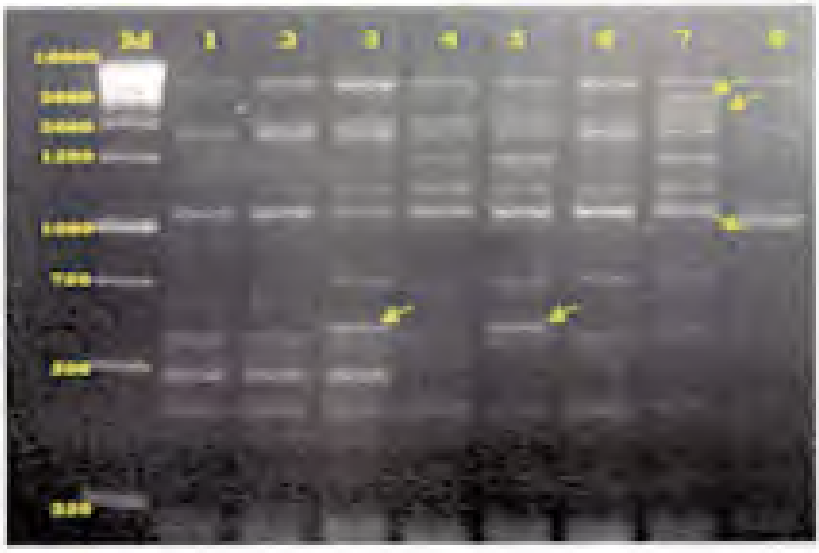
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 5. DNA amplification pattern generated with primer UBC 810

Mol. Size(bp)/ varieties	1	2	3	4	5	6	7	8
1600								
1250								
1200								
1000								
900								
700								
680								
630								
600								
520								
500								
480								
450								
430								
380								
370								

Colour codes for bands

Fig. 3. Colour chart developed using ISSR primer UBC 810



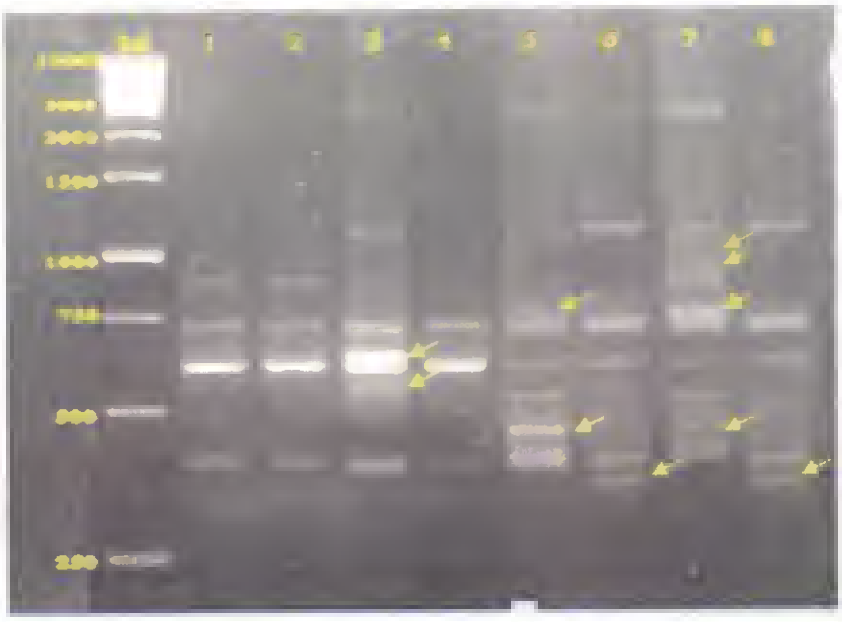
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 6. DNA amplification pattern generated with primer UBC 815

Mol. Size(bp)/ varieties	1	2	3	4	5	6	7	8
8000								
3500								
2500								
2000								
1900								
1500								
1250								
1120								
1050								
750								
730								
600								
590								
580								
490								
410								

Colour codes for bands

Fig. 4 Colour chart developed using ISSR primer UBC 815



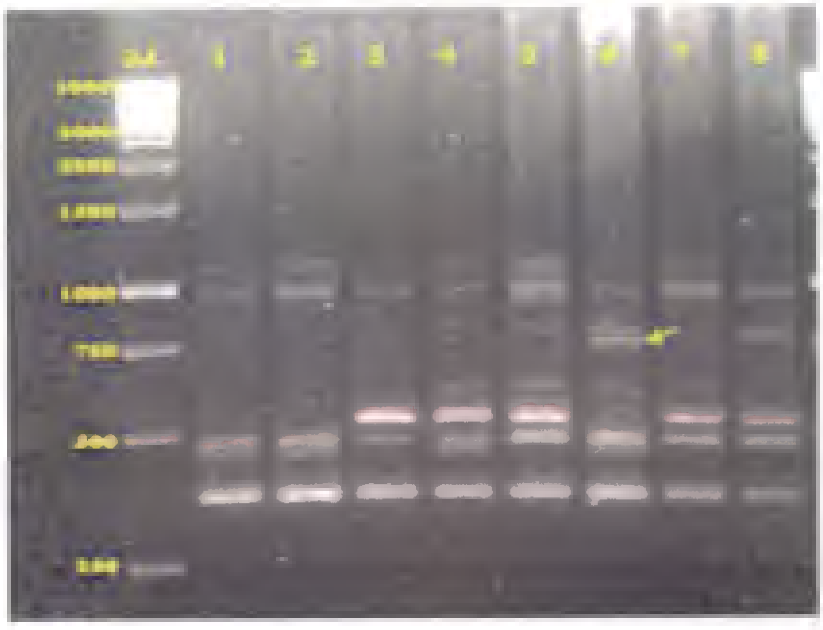
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 7. DNA amplification pattern generated with primer UBC 834a

Mol. Size(bp)/ varieties	1	2	3	4	5	6	7	8
2500								
1200								
1000								
950								
900								
760								
730								
640								
630								
580								
550								
450								
400								
370								

Colour codes for bands

Fig. 5 Colour chart developed using ISSR primer UBC 834a



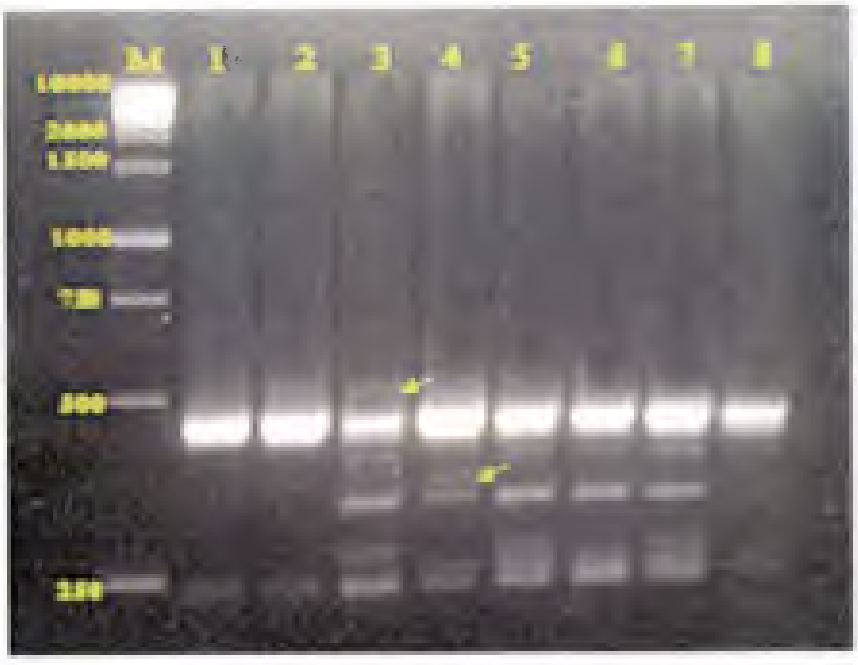
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 8. DNA amplification pattern generated with primer UBC 834b

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
1100								
950								
800								
760								
600								
550								
490								
470								
380								

Colour codes for bands

Fig. 6 Colour chart developed using ISSR primer UBC 834b



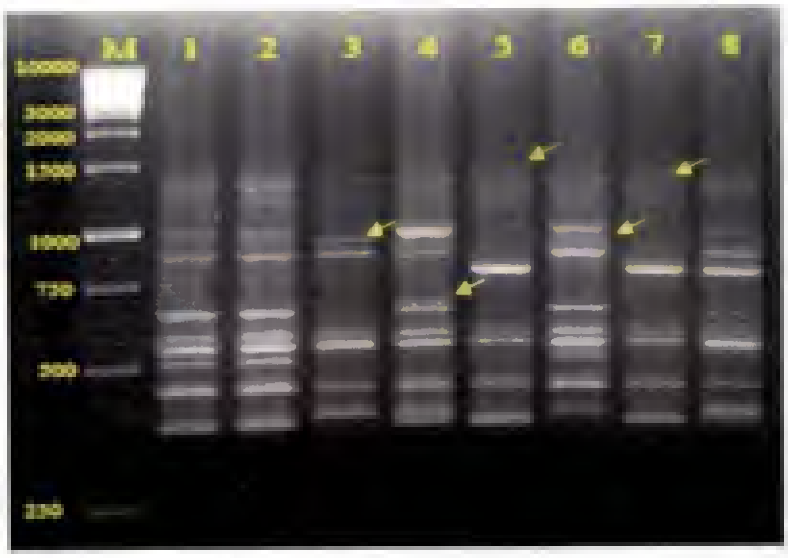
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 9. DNA amplification pattern generated with primer UBC 841a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
520								
450								
440								
410								
370								
350								
280								
260								
240								

Colour codes for bands

Fig. 7 Colour chart developed using ISSR primer UBC 841a



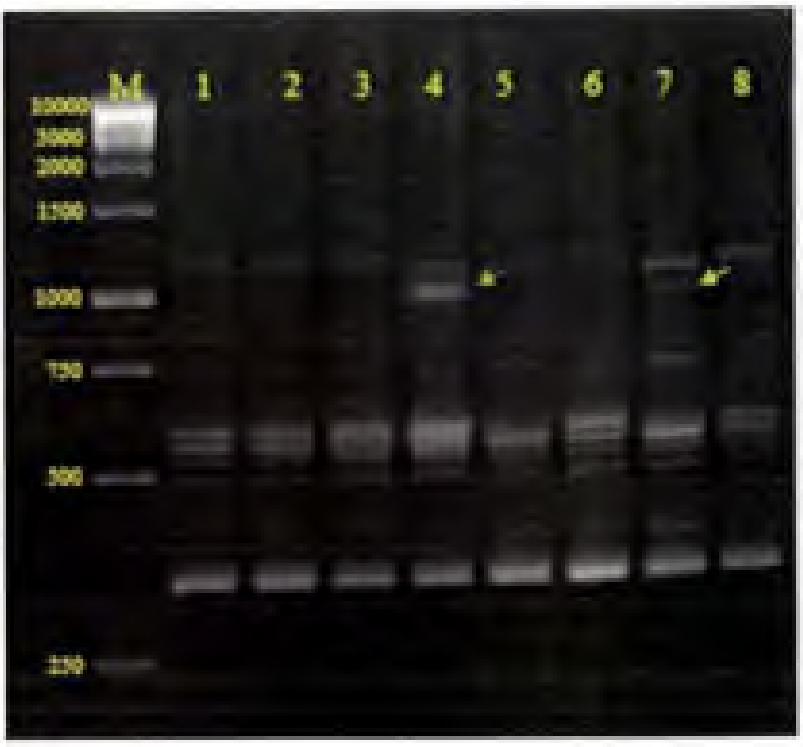
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 10. DNA amplification pattern generated with primer UBC 841b

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
1450								
1400								
1350								
1250								
1150								
1000								
950								
870								
820								
700								
680								
600								
550								
510								
450								
400								
380								

Colour codes for bands

Fig. 8 Colour chart developed using ISSR primer UBC 841b



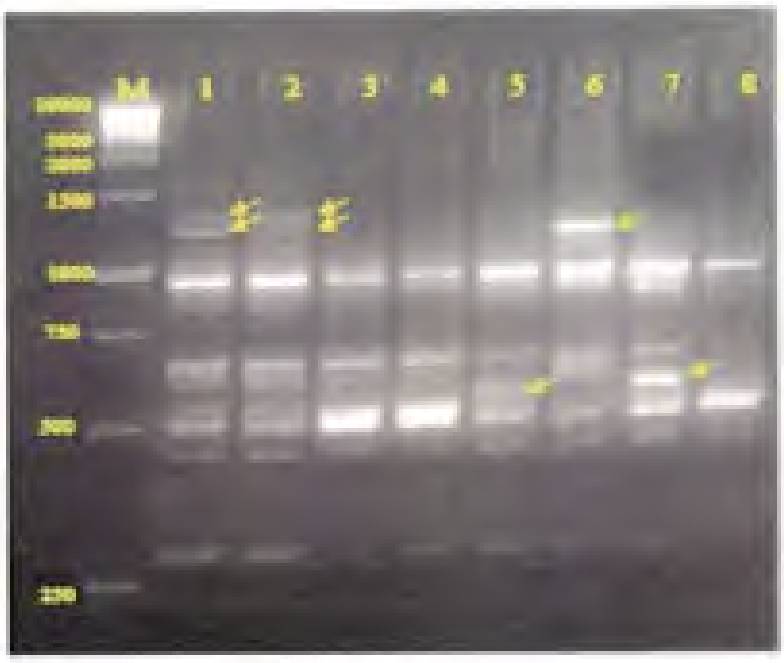
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 11. DNA amplification pattern generated with primer UBC 844a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
1200								
1190								
1050								
750								
650								
600								
570								
540								
500								
400								
350								

Colour codes for bands

Fig. 9 Colour chart developed using ISSR primer UBC 844a



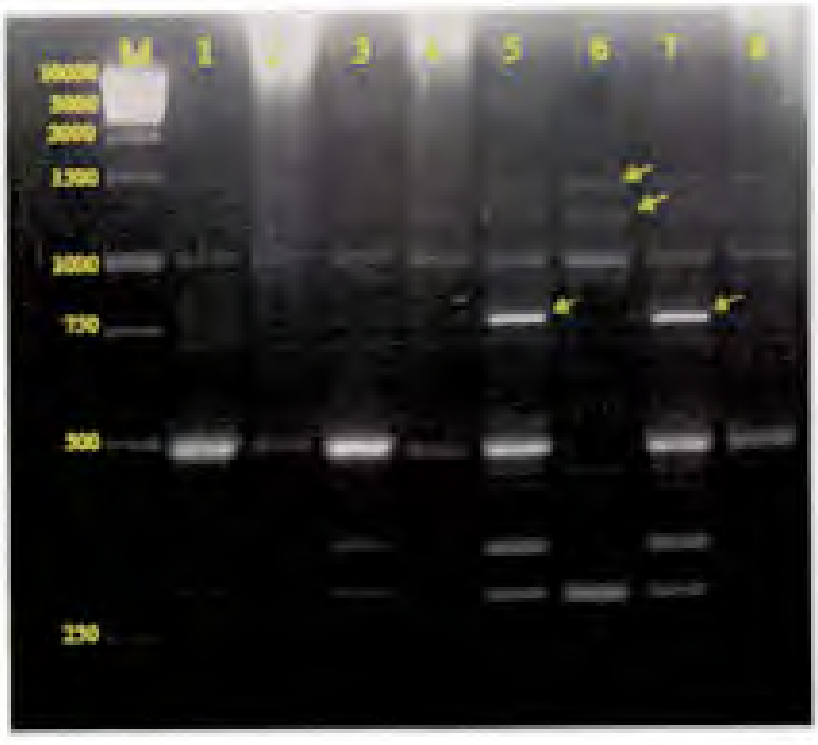
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 12. DNA amplification pattern generated with primer UBC 848a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
1400								
1370								
1300								
980								
950								
920								
680								
640								
600								
550								
500								
450								
300								

Colour codes for bands

Fig. 10 Colour chart developed using ISSR primer UBC 848a



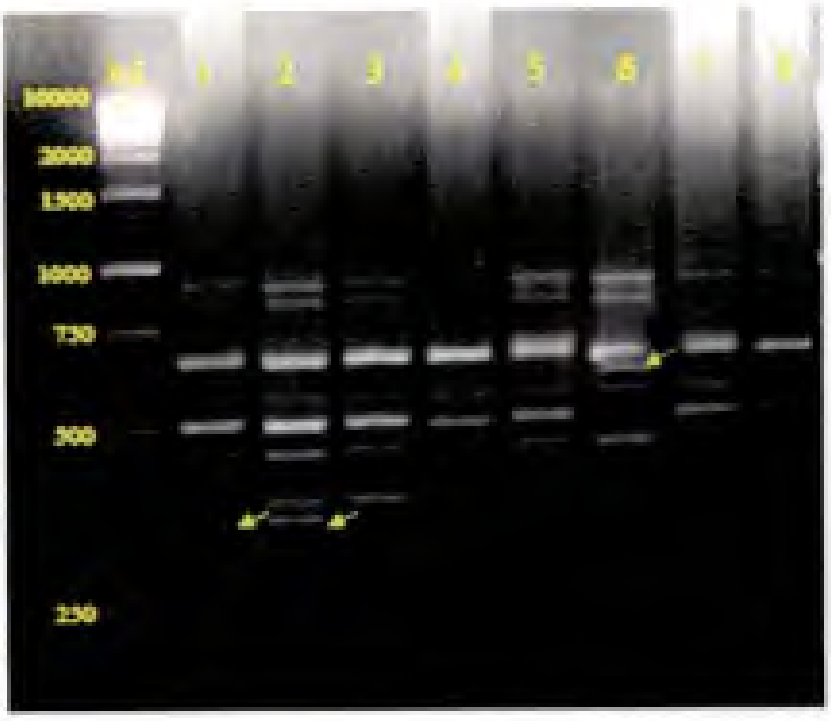
M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur.

Plate 13. DNA amplification pattern generated with primer UBC 857a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
1490								
1250								
1000								
800								
500								
470								
370								
300								

Colour codes for bands

Fig. 11 Colour chart developed using ISSR primer UBC 857a



M- Molecular weight marker (1 kb), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 14. DNA amplification pattern generated with primer UBC 857b

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
950								
900								
690								
680								
590								
500								
450								
380								
360								

Colour codes for bands

Fig. 12 Colour chart developed using ISSR primer UBC 857b

4.3.6 DNA amplification of selected SSR primers

Amplification of DNA samples from eight jackfruit genotypes were carried out using selected eleven SSR primers (Table 10) to develop fingerprints. Amplification pattern of each primer was studied to recognise polymorphic bands. The details of amplification with selected primers are described below.

4.3.6.1 mAaCIR 0049

Amplification pattern of eight jack genotypes with SSR primer mAaCIR 0049 was peformed (Plate 15). The molecular size of the amplicons generated varied from 100 bp to 110 bp. At 100 bp length, polymorphic bands were present in Kavanar varikka-1 and Muttom varikka (Fig. 13). Thus the primer mAaCIR 0049 can be used for identifying Kavanar varikka-1 and Muttom varikka.

4.3.6.2 mAaCIR 0078

Amplification of DNA samples of eight jack genotypes with SSR primer mAaCIR 0078 yielded two amplicons (Plate 16). Molecular size of the amplicons generated varied from 140 bp to 160 bp. Pathamuttom varikka-1 produced a unique bands at a lenght of 160 bp (Fig. 14). Hence, the primer mAaCIR 0078 can be used for generating fingerprint for Pathamuttom varikka-1.

4.3.6.3 mAaCIR 0115

Amplification pattern of eight jack genotypes with SSR primer mAaCIR 0115 (Plate 17) showed that a polymorphic band was shared by Pathamuttom varikka-1, Pathamuttom varikka-2 and Sindhur at 175 bp (Fig. 15). Thus the primer mAaCIR 0115 can be used for identifying Pathamuttom varikka-1, Pathamuttom varikka-2 and Sindhur from a mixed population.

4.3.6.4 mAaCIR 0127

SSR analysis of DNA samples using SSR primer mAaCIR 0127 generated polymorphic bands among the genotypes studied (Plate 18; Fig. 16). Molecular size of the amplicons generated varied from 180 bp to 200 bp. The band obtained at 200 bp with this primer set was found to be polymorphic and this was shared between Kavanar varikka-1, Chengalam varikka and Muttom varikka. Another amplicon

observed at 180 bp was not polymorphic. The amplicon obtained at 200 bp was further utilized for DNA fingerprinting.

4.3.6.5 mAaCIR 0134

Amplification pattern of eight jack genotypes with SSR primer mAaCIR 0134 was peformed (Plate 19). Molecular size of the amplicons generated varied from 195 bp to 240 bp. At length of 240 bp, Pathamuttom Varikka-2 showed a unique polymorphic band (Fig. 17). Hence the primer mAaCIR 0134 can be used for identifying Pathamuttom Varikka-2 and to create fingerprint of this genotype.

4.3.6.6 mAaCIR 0141

DNA samples from eight jack genotypes were amplified with SSR primer mAaCIR 0141 and Plate 20 shows the amplification pattern. Molecular size of the amplicons generated varied between 230 bp and 240 bp. On close observation, it is found that the variety Sindhur produced a unique polymorphic band at 230 bp (Fig. 18). Thus the primer mAaCIR 0141 can be used for identifying Sindhur.

4.3.6.7 MAA 54a

Out of the four amplicons developed using the primer MAA 54a (Plate 21), polymorphic band at 220 bp was shared between Kavanar Varikka-1, Muttom varikka and Sindhur (Fig. 19). Thus the primer MAA 54a can be used for identifying Kavanar Varikka-1, Muttom varikka and Sindhur genotypes of jackfruit. Useful amplification pattern were incorporated into individual DNA fingerprint.

4.3.6.8 MAA 105

SSR analysis of eight jack genotypes with the primer MAA 105 generated a polymorphic band at 290 bp which was shared by Kavanar Varikka-1, Muttom varikka and Sindhur (Plate 22; Fig. 20). Another polymorphic band was observed at 270 bp which was shared by Veloor varikka-1, Veloor varikka-2 and Chengalam varikka. Pathamuttom Varikka-2 showed a unique band at a length of 265 bp. Thus the primer MAA 105 can be used for identifying Veloor varikka-1, Veloor varikka-2, Pathamuttom Varikka-2, Kavanar Varikka-1, Chengalam varikka, Muttom varikka and Sindhur genotypes of jackfruit.

4.3.6.9 MAA122

Amplification pattern of eight jack genotypes performed with SSR primer MAA 122 was showed in Plate 23. Molecular size of the amplicons generated varied from 265 bp to 270 bp. At length of 270 bp, Pathamuttom varikka-1 generated a specific polymorphic band (Fig. 21). Thus the primer MAA 122 can be used for developing fingerprint of Pathamuttom varikka-1 selection.

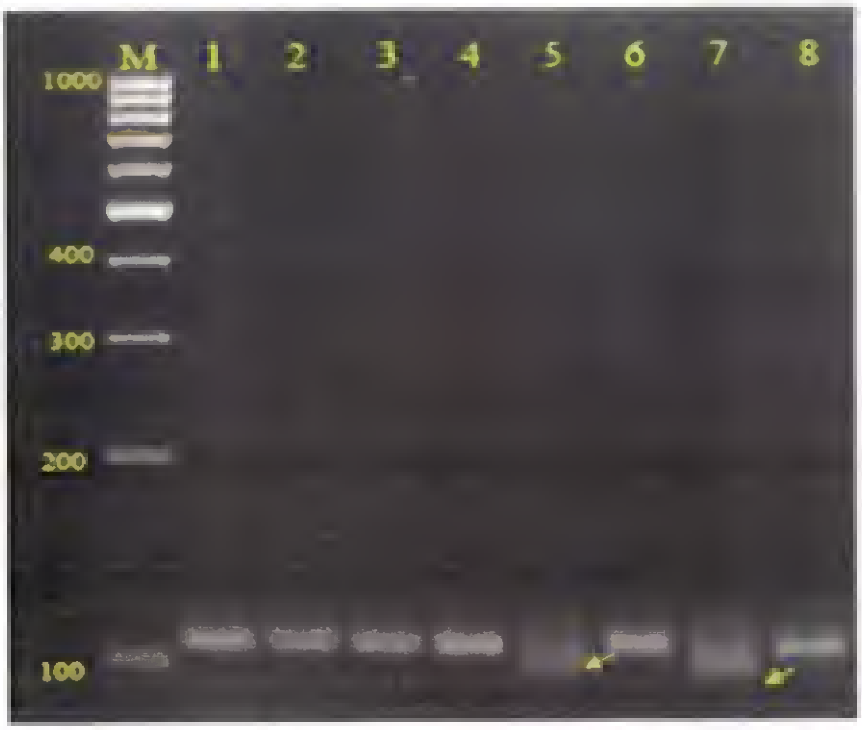
4.3.6.10 MAA 145

DNA samples from eight jack genotypes were amplified using SSR primer MAA 145 (Plate 24). Molecular size of the amplicons generated varied from 280 bp to 290 bp. A unique band was observed at 287 bp for Pathamuttom varikka-2 and at 280 bp for Chengalam varikka (Fig. 22). Thus the primer MAA 145 can be used for fingerprinting Pathamuttom varikka-2 and Chengalam varikka genotypes of jackfruit.

4.3.6.11 MAA 196a

Amplification pattern of eight jack genotypes with SSR primer MAA 196a was depicted in Plate 25. Molecular size of the amplicons generated ranged between 280 bp and 340 bp. The Pathamuttom varikka-2 and Sindhur developed unique bands at 340 bp and 320 bp respectively (Fig. 23). Pathamuttom varikka-2 and Sindhur also shares another band at 290 bp. Thus the primer MAA 196a can be used for identifying Pathamuttom varikka-2 and Sindhur.

In the present study, out of eleven SSR primers, six belonged to the series mAaCIR and five belonged to the series MAA. Among these selected primers, seven of them yielded unique bands which can acts as variety specific DNA fingerprints.



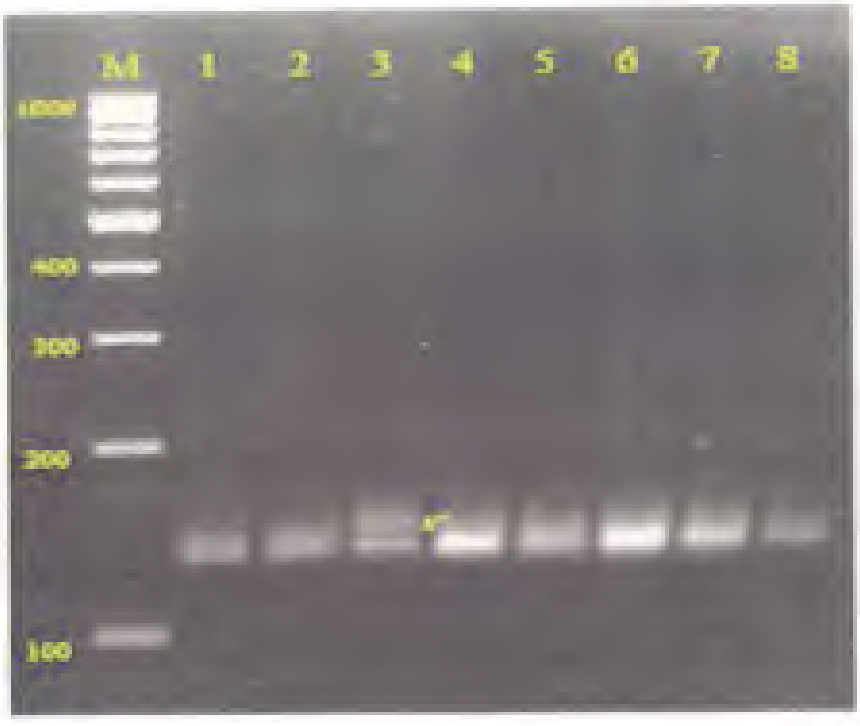
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 15. DNA amplification pattern generated with primer mAaCIR 0049

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
110								
100								

Colour codes for bands

Fig. 13 Colour chart developed using SSR primer mAaCIR 0049



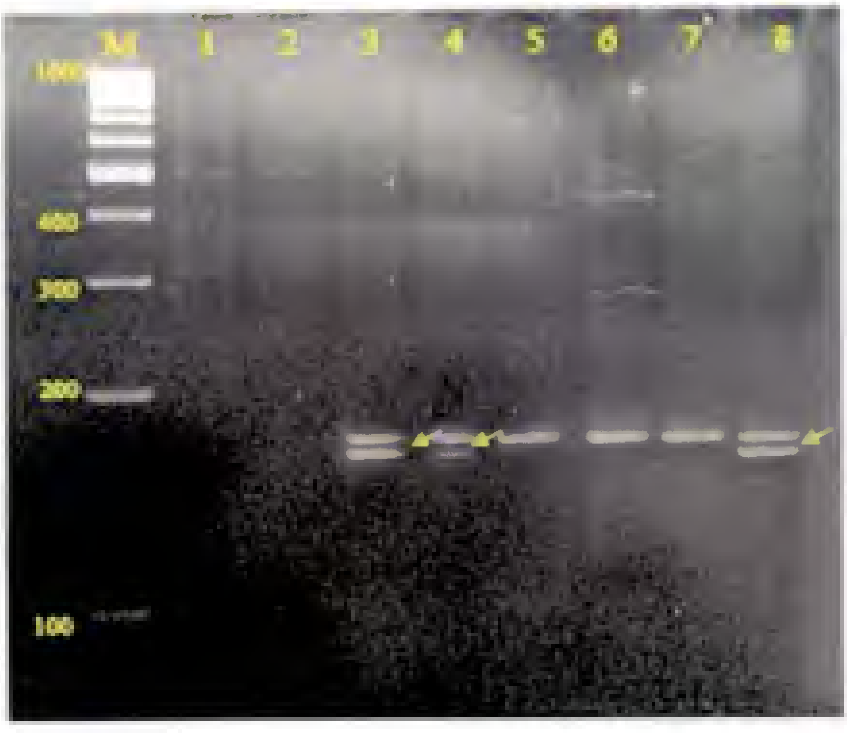
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 16. DNA amplification pattern generated with primer mAaCIR 0078

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
160								
140								

Colour codes for bands

Fig. 14 Colour chart developed using SSR primer mAaCIR 0078



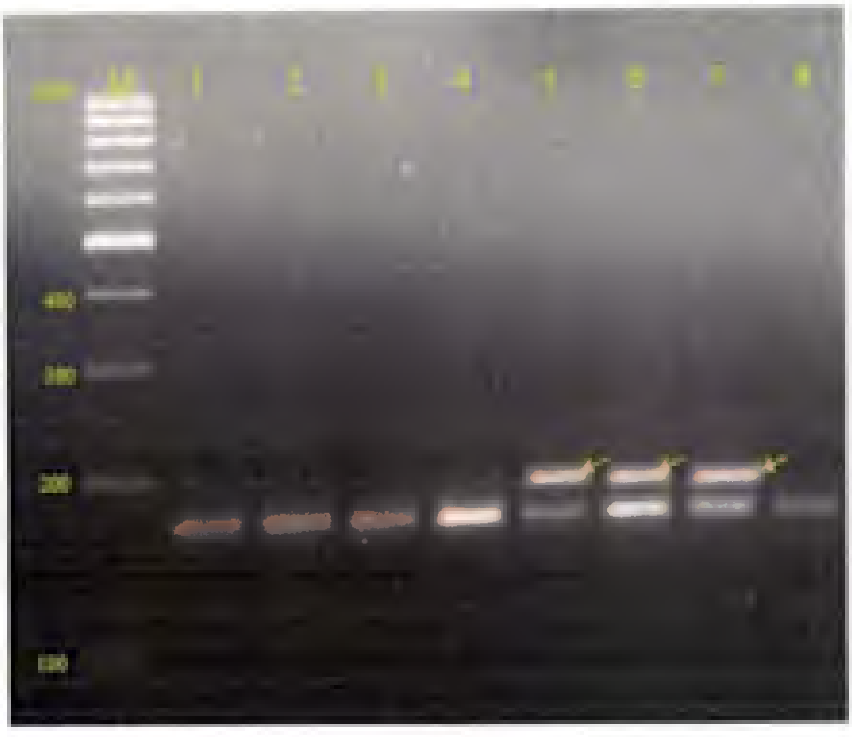
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 17. DNA amplification pattern generated with primer mAaCIR 0115

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
180								
175								

Colour codes for bands

Fig. 15 Colour chart developed using SSR primer mAaCIR 0115



M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 18. DNA amplification pattern generated with primer mAaCIR 0127

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
200								
180								

Colour codes for bands

Fig. 16 Colour chart developed using ISSR primer mAaCIR 0127

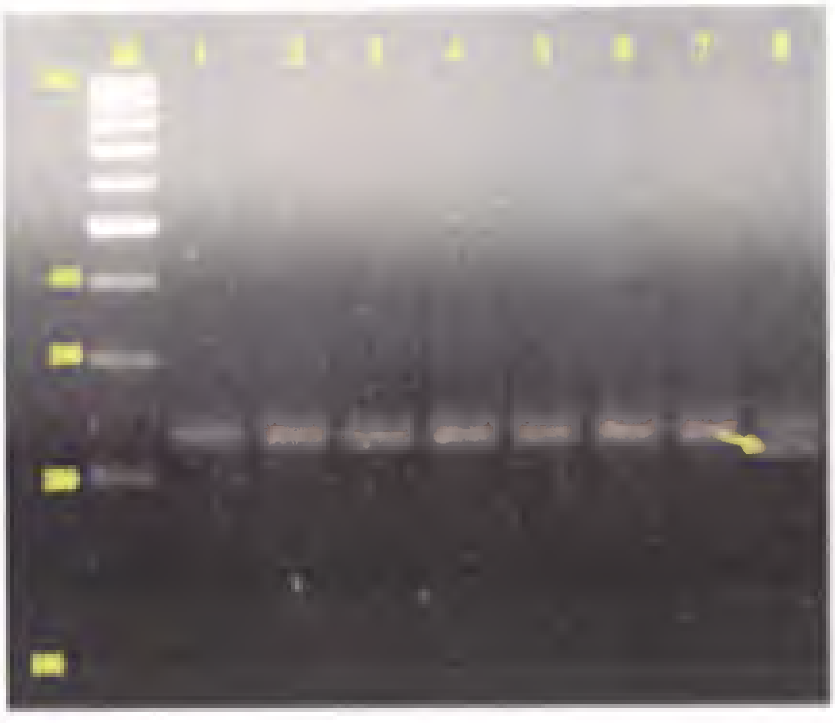
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

.Plate 19. DNA amplification pattern generated with primer mAaCIR 0134

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
240								
195								

Colour codes for bands

Fig. 17 Colour chart developed using ISSR primer mAaCIR 0134



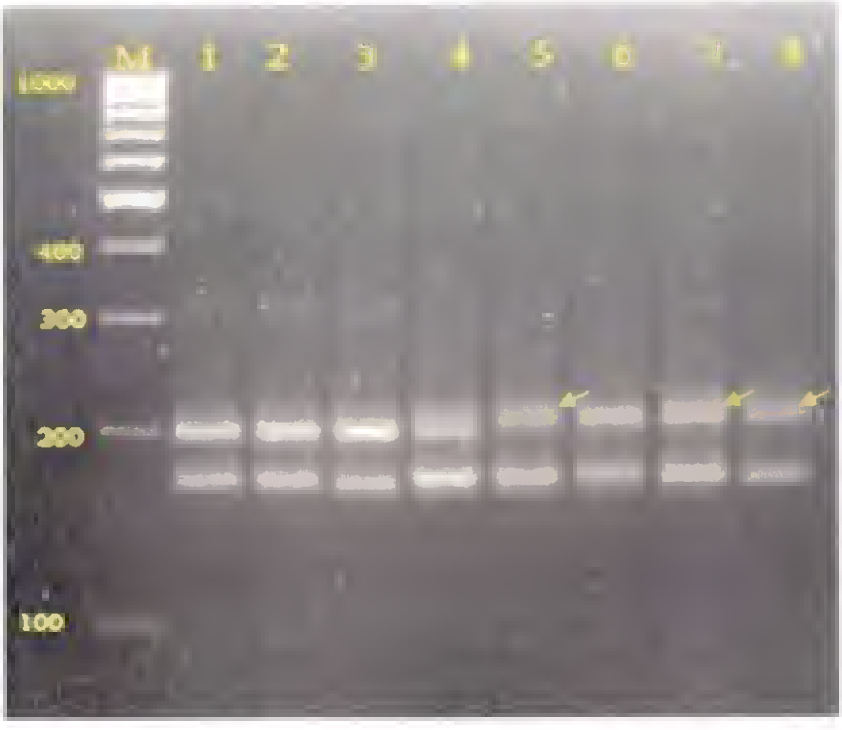
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 20. DNA amplification pattern generated with primer mAaCIR 0141

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
240								
230								

Colour codes for bands

Fig. 18 Colour chart developed using SSR primer mAaCIR 0141



M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 21. DNA amplification pattern generated with primer MAA 54a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
220								
210								
200								
180								

Colour codes for bands

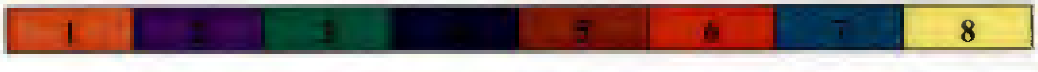
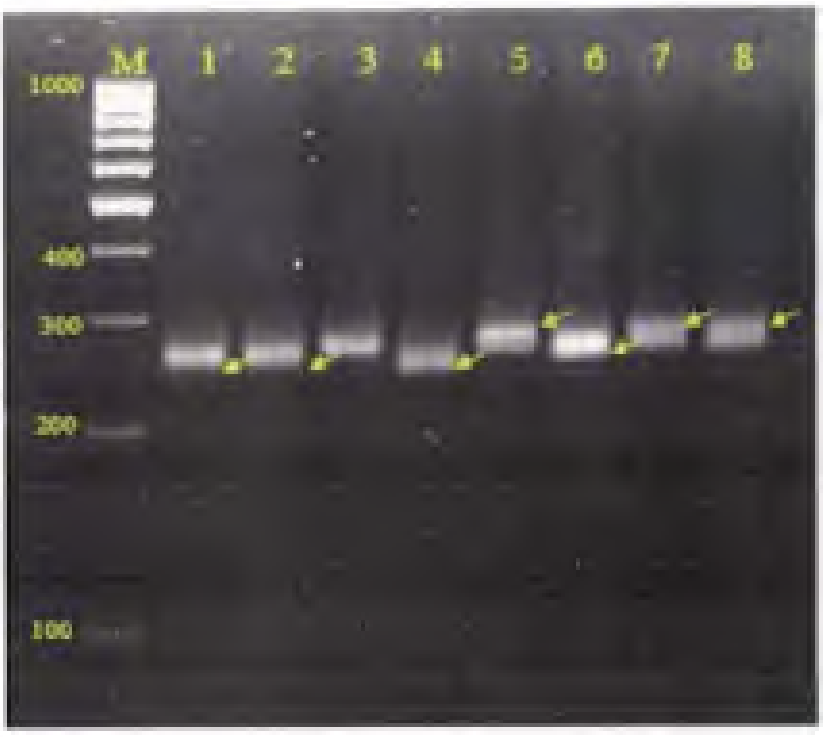


Fig. 19 Colour chart developed using SSR primer MAA 54a



M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 22. DNA amplification pattern generated with primer MAA 105

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
290								
280								
270								
265								

Colour codes for bands

Fig. 20 Colour chart developed using SSR primer MAA 105

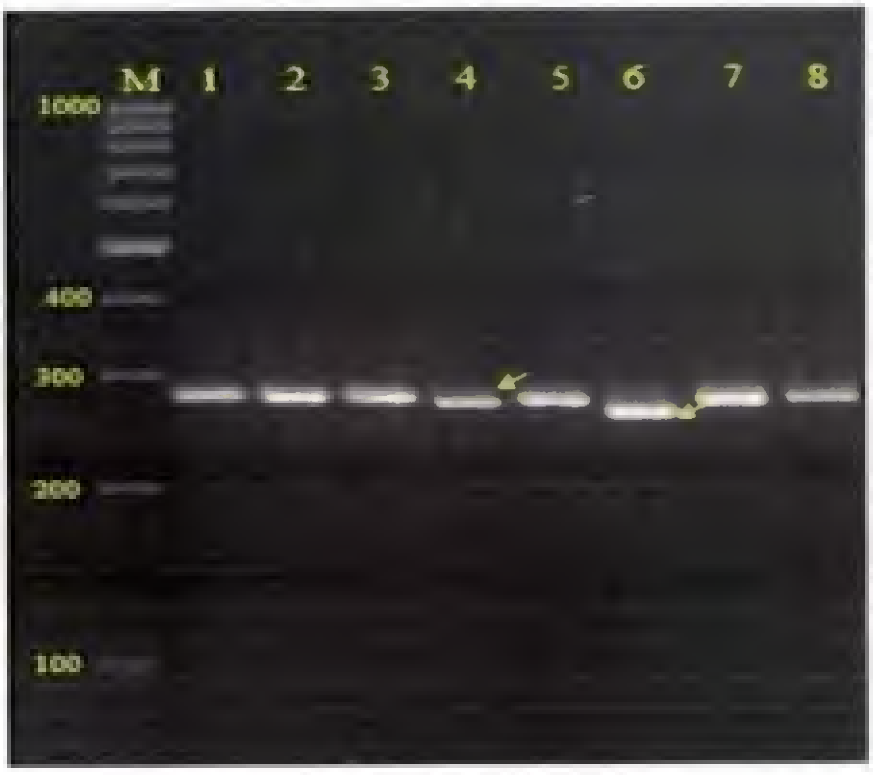
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 23. DNA amplification pattern generated with primer MAA 122

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
270								
265								

Colour codes for bands

Fig. 21 Colour chart developed using SSR primer MAA 122



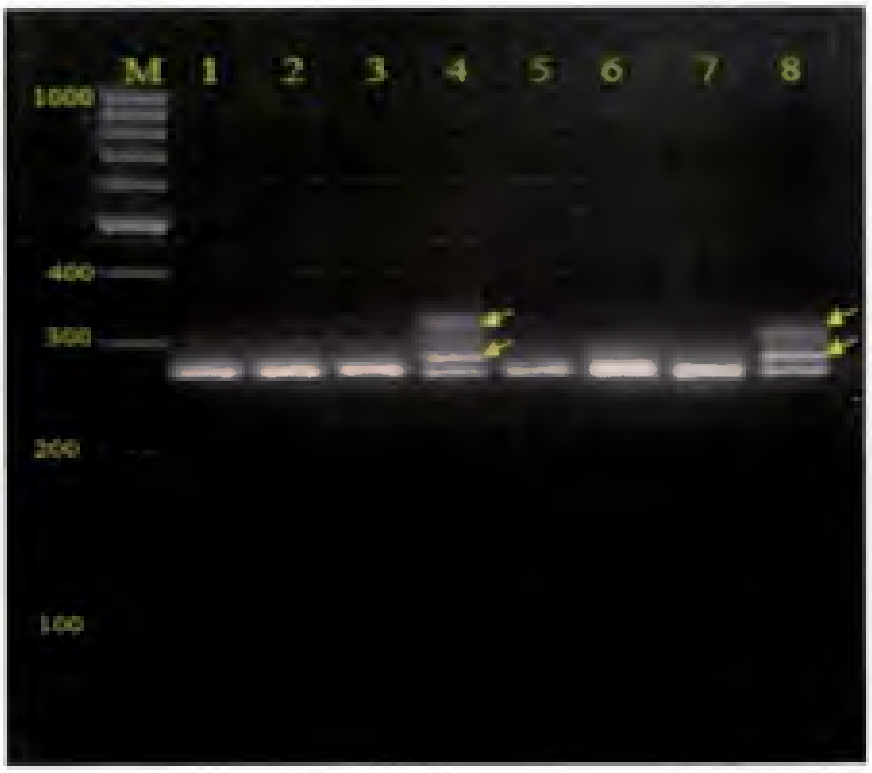
M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 24. DNA amplification pattern generated with primer MAA 145

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
290								
287								
280								

Colour codes for bands

Fig. 22 Colour chart developed using SSR primer MAA 145



M- Molecular weight marker (100bp), 1- Veloor varikka-1, 2- Veloor varikka-2, 3- Pathamuttom varikka-1, 4- Pathamuttom varikka-2, 5- Kavanar varikka-1, 6- Chengalam varikka, 7- Muttom varikka, 8- Sindhur

Plate 25. DNA amplification pattern generated with primer MAA 196a

Mol. Size(bp)/ Variety	1	2	3	4	5	6	7	8
340								
320								
290								
280								

Colour codes for bands

Fig. 23 Colour chart developed using SSR primer MAA 196a

4.3.7 Variety wise marker analysis using polymorphic amplicons in selected ISSR and SSR markers

Individual primer data obtained using selected 10 ISSR primes and 11 SSR primers were used to develop colour chart for each genotype of jackfruit. Polymorphic amplicons alone were used for developing colour charts. Genotype wise marker analysis are narrated below.

4.3.7.1 Veloor varikka-1

4.3.7.1.1 ISSR profile

Amplification with 10 ISSR primers yielded 39 distinct polymorphic bands in Veloor varikka-1. Molecular size of polymorphic bands ranged from 240 bp to 1400 bp (Fig. 24a). Maximum polymorphic bands were observed in UBC 848a (8) and the minimum (1) was yielded by UBC 834a, UBC 834b and UBC 841a. The amplicons developed by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp) and UBC 857b (at 360 bp) were considered for developing the fingerprint of the genotype.

4.3.7.1.2 SSR profile

Using selected 11 SSR primer sets, 3 distinct polymorphic bands were observed in Veloor varikka-1. Except MAA 54a, MAA 105 and MAA 145 all the other primers produced monomorphic bands. Molecular size of polymorphic bands ranged between 200 bp and 290 bp (Fig. 24b). The polymorphic band developed using MAA 105 at 270 bp was considered for producing the DNA fingerprint of the genotype.

4.3.7.2 Veloor varikka-2

4.3.7.2.1 ISSR profile

Selected 10 ISSR primers amplified the DNA of Veloor varikka-2 at 41 distinct loci which were polymorphic. Molecular size of polymorphic bands varied from 240 bp to 2000 bp (Fig. 25a). Maximum polymorphic bands (8) were observed in UBC 810 and UBC 848a. The primers UBC 834a, UBC 834b, UBC 841a and 857a developed only one polymorphic band with this genotype. The amplicons developed

by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp) and UBC 857b (at 360 bp) were considered for constituting fingerprint of the genotype.

4.3.7.2.2 SSR profile

Among the 11 SSR primers used for amplification, only MAA 54a, MAA 105 and MAA 145 yielded polymorphic bands in Veloor varikka-2. Molecular size of polymorphic bands ranges from 200 bp to 290 bp (Fig. 25b). The polymorphic band developed using MAA 105 at 270 bp was considered for developing fingerprint of the genotype.

4.3.7.3 Pathamuttom varikka-1

4.3.7.3.1 ISSR profile

Amplification with 10 ISSR primers produced 43 distinct polymorphic bands in Pathamuttom varikka-1. Molecular size of polymorphic bands ranged between 240 bp and 2500 bp (Fig. 26a). The primer UBC 815 developed maximum number of polymorphic bands (8) and the minimum number of bands (2) were yielded by UBC 834b and UBC 841b. Out of 43 polymorphic bands, six were useful for final fingerprinting. Among these six bands two produced by UBC 834a (at 580 bp and 640 bp), one each from UBC 810 (at 480 bp) and 841a (at 520 bp) were unique and served as specific fingerprints of the variety. Another two polymorphic bands produced by UBC 815 (at 600 bp) and UBC 841b (at 950 bp) were also considered for developing individual fingerprint of this genotypes.

4.3.7.3.2 SSR profile

On amplification with 11 SSR primers, five primers developed six distinct polymorphic bands in Pathamuttom varikka-1 (Fig. 26b). The bands at 150 bp and 270 bp developed by the primers mAaCIR 0078 and MAA 122 respectively were unique and hence will serve as specific fingerprint of this genotype. Another polymorphic band produced by mAaCIR 0115 at 175 bp was also considered for preparing fingerprint of this genotype.

4.3.7.4 Pathamuttom varikka-2

4.3.7.4.1 ISSR profile

Using 10 ISSR primers 39 distinct polymorphic bands were observed in Pathamuttom varikka-2. Molecular size of polymorphic bands ranged between 240 bp and 2000 bp (Fig. 27a). Except UBC 834a, all the other selected primers produced polymorphic bands. Maximum number of polymorphic bands (6) were observed in UBC 810, UBC 841b and UBC 844a. The primer UBC 857a developed minimum number of band (1). Out of the 39 polymorphic bands only three were useful polymorphism. Among these three bands, two bands developed by UBC 841a (at 370 bp) and UBC 841b (at 700 bp) were unique and specific fingerprints of the variety. The polymorphic band yielded by UBC 844a at 1050 bp was also considered for identifying this genotype.

4.3.7.4.2 SSR profile

Out of the 11 SSR primers, 6 primers produced distinct polymorphic bands (9) in Pathamuttom varikka-2. Molecular size of polymorphic bands varied from 175 bp to 340 bp (Fig. 27b). Among the 9 polymorphic bands observed, six were useful for fingerprinting. Four bands observed with mAaCIR 0134 (at 240 bp), MAA 105 (at 265 bp), MAA 145 (at 287 bp) and MAA 196a (at 340 bp) were unique and these bands acted as specific fingerprints of the variety. Other two polymorphic bands developed by mAaCIR 0115 (at 175 bp) and MAA 196a (at 290 bp) were also considered for identifying the genotype.

4.3.7.5 Kavanar varikka-1

4.3.7.5.1 ISSR profile

Amplification with 10 ISSR primers yielded 48 distinct polymorphic bands in Kavanar varikka-1. Molecular size of polymorphic bands ranged between 240 bp and 2500 bp (Fig. 28a). The primer UBC 815 developed highest number of polymorphic bands (8) and UBC 834b and UBC 844a developed lowest number (3). The primers UBC 834a (at 450 bp and 760 bp), UBC 810 (at 630 bp), UBC 815 (at 600 bp), UBC 848a (600 bp), UBC 857a (at 800 bp) and UBC 841b (at 1450 bp) produced useful

polymorphic amplicons and these were considered for developing individual fingerprint of the genotype.

4.3.7.5.2 SSR profile

Amplification with 11 SSR primers yielded seven distinct polymorphic bands in Kavanar varikka-1. Out of 11 primers, 5 produced only monomorphic bands. Molecular size of polymorphic bands ranged from 100 bp to 290 bp (Fig. 28b). The amplicons developed using the primers mAaCIR 0049 (at 100 bp), mAaCIR 0127 (at 200 bp), MAA 54a (at 220 bp) and MAA 105 (290 bp) were used for constituting DNA fingerprint of the genotype.

4.3.7.6 Chengalam varikka

4.3.7.6.1 ISSR profile

The selected 10 ISSR primers yielded 45 distinct polymorphic bands in Chengalam varikka. Molecular size of polymorphic bands varied between 240 bp and 2500 bp (Fig. 29a). The primer UBC 810 developed 7 polymorphic bands and was maximum while UBC 834a and UBC 857b developed minimum number (3) of bands. Out of the 45 polymorphic amplicons seven were useful polymorphisms. Among these seven amplicons, five were unique and were useful as specific fingerprints of the genotype. These unique amplicons were observed with the primers UBC 857a (at 1250 bp and 1490 bp), UBC 834 b (at 760 bp), UBC 848a (at 1300 bp) and UBC 857b (at 680 bp). Other two polymorphic bands yielded by UBC 834a (at 370 bp) and UBC 841b (at 950 bp) were also considered for identifying the genotypes.

4.3.7.6.2 SSR profile

Using selected with 11 SSR primers five distinct polymorphic bands were observed in Chengalam varikka. Out of 11 primers, only six produced polymorphic bands. Molecular size of polymorphic bands varied from 180 bp to 280 bp (Fig. 29b). Out of the five polymorphic bands, three were useful polymorphisms. Among these three bands, a band produced by MAA 145 at 280 bp was unique and served as specific fingerprint of the genotype. Other two polymorphic bands observed with mAaCIR 0127 (at 200 bp) and MAA 105 (at 270 bp) were also considered for identifying the genotypes.

4.3.7.7 Muttom varikka

4.3.7.7.1 ISSR profile

Amplification with 10 ISSR primers yielded 53 distinct polymorphic bands in Muttom varikka. Molecular size of polymorphic bands ranged from 240 bp to 3500 bp (Fig. 30a). The primer UBC 815 generated maximum number of polymorphic bands (9) and the UBC 834b and UBC 857 b produced minimum (3). Out of the 53 polymorphic bands 11 were useful polymorphisms. Among these 11 amplicons, four were unique and served as specific fingerprints of the variety. The unique amplicons were generated by UBC 815 (at 2500 bp and 3500 bp) and UBC 834a (at 950 bp and 1000bp). Other seven polymorphic bands yielded by UBC 810 (at 630 bp), UBC 834a (at 450 and 760 bp), UBC 841b (at 1450 bp), UBC 844a (at 1050 bp), UBC 848a (at 600 bp), and UBC 857a (at 800 bp) which were also considered for identifying the genotypes.

4.3.7.7.2 SSR profile

The selected 11 SSR primers yielded seven distinct polymorphic amplicons in Muttom varikka. Out of the 11 primers, only 6 produced polymorphic bands. Molecular size of polymorphic bands ranged from 100 bp to 290 bp (Fig. 30b). Out of the seven polymorphic bands, four produced by mAaCIR 0049 (at 100 bp), mAaCIR 0127 (at 200 bp), MAA 54a (at 220 bp) and MAA 105 (at 290 bp) were useful polymorphisms. These bands were considered for developing final DNA fingerprint of the cultivar.

4.3.7.8 Sindhur

4.3.7.8.1 ISSR profile

Amplification with 10 ISSR primers yielded 24 distinct polymorphic bands in the variety Sindhur. Molecular size of polymorphic bands varied between 370 bp and 3500 bp (Fig. 31a). The primers UBC 841a and UBC 848a produced only monomorphic bands. Maximum number of polymorphic bands (6) were observed in UBC 810 and minimum number of bands were yielded by UBC 857b (1). Out of the 24 polymorphic bands only two were useful. The unique polymorphic band produced by UBC 815 at 1050 bp was utilized as specific fingerprint of Sindhur. Similarly

another polymorphic band yielded by UBC 834a at 370 bp was also considered for identifying the variety.

4.3.7.8.2 SSR profile

The selected 11 SSR primers yielded 9 distinct polymorphic bands in Sindhur. Out of the 11 primers, 5 produced only monomorphic bands. Molecular size of polymorphic bands ranged from 175 bp to 320 bp (Fig. 31b). Out of the nine polymorphic bands, five were useful polymorphism. Among these five bands, two were unique and were considered as specific fingerprint of the variety. These unique bands were generated by primer sets mAaCIR 0141(at 230 bp) and MAA 196a (at 320 bp). Other polymorphic bands produced by mAaCIR 0115 (at 175 bp), MAA 54a (at 220 bp) and MAA 105 (at 290 bp) and MAA 196a (at 290 bp) were also considered for identifying the variety.

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
1400										
1370										
1200										
1150										
1000										
950										
920										
900										
750										
680										
650										
640										
600										
580										
550										
520										
510										
500										
490										
470										
450										
430										
410										
400										
380										
370										
360										
300										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	MAA 54a	MAA 105	MAA 145
290			
270			
200			

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 24 Colour chart developed using polymorphic bands in selected ISSR and SSR primers- Veloor varikka-1

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
2000										
1400										
1370										
1250										
1200										
1150										
1000										
950										
920										
900										
750										
680										
650										
640										
600										
580										
550										
520										
510										
500										
490										
470										
450										
430										
410										
400										
380										
370										
360										
300										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	MAA 54a	MAA 105	MAA 145
290			
270			
200			

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 25 Colour chart developed using polymorphic bands in selected ISSR and SSR primers- Veloor varikka-2

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
2500										
2000										
1250										
1200										
950										
900										
800										
750										
730										
680										
650										
640										
600										
580										
550										
520										
500										
490										
480										
450										
410										
400										
380										
370										
350										
300										
280										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0078	mAaCIR 0115	MAA 54a	MAA 122	MAA 145
290					
270					
200					
180					
175					
150					

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 26 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Pathamuttom varikka-1

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
2000									
1500									
1250									
1190									
1150									
1050									
1000									
950									
800									
750									
700									
680									
650									
640									
600									
550									
520									
500									
470									
450									
410									
400									
380									
370									
350									
300									
260									
240									

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0115	mAaCIR 0134	MAA 54a	MAA 105	MAA 145	MAA 196a
340						
290						
287						
265						
240						
210						
200						
180						
175						

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 27 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Pathamuttom varikka-2

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
2500										
2000										
1500										
1450										
1400										
1250										
1200										
1190										
950										
900										
920										
820										
800										
760										
750										
730										
680										
630										
600										
590										
550										
500										
470										
450										
410										
400										
380										
370										
350										
300										
280										
260										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0049	mAaCIR 0115	mAaCIR 0127	MAA 54a	MAA 105	MAA 145
290						
220						
210						
200						
180						
100						

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 28 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Kavanar varikka-1

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
2500										
2000										
1490										
1300										
1250										
1200										
1190										
1150										
1000										
950										
900										
800										
760										
750										
730										
680										
640										
600										
590										
520										
510										
470										
450										
410										
400										
380										
370										
350										
300										
260										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0115	mAaCIR 127	MAA 54a	MAA 105	MAA 145
280					
270					
210					
200					
180					

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 29 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Chengalam varikka

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	UBC 857b
3500										
2500										
2000										
1500										
1450										
1400										
1250										
1200										
1190										
1050										
1000										
950										
920										
900										
820										
800										
760										
750										
730										
680										
630										
600										
590										
550										
520										
500										
470										
450										
410										
400										
380										
370										
350										
300										
280										
260										
240										

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0049	mAaCIR 0115	mAaCIR 0127	MAA 54a	MAA 105	MAA 145
290						
220						
210						
200						
180						
100						

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 30 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Muttom varikka

Mol. Size(bp)/ Primer	UBC 810	UBC 815	UBC 834a	UBC 834b	UBC 841b	UBC 844a	UBC 857a	UBC 857b
3500								
2500								
2000								
1250								
1200								
1190								
1050								
1000								
900								
800								
680								
600								
550								
500								
470								
450								
400								
380								
370								

a. ISSR DNA amplification pattern

Mol. Size(bp)/ Primer	mAaCIR 0115	mAaCIR 0141	MAA 54a	MAA 105	MAA 145	MAA 196a
320						
290						
230						
220						
210						
180						
175						

b. SSR DNA amplification pattern

Colour codes for bands

Fig. 31 Colour chart developed using polymorphic bands in selected ISSR and SSR primers - Sindhur

4.4 DNA fingerprints with selected amplicons

4.4.1 Selected ISSR amplicons for DNA fingerprinting in jackfruit genotypes

The analyses of amplification pattern generated by 10 ISSR primers were conducted and useful distinct polymorphic amplicons identified were depicted in a colour chart (Fig. 32 a). In order to give polymorphism coverage to all eight varieties polymorphic bands which were shared among maximum two varieties were selected for developing individual fingerprint. All the selected primers generated at least one useful polymorphic band.

Amplification with 10 ISSR primers yielded 44 useful distinct polymorphic bands over eight jackfruit genotypes studied. Molecular size of selected amplicons ranged from 360 bp to 3500 bp. Among these, 16 amplicons were unique and 14 of them were shared by two genotypes. Maximum number of useful amplicons (11) were present in the cultivar Muttom varikka, whereas, the variety Sindhur generated only two useful amplicons. The primer UBC 834a generated maximum number of useful amplicons (10), whereas, the primer UBC 834b generated only one amplicon.

4.4.2 Selected SSR amplicons for DNA fingerprinting in jackfruit genotypes

Distinct polymorphic bands generated for eight jackfruit genotypes using selected eleven SSR primer sets were consolidated and useful bands were depicted in the colour chart (Fig. 32b). The fingerprint was generated using polymorphic bands which were shared among maximum three genotypes. All the selected SSR primers yielded at least one useful polymorphic band.

Amplification with 11 SSR primers yielded 28 useful distinct polymorphic bands over eight jackfruit genotypes. Molecular size of these selected bands ranged from 100 bp to 340 bp. Among these, 9 bands were unique, two of them were shared by two genotypes and five of them were shared by three genotypes. Maximum number of useful amplicons (6) was observed in Pathamuttom varikka-2 and Sindhur, whereas, the genotypes Veloor varikka-1 and Veloor varikka-2 had only one useful amplicon. The primer MAA 105 generated maximum number of useful amplicons (7). The unique bands observed were served as specific fingerprints of the genotypes.

Mol. Size(bp)/ Primer	Veloor varikka-1	Veloor varikka-2	Pathamuttom vaikka-1	Pathamuttom varikka-2	Kavanar varikka-1	Chengalam varikka	Muttom varikka	Sindhur
3500								
2500								
1490								
1450					UBC 841b		UBC 841b	
1400	UBC 848a	UBC 848a						
1370	UBC 848a	UBC 848a						
1300								
1250								
1050				UBC 844a			UBC 844a	UBC 815
1000								
950			UBC 841b			UBC 841b		
800					UBC 857a		UBC 857a	
760					UBC 834a		UBC 834a	
700								
680								
640								
630								
600			UBC 815					
580								
520								
480								
450								
430	UBC 810	UBC 810						
370								
360								

a. Colour chart developed using selected amplicons in ISSR primers

Mol. Size(bp)/ Primer	Veloor varikka-1	Veloor varikka-2	Pathamuttom vaikka-1	Pathamuttom varikka-2	Kavanar varikka-1	Chengalam varikka	Muttom varikka	Sindhur
340								
320								
290				MAA 196a	MAA 105		MAA 105	MAA 196a
287								
280								
270	MAA 105	MAA 105	MAA 122			MAA 105		
265								
240								
230								mAa 0141
220					MAA 54a		MAA 54a	MAA 54a
200					mAa 0127	mAa 0127	mAa 0127	
175			mAa 0115	mAa 0115				mAa 0115
150								
100								

b. Colour chart developed using selected amplicons in SSR primers

Colour codes for bands

Fig. 32 DNA fingerprints with selected amplicons

4.5 DNA fingerprinting of different jackfruit genotypes using ISSR and SSR marker system

Individual primer data obtained using selected 10 ISSR primes and 11 SSR primers were used to develop fingerprint for each genotype of jackfruit. Individual colour charts were developed using selected polymorphic amplicons. To give polymorphism coverage to all eight genotypes amplicons which were distinct and shared among maximum of two accessions in ISSR and three accessions in SSR were selected for developing individual fingerprints. Genotype wise DNA fingerprinting details are narrated below.

4.5.1 Veloor varikka-1

Amplification with 10 ISSR and 11 SSR primers yielded 5 useful polymorphic amplicons in Veloor varikka-1. Molecular size of these polymorphic amplicons ranged between 270 bp and 1400 bp (Fig. 33a). The amplicons developed by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) and MAA 105 (at 270 bp) were considered for generating fingerprint of the genotype.

4.5.2 Veloor varikka-2

Selected 10 ISSR and 11 SSR primers on amplification with the DNA of Veloor varikka-2 generated 5 useful polymorphic amplicons. Molecular size of these polymorphic bands varied from 270 bp to 1400 bp (Fig. 33b). The amplicons yielded by ISSR primers UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) and SSR primer MAA 105 (at 270 bp) were used for constituting fingerprint of the genotype.

4.5.3 Pathamuttom varikka-1

Five ISSR and three SSR primers produced useful polymorphic bands in Pathamuttom varikka-1. Molecular size of these polymorphic bands ranged between 150 bp and 950 bp (Fig. 33c). Six polymorphic bands from ISSR primers and three polymorphic bands from SSR primers were used for individual fingerprinting. Among these bands, two produced by UBC 834a (at 580 bp and 640 bp) and one each from UBC 810 (at 480 bp), UBC 841a (at 520 bp), mAaCIR 0078 (at 150 bp), MAA 122 (at 270 bp) were unique and served as specific fingerprints of the genotypes.

4.5.4 Pathamuttom varikka-2

Selected ISSR and SSR primers produced 9 useful polymorphic amplicons in Pathamuttom varikka-2. Molecular size of these polymorphic bands ranged between 175 bp and 1050 bp (Fig. 33d). Out of the 9 polymorphic bands, ISSR primers produced two unique bands (UBC 841a at 370 bp and UBC 841b at 700 bp) and SSR primers produced four unique bands (mAaCIR 0134 at 240 bp, MAA 105 at 265 bp, MAA 145 at 287 bp and MAA 196a at 340 bp). These unique bands served as specific fingerprints of the genotype.

4.5.5 Kavanar varikka-1

Six ISSR and four SSR primers produced 11 useful polymorphic bands in Kavanar varikka-1. Molecular size of these polymorphic bands ranged between 100 bp and 1450 bp (Fig. 33e). Unique bands were not obtained for this genotype. The ISSR primers UBC 834a (at 450 bp and 760 bp), UBC 810 (at 630 bp), UBC 815 (at 600 bp), UBC 848a (600 bp), UBC 857a (at 800 bp), UBC 841b (at 1450 bp) and SSR primers mAaCIR 0049 (at 100 bp), mAaCIR 0127 (at 200 bp), MAA 54a (at 220 bp), MAA 105 (290 bp) produced useful amplicons which were considered for DNA fingerprint of the genotype.

4.5.6 Chengalam varikka

The selected ISSR and SSR primers produced 10 useful amplicons in Chengalam varikka (Fig. 33f). Among these ten amplicons, six were unique and were useful as specific fingerprints of the genotypes. The unique amplicons were observed with the ISSR primers UBC 857a (at 1250 bp and 1490 bp), UBC 834b (at 760 bp), UBC 848a (at 1300 bp), UBC 857b (at 680 bp) and SSR primer MAA 145 (at 280 bp).

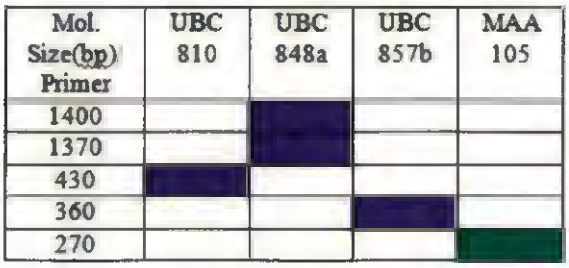
4.5.7 Muttom varikka

Amplification with 10 ISSR and 11 SSR primers yielded 15 distinct useful polymorphic bands in Muttom varikka. Molecular size of polymorphic bands ranged from 100 bp to 3500 bp (Fig. 33g). Among these 15, four amplicons produced by ISSR primers were unique and served as specific fingerprints of the variety. The unique amplicons were generated by UBC 815 (at 2500 bp and 3500 bp) and UBC 834a (at 950 bp and 1000 bp). Seven shared bands generated by ISSR primers and

four shared bands generated by SSR primers were also considered for identifying the genotypes.

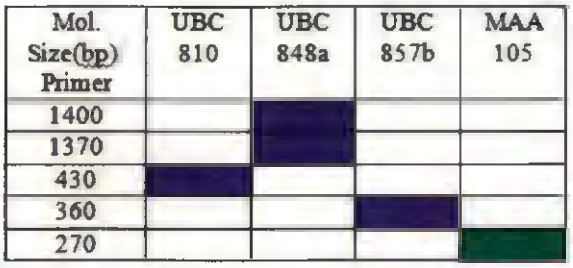
4.5.8 Sindhur

Useful polymorphic amplicons produced by ISSR primers UBC 815 (at 1050 bp), UBC 834a (at 370 bp) and SSR primers mAaCIR 0141(at 230 bp), mAaCIR 0115 (at 175 bp), MAA 54a (at 220 bp), MAA 105 (at 290 bp) and MAA 196a (at 320 bp and (at 290 bp) were used for developing DNA fingerprints in Sindhur (Fig. 33h). The unique amplicons observed with the primers UBC 815 (at 1050 bp), mAaCIR 0141 (at 230 bp) and MAA 196a (320 bp) were considered as specific fingerprint of variety.



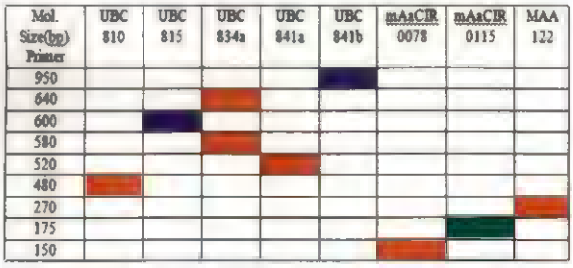
Mol. Size(bp) Primer	UBC 810	UBC 848a	UBC 857b	MAA 105
1400				
1370				
430				
360				
270				

a. DNA fingerprint of Veloor varikka-1



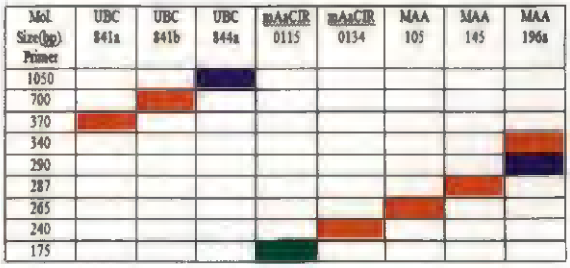
Mol. Size(bp) Primer	UBC 810	UBC 848a	UBC 857b	MAA 105
1400				
1370				
430				
360				
270				

b. DNA fingerprint of Veloor varikka-2



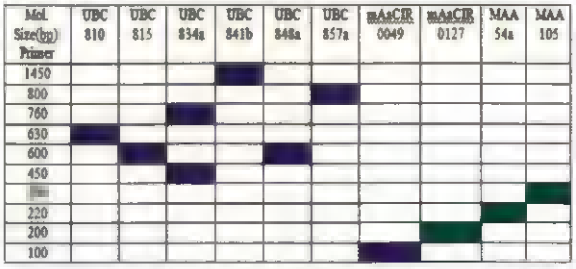
Mol. Size(bp) Primer	UBC 810	UBC 815	UBC 834a	UBC 841a	UBC 841b	mAsCIR 0078	mAsCIR 0115	MAA 122
950								
640								
600								
580								
520								
480								
270								
175								
150								

c. DNA fingerprint of Pathamuttom varikka-1



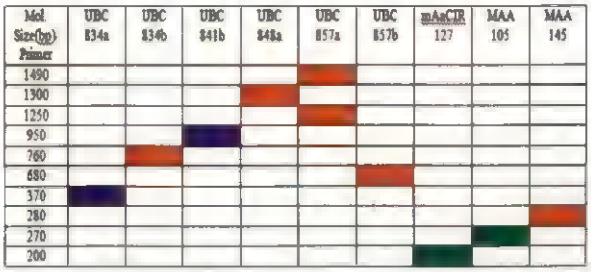
Mol. Size(bp) Primer	UBC 841a	UBC 841b	UBC 844a	mAsCIR 0115	mAsCIR 0134	MAA 105	MAA 145	MAA 196a
1050								
700								
370								
340								
290								
287								
265								
240								
175								

b. DNA fingerprint of Pathamuttom varikka-2



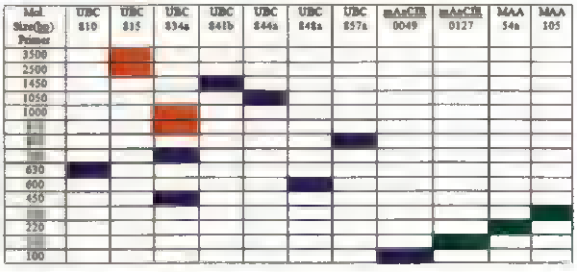
Mol. Size(bp) Primer	UBC 810	UBC 815	UBC 834a	UBC 841b	UBC 848a	UBC 857a	mAsCIR 0049	mAsCIR 0127	MAA 54a	MAA 105
1450										
800										
760										
630										
600										
450										
220										
200										
100										

e. DNA fingerprint of Kavanar varikka-1



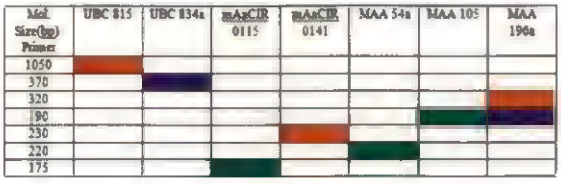
Mol. Size(bp) Primer	UBC 834a	UBC 834b	UBC 841b	UBC 848a	UBC 857a	UBC 857b	mAsCIR 127	MAA 105	MAA 145
1490									
1300									
1250									
950									
760									
680									
370									
280									
270									
200									

f. DNA fingerprint of Chengalam varikka



Mol. Size(bp) Primer	UBC 810	UBC 815	UBC 834a	UBC 841b	UBC 844a	UBC 848a	UBC 857a	mAsCIR 0049	mAsCIR 0127	MAA 54a	MAA 105
3500											
2500											
1450											
1050											
1000											
630											
600											
450											
220											
100											

g. DNA fingerprint of Muttom varikka



Mol. Size(bp) Primer	UBC 815	UBC 834a	mAsCIR 0115	mAsCIR 0141	MAA 54a	MAA 105	MAA 196a
1050							
370							
320							
190							
230							
220							
175							

h. DNA fingerprint of Sindhur

Colour codes for bands

Fig. 33 DNA fingerprints of eight jackfruit genotypes developed using selected amplicons in ISSR and SSR marker system

4.6 Diversity analysis

Cluster analysis was performed using Fruit characters and molecular data for determining the variability within eight jackfruit genotypes.

4.6.1 Diversity analysis based on fruit characters of different jackfruit genotypes

4.6.1.1 Cluster analysis based on qualitative traits

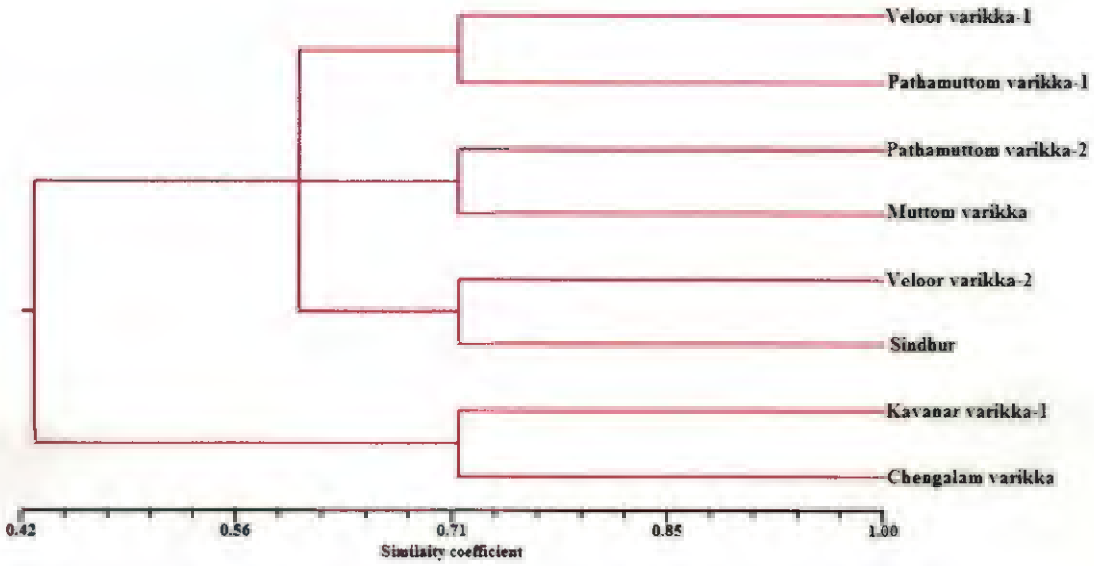
An UPGMA dendrogram was constructed using seven qualitative fruit character of jackfruit based on Jaccard's similarity co-efficient (Fig. 34a). At 0.71 similarity coefficient, the jackfruit genotypes were clustered into four groups (Table 13) with two genotypes each. Cluster I consisted of Veloor varikka-1 and Pathamuttom varikka-1, Cluster II comprised of Pathamuttom varikka-2 and Muttom varikka, Cluster III included Veloor varikka-2 and Sindhur and Cluster IV comprised Kavanar varikka-1 and Chengalam varikka.

4.6.1.2 Cluster analysis based on quantitative traits

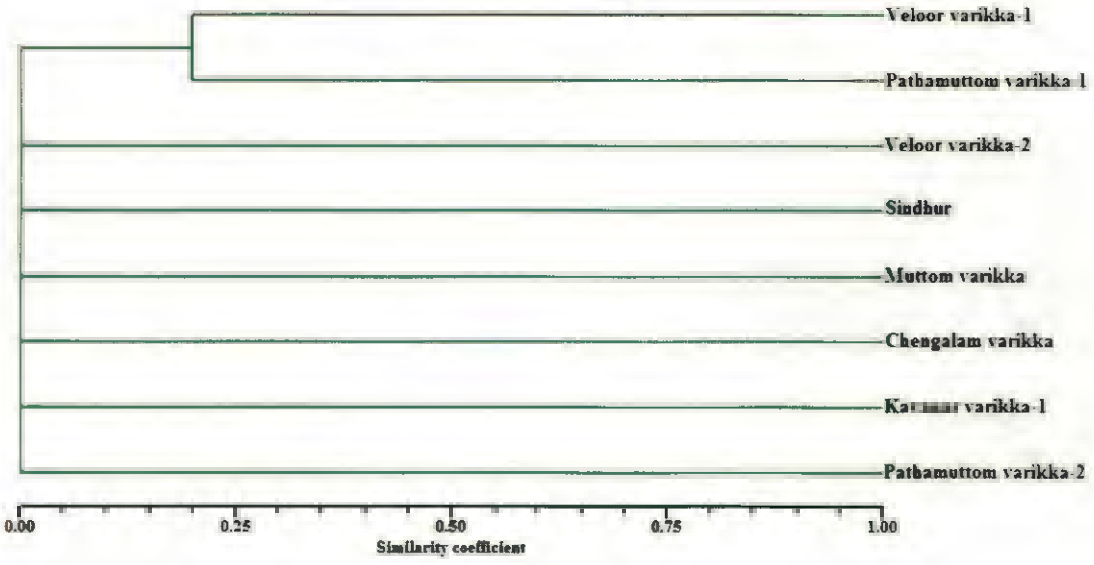
An UPGMA dendrogram was constructed with five quantitative fruit characters of jackfruit based on Jaccard's similarity co-efficient (Fig. 34b). At 0.78 similarity coefficient, the jackfruit genotypes were clustered into eight groups (Table 14) with each genotype forming a separate group. The jackfruit genotypes Veloor varikka-1 and Pathamuttom varikka-1 were clustered together with 20 % similarity.

4.6.1.3 Clustering based on biochemical characters

Agglomerative hierarchical Cluster analysis was carried out based on Jaccard's similarity co-efficient and UPGMA dendrogram was constructed with six biochemical character of jackfruit (Fig. 35). At 0.78 similarity coefficient, the jackfruit genotypes were grouped into eight groups (Table 15) with each genotype forming a separate cluster. However at 0.16 similarity coefficient, the jackfruit genotypes Veloor varikka-2 and Pathamuttom varikka-1 were clustered together into one group with 16 % similarity, whereas Chengalam varikka and Muttom varikka were clustered together with 33 % similarity.



a. Dendrogram based on qualitative data using NTsys



b. Dendrogram based on quantitative data using NTsys

Fig. 34 Dendrogram based on similarity coefficient for qualitative and quantitative data of jackfruit genotypes

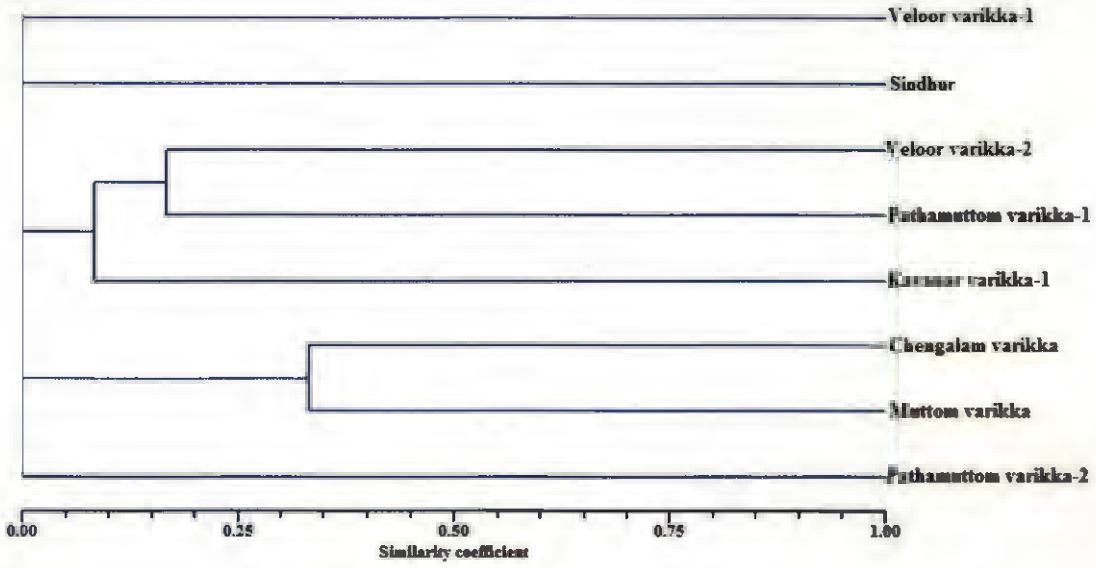


Fig. 35 Dendrogram based on similarity coefficient for biochemical analysis of jackfruit genotypes

Table 13. Clustering based on qualitative data

Cluster no.	No. of cluster members	Cluster members
I	2	Veloor varikka-1 Pathamuttom varikka-1
II	2	Pathamuttom varikka-2 Muttom varikka
III	2	Veloor varikka-2 Sindhur
1V	2	Kavanar varikka-1 Chengalam varikka

Table 14. Clustering based on quantitative data

Cluster no.	No. of cluster members	Cluster members
I	1	Veloor varikka-1
II	1	Pathamuttom varikka-1
III	1	Veloor varikka-2
IV	1	Sindhur
V	1	Muttom varikka
VI	1	Chengalam varikka
VII	1	Kavanar varikka-1
V1II	1	Pathamuttom varikka-2

Table 15. Clustering based on biochemical data

Cluster no.	No. of cluster members	Cluster members
I	1	Veloor varikka-1
II	1	Sindhur
III	1	Veloor varikka-2
IV	1	Pathamuttom varikka-1
V	1	Kavanar varikka-1
VI	1	Chengalam varikka
VII	1	Muttom varikka
VIII	1	Pathamuttom varikka-2

4.6.2 Diversity analysis based on molecular characters of different jackfruit genotypes

4.6.2.1 Cluster analysis based on ISSR data

A dendrogram was constructed using the amplification data generated by selected ISSR primers (Fig. 36a). At 0.78 similarity coefficient, the eight jackfruit genotypes were clustered into six groups (Table 16). Cluster I and V were formed with two members each *i.e.*, cluster I with Veloor varikka-1 and Veloor varikka-2 and Cluster V with Kavanar varikka-1 and Muttom varikka. Other four clusters had only one member *i.e.*, Cluster II, III, IV and VI with Pathamuttom varikka-2, Chengalam varikka, Pathamuttom varikka-1 and Sindhur respectively. Maximum similarity was seen in between Veloor varikka-1 and Veloor varikka-2 (95%) and maximum variability observed was 45 percent for the variety Sindhur.

4.6.2.2 Cluster analysis based on SSR data

Polymorphism generated with SSR primers for the eight jack genotypes were used for constructing dendrogram (Fig. 36b). At 0.78 similarity coefficient, the jackfruit genotypes were clustered into six groups (Table 17). Cluster I and III were formed with two members each *i.e.*, cluster I with Veloor varikka-1 and Veloor varikka-2 and Cluster III with Kavanar varikka-1 and Muttom varikka. Other four clusters were formed with only one member *i.e.*, Cluster II, IV, V and VI with Pathamuttom varikka-1, Sindhur, Chengalam varikka and Pathamuttom varikka-2 respectively. Maximum variability observed was 48 percent for the genotype Pathamuttom varikka-2.

4.6.2.3 Cluster analysis based on combined ISSR and SSR data

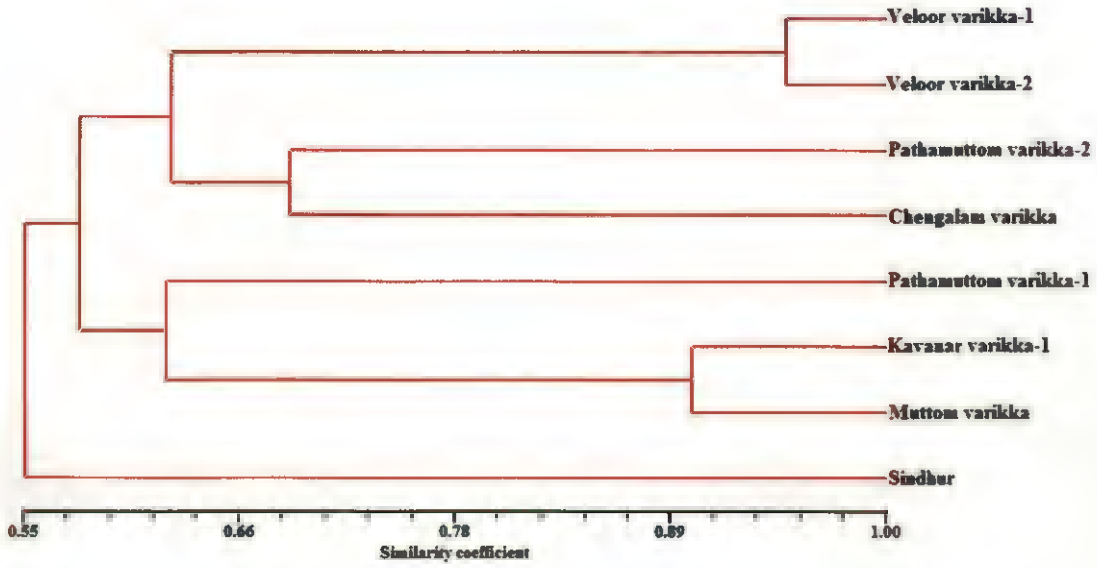
Using combained ISSR and SSR primer data, dendrogram was constructed (Fig. 37). At 0.78 similarity coefficient, the jackfruit genotypes were clustered into six groups (Table 18). Cluster I and V were formed with two members each *i.e.*, cluster I with Veloor varikka-1 and Veloor varikka-2 and Cluster V with Kavanar varikka-1 and Muttom varikka. Other four clusters were found to have only one member *i.e.*, Cluster II, III, IV and VI with Pathamuttom varikka-2, Chengalam varikka, Pathamuttom varikka-1 and Sindhur respectively. Maximum similarity was seen

between Veloor varikka-1 and Veloor varikka-2 (95%) and maximum variability observed was 44 percent for the variety Sindhur.

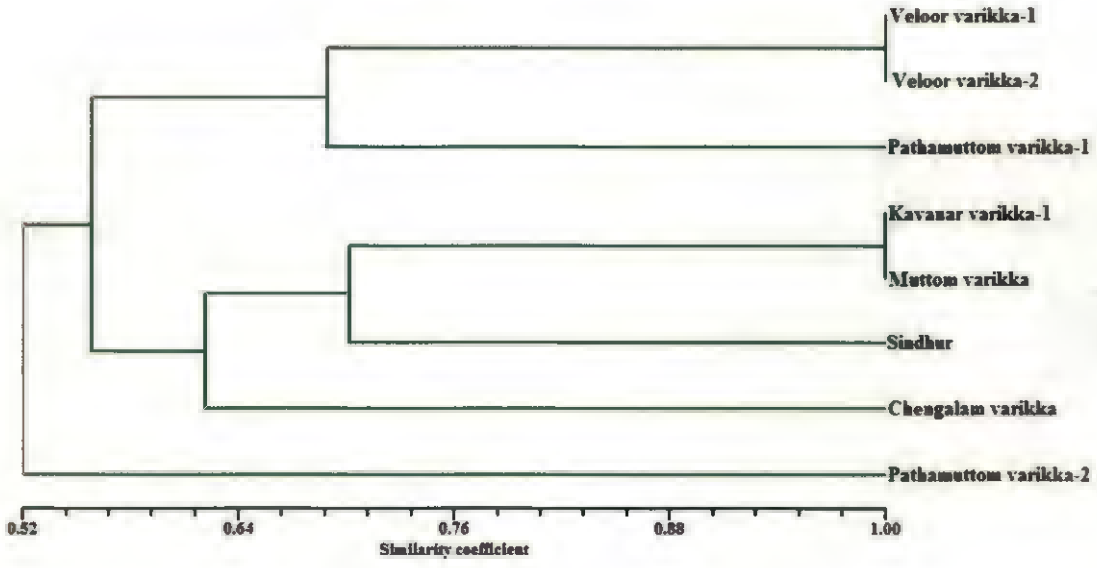
Combined ISSR – SSR clustering and ISSR based clustering produced same clustering pattern. In all clustering analysis, Veloor varikka-1 and Veloor varikka-2 grouped together in one cluster and Kavanar varikka-1 and Muttom varikka grouped together in another cluster. Based on SSR clustering Veloor varikka-1 and Veloor varikka-2; Kavanar varikka-1 and Muttom varikka were exactly similar (100%) but when ISSR clustering was done they showed 95% and 90% similarity respectively.

4.6.3 Comparative clustering based on quantitative and molecular characters

In these two clustering analysis, number of clusters and distribution patterns were different. The combined IISR – SSR cluster data of eight jack genotypes was compared with quantitative clustering pattern (Table. 14; Table 18). In both of these cluster analysis the number of clusters and distribution pattern were different. This may be because of the actual coding sequence for quantitative characters were not used for molecular clustering. Homology has to be worked out between expressed gene sequence and quantitative data for obtaining more precise result.



a. ISSR Dendrogram using NTsys



b. SSR Dendrogram using NTsys

Fig. 36 Dendrogram based on similarity coefficient for ISSR and SSR analysis of jackfruit genotypes

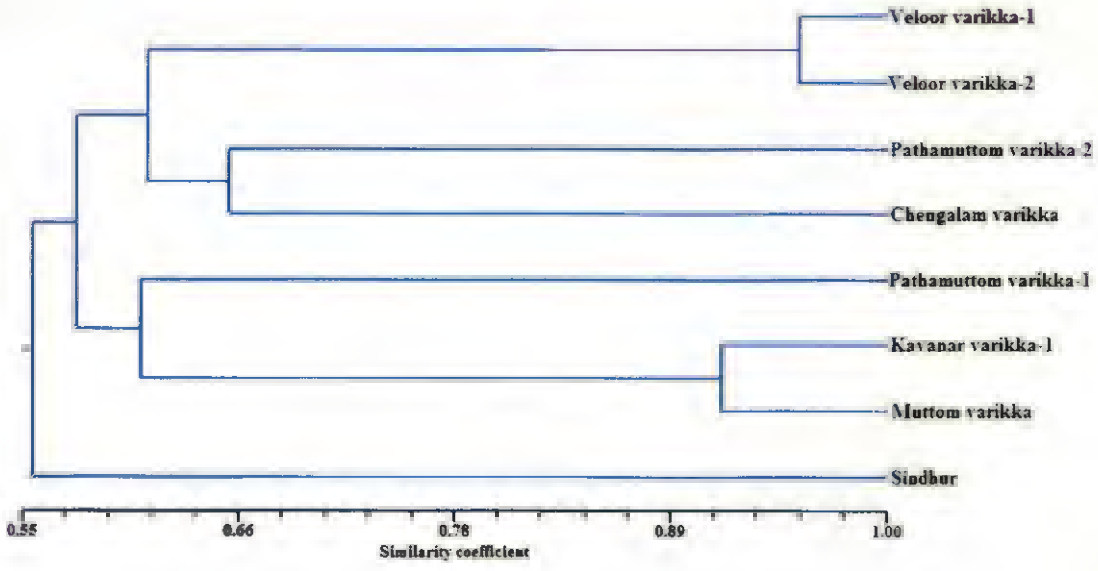


Fig. 37 Combined dendrogram based on similarity coefficient for ISSR and SSR analysis of jackfruit genotypes

Table 16. Clustering based on ISSR data

Cluster no.	No. Of cluster members	Cluster members
I	2	Veloor varikka-1 Veloor varikka-2
II	1	Pathamuttom varikka-2
III	1	Chengalam varikka
IV	1	Pathamuttom varikka-1
V	2	Kavanar varikka-1 Muttom varikka
V1	1	Sindhur

Table 17. Clustering based on SSR data

Cluster no.	No. Of cluster members	Cluster members
I	2	Veloor varikka-1 Veloor varikka-2
II	1	Pathamuttom varikka-1
III	2	Kavanar varikka-1 Muttom varikka
IV	1	Sindhur
V	1	Chengalam varikka
V1	1	Pathamuttom varikka-2

Table 18. Clustering based on ISSR and SSR combined data

Cluster no.	No. Of cluster members	Cluster members
I	2	Veloor varikka-1 Veloor varikka-2
II	1	Pathamuttom varikka-2
III	1	Chengalam varikka
IV	1	Pathamuttom varikka-1
V	2	Kavanar varikka-1 Muttom varikka
V1	1	Sindhur

Discussion

5. DISCUSSION

Jackfruit is scientifically known as *Artocarpus heterophyllus* Lam. and belongs to the family moraceae. Due to the rich jack diversity, it is believed to have originated in the Western Ghats of India (Rowe-Dutton, 1985). However, well defined jack varieties are not as much in Kerala. Moreover, local varieties are available in plenty with different names based on shape of their fruits, flake colour *etc*. Cultivar identification and estimation of genetic diversity using morphological data has limitation, as they are affected by environment. However, molecular markers can be effectively utilized for this purpose. Information on the molecular aspects of jack using markers are less as scanty research has been conducted based on this in this crop. DNA fingerprinting data will serve as a tool for identifying the genotypes and settling IPR issues. The present work will help to characterize the released jack variety Sindhur, cultivar Muttom varikka and superior jack selections identified at RARS, Kumarakom.

5.1 Fruit characters

Morphological characterisation is a simple marker technique, which is used for plant germplasm assessment (Gottlieb, 1984; Hilu, 1984), for choosing desirable traits in plant breeding programs (Khalid *et al.*, 2010) and diversity studies (Ganesan *et al.*, 2014). Several morphology based diversity studies have been reported in jackfruit (Hossain, 1996; Saha *et al.*, 1996; Jagadees *et al.*, 2007) and some studies are also conducted in Kerala Agricultural University (KAU) (Ashwini, 2015; Krishnan *et al.*, 2015a).

Ashwini (2015) conducted a study on morphological characterization of twenty accessions and three varieties of jackfruits, including Muttom varikka, Sindhur and Thamarachakka. She evaluated five tree characters, five leaf characters, six inflorescence characters and twenty four fruit characters for assessing variability among varieties. Krishnan *et al.* (2015a) identified 10 promising firm fleshed jack genotypes from Kuttanadu region. Different quantitative, qualitative and biochemical characters were recorded in this study.

One KAU released jack variety (Sindhur), one cultivar (Muttom varikka) and six superior jackfruit selections (Veloor varikka-1, Veloor varikka-2, Pathamuttom

varikka-1, Pathamuttom Varikka-2, Kavanar Varikka-1and Chengalam varikka) identified at RARS, Kumarakom (Krishnan *et al.,* 2015a) were characterized in present study. Standard descriptors prescribed by Bioversity International (2006) were used as guideline to describe the qualitative and quantitative characters of jackfruit.

Very low variability was observed for qualitative fruit characters among the selected jackfruit genotypes (Table 3). The pulp colour of Pathamuttom varikka-1, Pathamuttom varikka-2 and Sindhur was coppery red (Plate 2) which has high fancy value in the market. The pulp colour of all the other genotypes were different shades of yellow. These accessions had firm flush. Except Kavanar varikka-1 and Chengalam varikka, fruits of all the other genotypes had strong pulp flavour. All genotypes except Kavanar varikka-1 have thick fruits. All the accessions pocessed elongated seed. Pathamuttom Varikka-2 and Muttom varikka produced fruits which were irregular in shape. Veloor varikka-1 and Pathamuttom vaikka-1 produced oblong fruit. Apart from these four varieties, all other had fruits with ellipsoid shape. Flake shape showed high variability among these genotypes.

High variation could be seen in the case of quantitative characters (Table 4). The total fruit weight varied between 5.93 to 21.52 kg. Fruit weight was highest for Pathamuttom varikka-1 and followed by Veloor varikkka-1. The flake thickness was highest for Muttom varikka (0.63 cm) whereas Veloor varikka-2 recorded lowest value for flake thickness (0.33 cm). The single bulb weight ranged between 15.12 to 39.87 g and Pathamuttom Varikka-2 showed superiority. The single seed weight and flake weight varied between 2.38 to12.89 g and 7.7 to 28.36 g respectively.

Published reports on jackfruit clones also indicated significant variation in fruit characters (Muralidharan *et al.*, 1997; Mitra and Mani, 2000; Reddy *et al.*, 2004; Jagadeesh *et al.*, 2010; Krishnan *et al.*, 2015a, Ashwini, 2015)

High variation could be observed in the case of biochemical characters also (Table 5). The values of Total soluble solids (TSS) varied between 18.8 to 32 °Brix. The TSS was maximum for Sindhur (32 °Brix) followed by Chengalam varikka (31.80 °Brix). The total sugar was highest for Chengalam varikka (19.81 %). Chengalam varikka and Muttom varikka (9.39 %) showed highest value for the percentage of reducing sugar. Percentage of non reducing sugar varied from 8.45 to 11.44 %. The ascorbic acid content ranged between 2.40 and 12.00 mg/100g and

Pathamuttom varikka-2 showed superiority. The acidity was highest for Veloor varikka-2 and Pathamuttom varikka-1 (1.02 %) whereas Kavanar Varikka-1 recorded lowest value for acidity (0.38 %).

Studies conducted by Gomez *et al.* (2015) also reported wide variation in biochemical attributes like total soluble solids, titratable acidity, TSS, ascorbic acid content etc. among eighteen jackfruit accessions including Muttom varikka and Sindhur collected from homestead gardens across Kerala.

Similar observations about significant variability in biochemical parameters of jackfruit accessions were reported by Azad (2000), Reddy *et al.* (2004) and Jagadeesh *et al.* (2010).

5.2 Molecular characterization

5.2.1 Isolation and quantification of DNA

In the present study tender emerging leaves were collected from each plant of jackfruit genotypes in early morning. DNA isolation was done as per the modified CTAB method described by Doyle and Doyle (1987).

Modified CTAB method of Doyle and Doyle (1987) was followed by many workers (Pushpakumara and Harris, 2007; Ashwini, 2015; Krishnan *et al.*, 2015b) for isolating genomic DNA from jackfruit genotypes. Shyamalamma *et al.* (2008) performed extraction of DNA from jackfruit leaf with a modified CTAB method described by Dellaporta *et al.* in 1983. Ying-zhi *el al.* (2010) extracted total DNA using a modified CTAB method as reported by Ye *et al.* (2005). De Bellis *et al.* (2016) used mixed alkyl trimethylammonium bromide (MATAB) protocol developed by Risterucci *et al.* (2000) for the isolation of genomic DNA from *Artocarpus* genus. Kits (QIAGEN DNeasy Plant Mini Kit) are also recommended for the isolation of DNA from jackfruit (Witherup *et al.*, 2013).

The quality of DNA was analysed by running DNA in 0.8 per cent agarose gel with ethidium bromide staining. For avoiding RNA contamination, DNA was treated with RNase A. Gallego and Martinez (1996) and Ravel *et al.* (1998) reported the use of RNase in the purification process.

In this study the DNA sample after RNase treatment showed a high molecular weight during electrophoresis. Hence the DNA under test was of good quality. According to Wettasingf and Peffley (1998) a DNA sample was considered as high quality if it had a band of high molecular weight with little smearing and low amount of RNA.

In order to assess purity of isolated DNA OD $_{260}$/OD$_{280}$ ratio was recorded using Nanodrop^{R} spectrophotometer (ND-1000). The absorbance ratio of DNA sample from different accessions was found to be in between 1.81 -1.96, which was considered as purest DNA (Gopalsamy *et al.*, 2012; Ashwini, 2015).

5.3 Molecular marker analysis

Molecular marker technologies act as a novel tool for germplasm characterisation, diversity analysis, DNA fingerprinting etc. Molecular markers are very efficient and environmentally unaffected techniques (Park *et al.*, 2009). PCR based markers are more common. They amplify specific regions on genomic DNA based on the primer used. Two PCR based marker techniques *i.e.,* ISSR and SSR were used for the present study.

Before conducting any molecular work, optimisation of reagent concentration and temperature regime used in PCR reactions are very essential. So these parameters were fixed by repeating the reactions. In the present study, DNA samples were diluted to 40 ng/µl. As per previous reports (Henegariu *et al.*, 1997) more than 30 ng/ µl concentration of genomic DNA gives good amplification in PCR.

5.3.1 Inter Simple Sequence Repeat (ISSR) analysis

In the present study fifty ISSR primers were used for sceening, among these 48 primers belongs to UBC (University of Columbia) series and two were TC10G and CT10A. Screening was done using bulked DNA isolated from eight samples. Out of the fifty primers, ten primers were selected based on polymorphism (Table 8). Occurrence of polymorphism in ISSRs is due to the deletion or insertion in between the SSRs, which modifies the distance between repeats. In the case of 5' anchored primers, variation in the length of the microsatellite is also a reason for the occurrence of polymorphism (Zietkiewicz *et al.*, 1994; Tsumara *et al.*, 1996; Nagaoka *et al.*,

1997). Reproducibility of selected ISSR primers was tested by repeating the PCR reactions for at least three times under same conditions (Nayak *et al.*, 2003).

In jackfruit, only a few studies were conducted on ISSR markers. Chunai *et al.* (2009) reported 24 ISSR primers in 76 accessions of jackfruit. Aswini (2015) conducted genetic diversity analysis in 20 accessions and three varieties of jack fruit using 50 ISSR primers and reported that only 10 were polymorphic.

5.3.2 Simple Sequence Repeat (SSR) analysis

In the present study fifty SSR primers were used for the screening of eight jackfruit genotypes. It included eight Mul SSR, two M2 SSR, twenty one mAaCIR and nineteen MAA series. Screening was done using bulked DNA from eight samples of jackfruit. Out of the fifty primers, eleven primers were selected for final fingerprinting based on polymorphism (Table 10). Occurrence of polymorphism in SSR depends on the number of repeated units (Weber, 1990) and only small amounts of DNA is required for the SSR analysis (Rafalski *et al.*, 1996). Russell *et al.* (1997) reported that SSR marker has high level of polymorphism, high information content, high discriminating power, reproducibility, codominant nature, mendelian inheritance and ease of genotyping through polymerase chain reaction (PCR) and electrophoretic methods.

In jackfruit, only a few SSRs markers were reported. Witherup *et al.*, (2013) reported 13 SSR markers and De Bellis *et al.* (2016) reported 21 SSR makers in jackfruit.

5.3.3 Polymorphic information content (PIC) and Marker index (MI) of selected ISSR and SSR primes

In order to understand the efficiency of primers PIC and MI value were calculated. In the present study, PIC value of selected ISSR primers ranged between 0.23 and 0.3 with an average of 0.27 (Table 11; Fig. 1a) and that of selected SSR primes ranged between 0.22 and 0.98 with an average of 0.75 (Table 12; Fig. 1b).

The PIC value helps to understand the usefulness of primer for characterizing a variety (Hollman *et al.*, 2005) and it is a reflection of allele diversity and frequency among genotypes (Sheeja *et al.*, 2013).

Mogalayi (2011) reported the values of ISSR Polymorphic information content (PIC) varied between 0.80 and 0.89 in black pepper. The PIC reported by Laxman (2013) in brinjal varieties varied between 0.83 to 0.96 for ISSR primers and zero for SSR primers. Similarly Meena (2014) reported PIC value in cashew, varied between 0.256 to 0.42 for ISSR primers and 0 to 0.50 for SSR primers. In cocoa, Sujith (2016) obtained PIC value between 0.17 to 0.68 for ISSR markers and 0.42 to 0.80 for SSR markers.

MI value of selected ISSR primers varied from 1.15 to 3.77 with an average of 2.38 (Table 11; Fig. 2a) and that of selected SSR primers ranged between 0.60 and 2.7 with an average of 1.18 (Table 12; Fig. 2b).

Marker index helps to understand the capacity of primer to detect polymorphic loci among varieties (Varshney *et al.* 2007). Naghavi *et al.* (2007) reported higher SSR MI value of 8.0 in *Aegilops tauschii*. Sheeja *et al.* (2013) reported the ISSR MI value in *Myristica* and related genera varies from 1.98 to 8.28 with an average of 5.3. Similarly, Kumar *et al.* (2014) also calculated ISSR MI value in *Justicia adhatoda*. It ranged from 2.06 to 6.09 with an average MI of 3.53 per primer.

Mandal *et al.* (2016) conducted discrimination analysis and obtained that MI was the most efficient parameter for the identification of most capable primer.

5.3.4 DNA amplification with selected ISSR primers

In the present study all the selected ten primes yielded at least one polymorphic band among the genotypes. All primers belonged to the UBC series (University of British Colombia). Annealing temperature of selected ISSR primers ranged between 50 °C and 52.5 °C.

Out of ten primers selected, nine of them generated unique bands which can acts as variety specific DNA fingerprints. The primer UBC 810 developed a unique band in Pathamuttom varikka-1 at 480 bp. Primer UBC 815 showed two distinct bands in Muttom varikka (at 2500 bp and 3500 bp) and one in Sindhur (at 1050 bp). Unique polymorphic bands were found in Muttom varikka at 950 bp and 1000 bp and in Pathamuttom varikka-1 at 580 bp and 640 bp with the primer UBC 834a. At 760 bp length, the primer UBC 834b yielded a unique band in Chengalam varikka. Primer UBC 841a produced unique band in Pathamuttom varikka-1 at 520 bp and

Pathamuttom varikka-2 at 370 bp. The primer UBC 841b generated specific polymorphic bands in Pathamuttom varikka-2 (at 700 bp) and UBC 848a yielded unique band in Chengalam varikka (at 1340 bp). Chengalam varikka generated two unique bands at 1250 bp and 1490 bp with the primer UBC 857a and one unique band at 680 bp with UBC 857b.

Apart from these unique bands, amplicons which were shared among maximum of two varieties were also selected for developing final ISSR fingerprints for each genotypes. Amplification with UBC 810 yielded polymorphic bands in Veloor varikka-1 and Veloor varikka-2 at 430 bp and in Kavanar varikka-1 and Muttom varikka at 630bp. Pathamuttom varikka-1 and Kavanar varikka -1 generated polymorphic bands at 600 bp when amplified with the primer UBC 815. Amplification with UBC 834a produced polymorphic bands in Kavanar varikka-1 and Muttom varikka at two sites, *i.e.*, 450 bp and 760 bp. Another polymorphic band at 370 bp was also observed in Chengalam varikka and Sindhur with this primer. The amplicones generated by the primer UBC 841b at 1450 bp in Kavanar varikka-1 and Muttom varikka, at 950 bp in Pathamuttom varikka-1 and Chengalam varikka were also polymorphic. Polymorphic band were observed at 1050 bp on amplication with the primer UBC 844a in Pathamuttom varikka-2 and Muttom varikka. The primer UBC 848a produced two shared bands in Veloor varikka-1 and Veloor varikka-2 at 1370 bp and 1400 bp and another band in Kavanar varikka-1 and Muttom varikka at 600bp. Amplification with UBC 857a generated polymorphic band at 800 bp in Kavanar varikka-1 and Muttom varikka-1. Primer UBC 857b yielded a shared band at 360 bp in Veloor varikka-1 and Veloor varikka-2.

Kalpana *et al.* (2012) conducted DNA fingerprinting of sixteen mulberries varieties using ISSR markers. In jackfruit ISSR markers were effectively utilized for genetic diversity analysis by Ashwini (2015).

5.3.5 DNA amplification with selected SSR primers

In the present study, out of eleven selected primers, six belonged to the series mAaCIR and five belonged to the series MAA. Annealing temperature of selected SSR ranged from 52 to 55°C.

Among these selected primers, seven of them produced specific bands which can acts as variety specific DNA fingerprints. Primers mAaCIR 0078 (at 150 bp) and MAA 122 (at 270 bp) produced unique band in Pathamuttom varikka-1. Amplification with mAaCIR 0134 (at 240 bp) and MAA 105 (at 265 bp) yielded unique bands in Pathamuttom varikka-2. At a length of 230 bp, primer mAaCIR 0141 poduced a specific band in Sindhur. In Pathamuttom varikka-2 and Sindhur unique bands were developed by the primer MAA 196a at 340 bp and 320 bp respectively. The primer MAA 145 generated unique band in Pathamuttom varikka-2 at 287 bp and Chengalam varikka at 280 bp.

Apart from these unique bands, amplicons which were shared among maximum of three varieties were also selected for developing final SSR fingerprints for each genotypes. Amplification with mAaCIR 0049 yielded polymorphic bands in Kavanar varikka-1 and Muttom varikka at 100 bp. A polymorphic band (at 175 bp) shared by Pathamuttom varikka-1, Pathamuttom varikka-2 and Sindhur, when amplified with the primer mAaCIR 0115. Polymorphic amplicones at 200 bp generated by the primer mAaCIR 0127 was shared by Kavanar varikka-1, Chengalam varikka and Muttom varikka. Polymorphic band at 220 bp obtained by amplification with the primer MAA 54a was observed in Kavanar Varikka-1, Muttom varikka and Sindhur. On amplification with the primer MAA 105, Kavanar Varikka-1, Muttom varikka and Sindhur shared a polymorphic band at 290 bp and Veloor varikka-1, Veloor varikka-2 and Chengalam varikka shared a band at 270 bp. Amplification with the primer MAA 196a developed a polymorphic band at 290 bp in Pathamuttom varikka-2 and Sindhur.

Literature on DNA fingerprinting using SSR markers of jackfruit cultivar were not available. Baraket *et al.* (2011) opined that SSR markers are suitable for diversity analysis and cultivar fingerprinting in *Ficus carica.* De Bellis *et al.* (2016) developed and validated 50 SSR markers in breadfruit (*Artocarpus altilis*) by next generation sequencing, which are polymorphic in 39 bread fruit accessions.

5.4 DNA fingerprinting of jackfruit genotypes

DNA fingerprinting is the molecular marker based techniques for the identification of cultivars. This is mainly based on the polymorphisms occurring at the molecular level (Archak, 2000) and they are unique to each individual (Bhat, 2001).

In plants, DNA profiling is used for the identification of gene diversity and variation, protection of biodiversity, identifying markers for traits (Archak, 2000).

In the present study, fingerprints were developed by locating useful polymorphic amplicons with selected 10 ISSR and 11 SSR primers.

5.4.1 Veloor varikka-1

DNA fingerprint of Veloor varikka-1 was developed using selected bands produced by both ISSR and SSR primers (Fig. 33a). The shared amplicons developed by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) and MAA 105 (at 270 bp) were considered for constituting fingerprint of the genotype. In this study the genotype Veloor varikka-1 could not be distinguished with unique bands. Fingerprint of Veloor varikka-1 was identical to Veloor varikka-2. We used only 10 ISSR and 11 SSR primers. If attempted with more number of primers, could bring out unique band and unique fingerprint in Veloor varikka-1

5.4.2 Veloor varikka-2

DNA fingerprint of Veloor varikka-2 was developed using selected amplicons developed with ISSR and SSR primers (Fig. 33b). There was no unique band observed in Veloor varikka-2. The amplicons yielded by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) from ISSR and MAA 105 (at 270 bp) from SSR were used for constituting fingerprint of the genotype. If attempted with more number of primers, unique band could be brought out in Veloor varikka-2.

5.4.3 Pathamuttom varikka-1

DNA fingerprint with useful amplicons observed in ISSR and SSR analysis was developed for Pathamuttom varikka-1 (Fig. 33c). Six polymorphic bands from ISSR primers and three polymorphic bands from SSR primers were used for individual fingerprinting. Among these 9 bands two produced by UBC 834a (at 580 bp and 640 bp) and each one from UBC 810 (at 480 bp), UBC 841a (at 520 bp), mAaCIR 0078 (at 150 bp), MAA 122 (at 270 bp) were unique and served as specific fingerprints of the variety. The fingerprint developed for this genotype was unique and different from all others. These can be effectively used to identify this genotype.

5.4.4 Pathamuttom varikka-2

Individual fingerprint of Pathamuttom varikka-2 was developed using selected amplicons observed with ISSR and SSR markers (Fig. 33d). Out of the 9 polymorphic bands, ISSR primers produced two unique bands (UBC 841a at 370 bp and UBC 841b at 700 bp) and SSR primers produced four unique bands (mAaCIR 0134 at 240 bp, MAA 105 at 265 bp, MAA 145 at 287 bp and MAA 196a at 340 bp). These bands acted as specific fingerprints of the variety. The fingerprint developed through combined ISSR and SSR assay was unique to distinguish the genotype Pathamuttom varikka-2 due to the presence of unique bands.

5.4.5 Kavanar varikka-1

DNA fingerprint of Kavanar varikka-1 was developed using both ISSR and SSR markers (Fig. 33e). The ISSR primers UBC 834a (at 450 bp and 760 bp), UBC 810 (at 630 bp), UBC 815 (at 600 bp), UBC 848a (600 bp), UBC 857a (at 800 bp), UBC 841b (at 1450 bp) and SSR primers mAaCIR 0049 (at 100 bp), mAaCIR 0127 (at 200 bp), MAA 54a (at 220 bp), MAA 105 (290 bp) produced useful amplicons from SSR primers. These amplicons were considered for preparing DNA fingerprint of the genotype. Both the marker system could not generate any unique band for this genotype, However the DNA fingerprint was unique for this genotype.

5.4.6 Chengalam varikka

Individual fingerprint of Chengalam varikka was developed using selected amplicons generated with ISSR and SSR markers (Fig. 33f). Among ten selected amplicons, five were unique and were useful as specific fingerprints of the genotype. The unique amplicons were observed with the primers UBC 857a (at 1250 bp and 1490 bp), UBC 834b (at 760 bp), UBC 848a (at 1300 bp), UBC 857b (at 680 bp) and MAA 145 (at 280 bp). Both the marker system could bring out unique bands in this genotype. The fingerprint developed for this genotype using useful amplicons obtained with ISSR and SSR markers were unique and can be used for identify this genotype.

5.4.7 Muttom varikka

DNA fingerprint of Muttom varikka was developed using both ISSR and SSR markers (Fig. 33g). Among the 15 amplicons, four amplicons produced by ISSR

primers were unique and served as specific fingerprints of the variety. The unique amplicons were generated by UBC 815 (at 2500 bp and 3500 bp) and UBC 834a (at 950 bp and 1000bp). However SSR markers could not bring out any unique bands. However, DNA fingerprints for this cultivar was unique and can be used for varietal identification.

5.4.8 Sindhur

DNA fingerprint of Sindhur was developed using selected bands in both ISSR and SSR markers (Fig. 33h). Out of the 8 polymorphic bands only 3 were unique. The unique amplicons observed with the primers UBC 815 (at 1050 bp), mAaCIR 0141(at 230 bp) and MAA 196a (320 bp) could act as specific fingerprints of this variety. The DNA fingerprint developed with both marker systems were unique and were useful for distinguishing this variety.

In the present investigation the genotypes Veloor varikka -1, Veloor varikka -2 and Kavanar varikka -1 could not be distinguished with unique band in both the marker systems. In this study only 10 ISSR and 11 SSR primers were used. If more primers were screened, unique bands could be brought out for these varieties also. However the DNA fingerprints developed were unique for all the genotypes except Veloor varikka-1 and Veloor varikka-2.

In this study ISSR marker system was found to be more efficient in fingerprinting of jack genotypes as it produced more number of unique bands in different genotypes studied as compared to SSR marker system.

In Kerala Agriculural University, DNA fingerprinting works were conducted in many plant species using ISSR and SSR markers, including black pepper (Mogalayi, 2011), brinjal (Laxman, 2013), ginger (Ghosh, 2013), cashew (Meena, 2014) and cocoa (Sujith, 2016) etc. All these study showed the effectiveness of SSR and ISSR makers in DNA fingerprinting work.

5.5 Diversity analysis

In molecular analysis, combined data clustering (ISSR+SSR) and ISSR based clustering produced same clustering pattern. In all clustering analysis, at 0.78 similarity coefficient, Veloor varikka-1 and Veloor varikka-2 grouped together in to

one cluster whereas, Kavanar varikka-1 and Muttom varikka grouped together in another cluster and all other varieties were placed in individual clusters. Based on SSR clustering (Fig. 36a) Veloor varikka-1 and Veloor varikka-2, Kavanar varikka-1 and Muttom varikka were exactly similar (100 %) but in ISSR clustering they showed some dissimilarity (Fig. 36b). This may be due to the nature of ISSR primers, which target highly variable microsatellite regions and produce large number of polymorphic fragments (Gupta *et al.*, 1994). In the present study jaccard's similarity coefficient values of SSR-ISSR cambined dendrogam varied from 0.56 to 0.95 (Fig. 37). Many previous workers used this coefficient for calculating similarity matrix. Meena (2014) in his work in Cashew, reported similarity coefficient ranged between 0.80 and 0.92 and Sujith (2016) reported that similarity coefficient was in the range of 0.75 to 0.95 in cocoa varieties.

While comparing quantitative data cluster analysis with combined (ISSR +SSR) clustering analysis, the number of clusters and distribution patterns are different. At 0.78 similarity coefficient, all jackfruit genotypes were clustered separately in quantitative clustering (Fig. 34b), but in SSR-ISSR combined data cluster analysis, Veloor varikka-1 and Veloor varikka-2 grouped together with 95% similarity in one cluster and Kavanar varikka-1 and Muttom varikka grouped together in another cluster with 91% similarity (Fig. 37).

Distribution of genotypes in various clusters were different in quantitative and molecular clustering. This may be because the molecular clustering was not done based on actual coding sequence for morphological traits, but it depends on non-coding sequence. Homology has to be found out between expressed gene sequence and morphological data to obtain more of accurate result. In a study on cocoa accessions, Sujith (2016) also obtained similar results for quantitative and molecular cluster analysis.

This investigation could fingerprint one jack variety, one cultivar and six selections using ISSR and SSR markers. The fingerprint generated in this study can be used for varietal identification. The extent of variability existing between these genotypes could also estimated.

In future more investigation are required to find out primers and marker systems which can generate unique bands in all the jackfruit genotypes studied.

Summary

6. SUMMARY

The study on "DNA fingerprinting of promising selections of jack (*Artocarpus heterophyllus* Lam.) using molecular markers" was carried out at Regional Agricultural Research Station, Kumarakom and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during the period 2015-2017. The objectives of the study were to characterize eight jackfruit genotypes including one KAU released jack variety (Sindhur), one cultivar (Muttom varikka) and six superior jackfruit selections (Veloor varikka-1, Veloor varikka-2, Pathamuttom varikka-1, Pathamuttom Varikka-2, Kavanar Varikka-1and Chengalam varikka) identified at RARS, Kumarakom using different molecular markers - ISSR and SSR and to develop DNA fingerprint.

The findings of the study were as follows.

1. For morphological characterization, seven qualitative and five quantitative fruit characters were recorded. For biochemical analysis, six characters were recorded.
2. DNA extraction was done with modified CTAB method by Doyle and Doyle (1987) with slight modification. RNase treatment helped to remove the RNA contamination.
3. Quantification of DNA was performed using spectrophotometer NanoDrop ND-1000. Good quality DNA with UV absorbance ratio (A260/A280) 1.81 - 1.96 was obtained and was used for further analysis.
4. Two of SSR and ISSR were used for molecular analysis. PCR conditions and master mix composition of primers were optimized.
5. Fifty each ISSR and SSR primers were screened with bulked DNA of jackfruit genotypes and selected 10 ISSR and 11 SSR primers with good polymorphism for further analysis.
6. Amplification patterns of selected primes were scored and colour chart were developed.
7. The amplicons shared by maximum two genotypes in ISSR and three genotypes in SSR were considered as useful polymorphism for generating DNA fingerprints of eight jack genotypes.

8. Veloor varikka-1:- Unique band was not observed for this selection. The shared amplicons developed by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) and MAA 105 (at 270 bp) were considered for constituting fingerprint of the genotype.
9. Veloor varikka-2:- The selected primers could not generate unique band in Veloor varikka-2. The amplicons yielded by UBC 810 (at 430 bp), UBC 848a (at 1370 bp and 1400bp), UBC 857b (at 360 bp) from ISSR and MAA 105 (at 270 bp) from SSR were used for generating fingerprint of the genotype
10. Pathamuttom varikka-1:- Nine bands were used for developing fingerprint. Among these 9 bands two produced by UBC 834a (at 580 bp and 640 bp) and each one from UBC 810 (at 480 bp), UBC 841a (at 520 bp), mAaCIR 0078 (at 150 bp), MAA 122 (at 270 bp) were unique and served as specific fingerprints of the variety.
11. Pathamuttom varikka-2:- DNA fingerprint was formed with nine bands. Among these 2 bands produced by ISSR primers (UBC 841a at 370 bp and UBC 841b at 700 bp) and 4 bands generated by SSR primers (mAaCIR 0134 at 240 bp, MAA 105 at 265 bp, MAA 145 at 287 bp and MAA 196a at 340 bp) were unique and served as specific fingerprints of the genotype.
12. Kavanar varikka-1:- Unique band was not observed in Kavanar varikka-1. The ISSR primers UBC 834a (at 450 bp and 760 bp), UBC 810 (at 630 bp), UBC 815 (at 600 bp), UBC 848a (600 bp), UBC 857a (at 800 bp), UBC 841b (at 1450 bp) and SSR primers mAaCIR 0049 (at 100 bp), mAaCIR 0127 (at 200 bp), MAA 54a (at 220 bp), MAA 105 (290 bp) produced useful amplicons. These amplicons were considered for developing DNA fingerprint of the genotype.
13. Chengalam varikka-1:- For fingerprinting this selection 10 useful amplicons were selected. Unique amplicons were observed with the primers UBC 857a (at 1250 bp and 1490 bp), UBC 834b (at 760 bp), UBC 848a (at 1300 bp), UBC 857b (at 680 bp) and MAA 145 (at 280 bp), which acted as specific fingerprint of this genotype.
14. Muttom varikka:- Among the 15 useful amplicons, four amplicons produced by ISSR primers were unique and served as specific fingerprints of the cultivar. The unique amplicons were generated by UBC 815 (at 2500 bp and

3500 bp) and UBC 834a (at 950 bp and 1000bp). DNA fingerprints for this cultivar was unique and can be used for varietal identification.

15. Sindhur:- Out of the 8 polymorphic bands used for fingerprinting, only 3 were unique. The unique amplicons were observed with the primers UBC 815 (at 1050 bp), mAaCIR 0141(at 230 bp) and MAA 196a (320 bp) which could act as specific fingerprints of this variety.
16. For checking the efficiency of a marker to detect the polymorphism within a population, the Marker Index (MI) and Polymorphic Information Content (PIC) were worked out for the selected primers. The Marker Index (MI) worked out for the ISSR primers ranged between 1.15 to 3.77 and 0.60 to 2.7 for SSR primers. The Polymorphic Information Content (PIC) varied from 0.23 to 0.3 in ISSR analysis and 0.22 to 0.98 in SSR analysis.
17. Clustering analysis was performed using the software NTSYS-Pc. Maximum variability observed was 44 percent for the variety Sindhur. The genotypes Veloor varikka-1 and Veloor varikka-2 collected from same locality indicated 95 percent similarity.
18. While comparing quantitative data cluster analysis with combined (ISSR +SSR) cluster analysis, the number of clusters and distribution patterns are different. This variation may be due to molecular clustering was done not based coding characters of morphological traits.

In future more investigation are required to find out the primers and marker systems which can generate unique bands in all the jackfruit genotypes studied. Characterization and sequencing of unique bands shall be carried out for identifying its coding character. For understanding the relation between molecular characters and morphological traits, more morphological character have to be screened and correlate with molecular marker study.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ANNEXURE 1

List of laboratory equipments used for the study

Refrigerated centrifuge	:	eppendorf 5430R
SureCycler 8800 PCR	:	Agilent Technologies
Electrophoresis unit	:	Bio-Rad, USA
Spectrophotometer	:	Nanodrop^{R} ND-1000, USA
Gel documentation system	:	Bio-Rad
Micropipettes	:	Eppendorf

ANNEXURE II

Reagents required for DNA isolation

1. **2X CTAB extraction buffer (100 ml)**

   CTAB : 2 g

   Tris base : 1.21 g

   EDTA: 0.745 g

   NaCl : 8.18 g

   PVP: 1.0 g

   Adjusted the pH to 8 and made up final volume up to 100 ml.

2. **Chloroform-Isolated alcohol (24:1 v/v)**

   To chlorofrorm (24 parts), isoamyl alcohol (1 part) was added and mixed properly.

3. **Chilled isopropanol**

   Isopropanol was stored in refrigerator at 0°C and was used for the study.

4. **Wash buffer**

   Ethyl alcohol 76 ml and distilled water 24 ml

   Ammonium acetate 0.077 g

   Add 0.077 g ammonium acetate in 100 ml 76 % ethyl alcohol and mixed well.

5. **Ethanol (70 %)**

   To the 70 parts of absolute ethanol (100 %), 30 parts of sterile distilled water was added to make 70 per cent ethanol

6. **TE buffer (pH 8, 100ml)**

   Tris HCl (10 mM) : 0. 1576 g

   EDTA (1 mM) : 0. 0372 g

(The solution was prepared, autoclaved and stored at room temperature)

ANNEXURE III

Composition of buffers and dyes used for gel electrophoresis

1. **TAE buffer 50 X**

   Tris base : 242 g

   Glacial acetic acid : 57.1 ml

   0.5 M EDTA (pH 8.0) : 100 ml

2. **Loading dye 6X**

   0.25 per cent bromophenol blue

   0.25 per cent xylene cyanol

   30 per cent glyceol in water

3. **Ethidium bromide**

   The dye was prepared as a stock solution of 10 mg/ml in water and was stored at room temperature in a dark bottle.

DNA FINGERPRINTING OF PROMISING SELECTIONS OF JACK (*Artocarpus heterophyllus* Lam.) USING MOLECULAR MARKERS

By

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(2015-11-106)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

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2017

ABSTRACT

The jack (*Artocarpus heterophyllus* Lam.) is the world's largest edible fruit crop which belongs to the family Moraceae. It was originated from Western Ghats of India. India is one of the major jackfruit producing country. It is an important component in the homestead gardens of Kerala. In India, Kerala is the largest producer. Kerala has only a few numbers of varieties in jack. Muttom varikka is a popular cultivar of jackfruit in Kerala. RARS, Kumarakom had identified few superior jackfruit genotypes from Kuttanad region. Fingerprint data are not available for these selections/variety/cultivar. Central Seed Committee established under the Seed Act (1966) insists for DNA fingerprint data for the varieties released or proposed to be released.

The study on "DNA fingerprinting of promising selections of Jack (*Artocarpus heterophyllus* Lam.) using molecular markers" was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), Vellanikkara and Regional Agricultural Research Station, Kumarakom during 2015-17. The objective of present study was to characterize popular jack variety Sindhur, cultivar Muttom varikka and six superior jack selections (Veloor varikka-1, Veloor varikka-2, Pathamuttom varikka-1, Pathamuttom Varikka-2, Kavanar Varikka-1and Chengalam varikka) identified at RARS, Kumarakom from Kuttanad tract using SSR and ISSR markers.

High variability was observed in qualitative, quantitative and biochemical characters of eight jack genotypes. The variety Sindhur and selections Pathamuttom varikka-1 and Pathamuttom varikka-2 showed coppery red colour for flake flesh which is a character having high market value. For molecular analysis, genomic DNA extraction was done using modified CTAB method (Doyle and Doyle, 1987). The RNA contamination was completely removed through RNase treatment. Good quality DNA with UV absorbance ratio (A260/A280) 1.80 - 1.90 was used for further analysis. Fifty each of ISSR and SSR primers were screened for amplification of jackfruit genomic DNA and those which showed reliable and distinct banding pattern were selected for further amplification and fingerprinting. Ten ISSR and eleven SSR primers were selected for developing fingerprints of jack varieties and promising selections. Only clear and distinct bands were used for developing DNA fingerprints. Specific fingerprints were developed by assigning different colour codes for unique and sharing bands.

Unique bands were observed in Pathamuttom varikka -1, Pathamuttom varikka -2, Chengalam varikka, Muttom varikka and Sindhur with ISSR marker system. The SSR marker system also could bring out unique bands in Pathamuttom varikka-1, Pathamuttom varikka -2, Chengalam varikka and Sindhur. These specific bands could be utilized for the identification of varieties and selections.

The Marker Index (MI) obtained for the selected ISSR primers ranged from 1.15 to 3.77 and for SSR primers it ranged from 0.60 to 2.7 which indicated the efficiency of primers selected. The Polymorphic Information Content (PIC) varied from 0.23 to 0.30 in ISSR analysis and 0.22 to 0.98 in SSR analysis. This is also an indication of suitability of primers. The computer package NTSYS-Pc was used for diversity analysis. Among the eight genotypes maximum diversity (44 %) was observed for the variety Sindhur. The varieties Veloor varikka-1 and Veloor varikka-2 collected from same locality indicated 95 percentage similarity. The number of clusters and distribution patterns were different in both quantitative data and combined ISSR-SSR data. This may be because the actual coding sequences for quantitative characters were not used for molecular clustering. Homology has to be worked out between expressed gene sequence and quantitative data for obtaining more precise result.

The specific DNA fingerprints developed for the jack variety Sindhur, cultivar Muttom varikka and promising selections Pathamuttom varikka-1, Pathamuttom varikka-2, Kavanar varikka-1 and Chengalam varikka could be utilized for varietal identification and settling IPR issues. Since the fingerprints obtained for Veloor varikka-1 and Veloor varikka-2 were identical more primers have to be screened for developing specific fingerprints for these selections.