"GENETIC DIVERSITY ANALYSIS OF *CRINUM VIVIPARUM*(LAM.) R. ANSARI & V. J. NAIR IN KERALA"

Dissertation submitted in partial fulfilment of the requirements for the award of the degree of "MASTER OF SCIENCE" in BOTANY

By

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ERNAKULAM

MAY 2023

CERTIFICATE

This is to certify that the dissertation entitled **"GENETIC DIVERSITY ANALYSES OF *CRINUM VIVIPARUM*(LAM.) R. ANSARI & V. J. NAIR IN KERALA"** is an authentic record of work carried out by SANIGA GEO CHERUVATHOOR under my supervision and guidance in the partial fulfilment of the requirement of the M.Sc. Degree of Mahatma Gandhi University, Kottayam. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

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SANIGA GEO CHERUVATHOOR

DECLARATION

This is to declare that the work which is being presented in the dissertation entitled "**GENETIC DIVERSITY ANALYSIS OF *CRINUM VIVIPARUM*(LAM.) R. ANSARI & V. J. NAIR IN KERALA"** in partial fulfilment of the requirements for the award of the degree of 'Master Of Science 'in Botany submitted to St.Teresa's College (Autonomous) Ernakulam is an authentic record of my work carried out during M.Sc period under the supervision of Smt. I.K. Nishitha

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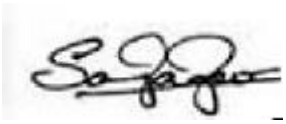

SANIGA GEO CHERUVATHOOR

CONTENTS

LIST OF TABLES

LIST OF FIGURES AND PLATES

ABSTRACT

Crinum viviparum (Lam.) R. ANSARI & V. J. NAIR IN KERALA commonly known as River Crinum Lilly, which is an extensively studied member of Amaryllidaceae family. The genetic diversity of *Crinum viviparum* was examined using molecular markers RAPD and ISSR. A numerical Taxonomy system for the personal computer (NTSYS) was used to estimate phylogenetic trees using UPGMA software.ISSR markers were found to be more efficient than RAPD markers regarding polymorphism detection.RAPD and ISSR marker systems were found to be useful for the genetic diversity study of *Crinum viviparum* (Lam.)R. ANSARI & V. J. NAIR IN KERALA and to identify the variations

INTRODUCTION

*Crinum viviparum*_is a herbaceous plant belonging to the family Amaryllidaceae. It is extensively widespread in Asian continent especially in the countries like India & Srilanka.

It usually grow submerged and in damp places ,and some variety are used in garden as ornamental plants all the effective conservation management and efficient utilisation of plant genetic resources. To understand basis of essential biological phenomena in plants in particular and adequate knowledge of existing genetic diversity where in plant population it is found and how to best utilise this can be known by using advanced molecular techniques these techniques have enabled the study of genetic variability at the DNA level which has significantly increased the accuracy in assessing the genetic diversity and identifying the cultivars the importance of plant genetic diversity is now being recognised as a specific area since exploding population with urbanization and decreasing cultivable land are the critical factors contributing to food insecurity in developing world agriculture scientist realised that can be captured and stored in the form of plant genetic resources such as genBank DNA library and so forth in the bio repositories which preserve genetic material for long period the concert could be utilised for crop plant improvement in order to meet future global challenges in relation to food medicinal and nutritional security.

Molecular markers with the development of polymerase chain reaction and numerous molecular technologies have been and still are being developed which can be used for the detection characterisation and evaluation of genetic diversity molecular markers which is an invention in advanced molecular technology have become an important tool in the studies of genetic diversity. The development and utilisation of molecular markers for the exploration and identification of plant genetic diversity is one of the most a development in the field of molecular genetic studies. Various types of techniques are used to estimate genetic diversity such as dominant markers and Random Amplified Polymorphic DNA. DNA amplification

fingerprinting, arbitrary primed polymerase chain reaction, inter simple sequence repeat and amplified fragment length polymorphism. simple sequence repeats extra molecular markers work by highlighting differences within nucleic acid sequence between different individuals. Molecular marker is a sequence of DNA which are located with the unknown position on the chromosome using phenotypic expression and is frequently distant and used to detect an individual or as a prop to mark a chromosome nucleus or locus. Markers show polymorphism which may arise due to alteration of nucleotide on mutation in the genome loci and make it possible to identify genetic differences between the individual organism. Molecular markers are used in many different areas such as genetic mapping pectinase test detect mutant genes which are connected to auditory diseases, cultivars identification marker-assisted breeding of crops population history ETC.

RANDOM AMPLIFIED POLYMORPHIC DNA

Of the common molecular markers RAPD (random amplified polymorphic DNA). Markers have the advantage of detecting polymorphism in nucleic acid sequence. It is a independent simple, quickly cost-effective and requires small amount of DNA. RAPD is pronounced as Rapid. Random amplification of polymorphic DNA is a type of PCR reaction what are the segments of DNA that are amplified are random. The RAPD analysis mainly used molecular marker in genetic diversity studies. No knowledge of the DNA sequence for the targeted gene is required as the primer will bind somewhere in the sequence. But it is not certain exactly where this makes. The method popular for comparing the DNA of biological system that have not had the attention of the scientific community or in a system in which relatively few DNA sequences are compared the main reason for the success is the game of a large number of genetic markers that required a small amount of DNA without the requirement for cloning Sequencing or any other form of the molecular characterization of the genome of the species in question. The RAPD Markers are more oligonucleotide primers usually 10 BP in the length of arbitrary sequence. To generate a band profile primer binds to the complementary sequence along the genome and PCR amplification coccus when the region between the opposite Primers sides is within the amplifiable distances.

ISSR MARKERS-INTER SIMPLE SEQUENCE REPEAT

Recently simple sequence repeat markers have emerged as an alternative system with the reliability and advantages of microsatellites. The technique involves the amplification of genomic segment plant inversely oriented and closely spaced microsatellite sequences by a single primer or a pair of primers based on SSRs are anchored 5'-3' with 14 purine or pyrimidine residues the sequences of repeats and anchor nuclei are arbitrarily selected coupled with separation of amplification product on polyacrylate made for agarose gels ISSR amplification can reveal a much larger number of fragments per Primer than RAPD. It is concluded that the ISSR technique provides a reliable and highly informative system for DNA fingerprinting. Markers are inherited in Mendelian mode and segregated as dominant markers. This technique has been widely used in the studies of cultivars identification genetic mapping and tagging genetic diversity evolution and molecular ecology(Easmin et al., 2008).

OBJECTIVES

- Survey and collection of *Criunum viviparum* (Lam) R.Ansari and V.J.Nair from different parts of Kerala.
- Isolation of genomic DNA from collected sample populations.
- PCR amplification of isolated genomic DNA using Random Amplified Polymorphic Dna (RAPD) and Inter Simple Sequence Repeat(ISSR) Markers.
- Analysis of genetic diversity in *Crinum viviparum* (Lam) R. Ansari and V.J. Nair.

REVIEW OF LITERATURE

TAXONOMY

AMARYLLIDACEAE

Bulbous (rarely rhizomatous), predominantly geophytic, perennials on land, sometimes aquatic or epiphytic, and abundant in family-specific alkaloids

Tungst bulbs. Annual or persistent leaves that are distichous or spirally arranged, sessile, linear, or lorate, or petiolate and lanceolate to broadly elliptic; occasionally basally sheathing and creating an aerial pseudostem; often glabrous; infrequently with trichomes.

Inflorescence spathaceous, pseudo umbellate (reduced helicoid cymes); scape occasionally completely underground and appearing obsolete; terminated by 2 or more spathaceous, obvolute or equitant, typically marcescent bracts that enclose the flowers in the bud (bracts rarely absent); inner bracteoles typically present and progressively shorter and narrower.

Flowers are actinomorphic or zygomorphic, 1-many, perfect, commonly large and showy, sessile or pedicellate, typically subtended by a bracteole, and typically protandrous.

Sanaa & Fadhel, 2010 conducted studies on *Pancratium maritimum* L. populations in Tunisia are currently threatened and only comprise a few dispersed individuals as a result of urbanization's degradation of coastal habitats and overharvesting for the plant's valuable decorative value.

Seven isozymes identified by starch gel electrophoresis were used to assess the genetic diversity and structural complexity of 19 populations growing in settings on the mainland and on islands.

In the study it was found that the low genetic divergence among populations and their high structuring indicate their recent isolation as a result of coastal habitat destruction by anthropic

pressures. The continuous eradication of *P. maritimum* populations reduced their size and contributed to enhancing their differentiation level unless in situ and ex-situ conservation measures are adopted very fast.

GENETIC DIVERSITY STUDY

Only rivers and streams in karst regions of southwest China are home to the endemic species *O. acuminata var. jingxiensis*, which is in danger of extinction. Using 12 ISSR markers, the genetic diversity of 10 naturally occurring populations that were taken from karst rivers was examined in this study. The findings showed that the populations had a moderate level of genetic variation. AMOVA, or the analysis of molecular variance, showed that the populations had a lot of genetic variation.(Li et al., 2019).

CRINUM VIVIPARUM (LAM.) R. ANSARI AND V. J. NAIR

A frequent aquatic plant on stony, shallow riverbeds is the river crinum lily. It belongs to the family Amaryllidaceae It may easily blossom under water and reaches heights of 0.5 to 1 bulb is 5-8 cm long, ovoid, and has a cylindrical neck that is 5–15 cm long. There are 6–8 leaves, each measuring 2–3 feet long, 3 cm broad, with a dark green tint.6–12 flowers appear in an umbel on a 40–75 cm tall, leafless scape. There are two white bracts with a lance-like shape that open and smell good at night. The flower tube is thin, and 6-8 cm long. There are six petals, each measuring 6 to 8 centimetres in length. Red filaments cover the six stamens, which are 4-6 cm long. flowering: September through October. (Tabish 2009)

MEDICINAL USES

Litton Skidel et al conducted a lab study on the pharmacological activities of *Crinum viviparum* composition of ethanolic extract of the plan and was determined and the antibacterial and the inflammatory and antidiarrheal activities were investigated by applying disc diffusion egg albumin close licences and castor oil-induced diarrhoea model in mice suggested that the ethanolic extract of the plant contain various phytochemicals including alkaloids tannins

flavonoids steroids and terpenoids they also found that the plant extract was antibacterial in cases of certain bacteria such as *Escherichia coli* and Staphylococcus aureus and Salmonella enterica also showed and inflammatory and thrombolytic activity in the desired concentration antidiarrheal in Swiss Albino mice

Ghane et al., 2018 examined the methanolic leaf extracts of the *Crinum* species for antioxidant and antidiabetic properties. This was the first comparative study account on anti-oxidant, phenolic concentration profiling and antidiabetic potential and also the quantification anti-Alzheimer and anticancer drugs obtained from the plant. The best technique for obtaining antioxidant components from *Crinum* species was methanolic extraction. The phytochemical analysis found that CAF2 showed significant antioxidant potential with a higher content of phenolics, flavonoids, tannins and alkaloids. Methanol extraction was the most suitable method for the isolation of antioxidant compounds from *Crinum* species.

Chahal et al., 2021 *Crinum* species still need pharmacological analysis. In addition, more extensive studies involving different biotechnological approaches are needed to ensure its sustainable use and long-term conservation. Further studies are needed to improve our current knowledge of bioactive components, toxicity, clinical relevance and shelf life**.** This review summarizes the phytochemistry, pharmacology and in vitro studies of Indian *Crinum*. Potential toxicity aspects**,** knowledge gaps and future perspectives are also discussed.

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

Freitas & Brehm, 2001 have explained the use of RAPD markers to assess the genetic diversity of liverworts which a special focus on the type *Porella* . The study mainly focused on the genetic variation of *Porella* species distributed across three continents European. African and American . Their use of RAPD markers has proved that *Porella* species that have been distributed across these three continents have some similar genetic makeup and RAPD markers have been useful in investigating the intraspecific variations, among the plants that are distributed across three continental areas, which is a comparatively large area can be used to infer a historic context to explain the patterns observed.

LIM et al., 1999has conducted studies on several species of *vanda orchidaceae* .Using RAPD and they summarized that Strap-leaf vanda species (including *Vanda sanderiana*) and *Ascocentrum miniatum* were more closely related than the pedunculated vanda species studied. RAPD analysis supports the proposal to place terete Vanda teres and *Vanda hookeriana* in a separate Papilionanthe genus, leaving *Vanda sanderiana* in his Vanda genus.

Susilo & Setyaningsih, 2018 have stated in their article the use od RAPD markers to analyze the genetic diversity and genome variations of four eggplant species *Solanum melongena* L.The research was undertaken to study the genetic variations of the eggplant species *Leuca, Tekokak, Gelatik and Kopek* using RAPD markers and the results showed that there is a close relationship between Tekokak and Kopek varieties of eggplant

LÁZARO & AGUINAGALDE, 1998) have conducted a study to find the genetic diversity of *Brassica oleraceae* (Cruciferae) and their genetic relationships to their wild varieties using RAPD markers. The analysis showed a high genetic variation between the cultivars and also they were able to establish a genetic relationship between the considered varieties.

Sa'eed et al., 2021 have conducted studies on the genetic relationship between several *Bellevalia lapyer* and *Ornithogalum* L using RAPD markers. The study evaluated the 11 wild varieties of two genera of the Asparagaceae family *Bellevalia lappyer* and *Ornithogalum* L They analysed the difference and similarities between these selected varieties using RAPD markers . From the studies, it was concluded that there are genetic similarities between some species and these species were arranged into different groups and those that showed variation were grouped into another subgroup.

P Arumugam et al in 2019 in a steady state mm the polymerase chain reaction-based molecular techniques random amplified polymorphic DNA is convenient in performance and does not need any information about the DNA sequence to be amplified has been used to an estimate the lytic diversity in various plant species RAPD a small oligonucleotide primer used Chilli then BP in

length arbitrary sequence to generate band profile bind to the complementary sequences along PCR amplification of colour then the region between the opposing Primus size are within amplifier distances amplified polymorphic DNA is a modified PCR technique based on the use of a single 10 base Primus of arbitrary sequence with 60% or more GC content PCR amplification would be expected only when the priming site occurs twice in opposite orientation with approximately 2,000 bases does correspondence among the amplified fragments of different species by same RAPD primer is natural.

Heider et al., 2007 have conducted studies on the RAPD variation of the North Vietnamese *Flemingia macrophylla* (Wild). They conducted these studies because the details of the genetic diversity of the plant are scarce and the conservation efforts of these plants are increasing.RAPD markers were used to analyse the genetic variation of 37 *F.marophyla* species and it was proposed that ex-situ conservation should be undertaken to those accessions that showed high genetic divergence and in-situ conservation should be done by establishing the populations, which in turn rehabilitate the endangered species.

Iqbal et al., 1997 have use RAPD markers to analyse the genetic diversity of elite commercial cotton varieties .22 varieties of *Gossypium hirstum* L and 1 variety of *G.arboreum* were considered for the study. The study was conducted using 5o random primers, among these 49 primers detected polymorphism, whereas one produced a monographic amplification profile. The study revealed that the genetic relationship of the species selected is related to the centre of origin and they had a narrow genetic base. The result obtained suggested that these relations can be used to select possible parents to create a mapping base and also the analyses proposed that the selected elite species of cotton has a relationship with old-world cotton.

Dwivedi et al., 2001 states that the genetic diversity assessment of crop plants are important and significant . their germplasm identification which have distinct DNA identification mark will be helpful ins selecting a suitable breeding programme. RAPD markers were analysed for 26 varieties of groundnut plant and some accession showed a great genetic diversity and these species were identified,for mapping and genetic enhancement of the ground nut .

Sharma et al., 1995 have conducted studies on the generic diversty analyses of wild varieties and cultivated varieties of lentils (*Lens culinaris)*. It was seen that many of the varieties showed a greater genetic diversity among the selected cultivated plants and wild varieties of lentils.RAPD was found to be more accurate and reliable than AFLP markers .It was suggested that RAPD was more suitable to construct genetic linkage map between closely rated lentils species .

Wilkie et al., 1993 conducted genetic diversity study of of *Allium cepa* and they determined whether using RAPD markers were suitable for diversity study of onion.7 varieties of onion was selected to check the polymorphism in the varieties and it was established that the previously established classification was true to its nature and the use of RAPD marker is a reliable approach .However it was also found that a certain species of allium were not in line with the current classification .

Chan & Sun, 1997 assessed the genetic diversity of different varieties of amaranthus using isozyme and RAPD markers .The study revealed high diversity level within the selected species Complementary approaches that use information from both isoenzymes and RAPD provide a more accurate picture of genetic diversity and relationships within and between crop species and their wild relatives than either dataset alone. has been shown to provide reasonable estimates.

INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

Metge & Burgermeister, 2008 studied the Intraspecific variation of isolates of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) revealed by ISSR and RAPD fingerprints They have conducted the study on pinewood nematode *Bursaphelenchus xylophilus* which is a causal agent of pine rot disease, in order to trace its origin they have studied genetic variation of *30B. xylophilus* which has been isolated from the United States, Canada, Japan, China, South Korea and Portugal. With two pcr techniques RAPD AND ISSR, their studies showed that the finger print obtained from the list of plants showed a limited variation in their genetics of isolated species

NAN et al., 2003 have assessed the genetic diversity in *Primula obonica* from central and south west china using ISSR markers .The study was conducted in 60 species of *P.obonica* .Two types

of populations was selected one from natural populations and one cultivated populations .it was found that the natural population varieties showed great genetic diversity than the cultivated varieties .using ISSR markers they were able to conduct a reliable and accurate population study of the *P.obonica* populations .

MELONI, 2006 conducted gentic variability study of 5 *Juniperus phoenicea* using ISSR markers .They reported that isolation of these plants is an important aspect for conserving efforts taken for the above mentioned plant.ISSR markers were selected because of their ability to reveal variation withour prior sequencing and they considered ISSR markers were the basic parameters .

Tikunov et al., 2003 detected variability and diversity in *Lycopersicon* .The ISSR markers were used to understand the level of polymorphism in 5 tomato species . Markers were used to create a phylogenetic tree to understand phylogenetic relationship between the species .Using of ISSR and rga were used to create fingerprints that were not analysed when ISSR was used alone.

Abou-Deif et al., 2013 characterised on 20 wheat varieties using ISSR markers .The main aim of the study was analyse the gentic diversity of wheat varieties which are hexapliod,tetraploid And diploid varieties .20 wheat genotypes was analysed using ISSR markers .The phylogenetic tree created using ISSR markers proved that it succeeded in distinguishing 20 wheat varieties had evolutionary significance and they are in accordance with their classification in placing tetraploid varieties in one group and hexapliod varities in one group

Thimmappaiah et al., 2009 assessed the diversity and genetic relationship of 100 cashew germplasm using rapd and ISSR markers .the cashew varieties NRC 142.NRC 12 were found to be highly divergent and NRC-231 and NRC-232 were genetically similar.they explained that use of molecular markers were more convenient to understand and manage the germplasm of a species .when compared ISSR markers were more reliable but complex process when compared to rapd markers which are simple in usage.

Luo et al., 2011 conducted the genetic diversity among mango cultivars using Scot and ISSR among 23 mango cultivars collected from Xinyang province of china it was assessed that SCoT and ISSR markers were applied to elucidate genetic diversity within Xiang Ya Mango type. The

SCoT markers detected higher polymorphisms than ISSR markers. The SCoT analysis better represents the actual relationships than ISSR analysis.

Terzopoulos & Bebeli, 2008 conducted genetic diversity studies on faba bean *Vicia faba* The Mediterranean faba beans were promising gene pool which provide high yeiding synthetic varieties. The study was conducted to describe the genetic diversity of faba beans using ISSR markers and also to classify the local population of the plant .

dos Santos et al., 2011 conducted genetic diversity studies in *Passiflora* .the main assessment was on sweet,purple and yellow passion fruit using ISSR markers .18 ISSR markers were used for 45 accessions .using variety of index markers it was concluded that the fruit germplasm showed a distinct genetic variation their findings suggest that ISSR can aid breeding and conservation methods, provide useful information for parental selection, and be used to study genetic diversity.

Galván et al., 2003 used ISSR markers to asses the genetic diversity of common bean and its genepool origin .In their experiment they describe how ten common bean cultivars created in Argentina and three materials from France were related using ISSR to measure genetic diversity.

MATERIALS AND METHODS

PLANT MATERIALS

A total of 10 genotypes of *Crinum viviparum* were collected from different regions of India (Table 1). These plants were successfully maintained in the aquatic plant conservatory at KSCSTE- Malabar Botanical Garden and Institute for Plant Science, Calicut, Kerala, India. Plant populations are named POP 1 *Crinum viviparum* to POP 17 *Crinum viviparum* The germplasm was well maintained with proper irrigation and under favourable conditions of growth. Thus, a healthy plant population of all 10 samples was available for study at any time.

Plate 1-*Crinum viviparum* single plant

Plate 2-*Crinum viviparum* :Habitat

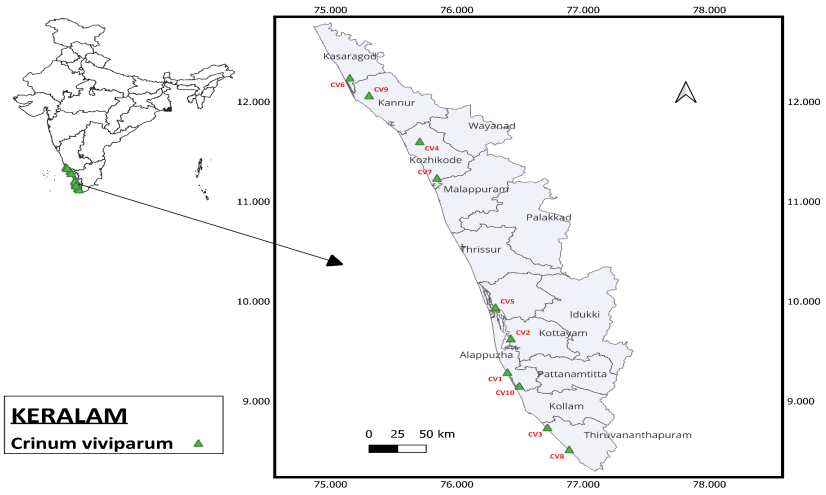

Plate 3- Map of Kerala showing the locations of 10 Accessions

Table1: Details of *Crinum viviparum* accession collected from different regions of India for genetic diversity analysis

Sl.No.	Plant ID	Location.	Latitude	Longitude	Altitude(msl)
1	CV1	Harippad	9.290222725	76.40018177	5
2	CV2	Kayamkulam	9.153465454	76.49471162	1
3	CV3	Kumarakom	9.627685728	76.42762846	1
4	CV4	Thevara	9.940107584	76.305116	70
5	CV5	Avala	11.6028529	75.70580607	1
6	CV6	Olavanna	11.23649251	75.8424199	21
7	CV7	Thumba	8.515128797	76.88950097	51
8	CV8	Varkala	8.736909569	76.71689754	1
9	CV9	Pariyaram	12.06430512	75.30420531	49
10	CV10	Nileswaram	12.24016414	75.15144046	10

PREPARATION OF GENOMIC DNA FROM PLANT TISSUE

Collect healthy and tender leaf samples from each accession. Wash thoroughly and dry using sterile filter paper. The leaf samples of kept in a zip lock cover with silica crystals for 4 days Weigh 1gm leaf sample and cut it into pieces into a pre-cooled mortar. Pre-warm the CTAB extraction buffer and add Polyvinylpyrolidine (PVP) prior to extraction. Grind the tissue into powder using a mortar and pestle and add 500-1000 µl extraction buffer, mix thoroughly to make it into a slurry, and transfer into a 2ml centrifuge tube. Incubate the tubes at 65c for 45 minutes in a water bath with occasional mixing at regular intervals. Cool the mixture to room temperature and add 70 µl Chloroform: Isoamyl alcohol mixture (24:1), mix thoroughly to form an emulsion, and centrifuge at 12000 rpm for 15 minutes at 25 ℃. Collect the upper aqueous phase to a fresh tube and add 1/10 volume of CTAB/ NaCl solution. Gently shake the mixture and add an equal volume of chloroform and centrifuge at 12000 rpm for 10 minutes at 4 ℃.

Collect the upper aqueous phase to a fresh eppendorf tube and an equal amount of chloroform added and centrifuged at 12000 rpm for 10 minutes at 4 ℃.Transfer the supernatant to a fresh Eppendorf tube and add 100% chilled isopropanol through the sides, gently mix by inverting the tubes. Keep the mixture at -20 ℃ for 1 hour. Centrifuge at 7850 rpm for 15 minutes at 4 ℃. Collect the pellets twice with cold 70% ethanol at 1000 rpm for 5 min and air dry the pellets. Suspend the pellets in 200 µl TE buffer. Add 4 µl RNase and incubate at 37 ℃ for 1 to 2 hours in a water bath.

Add 500 µl Phenol: Chloroform: Isoamyl mixture (24:25:1), mix gently and centrifuge at 10000 rpm for 10 minutes at 4 ℃. Collect the supernatant in a fresh Eppendorf tube and add 500 µl chloroform, mix gently and centrifuge at 1000 rpm for 10 minutes. Collect the supernatant in a fresh 1.5 ml Eppendorf tube and add double volume chilled ethanol and 0.1 volume 7.5 M Sodium acetate. Keep the sample overnight at -20 ℃. Collect the pellets by centrifugation at

10000 rpm for 15 minutes at 4 ℃.Wash the pellet in cold 75% ethanol and air dry it. Resuspend the pellet in about 50 µl TE buffer. The resuspended pellets were collected and stored at -20 ℃.

Table 2 : Stock solution required for Genomic DNA extraction

Solutions	Composition	Quantity
Tris buffer pH 8	Tris 1M H_2O	12.11 gm 100 ml
EDTA	Na_2EDTA H_2O	18.61 gm 100 ml
CTAB Extraction buffer pH 8.0 (stored at room temperature)	CTAB 2% W/V Tris buffer 100 mM Na_2EDTA 20 mM PVP 1% NaCl 1.4 M H_2O	2 gm 10 ml 4 ml 1 gm 8.2 gm 100 ml

TE buffer	Tris buffer 10mM Na_2EDTA 1M H_2O	1 ml 0.2 ml 100 ml
Sodium acetate	Sodium acetate 3 M H_2O	24.61 gm 100 ml

QUANTIFICATION AND VISUALIZATION OF DNA

DNA were quantified by measuring optical density (O.D) at A_{260} and A_{280} with a nanodrop spectrophotometer (Multiskan sky). The purity and concentration of the samples were noted using the Nanodrop (Thermo Scientific, USA) software. The DNA samples were diluted to the concentration of 50 ng/µl and stored at +20℃ for use. DNA samples were prepared by mixing 6X loading dye up to a final concentration of 1X. 1µl loading dye and 5µl isolated genomic DNA were loaded on 0.8% agarose gel stained with ethidium bromide and subjected to electrophoresis in 1xTAE buffer for 30 minutes at 100 V to check the quality of isolated DNA. The gels were photographed under a Gel Documentation system (Bio-Rad).

Table 3:**Stock solution required for Agarose gel electrophoresis**

Solution	Composition	Quantity
TAE Buffer (10x) pH 8	Tris base acetic acid Na_2EDTA 0.5Mm H_2O	21.6 gm 11 gm 8 ml 100 ml

Gel loading buffer	Bromo phenol blue 0.25% Xylene cyanole 0.25 % Sucrose 40%(w/v) H_2O	250 gm 250 gm 40 gm 100 ml
Ethidium bromide	Ethidium bromide H_2O	1gm 100 ml

PCR AMPLIFICATION

PCR amplification of 10 accessions of genomic DNA of *Crinum viviparum* was carried out using Random Amplified Polymorphic DNA (RAPD) primer and Inter Simple Sequence Repeats(ISSR), which were synthesized by Bioserve Biotechnologies as per the sequence of Operon technologies, Inc. USA, were used for amplification. PCR amplification reactions were carried out in 25 µl volume containing 1µl of template DNA, 12.5 µl Master Mix (Takara),1µl of 10 pmol primers, and Milli Q water to make it to 25 µl. All the reagents were mixed by giving a short spin. Amplification was performed by using a thermal cycler (Bio-Rad).

RAPD amplification was performed using 10-mer short oligonucleotide primers randomly from the operon series. RAPD amplification sequential steps involved, 1 cycle of 2 min at 95℃, 2 min at 35℃ and 2 min at 75℃ followed by 39 cycles of 1min at 36℃ and 2 min at 72℃. The last cycle was followed by 7 min extension at 72℃. samples were held at 12℃.

Table 4 : List of RAPD primers used in the study

Primer name	**Primer sequence (5′-3′)**	**G C content (%)**
OPD5	TGCCGAGCTG	70

OPB06	TGCTCTGCCC	70
OPC08	GTTTCGCTCC	60
OPB1	GGACTGGAGT	60
OPA5	GAGCCCTCCA	80

Table 5 :List of ISSR primers used in the study

Primer name	Primer sequence (5 ' -3 ')	G C content (%)
ISSR 1	AGAGAGAGAGAGAGAGT	47.05
ISSR2	AGAGAGAGAGAGAGAGC	52.2
ISSR3	AGAGAGAGAGAGAGAGG	52.2
ISSR4	GAGAGAGAGAGAGAGAT	47.05
ISSR5	GAGAGAGAGAGAGAGAC	52.2

Table 6 : Conditions for PCR reaction

PCR steps	Temperature	Time
Initial denaturation	94℃	2 min
Denaturation	95℃	2 min
Annealing	35℃	2min
Extension	72℃	2min

No. of cycles of denaturation, annealing, extension	39 cycles at 93℃,36℃,72℃	1min,1min, and 2min
Final extension	72℃	7 min

DATA ANALYSIS

Qualitative scoring of bands was done from gel photographs obtained from RAPD and ISSR analysis .Clearly reproducible bands from each accession were scored as unit character and date were recorded in a binary matrix (‘0’ for absence and ‘1’ for presence)sheet.both monomorphic and polymorphic bands were included in the data set to proved unbiased estimation of genetic variation .The binary data obtained from the marker systems were analyzed usingNTSYS (version 1.32)software package

The coefficient selected in NTSYS is ‘J’ coefficient,and for clustering we used UPGMA (Unweighted Pair Group Method with Arithmetic Mean) for generating dendrogram for data interpretation and to diversity patterns (Bhardwaj et al., 2010).

PIC (Polymorphism Information Content)

In dominant markers we elaborate a binary matrix of presence and absence of bands as a result of the polymorphism revealed by the marker .If we consider the frequency of bands present as *P ,* and the frequency of absents as *q,* we can establish the following general equation to estimate the Polymorphism Information Content (PIC)

PIC $1-(P^2+Q^2)$

(Lemos *et.*al,2019) .The frequency of an allele was obtained by dividing the number of alleles of ioslates where the band was found by the total number of isolates.PIC is a good index for genetic diversity evaluation .PIC value is often used to measure the informatives of a genetic marker for

linkage studies .PIC index can be used to evaluate the level of gene variation were PIC >0.5 indicates that the locus is of high diversity ,

PIC <0.25 indicates the locus is low diversity

PIC between 0.25 and 0.5 indicates the locus is a intermediate diversity

PIC analyses can be used to evaluate markers so that the most appropriate marker can be selected for genetic mapping and phylogenetic analysis (Anderson et al., 1993.Powell et al 1996)

EMR (Effective Multiplex Ratio)

EMR of a primer is defined as "the product of the fraction of polymorphic loci and the number of polumorphic loci of the individual assay"(Milbourne et al 1997).it is the product of the total number of fragments per primer (n) and the fraction of polymorphic fragments β(Prevost *et,al* 1999).

E=nβ

where n =total number of bands and β =total number of polymorphic DNA

MARKER INDEX

for determining the overall utility of a given marker system ,the marker index MI was calculated for all ten markers .The high MI is the reflection of the efficiency of marker to simultaneously analyse the larger number of bands rather than the level of polymorphismdetected (Powell *et.al* 1996).MI is the product of PIC and EMR (Varshney *et.al* .2005)

MI=PIC *EMR

Where PIC =Polymorphism information content and EMR =Effective Multiplex Ratio

RESULTS

In this study, 10 samples of *Crinum viviparum* were subjected to amplification using RAPD primers and ISSR primers in PCR thermal cycler. The banding pattern thus obtained by RAPD and ISSR primers clearly distinguished varieties into different clusters showing genetic diversity Diversity estimates provide useful information to understand the genetic structure of the plant using RAPD and ISSR primers. In the present study, 5 RAPD primers and 5 ISSR primers were screened for polymorphism survey in pooled DNA accessions of *Crinum viviparum* .

ISSR BAND ANALYSIS

The PCR analysis of these 10 samples taken in the study with 5 ISSR primers (Inter Simple Sequence Repeat) generated 53 scorable bands. An average of 10.6 bands per primer was generated. Among 5 ISSR primers.ISSR 3 produced more bands (14 in all varieties)followed by ISSR 5 (11). and ISSR 1(11), ISSR 4 (10), and ISSR 2 produced a minimum number of bands which was 7.

Out of 53 bands 39 bands were found to be polymorphic. They show 73.2 % polymorphism and the average number of polymorphic bands was 7.8. Different primers produce different levels of polymorphism among the different genotypes.ISSR1 and ISSR 2 produced the same level of polymorphism ie 85.7%, whereas ISSR 4 produce 70% and ISSR 5 produced a 72.7 % level of polymorphism .among the primers ISSR 1 produced the lowest level of polymorphism (54.5). the average level of polymorphism for 5 ISSR primers was 73.2%.

PIC values obtained for the primers ranged from 0.4-0.2.0.4 was found for ISSR 1 and the lowest value of PIC was seen for ISSR5, intermediate values were seen for ISSR2, ISSR3 and ISSR4. The EMR(Effective Multiplex Ratio) was found maximum for ISSR 3 and minimum for ISSR 2, intermediate values were found for ISSR 1 and ISSR 3, and ISSR 4 and MI(Marker Index) was also determined by multiplying EMR and PIC and it was found, maximum for ISSR 3 and lowest for ISSR 5 .

Using the UPGMA methods we were able to construct a dendrogram.. Between 10 samples, we could summarise that there was a relationship between genetic separation and genetic identity. Genetic distance is displayed below the diagonal of the table, and genetic identity is displayed above it.

According to the data computed the maximum genetic diversity was shown by the CV5 population with a value of 0.74 and the least diverse population is CV2 with a value of 0.4

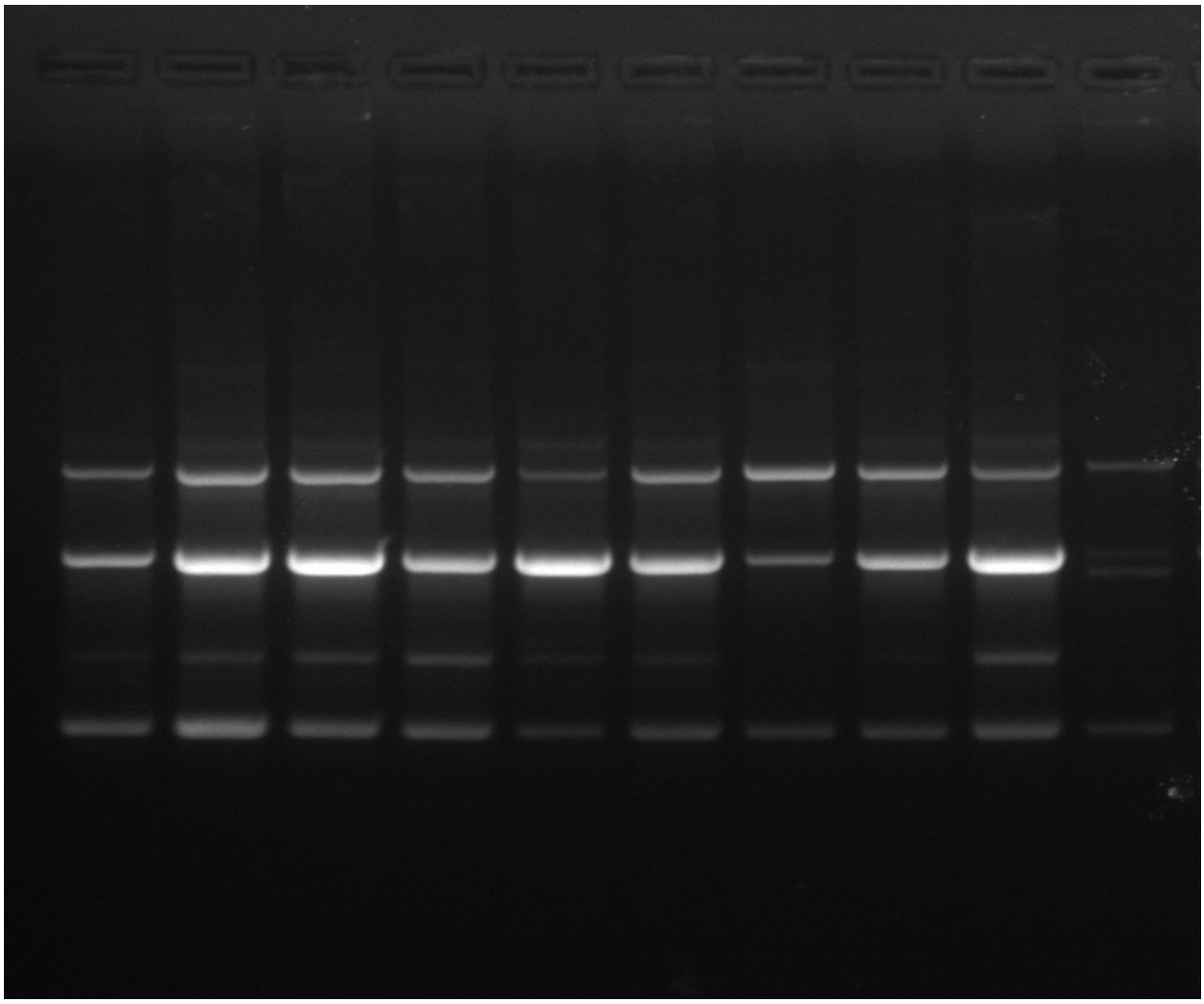

Figure 1: Band pattern of ISSR 1 markers

Table 7 : PIC score shown by ISSR primers

ISSR PRIMERS	NO.OF BANDS	NO.OF MONOMORPHIC BANDS	NO OF POLYMORPHIC BANDS	%OF POLYMORPHISM	PIC	EMR	MI
ISSR 1	11	5	6	54.5	0.40	66	26.4
ISSR 2	7	1	6	85.7	0.32	42	13.4
ISSR 3	14	2	12	85.7	0.36	168	60.4
ISSR 4	10	3	7	70	0.33	70	23.1
ISSR 5	11	3	8	72.7	0.23	88	2.02

TOTAL	53	14	39				
AVERAGE	10.6			73.2			

RAPD BAND ANALYSIS

The PCR analysis for 10 samples taken in the study with 5 RAPD primers (random Amplified Polymorphic DNA) primers generated 58 scorable bands . An average of 11.6 bands per primer was generated .among 5 RAPD primers OPD 5 produced a maximum number of bands(15) whereas OPC 08 produced more no of bands (8). The primers OPB06 produce 12 bands, and OPB 1 produced 9 bands respectively .

Out of 58 scoring bands 38 were found to be polymorphic and the average number of polymorphic bands was found to be 7.6.OPD 5 and OPA 5 were found to be having a higher number of polymorphic bands whereas OPC 08 and OPB 1 produced to be found have 4 polymorphic bands. the level of polymorphism was found high for OPA 5(85.7) and minimum for OPB 1 (44). The level of polymorphism was found identical for OPB06 and OPC 08 .the average level of polymorphism was found to be 61.94. The PIC value was found to be high range for OPD 5 and lowest for OPB08. The EMR was calculated by multiplying the of polymorphic bands and no bands. the PIC value was found to have higher for OPD 05 and lowest for OPC 08 .the EMR value of OPB06 and OPB 1 was found to be 72 and 36 respectively.The marker index was calculated by multiplying EMR and PIC. it is found to be maximum for OPD 5 and minimum for OPC 08 . the marker index of OPB06 and OPA 5 were 15.1 and 52.08 .

As same as that of ISSR markers , we have constructed dendrograms with the help pf UPGMA software . Between 10 samples the highest diverse population is CV 3 with a value of 0.5 and the least diverse population is CV 1 with a value of 0.04.

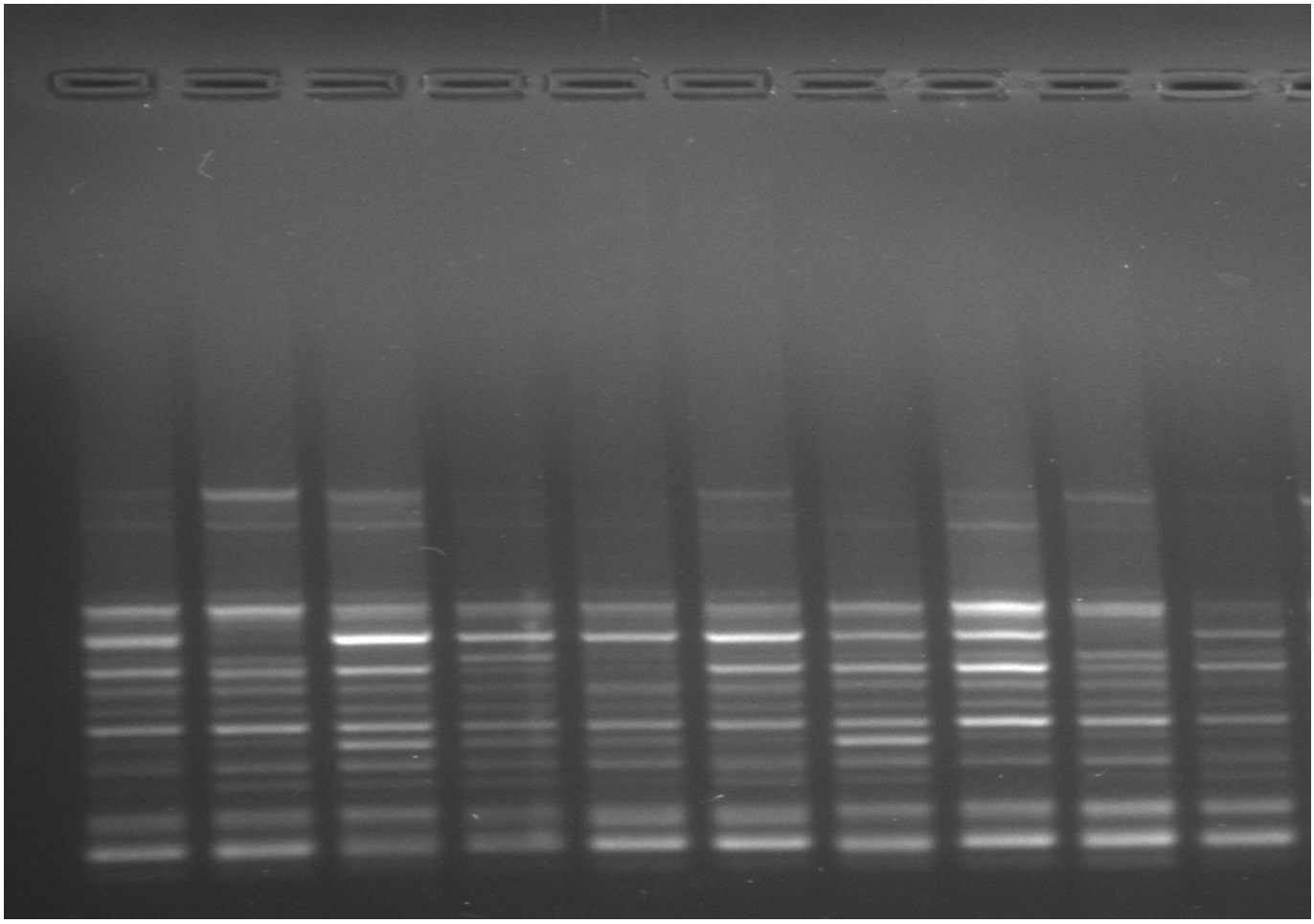

Figure 2: Band pattern shown by RAPD(OPB1) Markers

Table 8: PIC score shown by RAPD primers

RAPD PRIMERS	NO OF BANDS	NO OF MONOMORPHIC BANDS	NO OF POLYMORPHIC BANDS	%OF POLYMORPHISM	PIC	EMR	MI
OPD 5	15	3	12	80	0.33	180	59.4
OPB06	12	6	6	50	0.21	72	15.1
OPC 08	8	4	4	50	0.20	32	6.4
OPB 1	9	5	4	44	0.23	36	8.2
OPA5	14	2	12	85.7	0.31	168	52.08
TOTAL	58	20	38				

AVERAGE	11.6			61.94			

Table 9: ISSR similarity table

	CV1	CV2	CV3	CV4	CV5	CV6	CV7	CV8	CV9	CV10
CV1 |	1.00									
CV2 |	0.42	1.00								
CV3 |	0.38	0.44	1.00							
CV4 |	0.33	0.33	0.43	1.00						
CV5 |	0.44	**0.96**	0.45	0.30	1.00					
CV6 |	0.27	0.28	0.37	0.72	0.26	1.00				
CV7 |	0.33	0.33	0.43	1.00	0.30	0.72	1.00			
CV8 |	0.42	0.47	0.95	0.46	0.48	0.39	0.46	1.00		
CV9 |	0.27	0.28	0.37	0.72	**0.26**	1.00	0.72	0.39	1.00	
CV10	0.92	0.41	0.36	0.36	0.42	0.30	0.36	0.40	0.30	1.00

Table 10:RAPD similarity Table

	CV1	CV2	CV3	CV4	CV5	CV6	CV7	CV8	CV9	CV10
CV1	1.0000									
CV2	0.4808	1.0000								
CV3	0.6042	0.5217	1.0000							
CV4	0.6667	0.5208	0.5870	1.0000						
CV5	**0.4808**	1.0000	0.5217	0.5208	1.0000					
CV6	0.6863	0.5490	0.6458	0.6400	0.5490	1.0000				
CV7	0.6667	0.5208	0.5870	1.0000	0.5208	0.6400	1.0000			
CV8	0.6042	0.5217	1.0000	0.5870	0.5217	0.6458	0.5870	1.0000		
CV9	0.6863	0.5490	0.6458	0.6400	0.5490	1.0000	0.6400	0.6458	1.0000	
CV10	**0.9767**	0.5000	0.5918	0.6875	0.5000	0.7059	0.6875	0.5918	0.7059	1.0000

CLUSTER ANALYSIS

ISSR DENDROGRAM

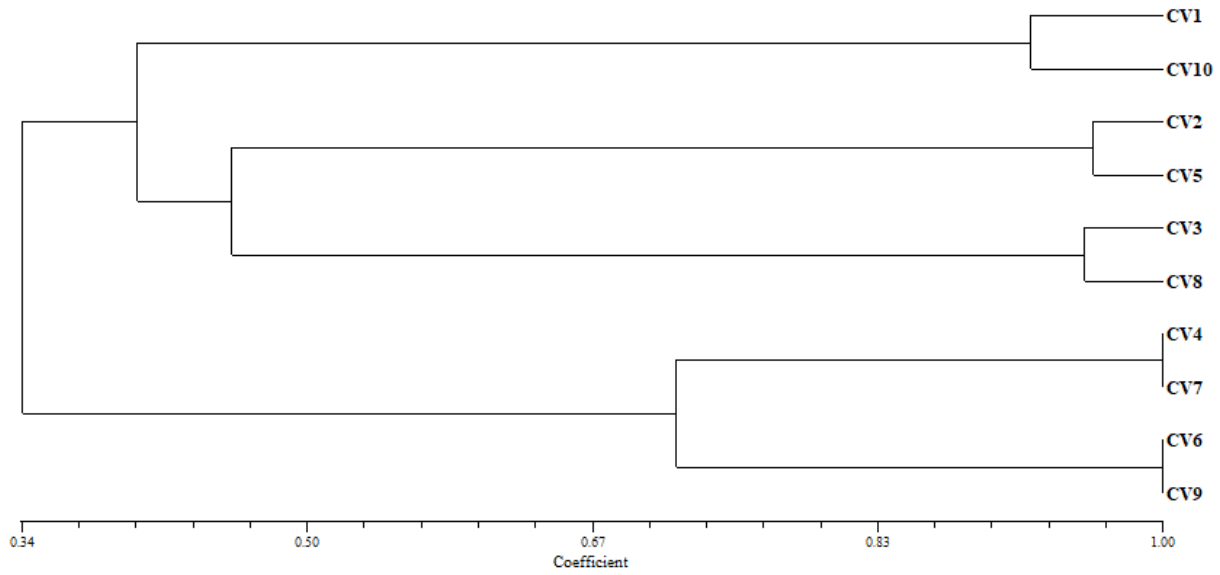

Figure 3: ISSR dendrogram of *Crinum viviparum* created by UPGMA software

The coefficient of dendrogram varies from the range 0.34- 1.00. The cluster analysis of the UPGMA software have revealed the relationship and diversity of the samples that are analysed by 5 ISSR markers. Of the 10 samples collected which were analysed by 5 ISSR markers revealed a well-constructed dendrogram, the cluster analyses of the UPGMA software visualised two major CLUSTERS-CLUSTER 1 and CLUSTER2 .

CLUSTER-1 was characterised by 6 samples - CV1, CV10, CV2, CV5, CV3,& CV 8. The clade was further divided into 2 sub-clusters SUBCLUSTER 1 and SUBCLUSTER 2 . These sub-clusters were further divided into groups which are basically similar in diversity.SUBCLUSTER 1 was divided into 3 groups G1.G2.& G3 and SUBCLUSTER 2 consist of 4 population CV4,CV7,CV6,CV9..The SUBCLUSETER 2 there 2 groups G4 and G5.From SUBCLUSTER 1 we can interpret that samples CV1 and CV 10 are more closely related and they have evolved much before all the other populations .. CV 2 and CV 5 tend to form a group of similarity and they are more closely related and theybhave similarity value of 0.96 their evolution is much after GROUP 1 and GROUP 3. In GROUP 3 CV3 and CV8 are more related to each other. In SUBCLUSTER 2 the GROUP 4 population CV4 and CV7 tend to form groups of similarity and they are more closely related to each other .and the populations CV6 AND CV9 tend to form a single group hence they show a close relationship and they have a similarity value

of 0.26.When comparing the similarity value we can infer that the groups which have the populations CV2 and CV5 is less diverse since they have high similarity value whereas CV6 and CV9 have low similarity value and hence they are more diverse.

RAPD CLUSTER ANALYSIS

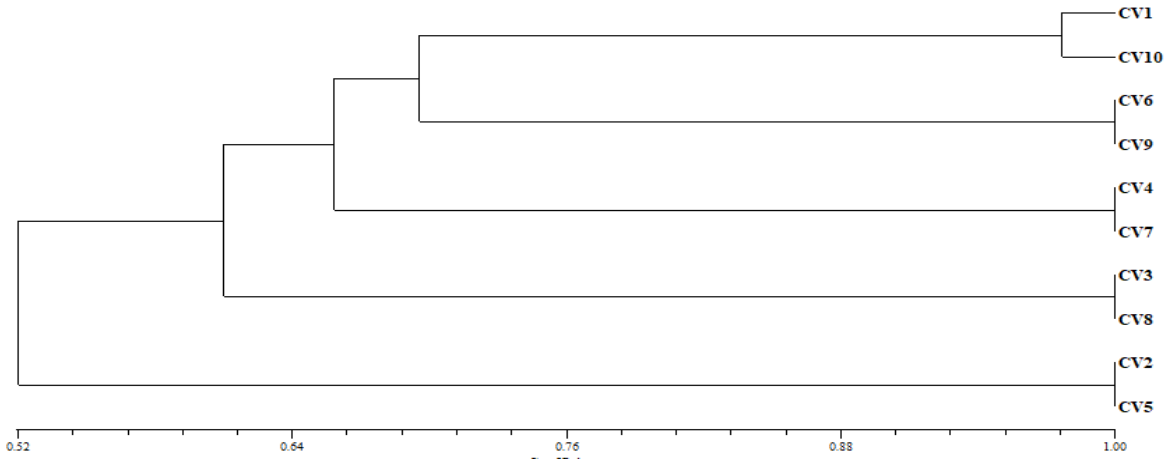

Figure 4: RAPD dendrogram of *Crinum viviparum* created by UPGMA software

The coefficient of dendrogram range from 0.52-1.00 The dendrogram has been formed by analysing 10 samples of *Crinum viivparum*, using 5 RAPD MARKERS. The dendrogram was created using UPGMA software . The dendrogram is divided into 2 main CLUSTERS -CLUSTER 1 and CLUSTER 2.In CLUSTER 1 there are 8 population-CV1,CV10,CV6,CV9,CV4,CV7,CV3,CV8. CLUSTER1 is divided into 2 SUBCLUSTERS,-SUBCLUSTER1 which contain 2 CLADES .-CLADE 1 &CLADE 2.In CLADE 1 have two GROUPS.G1 and G2 .G1 has CV1 and CV10 and they have a similarity value of 0.97 ..CLADE 2 has CV 6 and CV9 which are closely related .SUBCLUSTER 2 2 have two population CV4 and CV7 which are closely related . Subcluster 2 has two populations CV3 nad CV8 which directly originated from CLUSTER 1 . CLUSTER 2 has two populations CV2 and CV5 which are closely related in their diversity .The similarity value of CV1 And CV5 is 0.48 which is lowest value in the similarity table which indicates that CV1 and CV5 are highly diverse .

DISCUSSION

Crinum viviparum is known as “veluthapolathali” in Malayalam and “visha moongil” in Tamil, & “River crinum Lilly” in English is an aquatic plant belonging to the family Amaryllidaceae. It is widely distributed in India and Srilanka and is useful to treat many diseases .It is found to have antioxidant, and antidiabetic properties. It contains an alkaloid Galanthimine which is found to have anti-Alzheimer's properties .

The river crinum lily is a common aquatic plant on stony, shallow riverbeds. It belongs to the family Amaryllidaceae The bulb is 5-8 cm tall, ovoid, and has a cylindrical neck that is 5-15 cm long. It may readily bloom underwater and grows to heights of 0.5 to 1.

Each of the 6–8 leaves is 2-3 feet long, 3 cm wide, and has a dark green tinge. There is 6–8 leaves total. An umbel of 6–12 flowers rises to a height of 40–75 cm on a leafless scape. Two white bracts that resemble lances open at night provide a pleasant scent. The flower tube is 6-8 cm long and very thin. Each of the six petals measures 6 to 8 centimetres long. The six, 4-6 cm long stamens are covered in red filaments.

Population genetic studies are essential for conservation programmes and restoring the threatened population. The development in molecular genetics has laid the groundwork for genomics**.** Population ecology and plant demography are integrated research fields that provide insights into the performance of plant populations in the environment. In the context of conservation biology, it is important to understand how plant populations function. This knowledge is relevant to the potential for developing comprehensive and effective conservation plans for endangered plant species. On the other hand, because evolutionary change begins at the population level, studies of population ecology shed light on the forces that influence the survival and fertility of individual plants, and assess the fitness of all different variants of a population.

The introduction of novel molecular markers has increased the reliability and improvement of plant genetics research. These markers are highly accurate and provide more information to help us better understand plant germplasm. PCR-generated markers have been used since the 1990s, providing access to information about intraspecific genetic variation at the nuclear level. Several DNA markers are now used in genetic diversity studies of many plants. The most commonly used markers are RAPD and ISSR.

The main advantage of RAPD is that it can be tested quickly and easily. Since it is a PCR, only a small amount of template DNA is required. Random primers are commercially available and do not require sequence data for primer design. Moreover, RAPD has a very high genomic frequency and is randomly distributed throughout the genome.

A significant drawback of RAPD is its low reproducibility, which is sensitive to reaction conditions and thus requires highly standardised experimental procedures. RAPD analysis generally requires purified high-molecular-weight DNA and uses short random primers that can amplify DNA fragments from a variety of organisms, so care must be taken to avoid contamination of the DNA sample. Overall, inherent problems with reproducibility make RAPD an unsuitable marker for transferring or comparing results between research teams working on similar species or topics. As with most other multilocus techniques, RAPD markers are not locus-specific, banding profiles cannot be interpreted in terms of loci and alleles (marker dominance), and similarly sized fragments are not homologous.

ISSR's are DNA fragments of approximately 100-3000 bp located between adjacent microsatellite regions of opposite orientations. The ISSR is amplified by PCR using the microsatellite core sequence as primers and several selective nucleotides as anchors for a non-repetitive contiguous region (16-18 bp). Approximately 10-60 fragments are generated simultaneously from multiple loci, separated by gel electrophoresis, and the presence or absence of fragments of a given size is noted. Related techniques for ISSR analysis include single primer amplification reactions (SPAR) using a single primer containing only the microsatellite core motif and minisatellite region DNA amplification using a single primer containing only the core motif. directional amplification (DAMD). a contains minisatellites.

A major advantage of ISSR is that no sequence data is required for primer design. Since the analytical method involves PCR, only small amounts of template DNA are required. Moreover, ISSRs are randomly distributed throughout the genome.

Since ISSR is a multilocus method, it has the disadvantage that similarly sized fragments may lack homology. Furthermore, like RAPD, ISSR can also have reproducibility issues.

In the present, upon studying 10 populations of *C.viviparum* collected and primarily DNA isolation was done using the suitable protocol. There are different protocols used by different

workers. Good quality DNA is a prerequisite for all experiments of DNA manipulation. All plant DNA extraction protocols comprise the basic disruptions of the cell wall, cell membrane, and nuclear membrane to release the DNA into solution followed by precipitation of DNA while ensuring the removal of contaminant biomolecules such as the protein polysaccharides, and lipids. phenols, and other secondary metabolites. this is brought by disruptions of the tissue in mortar and pestle aided by liquid nitrogen and the various components of homogenizations or extraction buffer followed by the precipitating and purification method employed .since DNA can be extracted from various types of tissues such as seedlings, leaves, cotyledons, seeds, endosperm, tissue culture callus, roots etc. the tissue type along with the concentration of DNA finally determine the methodology of DNA extraction to followed by the experimenter. the most commonly used basic plant DNA extraction protocols are those of Murray M.G.and Thompson.W.(1980) along with the many others that are modifications of the components of these protocols to suit a particular tissue type or downscaling them for miniprep.

The protocol adopted in this work is ideal for isolating a considerable amount of DNA which was quantified in a spectrophotometer. the isolated DNA was treated with 5 ISSR primers ie ISSR1, ISSR2, ISSR3, ISSR4, ISSR5 and 5 RAPD primers ie OPD-05, OPB-06, OPC-08, OPB1, OPA-5 In PCR thermocycler with appropriate conditions amplified fragments of DNA produced by the PCR were subjected to gel electrophoresis to separate the bands according to their size and charge. a clear banding pattern was obtained by viewing the gel under the gel imager.. The data of scored bands from amplified products of PCR revealed the polymorphism exhibited by these populations.

The binary scored data used NTSYS software and it generated the genetic distance and genetic similarity. A dendrogram was generated from UPGMA (**Unweighted Pair Group Method with Arithmetic Mean)** cluster analysis of RAPD and ISSR primers in which plant populations were grouped into certain clusters .

When the results were compared the efficiency of both markers based on their average percentage of polymorphism , it revealed that ISSR markers (73.2) are more efficient that RAPD (61.94) markers .

SUMMARY AND CONCLUSION

The present study was aimed at developing RAPD and ISSR-based molecular markers for studying genetic diversity among the different accessions of *Crinum viviparum* plant populations collected from various geographical regions of India. *Crinum viviparum*_is a herbaceous plant belonging to the family Amaryllidaceae.Iti s extensively widespread in Asian continent especially in the countries like India & Srilanka. It usually grow submerged and in damp places ,and some variety are used in garden as ornamental plants

The importance of plant genetic diversity (PGD) is now being recognized as a specific area since exploding population with urbanization. Common marshy lands and other natural habitats of many plants are at the edge of destruction. Diversity helps in increasing the resistance of ecosystem productivity to climatic extremes. If ecosystems have value because they provide services to humans and ecosystems function better when they contain more species, then the loss of species could diminish the value of ecosystems. It is cruciate to know the existing variability among *Crinum* plant species in different habits for the effective utilization and conservation of these plants.

The present work deal with the isolation of genomic DNA from *Crinum viviparum* obtained from 10 population and then the amplification of this DNA in PCR using Random Polymorphic DNA Markers. 5 primers of RAPD and ISSR were used in the study and they are 0PD 5, OPA5, OPB06, OPC08, OPB1, ISSR1, ISSR2, ISSR3, ISSR4, and ISSR 5. The amplified products are then visualized using gel electrophoresis and a qualitative scoring band was done using gel photographs. The binary data obtained here from the marker system were analyzed using software called NTSYSpc version 2.2. NTSYSpc can be used to discover patterns and structures in multivariate data. The UPGMA method is used for constructing dendrograms. The genetic parameters viz, the polymorphism among the population (PIC), Effective multiplex ratio (EMR), and Marker Index(MI) were calculated using the same program. RAPD and ISSR markers could reveal the genetic characteristics of each population.

And the comparison of the genome diversity among *Crinum viviparum* of different geographical locations was carried out in the study.

Both RAPD and ISSR markers might detect non coding and therefore more polymorphic DNA by exploiting the different regions of the genome . The differences found among the dendrograms generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analyzed . Another explanation could be the low reproductibility of RAPD.

DNA technologies are reliable and powerful tools for the identification of taxa at various taxonomic levels as they provide consistent results irrespective of age, tissue, origin, physiological conditions, environmental factors, harvest, storage, and processing of samples. With the increasing demand for high-quality herbs, the need for DNA authentication will accelerate for ensuring therapeutic effectiveness, fair trade of drugs, and raise consumer confidence. However, for the modernization of traditional medicines, it is inevitable in the future to compile a comprehensive database for all investigated medicinal taxa with reference information on nomenclature, phylogenetic relationships, macroscopic and microscopic features, chemical constituents, and profiling, toxicity and voucher specimens in herbaria or museums. Through this work, it is proved that RAPD and ISSR markers are a good choice among other molecular markers for the assessment of the genetic diversity of *Crinum viviparum* .

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