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Genetic Diversity Assessment of Selected *Annona muricata* L. Germplasm in Sri Lanka

S. H. M. R. N. P. Samaradiwakara[1*], W. L. G. Samarasinghe[2], P. G. S. Shantha[3], K. G. C. N. Jayarathna[2], P. Dehigaspitiya[4] and N. M. Ubeysekera[2]

[1]Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka.
[2]Plant Genetic Resources Centre, Department of Agriculture, Peradeniya, 30400, Sri Lanka.
[3]Agriculture Research Station, Department of Agriculture, Girandurukotte, 90750, Sri Lanka.
[4]Faculty of Agriculture, University of Ruhuna, 81100, Sri Lanka.

Authors' contributions

This work was carried out in collaboration among all authors. Author SHMRNPS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WLGS, PGSS and PD managed the analyses of the study. Authors KGCNJ and NMU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Annona muricata L. commonly known as soursop is an underutilized fruit crop species in Sri Lanka gaining much importance in the recent past due to its high nutritional and medicinal value. Soursop germplasm collections are available within the country and assessing the genetic diversity is needed to proceed with conservation, detecting promising lines and breeding programs. This study was conducted to assess the genetic diversity of 50 soursop individuals using Inter-Simple Sequence Repeat (ISSR) markers. The study was conducted at Plant Genetic Resources Centre of the Department of Agriculture in Gannoruwa during 2017 to 2019. DNA of the 50 soursop samples were extracted using CTAB method and Polymerase Chain Reaction (PCR) was carried using 13 Inter Simple Sequence Repeat (ISSR) Markers. PCR products were visualized using 1.5 percent Agarose gel electrophoresis under the Biorad Gel documentation system and analyzed using POPGENE 1.31. PCR amplified 139 bands from 13 ISSR markers among which 118 were found to be polymorphic. The polymorphic band percentage was 85 percent while as the average

**Corresponding author: Email: nethmips@gmail.com;*

number of bands observed (Na) was 1.8489 and the effective allele number (Ne) was 1.5377. The Nei's gene diversity index (h) was 0.3079. The Shannon Information Index (I) found to be 0.4556. Dendrogram constructed based on the UPGMA method clustered the studied accessions into four major clusters at 80 percent similarity level. Results revealed considerable degree of genetic diversity existed within the studied soursop germplasms at Sri Lanka. Existing genetic diversity within soursop individuals will serve as germplasm bank to identify and utilize potential germplasm resources for conservation and future breeding programs to develop quality soursop varieties in Sri Lanka.

Keywords: Molecular characterization; soursop; ISSR markers; underutilized fruits; germplasm conservation.

1. INTRODUCTION

Annona muricata L. is a specie belongs to family Annonaceae, [1] commonly known as soursop. It is a tropical fruit specie native to Central America and Caribbean presently distributed in tropics and sub tropics [2]. In Sri Lanka, the fruit is unexploited as it is mostly confined to home gardens and commonly known as “Katu Anoda" or "Katu Artha" [3]. Lack of awareness among people and unavailability of commercial cultivations has made the fruit to be categorized as an underutilized fruit species in Sri Lanka [3]. Soursop gained much importance in the recent past due to its rich nutritional status containing vitamins, minerals & bioactive chemical substances [2,4]. These compounds including acetogenins have anti-tumoral insecticidal, antibacterial, immune-suppressant, pesticidal or anthelminthic properties [5,6] with a significance towards its medicinal value. The Soursop fruits are consumed widely as fresh form and also used in other industries such as wine, jellies, beverages, jam, etc [7].

Assessing the genetic diversity of a plant species aids and contributes in exploring biological evolution and biodiversity, adaptation potential, conservation and its use as genetic resources [8]. Knowledge generated on genes, individuals, species and communities will support towards better understanding of biodiversity and conservation of environmental resources using adequate strategies [8]. Likewise, furthering characterized information will maximize the effective utilization of germplasms by the plant scientists.

Morphological characterization will not be most effective in germplasm characterization as they are significantly affected by environmental influences and changing climatic conditions [9]. Molecular characterization to assess the genetic diversity using molecular markers contributes more towards conservation and utilization of biological resources [8], aids in management and conservation of biodiversity, being an efficient alternative to the conventional diversity analyses [10]. Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP) are DNA based markers which have been widely used in assessing genetic diversity and genetic variations detected by Random Amplified polymorphic DNA (RAPD) were also quite efficient [11,12,13]. Inter Simple Sequence Repeat (ISSR) markers were recently introduced as a rapid inexpensive, repeatable marker generating higher polymorphism compared to other marker techniques [14]. ISSR markers are PCR based and are identified as a marker which can rapidly differentiate between closely related individuals [15]. Marker is much informative for species where genome sequences are unavailable [16].

ISSR analysis is used in studies involved in genetic identity, parentage, clone and strain identification and taxonomic studies of closely related species due to its ability to produce multi locus finger printing profiles [17]. ISSR markers have also reported to be suitable for characterization of fruit species in combination with other markers [18]. Many reports are available on assessing the genetic diversity using DNA markers among species of several horticultural crops thus, it appeared that ISSR markers are useful in elucidating the genetic diversity of species towards their efficient breeding and genetic resource management. Due to the above-stated advantages, ISSR markers are selected to assess the genetic diversity of *Annona muricata* (L) germplasm in present study.

Germplasm collections of soursop are available in major research stations of the Department of Agriculture, Sri Lanka. Assessment of the genetic diversity of these genotypes have not been

conducted properly. Currently there are no developed cultivar or variety for soursop in Sri Lanka and assessing the genetic diversity of the existing germplasms will immensely support towards selection, breeding and Marker Assisted Selection (MAS) in development of high quality soursop cultivars and varieties as well as in future germplasm conservation strategies. Therefore, this research study employed 13 ISSR markers to characterize selected soursop germplams and determine the degree of genetic diversity. This is the first report on comprehensive molecular characterization of soursop using ISSR markers to assess the genetic diversity of *Annona muricata* (L) in Sri Lanka.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaf samples were collected from 50 selected soursop individuals distributed in central, Northern, Sabaragamuwa, and Uva provinces in Sri Lanka. These samples were selected to represent a wide geographical range where the soursop plants are abundantly available. Among these areas where samples have been collected, Girandurukotte, Horana, Gannoruwa and Ambatenna represents the maintained germplasm collections of soursop under the Department of Agriculture, Sri Lanka. Young tender leaves from each soursop genotype were collected and processed for DNA extraction. Details of the collected soursop germplasm have been indicated in Table 1 and Fig. 1.

2.2 DNA Extraction & Detection

Genomic DNA was extracted from 2 g of fresh leaves by Cetyl-Tri-methyl Ammonium Bromide (CTAB) method [19] with a few modifications (modified CTAB method). Leaves were grounded in liquid nitrogen to form a fine powder and transferred into a microtube containing 4 ml of preheated (65°C) 2 percent CTAB Buffer (5M NaCl, 0.5M EDTA, 1M Tris HCl, Distilled water) at a water bath. 20 µl 0.2% beta-mercaptoethanol was added together with a pinch of Poly Vinyl Pyrrolidine (PVP). The samples were incubated at 65°C for 45 minutes while shaking the tubes. Samples were kept for cooling and 4 ml of chloroform: Iso Amyl Alcohol (24:1) was added. Tubes were shaken slowly for 10 minutes and centrifuged at 13,000 rotation per minute (rpm) for 15 minutes. The supernatant was separated into a new tube and pellet was discarded. Chloroform: Iso Amyl Alcohol step and centrifugation step was repeated once again. The upper aqueous layer was separated and 2/3 of Ice Cold 100 percent Ethanol was added and mixed slowly. Samples kept at -20°C for 2 hours or overnight. Samples were centrifuged again at 10000 rpm at 4°C for 10 minutes. The pellet obtained was washed two times with 70 percent Ethanol and the pellet was air-dried. Finally, the DNA pellets were dissolved in TE Buffer (50Mm Tris HCl, 10 Mm EDTA). The DNA quality was detected by 0.8 percent Agarose gel electrophoresis run at 60 Volt for 30 minutes. Raw DNA image was detected after ethidium bromide staining and visualization through the Bio-rad gel documentation system. Raw DNA concentration was determined against Lambda 50 standard. DNA was diluted to 45 ng/ µl with TE (Tris, EDTA) and stored at -20°C until brought into use.

2.3 ISSR- PCR amplification

Thirteen ISSR markers (University of British Columbia, Canada) were used for PCR amplification and genetic diversity assessment of soursop (Table 2).

PCR amplification was carried out in a 10.00 µl reaction volume containing 0.25 mM dNTP Mix, 2 mM $MgCl_2$, 0.75 Units Taq DNA polymerase, 1x PCR Buffer, 1.00 µM Primer 4.30 µl of distilled water with 45 ng of genomic DNA. The amplification was performed in a Veriti thermal cycler (96 wells) of Applied Biosystems with reaction conditions programmed as initial denaturation at 94°C for 5 minutes followed by second denaturation at 94°C for 45 seconds, annealing at 52°C for 1 minute, extension at 72°C 2 minutes and a final Extension was done for 10 minutes at 72°C. Amplicons were separated by performing electrophoresis using TBE (Tris-Boric-EDTA) buffer and using a 1X buffer to have a final concentration of 1.5% in the Agarose gel. As a reference, 100 base pair (bp) DNA ladder (Promega, USA) was included in all gels to estimate the size of the bands. The gel was stained with ethidium bromide and visualized under UV light using the BIORAD gel documentation system (Bio Rad Laboratories, Hercules, CA). Image editing was done using Quantity One software.

Table 1. Selected germplasm accession and their sites of collection along with their codes for genetic diversity analysis through ISSR Markers in soursop (*Annona muricata* L.)

Name of the germplasm collection site	District	Name of the germplasm accession	Code
FRDI, Horana	Kaluthara	H-01	01
FRDI, Horana	Kaluthara	H-02	02
FRDI, Horana	Kaluthara	H-19	03
FRDI, Horana	Kaluthara	H-30	04
FRDI, Horana	Kaluthara	H-34	05
FRDI, Horana	Kaluthara	H-44	06
FRDI, Horana	Kaluthara	H-36	07
FRDI, Horana	Kaluthara	H-24	08
FRDI, Horana	Kaluthara	H-13	09
FRDI, Horana	Kaluthara	H-M2	10
IFCO Farm, Badulla	Badulla	RW 2899	11
IFCO Farm, Badulla	Badulla	RW 2898	12
IFCO Farm, Badulla	Badulla	RW 2918	13
IFCO Farm, Badulla	Badulla	RW 2921	14
IFCO Farm, Badulla	Badulla	RW 654	15
IFCO Farm, Badulla	Badulla	SSV 659	16
IFCO Farm, Badulla	Badulla	SSV 640	17
IFCO Farm, Badulla	Badulla	SSV 649	18
AF, HORDI, Ambatenna	Kandy	Am1	19
AF, HORDI, Ambatenna	Kandy	Am2	20
FCRDS, Gannoruwa	Kandy	G-01	21
FCRDS, Gannoruwa	Kandy	G-325	22
FCRDS, Gannoruwa	Kandy	G-A	23
FCRDS, Gannoruwa	Kandy	G-137	24
FCRDS, Gannoruwa	Kandy	G-AS	25
FCRDS, Gannoruwa	Kandy	G-41	26
ARS, Girandurukotte	Badulla	GK-175	27
ARS, Girandurukotte	Badulla	GK-159	28
ARS, Girandurukotte	Badulla	GK-170	29
ARS, Girandurukotte	Badulla	GK-197	30
ARS Girandurukotte	Badulla	GK-90	31
ARS Girandurukotte	Badulla	GK-167	32
ARS, Girandurukotte	Badulla	GK-168	33
ARS, Girandurukotte	Badulla	GK-29	34
ARS, Girandurukotte	Badulla	GK-25	35
ARS, Girandurukotte	Badulla	GK-195	36
ARS, Girandurukotte	Badulla	GK-68	37
ARS, Girandurukotte	Badulla	GK-172	38
ARS, Girandurukotte	Badulla	GK-30	39
ARS, Girandurukotte	Badulla	GK-171	40
Jaffna	Jaffna	J-1	41
Jaffna	Jaffna	J-2	42
Peradeniya	Kandy	PD	43
Kegalle	Kegalle	KG	44
Awissawella	Colombo	AW	45
Gampola	Kandy	GAM	46
Medawala	Kandy	MED	47
Kundasale	Kandy	KUN	48
Kadugannawa	Kandy	KAD	49
Pilimathalawa	Kandy	PIL	50

FRDI- Fruit Research & Development Institute; AF,HORDI-Ambatenna Farm, Horticultural Crop Research & Development Institute; FCRDS-Fruit Crop Research & Development Station; ARS- Agriculture Research Station

Fig. 1. The map showing the districts of selected germplasm collection sites of accessions for genetic diversity analysis through ISSR Markers in soursop (*Annona muricata* L.)

Table 2. ISSR markers used for screening of Soursop germplasms during 2017-2019

Marker name	Nucleotide Sequence	Melting temperature	Annealing temperature
UBC 812	GAG AGA GAG AGA GAG AA	47.1°C	52°C
UBC 841T	GAG AGA GAG AGA GAG ATC	48.5°C	52°C
UBC 841Y	GAG AGA GAG AGA GAG ACC	45.7°C	52°C
UBC 873	GAC AGA CAG ACA GAC A	47.4°C	52°C
UBC 888	(C, G or T) (A, G or T) (C, G or T) CAC ACA CAC ACA CA	52.4°C	52°C
UBC 807	AGA GAG AGA GAG AGA GT	48.9°C	52°C
UBC 809	AGA GAG AGA GAG AGA GG	51.3°C	58°C
UBC 840a	GAG AGA GAG AGA GAG ACT	51.3°C	58°C
UBC 810	GAG AGA GAG AGA GAG AT	48.9°C	50°C
UBC 848a	CAC ACA CAC ACA CAC AAG	51.3°C	52°C
UBC 850a	GTG TGT GTG TGT GTG TCC	51.3°C	56°C
TC 10G	TCT CTC TCT CTC TCT CTCTCG	51.3°C	58°C
UBC 855	ACA CAC ACA CAC ACA CT	49.8°C	50°C

2.4 Data Analysis

Amplified PCR products which resulted clear and reproducible bands were selected to generate a score file based on their presence (1) and absence (0). The size of the amplicons were estimated by a 100 bp DNA ladder. The genetic relationship between selected accessions and percentage of polymorphism was analyzed using the Popgene version 1.31 software [20]. Genetic

diversity was evaluated by calculating the overall gene frequency, *Nei's* Genetic identity & distance, *Nei's* (1978) Gene diversity (h), Shannon's Information Index (I), percentage of polymorphic bands & Dendrogram based on *Nei's* (1972) genetic distance evaluated by Unweighed Pair Group Method using Arithmetic averages (UPGMA) [21]. Nei-Li similarity coefficient [22] was used to describe the relationships among the accessions and to generate UPGMA dendrogram. The Polymorphic Information Content (PIC) was calculated with the following formula: PIC $_{j=}$ 1- $\Sigma^{n} P_i^2$ where *i* is the i-th allele of the *j*-th marker, *n* is the number of the *j*-th marker's alleles, *P* is allele frequency [23].

3. RESULTS

Thirteen ISSR markers were used to assess the genetic diversity of fifty soursop genotypes. Band amplification results yielded one hundred thirty nine bands out of which one hundred eighteen bands found to be polymorphic resulting 85 percent polymorphism. The size of the amplified products ranged between 100 to 1500 base pair (bp) compared to a 100 base pair ladder (Fig. 2). The number of bands amplified by thirteen ISSR markers ranged from eight (UBC 840a) to thirteen (UBC 809) bands and the number of polymorphic bands ranged from 4 (UBC 855) to 12 (UBC 809) where UBC 809 primer generated highest number of bands (Table 3). The highest percentage of polymorphic loci was generated from primer UBC 841T, UBC 807 & UBC 840a resulting 100 percent of polymorphism. The polymorphism percentage for primers ranged from 44.44 percent to 100 percent with an average of 85.12 percent (Table 3).

PIC values ranged from 0.201 to 0.401 with an average of 0.305. Primer UBC 840a was the most informative generating the highest PIC value of 0.401 an indication of the effectiveness of primers used in assessing genetic diversity of soursop. The lowest PIC value belonged to ISSR UBC 855 (0.179). The average gene diversity ranged from 0.176 to 0.401. The average effective allele number (Ne) was 1.5377 & the average Nei's gene diversity index (h) was 0.3079. The average Shannon information index (I) was 0.4556 indicating moderate level of genetic diversity. The fragment size of the thirteen primers ranged from 150 bp to 1500 bp (Table 3).

The highest genetic identity coefficient of 0.9856 recorded between 22 (325) & 23 (A) genotypes collected from FCRDS, Gannoruwa and between 06 (36) & 07 (44) genotypes collected from FRDI, Horana and this seemed to depend on the similar location and environmental conditions. Moreover, the highest genetic distance coefficient of 0.6440 with a maximum distance was observed between genotype 13 (RW 2918) from IFCO farm, Badulla and 43 (J-2) genotype collected from Jaffna. This might be due to variability in environmental conditions and the originality of the genotypes.

Table 3. ISSR data obtained for 50 genotypes including number of polymorphic bands, percentage of polymorphism, gene diversity, Shannon's information index and polymorphic information content (PIC) values

ISSR marker	Number of amplified bands	Number of polymorphic bands	Percentage of polymorphism %	Gene diversity	Shannon's information index	PIC
UBC 841T	11	11	100	0.373391	0.543109	0.373
UBC 841 Y	12	11	91.66	0.315367	0.472742	0.314
UBC 812 *	11	8	72.72	0.244936	0.369745	0.243
UBC 873	11	8	72.72	0.229145	0.348355	0.227
UBC 888	12	11	91.66	0.309183	0.467075	0.309
UBC 807	11	11	100	0.363191	0.534964	0.365
UBC 809	13	12	92.30	0.365954	0.529431	0.367
UBC 840a	8	8	100	0.401588	0.587213	0.401
UBC 810	10	9	90	0.32687	0.48026	0.327
UBC 848a	10	9	90	0.2021	0.32615	0.201
UBC 850a	12	10	83.33	0.34915	0.5045	0.349
TC 10G	9	7	77.77	0.326767	0.473822	0.320
UBC 855	9	4	44.44	0.176589	0.258722	0.179

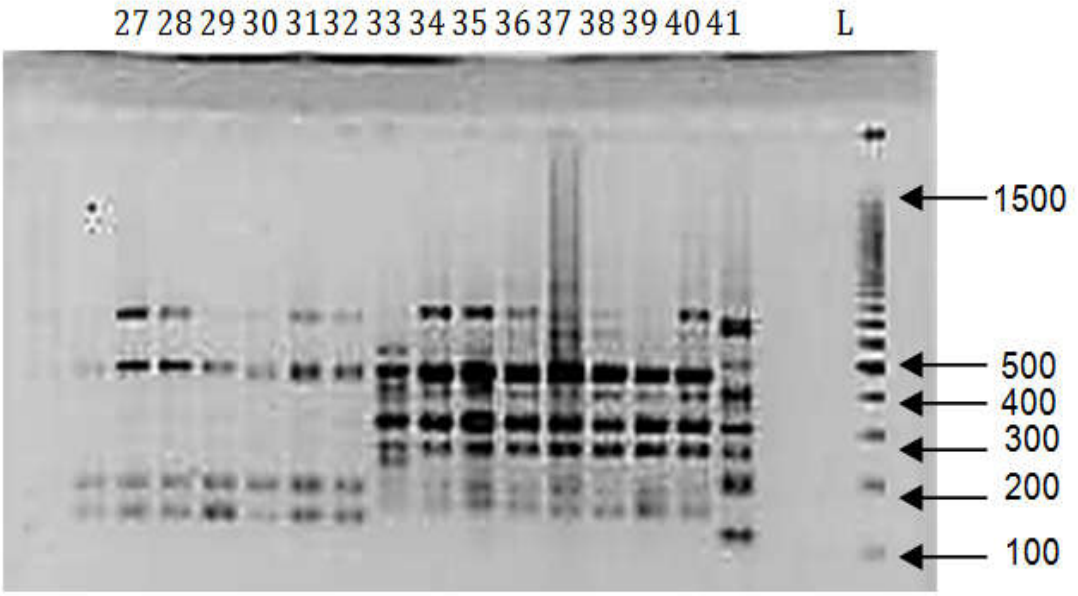

Fig. 2. Profile of the ISSR fragments amplified by primer UBC 841a for soursop (*Annona muricata* L.) genotypes belonging to germplasm collections and different locations in Sri Lanka. L indicates the 100 base pair DNA ladder (Promega, USA) and the number well indicated each genotype of soursop

(27-GK-175, 28-GK-159, 29-GK-170, 30-GK-197, 31-GK-90, 32-GK-167, 33-GK-168, 34-GK-29, 35-GK-25, 36-GK-195, 37-GK-68, 38-GK-172, 39-GK-30, 40-GK-171, 41-J-1)

A dendrogram based on UPGMA analysis is presented in Fig. 3. According to the dendrogram, fifty soursop genotypes were separated into four major clusters at 80% genetic similarity level (Fig. 3). In general, the samples collected from major germplasm locations in IFCO Farm, AF, HORDI, ARS, Girandurukotte and FRDI, Horana formed distinct clusters in the dendrogram exhibiting the geographical similarity within the genotypes and it seemed that they have the same genetic origin. The samples collected from different geographical locations and FCRDS, Gannoruwa genotypes grouped together indicating genetic relatedness among the genotypes.

Distinct separation of each genotype was exhibited by the cluster analysis where the dendrogram divided the fifty individuals in to four major clusters. Cluster one composed of genotypes from FRDI, Horana and divided into two sub clusters (A1 & A2). Sub cluster A1 consisted of genotype M2 (10) and genotype 01 (01) obtained from FRDI, Horana and sub cluster A2 with rest of the genotypes obtained from the same germplasm. The division within the same cluster may be due to unique morphological characters such as large fruits with higher brix values. These characters were prominently expressed by the two genotypes (M2 and 01) in sub cluster A1 compared to rest of the genotypes clustered in the sub cluster A2 with similar morphological characters such as leaf shape, number of fruits, size of the fruits etc. within the same germplasm. Cluster two composed of accessions from FCRDS, Gannoruwa together with genotypes collected from Jaffna (41, 42), Peradeniya (43), Kegalle (44), Awissawella (45), Gampola (46), Medawala (47), Kundasale (48), Kadugannawa (49), and Pilimathalawa (50) irrespective of the location where samples collected. Cluster two divided into two sub clusters as B1 with genotype 43 (Peradeniya) & B2 with genotypes obtained from germplasm collection at FCRDS, Gannoruwa (21, 22, 23, 24, 25, and 26) and genotypes obtained from Pilimathalawa (50), Medawala (47), Gampola (46), Awissawella (45) and Kegalle (44). These genotypes were obtained from home gardens representing different agro ecological regions of the country where soursop plants are abundantly available.

Cluster three included fourteen genotypes collected from ARS, Girandurukotte (27,28,29,30,31,32,33,34,35,36,37,38,39,40) grouped according to the geographical origin of the genotypes. The cluster provided evidence for

the close proximity and similar morphology (leaf type, fruit size, pulp colour, brix value) of the genotypes within the same geographical location sharing a common origin. Cluster four composed of genotypes collected from IFCO Farm, Badulla (11, 12, 13, 14, 15, 16, 17, 18) and AF, HORDI (19, 20) which are distant locations yet grouping together within the same cluster. Cluster four divided in to two sub clusters as sub cluster D1 and D2 with genotype 11 (RW 2899), 19 (Am1) in cluster D1 and genotype 12 (RW 2898), 13 (RW 2918) 14 (RW 2921), 15 (RW 654), 16 (SSV 659), 17 (SSV 640), 18 (SSV 649), 20 (Am 2) in Sub cluster D2. These soursop genotypes may be genetically distant relatives placed in the adjacent genetic groups.

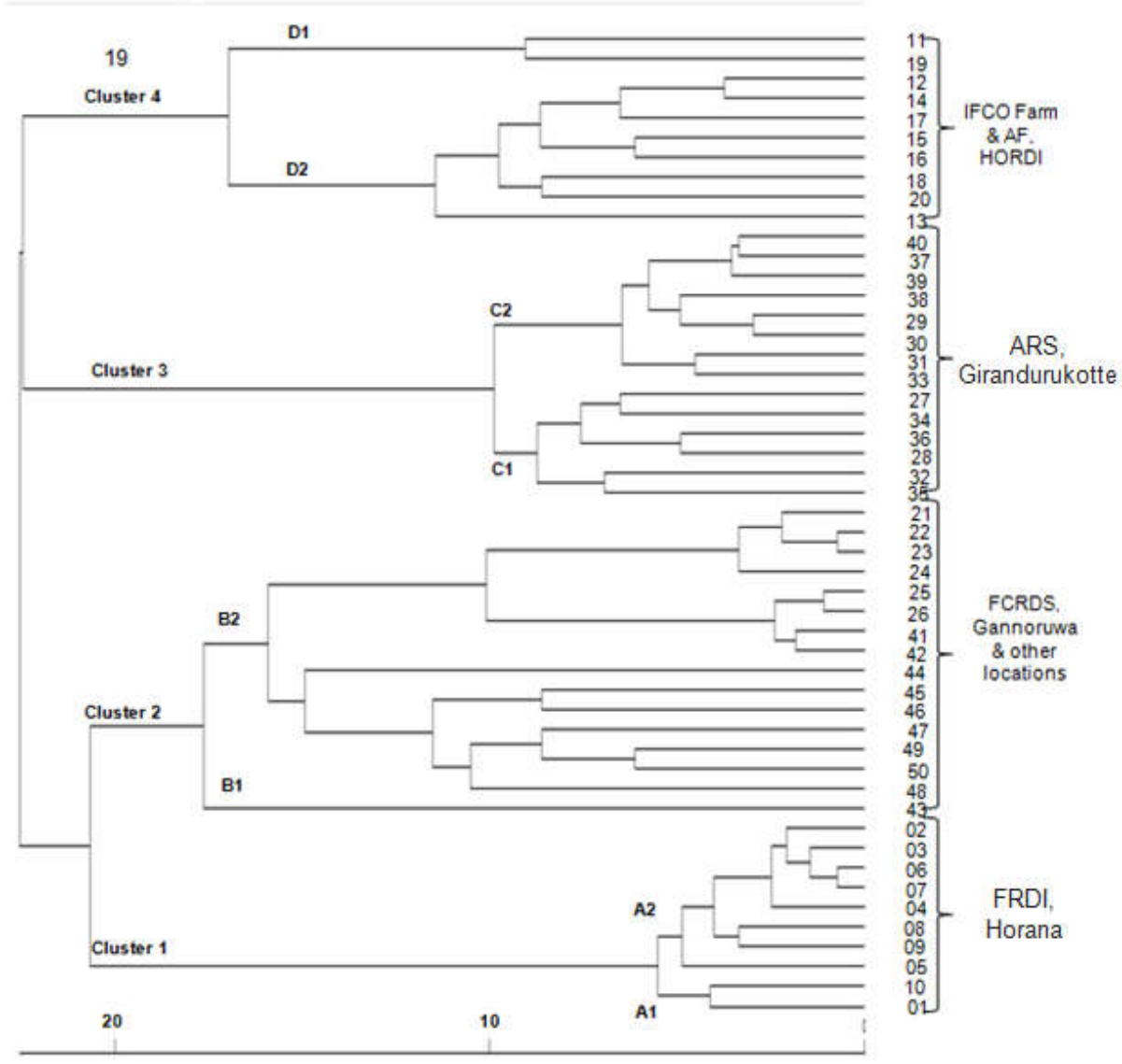

Fig. 3. Dendrogram constructed with UPGMA clustering method among 50 germplasm accessions of *Annona muricata* (L) using 13 ISSR markers

ARS-Girandurukotte;
FRDI-Horana
{Samples-IFCO Farm, Badulla –RW 2899(11),RW2898(12),RW2918(13),RW2921(14),RW654(15),SSV659(16),SSV640(17),SSV649(18),AF,HORDI-Am 01(19),Am 02(20), FRDI, Horana – H-01 (01),H-02 (02), H-M2 (10),H-19 (03),H-34 (05),H-24 (08),H-44 (06),H-36 (07),H-13 (09),H-30 (04) FCRDS,Gannoruwa –G-01(21),G-325(22),G-A(23),G-137(24),G-AS(25),G-41(26) J1(41), J2(42), PIL (50), AW(45), KG(44) , GAM (46),MED (47),KUN (48),KAD (49),PD (43) ARS,Girandurukotte-GK-171 (40), GK-170 (29), GK-175 (27),GK-197 (30), GK-90 (31), GK-167 (32), GK-159 (28),GK-168 (33),GK-172 (38), GK-29 (34), GK-195 (36), GK-68 (37),GK-30 (39),GK-25 (35)}

4. DISCUSSION

Tree species which are tropical and sub-tropical, are still in the primitive stage of domestication where their genetic resources are present in situ or ex situ on farms, home gardens, orchards or in natural populations [24]. Many of these plant species are a source towards achieving food and nutritional security [25] yet due to lack of attention, these plant species remain inadequately characterized and neglected by research and conservation [25]. Soursop is an underutilized fruit species in Sri Lanka [3] mostly confined to home gardens. The present study is the first attempt in comprehensive assessment of genetic diversity of available soursop germplasms using ISSR markers conducted in Sri Lanka. In the present study, thirteen primers of ISSR used to assess genetic diversity of fifty genotypes. The results revealed higher degree of genetic variation among soursop genotypes with 85% polymorphism. Diversity of a species plays a major role in adapting to environmental changes [26] yet genetic diversity revealed by the present study of soursop genotypes provided important insights of the existing genetic diversity prevailing within Sri Lanka.

The obtained dendrogram were able to display the grouping of soursop genotypes in an effective manner where no duplicates were identified within the studied genotypes. Discrepancies were evident with genotypes from different sites of collection found clustering together indicating no correlation between ISSR markers based clustering and their geographical origin. The first and third clusters were grouped in accordance with the geographical location describing the close genetic proximity of genotypes. Grouping of samples together in each germplasm collections may be due to the presence of a common genetic origin among the samples tested despite their geographical region. The results provided a clear evidence for the genetic diversity prevailed within the studied genotypes of *A. muricata*.

The polymorphic information content value provides the discriminating power of the locus using expressed number of alleles and the frequency of those alleles [27] being an important tool in representing the genetic diversity. Accordingly, the maximum PIC value for a dominant marker is 0.5 [28] and the PIC value generated in the present study by primer UBC 840a was 0.401. The higher PIC value represented useful information in screening the diversity and polymorphism among the studied soursop genotypes. The Shannon's Information Index value of 0.4556 and gene diversity value of 0.3079 also provided evidence for moderate to higher degree of diversity within the studied soursop genotypes. Soursop genotypes belonged to same germplasm collection site indicated highest genetic identity coefficient of 0.9856 between 22 (325) & 23 (A) genotypes collected from FCRDS, Gannoruwa and between 06 (36) & 07 (44) genotypes collected from FRDI, Horana. A study evaluating the genetic diversity of *Annona crassiflora* by Telles et al. (2003) reported that lower genetic distance among individuals within the same location may be due to sharing a common ancestor [29].

Assessing the genetic diversity of a species can aid in identifying alleles that might affect in the ability of an organism to survive or enable to prevail in its existing environment or in a more diverse habitat. Revealing such variations is valuable for germplasm conservation, individual population, variety and breed identification [30]. Annona species were able to generate moderate to higher degree of diversity in the previous studies conducted. Ronning et al. [31] evaluated genetic diversity of atemoya, sugar apple and cherimola using 15 RAPD primers and observed highly distinct polymorphic patterns. Five RAPD primers were also utilized to assess the genetic diversity of 11 sugar apple genotypes by Bharad et al. [32] where 73% of polymorphism resulted. Another study using thirty ISSR markers to reveal the genetic diversity of four annona species generated 58% polymorphism dividing in to three major clusters [33].

Presently ISSR markers are routinely used in ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences [34,35]. The utility of ISSR markers in estimating the genetic diversity has been demonstrated in many crops as well as in underutilized crop species. Thirty ISSR markers have been able to generate 58 percent polymorphism and effective clustering of the Annona species in South Andamans and proved to be suitable in characterization of annona species as demonstrated by Ahmad in 2010 [33]. Similar studies have been conducted in assessing the genetic diversity in *Annonaceae* species. As a comparison, 15 selected primers were used to evaluate genetic diversity of *Annona crassiflora* and these primers generated 140 bands of which 123 (87.8 percent) were polymorphic [36]. In contrast, 15 primers were

selected to analyze genetic variation between *A. cherimola, A. squamosa* and their hybrids where a total of 92 bands were produced while 86 bands (93.5 percent) were polymorphic [24].

Similarly in the present study, 13 ISSR markers were able to generate 139 bands where 118 found to be polymorphic with 86 percent polymorphism. The amplification may depend on the random marker sequence and their compatibility within the genomic DNA [37] which revealed a higher degree of polymorphism among the studied genotypes. ISSR markers were able to exhibit a unique banding pattern for each marker generating different amplification results and it highlighted the efficiency of ISSR markers in identifying the genetic diversity and determining polymorphisms in soursop genotypes.

Different results were also reported in previous studies in revealing the genetic diversity of soursop. This may be due to the variability in marker efficiency which may significantly vary in different plant types. The extent of genetic variation of the studied plant species and the primer sequence may affect the degree of polymorphism resulted from each marker. Except for one primer (UBC 855) all the other 12 primers were able to generate more than 70 percent polymorphism in the studied soursop genotypes in Sri Lanka. The clustering pattern also revealed genetic diversity existed among soursop genotypes effectively. None of the evaluated accessions of soursop were previously evaluated for genetic diversity.

Plant genetic resources plays an important role in biodiversity conservation by providing valuable genetic variations which allows to develop new and improved cultivars [38] yet many tree species are increasingly vulnerable to loss of genetic variability with the changes of climatic factors [39]. Advantage of species with rich genetic diversity like *Annona muricata* is that it can easily adapt and conform to a wide range of environmental conditions in comparison to none genetically diverse species as those of the domesticated crops [33]. The determination of genetic variability is important for breeders when developing varieties with better characteristics and productivity [40]. The results generated from this study can be combined with a detailed morphological characterization of soursop and this information can be used in future breeding programs to develop elite soursop cultivars and varieties in Sri Lanka.

Previously Simple Sequence Repeat (SSR) molecular markers developed for *A. cherimola* were used to assess the genetic diversity of soursop germplasm resources where no significant variation was observed [41]. The present study utilized ISSR molecular markers to reveal the genetic diversity among selected soursop germplasm within Sri Lanka and to our knowledge it is the first such study conducted to identify the genetic diversity among the soursop genotypes. Higher degree of genetic variation was observed among the studied genotypes of soursop.

Studying the genetic diversity of *Annona muricata* germplasm in Sri Lanka carried out using ISSR markers has been effective in generating considerable degree of polymorphism. As the ISSR markers correspond to gene rich regions linking coding regions [42], incorporation of a relatively higher number of ISSR markers will be effective in revealing a higher degree of genetic diversity within a species and in between species. This approach will be useful in future work with relation to genetic diversity assessment in soursop.

5. CONCLUSION

Characterization of the available germplasm of soursop is an important step towards future breeding, varietal development and genetic resources conservation programs in Sri Lanka. As the first approach, this study utilized fifty genotypes selected from different germplasms and locations to identify the existing genetic diversity within the species. In the present study, limited number of samples and ISSR markers were able to generate a considerable degree of polymorphism giving insights towards an intense characterization program aiming crop development. Future studies can be aimed at a comprehensive phenotypic assessment and incorporating more molecular tools and techniques as whole genome sequencing for soursop germplasm characterization. This approach would widen the scope towards breeding of elite soursop varieties in Sri Lanka.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Suratman S, Pitoyo A, Mulyani S, Suranto S. Assessment of genetic diversity among soursop (*Annona muricata*) populations from Java, Indonesia using RAPD markers. Biodiversitas. 2015;16(2):247-253.
DOI: 10.13057/biodiv/ d160220
2. Badrie N, Schauss AG. Soursop (*Annona muricata* L.): Composition, nutritional value, medicinal uses and toxicology. Bioactive Foods in Promoting Health. In: Watson RR, Preedy VR (Eds.). Academic Press, Oxford. 2009;621–643.
3. Dahanayake N. Some neglected and underutilized fruit-crops in Sri Lanka. Sri Lanka International Journal of Scientific and Research Publications. 2015;5(2): 2250-3153.
4. Padmini SMPC, Pushpakumara DKNG, Samarasekera R. Morphological variation in selected *Annona muricata* L. (Soursop) germplasm in Sri Lanka. Tropical Agricultural Research. 2013;24:362-374.
DOI: 10.4038/tar.v24i4.8021
5. Kim GS, Zeng L, Alali F, Rogers LL, Wu FE, McLaughlin JL. Muricoreacin and murihexocin C, mono-tetrahydrofuran acetogenins, from the leaves of *Annona muricata* in honour of professor GH Neil Towers 75th Birthday. Phytochemistry. 1998;49:565–571.
DOI: 10.1016/S0031-9422(98)00172-1
6. Yu J, Gui H, Luo X, Sun L, Zhu P, Yu Z. Studies on the chemical constituents of *Annona muricata*. Yao Xue Xue Bao. 1997;32:431–437.
7. Abbo ES, Olurin T, Odeyemi G. Studies on the storage stability of Soursop (*Annona muricata* L.) juice. African Journal of Biotechnology. 2006;5:108-112.
8. Bianca W. Bertoni, Mariana P. de C. Telles, Milena G. Malosso, Simone C. Z. Torres, José O. Pereira, Mirian V. Lourenço, Suzelei de C. França, Ana M. S. Pereira. Genetics and Molecular Biology. 2010;33(3):532–538.
DOI: 10.1590/S1415-47572010005000068
9. Kumar Mukesh, Fougat Ranbir, Sharma Anil, Kulkarni Kalyani, Ramesh, Mistry Jigar, Sakure Amar, Kumar Sushil. Phenotypic and molecular characterization of selected species of Plantago with emphasis on *Plantago ovata*. Australian Journal of Crop Science. 2014;8:1639–1647.
10. Gajera BB, Kumara N, Singha AS, Punvara BS, Ravikirana R, Subhasha N, Jadeja GC. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Industrial Crops and Products. 2010;32:491–498.
DOI:https://doi.org/10.1016/j.indcrop.2010.06.021
11. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research. 1990;18:7213–7218.
DOI: 10.1093/nar/18.24.7213
12. Gwanama C, Labuschagne MT, Botha AM. Analysis of genetic variationin *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. Euphytica. 2000;113(1):19–24.
DOI: 10.1023/a:1003936019095
13. Kapteyn J, Simon JE, Janick J, Whipkey A. The use of RAPDs for assessment of identity, diversity and quality of Echinacea. Trends in New Crops and New Uses. ASHS Press, Alexandria, VA. 2002;509–513.
14. Cong-Ying Yuan, Ping Wang, Pang-Pang Chen, Wen-Jun Xiao, Cheng Zhang, Shuai Hu, Ping Zhou, Hong-Ping Chang, Zhuang He, Rong Hu, Xiu-Tao Lu, Jia-Zhuo Ye, Xin-Hong Guo. Genetic diversity revealed by morphological traits and ISSR markers in 48 Okras (*Abelmoschus escullentus* L.). Physiologyand Molecular Biology Plants. 2015;3:359–364.
DOI: 10.1007/s12298-015-0303-5
15. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20(2):176-83.
DOI: 10.1006/geno.1994.1151
16. Meloni Marilena. Genetic variation in five Mediterranean populations of *Juniperus*

phoenicea as revealed by inter-simple sequence repeat (ISSR) markers. Annals of Botany. 2006;97(2):299-304.
DOI: 10.1093/aob/mcj024

17. Godwin ID, Aitken EA, Smith LW. Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis. 1997;18(9):1524-1528.
DOI: 10.1002/elps.1150180906
18. Sarmiento L, Perez-Almeida L, Diaz B, Diaz B, Alvarez H, Viera W. Molecular marker based characterization of Ecuadorian dry forest tamarind plus trees. Bioagro. 2017;29(3):153-162.
19. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12(1):13-15.
20. Yeh FC, Yang RC, Boyle T. POPGENE Version 1.32: Microsoft window-based freeware for population genetics analysis. University of Alberta, Edmonton; 1999.
21. Sneath PHA, Sokal RR. Numerical taxonomy. W.H. Freeman and Company, San Francisco; 1973.
22. Nei M, Li W. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci.1979;76:5269–5273.
23. Chesnokov YV, Artemyeva A. Evaluation of the measure of polymorphism information of genetic diversity. Agric Biol. 2015;50(5):571-578.
24. Van Zonneveld M, Scheldeman X, Escribano P, Viruel MA, Van Damme P, et al. Mapping genetic diversity of cherimoya (*Annona cherimola* Mill.): Application of spatial analysis for conservation and use of plant genetic resources. PLoS ONE. 2012;7(1):29845.
DOI: 10.1371/journal.pone.0029845
25. Dansi A, Vodouhe R, Azokpota P, Yedomonhan H, Assogba P, Adjatin A, Loko YL, Dossou-Aminon I, Akpagana K. Diversity of the neglected and underutilized crop species of importance in Benin. The Scientific World Journal. 2012;932947:19.
DOI: 10.1100/2012/932947
26. Dawson IK, Lengkeek A, Weber JC, Jamnadass R. Managing genetic variation in tropical trees; linking knowledge with action in agroforestry ecosystems for improved conservation and enhanced livelihoods. Biodiversity and Conservation. 2009;18:969-986.
27. Akcali Giachino RR. Investigation of the genetic variation of anise (*Pimpinella anisum* L.) using RAPD and ISSR markers. Genetic Resources and Crop Evolution. 2020;67:763-780.
DOI: 10.1007/S10722-019-00861-Y
28. Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome. 2001;44(5):763–772.
29. Telles MPC, Valva FD, Bandeira LF, Coelho ASG. Characterizacao genetica de populacoes naturais de araticunzeiro (*Annona crassiflora* Mart.-Annonaceae) no Estado de Goias. Revista Brasileira de Botanica. 2003;26:123-129.
30. Duran C, Appleby N, Edwards D, Batley J. Molecular genetic marker: Discovery, applications, data storage and visualization. Current Bioinformatics. 2009;4:16-27.
31. Ronning CM, Schnell RJ, Gazit S. Using Randomly Amplified Polymorphic DNA (RAPD) markers to identify annona cultivars. Journal of the American Society for Horticultural Science. 1995;120(5):726-729.
DOI: 10.21273/JASHS.120.5.726
32. Bharad SG, Kulwal PL, Bagal SA. Genetic diversity study in *Annona squamosa* by morphological, biochemical and RAPD markers. Acta Horticulturae. 2009;839:615-623.
33. Ahmad I, Bhagat S, Sharma TVRS, Kumar K, Smachalam P, Srivastava RC. ISSR and RAPD marker based DNA fingerprinting and diversity assessment of Annona species in South Andamans. Indian Journal of Horticulture. 2010;67(2): 147-151.
34. Iqbal MJ, Aziz N, Saeed N, Zafar A, Malik Y. Genetic evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 1997;94:139-44.
35. Escibano P, Viruel MA, Hormaza JI. Characterization and cross species amplification of microsatellite markers in cherimola Mill. Annonaceae. Mol. Ecol. Notes. 2011;4:746-48.
36. Cota LG, Vieira Fabio, Junior AF, Brandao Murilo, Santana KNO, Lisboa Guedes, Marcio Oliveira Dario. Genetic diversity of *Annona crassiflora* (Annonaceae) in

northern Minas Gerais state. Genetics and Molecular Research. 2011;10(3):2172-2180.
DOI: 10.4238/vol10-3gmr1188

37. Upadhyay A, Jayadev K, Manimekalai R, Parthasarathy VA. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. Scientia Horticulturae. 2004;99(3-4):353-362.
DOI: 10.1016/S0304-4238(03)00103-1

38. Halewood Michael, Chiurugwi Tinashe, Hamilton Ruaraidh, Kurtz Brad, Marden Emily, Welch Eric, Michiels Frank, Mozafari Javad, Sabran Muhamad, Patron Nicola, Kersey Paul, Bastow Ruth, Dorius Shawn, Dias Sonia, McCouch Susan, Powell Wayne. Plant genetic resources for food and agriculture: Opportunities and challenges emerging from the science and information technology revolution. New Phytologist. 2018;217.
DOI: 10.1111/nph.14993

39. Dawson IK, Vinceti B, Weber JC, Neufeldt H, Russell J, et al. Climate change and tree genetic resource management: Maintaining and enhancing the productivity and value of smallholder tropical agroforestry landscapes. A review. Agroforestry Systems. 2011;81:67-78.

40. Kumar M, Ponnuswami V, Rajamanickam C, Preethi T. Assessment of genetic diversity in tamarind (*Tamarindus indica* L.) using random amplified polymorphic DNA markers. SAARC Journal of Agriculture. 2015;13(1):27-36.
DOI: 10.3329/sja.v13i1.24177

41. Thanachseyan P, Smarasinghe WLG, Thirukkumaran G. Diversity of *Annona muricata* L. through molecular characterization using SSR markers. International Journal of New Technology and Research. 2017;3(9):57-62.

42. Kojima T, Nagaoka T, Noda K, Ogihara Y. Genetic linkage map of ISSR and RAPD markers in Eincorn wheat in relation to that of RFLP markers. Theoretical and Applied Genetics. 1998;96:37-45.
DOI: 10.1007/s001220050706

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